Original Article

Relationship Between *Staphylococcus aureus* Biofilm Formation and Antibiotic Resistance that Isolated from Infections of the Urinary Tract in Babylon Province, Iraq

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Abstract

Background: Hospital and community-acquired *Staphylococcus aureus* is a major concern for healthcare costs in addition to severe morbidity and mortality. *Staphylococcus aureus* biofilm generation contributes considerably to treatment failures. **Objective:** The current study intends to determine the association between biofilm formation and antibiotic resistance in isolated *S. aureus*. **Materials and Methods:** During the period from June 2022 to October 2022, 80 clean, voided, a midstream specimen of urine (MSU) were obtained from patients who attended the AL-Hashimiyah General Hospital. Cultured assays and conventional biochemical tests were performed to isolate and identify *S. aureus* and then confirmed by using VITEK2 system. **Results:** The results of the antimicrobial resistance test by the disc diffusion method for nine antibiotics from different groups showed Erythromycin 36)66.66%(, Trimethoprim 31)57.4%(, Amoxicillin-clavulanic acid 30 (55.55%), Ceftriaxone 23 (42.59%), Meropenem 17 (31.48), Ciprofloxacin 10 (18.51%), Nitrofuranate 10 (18.51%), Imipenem 5 (9.25%) while the less resistant showed with antibiotic Vancomycin 3 (5.55%). Through this study, it was found that women are more susceptible to urinary tract infection, where the ratio was (38/54) 70.37% compared to men (16/54) 29.62% susceptible to infection. Monitoring the resistance and spread of *Staphylococcus aureus* is of paramount importance in clinical management. The prevalence of biofilm producer isolated bacteria was 43 (79.6%) that 18 (41.8%) produced strong biofilm, 25 (58.1%) produced moderate biofilm, and 11 (20.3%) isolates produced non/weak biofilm. **Conclusions:** The study's findings indicate that a high frequency of urinary tract infections is associated with *Staphylococcus aureus* also this pathogen highly biofilm-producing is developing resistance to numerous antibiotics used in this study and thus decreasing their value in the empirical management of simple UTIs. Therefore, it's imperative to implement fresh approaches to combat antibiotic resistance. The high prevalence of *S. aureus* has been a serious health concern in urological patients.

Keywords: Antibiotic resistance and biofilm formation, *Staphylococcus aureus*, urinary tract infections

Introduction

Urinary tract infections (UTIs) are among the most prevalent bacterial illnesses, and they make up a sizable portion of the workload in clinical microbiology labs.[1] Among the most common infectious diseases in humans, UTIs pose a serious threat to public health and have a substantial financial impact. In the United States, UTIs are responsible for more than 7 million doctor visits annually and 15% of all antibiotics prescribed by the community. In uncomplicated renal and cystitis.[2] The most prevalent form of infection is a UTIs, which affects 40% of American women over the course of their lifetimes.[3] The use of antibiotics to treat UTIs eliminates the microbes that cause them, but they negatively affect the intestines because they kill existing bacteria.[4] and researchers expect an alarming increase in UTIs and believe that they will affect

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more than 100 million people worldwide each year one[5] in 2020 researcher Ibrahim, and colleagues showed that the highest percentage isolated from urine after pus was *Staphylococcus aureus* was 30%.[6] Globally, *Staphylococcus aureus* is among the most common and clinically important pathogens. From minor skin eruptions to potentially fatal infections like bacteremia, endocarditis, pneumonia, and toxic shock syndrome, it can cause a wide range of illnesses.[7] This pathogen's ability to develop antibiotic resistance traits through frequently poorly understood mechanisms is evidence of its success.[8] There is no benefit from the use of penicillin treatment at the present time, as the rate of resistance of *S. aureus* to penicillin has become more than 90%.[9] By growing the organism with increasing concentrations of penicillin over a long period, it was possible to make the organism resistant to penicillin. Similar degrees of increased resistance were found in four *Staphylococcus* strains isolated during penicillin treatment of topical infections.[10] One of the main causes of nosocomial infections is *Staphylococcus aureus* particularly methicillin-resistant bacteria (MRSA) because of this resistance, it has become difficult to fight and eliminate it, thus increasing its spread.[11] *Staphylococcus aureus* is a major pathogen in human infections. Bacterial biofilm development is a significant component in treatment failures. Biofilms are highly resistant to antibiotics than planktonic cells due to the extracellular matrix's multi-level protection (which inhibits antibiotic penetration), altered metabolic states, and growth rate.[12,13]

