Ukrainian Journal of Nephrology and Dialysis
Scientific and Practical, Medical Journal

Founder:
- National Kidney Foundation of Ukraine

ISSN 2304-0238;
eISSN 2616-7352

Journal homepage: https://ukrjnd.com.ua

Research article

doi: 10.31450/ukrjnd.3(87).2025.06

Sanaa Ghali Jabur

Molecular surveillance of *Staphylococcus aureus* and *Staphylococcus saprophyticus* isolates from pregnant women with asymptomatic bacteriuria in Thi-Qar province

College of Science, University of Thi-Qar, Thi-Qar, Iraq

Citation: Jabur SG. Molecular surveillance of Staphylococcus aureus and Staphylococcus saprophyticus isolates from pregnant women with asymptomatic bacteriuria in Thi-Qar province. Ukr J Nephrol Dialys. 2025;3(87):53-61. doi: 10.31450/ukrjnd.3(87).2025.06.

Abstract. *Asymptomatic bacteriuria (ASB) is a common condition during pregnancy, increasing the risk of maternal complications and adverse fetal outcomes. The number of pregnant women infected with Staphylococcus aureus has risen in recent years, while Staphylococcus saprophyticus is detected in up to 40% of sexually active young women as part of the normal genitourinary flora. Maternal infections during pregnancy require particular attention, as they may be vertically transmitted to the fetus, leading to unfavorable outcomes. This study aimed to investigate the prevalence and frequency of S. aureus and S. saprophyticus in pregnant women with ASB in Thi-Qar Province, considering gestational stage, maternal age, and molecular identification.*

Methods. Midstream urine samples were collected from pregnant women between January 2023 and December 2024. A total of 235 samples were analyzed: 116 from pregnant women with ASB and 119 from non-pregnant women who served as controls. Diagnosis was performed using standard biochemical phenotypic tests, and molecular confirmation of S. aureus and S. saprophyticus was carried out by PCR targeting the 16S rRNA gene.

Results. Among pregnant women, the prevalence of S. aureus and S. saprophyticus was 25.0% and 60.3%, respectively, significantly higher than in the control group (5.8% and 10.0%; χ^2=13.4 and 41.0; $p<0.05$). The frequency of isolation was 30.6% for S. aureus and 43.6% for S. saprophyticus in pregnant women, compared with 3.4% and 22.5% in controls (χ^2=10.6 and 157.3; $p<0.05$). Gestational age influenced bacterial detection: S. aureus peaked in months II–IV (40–46.6%) but declined to 0% after the seventh month ($p<0.05$), while S. saprophyticus gradually increased, reaching 64.2–100% in months II–IX (χ^2=120, $p<0.05$). Maternal age also correlated with infection rates: S. aureus was highest in women <20 years (45.3%), whereas S. saprophyticus dominated in women aged 30–39 years (68.0%) (χ^2=453.7 and 1249.7; $p<0.05$).

Conclusions. S. aureus and S. saprophyticus are prevalent etiological agents of asymptomatic bacteriuria in pregnant women, with clear associations to gestational stage and maternal age. S. saprophyticus predominates in later months of pregnancy and among women of middle age, whereas S. aureus is more frequent in early pregnancy and younger women.

Keywords: *asymptomatic bacteriuria, pregnancy, Staphylococcus aureus, Staphylococcus saprophyticus, molecular identification, prevalence.*

Article history:
Received April 4, 2025
Received in revised form June 25, 2025
Accepted June 26, 2025

Conflict of interest. The author declares no conflict of interest.

Correspondence should be addressed to Sanaa Ghali Jabur: sanaaghali@sci.utq.edu.iq

УДК 618.3-06:616.61/63-022.7]-036-039.1

Санаа Галі Джабур

Молекулярний моніторинг ізолятів *Staphylococcus aureus* та *Staphylococcus saprophyticus* у вагітних жінок з безсимптомною бактеріурією в провінції Ті-Кар

Коледж наук, Університет Ті-Кар, Ті-Кар, Ірак

Резюме. *Безсимптомна бактеріурія (ББ) є частим явищем під час вагітності, підвищуючи ризик материнських ускладнень та несприятливих наслідків для плода. Кількість вагітних жінок, інфікованих Staphylococcus aureus, останніми роками також зросла, тоді як Staphylococcus saprophyticus виявляється майжу 40% сексуально активних молодих жінок, як складова нормальної мікрофлори сечостатевого тракту. Інфекції у матері під час вагітності потребують особливої уваги, оскільки можуть передаватися плоду, спричиняючи несприятливі наслідки.*

Метою цього дослідження було вивчити поширеність та частоту ізоляції Staphylococcus aureus і Staphylococcus saprophyticus у вагітних жінок з ББ в провінції Ті-Кар з урахуванням терміну вагітності, віку матері та результатів молекулярної ідентифікації.

