Original Article

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Bacterial Profile and Biofilm Detection in Burn Wound Isolates Comparing Three Phenotypic Methods: A Cross-sectional Study from a Tertiary Care Hospital, Jamnagar, Gujarat, India

Microbiology Section

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ABSTRACT

Introduction: Burn wounds are highly susceptible to bacterial infections, particularly from organisms that produce biofilms and exhibit multidrug resistance. These biofilms make infections more difficult to treat by protecting bacteria from antibiotics and slowing the healing process. Identifying the microorganisms involved and understanding their resistance patterns are essential for guiding treatment and improving outcomes in burn patients.

Aim: To investigate the microbial profile, antibiotic susceptibility, and biofilm production in bacterial isolates from burn wound infections in patients at a tertiary care hospital.

Materials and Methods: This cross-sectional study was conducted in the Department of Microbiology, Shri M.P. Shah Government Medical College, Jamnagar, Gujarat, India, over a one-year period (October 2018-September 2019). A total of 100 samples were collected from patients with burn wounds, yielding 48 bacterial isolates. Swab samples were cultured for bacterial growth, identified using standard biochemical methods, and tested for antibiotic susceptibility using the Kirby-Bauer disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Biofilm production was assessed using the Tissue Culture Plate (TCP), tube adherence, and Congo Red Agar (CRA) methods. Demographic details including age, sex, type, and extent of burns were recorded. Data were analysed using Statistical Package for the Social Sciences (SPSS) version 24.0, applying the Chi-square test, with p-values <0.05 considered statistically significant.

Results: Of the 100 burn wound specimens, 40 (40.0%) showed positive cultures, yielding a total of 48 bacterial isolates. The predominant pathogen was *Pseudomonas aeruginosa* (17; 35.4%), followed by *Staphylococcus aureus* (12; 25.0%), with methicillin resistance identified in 7 (58.3%) of the *S. aureus* isolates. Biofilm formation by the TCP method was detected in 38 (79.2%) isolates. Multidrug resistance (MDR) was observed in 34 (70.8%) isolates, of which 33 (97.1%) were biofilm producers, compared with 3 (21.4%) among non Multidrug Resistant Organisms (MDROs) (χ^2=30.25, p-value <0.001). Deep burns accounted for 25 (52.1%) culture-positive cases and superficial burns for 23 (47.9%), with biofilm positivity rates of 20 (80.0%) and 18 (78.3%), respectively.

Conclusion: *Pseudomonas aeruginosa* and *Staphylococcus aureus* were the most common bacterial isolates from burn wound infections, with a notable prevalence of MDR strains. Biofilm production was frequently observed, particularly among *Pseudomonas aeruginosa* isolates, and showed a strong association with multidrug resistance. These findings highlight the importance of considering biofilm formation when managing burn wound infections, as it plays a critical role in antimicrobial resistance and may contribute to treatment failure.

Keywords: Antibacterial agents, Bacterial, Biofilms, Drug resistance, Multiple, Wound healing

INTRODUCTION

Burn injuries are among the most severe forms of trauma, contributing significantly to morbidity and mortality worldwide. Their pathophysiology involves destruction of the skin, a vital barrier against environmental pathogens. Once this protective layer is compromised, patients become highly susceptible to infections due to continuous exposure to microorganisms in the environment. The risk and severity of infections increase with the extent and depth of the burn wound and remain a major cause of mortality in burn patients. Mortality rates rise proportionally with deeper tissue involvement and larger burn surface areas [1]. Burn injuries may result from heat, electricity, chemicals, friction, or radiation, each causing different levels of tissue damage. They account for approximately 180,000 deaths annually worldwide, with the majority occurring in low- and middle-income countries where access to specialised care is limited [2]. The treatment of burns imposes a substantial financial burden due to prolonged recovery, multiple procedures, and loss of productivity, underscoring the need for improved prevention and management strategies.