Staphylococcus aureus may elude human defenses and develop resistance to the action of antimicrobial medicines while growing in biofilms, making biofilm infections extremely difficult to eliminate.[14,15]

Materials and Methods

Bacterial isolates

About 86 mid-stream morning urine samples were collected from patients attending clinics for consultation at AL-Hashimiyah General Hospital, and the urine samples were cultured on Blood agar, MacConkey Agar, and mannitol salt agar then incubated at 37°C for 24 h. *Staphylococcus* isolates were identified by microscopic examination involving bacterial cell morphology by Gram-stain to determine Gram-stain reactivity, shape, and cell arrangement. further biochemical tests were carried out to identity of the isolates. The results of these biochemical tests were used to identify the *S. aureus* also, the diagnosis was confirmed by using the Vitek 2 device.

Antimicrobial susceptibility testing (AST)

AST of the isolates was performed by using Vitek2 system.

Biofilm formation of *S. aureus*

The biofilm formation was detected for all isolates of *S. aureus* by using the microtiter plate assay.

Isolates of *S. aureus* were cultivated for one night in Tryptic soy broth (TSB) with 1% glucose added as a supplement. Bacterial cultures were diluted into 5 mL of sterile normal saline (NaCl 0.85%), mixed thoroughly, and adjusted to the McFarland turbidity standard (0.5). Bacterial isolates (1.5×10^8 CFU/mL) from each culture were placed in three wells (triplicate testing) of a polystyrene microtiter plate (180 µL from TSB supplemented with 1% glucose and 20 L from Bacterial cultures) and incubated at 37°C for 18–24 h.

Ethical approval

The study was carried out in compliance with the moral guidelines found in the Helsinki Declaration. Before taking a sample, the patient's verbal and analytical consent was obtained. According to the document number dated March 1, 2022, To get this approval, the study protocol, subject data, and consent form were examined and approved by a local ethics committee.

Results

In the present investigation, 86 urine samples from UTI patients were obtained who attended the Al-Hashimiyah General Hospital in Babil Governorate from June 2022 to October 2022. Of the total samples studied, 54 (62.79%) were identified *S. aureus*. We also found that the infection rate in women infected with *S. aureus* was 70.37% which was significantly higher than the infection rate in men (29.62%) [Table 1].

Antibiotic Resistance Profile (Disc Diffusion method)

The drug sensitivity of *S. aureus* isolates under study was tested for nine different antibiotics belonging to the groups (Macrolides, Penicillin, Cephalosporin, Fluoroquinolones, Carbapenems, Glycopeptide) and the percentage and resistance were calculated by measuring the inhibition area around the discs used and then compared with the approved standard tables (CLSI 2021).

The results in Table 2 demonstrated that the greatest resistance rate of *S. aureus* isolates Erythromycin (E) (36/54) 66.66% followed by Trimethoprim (31/54) 57.4%, Amoxicillin-clavulanic acid (30/54) 55.55%, and the rest of the antibiotics they are Ceftriaxone (23/54) 42.59%, Meropenem (17/54) 31.48%, Nitrofuranate (10/54) 18.15%, Ciprofloxacin (CIP) (10/54) 18.15%, Imipenem (5/54) 9.25% and the lowest percentage of resistance to vancomycin with a value of (3/54) 5.55%.

