International Journal of Biomedicine 15(1) (2025) 192-195
http://dx.doi.org/10.21103/Article15(1)_OA24

ORIGINAL ARTICLE

Antimicrobial Resistance

INTERNATIONAL JOURNAL OF BIOMEDICINE

Virulence Factors and Antibiotic Susceptibility in *Staphylococcus aureus* Isolated from Burn Infections

Mustafa S. Al-Salmani[1], Saja A. Shareef[1], Sama Fakhri Ali[2], Sabreen Ali Hadi[3], Hasan A. Aal Owaif[3*]

[1]*Department of Molecular and Medical Biotechnology, College of Biotechnology, Al-Nahrain University, Baghdad, Iraq*
[2]*Dijlah University College, Baghdad, Iraq*
[3]*Department of Plant Biotechnology, College of Biotechnology, Al-Nahrain University, Baghdad, Iraq*

Abstract

Background: Burn injuries constitute a significant public health concern. A burn compromises the skin's protective barrier, resulting in the downregulation of local and systemic immunological responses.

Staphylococcus aureus is among the most prevalent causes of burn wound infections. We conducted this study to detect the virulence genes, antibiotic susceptibility, and biofilm production of locally isolated S. aureus from burn infections.

Methods and Results: We obtained 120 swabs from burn patients at several hospitals in Baghdad between December 2023 and March 2024. The *S. aureus* isolates were identified using biochemical assays and the VITEK-2 system. The Kirby Bauer test was used for antimicrobial susceptibility, and the result was evaluated based on the guidelines of CLSI 2023. The microtiter-plate assay was conducted to measure the biofilm formation at OD570 using an ELISA reader. The icaA, icaD, ebpS, fnbB, and fib genes were detected by PCR. Fifty-four (45.8%) of S. aureus isolates were identified from 120 swabs of burn infections. The results demonstrated that all isolates (100%) were resistant to penicillin, 75.9% were resistant to erythromycin, 63% to tetracycline, 61.1% to oxacillin, 48.2% to imipenem, 42.6% to gentamicin, 27.8% to ciprofloxacin, 26% to vancomycin, and 9.2% to rifampicin. The rate of multidrug resistance in S. aureus isolates was 48%. The results indicated that 48.2%, 33.3%, and 18.5% of the isolates produced high, intermediate, and low biofilms, respectively. The biofilm-related genes icaA, icaD, ebpS, fnbB, and fib were detected in 87%, 77.7%, 42.5%, 33.3%, and 51.8% of the isolates, respectively.

Conclusion: Our study indicated that biofilm formation is correlated with antibiotic resistance and genes related to biofilm development. It is important to address genetic variables that affect resistance mechanisms and microbial behavior to reduce the spread of resistant strains and enhance treatment results.(**International Journal of Biomedicine. 2024;15(1):192-195.**)

Keywords: *Staphylococcus aureus* • virulence genes • antibiotic resistance • biofilm

For citation: Al-Salmani MS, Shareef SA, Ali SF, Hadi SA, Owaif HAA. Virulence Factors and Antibiotic Susceptibility in *Staphylococcus aureus* Isolated from Burn Infections. International Journal of Biomedicine. 2024;15(1):192-195. doi:10.21103/Article15(1)_OA24

Introduction

Burn injuries constitute a significant public health concern. A burn compromises the skin's protective barrier, resulting in the downregulation of local and systemic immunological responses. Consequently, burn wounds provide an optimal environment for microbial proliferation.[1,2] The length of hospitalization following a burn injury correlates closely with the type of bacteria that infect patients. *Staphylococcus aureus* represents the main cause of such infections.[3] *S. aureus* is the most prevalent opportunistic bacterium, and is responsible for several superficial and potentially fatal infections.[4] It can lead to several illnesses, such as skin and soft tissue infections, toxin-mediated complications, and acute infections.[5] It is a major pathogenic agent in both community and hospital settings because it produces many virulence factors and develops multidrug resistance to several antibacterial therapies.[6] Several virulence

factors, including biofilms, surface proteins, exotoxins, exoenzymes, and exfoliative toxins, correlate with *S. aureus's* capacity to induce various illnesses. These substances enable bacteria to adhere to tissues, as a result developing disease and infiltrating the immune system, leading to toxicity.[7] The biofilm formation by *S. aureus* provides a distinct potential for persistent infection, resistance to antibiotics, and immune system evasion.[8] *S. aureus*-producing biofilm is infamous for its capacity to resist treatment because biofilms are built on implanted medical devices, leading to chronic infections.[9] Furthermore, *S. aureus* strains in the biofilm often exhibit a diminished response to antibiotics due to inadequate drug penetration into the biofilm matrix and to the immune system and the obstruction of recognized antigens on bacterial cells. [10] We conducted this study to detect the virulence genes, antibiotic susceptibility, and biofilm production of locally isolated *S. aureus* from burn infections.

