

## Role of Biofilms in Chronic Surgical Wound Infections

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### Abstract

Chronic surgical wound infections (CSWIs) remain a major clinical challenge due to biofilm formation, which contributes to antibiotic resistance and delayed healing. Despite growing recognition of biofilms in persistent infections, limited data exist on their prevalence and impact in low-resource settings. This prospective, cross-sectional study was conducted at 250-bedded district hospital and Sreepur Upazilla Health Complex in Magura district of Bangladesh, between July 2024 and June 2025, to evaluate the role of biofilms in CSWIs, assessing their association with wound chronicity, microbial resistance, and clinical outcomes. A total of 287 patients with CSWIs were enrolled via purposive sampling. Wound swabs were analyzed using culture, Gram staining, and microscopy to detect biofilms. Clinical and microbiological data were recorded and analyzed. Among 287 CSWI patients (mean age  $52.4 \pm 12.8$  years), biofilm prevalence was 78.4%, significantly higher in diabetics (84.6% vs 68.2%,  $p=0.003$ ). *Staphylococcus aureus* (32.4%) and *Pseudomonas aeruginosa* (24.7%) dominated biofilm-positive isolates, showing 85.2% antibiotic resistance versus 41.9% in biofilm-negative cases ( $p<0.001$ ). Biofilm-associated wounds required longer healing ( $42.6 \pm 18.3$  vs  $28.5 \pm 14.7$  days,  $p<0.05$ ) and showed 3.1-fold increased recurrence risk ( $p<0.05$ ). Diabetes mellitus (OR=2.6) and poor wound care (OR=3.1) predicted biofilm formation. Biofilms were highly prevalent in CSWIs, strongly associated with antibiotic resistance and delayed healing. Diabetes mellitus and inadequate wound care emerged as key risk factors. These findings underscore the need for biofilm-targeted management strategies in surgical wound care, particularly for high-risk populations in resource-limited settings.

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### Introduction

Chronic surgical wound infections (CSWIs) represent a significant burden in clinical practice, leading to prolonged hospitalization, increased healthcare costs, and substantial patient morbidity.<sup>1</sup> Despite advances in aseptic techniques and antimicrobial therapy, a considerable proportion of surgical wounds fail to heal, often due to persistent microbial infections facilitated by biofilm formation.<sup>2</sup> Biofilms are structured microbial communities encased in a self-produced extracellular matrix, which enhances bacterial resistance to antibiotics and host immune responses.<sup>3,4</sup> This resistance complicates treatment, contributing to recurrent and chronic infections.<sup>5</sup> The role of biofilms in CSWIs has gained increasing attention, with studies indicating that 60–80% of chronic wounds harbor biofilm-forming bacteria.<sup>6</sup> These biofilms impair wound healing by sustaining inflammation, delaying tissue repair, and promoting antimicrobial resistance.<sup>7</sup> Common pathogens such as *Staphylococcus aureus*, *Pseudomonas*

*aeruginosa*, and *Escherichia coli* frequently form biofilms, further complicating infection management.<sup>8</sup> In low-resource settings, where access to advanced diagnostics and targeted therapies is limited, biofilm-associated infections pose an even greater challenge.<sup>9</sup> Several factors contribute to biofilm

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formation in surgical wounds, including poor wound care, prolonged antibiotic use, and underlying comorbidities like diabetes mellitus.<sup>10</sup> Diabetic patients, in particular, exhibit a higher susceptibility to biofilm infections due to impaired immune function and microvascular complications.<sup>11</sup>

Additionally, inappropriate empiric antibiotic use accelerates resistance, making biofilm eradication more difficult.<sup>12</sup> Despite these challenges, routine detection of biofilms remains uncommon in many healthcare facilities, particularly in resource-constrained regions like Bangladesh.<sup>13</sup>

This study aimed to investigate the prevalence, microbiological profile, and clinical impact of biofilms in CSWIs at two healthcare facilities in Magura, Bangladesh. By analyzing biofilm-associated antibiotic resistance patterns and risk factors, we seek to provide evidence for improved wound management strategies. The findings may guide clinicians in adopting early biofilm detection and targeted therapies to reduce chronic wound complications.