Infections in burn wounds are often complicated by the formation of biofilms-clusters of microorganisms attached to a surface and enclosed within a matrix rich in polysaccharides and extracellular DNA. Biofilm formation confers inherent resistance to antimicrobial agents and provides greater protection against host immune responses [3]. Organisms within biofilms typically exhibit altered growth patterns, reduced metabolic activity, and increased expression of virulence factors, making them significantly less susceptible to standard treatments than their free-floating (planktonic) counterparts [3]. In burn wounds, biofilms can sustain infection, prolong hospitalisation, increase healthcare costs, and raise the risk of severe complications such as sepsis and multiorgan failure [3]. Therefore, recognising and addressing biofilm formation is crucial when developing effective treatment strategies.

Burn wound infections remain a major cause of illness and death, particularly in resource-limited settings, emphasising the need for improved diagnostic approaches to guide patient care.

This study aimed to investigate the microbial profile, antibiotic susceptibility, and biofilm production in bacterial isolates from burn

wound infections, and to compare three phenotypic methods for biofilm detection. The primary objective was to determine the microbial profile of bacterial isolates from pus samples of burn wound infections. The secondary objectives were to assess biofilm production using the microtitre plate method, tube adherence method, and CRA method, and to compare the effectiveness of these methods to identify the most reliable approach.

MATERIALS AND METHODS

This was a cross-sectional study carried out in the Department of Microbiology, Shri M.P. Shah Government Medical College, Jamnagar, Gujarat, India, over a one-year period from October 2018 to September 2019. Ethical approval was obtained from the Institutional Ethics Committee (IEC) of Shri M.P. Shah Government Medical College, Jamnagar (IEC/Certi/152/09/2018). Written informed consent was obtained from all participants or their legal guardians prior to enrolment. Patient confidentiality and anonymity were maintained throughout the study.

Sample size: This was a time-bound observational study in which all patients admitted to the burns ward during the study period (October 2018 to September 2019) were included. A total of 100 burn wound samples were collected, yielding 48 bacterial isolates. As the study was time-bound, no formal sample size calculation was performed; instead, all eligible patients admitted during the study period were included.

Inclusion criteria: All patients admitted to the burns ward during the study period were eligible for inclusion, irrespective of age or sex. Patients with burn wounds of any degree—first, second, or third—were included, provided that they (or their legal guardians, in the case of minors) were willing to provide written informed consent or assent.

Exclusion criteria: Patients with non burn-related infections; those who had received systemic antibiotics within 48 hours prior to sample collection; and individuals with immunocompromised conditions or undergoing treatment for chronic systemic illnesses such as cancer or immunosuppressive therapy were excluded. Patients who declined to provide written informed consent were also excluded from the study.

Study Procedure

1. **Data collection:** A predesigned proforma [Annexure I] was used to document patient information, including:

- **Demographic details:** Name, registration number, age, sex, date of admission, date of discharge, and duration of hospital stay.
- **Clinical details:** Type and degree of burn, Total Body Surface Area (TBSA) involved, and treatment received.

2. **Sample collection:** Burn wound swabs were collected using sterile cotton swabs after removing the gauze bandage and before routine dressing. Samples were immediately transported to the microbiology laboratory to minimise contamination.
3. **Laboratory procedures:** All laboratory procedures were performed according to the methods described by Koneman et al., and the Clinical and Laboratory Standards Institute (CLSI) guidelines [4,5].

Day 1

- **Direct microscopy:** Pus samples collected from burn wounds were used for microbiological analysis. Smears were prepared from each sample, Gram-stained, and examined to assess bacterial morphology and the presence of pus cells.
- **Culture:** Samples were inoculated onto nutrient agar, MacConkey agar, and blood agar. If fastidious organisms were suspected based on clinical or microscopic findings, chocolate agar was also used. All plates were incubated at 37°C for 24-48 hours.