Методи. Зразки середньої порції сечі були зібрані у вагітних жінок з січня 2023 року по грудень 2024 року. Проаналізовано 235 зразків: 116 від вагітних жінок та 119 від невагітних жінок, які слугували контрольною групою. Діагностику здійснювали із застосуванням стандартних біохімічних фенотипових тестів, а молекулярне підтвердження S. aureus та S. saprophyticus проводили методом ПЛР з таргетуванням гена 16S rRNA.

Результати. Серед вагітних жінок поширеність Staphylococcus aureus та Staphylococcus saprophyticus становила 25,0% і 60,3% відповідно, що було достовірно вище, ніж у контрольній групі (5,8% і 10,0%; χ^2=13,4 і 41,0; p<0,05). Частота ізоляції серед вагітних жінок дорівнювала 30,6% для S. aureus та 43,6% для S. saprophyticus, тоді як у контрольній групі – лише 3,4% та 22,5% (χ^2=10,6 і 157,3; p<0,05). Гестаційний вік впливав на виявлення ізолятів: S. aureus найчастіше зустрічався у II–IV місяцях (40–46,6%), але знижувався до 0% після VII місяця (p<0,05), тоді як S. saprophyticus поступово зростав і стабілізувався на рівні 64,2–100% у II–IX місяцях вагітності (χ^2=120, p<0,05). Вік матері також асоціювався з частотою інфекцій: для S. aureus найвищий рівень був у жінок <20 років (45,3%), тоді як для S. saprophyticus — у віковій групі 30–39 років (68,0%) (χ^2=453,7 і 1249,7; p<0,05).

Висновки. S. aureus і S. saprophyticus є поширеними етіологічними агентами безсимптомної бактеріурії у вагітних жінок, з чіткими залежностями від терміну вагітності та віку матері. S. saprophyticus переважає у пізніх гестаційних місяцях та серед жінок середнього віку, тоді як S. aureus частіше ізолюється на ранніх термінах та у молодших жінок.

Ключові слова: *безсимптомна бактеріурія, вагітність, Staphylococcus aureus, Staphylococcus saprophyticus, молекулярна ідентифікація, поширеність.*

Introduction. Pregnancy alters the function, structure, and physiology of the urinary tract, predisposing women to urinary tract infections (UTIs). UTIs are among the most common medical complications of pregnancy and represent a significant challenge for treatment because of the risks they pose to both mother and fetus. A frequent manifestation of UTI in pregnancy is asymptomatic bacteriuria (ASB), defined as the presence of one or more bacterial species in urine at counts $\geq 10^5$ to $\geq 10^8$ CFU/l, regardless of pyuria and in the absence of symptoms related to UTI [**1, 2**].

Sanaa Ghali Jabur:
sanaaghali@sci.utq.edu.iq

The microorganisms that cause infections during pregnancy are generally similar to those in the general population. Most cases are due to *Enterobacteriaceae*, primarily *Escherichia coli*, followed by coagulase-negative *staphylococci*, with *Staphylococcus aureus* (*S. aureus*) accounting for many cases, and group B streptococci as additional pathogens [**3**].

In recent years, the incidence of infections caused by *S. aureus* among pregnant women, postpartum women, and neonates has increased. *S. aureus* has become a major source of healthcare-associated infections over the past decade [**4, 5**]. *Staphylococcus saprophyticus* (*S. saprophyticus*), on the other hand, is detected in up to 40% of sexually active young women as part of the normal genitourinary flora and is considered the second most common cause of community-acquired UTIs after *E. coli*. It can cause complications such as acute pyelonephritis, epididymitis, prostatitis, and urethritis. Overall, about 50% of women will experi-

ence a UTI during their lifetime, and 5–20% of hospitalized patients may develop infections associated with *S. saprophyticus* [6].

Several host factors influence susceptibility to UTI during pregnancy. Hormonal changes across gestation affect microbial colonization and may increase UTI-related complications [7, 8]. Maternal age also plays an important role: older pregnant women face higher risks of adverse outcomes, including gestational diabetes and hypertensive disorders, partly due to reduced immunity and increased susceptibility to microbial infections [2, 8].

Maternal infections during pregnancy are of particular concern because they can be vertically transmitted to the fetus, resulting in serious complications. Molecular diagnostic methods, especially PCR-based identification of *S. aureus*, have been shown to improve diagnostic accuracy [9]. Microbial prevalence (appearance rate) reflects the distribution of specific organisms in a population, whereas frequency (microbial profile) provides insight into the intensity of colonization or infection [10].