## Methods

This prospective, cross-sectional study was conducted at a 250-bedded district hospital and Sreepur Upazilla Health Complex in Magura district of Bangladesh, from July 2024 to June 2025. The study population comprised 287 patients with chronic surgical wound infections (CSWIs) lasting >4 weeks, purposively sampled to ensure representation of diverse wound types and severities.

**Inclusion criteria:** Patients aged  $\geq 18$  years with clinically diagnosed CSWIs (pus/discharge, delayed healing, erythema) were included. Those with a history of surgical intervention within the past 4 weeks and a willingness to provide informed consent were

eligible. Pregnant women and immunocompromised patients (e.g., HIV/AIDS) were excluded to avoid confounding effects.

**Exclusion criteria:** Individuals with non-surgical wounds (e.g., traumatic, burns), acute infections (<4 weeks), or those receiving systemic antibiotics within 48 hours before sampling were excluded. Patients unwilling to participate or with incomplete follow-up data were also omitted.

Wound swabs were collected aseptically and processed for microbiological analysis (Gram staining, culture, biofilm detection via Congo red assay). Demographic, clinical, and treatment data were recorded using a structured questionnaire.

Data was recorded in the data sheet and analyzed using SPSS version 23.0. Descriptive statistics, Student's independent t-tests and Chi-square tests were done to observe the differences. Logistic regression determined associations between biofilm presence, antibiotic resistance, and wound outcomes. A p-value  $<0.05$  was considered statistically significant.

## Results

In the present study, microbiological analysis identified *Staphylococcus aureus* (32.4%) and *Pseudomonas aeruginosa* (24.7%) as the predominant pathogens, with 78.4% of wounds showing biofilm formation (Table-I). Notably, length of days to heal was found much higher in biofilm-positive group ( $p<0.05$ ). Diabetic patients demonstrated significantly higher biofilm prevalence (84.6%) compared to non-diabetics (68.2%) ( $p<0.05$ ). Biofilm-positive isolates exhibited substantially greater antibiotic resistance (85.2%) compared to non-biofilm producers (41.9%) ( $p<0.001$ ) (Table-II). The most frequent resistances occurred against

ciprofloxacin (69.3%), ceftriaxone (63.1%), and gentamicin (56.9%) in biofilm-associated bacteria (Table-III). Multivariate analysis identified three independent risk factors for biofilm development: diabetes mellitus (OR=2.6, 95%CI=1.4-4.8; p<0.05), inadequate wound care (OR=3.1, 95%CI=1.7-5.6; p<0.001), and prior antibiotic use (OR=2.2, 95%CI=1.2-4.0; p<0.05) (Table-IV). Clinical outcomes were significantly worse in biofilm-positive cases, demonstrating extended healing times (42.6±18.3 vs. 28.5±14.7 days; p<0.001), longer hospital stays (14.2±6.8 vs 9.5±4.3 days, p<0.001), and higher rates of surgical debridement (43.6% vs. 19.4%; p<0.001). Additionally, biofilm-positive wounds showed nearly triple the recurrence rate (29.8% vs. 12.9%; p<0.05) (Table-V).

**Table-I:** Microbial isolates from chronic surgical wounds

Microorganism	Total (Percentage)	Biofilm-Positive (Percentage)
<i>Staphylococcus aureus</i>	93 (32.4%)	81 (87.1%)
<i>Pseudomonas aeruginosa</i>	71 (24.7%)	58 (81.7%)
<i>Escherichia coli</i>	45 (15.7%)	32 (71.1%)
Others	78 (27.2%)	44 (56.4%)

**Table-II:** Association between biofilm presence and wound characteristics

Characteristic	Biofilm		p-value
	Positive	Negative	
	(n=225)	(n=62)	
Healing duration (days)	42.6±18.3	28.5±14.7	<0.05
Diabetic patients	117 (84.6%)	21 (15.4%)	<0.05
Antibiotic resistance	192 (85.2%)	26 (41.9%)	<0.001

Independent t-test and chi-square test were applied

**Table-III:** Antibiotic resistance patterns in biofilm vs. non-biofilm isolates

Antibiotic	Biofilm		p-value
	Positive Frequency (Percentage)	Negative Frequency (Percentage)	
Ciprofloxacin	156 (69.3%)	18 (29.0%)	<0.001
Ceftriaxone	142 (63.1%)	15 (24.2%)	<0.001
Gentamicin	128 (56.9%)	12 (19.4%)	<0.001

Chi-square test was applied.

