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Research Article

Biofilm Formation of *Staphylococcus saprophyticus* Urinary Tract Infection and Cytokine Response in Reproductive Age Women

Samira Ayoob Hameed[1], Rafal Khaleel Farhan[2]

[1,2]Department of medical microbiology, Tikrit University, Salaheddin, Iraq.
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I N F O

Corresponding Author:
Samira Ayoob Hameed, Department of medical microbiology, Tikrit University, Salaheddin, Iraq.
E-mail Id:
samira.ayoob.2022@st.tu.edu.iq
Orcid Id:
https://orcid.org/0009-0002-3478-5179
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A B S T R A C T

Introduction: A cross-sectional study was conducted during the period extending from the first of November 2022 to the end of Jun 2023. In this study, 425 women aged between 15-45 years old were selected.

Material and methods: Urine samples were cultured and IL17 and CXCL1 levels were determined by using the enzyme-linked Immunosorbent assay (ELISA) technique.

Results: The urine culture revealed that 65.65% of urine samples were positive for bacteria while 34.35% were negative. The study showed that urinary tract infection *Staphylococcus saprophyticus* is most common in the age group of 15-25 years old (45.2%) and in the age group 26-35 years old it was 38.1% while the lowest rate was within the age group of 36-45 years old (16.7%). Biofilm formation was 92.9%. The current study shows that the mean urine IL-17 level among the study group had non-significant differences compared with that of the control group.

Conclusion: The study shows that urine CXCL1 mean level of reproductive age women with UTI where statistically significant as compared to the control group.

Keywords: UTI, *Staphylococcus saprophyticus*, Biofilm formation, cytokine

Introduction

Urinary tract infection (UTI) is the most common infectious disease of the urinary system caused by diverse uropathogens, affecting females and males of all ages.[1] It affects around 150 million people worldwide annually.[2] Bacteria and fungi are the causative agents of UTIs, which can be found in the urine of an infected person .[3] Although UTI is caused by a range of pathogens, gram-negative bacteria, such as *Escherichia coli* (*E. coli*), are the main causative agents of UTIs, accounting for up to 80% of community-acquired uncomplicated UTIs (CAUTI), followed by *Klebsiella pneumonia, Enterobacter*, and *Proteus species*.[4] *Pseudomonas aeruginosa* accounts for more than 40% of the cases, followed by the gram-positive organisms *Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis*[5] and *Staphylococcus saprophyticus*.[6]

UTI is the most common type of Hospital Acquired Infection (HAI) that poses serious challenges in patient care. It is also common among young, sexually active, and premenopausal women. The majority of UTIs are biofilm-associated infections, wherein pathogenic bacterial strains colonise both the tissues of the urinary tract and indwelling devices such as surgical catheters.[6]

Staphylococcus saprophyticus is a uropathogen associated with 10–20% of urinary tract infections (UTIs) in sexually active young women worldwide. The success of S. *saprophyticus* as a uropathogen is due to its ability to survive in harsh and toxic conditions.[7]

Some strains of *S. saprophyticus* can create biofilms, and increase their virulence.[8,9] The bladder immune responses to invading uropathogens have certain similarities but also show differences depending on the type of uropathogens.[10,11] During UTI, macrophages and uroepithelial cells produce pro-inflammatory cytokines and chemokines that attract neutrophils to the site of infection and regulate antibacterial defenses, including, gamma interferon (IFN-γ) and interferon-17 (IL-17).[12]

Materials & methods

Study Groups

A hospital-based cross-sectional study was carried out from November 2022 to June 2023. The study included 425 female patients, aged between 15 and 45 years and 40 samples as control group without any disease . Patients who had taken antibiotics within 3 days before attending the hospital were excluded from the study. Women were told to clean the area around the urethral opening with soap and clean water before urination for sample collection.[13]

Questionnaire

Each patient with UTI was assessed using a structured questionnaire including the name, age, gender, occupation, socio-economic status, chronic illness, medications, pregnancy, duration of pregnancy, and number of births.

Study plan

Urine samples (about 10 mL) were collected from each of the 425 women of the reproductive age group. Culture and ELISA were performed on each sample. The IL-17 and CXCL1 mean levels of these samples were assessed using the ELISA technique.

