RESEARCH ARTICLE

# Drug susceptibility, Prevalence and effect of *Pseudomonas aeruginosa* on wound healing of burn patients

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## Abstract

**Objective:** To determine the prevalence, antibiotic susceptibility and effect of Pseudomonas aeruginosa in relation to burn patients.

**Method:** The cross-sectional study was conducted at the Centre for Advanced Studies in Vaccinology and Biotechnology, University of Balochistan, Quetta, Pakistan, from March 2018 to May 2021, and comprised pus swab cultures were isolated from inpatients with 2nd and 3rd degree burns aged up to 60 years at Bolan Medical Complex Hospital and Sandeman Provinical Hospital, the two main government tertiary care hospitals in Quetta. The samples were immediately cultured, and evaluated using biochemical tests, antibiotic susceptibility and molecular identification using polymerase chain reaction. Data was analysed using SPSS 20.

**Results:** Of the 720 burn wound samples, 424(58.9%) were positive for *Pseudomonas aeruginosa*; 304(42%) males and 120(16%) females ($p$<0.02). The overall mean age of the patients was 27.7±6.2 years (range: 1-60 years). The mean total burn surface area was not significantly different in positive 29.6±6.2% and negative 30.3±6.2% cases ($p$>0.05). The time leading to skin grafts in positive patients was 29.5±6.5 days compared to 22.3±6.3 days for negative patients ($p$< 0.007), and the time required for wound healing was 25.0±4.7 days and 16.7±5.2 days, respectively ($p$<0.001). Length of hospital stay of Pseudomonas aeruginosa positive patients was 38.0±7.8 days compared to 32.1±6.8 days for negative patients ($p$<0.001).

**Conclusion:** Nosocomial infections and multidrug resistance species were observed frequently at the burn wound site. *P. aeruginosa.*

**Keywords:** Antibiotics, antimicrobial resistance, burn wounds, nosocomial infection, *Pseudomonas aeruginosa.*

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## Introduction

Nosocomial or healthcare-associated infections (HAIs) are usually acquired during prolonged hospital stay, and represent a major problem worldwide.[1] The problem is more acute when the infecting pathogen is resistant to antimicrobial agents. *Enterococcus (E.) faecium, Staphylococcus (S.) aureus, Klebsiella (K.) pneumoniae, Acinetobacter (A.) baumannii, Pseudomonas (P.) aeruginosa* and *Enterobacter specie (spp.)*, commonly called the ESKAPE bacterial pathogens, have increased levels of resistance towards multiple classes of first-line and last-resort antibiotics, and these are the major cause of life-threatening HAIs.[2,3]

*P. aeruginosa* infections are frequently associated with high mortality rates. The intrinsically high resistance to many antibiotics and multidrug resistance (MDR) status in a hospital setting is considered to be among the reasons for high pathogenicity of *P. aeruginosa*.[4] *P. aeruginosa* is an opportunistic pathogen which causes HAIs and sepsis in intensive care units (ICUs).[5,6] The assessed ICU-acquired infections were 23% and 7.1-7.3% of HAIs. *P. aeruginosa* causes 10% of catheter-associated urinary tract infection (UTIs) and ventilator-associated *pneumonia* (VAP).[7] Aspects of hospital environment that contribute to increase in infection of drug resistant *P. aeruginosa* include ability to survive in humid environment and hospital equipment/utensils, selective pressure of antimicrobials, surgical procedures, long periods of hospitalisation, use of catheters, mechanical ventilation and previous antibiotic therapy.[8]

In developing countries, the prevalence of nosocomial infections surpasses 25% and it is up to 15% in developed countries, which causes the death of approximately 40,000 hospitalised patients worldwide.[9] Due to lack of facilities and poor management, control of burn events in low- and middle-income countries (LMICs) has been reported to be 90% and in Southeast Asia and Middle East, 300,000 people die from fire-related injuries every year, according to the World Health Organisation (WHO).[10]

The treatment and prevention of hospital-acquired microorganisms are challenging due to prolonged

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hospitalisation, high healthcare costs and poor clinical outcomes.[11] The WHO has listed *P. aeruginosa* and Acinetobacter species as dangerous pathogens.[12] The new therapeutics are immediately required because the infection of these dangerous microorganisms are considered a global threat.[13]

The Balochistan province of Pakistan lacks elementary healthcare facilities, especially in the less-privileged rural areas. Consequently, infection in overcrowded burn care centres is a major risk factor and remains a challenging problem.