Day 2

- **Culture examination:** Plates were examined for bacterial growth, including colony size, shape, surface texture, opacity, and haemolysis.
- **Gram staining of colonies:** Performed to confirm bacterial morphology.
- **Biochemical identification:** Bacterial isolates were identified using standard biochemical tests.
- **Motility testing (Hanging drop method):**
  - *E. coli*: Motile
  - *K. pneumoniae*: Non motile
  - *P. mirabilis*: Highly motile
  - *P. vulgaris*: Highly motile
  - *P. aeruginosa*: Motile
  - *S. aureus*: Non motile

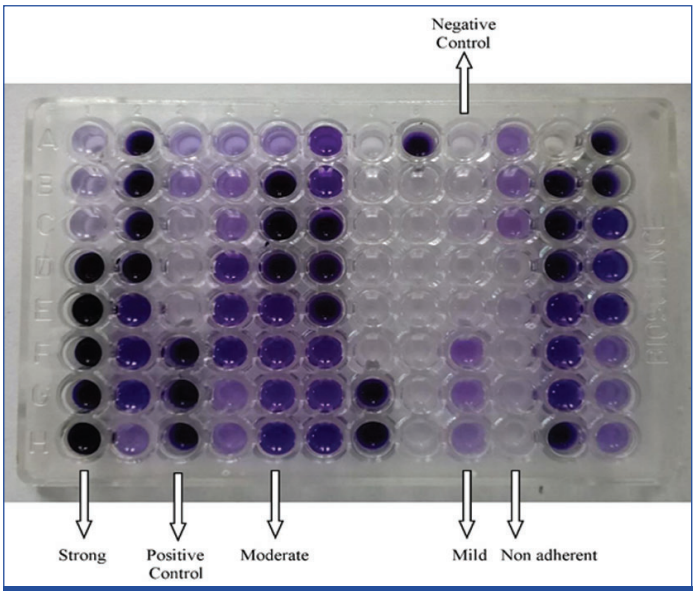
Day 3

Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method, and results were interpreted according to CLSI guidelines, 28th edition (2018) [5]. Zones of inhibition were measured, and isolates were classified as sensitive, intermediate, or resistant.

Multidrug-resistant organisms (MDROs) were defined as isolates that were non susceptible to at least one agent in three or more antimicrobial categories [6].

4. **Biofilm detection methods:** Biofilm formation by bacterial isolates was assessed using the following methods [7-10]:

a. **Tissue Culture Plate Method (TCP) [Table/Fig-1]**

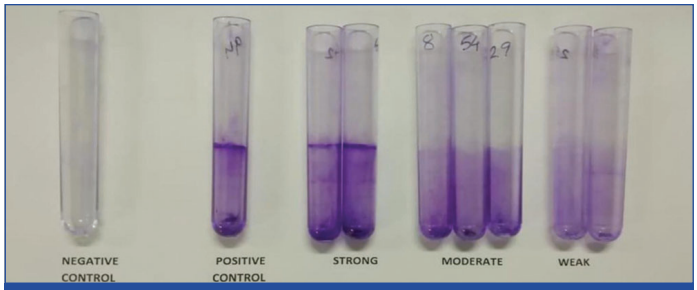


[Table/Fig-1]: Biofilm formation detected by Tissue Culture Plate (TCP) method.

- Bacterial isolates were grown in nutrient broth at 37°C for 24 hours.
- Two hundred microlitres of culture were transferred into sterile 96-well flat-bottom tissue culture plates (TCP).
- Wells were washed three times with phosphate-buffered saline (PBS, pH 7.2) to remove planktonic cells.
- Adherent cells were stained with 0.1% crystal violet for 15 minutes at room temperature.
- Excess stain was removed with distilled water, and the bound dye was eluted using 200 µl of 95% ethanol for 15 minutes.
- Optical density (OD) was measured at 540 nm [9]; higher OD values indicated greater biofilm biomass.
- The cut-off OD (ODc) was defined as the mean OD of the negative control plus three standard deviations.