Urine Sample

The urine samples were divided into 2 parts:

1. Five mL of the sample was centrifuged for 20 minutes at 3000 rpm, and 2 mL supernatants were stored in a deep freezer at -80 ^{O}C for interleukin assay.
2. One to five mL of urine was used for culture.

Culture Media Preparation

Media were prepared and sterilised according to the recommended instructions by the manufacturing company and then sterilised by autoclaving at 121 ^{O}C for 15 minutes. They were investigated for no contamination and the dishes were overturned and incubated at 37 °C for 24 hours and then stored in the refrigerator at 4 °C.[14]

Blood Agar Medium

About 40 g of media powder was suspended in 1000 mL of distilled water. The suspension was heated to boiling to dissolve the medium completely. It was sterilised by autoclaving at 15 lbs pressure (121 °C) for 15 minutes and was cooled to 50 °C, after which 50 mL of sterile defibrinated blood was aseptically added. It was then mixed well and poured into sterile Petri plates.[15]

Biofilm Formation Agar

About 2 g of media powder agar and 3.9 g of biofilm formation medium were suspended in 90 ml of distilled water. The suspension was heated to boiling to ensure that the medium dissolved completely. It was sterilised by autoclaving at 15 lbs pressure (121 °C) for 15 minutes and was cooled to 50 °C, after which 10 mL of biofilm formation supplement (Congo red agar) was aseptically added. Finally, it was mixed well and poured into sterile Petri plates.[16]

MacConkey Agar

About 50 g of media powder was suspended in 1000 mL distilled water. The suspension was heated to boiling to dissolve the medium completely, sterilised by autoclaving at 15 lbs pressure (121 °C) for 15 minutes, and poured into sterile Petri plates.[17]

Mannitol Salt Agar

About 111g of media powder was suspended in 1000 mL distilled water. The suspension was heated to boiling to dissolve the medium completely. Further, it was sterilised by autoclaving at 15 lbs pressure (121 °C) for 15 minutes, and poured into sterile Petri plates [18]

Nutrient Agar

About 28 g of media powder was suspended in 1000 mL distilled water. It was then heated to boiling to ensure that the medium dissolved completely. It was further sterilised by autoclaving at 15 lbs pressure (121 °C) for 15 minutes and then poured into sterile Petri plates [19]

Identification of Bacterial Isolates

Biochemical Tests

Catalase Production Test: A small amount of pure culture was transferred to a clean slide using a sterile wooden stick. A few drops of 3% H_2O_2 were placed on a portion of the colony on the slide and rubbed. The appearance of gas bubbles is an indication of a positive test.[20]

Oxidase Production Test

Strip Method: The test was performed using a filter paper saturated with oxidase reagent (1% tetramethyl-p-phenylenediamine dihydrochloride) which was placed on a Petri dish. A small portion of the organism was removed using a sterile wooden stick and was placed on the filter

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paper and rubbed. The development of a dark purple colour within 10 seconds indicated a positive reaction.[21]

Coagulase Test : Coagulase causes plasma clots by converting fibrinogen to fibrin. It is generated by most strains of *S. aureus*. Free coagulase converts fibrinogen to fibrin by activating a coagulase-reacting factor present in plasma. Free coagulase is detected by the formation of a clot in the tube test. Two drops of sterile saline were added to each area of the divided slide. The bacterial colonies were then transferred to make a suspension and were treated with undiluted plasma.[22] The result was observed as clumping within 10–20 seconds of the bacterial suspension.[23]

Virulence Factor: Biofilm Formation

1. **Congo Red Agar Method:** The Congo red agar (CRA) was streaked with bacterial culture and incubated at 37 °C. After 24 hours, the results were read. A change in the colour of the medium from red to black indicates a positive result.[24]
2. **Biofilm Formation Assay:** The quantitative determination of biofilm formation was using a colourimetric microtiter plate assay.[25]

The bacterial isolates in this study were identified using conventional methods. A VITEK 2 system (BioMérieux, France) was used according to the manufacturer's instructions for the definitive detection of isolated bacteria and antibiotic sensitivity.