The current study was planned to determine the prevalence, antibiotic susceptibility and effect of *P. aeruginosa* in relation to burn patients in a public-sector setting in Balochistan.

## Materials and Methods

The cross-sectional study was conducted at the Centre for Advanced Studies in Vaccinology and Biotechnology, University of Balochistan, Quetta, Pakistan, from March 2018 to May 2021, and comprised pus swab cultures were isolated from burn wound inpatients at Bolan Medical Complex Hospital and Sandeman Provinical Hospital, the two main government tertiary care hospitals in Quetta with well-established and proper burn care centres.

After approval from the institutional ethics review committee, the sample size was calculated using the Cochran formula n = z2 p (1- p)/ d2, where n was the required sample size, Z was level of confidence 95% which was 1.96, P was 50% proportion which was 0.5 d was the margin of error which was 0.05.[14]

Sampling was done by scrubbing the pus of burn wound patients with the help of sterilized cotton swab. A single swab sample was taken from of each admitted patient, and it was then transported straight to the laboratory. Patients with only 2nd and 3rd degree burns and aged from 2 months to 60 years were included. Those outside the age range, pregnant women's, victims of high electric voltage, and those with comorbidities were excluded.

Data was recorded about the patient's demography, including age, gender, socioeconomic status (SES), and clinical details, such as aetiology of burn, total burn surface area (TBSA), length of hospital stay and healing time.

The swab samples were inoculated with brain heart infusion (BHI) liquid medium and incubated at 37$^{o}$C for 24 hours. The overnight bacterial culture was streaked into specific medium Cetrimide agar for the isolation of P. aeruginosa, and incubated at 37$^{o}$C for 24 hours. After a third passage, pure isolated colonies were picked up for gram staining, biochemical tests and polymerase chain reaction (PCR). Biochemical tests and sugar fermentation tests were used to confirm the identity of *P. aeruginosa* isolates.

Antibiotic susceptibility testing was performed on *P. aeruginosa* isolates. A 0.5 MacFarland standard bacterial culture was prepared and spread on Mueller-Hinton agar plates (Oxoid, United Kingdom) and antibiotic discs were placed at equal distances. Then the plates were incubated at 37$^{o}$C for 24 hours. The zones of inhibition were measured and interpreted, according to Clinical and Laboratory Standards Institute (CLSI) guidelines.[15] Sensitivity and resistivity of the isolates were evaluated against oxytetracycline (30µg), amoxicillin (25µg), ceftazidime (30µg), erythromycin (15µg), imipenem (10µg), meropenem (10µg), colistin (25µg), levofloxacin (5µg), cefepime (30µg), ciprofloxacin (5µg), gentamycin (10µg), amikacin (30µg) and vancomycin (30µg).

Molecular identification of isolates was achieved by PCR amplification of the *P. aeruginosa* 16Sr ribonucleic acid (RNA) gene. An overnight bacterial culture was used for deoxyribonucleic acid (DNA) extraction using a DNA purification kit (Gene All ®Biotechnology Seoul, South Korea). After DNA extraction, the DNA templates were stored at -20$^{o}$C for later use. Primer sequences used for 16SrRNA gene amplification were F: (GGGGGATCTTCGGACCTCA) and R: (TCCTTAGAGTGCCCACCCG). PCR reaction mixtures had a total volume of 25µl, containing 11µl of PCR master mix (amaR PCR GeneDirex SIMPLYTM Seoul, South Korea), 10µl of PCR water, 1µl of forward primer, 1µl of reverse primer and 2µl of DNA. PCR thermal cycling included initial denaturation at 95$^{o}$C for 2 minutes, denaturation at 94$^{o}$C for 30 seconds, annealing at 58°C for 30 seconds, extension at 72$^{o}$C for 40 seconds and final extension at 72$^{o}$C for 1 minute set for 25 cycles. The final PCR products were examined by gel electrophoresis using an ultraviolet (UV) light transilluminator for visualisation.