- Biofilm production was categorised as follows [9]:
  - Non adherent: OD ≤ ODc
  - Weak: ODc < OD ≤ 2×ODc
  - Moderate: 2×ODc < OD ≤ 4×ODc
  - Strong: OD > 4×ODc
- *Staphylococcus epidermidis* ATCC 35984 (biofilm-positive) and *S. epidermidis* ATCC 12228 (biofilm-negative) served as positive and negative controls, respectively. All assays were performed in triplicate to ensure reproducibility [9].

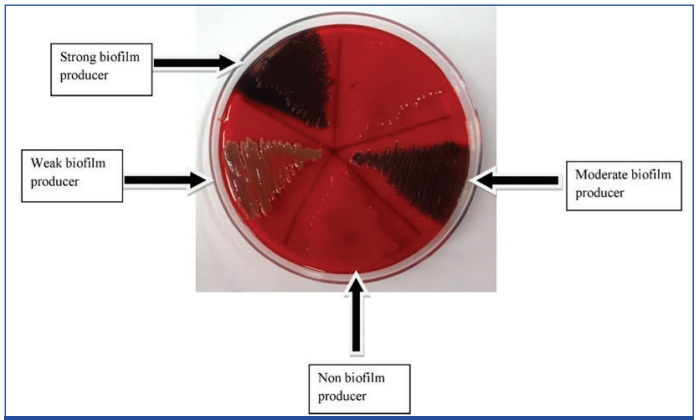
b. Tube Method (TM) [Table/Fig-2]



[Table/Fig-2]: Biofilm formation detected by tube method.

- Ten millilitres of Tryptic Soy Broth (TSB) were inoculated with a loopful of the test organism from overnight culture and incubated at 37°C for 24 hours.
- The broth was decanted, and tubes were washed with PBS (pH 7.3), air-dried, stained with 0.1% crystal violet for 10 minutes, rinsed with distilled water, and dried in an inverted position.
- Biofilm formation was considered positive when a visible film lined the walls and bottom of the tube.
- Biofilm intensity was scored as: 0 - absent, 1 - weak, 2 - moderate, 3 - strong.
- Tests were performed in triplicate and repeated three times.

c. Congo Red Agar Method (CRA) [Table/Fig-3]



[Table/Fig-3]: Biofilm formation detected by Congo Red Agar (CRA) method.

- Congo Red Agar was prepared using brain-heart infusion broth (37 g/L), sucrose (50 g/L), agar (10 g/L), and Congo red (0.8 g/L).
- Congo red was autoclaved separately at 121°C for 15 minutes and added to the medium after cooling to 55°C.
- Plates were inoculated and incubated aerobically at 37°C for 24-48 hours.
- Strong biofilm producers formed dark black, dry, crystalline colonies; moderate producers formed black colonies; weak producers showed slight darkening; and non producers remained red or pink.
- All experiments were performed in triplicate and repeated three times.

STATISTICAL ANALYSIS

Data were analysed using SPSS version 24.0. Descriptive statistics (frequencies, percentages, mean, standard deviation) were calculated. The Chi-square (χ^2) test was used to assess associations between categorical variables, with p-value <0.05 considered statistically significant.