S. saprophyticus has recently attracted more attention due to the lack of studies in Iraq, particularly in Thi-Qar Governorate, concerning its role in pregnant women and its potential plasmid-mediated virulence. The evolution of bacterial strains from commensal to pathogenic forms often occurs through the horizontal transfer of virulence genes, including those from *S. aureus*, via plasmids or other mobile genetic elements. This poses a clinical risk, as virulence determinants may be disseminated in clinical environments where multiple bacterial species coexist.

Based on these considerations, **this study aimed** to investigate the prevalence and frequency of *S. aureus* and *S. saprophyticus* in pregnant women with ASB in Thi-Qar Province, considering gestational stage, maternal age, and molecular identification.

Materials and methods. *Sample collection.* A total of 235 midstream urine specimens (5 mL each) were collected according to the protocol of Abbas and Al-Mathkhury [11]. The study group consisted of 116 pregnant women diagnosed with ASB, while 119 samples were obtained from married, non-pregnant, healthy women who served as the control group. Pregnant women with symptomatic urinary tract infections were excluded. Age distribution was randomized across both groups.

Samples were collected between January 2023 and December 2024 at Al-Habboby Hospital and Al-Hussein Teaching Hospital in Thi-Qar Governorate, Iraq. Within two hours of collection, specimens were transported in Cary Blair transport medium to the Department of Pathological Analysis, College of Science, University of Thi-Qar. Each specimen (1 mL) was inoculated into tryptic soy broth and incubated overnight at 37°C, followed by sub-culturing on selective media, including mannitol salt agar. Bacterial quantification was expressed as colony-forming units per milliliter (CFU/mL), following WHO standards [11].

Clinical and demographic data collected from participants included gestational month, maternal age, antibiotic use, and medical history. Supplementary information was obtained from attending physicians and nurses.

Isolation and phenotypic identification. Urine specimens (1 mL) were plated on brain–heart infusion agar, blood agar, and MacConkey agar, and incubated at 37°C for 24–48 h. Positive growth was initially characterized based on colony morphology and Gram staining. Suspected *Staphylococcus* isolates were further subcultured on mannitol salt agar and tested with the coagulase assay. Species-level identification of Gram-positive isolates was performed using the Vitek automated system (bioMérieux, France) according to the manufacturer's instructions. Confirmed isolates were preserved at –80°C in 25% glycerol [12].

Molecular analysis. DNA extraction and quantification. Genomic DNA was extracted from Staphylococcus isolates using the Genomic DNA Mini Kit (Favorgen, China) according to the manufacturer's instructions. DNA samples were stored at –80°C until further analysis. Concentration and purity were measured with a NanoDrop spectrophotometer (Thermo Scientific, USA), and DNA quality was assessed using the 260/280 nm absorbance ratio.

PCR amplification. Species-specific PCR assays targeting the 16S rRNA gene were used for molecular confirmation of *S. aureus* and *S. saprophyticus*. Primer sequences and expected product sizes are shown in Table 1.

Table 1

Primers used for PCR amplification of *S. aureus* and *S. saprophyticus*

Gene target	Primer sequence	Product size (bp)	Reference
S. aureus 16S rRNA	F: 5'-TCA ACC GTG GAG GGT CAT TG-3' R: 5'-GTT TGT CAC CGG CAG TCA AC-3'	556	[4]
S. saprophyticus 16S rRNA	27F: 5'-AGAGTTTGATCCTGGCTCAG-3' 1492R: 5'-GGTTACCTTGTTACGACTT-3'	1500	[16, 17]

PCR cycling conditions for each species are summarized in Tables 2 and 3.

Table 2

PCR conditions for 16S rRNA amplification of *S. aureus*

Step	Temperature (°C)	Time	Cycles
Initial denaturation	94	30 s	1
Denaturation	94	30 s	30
Annealing	59	30 s	30
Extension	68	45 s	30
Final extension	68	5 min	1
Hold	4	∞	

Table 3

PCR conditions for 16S rRNA amplification of *S. saprophyticus*

Step	Temperature (°C)	Time	Cycles
Initial denaturation	95	5 min	1
Denaturation	94	30 s	30
Annealing	52	30 s	30
Extension	72	1 min 25 s	30
Final extension	72	10 min	1
Hold	4	∞	

Gel electrophoresis. PCR products were resolved by agarose gel electrophoresis using TBE buffer and visualized under UV light, following the protocol of [13].

Definitions. The appearance rate (prevalence) was defined as the proportion of urine samples positive for a given bacterial species. The frequency (microbial profile) was defined as the proportion of colonies of each species among all *Staphylococcus* isolates obtained.