Results

Frequency of Urinary Tract Infection

Four hundred and twenty-five urine samples were collected from women of reproductive ages for this study. Their ages ranged from 15 to 45 years. It was found that 65.65% (279/425) of cases were positive in bacteriological culture while 34.35% (146/425) were negative (Figure 1).

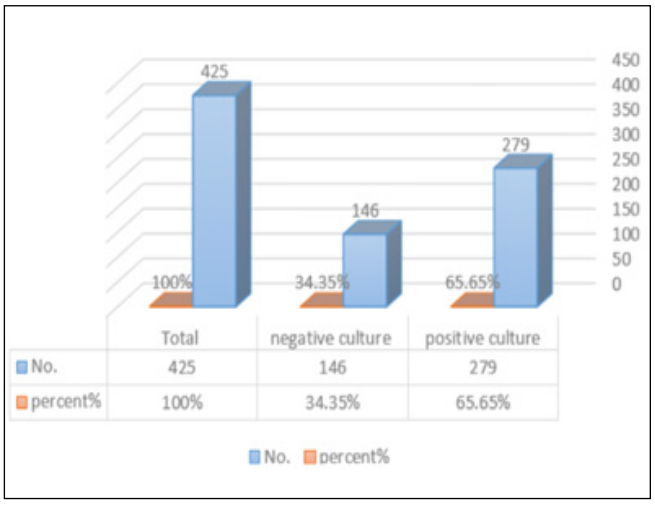

Figure 1.Frequency of Urinary Tract Infection

Identification of Bacterial Causes of UTI

The bacterial identification was for all (279) isolates of gram-positive and gram-negative bacteria detected in urine specimens. The study group showed that out of 279 (65.65%) specimens from reproductive-age group women with positive bacterial culture, 132 (47%) bacterial isolates were gram-positive, while 147 (53%) were gram-negative.

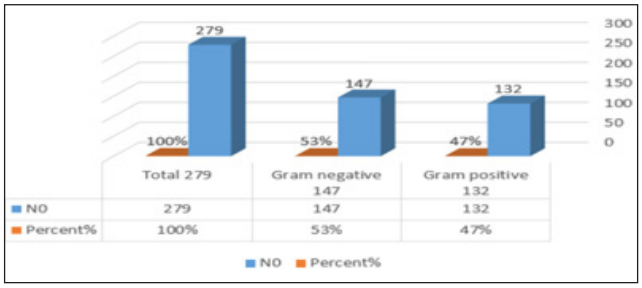

Figure 2. Bacterial Causes of UTI

Distribution of Isolated Bacteria among Study Groups

According to the distribution of the isolated bacteria in the study group, the commonest isolated bacteria among the reproductive age women with UTI was Escherichia coli (91, 33.0%) followed by *Staphylococcus saprophyticus* (42, 15%), *Staphylococcus aureus* (31, 11%), *Enterococcus faecalis* (29, 10%), *Pseudomonas aeruginosa* (25, 9%), *Klebsiella pneumonia* (19, 7%), *Staphylococcus haemolyticus* (17, 6%), *Proteus mirabilis* (6, 2%), *Micrococcus luteus* (13, 5%), and more than one spp. (6, 2%).

Distribution of *Staphylococcus saprophyticus* Isolates among Reproductive Age Women according to Age Groups

The patients in this study with *Staphylococcus saprophyticus* infection were divided into three age groups. Data from Table 1 showed that the highest rate of bacteria growth among females with UTI was in the age group of 15–25 years (45.2%), followed by the age group of 26–35 years (38.1%), while the lowest rate was in the age group of 36–45 years (16.7%).

Distribution of *Staphylococcus saprophyticus* Isolates among Married Women of Reproductive Age according to Pregnancy Status

The distribution of married patients suffering from UTI (*Staphylococcus saprophyticus*) according to their pregnancy status showed that most (56.5%) of them were pregnant while 43.5% were not pregnant (Table 2).