Materials and Methods

We obtained 120 swabs from burn patients at several hospitals in Baghdad between December 2023 and March 2024. The *S. aureus* isolates were identified using biochemical assays and the VITEK-2 system. The Kirby Bauer test was used for antimicrobial susceptibility, and the result was evaluated based on the guidelines of CLSI 2023. The following antimicrobial agents were used: imipenem (10µg), erythromycin (15 µg), rifampicin (5 µg), tetracycline (30 µg), cefazoline (1 µg, penicillin (10 µg), ciprofloxacin (5 µg), vancomycin (30 µg), and gentamicin (10 µg). The microtiter-plate assay was conducted to measure the biofilm formation at OD570 using an ELISA reader.[11] The *icaA*, *icaD*, *ebpS*, *fnbB*, and *fib* genes were detected by PCR (Table 1).

Table 1.

PCR primer sequence.

Gene	Sequence: 5' ⟶ 3'	Product size(bp)	Ref.
icaA	F: TCTCTTGCAGGAGCAATCAA R: TCAGGCACTAACATCCAGCA	188	12
icaD	F: ATGGTCAAGCCCAGACAGAG R: CGTGTTTTCAACATTTAATGCAA	198	12
ebpS	F:CATCCAGAACCAATCGAAGAC R:CTTAACAGTTACATCATCATGTTTATCTTTG	186	13
fnbB	F:GTAACAGCTAATGGTCGAATTGATACT R:CAAGTTCGATAGGAGTACTATGTTC	524	13
fib	F:CTACAACTACAATTGCCGTCAACAG R:GCTCTTGTAAGACCATTTTCTTCAC	404	13

Results

Fifty-four (45.8%) of *S. aureus* isolates were identified from 120 swabs of burn infections. The results demonstrated that all isolates (100%) were resistant to penicillin, 75.9% were resistant to erythromycin, 63% to tetracycline, 61.1% to oxacillin, 48.2% to imipenem, 42.6% to gentamicin, 27.8% to ciprofloxacin, 26% to vancomycin, and 9.2% to rifampicin (Table 2). The rate of multidrug resistance in *S. aureus* isolates was 48%. The results indicated that 48.2%, 33.3%, and 18.5% of the isolates produced high, intermediate, and low biofilms, respectively. The biofilm-related genes *icaA*, *icaD*, *ebpS*, *fnbB*, and *fib* were detected in 87%, 77.7%, 42.5%, 33.3%, and 51.8% of the isolates, respectively (Figure 1). Table 3 shows the relationship between antibiotic resistance and biofilm production. Table 4 represents the relationship between biofilm-related genes and biofilm formation.

Table 2.

Antimicrobial resistance of S. aureus isolates.

Antibiotic	Sensitive n (%)	Intermediate n (%)	Resistance n (%)
Imipenem	20 (37)	8 (14.8)	26 (48.2)
Erythromycin	9 (16.7)	4 (7.4)	41 (75.9)
Rifampicin	49 (90.8)	0 (0)	5 (9.2)
Tetracycline	13 (24)	7 (13)	34 (63)
Oxacillin	18 (33.3)	3 (5.6)	33 (61.1)
Penicillin	0 (0)	0 (0)	54 (100)
Ciprofloxacin	37 (68.5)	2 (3.7)	15 (27.8)
Vancomycin	40 (74)	0 (0)	14 (26)
Gentamicin	29 (53.7)	2 (3.7)	23 (42.6)

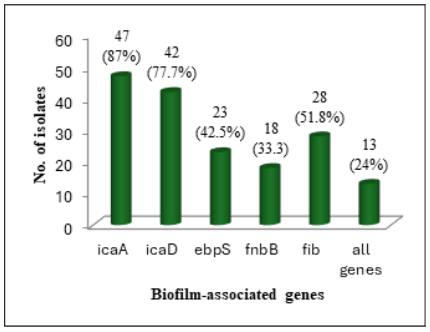

Fig. 1. *Biofilm-related genes among S. aureus isolates.*

Table 3.

