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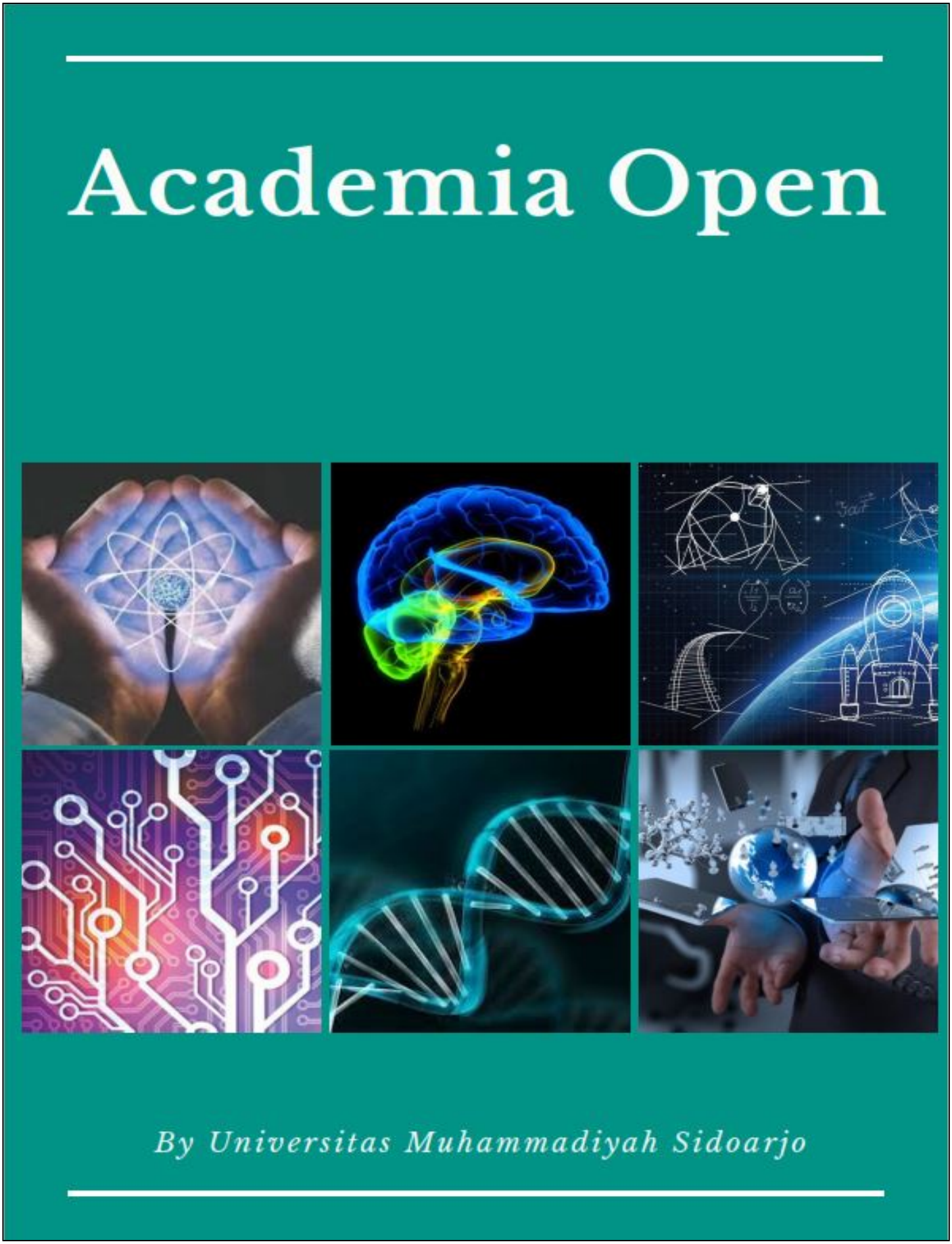

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Biofilm Formation and Antibiotic Resistance of Staphylococcus aureus Isolates

Pembentukan Biofilm dan Resistensi Antibiotik pada Isolat Staphylococcus aureus

Ahmed Ramadan Hadabi Al-Khamis, ahmedramadanalaskary@gmail.com, (1)
Department of Laboratory, Thi-Qar Health Directorate, Iraqi Ministry of Health, Iraq

Jasim Mohammed Abed, jasim@gmail.com, (0)
Department of Laboratory, Thi-Qar Health Directorate, Iraqi Ministry of Health, Iraq

Hamid Kadhim Sameer, hamid@gmail.com, (0)
Department of Laboratory, Thi-Qar Health Directorate, Iraqi Ministry of Health, Iraq