Statistical analysis. Statistical analyses were performed using IBM SPSS Statistics v.20 and Microsoft Excel 2010. The Chi-square test was applied to compare categorical variables between groups, with $p < 0.05$ considered statistically significant. Appearance rates and frequencies were calculated according to standard epidemiological formulas [14].

Results. *Distribution of samples.* A total of 235 urine specimens were collected, comprising 116 from pregnant women with asymptomatic bacteriuria (ASB) and 119 from non-pregnant married women without urinary symptoms (control group) (Table 4).

Table 4

Distribution of study samples

Group	n	%
Pregnant women with ASB	116	49.3
Non-pregnant women (control)	119	50.6
Total	235	100

Phenotypic and molecular identification. Phenotypic and molecular (PCR) identification results were fully concordant. Of the 2,337 colonies analyzed, 712 (30.4%) were identified as *Staphylococcus aureus* and 1,019 (43.6%) as *Staphylococcus saprophyticus* (Table 5).

Table 5

Phenotypic and molecular identification of isolates

Method	Total colonies (n=2337)	*S. aureus* n (%)	*S. saprophyticus* n (%)
Phenotypic identification	2337	712 (30.4)	1019 (43.6)
Molecular identification	2337	712 (30.4)	1019 (43.6)

Representative PCR results are shown in Figures 1 and 2.

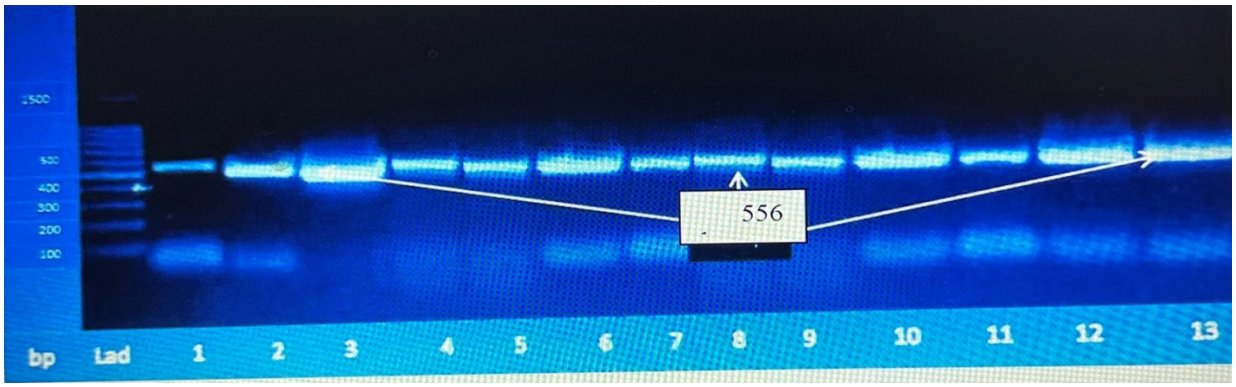

Fig. 1. Agarose gel electrophoresis showing amplification of the 16S rRNA gene (556 bp) specific to *Staphylococcus aureus* from urine samples of pregnant women. Lane M: 1500 bp DNA ladder; lanes 1–13: positive PCR products. Electrophoresis was performed on 1% agarose gel in 1× TBE buffer at 100 V for 30 min, followed by 50 V for 45 min, stained with RedSafe, and visualized under a UV transilluminator.