Distribution of *Staphylococcus saprophyticus* Isolates according to Area of Residence

The distribution of *Staphylococcus saprophyticus* UTI patients according to the residence showed that 78.6% of cases belonged to urban areas while 21.4% were from rural areas as shown in Table 3.

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Distribution of *Staphylococcus saprophyticus* Isolates according to Biofilm Formation

Biofilm detection was done by the CRA method and a microtiter plate (MTP) assay. The results of the CRA method showed that 26.19% of isolates were biofilm formers, while 39/42 (92.9%) isolates were detected as biofilm formers using microtiter plate assay. Among these 39 isolates, 2 (5.1%) isolates showed strong biofilm formation, 17 (43.6%) showed moderate biofilm formation, and 20 (51.3%) were weak biofilm producers. The remaining 3 isolates among the 42 isolates (7.14%) could not form any detectable biofilm.

Evaluation of IL-17 Level in Urine of Reproductive Age Women with *Staphylococcus saprophyticus* Urinary Tract Infection

The current study showed that the mean urine IL-17 level in the study group had a non-significant association with that of the control group, as shown in Table 4.

Evaluation of CXCL1 Levels in Urine of Reproductive Age Women with *Staphylococcus saprophyticus* Urinary Tract Infection

The current study showed that the mean CXCL1 value (60.5405 pg/mL) of women with UTI was statistically significant (p = 0.0001) when compared with that of the control group (28.2750 pg/mL) as shown in Table 5.

Association between Virulence Factors and Cytokine Production

The current study revealed no significant association between biofilm and CXCL1 (p = 0.246).

Table 1.Distribution of Isolates of *Staphylococcus saprophyticus* according to Age Groups

Age (Years)	Frequency	Percentage
15–25	19	45.2
26–35	16	38.1
36–45	7	16.7
Total	42	100.0

Table 2.Distribution of *Staphylococcus saprophyticus* Isolates according to Pregnancy Status of Married Participants

Status of Pregnancy	Frequency	Percentage
Not pregnant	10	43.5
Pregnant	13	56.5
Total	23	100.0

Table 3.Distribution of *Staphylococcus saprophyticus* Isolates according to Area of Residence

Area of Residence	Frequency	Percentage
Rural	9	21.4
Urban	33	78.6
Total	42	100.0

Table 4.Level of Urine IL-17 in Urine Patients

Study Group	Mean Urine IL-17 Level	Unpaired t Test	Degree of Freedom	SE	95% Confidence Interval	p Value
Patient group (n = 42)	82.1619	1.251	80	8.12265	-6.00267–26.32648	0.215
Control group (n = 40)	72.0000					

Table 5.Level of Urine CXCL1 in Urine Patients

Study Group	Mean Urine CXCL1 Level	Unpaired t Test	Degree of Freedom	SE	95% Confidence Interval	p Value
Patient group (n = 42)	60.5405	13.146	50.669	2.45432	27.33744–37.19351	0.0001
Control group (n = 40)	28.2750					

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Discussion

Frequency of Urinary Tract Infection

Among the suspected reproductive age women with UTI in our study, a definite positive urine culture was seen in 66% of the sample. This is in agreement with previous studies of Naji & Awadh in Tikri City,[26] Zavala-Cerna et al. in Mexico,[27] Sharma et al. in India,[28] Hussien & Makhrmash in Wasit, Iraq,[29] and Simon-Oke et al. in Nigeria,[30] in which the values were 77.2%, 62.8%, 65.45%, 60.0%, and 61.0%, respectively. The minor variation in the results is perhaps due to the difference of site of study and the type of community and maybe the bacterial growth has been suppressed by antibiotic therapy.

Identification of Bacterial Causes of UTI

In the current study, 52.7% of the isolates turned out to be gram-negative bacteria and 47.3% gram-positive bacteria. This result agrees with those of Al-Obaidi & Mohammed which showed that isolated gram-negative bacteria (59.4%) were more common than gram-positive bacteria (40.6%).[31] However, this result disagrees with that of a study conducted by Naji & Awadh in Tikrit City, which showed that gram-positive bacteria (66.7%) were more common than gram-negative bacteria (33.3%).[32] This may be due to the different age groups included in the two studies, besides the differences in periods of both studies.

