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Prevalence and Antimicrobial Susceptibility Pattern of *Staphylococcus Species* Causing Urinary Tract Infections in Women of Reproductive Age: 5 Years Retrospective Study

Üreme Çağındaki Kadınlarda İdrar Yolu Enfeksiyonlarına Neden Olan Stafilokok Suşlarının Prevalansı ve Antimikrobiyal Duyarlılık Paterni: 5 Yıl Retrospektif Çalışma

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Abstract

Aim Infections of the urinary tract are amongst the most prevalent infections in women, females at child-bearing age have a higher predisposition to urinary tract infections. Approximately 13% of health-care-associated urinary tract infections occur due to Coagulase Negative *Staphylococci* (CNS); this species' resistance rate is alarming. The study aims to describe urinary tract infections of women of reproductive age through 5 years of results, emphasizing *Staphylococcus species* (*Staphylococcus aureus, Staphylococcus saprophyticus* ve other CNS) as an etiological agent and their patterns of antimicrobial susceptibility.

Material and Method In the study, 4562 urine samples sent between November 2017 and November 2022 were retrospectively analyzed. Routine culture methods were used to isolate bacteria from urine specimens. According to the European Committee on Antimicrobial Susceptibility Testing (version 8.0-12.0) recommendations, an antimicrobial susceptibility test was performed using the disc diffusion technique. Obtained data analysis was achieved by Statistical Package for Social Sciences (SPSS 20.0), Categorical variables were presented as frequency and percentage however continuous variable was described as mean ± Standard deviation (SD), and binary logistic regression test was done to determine the association with statistical significance of ($p<0.05$).

Results Of the 4562 urine samples taken from women of reproductive age, 166 (3.6%) had a UTI due to *Staphylococcus species*. The most common species was other CNS 110 (66.2%). Followed by, S. aureus which was 42 (25.3%), and *S. saprophyticus* 14 (8.4%). All isolate species showed susceptibility to tigecycline and linezolid (100%). The highest level of antibiotic resistance was showed by *S. aureus* and other CNS against penicillin-G which was (83.30%) and (58.1%) respectively, while the majority of *S. saprophyticus* showed resistance against Erythromycin (64.2%). Other CNS displayed the highest oxacillin resistance (20.90%) among *Staphylococcus species*.

Conclusion The study illustrates the significance of *Staphylococcus species* as a pathogen of the urinary tract, especially in women of reproductive age. There is clear evidence of the resistance of isolates to penicillin-G which may suggest the production of penicillin-binding protein 2a. Regular surveillance of the frequency and resistance pattern of *Staphylococcus species* causing urinary tract infections in local regions, especially keeping in mind the high-risk patients mentioned in our study should be monitored.

Keywords : Reproductive age, *Staphylococcus species*, urinary tract infection, women.

Özet

Amaç *İdrar yolu enfeksiyonları kadınlarda en sık görülen enfeksiyonların arasındadır. Doğurganlık çağındaki kadınların idrar yolu enfeksiyonlarına yatkınlığı daha fazladır. Koagülaz Negatif Stafilokoklar (KNS), yatan hastalarda idrar yolu enfeksiyonlarının (İYE) yaklaşık %13'ünden sorumludur. Bu patojen grubunun antimikrobiyallerine karşı direncin artması da endişe vericidir. Bu çalışmada, üreme çağındaki kadınların idrar yolu enfeksiyonlarının etiyolojik bir ajanı olan Stafilokok türlerini (Staphylococcus aureus, Staphylococcus saprophyticus ve diğer KNS) ve bunların antimikrobiyal duyarlılık paternlerinin araştırması amaçlanmıştır.*

Gereç ve Yöntem *Bu çalışmada, Kasım 2017-Kasım 2022 tarihleri arasında toplanan 4562 adet idrar örneği retrospektif olarak incelenmiştir. Bakterileri idrar örneklerinden izole etmek için rutin kültür yöntemleri kullanıldı. European Committee on Antimicrobial Susceptibility Testing (versiyon 8,0-12,0) önerilerine göre disk difüzyon tekniği kullanılarak antimikrobiyal duyarlılık testi yapıldı Veriler Statistical Package for Social Sciences (SPSS 20,0) programıyla analiz edildi. Kategorik değişkenler frekans ve yüzde olarak ifade edildi, sürekli değişkenler ise ortalama ± standart sapma (SS) şeklinde verildi. Tüm karşılaştırmalarda $P<0,05$ istatistiksel anlamlılık düzeyi olarak kabul edildi.*

Bulgular *Doğurgan çağındaki kadınlardan elde edilen 4562 idrar örneğinin 166'sında (%3,6) stafilokok türlerine bağlı İYE saptandı. En sık karşılaşılan stafilokok türü diğer KNS olup, 110 adet (%66.2) idrar örneğinde etken olarak tespit edildi. Bunu takiben, 42 adet S. aureus (%25.3) ve 14 adet (%8.4) S. saprophyticus etken olarak saptandı. Tüm izolatlar tigesiklin ve linezolide karşı (%100) duyarlıydı. En yüksek antibiyotik direnci, penisilin-G'ye karşı S. aureus (%83,30) ve diğer KNS (%58,1) tarafından gelişirken, S. saprophyticus'un büyük çoğunluğu eritromisin'e (%64,2) karşı direnç göstermiştir. Diğer KNS, stafilokok türleri arasında en yüksek oksasilin direncini (%20,90) göstermiştir.*