Data was analysed using SPSS 20. Data normality was checked, and Kolmogorov-Smirnov test was used because the data exceeded the stated limit. Data was found to have normal distribution. Parametric statistics were then applied. $P \leq 0.05$ was considered significant.

## Results

Of the 768 samples evaluated, 720(93.75%) were analysed. Of them, 424(58.9%) samples were positive for P. aeruginosa and 296(41.1%) were negative. Among the positive samples, 304(42%) were related to male patients and 120(16%) to females ($p<0.02$). The overall mean age of the patients was 27.7±6.2 years (range: 1-60 years), while

**Table-1:** Demographic data and associated risk factors of burn wound patients.

| Possible risk factors | Culture results Positive n (%) | Negative n (%) | Total n (%) | *p*-value |
|---|---|---|---|---|
| **Hospital** | | | | |
| BMCH | 382 (53) | 125 (17) | 507 (70) | 0.001* |
| SPH | 42 (5.8) | 171 (24) | 213 (29.8) | |
| **Gender** | | | | |
| Male | 304 (42) | 190 (26) | 494 (68.6) | 0.02* |
| Female | 120 (16) | 106 (14.7) | 226 (30.7) | |
| **Age** (years) | | | | |
| 0-10 | 144 (20) | 71 (9.8) | 215 (29.8) | 0.002* |
| 11-20 | 42 (5.8) | 72 (10) | 114 (15.8) | |
| 21-30 | 66 (9) | 80 (11) | 146 (20) | |
| 31-40 | 20 (2.7) | 16 (2) | 36 (5) | |
| 41-50 | 50 (6.9) | 37 (5) | 87 (12) | |
| >50 | 102 (14) | 20 (2.7) | 122 (16.9) | |
| **Socio-economic Status** | | | | |
| Higher | 59 (8) | 102 (14) | 161 (22) | 0.001* |
| Middle | 140 (19) | 92 (12.7) | 232 (32) | |
| Lower | 225 (31) | 102 (14) | 327 (45) | |
| **Season** | | | | |
| Spring | 174 (24) | 107 (14.8) | 281 (39) | 0.12 |
| Autumn | 99 (13.7) | 71 (9.8) | 170 (23) | |
| Winter | 109 (15) | 54 (7.5) | 163 (22) | |
| Summer | 42 (5.8) | 64 (8.8) | 106 (14.7) | |
| **Source of burn** | | | | |
| Scald | 197 (27) | 65 (9) | 262 (36) | 0.01* |
| Flame | 87 (12) | 64 (8.8) | 151 (20.9) | |
| Electric | 106 (14.7) | 89 (12) | 195 (27) | |
| Chemical | 34 (4.7) | 78 (10.8) | 112 (15.5) | |
| **Total** | 424 (58.8) | 296 (41) | 720 (100) | |

BMCH: Bolan Medical Complex Hospital; SPH: Sandeman Provincial Hospital; P<0.05 was considered significant.

**Table-2:** Characteristics of Pseudomonas (P.) aeruginosa infected and non-infected burn wound patients (n=720).

| *P. aeruginosa* | n | Mean±SD | SEM | *p*-value |
|---|---|---|---|---|
| **Mean TBSA** (%) | | | | |
| Positive | 424 | 29.5896±6.19131 | 0.30068 | 0.365 |
| Negative | 296 | 30.2770±6.17411 | 0.35886 | |
| **Hospital stay** (days) | | | | |
| Positive | 424 | 38.0212±7.75494 | 0.37661 | 0.001* |
| Negative | 296 | 32.1047±6.79575 | 0.39499 | |
| **Weight at day** 1 (kg) | | | | |
| Positive | 424 | 51.6297±30.05263 | 1.45948 | 0.458 |
| Negative | 296 | 56.0169±32.00450 | 1.86022 | |
| **Weight at discharge** (kg) | | | | |
| Positive | 424 | 50.4882±30.18336 | 1.46583 | 0.364 |
| Negative | 296 | 54.6689±31.91034 | 1.85475 | |
| **Skin ready for grafting** (days) | | | | |
| Positive | 424 | 29.4953±6.48657 | 0.31502 | 0.007* |
| Negative | 296 | 22.3311±6.34370 | 0.36872 | |
| **Healing time** (days) | | | | |
| Positive | 424 | 24.9788±4.72827 | 0.22963 | 0.001* |
| Negative | 296 | 16.7061±5.18846 | 0.30157 | |