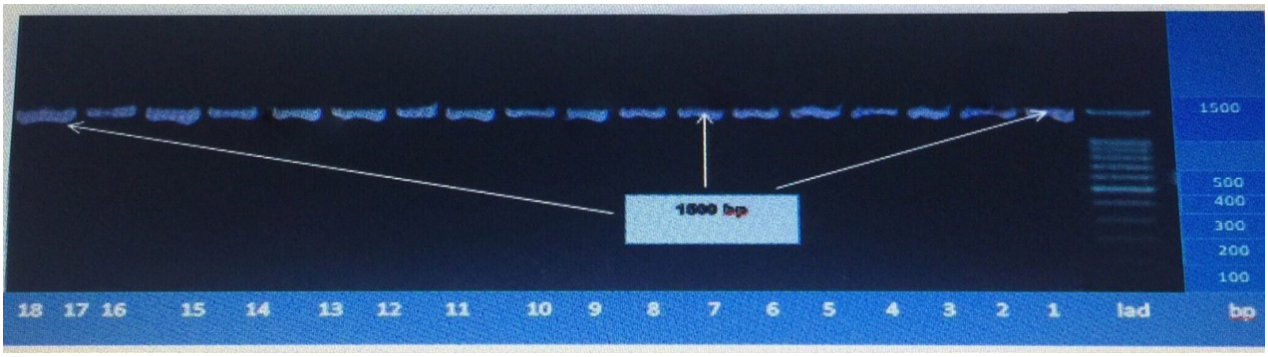

Fig. 2. Agarose gel electrophoresis showing amplification of the 16S rRNA gene (1500 bp) specific to *Staphylococcus saprophyticus* from urine samples of pregnant women using universal primers (27F/1492R). Lane M: 1500 bp DNA ladder; lanes 1–18: positive PCR products. Electrophoresis was performed on 1% agarose gel in 1× TBE buffer at 100 V for 30 min, followed by 50 V for 45 min, stained with RedSafe, and visualized under a UV transilluminator.

Prevalence. Among pregnant women, the prevalence of *S. aureus* and *S. saprophyticus* was 25.0% and 60.3%, respectively, compared with 5.8% and 10.0% in the control group (χ^2=13.4 and 41.0, p<0.05) (Table 6).

Table 6

Prevalence of *S. aureus* and *S. saprophyticus* in pregnant and non-pregnant women

Group	n	*S. aureus* n (%)	*S. saprophyticus* n (%)	χ^2	p-value
Pregnant women	116	29 (25.0)	70 (60.3)	13.4 / 41.0	<0.05
Control	119	7 (5.8)	12 (10.0)	–	–
Total	235	36 (30.8)	82 (70.3)	–	–

Frequency. The frequency of isolates among all *Staphylococcus colonies* was 30.6% for *S. aureus* and 43.6% for *S. saprophyticus* in pregnant women, compared with 3.4% and 22.5% in the control group (χ^2=10.6 and 157.3, p<0.05) (Table 7).

Table 7

Frequency of *S. aureus* and *S. saprophyticus* among total isolates

Group	Total colonies	*S. aureus* n (%)	*S. saprophyticus* n (%)	χ^2	p-value
Pregnant women	1731	712 (30.6)	1019 (43.6)	10.6 / 157.3	<0.05
Control	606	80 (3.4)	526 (22.5)	–	–
Total	2337	792 (34.0)	1545 (66.1)	–	–

Effect of Gestational Month. The gestational stage significantly affected bacterial prevalence. *S. aureus* was most common in months II–IV (40–46.6%) but decreased progressively to 0% from month VII onward. In contrast, *S. saprophyticus* increased from 53.3% in month II to a stable 64.2–100% from months VI–IX (χ^2=120.0, p<0.05) (Table 8).

Table 8

Prevalence of *S. aureus* and *S. saprophyticus* by gestational month

Month	n	*S. aureus* n (%)	*S. saprophyticus* n (%)	χ^2	p-value
II	15	6 (40.0)	8 (53.3)	1.4	ns
III	15	7 (46.6)	8 (53.3)	1.8	ns
IV	15	7 (46.6)	9 (60.0)	1.8	ns
V	15	5 (33.3)	9 (60.0)	3.2	ns
VI	14	4 (28.5)	9 (64.2)	1.9	ns
VII	14	0 (0.0)	9 (64.2)	11.0	<0.05
VIII	14	0 (0.0)	9 (64.2)	11.0	<0.05
IX	14	0 (0.0)	9 (64.2)	11.0	<0.05
Total	116	29 (25.0)	70 (60.3)	120.0	<0.05

Similarly, gestational month influenced microbial frequency. *S. aureus* peaked at 44.4% in month II and declined to 0% after month VII, whereas *S. saprophyticus* progressively increased, reaching 100% from month VII onward (χ^2=349, p<0.05) (Table 9).

Table 9

Frequency of *S. aureus* and *S. saprophyticus* by gestational month.

Month	Total colonies	*S. aureus* n (%)	*S. saprophyticus* n (%)	χ^2	p-value
II	430	191 (44.4)	239 (75.0)	5.3	<0.05
III	362	193 (53.3)	169 (49.2)	1.5	ns
IV	266	135 (50.7)	131 (75.0)	0.0	ns
V	219	99 (45.2)	120 (80.4)	2.0	ns
VI	203	94 (46.3)	109 (83.4)	1.1	ns
VII	92	0 (0.0)	92 (100)	92.0	<0.05
VIII	81	0 (0.0)	81 (100)	81.0	<0.05
IX	78	0 (0.0)	78 (100)	78.0	<0.05
Total	1731	712 (33.8)	1019 (43.5)	349.0	<0.05

Effect of maternal age. The prevalence of infection varied with age group. Among women <20 years, *S. aureus prevalence* was 51.7% compared to 86.2% for *S. saprophyticus*. In contrast, in women aged 30–39 years, *S. aureus* prevalence was only 10.3%, whereas *S. saprophyticus* remained high at 51.7% (Table 10).