For all of the *S. aureus* isolates that were being studied, the Multiple Antibiotic Resistance Index (MAR) was determined. The findings revealed that all isolates 53 had extremely high MAR index values of greater than 0.2, as shown in Figure 1. These findings suggest that antibiotics

Table 1: Distribution of *S. aureus* isolated per age group (%)

Age groups years	Percentage (%) of *S. aureus* isolates		
	No. (%)	Male	Female
0–9	4 (7.4)	2 (3.7)	2 (3.7)
10–19	12 (22.2)	2 (3.7)	10 (18.5)
20–29	15 (27.7)	3 (5.6)	12 (22.2)
30–39	8 (14.8)	3 (5.6)	5 (9.25)
40–49	7 (12.9)	4 (7.4)	3 (5.6)
50–59	5 (9.25)	2 (3.7)	3 (5.6)
>60	3 (5.6)	0	3 (5.6)
Total	54	16 (29.62)	38 (70.37)

Table 2: Table showing antibiotic resistance of *S. aureus* isolates by disc diffusion test

Antibiotic class	Antibiotic	Sensitive	Resistance
Beta-Lactams	Amoxillin – clavulanic acid(AUG)	24 (44.44%)	30 (55.5%)
	Ceftriaxone (Cro)	31 (57.4%)	23 (42.59%)
Carbapenems	Imipenem (Imp)	49 (90.7%)	5 (9.2%)
	Meropnem (Mem	37 (68.5%)	17 (31.4%)
Quinolones	Ciprofloxacin	44 (81.4%)	10 (18.5%)
Macrolides	Erythromycin (Ery)	18 (33.33%)	36 (66.66%)
Glycopeptid	Vancomycin (Va)	51 (94.4%)	3 (5.55%)
Sulfonamide	Trimethoprim	23 (42.5%)	31 (57.4%)
Nitrofurans	Nitrofurantoin	44 (81.4%)	10(18.5%)

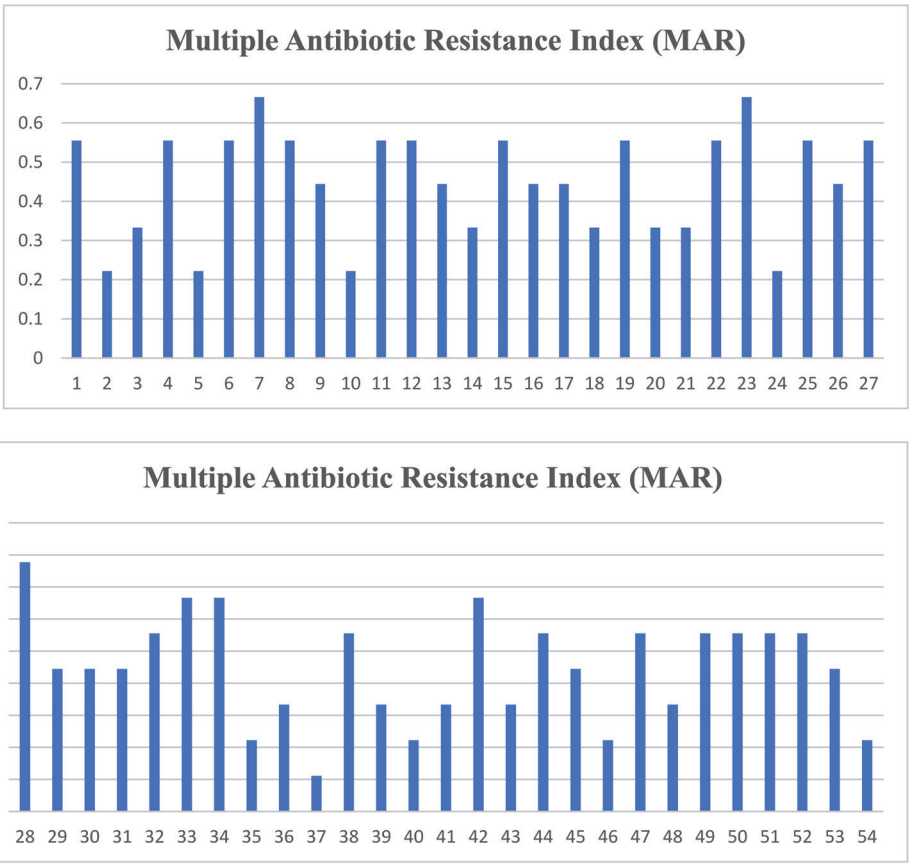

Figure 1: MAR index values for 54 *S. aureus* isolates

Table 3: Number and percentage of *S. aureus* and the degree of their biofilm formation by microtiter plate

Biofilm production	No. Percentage (%)
Strong	18 (33.33)
Moderate	25 (46.2)
Non/weak	11 (20.3)

are being used excessively outside of healthy controls and need for tighter health regulation and legalization of usage.