TBSA: Total burn surface area; n: Number; SD: Standard deviation; SEM: Standard error mean; P<0.05 was considered significant.

*P. aeruginosa* infection was significantly more prevalent among paediatric patients aged 0-10 years ($p<0.001$). Scald burn was the most dominant aetiology 197(27%) ($p<0.001$) (Table 1).

The mean TBSA was not significantly different in positive 29.6±6.2% and negative 30.3±6.2% cases ($p>0.05$). The time leading to skin grafts in positive patients was 29.5±6.5 days compared to 22.3±6.3 days for negative patients ($p< 0.007$), and the time required for wound healing was 25.0±4.7 days and 16.7±5.2 days, respectively ($p<0.001$). Length of hospital stay of *Pseudomonas aeruginosa* positive patients was 38.0±7.8 days compared to 32.1±6.8 days for negative patients ($p<0.001$). *P. aeruginosa* positive patients had a higher incidence of septic shock, respiratory failure, and renal failure compared to the negative group. Mean body weight at baseline and discharge was not significantly different between the positive and negative groups (Table 2).

The isolated *P. aeruginosa* was an active fermenter of glucose and mannitol, but did not ferment dextrose, sorbitol, trehlose, inositol and mannose sugars (Table 3).

*P. aeruginosa* isolates had highest antibiotic resistance against cefepime 394(92.9%), followed by ceftazidime 382(90%), oxytetracycline 354(83.9%), erythromycin 341(80%) and levofloxacine 320 (75%). Colistin 183 (43%), imipenem 7417%) and meropenem 59(13.9%) showed high level of sensitivity (Figure 1).

Molecular analysis showed that all isolates harboured the 16SrRNA gene of size 956bp (Figure 2).

**Table-3:** Biochemical tests and sugar fermentation of *Pseudomonas (P.) aeruginosa*.

| *P. aeruginosa* | Critera |
|---|---|
| Citrimide agar | Yellow-Green |
| Gram staining | Gram-negative |
| Shape | Rod |
| Size | 1–5µm long and 0.5–1.0µm wide |
| **Biochemical tests** | |
| Catalase | +ve |
| Oxidase | +ve |
| Indole | -ve |
| Methyl red | -ve |
| Voges-proskauar | -ve |
| Citrate | +ve |
| Urease | -ve |
| **Sugar fermentation tests** | |
| Glucose | +ve |
| Dextrose | -ve |
| Sorbitol | -ve |
| Trehlose | -ve |
| Mannitol | +ve |
| Inositol | -ve |
| Mannose | -ve |