RESULTS

Out of 100 clinical specimens processed, 40 (40%) yielded positive cultures, resulting in a total of 48 bacterial isolates. Among the culture-positive cases, single bacterial growth was observed in 32 (80%), while 8 (20%) showed mixed growth, contributing 16 additional isolates. The highest culture positivity was seen in the 41-50-year age group (14 cases, 35%), followed by 51-60 years (8 cases, 20%) and 31-40 years (6 cases, 15%). The remaining 12 cases (30%) were distributed across other age groups. Males accounted for 24 (60%) of the culture-positive cases, while females constituted 16 (40%). Among the 48 bacterial isolates, *Pseudomonas aeruginosa* was the predominant species (17 isolates, 35.42%), followed by *Staphylococcus aureus* (12 isolates, 25%), *Proteus mirabilis* (5 isolates, 10.42%), *Klebsiella* spp. (5 isolates, 10.42%), *Acinetobacter* spp. (3 isolates, 6.25%), *Escherichia coli* (3 isolates, 6.25%), and coagulase-negative staphylococci (CONS) (3 isolates, 6.25%). Among the *S. aureus* isolates, 7 (58.33%) were MRSA. Deep burn wounds contributed 25 (52.08%) isolates, while superficial wounds accounted for 23 (47.92%).

Biofilm formation assessed using the TCP method was detected in 38 (79.17%) isolates. All *P. aeruginosa* (17/17, 100%) and *Acinetobacter* spp. (3/3, 100%) were biofilm-positive. High biofilm positivity was also recorded in *Klebsiella* spp. (4/5, 80%) and *Proteus mirabilis* (4/5, 80%). Moderate biofilm formation occurred in *E. coli* (2/3, 66.67%) and *S. aureus* (8/12, 66.67%), while no biofilm production was observed in CONS (0/3, 0%). Chi-square analysis showed a statistically significant association between bacterial species and biofilm formation (χ^2=18.09, df=6, p=0.0060). The distribution of isolates and biofilm-positive cases is summarised in [Table/Fig-4].

Microorganism	Total isolates n (%)	Biofilm formation TCP n (%)	Tube method n (%)	CRA method n (%)	χ^2	p-value
Pseudomonas aeruginosa	17 (35.42)	17 (100.00)	14 (82.35)	12 (70.59)		
Staphylococcus aureus	12 (25.00)	8 (66.67)	8 (66.67)	4 (33.33)		
Proteus mirabilis	5 (10.42)	4 (80.00)	3 (60.00)	1 (20.00)		
Klebsiella spp.	5 (10.42)	4 (80.00)	4 (80.00)	2 (40.00)		
Acinetobacter spp.	3 (6.25)	3 (100.00)	3 (100.00)	2 (66.67)		
Escherichia coli	3 (6.25)	2 (66.67)	0	0		
CONS	3 (6.25)	0	0	0		
Total	48 (100.00)	38 (79.17)	32 (66.67)	21 (43.75)	13.32	0.00128

[Table/Fig-4]: Distribution of bacterial isolates and biofilm formation by different methods.
$\chi^2(2)$=13.32, p=0.00128 refers to comparison of biofilm-positive vs negative counts across the three methods (TCP, Tube, CRA). Chi-square test applied; p<0.05 considered statistically significant.

Among the 48 bacterial isolates, biofilm production was evaluated using three different methods. By TCP method, biofilm formation was detected in 38 (79.17%) isolates, of which 15 (39.47%) were classified as strong producers, 16 (42.11%) as moderate producers, and 7 (18.42%) as weak producers.The grading of biofilm formation based on the TCP method is presented in [Table/Fig-5]. Using

the tube method, biofilm formation was observed in 32 (66.67%) isolates, comprising 13 (40.63%) strong, 15 (46.88%) moderate, and 4 (12.50%) weak biofilm producers. The Congo red agar (CRA) method detected biofilm production in 21 (43.75%) isolates, including 12 (57.14%) strong, 5 (23.81%) moderate, and 4 (19.05%) weak producers.