Table 10

Prevalence of *S. aureus* and *S. saprophyticus* by maternal age

Age group (years)	n	*S. aureus* n (%)	*S. saprophyticus* n (%)	χ^2	p-value
<20	29	15 (51.7)	25 (86.2)	2.5	ns
20–29	29	10 (34.4)	20 (68.9)	3.2	ns
30–39	29	3 (10.3)	15 (51.7)	8.0	<0.05
40–49	29	1 (3.4)	10 (34.4)	7.2	<0.05
Total	116	29 (25.0)	70 (60.3)	16.8	<0.05

Frequency analysis confirmed significant age-related variation. *S. aureus* was most frequent in women <20 years (45.3%), while *S. saprophyticus* was most frequent in women aged 30–39 years (68.0%) (χ^2=453.7 and 1249.7, p<0.05) (Table 11).

Table 11

Frequency of *S. aureus* and *S. saprophyticus* by maternal age

Age group (years)	Total colonies	*S. aureus* n (%)	*S. saprophyticus* n (%)	χ^2	p-value
<20	300	136 (45.3)	164 (54.7)	2.6	ns
20–29	338	121 (35.8)	217 (64.2)	27.2	<0.05
30–39	495	158 (31.9)	337 (68.0)	64.6	<0.05
40–49	598	214 (36.1)	384 (64.2)	48.2	<0.05
Total	1731	712 (24.4)	1019 (67.7)	453.7 / 1249.7	<0.05

Discussion. Molecular analysis confirmed that PCR targeting 16S rRNA is a highly sensitive and specific method for the identification of *Staphylococcus* spp., supporting previous studies that recognized PCR as the most reliable approach for species-level differentiation of staphylococci [15, 16].

The relatively high proportions of *S. aureus* and *S. saprophyticus* observed in pregnant women with asymptomatic bacteriuria in Thi-Qar are consistent with their role as important uropathogens. Their presence in the control group of non-pregnant women may be explained by their role as components of the normal gastrointestinal and genitourinary flora, which under certain conditions may migrate to inappropriate sites and cause infection [1, 4, 16]. The association between coagulase-negative staphylococci, including *S. saprophyticus*, and urinary tract infections has been reported previously [16].

The higher prevalence among pregnant women compared with controls may reflect both the increased susceptibility to infection during pregnancy and potential healthcare-associated exposures. Pregnancy is accompanied by profound hormonal, metabolic, and immunological changes that predispose women to urinary tract infections [17]. Pregnant women are often considered relatively immunocompromised, and the physiological changes of gestation increase the risk of infection and colonization.

Our findings regarding *S. aureus* are in line with previous reports showing that rectovaginal colonization is associated with a measurable risk of maternal infection, with colonization rates ranging from 4–22% in pregnant women and MRSA carriage reported at 0.5–10% [15]. For *S. saprophyticus*, its persistence and clonal expansion, as shown in previous pulsed-field gel electrophoresis studies [18], suggest that this species may be particularly well adapted to colonization in women of reproductive age.

Gestational age strongly influenced bacterial distribution. The decline of *S. aureus* after the seventh month of pregnancy and its complete absence in late gestation may be linked to changes in the vaginal microbiome. During pregnancy, microbial diversity and richness decline compared with non-pregnant women, with a predominance of *Lactobacillus* spp. [7, 19]. These organisms produce lactic acid, lowering vaginal pH, which likely inhibits *S. aureus*, a species relatively sensitive to weak organic acids [19–21]. In contrast, *S. saprophyticus* may thrive under these conditions due to its ability to produce siderophores, conferring resistance to acidic environments [20].

Age-related effects were also observed. In women aged 30–39 and 40–49 years, *S. saprophyticus* predominated, whereas *S. aureus* prevalence decreased. This may again be explained by microbiome shifts, where *Lactobacillus*-dominated communities create an acidic milieu unfavorable for S. aureus but tolerated by *S. saprophyticus* through siderophore-mediated adaptations [19, 21]. In younger women (<20 years), however, *S. aureus* prevalence and frequency were higher, which may reflect increased biological susceptibility as well as possible interactions with group B *Streptococcus* (GBS). GBS colonization is a known risk factor for *S. aureus* carriage, as competitive inhibition between streptococci and staphylococci is less pronounced than between streptococci and other species [22]. Moreover, up to 40% of sexually active young women may harbor *S. saprophyticus* as part of their normal genitourinary microbiota, and this species contributes to more than 40% of uncomplicated urinary infections in women aged 16–25 years [)].