**Table-IV:** Multivariate analysis of biofilm risk factors

Factors	Adjusted OR (95% CI)	p-value
Diabetes mellitus	2.6 (1.4-4.8)	<0.05
Inadequate wound care	3.1 (1.7-5.6)	<0.001
Prior antibiotic use	2.2 (1.2-4.0)	<0.05

Logistic regression analysis was performed.

**Table-V:** Comparison of wound outcomes by biofilm status

Outcome	Biofilm-Positive	Biofilm-Negative	p-value
Hospital stays (days)	14.2 ± 6.8	9.5±4.3	<0.001
Surgical debridement	98 (43.6%)	12 (19.4%)	<0.001
Infection recurrence	67 (29.8%)	8 (12.9%)	<0.05

Chi-square test and Student's t-test were applied.

## Discussion

Our study provides compelling evidence about the critical role of biofilms in chronic surgical wound infections (CSWIs) within a Bangladeshi healthcare setting. Our findings demonstrate that 78.4% of CSWIs harbored biofilms, aligning with global reports indicating 60-80% prevalence in chronic wounds.<sup>1,2</sup> The predominance of *Staphylococcus aureus* (32.4%)

and *Pseudomonas aeruginosa* (24.7%) as biofilm formers corroborates existing literature identifying these as key pathogens in persistent wound infections.<sup>3,8</sup> Notably, our observed biofilm prevalence was significantly higher in diabetic patients (84.6% vs 68.2%), supporting the well-established link between diabetes and impaired wound healing.<sup>11</sup> The striking antibiotic resistance patterns we observed (85.2% in biofilm-positive vs 41.9% in biofilm-negative isolates) reinforce the protective role of biofilm matrices against antimicrobial agents.<sup>7</sup> Our resistance rates for ciprofloxacin (69.3%), ceftriaxone (63.1%), and gentamicin (56.9%) in biofilm-associated bacteria exceed those reported in similar studies from neighboring countries<sup>13</sup> potentially reflecting regional antibiotic prescribing practices. These findings underscore the urgent need for antimicrobial stewardship programs in wound care management.<sup>14</sup>

Our clinical outcome data revealed that biofilm-positive wounds required 50% longer healing times (42.6 vs 28.5 days) and had triple the recurrence rates (29.8% vs 12.9%). These results mirror findings from European cohorts<sup>5</sup> and validate biofilms as a key contributor to wound chronicity. The extended hospital stays (14.2 vs 9.5 days) associated with biofilm infections highlight their substantial economic burden, particularly relevant for resource-limited settings.<sup>9</sup> The identified risk factors were: diabetes (OR=2.6), inadequate wound care (OR=3.1), and prior antibiotic use (OR=2.2); this report provides actionable targets for prevention strategies. Our diabetes association aligns with known pathophysiological mechanisms including impaired neutrophil function and microangiopathy.<sup>10</sup> The strong link with inadequate wound care emphasizes the importance of proper wound management protocols in preventing biofilm formation.<sup>6</sup>

This study was conducted at two centers in Bangladesh, potentially limiting generalizability. The purposive sampling may introduce selection bias, and biofilm detection relied on conventional methods rather than advanced molecular techniques. Additionally, the cross-sectional design precludes assessment of causal relationships between biofilm formation and clinical outcomes.

## Conclusion

This study demonstrates that biofilms are highly prevalent (78.4%) in chronic surgical wound infections in Bangladesh, significantly contributing to antibiotic resistance and delayed healing. The strong association with diabetes and poor wound care underscores the need for biofilm-targeted strategies in wound management. Routine biofilm detection and antimicrobial stewardship should be prioritized in resource-limited settings to improve outcomes. These findings call for further research into cost-effective anti-biofilm interventions tailored for developing healthcare systems. Healthcare facilities should implement routine biofilm screening for chronic wounds using affordable diagnostic methods. Diabetic patients require prioritized wound surveillance and targeted anti-biofilm therapies. Antimicrobial stewardship programs should be strengthened to combat resistance. Future research should develop cost-effective biofilm disruption techniques for resource-limited settings.

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