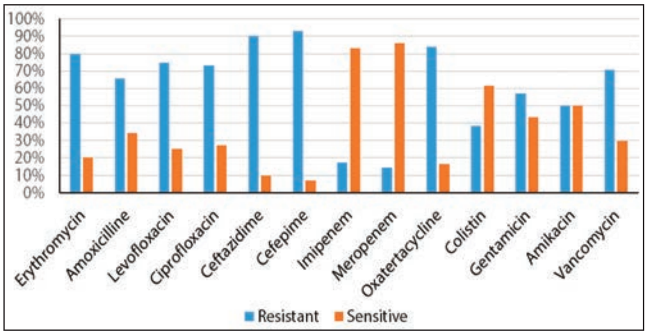


**Figure-1:** Antibiotic resistance pattern of *Pseudomonas (P.) aeruginosa*.

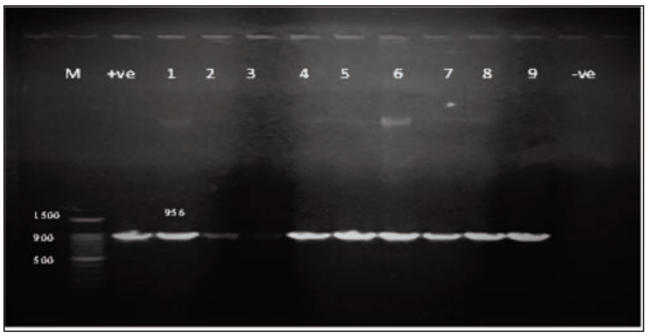


**Figure-2:** Gel electrophoresis analysis of polymerase chain reaction (PCR) products showing positive amplification of the 16Sr ribonucleic acid (RNA) gene. Lane M represents a 100bp deoxyribonucleic acid (DNA) ladder, lane +ve is positive control, lanes 1-9 are positive isolates of the 16SrRNA gene of size 956bp and lane -ve is negative control.

## Discussion

Burn wounds are one-third of nosocomial infections associated with morbidity and mortality along with enduring modification in the developing world. Burns can affect anyone, anytime and anywhere, causing physical and psychosocial disturbance.[16] The present study analysed the drug susceptibility and effects of *P. aeruginosa* on burn wound healing.

*Pseudomonas spp.* are commonly isolated pathogens in burn patients, and in the present study their prevalence was 58.8%, which is somewhat alarming. Shukla et al. reported 37.5%[17] and it was 29.2% in a study by Mehta Isha et al.[18]

The current results showed that male cases (42%) were higher than females (16%), which may be due to the fact that males are more involved in outdoor activities and more exposed to open flame injury. The results were similar to those presented by Hashemi et al.[19] The paediatric patients were a major risk group in the current study, with those aged 0-10 years having the highest number of cases (20%). The risk of morbidity and mortality is increased in children due to their thinner and smaller skin which causes deep burn injury and more extensive skin loss compared to the adults.[20] The current results demonstrated that scalding was the most common cause of burn, which was similar to the finding of Kelly C. Jordan et al.[21]

In the present study, the presence of *P. aeruginosa* in burn wounds resulted in more severe effects on wound healing. One study showed that burn wound infections caused by gram-negative bacteria represented 57.9% compared to the gram-positive bacteria 36.6%.[22] Wounds infected with microorganisms do not compare with the number and population of microorganisms, thus wound infections can be mono-microbial or poly-microbial depending on the patient's previous treatment and state of the wound.[23] The wound healing process of the skin is significantly coordinated with the recruitment of dermal cells and active molecules to quickly close the injured area and restore the damage tissues.[24] The findings in the study and from other studies demonstrate a greater compromise to health for burn wound patients with *P. aeruginosa* infection compared to those without such an infection. A more rapid deterioration has been observed in patients with burn wounds associated with *P. aeruginosa* that ultimately results in increased death rates due to consequent sepsis.[25]

*P. aeruginosa* infections' appropriate treatment and detection is a major public health concern considered challenging because of the emergence of antibiotic resistance to several antibiotic that can lead to greater incidence of pan-drug-resistant (PDR), MDR and extensively drug-resistant (XDR) isolates of *P. aeruginosa*. Carbapenems imipenem, meropenem are the most effective treatments of MDR and XDR isolates, but bacteria have developed resistance to these drugs primarily due to their inadequate excessive use.[26]

The current study found that the meropenem, imipenem and colistin were sensitive against *P. aeruginosa*, which is in agreement with another study which also noted that in the past few years, *P. aeruginosa* has shown resistance against such antibiotics, and most recently colistin or polymyxin B has been considered a treatment option in XDR Cases even though colistin resistance has also been recognised against *P. aeruginosa*.[27] It is, therefore, important that unnecessary antibiotic treatments are avoided, which may otherwise lead to the emergence of further antibiotic-resistant pathogens.