Distribution of Isolated Bacteria among Study Groups

According to the VITEK 2 diagnostic system, bacterial isolates were identified as follows: E. coli was the main pathogen in UTI patients with a percentage of 33.0%. This result is in agreement with those of a study conducted by Seid et al.[33] in Ethiopia who reported that *E. coli* accounted for 35.48% of all bacterial isolates. This is also in agreement with the results of studies done by Medina & Castillo-Pino,[34] Odoki et al.,[35] Alotaibi et al.,[36] Johnson et al.,[13] and Simon-Oke et al.[30] The E. coli percentages observed in these studies were 39.7%, 40.9%, 38.1%, 28.78%, and 31.7%, respectively.

On the contrary, these results are not in agreement with that of Rehman & Shrivastva in Ghaziabad[37] and Czajkowski et al. in Poland[38] which showed that *E. coli* was found in 79% and 69% of isolates, respectively.

S. saprophyticus appeared in 15% of samples in the present study and this is in agreement with the study of Rehman & Shrivastva,[37] in which *Staphylococcus saprophyticus* was found in 11% of samples. This is also in concordance with the results of the studies conducted by Naderi et al.[39] and Gajdács et al.[40] in which the values were 13.82% and 9.2%, respectively. However, these results are not in agreement with the studies conducted by Isberg et al. (6%),[41] Baba et al. (55.1%),[42] and Arends et al. (81.1%).[43]

S. aureus was found in 11.11% of samples in the present study. This result is similar to those of studies conducted by Simon-Oke et al. (14.8%),[30] Ali et al. (13%),[44] Belete & Saravanan (8.3%),[45] and Omidifar et al. (6.3%)[46]. On the contrary, this is not in agreement with the result of the study done by Baba et al. (28.6%).[42]

The present study showed that *E. faecalis* was found in 10.39% of samples, which is in relative agreement with the result of Sibi et al.'s study (6.7%),[47] but is not in agreement with the study conducted by Odoki et al. in Uganda (1.5%)[35].

P. aeruginosa was observed in 8.96% of samples in the present study, which is similar to the results of the study conducted by Nahab et al. (8.8%),[48] Ali et al. (7.2%),[44] Hussein et al. (5.1%),[49] Johnson et al. (5.04%),[13] and Imade et al. (4.4%),[50] but does not agree with the study done by Omidifar et al. (1.8%)[46]. *Klebsiella* appeared in 6.81% of samples in the present study, which is similar to the results observed by AL-Tikrity et al. (5%),[51] Nahab et al. (4.9%),[48] Ali *et al*. (8.7%),[44] and Khanal et al. (9.6%).[52]

Similar to the occurrence of *S. haemolyticus* (6.1%) in the present study, Hussien & Makhrmash observed 8.3% occurrence in their study,[29] while Haque et al. observed an occurrence of 80.76%.[53]

Proteus mirabilis was found in 2.5% of samples in the present study, which is relatively similar to the results of the study conducted by Odoki et al. in Uganda (3%).[35] This is also in agreement with the results observed by Rehman & Shrivastva,[37] Ali et al.,[44] Belete & Saravanan,[45] and Khanal et al.[52] with the values in these studies being 2.0%, 5.8%, 3.2%, and 1.8%, respectively.

Micrococcus luteus was found in 4.6% of samples in the current study, which is similar to the results of the study conducted by Younis & Ali (1.42%),[54] but is not in agreement with the results observed by Hammad et al. in Sudan (11%).[55]

Distribution of Urinary Tract Infection among Reproductive Age Women according to Age Groups

The ages of UTI patients in the present study ranged between 15 years and 45 years. The highest percentage (45%) of patients belonged to the age group of 15–25 years, followed by the age group of 26–35 and 36–45 years, with the percentages being 38.1% and 16.7%, respectively. This is in accordance with a study conducted by Raz et al. (with the highest rate of *S. saprophyticus* infection of 42.3% found in women aged 16–25 years).[56] Similar results were seen in studies conducted by Adeghate et al.,[57] and Turpin et al.[58] who reported that the age group of 15–25 years was most susceptible to UTI.