Phenotype detection of the biofilm production (microtiter plate)

The results showed that a high percentage of bacteria were biofilm producers in varying proportions, with 18 (33.33%) isolates possessing the capacity to create robust biofilm, 25 (46.2%) bacteria being able to generate a moderate amount of biofilm, and 11 (20.3%) isolates not producing or weakly producing biofilms as shown in Table 3.

Discussion

In contrast to earlier research, the current study's results indicate that *Staphylococcus aureus* plays a significant role in causing urinary tract infections, with a percentage of 62.79%.[16-18] which showed the prevalence of *S. aureus* in UTIs as 8.8%, 11.1%, 0.5%, respectively.

The main isolates involved in UTIs were *Escherichia coli*, followed by *S. aureus* 31.5% and 26.56%, respectively, *Klebsiella* (13.9%) was isolated with 12/86 isolates, *E. coli* was isolated from 4/86 patients with 4.65% and both were *Proteus mirabilis* and *Staphylococcus saprophyticus* were isolated. From 3/86 patients 3.48% whereas the rest 10/86 samples 11.62% were from other microorganisms.

Chinampedu *et al*. found that the percentage of *S. aureus* was isolated from urine samples in women, with a percentage of (69.5%) which is a result close to that reached in our current study[18] Methicillin-resistant *Staphylococcus aureus* (MRSA) in pregnant women increases the risk transmission of infection to the fetus in the perinatal period in addition to fatal skin and soft tissue infections.[19] MRSA colonization of the vagina is seen in 14%–22% of pregnant women.[20] A study done by Akortha and Ibadin revealed *S. aureus* to be the most common organism isolated from urine, 39 (72.3%) *S. aureus* isolates were isolated from female infection and 15 (27.7%) *S. aureus* were isolated from males.[21] The frequency of UTIs in women compared to men is due to the shortness of the urethra and the proximity to the anus, which leads to easy contamination of the urinary tract with fecal microbes.[22] The shortening and expansion of the urinary tract in females facilitates the transmission of fecal microorganisms to the urinary tract and increases the incidence of UTIs as well as during sexual intercourse, pregnancy and childbirth,[23] according to study by Guinan *et al*., revealed the association between *S. aureus* in the vagina and its presence in the anterior openings and labia minora, two additional sites. These results raises concerns about the way *S. aureus* enters the vagina. After contact with one of these sites of transmission, fingers and hands may become infected, and inserting contaminated fingers into the vagina may be a method by which this organism enters the body. The finding that hygiene and contraceptive procedures involving the insertion of fingers into the vagina appear to increase the likelihood of vaginal infection Female diaphragm or IUD users have the highest rates of vaginal infections.[24]

The persistent abuse of antibiotics has resulted in the rise of bacterial strains that are resistant to multiple drugs, and thus antibacterial drugs have lost some of their effectiveness or may have become useless, leading to a widening global health security crisis that goes beyond current treatment options. The proliferation of a very limited number of extremely effective antibiotic resistant lineages is the primary cause of the rise in antibiotic resistance in many diseases. The effective acquisition of uncommon antibiotic-resistance genes by mutation or horizontal gene transfer by these lineages is one explanation for this trend. Alternately, it's possible that some bacterial strains are more prone than others to develop resistance, for instance because they have a high rate of mutation or because they possess "potentiator" genes that open up new genetic pathways for resistance development.[25] The researcher considered that the presence of the *blaZ* gene explains the high resistance to beta-lactam antibiotics, which was found in 65% of *S. aureus*.[17]

As for the carbapenems, which were represented in this study by the antibiotic Imipenem and the antibiotic Meropenem. The resistance rate of isolates in this study to imipenem was (9.25%). The results of this study converged with the results of the local study conducted by Al-Hassnawi *et al*. revealed the percentage of resistant isolates was 6.8%.[26] It differed with another study by Akanbi *et al*. showed the percentage of resistant isolates was 96.7%.[27]

In this study, 17 isolates in percentage (31.48%) were resistant to the antibiotic Meropenem, and this result differs from the result in the local study conducted by Al-Hassnawi *et al*. showed the resistance to Meropenem about 11.3%.[26]

In this study, the percentage of *S. aureus* isolates resistant to the Ceftriaxone and amoxicillin/clavulanic acid was 42.59% and 55.55%, respectively, and these results agree and converge with other studies result in Nigeria by Onanuga and Awhowho with percentage 54.3% and 69.6%, respectively, resistance to Ceftrixone.[28] Also, the resistance to Ceftriaxone is similar to the study result conducted by Ali *et al*.[29] with resistance rate about 52.7%.

