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Relationship between Some Virulence Factors of *Staphylococcus. saprophyticus* associated with urinary tract infection and Interferon Gamma In Reproductive Age Women In Samarra City

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Virulence Factors, *Staphylococcus Saprophyticus*, UTI, IFN-gamma

ABSTRACT
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.

Cross-sectional study which was conducted during the period extending from the first of November 2022 to the end of Jun 2023.

Background: The total number of samples in this study was 425 reproductive age women. Whose age were between 15-45 years old .

Aim: To look for some virulence factors of Staphylococcus saprophyticus causing urinary tract infections and cytokine response in reproductive age women in Samarra City.

Methods :Type of samples were urine collected from women ,and then conducting microbiological examination and located of IFN gamma amount by using (ELISA) technique .

Results :When laboratory culture was performed ,it was found (65.65%) samples G+ve in laboratory and (34.35%) G -ve . Through this study , the percentage of negative bacteria higher than positive bacteria when performing gram stain. The commonest type of bacteria isolates among reproductive age women with urinary tract infection was *E. coli* which constitutes 91 (33.0%) followed by *S. saprophyticus* 42 (15.0%), *S. aureus* 31(11%) , *Enterococcus faecalis* 29 (10%), *P. aeruginosa* 25 (9%), *Klebsiella pneumonia*19 (7%) , *Staphylococcus haemolyticus* 17 (6%), *Proteus mirabilis* 6 (2%), *Micrococcus luteus* 13(5%), and mixed 6(2.0%). The current study showed that UTI *staphylococcus saprophyticus* is more frequent in the age 15-25 years old about (45.2%) and the age group 26-35 years old of about (38.1%) while the lowest percentage was within the age group of (36-45)years old which constitutes16.7%. The urease enzyme produce by *staphylococcus saprophyticus* prevalence was 100% , and lipase enzyme about 38.1%..

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epithelial cells [14]. And other virulence factors [15] .

Urease production

A.The enzyme urease produced by bacteria is considered one of the important virulence factors, as this enzyme works to break down urea and release ammonia, which raises the pH of the medium, which leads to a change in the color of the phenol red reagent to pink [16]. Urease is a most common virulent issue initiate in pathogenesis of *S. saprophyticus* which lead to stone formation in urine , kidney infection, and human health[17)] .

B. Lipase

The lipase production hydrolyzes triacylglycerol into free fatty acids and glycerol of the uroepithelial membrane, thus promoting bacterial survival or invasion[18].

Immune response to urinary tract infection

Interferon-γ (IFN-γ)

IFN-γ is the lone member of type II IFN family [19]. IFN-γ is a protein that act as against bacteria by activated immune cells [20]. The cells were responsible for IFN-γ is natural killer (NK) and natural killer T (NKT) cells CD8+ , CD4+ T-cells [21].

Aim: To look for some virulence factors of Staphylococcus saprophyticus causing urinary tract infections and cytokine response in reproductive age women in Samarra City.

Materials & Methods

Study Groups

Cross sectional study which done between November 2022 to June 2023 , which contains 425 female, aged between 15 to 45 years and 40 samples as control which do not suffer from any diseases. Give women instructions to clean the area before collecting the sample.

INTRODUCTION

(UTI) is the most prevalent infectious illness of the urinary system caused by diverse uropathogens , affecting females and males of all ages [1], 150 million people are infected annually worldwide [2]. Bacteria and fungi are the causative agents of urinary tract infections, which can be found in the urine of someone who is indicated to be suffering from a urinary tract infection [3]. Although UTI is caused by a range of pathogens such as *Escherichia coli* (*E. Coli*), are the common causal of UTIs, reported for up to 80% of (CAUTI), then *Klebsiella pneumonia*, *Enterobacter*, *Proteus* species [4]*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*[5] *and Staphylococcus saprophyticus* [6].

Staphylococcus saprophyticus is a pathogen that causes UTI about 10-20% of (UTI) in active women . The ability of *S. saprophyticus* as a pathogen in urine organ is due to its could to adaptation in difficult environment [7] .

Some strains of *S.saprophyticus* have the ability to create biofilms, increasing their virulence [8,9]. The host defenses to microorganisms show vary dependent on the kind of bacteria [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] .

Staphylococcus saprophyticus is can to survive in difficult environments, it is have genetic determinants to survive types of materials [13]. Moreover, *S. saprophyticus* has some virulence factors to be capacity to adhere to

for 15 min, pour into sterile Petri plates(**Liofilchem**) [25].

Nutrient agar Mix twenty eight grams with one liter of aquatic and heated the medium. uses autoclaving at 15 lbs pressure (121°C) for 15 min to Pure the medium , pour into sterile Petri plates(**Liofilchem**) [26].