Microorganism	Strong n (%)	Moderate n (%)	Weak n (%)	Total n (%)	χ^2	p-value
Pseudomonas aeruginosa	10 (58.82)	3 (17.65)	4 (23.53)	17 (100)		
Staphylococcus aureus	3 (37.50)	4 (50)	1 (12.50)	8 (100)		
Proteus mirabilis	1 (25)	2 (50)	1 (25)	4 (100)		
Klebsiella spp.	1 (25)	3 (75)	0	4 (100)		
Acinetobacter spp.	0	3 (100)	0	3 (100)		
Escherichia coli	0	1 (50)	1 (50)	2 (100)		
Total	15 (39.47)	16 (42.11)	7 (18.42)	38 (100)	12.92	0.228

[Table/Fig-5]: Grading of biofilm production by the Tissue Culture Plate (TCP) method among biofilm-positive isolates (n=38).
Chi-square test applied; p>0.05 considered not statistically significant

A Chi-square test comparing biofilm grades (strong, moderate, weak) across species yielded χ^2=12.92, p-value=0.228, indicating no statistically significant difference (p-value>0.05).

- Comparison of biofilm detection methods demonstrated that the TCP method, considered the reference standard, detected biofilm formation in 38 (79.2%) of the 48 isolates. Using TCP as the benchmark, the tube method showed a sensitivity of 31/38 (81.6%), a specificity of 9/10 (90.0%), a positive predictive value (PPV) of 31/32 (96.9%), a negative predictive value (NPV) of 9/16 (56.3%), and an overall accuracy of 40/48 (83.3%).
- In contrast, the Congo red agar (CRA) method detected biofilm formation in 21 (43.8%) isolates, corresponding to a sensitivity of 21/38 (55.3%), a specificity of 10/10 (100.0%), a PPV of 21/21 (100.0%), an NPV of 10/27 (37.0%), and an overall accuracy of 31/48 (64.6%).

The distribution of test results relative to TCP is shown in [Table/Fig-6], and comparative diagnostic performance in [Table/Fig-7].

Tube method	Reference positive	Reference negative	Total
Test positive	31 (TP)	1 (FP)	32
Test negative	7 (FN)	9 (TN)	16
Total	38	10	48
CRA method			
Test positive	21 (TP)	0 (FP)	21
Test negative	17 (FN)	10 (TN)	27
Total	38	10	48

[Table/Fig-6]: Contingency tables for the Tube method and CRA method in comparison with the reference standard.
Abbreviations: TP: True positive; FP: False positive; TN: True negative; FN: False negative

Method	Sensitivity n/N (%)	Specificity n/N (%)	PPV n/N (%)	NPV n/N (%)	Accuracy n/N (%)
Tube method	31/38 (81.6)	9/10 (900)	31/32 (96.9)	9/16 (56.3)	40/48 (83.3)
CRA method	21/38 (55.3)	10/10 (100)	21/21 (100)	10/27 (37)	31/48 (64.6)

[Table/Fig-7]: Diagnostic performance of biofilm detection methods.

Biofilm production and antimicrobial resistance: Of the 48 isolates, 34 (70.8%) were identified as MDROs. Among these, 33 (97.1%) were biofilm producers. In contrast, only 3/14 (21.4%) non MDRO isolates formed biofilms.

This association was highly significant (χ^2=30.25, df=1, p=3.79 × 10^{-8}; Fisher's exact p=1.79 × 10^{-7}). [Table/Fig-8] presents the distribution of MDRO and non-MDRO isolates according to biofilm formation.

Microorganism	Total isolates n (%)	MDRO n (%)	Biofilm formation among MDRO n (%)	Biofilm formation among non-MDRO n (%)	χ^2	p-value
Pseudomonas aeruginosa	17 (35.4)	13 (76.5)	13 (100)	0		
Staphylococcus aureus	12 (25)	7 (58.3)	7 (100)	0		
Proteus mirabilis	5 (10.4)	4 (80)	4 (100)	0		
Klebsiella spp.	5 (10.4)	5 (100)	4 (80)	-		
Acinetobacter spp.	3 (6.3)	3 (100)	3 (100)	-		
Escherichia coli	3 (6.3)	2 (66.7)	2 (100)	0		
CONS	3 (6.3)	0	-	3 (100)		
Total	48 (100)	34 (70.8)	33 (97.1)	3 (21.4)	30.25	3.79 × 10^{-8}

[Table/Fig-8]: Incidence of Multidrug-Resistant Organisms (MDROs) and biofilm formation.
Chi-square test applied; p<0.05 considered statistically significant

With respect to wound depth, superficial burns accounted for 23 (47.9%) of the culture-positive cases, of which 18 (78.3%) demonstrated biofilm formation by the TCP method. Deep burns comprised 25 (52.1%) of the cases, with biofilm formation detected in 20 (80.0%) of these.