Taken together, these results indicate that both gestational age and maternal age strongly influence the prevalence and frequency of *Staphylococcus* spp. in pregnant women with asymptomatic bacteriuria. The increase of *S. saprophyticus* in late pregnancy, contrasted with the decline of *S. aureus*, suggests important ecological interactions between the host, microbiota, and pathogen.

This study has some limitations. Patient selection in both the pregnant and control groups was based on hospital attendance, which may introduce bias, and the sample size, although adequate, may not capture the full diversity of the population. Clinical outcomes of infected patients were not evaluated, as the focus was limited to prevalence, frequency, gestational month, and age. These aspects should be addressed in future studies.

Conclusions. The present study demonstrates that *S. aureus* and *S. saprophyticus* are important etiological agents of asymptomatic bacteriuria among pregnant women in Thi-Qar Province. Their prevalence and frequency were significantly higher in pregnant women compared with non-pregnant controls and showed distinct associations with gestational stage and maternal age. *S. aureus* was more common in early pregnancy and among younger women, whereas *S. saprophyticus*

predominated in later gestational months and in women of middle age. These results suggest that changes during pregnancy, including hormonal and immune shifts, play a role in which bacteria are most likely to cause infection. Regular monitoring of urinary infections in pregnant women is important, and molecular methods such as PCR can provide fast and reliable identification of the bacteria involved.

Ethical compliance. This study was conducted in accordance with the ethical standards of the institutional and national research committees and with the 1964 Helsinki Declaration and its later amendments. The study protocol was reviewed by the Ethics Committee of the Department of Pathological Analysis, Science College, University of Thi-Qar approved this study (no.015 on 12/2/2022), confirming that formal written approval and protocol registration were not required for this type of research. Oral informed consent was obtained from all participants during their hospital visits prior to urine sample collection. Participants were assured of confidentiality, and their participation was entirely voluntary.

Author's contributions. The author was solely responsible for the conception and design of the study, data collection, laboratory analysis, data interpretation, and manuscript preparation.

Funding. This research received no external funding.

Conflicts of interest. The author declares no conflict of interest.

Data availability. The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

References:

1. *Nicolle LE, Gupta K, Bradley SF, Colgan R, DeMuri GP, Drekonja D, et al.* Clinical practice guideline for the management of asymptomatic bacteriuria: 2019 update by the Infectious Diseases Society of America. Clin Infect Dis. 2019;68(10):e83-110. doi: 10.1093/cid/ciy1121.
2. *Bonkat G, Bartoletti R, Bruyere F, Cai T, Geerlings SE, Köves B, et al.* EAU guidelines on urological infections. European association of urology. [Internet].2017;18:22-6. Available from: https://d56bochluxqnz.cloudfront.net/documents/EAU-Guidelines-Urological-infections-2017.pdf.
3. *Matuszkiewicz-Rowińska J, Małyszko J, Wieliczko M.* State of the art paper Urinary tract infections in pregnancy: old and new unresolved diagnostic and therapeutic problems. Archives of Medical Science.2015;11(1):67-77. doi: 10.5114/aoms.2013.39202.
4. *Khudher KK, Jabur SG.* The prevalence of some virulence plasmid genes in Staphylococcus aureus infection. Plant Archives. [Internet]. 2020;20(2): 8851-62. Available from: https://www.researchgate.net/profile/Khudher-Khudher-2/publication/347011330_THE_PREVALENCE_OF_SOME_VIRULENCE_PLASMID_GENES_IN_STAPHYLOCOCCUS_AUREUS_INFECTION/links/5fd788de92851c13fe862731/THE-PREVALENCE-OF-SOME-VIRULENCE-PLASMID-GENES-IN-STAPHYLOCOCCUS-AUREUS-INFECTION.pdf.
5. *Natsis NE, Cohen PR.* Coagulase-negative Staphylococcus skin and soft tissue infections. Am J Clin Dermatol. 2018;19(5):671-677. doi: 10.1007/s40257-018-0362-9.
6. *Zhang K, Potter RF, Marino J, Muenks CE, Lammers MG, Dien Bard J, et al.* Comparative genomics reveals the correlations of stress response genes and bacteriophages in developing antibiotic resistance of Staphylococcus saprophyticus. mSystems. 2023;8(6):e00697-23. doi: 10.1128/msystems.00697-23.
7. *Stout MJ, Zhou Y, Wylie KM, Tarr PI, Macones GA, Tuuli MG.* Early pregnancy vaginal microbiome trends and preterm birth. Am J Obstet Gynecol. 2017;217(3):356.e1-356.e18. doi: 10.1016/j.ajog.2017.05.030.
8. *Zhou Y, Yin S, Sheng Q, Yang J, Liu J, Li H, et al.* Association of maternal age with adverse pregnancy outcomes: a prospective multicenter cohort study in China. J Glob Health. 2023;13:04161. doi: 10.7189/jogh.13.04161.
9. *Fadhil H, Mohammed BJ.* Molecular and Classical identification of Staphylococcus aureus, isolated from Iraqi Patients with recurrent tonsillitis. Medico-legal Update.2022;22(1):23. doi: 10.37506/mlu.v22i1.3176.
10. *Abdelwahab MA, Amer WH, Elsharawy D, Elkolaly RM, Helal RA, El Malla DA, et al.* Phenotypic and genotypic characterization of methicillin resistance in Staphylococci isolated from an Egyptian University Hospital. Pathogens.2023;12(4):556. doi: 10.3390/pathogens12040556.
11. *Abbas HM, Al-Mathkhury HJ.* Isolation and identification of bacteria from Iraqi women with recurrent urinary tract infection. Plant Archives. 2020;20(2):1838-42. doi: 10.1007/s00192-014-.
12. *Pugazhendhi A, Michael D, Prakash D, Krishnamurthy PP, Shanmuganathan R, Al-Dhabi NA, et al.* Antibiogram and plasmid profiling of beta-lactamase producing multi-drug resistant Staphylococcus aureus isolated from poultry litter. J King Saud University-Science. 2020;32(6):2723-7. doi: 10.1016/j.jksus.2020.06.007.
13. *Alao FO, Smith SI, Omonigbehin EA, Adeleye IA.* Genetic Diversity of Staphylococcus Saprophyticus Strains Causing Urinary Tract Infections in Lagos