Mohammed Ali Hassan, ali@gmail.com, (0)
Department of Laboratory, Thi-Qar Health Directorate, Iraqi Ministry of Health, Iraq

(1) Corresponding author

Abstract

General Background: Biofilm-associated infections represent a major clinical challenge due to their persistence and resistance to antibiotics. **Specific Background:** *Staphylococcus aureus* is a common pathogen implicated in chronic and hospital-acquired infections, particularly due to its biofilm-forming capability. **Knowledge Gap:** Despite global reports on S. aureus resistance, limited data exist regarding its biofilm formation and antibiotic susceptibility in clinical isolates from Mashhad Hospital. **Aims:** This study aimed to investigate the prevalence, biofilm formation, and antibiotic resistance profile of S. aureus isolated from hospitalized patients. **Results:** A total of 150 clinical samples (blood, urine, wounds, and secretions) were collected from 95 male and 55 female patients, yielding 70 S. aureus isolates. All isolates were biofilm-positive. Antibiotic susceptibility testing revealed 100% resistance to ampicillin, 80.3% to azithromycin, and 70.7% to cefoxitin, while all isolates remained sensitive to vancomycin and clarithromycin. Statistical analysis showed significant associations ($p < 0.05$) between patient sex and both biofilm formation and antibiotic resistance patterns. **Novelty:** This study provides updated, localized resistance data and highlights the universal biofilm-forming potential of S. aureus in this region. **Implications:** The findings underscore the need for enhanced infection control strategies and the prudent use of antibiotics to mitigate biofilm-related resistance in hospital settings.

Highlights:

1. S. aureus causes resistant, biofilm-related hospital infections.
2. 70 isolates: all biofilm-positive, high resistance to common antibiotics.
3. Requires improved antibiotic stewardship and infection control measures.

Keywords: S. aureus, Antibiotic Resistance, Biofilm, Clinical samples

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Introduction

Staphylococcus aureus infection depends on the synthesis of surface proteins that facilitate bacterial adhesion to host tissues, the release of extracellular toxins and enzymes that damage host cells and tissues, and the evasion or inactivation of the host immune response, enabling bacterial proliferation and dissemination within host cells and tissues [1]. Staphylococcus aureus synthesizes and releases enzymes such as coagulase, hyaluronidase, deoxyribonuclease, and lipase to augment its pathogenicity [2]. Moreover, enterotoxins, toxic shock syndrome toxin 1 (TSST-1), exfoliating toxins (ETs), hemolysins, epidermal differentiation inhibitors (EDINs), and Pantone-Valentine leukocidin (PVL) have been recognized as extracellular protein toxins that augment pathogenicity [3]. Some toxins were detected more frequently in MRSA infections compared to non-MRSA cases [4].

Staphylococcal infection-related hospitalizations are common, resulting in elevated mortality rates and healthcare expenditures [5]. The ability of Staphylococcus aureus to produce enzymes that neutralize antibiotics complicates antimicrobial therapy, leading to multiple drug resistances [6]. Antibiotic resistance enzymes significantly contribute to bacterial resistance against antibiotic pressure, influencing diversity, evolution, and expansion. Antibiotic-producing bacteria necessitate mechanisms to mitigate the harmful effects of chemicals through the production of degrading enzymes [7]. The selection pressure from the extensive use of antibiotics in humans and animals has increased the prevalence of resistant bacterial clones.

Antibiotic resistance in Staphylococcus aureus is advancing rapidly, and the rise of multidrug-resistant strains presents a significant challenge. The annual mortality rate from antibiotic-resistant diseases has surpassed 10 million individuals, and projections indicate that by 2050, deaths attributable to cancer will exceed this figure. The implications of morbidity and mortality underscore the pressing necessity for the identification of novel effective solutions, given the limitations of conventional antibiotics [8]. Consequently, alternative therapies constitute a significant area of investigation given the scarcity of novel antibiotic classes. Multiple strategies have been implemented, particularly those involving drug design with synthetic analogues that inhibit virulence factors. Nonetheless, these studies have not yielded favorable outcomes owing to issues of toxicity and/or low bioavailability. Current research is examining new options that target molecules or biological compounds to disrupt toxins or genes that regulate toxins, representing a novel generation of potential anti-staphylococcal therapies [9].

Methods

Sample Collection

During a four-month period, 150 samples were collected from patients presenting with urinary tract infections, unexplained fever, wounds, and ear discharge at Mashhad Hospital. The sample included 95 males and 55 females.