DISCUSSION

The culture positivity rate in the present study (40.0%) falls at the lower end of the spectrum when compared with earlier reports. Singh B et al., and Kulayata K et al., documented considerably higher positivity rates of 82% in general wound infections, while Kunwar A et al., reported 71% and Banu A et al., an even higher 90% in burn wound cases [11-14]. In contrast, Natsha S et al., observed a 35% positivity rate in paediatric burn injuries—predominantly scald-related—which closely aligns with the current findings [15]. The lower positivity rate in the present study may be attributable to differences in patient selection (including both superficial and deep wounds), variations in the timing of sample collection, prior antibiotic exposure, and institutional infection control practices. Weinand C; Mandal A and Das S also investigated wound and burn infections but did not specify culture positivity rates, limiting direct quantitative comparison [16,17].

Regarding the pathogen profile, *Pseudomonas aeruginosa* was the predominant isolate in the current study (35.42%), consistent with previous findings by Singh B et al., Banu A et al., Weinand C; Mandal A and Das S reaffirming its central role in chronic wound and burn infections [11,14,16,17]. *Staphylococcus aureus* was the second most common pathogen (25.0%), of which 58.3% were methicillin-resistant (MRSA). This finding was in line with reports by Kulayata K et al., Weinand C; Mandal A and Das S who similarly highlighted its clinical significance [12,16,17]. *Proteus mirabilis* and *Klebsiella* spp. were each isolated in 10.42% of cases, comparable to frequencies reported by Singh B et al., Banu A et al., and Mandal A and Das S [11,14,17]. *Acinetobacter* spp. and *Escherichia coli* each accounted for 6.25% of isolates, reflecting the moderate prevalence noted in earlier studies by Singh B et al., Kulayata K et al., Weinand C; Mandal A and Das S [11,12,16,17]. Coagulase-negative staphylococci (CoNS) were also detected (6.25%), though at a lower proportion than the 13% reported by Kulayata K et al., yet still indicating their emerging opportunistic role [12].

Biofilm production was notably high in the present study, with 79.2% positivity by the TCP method. This exceeds the 56.8% reported by Singh B et al., the 62.2% documented by Kulayata K et al., and the 46.6% observed by Banu A et al., but approximates the 61.9% reported by Basnet A et al., [11,12,14,18]. All *P. aeruginosa* and *Acinetobacter* spp. isolates in the present study were biofilm producers—a finding consistent with their known virulence in earlier literature. The proportion of strong biofilm producers (39.47%) was substantially higher than the 8.5% reported by Kulayata K et al., suggesting differences in strain virulence or regional epidemiology [12]. As with Kunwar A et al., detection rates varied across methods, with the TCP method outperforming both the tube method (82.0% sensitivity, 90.0% specificity) and CRA (55.3% sensitivity, 100% specificity), highlighting the influence of methodology on reported prevalence [13].

Multidrug resistance was alarmingly high at 70.8%, with nearly all MDR isolates (97.1%) exhibiting biofilm production. This MDR rate surpasses the 48.4% reported by Kulayata K et al. and was higher than figures reported in other regional studies [12]. While Singh B et al., and Natasha S et al., documented the presence of MDR organisms—including *P. aeruginosa*, *Acinetobacter* spp., MRSA, and ESBL-producing *Klebsiella* spp.—they did not report comparable overall MDR prevalence [11,15]. Unlike the non-significant association between MDR and biofilm formation reported by Kunwar A et al., the present study demonstrates a strong correlation, suggesting potential differences in local resistance dynamics or infection control practices [13]. The significance of biofilm-forming MRSA observed by Banu A et al., and the frequent isolation of MDR Pseudomonas spp. reported by Mandal A and Das S are consistent with the current findings [14,17].