Trimethoprim is an antibiotic used for UTIs. In our current study, it was used against *S. aureus*, and the resistance rate was 57.4%, which is a disagreement with the local study result conducted by Ali *et al.*[29] and Al-Hassnawi *et al*.[26] are 17.7% and 13.6%, respectively.

Staphylococcus aureus in current result revealed high resistance to erythromycin with 66. 66% and this is similar to the result of the local study by Al-Saadi and Abd Al-Mayahi with resistance rate 70%.[30]

Another antibiotic Nitrofuran and Ciprofloxacin is used in UTIs, and its resistance was 18.51%. This resistance in the current study is not consistent with the results of Onanuga and Awhowho,[28] Ali *et al.*,[29] and Al-Jebouri and Mdish[17] was the percentage of resistance was recorded at 39.1%, 60.3%, and 45%, respectively, resistance for Nitrofuran.

Another two previous studies by Al-Saadi and Abd Al-Mayahi[30] and Rasheed and Hussein[31] in Iraqi Kurdistan study reported that the resistance rate for Ciprofloxacin was 12.5% and 9.19%, respectively.

In this study, vancomycin was the most effective antibiotic against *S. aureus* at 5.55% which is consistent with the study of Rasheed and Hussein in Iraqi Kurdistan the percentage of resistance to vancomycin was 7.56%, respectively,[30] and differs significantly from the result another study where they reported vancomycin resistance by 69.6% and 28%, respectively.[28,32] Correct treatment and removal of urinary pathogenic bacterial strains from individuals with bacterial UTIs depends on accurate diagnosis and understanding patterns of antibiotic susceptibility.

Multidrug antibiotic resistance (MAR) of *S. aureus*

The MDR coefficient was calculated for all *S. aureus* isolates under study, the results showed that the most isolates 43 (79.62%) were shown in Table 2, and these results indicate that there is excessive use of antibiotics outside the health controls, and require more strict health control and rationing of use.[33] The emergence of MDR *S. aureus* has raised health concerns around the world at the moment, biofilms are thought to be the cause of 80% of bacterial infections and more than 65% of all nosocomial infections.[19]

The presence of most, if not all, antibiotics on the market and which can be obtained without a prescription has led to widespread use in many cases to a general rise in the emergence of resistant bacteria.

The energy metabolism of the bacteria is impacted when the cell membrane permeability diminishes, which in turn affects medication absorption and causes drug resistance for instance, a decrease in membrane permeability contributes to *S. aureus'* resistance to aminoglycosides, which ultimately leads to a reduction in drug intake.[34]

High rate of biofilm formation in *S. aureus* bacteria that converged and concurred with the findings of the current study, including the findings Tawfeeq study where the proportion of bacterial isolates produced for biofilm was, respectively, 80.6% and 90%.[35]

According to a study by Mashaly and Badr about 76.5% of isolates in Egypt have the ability to form biofilms.[36] This finding, however, conflicts with research from Belbase *et al*.[37] and Pandey *et al*.,[38] which found that only 25.6% of isolates in those studies were capable of producing biofilms.

The innate and acquired host immune systems may become active at the same time during a biofilm infection; neither of these immune systems is able to eradicate the biofilm pathogen but instead speeds up collateral tissue damage.[39]

Most bacteria live by forming biofilms, which give them stability, catalytic abilities, increased chances of passing along genetic material and antibiotic resistance, participation in cellular communication processes, and protection from harsh and unpredictable environmental conditions, all of which aid in the bacteria's successful colonization of the host.[40-41]

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Conflicts of interest

There are no conflicts of interest.

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