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Distribution of *Staphylococcus saprophyticus* Isolates among Reproductive Age Women according to Pregnancy Status of Married Participants

The present study showed most females suffering from UTI were pregnant (56.5%) while 43.5% were not pregnant. This result was relatively similar to that reported by Obeagu et al.[59] who mentioned that UTI is the most common health problem among pregnant women. This may be due to physiological changes in women during pregnancy.

Distribution of *Staphylococcus saprophyticus* Isolates among Reproductive Age Women according to Area of Residence

The present study showed that among the women with UTI, 78.6% belonged to urban areas and 21.4% were from rural areas. Similar results were obtained in a study by Almukhtar, in which a greater percentage of women who lived in urban areas suffered from UTIs.[60] This may be because the number of samples collected from urban areas was higher than those collected from rural areas.

Distribution of *Staphylococcus saprophyticus* Isolates among Reproductive Age Women according to Biofilm Formation

Biofilm detection by the CRA method showed that 26.2% of isolates were biofilm formers, while 92.9% of isolates were detected as biofilm formers by microtiter plate assay. This result was similar to those reported by Lawal et al.[7] and Rafiee & Ghaemi[61]. In contrast, Martins et al.[62] and Hashemzade et al.[63] reported that 70% and 63% of these strains, respectively, were biofilm producers.

Evaluation of IL-17 Level in Urine of Reproductive Age Women with *Staphylococcus saprophyticus* Urinary Tract Infection

IL-17 is a family of pro-inflammatory cytokines. Numerous immune regulatory functions have been reported for the IL-17 family of cytokines, presumably due to their induction of many immune signalling molecules. The most notable role of IL-17 is its involvement in inducing and mediating pro-inflammatory responses.[64] The detection of cytokines in urine has been used in the diagnosis and monitoring of various urological diseases. However, in the current study, the urine IL-17 mean levels (82.1619 pg/mL) of women with UTI were non-significant (p = 0.215) when compared with the control group (72.0000 pg/mL). These findings are in agreement with those of Sivick et al.[65] and Jones-Carson et al.[66] IL-17 is expressed by T cells that contribute to the up-regulation of additional pro-inflammatory cytokines. It does not appear to play a role in the development of adaptive immune responses to the bacteria, suggesting that T cells contribute to innate defences against infection in the bladder. Two studies demonstrated that gram-negative Uropathogenic *Escherichia coli* (UPEC) infection is highly inflammatory, inducing cytokines such as tumour necrosis factor-a, IL-6 and IL-17, whereas the response to the gram-positive organism *S. saprophyticus* is comparatively silent in the bladder.[67,68] The reasons for this are unclear, but likely bacterial colonisation is required to trigger an inflammatory response in the bladder, for example, *S. saprophyticus* infection predominantly occurs in the kidneys, with infection in the bladder being a transient event.

Evaluation of CXCL1 Level in Urine of Reproductive Age Women with *Staphylococcus saprophyticus* Urinary Tract Infection

The chemokine (C-X-C motif) ligand 1 (CXCL1) is a small peptide belonging to the CXC chemokine family that acts as a chemo-attractant for several immune cells, especially neutrophils or other non-haematopoietic cells to the site of injury or infection and plays an important role in the regulation of immune and inflammatory responses.

The current study showed that the urine CXCL1 mean level in the women group with UTI (60.5405 pg/mL) was highly significant in comparison with the control group (28.2750 pg/mL). This finding is in agreement with that of Rodhe et al. which demonstrated that urinary levels of CXCL1 and IL-6 were significantly higher in acute cystitis patients than in the control group.[69]

Association between Virulence Factors and Cytokine Production

The current study results showed no significant association between biofilm and CXCL1 (p = 0.246), while another study reported by Engelsöy et al.[70] found that all tested proinflammatory cytokines (TNF-α, IL-1β, IL-6, IL-8, and IFN-γ), and biofilm formation and haemolytic activity was reduced in the presence of all proinflammatory cytokines.

Conclusion

The study shows that urine CXCL1 mean level of reproductive age women with UTI where statistically significant as compared to the control group.

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Conflict of Interest: None

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