## Conclusion

Nosocomial infections and MDR species were observed frequently at the burn wound site. HAIs and antibiotic resistance have very serious consequences and may lead to complications. The eradication of microorganisms, especially *P. aeruginosa*, are difficult to control in hospitals. The resolution of infection followed by proper therapeutic treatment, promoting wound healing, would limit the spread of antibiotic resistance.

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## References

1. Haque M, Sartelli M, McKimm J, Abu Bakar M. Health care-associated infections - an overview. Infect Drug Resist 2018;11:2321-33. doi: 10.2147/IDR.S177247.
2. Mulani MS, Kamble EE, Kumkar SN, Tawre MS, Pardesi KR. Emerging Strategies to Combat ESKAPE Pathogens in the Era of Antimicrobial Resistance: A Review. Front Microbiol 2019;10:e539. doi: 10.3389/fmicb.2019.00539.
3. De Oliveira DMP, Forde BM, Kidd TJ, Harris PNA, Schembri MA, Beatson SA, et al. Antimicrobial Resistance in ESKAPE Pathogens. Clin Microbiol Rev 2020;33:e00181-19. doi: 10.1128/CMR.00181-19.
4. Rashid Mahmood A, Mansour Hussein N. Study of Antibiotic Resistant Genes in Pseudomonas aeroginosa Isolated from Burns and Wounds. Arch Razi Inst 2022;77:403-11. doi: 10.22092/ARI.2021.356681.1893.
5. Horcajada JP, Montero M, Oliver A, Sorlí L, Luque S, Gómez-Zorrilla S, et al. Epidemiology and Treatment of Multidrug-Resistant and Extensively Drug-Resistant Pseudomonas aeruginosa Infections. Clin Microbiol Rev 2019;32:e00031-19. doi: 10.1128/CMR.00031-19.
6. Abd El-Baky RM, Mandour SA, Ahmed EF, Hashem ZS, Sandle T, Mohamed DS. Virulence profiles of some Pseudomonas aeruginosa clinical isolates and their association with the suppression of Candida growth in polymicrobial infections. PLoS One 2020;15:e0243418. doi: 10.1371/journal.pone.0243418.
7. Lemiech-Mirowska E, Kiersnowska ZM, Michałkiewicz M, Depta A, Marczak M. Nosocomial infections as one of the most important problems of healthcare system. Ann Agric Environ Med 2021;28:361-6. doi: 10.26444/aaem/122629.
8. Ponce de Leon A, Merchant S, Raman G, Avendano E, Chan J, Tepichin Hernandez G, et al. Pseudomonas infections among hospitalized adults in Latin America: a systematic review and meta-analysis. BMC Infect Dis 2020;20:250. doi: 10.1186/s12879-020-04973-0.
9. Reynolds D, Kollef M. The Epidemiology and Pathogenesis and Treatment of Pseudomonas aeruginosa Infections: An Update. Drugs 2021;81:2117-31. doi: 10.1007/s40265-021-01635-6.
10. Mirza Aghazadeh A, Lotfi M, Ghahramanian A, Ahadi F. Lethal Area 50 in Patients with Burn Injuries in North West, Iran. J Caring Sci 2018;7:53-8. doi: 10.15171/jcs.2018.009.
11. Huang H, Chen B, Liu G, Ran J, Lian X, Huang X, et al. A multi-center study on the risk factors of infection caused by multi-drug resistant Acinetobacter baumannii. BMC Infect Dis 2018;18:11. doi: 10.1186/s12879-017-2932-5.
12. Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis 2018;18:318-27. doi: 10.1016/S1473-3099(17)30753-3.
13. Shlaes DM, Bradford PA. Antibiotics-From There to Where?: How the antibiotic miracle is threatened by resistance and a broken market and what we can do about it. Pathog Immun 2018;3:19-43. doi: 10.20411/pai.v3i1.231.
14. Daniel WW. Biostatistics: A Foundation for Analysis in the Health Sciences, 7th ed. Hoboken, New Jersey: John Wiley and Sons; 1999.