Lipase agar 7.5 grams lipase agar were mixed in 90 ml water. Suspension was Heated to dissolve the medium totally. uses autoclaving at 15 lbs pressure (121°C) for 15 minutes to Sterilized the medium , pour into sterile Petri plates. Cool to 50°C and a add 10 ml lipase supplement media. Mixed well and pour into sterile Petri plates(**midi**) [27].

Muller Hinton agar

Mueller -Hinton Agar: 38 grams agar mixed with 1 L water then Heated to dissolved the media .used auto-claving at 15 lbs pressure (121°C) for 15 min to pure the media . Cool the medium to 45-50°C and mixed well and poured into sterile Petri plates [28].

Urease Activity Test Medium

2.4 Grams agar were add in 95 ml water. Suspension was Heated to boiling to dissolve the medium totally. Sterilized by auto-claving at 15 lbs pressure (121°C) for twenty min. Cooled to fifty °C and aseptically add five ml Urea Solution (SR20) . Mixed well and pour into sterile tube [29].

Biochemical tests

a. Catalase enzyme test

A small amount of colony was transferred to slide mixed with drops of hydrogen peroxide then rubbed. Bubbles appear refers to positive results [30].

b. Oxidase production test

Strip method

The strip contain Oxidase reagent , small amount of colony placed on strip and rubbed. Convert the color of strip to deep blue appear in about ten

Exclusion :Any patients that taken antibiotics in 3 days from done analysis well exclusion . [22]

Questionnaire

Each patient with UTI is assessed by a prepared questionnaire including the name, age, gender, occupation, socio-economic status, chronic illness, taking medicines, pregnancy, duration of pregnancy, number of births.

Pilot Study

About 10 ml urine were collected from 425 reproductive age women. For culture and ELISA. By using ELISA technique, these urine samples were used to assess the IFN gamma mean levels in order to use the data to compare it with that of study group later .

Study samples

Urine samples were collected to stored 2ml for immunological examination and some amount for microbiological culture.

Culture media preparation

Blood agar medium

40.0 g were suspended in 1 L water. Suspension was Heated to hot to melt the media totally. Sterilized by auto-claving at 15 lbs pressure (121°C) for 15 min. Cooled to 50°C and aseptically add 50 ml sterile defibrinated blood. Mixed well and pour into sterile Petri plates [23].

MaCconky agar

50 grams were suspended by 1L water. Suspension was Heated to boiling to dissolve the medium completely. Sterilized by auto-claving at 15 lbs pressure (121°C) for 15 minutes, pour into sterile Petri plates(**Liofilchem**) [24].

Mannitol salt agar .

111 g suspended in 1 L water. Suspension was Heated to boiling to dissolve the media totally. Treated by auto-claving at 15 lbs pressure (121°C)

within 10- 20 second of the bacterial suspension [34].

e. Lipase test

The isolated bacteria inoculate into lipase activity agar and the growth colonies had the opacity after 24-48hour incubation time at 37ºC defined the positive result test [35].

RESULTS

Frequency of urinary tract infection

four hundred twenty five urine sample have been collected from reproductive age women. Their age ranged from 15 to 45 years old.65.65% [279/425] were positive while 34.35% [146/425] were negative. **figure (1)**

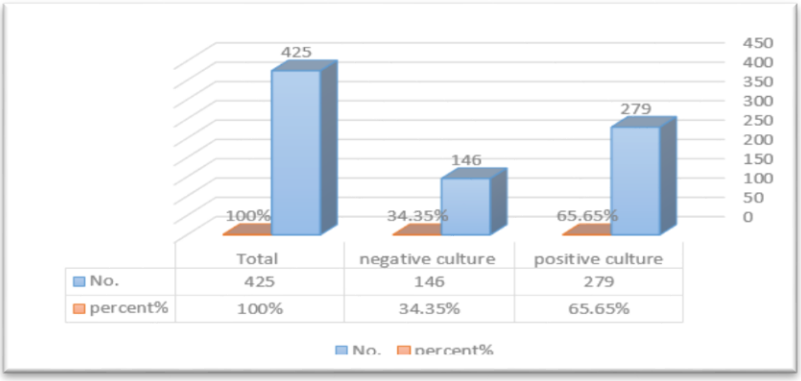

Fig (1) : Frequency of urinary tract infection

Table (1) :Distribution of Isolated Bacteria Among Study Groups

Bacterial isolates	**No**	**Percent**
Escherichia coli	91	33.0%
Staphylococcus saprophyticus	42	15.0%
Pseudomonas aeruginosa	25	9%
Klebsiella pneumonia	19	7%
Staphylococcus haemolyticus	17	6%
Proteus mirabilis	6	2%
Micrococcus luteus	13	5%
Enterococcus faecalis	29	10%
Staphylococcus aureus	31	11%
Mixed	٦	2.0
Total	279	100%

c .Urease enzyme test

To detect the urease enzyme were streaked amount of bacterial culture on slant urease agar and incubated at 37°C for 24 hours .Color changing of the medium indicate a positive (Color changing from yellow to purple- pink) [30,32].

d. Coagulase test

Coagulase enzyme produced via most strain of S. aureus .causes clotted of plasma via converting fibrinogen to fibrin. the slide method was 2 droplets of saline on all part of the separated slide. Formerly mix the bacterial colonies with normal saline [33]. Observed the result by clumping seconds which mean that the test is positive [31] .