Metropolis. 2020:1-14, PREPRINT (Version 1). doi: 10.21203/rs.3.rs-117287/v1.

14. *Jabur SG.* Studying Virulence Plasmids and Endotoxin of Salmonella enteric Serovar Typhimurium Isolated from Chickens in Al-Najaf Al-Ashraf Dissertation. 2014. Jul. Dissertation submitted to the council of College of Science/ University of Kufa in Partial fulfillment of the requirements for the (PhD) degree of philosophy in microbiology/molecular biology. doi: 10.13140/RG.2.2.33615.25768.
15. *Lee TF, Lee H, Chen CM, Du SH, Cheng YC, Hsu CC, et al.* Comparison of the accuracy of matrix-assisted laser desorption ionization–time of flight mass spectrometry with that of other commercial identification systems for identifying Staphylococcus saprophyticus in urine. J Clin Microbiol.2013;51(5):1563-6. doi: 10.1128/JCM.00261-13.
16. *Paiva-Santos W, Barros EM, de Sousa VS, Laport MS, Giambiagi-deMarval M.* Identification of coagulase-negative Staphylococcus saprophyticus by polymerase chain reaction based on the heat-shock repressor encoding hrcA gene. Diagn Microbiol Infect Dis.2016;86(3):253-256. doi: 10.1016/j.diagmicrobio.2016.08.006.
17. *Yeruva T, Rajkumar H, Donugama V.* Vaginal lactobacilli profile in pregnant women with normal and abnormal vaginal flora. Indian J Med Res. 2017;146(4):534-540. doi: 10.4103/ijmr.IJMR_774_16.
18. *Phillip S, Mushi MF, Decano AG, Seni J, Mmbaga BT, Kumburu H, et al.* Molecular characterizations of the coagulase-negative Staphylococci species causing urinary tract infection in Tanzania: a Laboratory-Based Cross-Sectional Study. Pathogens. 2023;12(2):180. doi: 10.3390/pathogens12020180.
19. *Amir M, Brown JA, Rager SL, Sanidad KZ, Ananthanarayanan A, Zeng MY.* Maternal microbiome and infections in pregnancy. Microorganisms. 2020;8(12):1996. doi: 10.3390/microorganisms8121996.
20. *Sivalingarajaha R, Balasinghamb B, Camilas JC.* Asymptomatic bacteriuria in pregnancy. Sri Lanka Journal of Obstetrics and Gynaecology.2023;45(1):21-26. doi: 10.4038/sljog.v45i1.8091.
21. *Zhou C, Fey PD.* The acid response network of Staphylococcus aureus. Curr Opin Microbiol.2020;55:67-73. doi: 10.1016/j.mib.2020.03.006.
22. *Top KA, Buet A, Whittier S, Ratner AJ, Saiman L.* Predictors of Staphylococcus aureus rectovaginal colonization in pregnant women and risk for maternal and neonatal infections. Journal of the Pediatric Infectious Diseases Society. 2012;1(1):7-15. doi: 10.1093/jpids/pis001.