The relationship between biofilm development and antibiotic resistance in S. aureus isolates.

Antibiotic	High (n=26)	Inter-mediate (n=18)	Low (n=10)
	n (%)	n (%)	n (%)
Imipenem	19 (73)	5 (27.7)	2 (20)
Erythromycin	24 (92.3)	14 (77.7)	3 (30)
Rifampicin	3 (11.5)	2 (11.1)	0 (0)
Tetracycline	22 (84.6)	9 (50)	3 (30)
Oxacillin	23 (88.4)	6 (33.3)	4 (40)
Penicillin	26 (100)	18 (100)	10 (100)
Ciprofloxacin	11 (42.3)	4 (22.2)	0 (0)
Vancomycin	9 (34.6)	3 (16.6)	2 (20)
Gentamicin	16 (61.5)	6 (33.3)	1 (10)

Table 4.

Relationship between biofilm-related genes and biofilm formation in S. aureus isolates.

Gene	High (n=26)	Inter-mediate (n=18)	Low (n=10)
	n (%)	n (%)	n (%)
icaA	26 (100)	16 (88.8)	5 (50)
icaD	23 (88.4)	17 (94.4)	2 (20)
ebpS	15 (57.6)	4 (22.2)	4 (40)
fnbB	13 (50)	5 (27.7)	0 (0)
fib	17 (65.3)	8 (44.4)	3 (30)
All genes	9 (34.6)	4 (22.2)	0 (0)

Discussion

This study demonstrated the prevalence of *S. aureus*, a gram-positive bacterium considered a major cause of burn infections. Our results agree with a study in Egypt, which showed a high incidence of *S. aureus* isolated from various wound infections.[14] In our study, all isolates were penicillin resistant. Resistance to penicillin can result from the hydrolysis of the penicillin β-lactam ring by the penicillinase produced by *S. aureus*.[15] The isolates were highly sensitive to rifampicin, 90.8%, consistent with Chan et al.'s findings that 99% of *S. aureus* isolates were rifampicin sensitive.[16] The results showed that out of 54 isolates, 26(48%) had multidrug resistance, and this agreed with a study in Iran, which revealed that 42.3% of the isolated *S. aureus* were susceptible to all antibiotics under study.[17] Transferable plasmids harboring resistant genes passed among pathogenic bacteria may be associated with multiple antibiotic resistance in bacteria.[18,19] Changes in bacterial enzymes and patients' overuse and abuse of antibiotics may be linked to antibiotic resistance.[20]

In our study, the biofilm-related genes *icaA*, *icaD*, *ebpS*, *fnbB*, and *fib* were detected in 87%, 77.7%, 42.5%, 33.3%, and 51.8% of the isolates. Other studies showed 49.2% for *icaA*, 54.8% for *icaD*, 26.5% for *ebpS*, 46.6% for *fnbB*, and 39.9% for *fib*.[14,21]

The complex relationship between biological and environmental factors is reflected in the variation in detecting virulence genes. Our study revealed a relationship between biofilm formation and antibiotic resistance. Bacteria in biofilms resist antimicrobial agents by several mechanisms, such as integrons, reduced antibiotic penetration, chemical changes of antibiotic, efflux pump, damage of antibiotic, and target protection.[22,23] Antibiotics can aggregate and contribute to the weight of the biofilm matrix. This matrix acts as a barrier to antibiotic penetration into the deeper layer of bacteria in the biofilm. The matrix reduces antibiotic mobility, resulting in insufficient entrance of antibiotics to the inner layer.[24,25] The results showed a correlation between biofilm-related genes and biofilm formation. Although all isolates could form biofilm to varying degrees, the frequency of biofilm-associated genes varied. The predominance of these genes indicates that several factors may play a role in different stages of biofilm development.[21,26]

Conclusion

Our study indicated that biofilm formation is correlated with antibiotic resistance and genes related to biofilm development. It is important to address genetic variables that affect resistance mechanisms and microbial behavior to reduce the spread of resistant strains and enhance treatment results.

Competing Interests

The authors declare that they have no competing interests.

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***Corresponding author:** Hasan A. Aal Owaif. Department of Plant Biotechnology, College of Biotechnology, Al-Nahrain University, Baghdad, Iraq. E-mail: hasan.abdulhadi@nahrainuniv.edu.iq*