Table (2):Distribution of *Staphylococcus Saprophyticus* among Reproductive Age Women According to Age Groups

Age	Frequency	Percent
15-25	19	45.2
26-35	16	38.1
36-45	7	16.7
Total	42	100.0

Distribution of *Staphylococcus Saprophyticus* among Reproductive Age Women According to seasonal

Most *Staphylococcus saprophyticus* excommunicate in the summer (73.8%), (2.4%, 23.8%) and in winter and spring seasons respectively.

Distribution of *Staphylococcus Saprophyticus* among Reproductive Age Women According to urease production

S. saprophyticus produce urease enzyme about (100)%.

Distribution of *Staphylococcus Saprophyticus* among Reproductive Age Women According to lipase production

In the present study, (38.1%)patient with UTI cases were positive lipase while (61.9%) were negative .

Distribution of *Staphylococcus Saprophyticus* among Reproductive Age Women According to marital status

The present study cleared the distribution of UTI patients related to marital status and show most of UTI patient were married (sexually active) (54.8%) while (45.2%) un-married.

Distribution of *Staphylococcus Saprophyticus* among Reproductive Age Women According to pregnancy status

The present study cleared the distribution of UTI patients related to pregnancy status and show most of UTI female were pregnancy (56.5%) while (43.5%) non-pregnancy.

Distribution of *Staphylococcus Saprophyticus* among Reproductive Age Women According to Residency

The present study cleared the distribution of UTI patients related to residence shown that (78.6%) was urban , while (21.4%) was rural.

Evaluation of IFN gamma level in urine of reproductive age women with *Staphylococcus saprophyticus* urinary tract infection.(Table. 3)

Table (3): INF- gamma level in urine of UTI patient's.

Groups of study	Mean urine IFN-γ level	Un-paired T-test	Degree of freedom	SE of difference	95%Confidence Interval	p-value
Patients group (n=42)	٥٣١,٩٨٨١	١٧,٤١٩	٤٢,٦٧٥	١٨,٠٨٢٩١	٢٧٨,٥١٢٤٠ **To**	٠,٠٠٠١
Control group (n=40)	٢١٧				**351.46379**	

difference of study and the kind of community and may be type of bacterial growth has been inhibited by antibiotic therapy.

Distribution of Isolated Bacteria Among Study Groups

when uses Vitek 2 diagnostic system bacterial isolates were Identified as following: *E.coli* was the main pathogen in UTI patients with percentage reached (33.0%), this result was agree with the study of Seid et al[41] in Ethiopia who reports that *E.coli* accounted for (35.48%) of all bacterial isolates· Also this is in agreement with the results of Medina [42], Odoki[43], Alotaibi [44], Johnson[45]and Simon [40] percentage of isolation of these studies are (39.7%, 40.9%, 38.1%, 28.78%, 31.7%) respectively. While this results are not in agreement with Rehman[46] in Ghaziabad which found that *E.coli* (79%) and (Czajkowski et al [47]in Poland which reported that *E.coli* (69%). *Staph .saprophyticus* appered

The Objectives of the study

This study attempts to
1- Isolate and identify *Staphylococcus saprophyticus* in women with
UTI.
2-Clarify virulence factors of *Staphylococcus saprophyticus*.
3-Clarify immune response represented by cytokines production against *Staph.saprophyticus*.

Discussion

Occurrence of UTI

Among the female with UTI the frequency of positive urine culture in our study (66 %) was in contract with other studies [36] of Safa in Tikri city, Zavala et al [37] in Mexico, Sharma et al in India[38], Hussien in Wasit, Iraq[39], Simon et al in Nigeria[40] which were (77.2 %, 62.8%, 65.45%, 60%, 61.0%) resspectively. The explanation of this difference of the outcomes is maybe because of the

[57], Ali [53], Khanal [61](4.9% ,8.7%, 9.6%) respectively. *Staph. haemolyticus* appeared in (6.1%) at present study and this relatively agreed with Hussien in Wasit, Iraq (8.3%)[49]. While this results are not in agreement with Sarker in Bangladesh (80.76%)[62] .