S. aureus Identification

The samples were initially cultured on blood agar, chocolate agar, and MacConkey agar. Following bacterial growth on blood agar, they were subsequently cultured on Mannitol Salt agar. Identification of S. aureus was performed using coagulase test, catalase test, and Gram staining.

Antibiotic Susceptibility

Before tested the bacteria for antibiotics susceptibility the bacteria were tested their ability for biofilm formation, and tested biofilm positive *S. aureus*.

Statistical Analysis

The current study data was statistically analysis by using SPSS version 26, based in using chi-square at p. value <0.05 [10].

Result and Discussion

Result

Prevalence of patients According to Sex

Out of a total of 150 patient (95 male and 55 female) from blood, urine, wound and secretion samples of patients hospitalized in one of the hospitals in Mashhad, 70 *S. aureus* samples were isolated and identified using different tests, the result was showed a significant difference at p. value <0.05.

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Samples	Male		Female	
	No.	%	No.	%
Blood	26	27.37	26	47.27
Urine	35	36.84	14	25.45
Wound and Exudate	34	35.79	15	27.27
Total	95	61.29	55	35.48
p. value 0.011				

Table 1. *Prevalence of patients according to sex*

Identification of *S. aureus*

Characteristics of *S. aureus* on Blood Agar and Gram Stain

S. aureus colonies were cultured on blood agar medium and grew after 24 hours at 37 degrees, and the results of all cultures were positive, and by gram-stain the bacteria were arranged as cluster, as in figure 1.

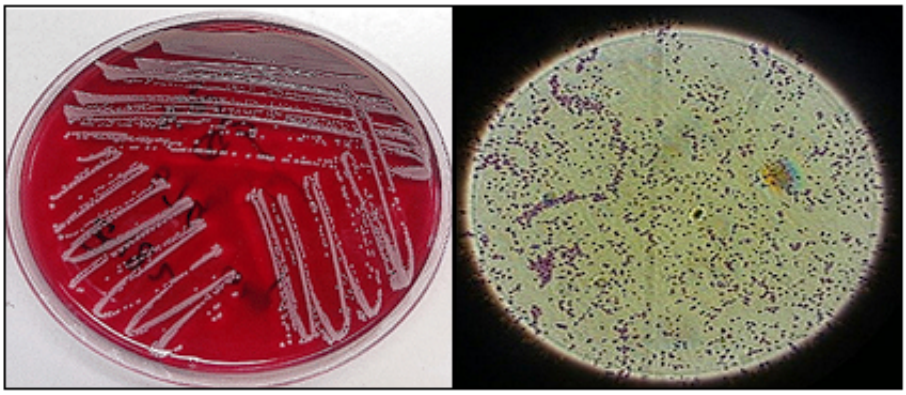

Figure 1. *Growth of S. aureus on blood agar and under gram stain*

Catalase test , Slide and T ube Coagulase T est

All strains of bacteria were rapidly formed bubbles after the addition of H_2O_2, resulting in a positive catalase test for all *S. aureus* strains. With regard slide coagulase results were positive for all strains after maxing the colonies with plasma. Regarding tube coagulase the results showed all strain showed positive coagulase test after mixing and incubation the colonies with plasm in test tube under 37 C, as in figure.

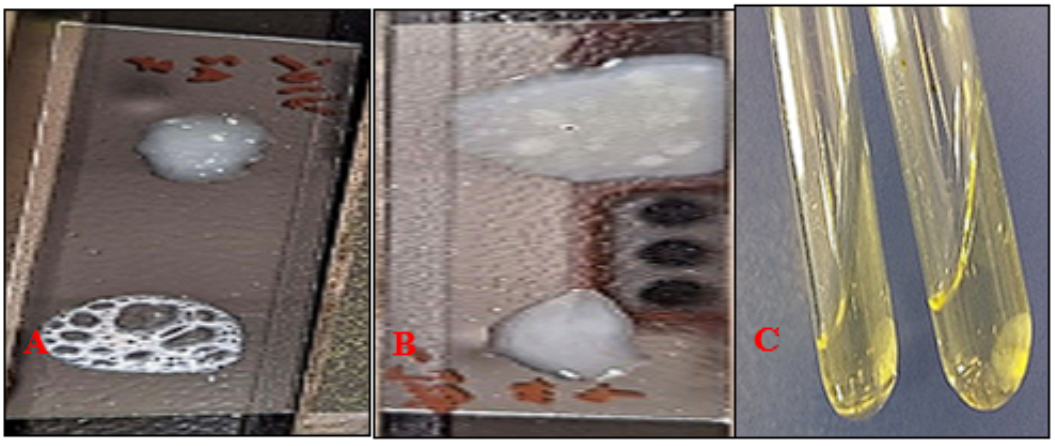

Figure 2. *S. aureus catalase test: positive*

Antibiotics Susceptibility

The present study was recorded a significant difference at p. value <0.05, was recorded the isolated *S. aureus* were

100% resistant for Ampicillin activity, 80.3% for Azithromycin, and 70.7% for Cefoxitin activity, whereas the study noted all isolated *S. aureus* were sensitive for both Vancomycin and Clarithromycin, as in table 2.