15. Clinical and Laboratory Standards Institute (CLSI). CLSI supplement M100: Performance Standards for Antimicrobial Susceptibility Testing, 30th ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2020.
16. Jeschke MG, van Baar ME, Choudhry MA, Chung KK, Gibran NS, Logsetty S. Burn injury. Nat Rev Dis Primers 2020;6:11. doi: 10.1038/s41572-020-0145-5.
17. Shukla V, Khare A, Bisen J, Darshan BY. Antimicrobial Susceptibility Pattern of Bacterial Isolates in Burn Patients in a Tertiary Care Center: A Prospective Study. Int J Sci Study 2020;7:113-20.
18. Isha M, Chandani S, Krunal M, Summaiya M. Prevalence of Multidrug Resistant Pseudomonas Aeruginosa Infection in Burn Patients at a Tertiary Care Centre Jamnagar, Gujarat, India. Saudi J Pathol Microbiol 2019;4:210-4. DOI:10.21276/sjpm.2019.4.3.13.
19. Hashemi SS, Mahmoodi M, Tohidinik HR, Mohammadi AA, Mehrabani D. The Epidemiology of Burn and Lethal Area of Fifty Percentage (LA50) in Children in Shiraz, Southern Iran. World J Plast Surg 2021;10:66-70. doi: 10.29252/wjps.10.1.66.
20. Meng F, Zuo KJ, Amar-Zifkin A, Baird R, Cugno S, Poenaru D. Pediatric burn contractures in low- and lower middle-income countries: A systematic review of causes and factors affecting outcome. Burns 2020;46:993-1004. doi: 10.1016/j.burns.2019.06.001.
21. Jordan KC, Di Gennaro JL, von Saint André-von Arnim A, Stewart BT. Global trends in pediatric burn injuries and care capacity from the World Health Organization Global Burn Registry. Front Pediatr 2022;10:e954995. doi: 10.3389/fped.2022.954995.
22. Puca V, Marulli RZ, Grande R, Vitale I, Niro A, Molinaro G, et al. Microbial Species Isolated from Infected Wounds and Antimicrobial Resistance Analysis: Data Emerging from a Three-Years Retrospective Study. Antibiotics (Basel) 2021;10:1162. doi: 10.3390/antibiotics 10101162.
23. Upreti N, Rayamajhee B, Sherchan SP, Choudhari MK, Banjara MR. Prevalence of methicillin resistant Staphylococcus aureus, multidrug resistant and extended spectrum β-lactamase producing gram negative bacilli causing wound infections at a tertiary care hospital of Nepal. Antimicrob Resist Infect Control 2018;7:e121. doi: 10.1186/s13756-018-0408-z.
24. Hassan MA, Tamer TM, Valachová K, Omer AM, El-Shafeey M, Mohy Eldin MS, et al. Antioxidant and antibacterial polyelectrolyte wound dressing based on chitosan/hyaluronan/phosphatidylcholine dihydroquercetin. Int J Biol Macromol 2021;166:18-31. doi: 10.1016/j.ijbiomac.2020.11.119.
25. Turner KH, Everett J, Trivedi U, Rumbaugh KP, Whiteley M. Requirements for Pseudomonas aeruginosa acute burn and chronic surgical wound infection. PLoS Genet 2014;10:e1004518. doi: 10.1371/journal.pgen.1004518.
26. Ugwuanyi FC, Ajayi A, Ojo DA, Adeleye AI, Smith SI. Evaluation of efflux pump activity and biofilm formation in multidrug resistant clinical isolates of Pseudomonas aeruginosa isolated from a Federal Medical Center in Nigeria. Ann Clin Microbiol Antimicrob 2021;20:11. doi: 10.1186/s12941-021-00417-y.
27. Lescat M, Poirel L, Tinguely C, Nordmann P. A Resazurin Reduction-Based Assay for Rapid Detection of Polymyxin Resistance in Acinetobacter baumannii and Pseudomonas aeruginosa. J Clin Microbiol 2019;57:e01563-18. doi: 10.1128/JCM.01563-18.

**Author Contribution:**

SA: Literature search, manuscript writing and performed the experiments.
MZM: Supervised and designed research work
MKT: Approved the study design and concept
MAM: Analyzed the data
MAM: Conceived the idea
AH: Drafted the final manuscript.