Proteus mirabilis appeared in (2.5%) at present study and this relatively agreed with Odoki in Uganda (3%)[43] . Also this is in agreement with the results of Rehman[46], Ali[53], Belete[54], Khanal[61],(2%,5.8%,3.2%,1.8%,) respectively. Micrococcus luteus appeared in (4.6%) at present study and this relatively agreed with Younis et al in Tikrit city (1.42%) [63]. While this results are not in agreement with Hammad in sudan (11%) [64].

Distribution of urinary tract infection among Reproductive Age Women According to Age Groups

Age of UTI patients in the present study was range between 15 years old to 45 years old. The highest percentage of patient were in the age group (15-25) in percentage of (45%), followed by age group (26-35), (36-45) respectively and with percentage (38.1%), (16.7%) respectively, this relatively agreed with Raz (The highest rate of *S. saprophyticus* infection was 42.3%, among women aged 16–25 years included in the study[65].this had agreement with other studies of Adeghate *et al* [66], Turpin [67] who was reported that age (15-25) most susceptible to urinary tract infection.

in (15%) at present study and this relatively agreed with Rehman [46.] in Ghaziabad which found that Staphylococcus *saprophyticus* (11%).Also this is in agreement with the results of Naderi in iran (13.82%) [48] and Gajdács in Györgyi (9.2%)[49] . While this results are not in agreement with Kornfält et al [50] in Sweden (6%),Baba et al (55.1%)in Nigeria [51] and Arends in United States (81.1%) [52] .

S.aureus appeared in (11.11%) at present study and this relatively agreed with Simon in Nigeria (14.8%),[40],Ali *et al* in Somaliland [53] (13%) , Belete in all the specific countries in Asia and Africa regions *(8.3%)*[54], Omidifar in Iran (14, 6.3%) [55]. While this results are not in agreement with Baba et al in Nigeria (28.6%) [51].

Entero. faecalis appeared in (10.39%) at present study and this relatively agreed with Sibi in India (6.7%) [56] . While this results are not in agreement with Odoki in Uganda [43] (1.5%) .

p. aeruginosa appeared in (8.96%) at present study and this relatively agreed with Nahab in Al Samawa City of Iraq (8.8%) [57]. Also this is in agreement with the results of Ali [53], Hussein [58], Johnson[45] ، Imade[59] (7.2%, 5.1%, 5.04%,4.4%) respectively . While this results are not in agreement with Omidifar in Iran (1.8%)[55]. Klebsiella appeared in (6.81%) at present study and this relatively agreed with AL-Tikrity *et al* in Tikrit city(5%) [60]. Also this is in agreement with the results of Nahab

urban areas suffered from UTIs**.** This may be due to the number of sample collection from the urban was higher than rural.

Distribution of *Staphylococcus Saprophyticus* among Reproductive Age Women According to seasonal .

The results of the current study have been showed that most *Staphylococcus saprophyticus* isolates in the summer (73.8%). This result agree with Rafiee et al [71] (68.6%) [72] , Eriksson [73] , Adeghate [66] .

Distribution of *Staphylococcus Saprophyticus* among Reproductive Age Women According to lipase production .

In the present study, (38.1%) patient with UTI cases were positive lipase, while (61.9%) were negative, while In previous studies reported different results by Rafiee *et al.,* in Iran [74] .. Although .*S.sp* gene shows important a role in the lipolytic activity of *S. saprophyticus*, previous studies reported different results lipolytic activity. .

Evaluation of IFN gamma level in urine of reproductive age women with *Staphylococcus saprophyticus* urinary tract infection .

IFN-γ plays an important role in both innate and adaptive immunity.

Type II IFN is primarily secreted by adaptive immune cells, more specifically CD4+ T helper 1 (Th1) cells, natural killer (NK) cells, and CD8+ cytotoxic T cells [75].

Distribution of urinary tract infection among Reproductive Age Women According to marital status.

The present study cleared the distribution of UTI patients related to marital status and show most of UTI patient were married (sexually active)(54.8)% while (45.2)% un married . this had agreement with other studies of Almukhtar[68], Alsamarai [69] who was reported that married most susceptible to urinary tract infection. The main reason may be due to sexually intercourse and poor hygiene.

Distribution of *Staphylococcus Saprophyticus* among Reproductive Age Women According to pregnancy status

The present study cleared the distribution of UTI patients related to pregnancy status and show most of UTI female were pregnancy(56.5)% while (43.5)% non-pregnancy. this result was relatively similar to those reported by Obeagu et al [70]that Urinary tract infection is the commonest health problem among pregnant women. This may be change in physiological effects.

Distribution of *Staphylococcus Saprophyticus* among Reproductive Age Women According to Residency .

The present study cleared the distribution of UTI patients related to residence that (78.6%) was urban and (21.4%)was rural, this result was relatively similar to those reported by Almukhtar et al [68]**.** A greater percentage of women who lived in

Conclusion :

The current study show that the mean urine IFN gamma mean level of reproductive age women with UTI where statistically significant when compared with that of control group.

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