Antibiotics	Resistance %	Sensitivity %
Ampicillin	100.0	0
Ciprofloxacin	63.3	36.7
Gentamicin	38.7	61.3
Azithromycin	80.3	19.8
Cefoxitin	70.7	29.3
Oxacillin	69.3	30.7
Vancomycin	0.0	100
Clarithromycin	0.0	100
% of Susceptibility	52.79	47.21
p. value <0.01		

Table 2. *Antibiotic resistance in Staphylococcus aureus strains*

Discussion

Biofilm formation poses considerable health and economic implications. Approximately 65% of hospital infections in the United States are linked to biofilm formation, with economic losses attributed to biofilms surpassing one billion dollars annually [11]. A significant quantity of bacteria proliferates in the biofilm phase within the bodies of livestock. The involvement of biofilms in numerous chronic and resistant infections has been established. Staphylococcus aureus infections are prevalent and exhibit significant resistance, making them among the most common long-term infections. Biofilm formation consists of two stages: the initial stage involves the adhesion of cells to a surface, aided by cell wall attachment factors. The second stage encompasses cell proliferation and the development of a mature structure comprising numerous distinct cell layers interconnected by polysaccharide intracellular junctions [12].

This study involved the collection of 150 samples (95 male and 55 female) from blood, urine, wound, and secretion sources of patients hospitalized in a Mashhad hospital. A total of 70 S. aureus samples were isolated and identified through various tests. Of the 150 samples, 63.33% were from male patients and 66.36% from female patients, with samples obtained from blood (26), urine (35), and wounds and secretions (34) of male patients. The clinical samples from women included blood (16 samples), urine (14 samples), and wounds and secretions (15 samples). The frequency of blood samples was 17% for both women and men, while urine and wound samples accounted for 9.33%, and women's secretions represented 10%. Additionally, men's samples comprised 23% of clinical urine and wound samples and 22.66% of secretions.

Following the assessment of drug resistance via the disk diffusion method, it was found that ampicillin exhibited the highest resistance rate at 100%, while vancomycin and clarithromycin demonstrated the lowest resistance rate at 0%. The resistance level to fluoroquinolone antibiotics, specifically ciprofloxacin, was recorded at 63%, while the resistance level to macrolides, such as azithromycin, was noted at 80%. The resistance rate to gentamicin was 38%.

Ghaderi et al. [13] conducted a study involving 200 samples in Tehran, revealing antibiotic resistance rates of 94% for penicillin, 72% for tetracycline, 54% for ampicillin, and 51% for cefoxitin. The resistance rate observed in this study is elevated. A 2018 study by Mashaiekhi et al. involving 117 hospital samples indicated that the highest levels of antibiotic resistance were associated with penicillin, gentamicin, and amikacin, consistent with the findings of Torki Baghbaderani et al. [14].

In a 2016 study by Tel et al. [15] involving 112 Staphylococcus aureus isolates, the resistance rates observed for penicillin, ampicillin, tetracycline, erythromycin, trimethoprim-sulfamethoxazole, enrofloxacin, and amoxicillin-clavulanic acid were 45.5%, 39.3%, 33%, 26.8%, 5.4%, 0.9%, and 0.9%, respectively. All isolates demonstrated susceptibility to vancomycin and gentamicin [16], indicating a significantly lower resistance rate compared to the current study. These differences may arise from variations in sampling methods, sample size, and geographical location.

Conclusion

This study highlights the high prevalence and significant antibiotic resistance patterns of Staphylococcus aureus isolated from hospitalized patients, with a marked resistance to commonly used antibiotics such as ampicillin (100%), azithromycin (80.3%), and cefoxitin (70.7%), while showing complete sensitivity to vancomycin and clarithromycin. The presence of biofilm-forming strains further complicates treatment, as biofilms contribute to persistent infections and enhanced resistance. These findings underscore the urgent need for continuous

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antimicrobial resistance surveillance and the development of targeted therapeutic strategies, especially in clinical settings with rising multidrug-resistant S. aureus cases. Future research should focus on novel anti-virulence therapies, biofilm-disrupting agents, and the molecular characterization of resistance genes to inform more effective treatment protocols.

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