Overall, when viewed alongside previous research, the present study highlights the persistent challenge posed by *P. aeruginosa*, MRSA, and other biofilm-forming MDR organisms in wound infections. The high prevalence of these pathogens emphasises the need for early and precise microbiological diagnosis, including routine biofilm detection, to enable timely and targeted antimicrobial therapy. Treatment protocols should incorporate strategies specifically aimed at biofilm disruption, as conventional antibiotic regimens alone are often inadequate. The increasing rates of multidrug resistance among burn wound pathogens further underscore the necessity of robust antibiotic stewardship and stringent infection control measures in burn units.

The increasing rates of MDR among burn wound pathogens further strengthen the case for robust antibiotic stewardship and stringent infection control measures within burn units.

Future research should prioritise the development of novel anti-biofilm agents, explore the efficacy of combination therapies, and evaluate adjunctive approaches such as bacteriophage therapy, quorum-sensing inhibitors, and nanoparticle-based drug delivery systems. Large-scale, multicentric studies employing standardised biofilm detection methods are essential to generate reliable epidemiological data, refine clinical guidelines, and ultimately improve patient outcomes in burn wound management.

A key strength of this study lies in its systematic evaluation of burn wound infections, combining microbial isolation patterns with biofilm production and MDR profiling using validated laboratory methods. The use of multiple biofilm detection techniques adds robustness to the findings, while the focus on clinical isolates from a high-risk patient group addresses a recognised gap in regional research. Importantly, the assessment of the relationship between biofilm formation and multidrug resistance provides insights directly relevant to infection control strategies.

Limitation(s)

The single-centre design may limit the generalisability of the results to other healthcare settings or regions. The moderate sample size, although adequate for observational purposes, may not capture the full diversity of microbial flora and resistance patterns in broader populations. The cross-sectional design restricts the ability to assess temporal changes in pathogen prevalence or treatment response. Additionally, the absence of molecular characterisation of resistance mechanisms and biofilm-associated genes constrains understanding of the underlying biological processes. Future studies should adopt multicentric designs, include larger patient cohorts, and incorporate molecular nalyses to strengthen and expand upon these findings.

CONCLUSION(S)

Burn wound infections present a complex microbiological landscape dominated by opportunistic pathogens with strong biofilm-forming capabilities and high rates of MDR. These patterns reflect the dynamic nature of infectious agents in burn patients and highlight the need for continuous surveillance and adaptable management strategies. Enhancing diagnostic precision, promoting rational antimicrobial use, and advancing research into novel therapeutic interventions are essential in improving clinical outcomes. Addressing these challenges requires a coordinated, multidisciplinary approach that integrates clinical practice, microbiology, and pharmacology to ensure both immediate patient benefit and long-term reduction in the burden of burn wound-associated infections.

Authors' contribution: All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

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[ANNEXURE-I]

Proforma for Data Collection

Patient Information

1. Name: _____
2. Registration Number: _____
3. Age: _____ years
4. Sex: ☐ Male ☐ Female
5. Date of Admission: ____ / ____ / _____
6. Date of Discharge: ____ / ____ / _____
7. Duration of Hospital Stay: _____ days

Clinical Details

1. Type of Burn: ☐ Flame ☐ Scald ☐ Electrical ☐ Chemical ☐ Other (specify) _____
2. Degree of Burn: ☐ First Degree ☐ Second Degree ☐ Third Degree
3. Total Body Surface Area (TBSA) Involved: _____ %
4. Treatment Administered: _____