Sonuç *Stafilokok türleri, özellikle üreme çağındaki kadınlarda idrar yolu patojeni olarak önemini göstermektedir. İzolatların penisilin-G'ye karşı direncine açık kanıtlar vardır. Bu direncin gelişmesine penisilin bağlayıcı protein 2a üretimi yol açmış olabilir. İdrar yolu enfeksiyonlarına neden olan stafilokok türlerinin sık görülen prevalansı ve duyarlılık paternleri, özellikle çalışmamızda ele alınan yüksek riskli hastaları tedavi ederken göz önünde bulundurulmalıdır.*

Anahtar Kelimeler *Doğurgan çağ, Stafilokok suşları. idrar yolu enfeksiyonu, kadınlar*

INTRODUCTION

Infections of the Urinary tract (UTI) are defined as the existence and growth of microbes in the different parts of the urinary system from the kidney, ureter, bladder, and urethra, which may or may not be accompanied by clinical symptoms and pyuria.[1] It is one of the most common infections known to be caused by bacteria, with an incidence rate of 150 million,[2] and causes almost 85,000 deaths annually worldwide according to the reports of the World Health Organization.[3] Reports from Turkey show that 15.7% of infections that occur in healthcare settings are UTIs.[4] It comes in the second ranking of the highest prevalent infections after respiratory.[5] Women have a high predisposition for UTI, females are 35 times more susceptible to UTI than males, especially between the age of 16–35.[6] Once in a lifetime, nearly 60% of women will be diagnosed with a symptomatic infection of the lower urinary tract,1 10% of females get infected yearly,[7] and 30% - 44% of women will have a recurrence.[8] The higher predisposition to infection in women is influenced by structural factors, the shortness of the urethra and its closeness to bacterial normal flora present in the rectum and vagina that allows easier colonization of those bacteria,[6] as well as behavioral factors e.g., sexual intercourse, spermicide exposure, and intrauterine devices. Furthermore, the previous history of UTI, the presence of a history of UTI in the mother, and the existence of UTI in childhood are also considered predisposing factors.[9]

Infections in the urinary tract can be attributable to various microbes, among which bacterial UTIs are highly dangerous and frequently occur in both inpatient and outpatient.[10] The most frequent isolated causative agents of UTI are members of *Enterobacterales*, including *Escherichia coli, Klebsiella spp., Enterobacter spp*., and *Proteus spp.*, commonly isolated Gram-positive bacteria comprehend *Enterococcus faecalis, Streptococcus agalactiae* and *Staphylococcus spp*..[5] *S. aureus* is a ubiquitous causative agent of UTI, with a growing prevalence rate.[11] *S. saprophyticu*s is one of the main causal pathogens of UTI in females and a species of the CNS which is categorized as the second most significant uropathogenic after *E. coli* representing 10–15% of cases.[12] CNS generally displays vague pathogenicity for the urinary system. Nonetheless, several studies have assessed the existence of CNS members (*S. haemolyticus, S. simulans, S. warneri*, and *S.hominis*) which have a clinically significant part in UTI. These species are important causes of UTI in young females, the elderly, hospital inpatients, and patients with a urinary tract surgery history.[13]

Nearly 50% to 60% of females of fertility age develop UTI.[14] Women reach sexual maturity between the ages of 15 and 49, in this period the fertile functions develop with which the risk of genital infections increases, subsequently UTI.[15] Increasing antimicrobial resistance in the control of UTI is a serious public health concern globally.[1] Reasoning from the fact that in most cases medications are prescribed empirically, it is crucial to identify the pathogens as well as the antibiotic susceptibility in urinary tract infections to ensure successful treatment.[16] The significance of *Staphylococcus species* is not merely as a human pathogen, but also because its resistance to antibiotics has increased.[13]

This retrospective study aimed to describe UTIs of women of reproductive age diagnosed in the University Hospital in Konya through 5 years of results, with a particular emphasis on *Staphylococcus species* (*S. aureus, S. saprophyticus*, and other CNS) as an etiological agent and their patterns of antimicrobial susceptibility.

MATERIALS and METHODS

1. Study design

This descriptive retrospective study was conducted at University Hospital in Konya, Türkiye. Ethical clearance was obtained from the Ethics Committee for Non-Medical and Medical Devices Research with the date of 20 January 2023 (Decision no. 2023/4166). All the medical records of analyzed urine samples of female patients admitted to the several clinics of the hospital between the period of November 2017 and November 2022 were reviewed. *Staph-*

ylococcus species grown in the urine culture of women of reproductive age (15-49 years) were grouped as *S. aureus, S. saprophyticus*, and other CNS, considering their importance for this population. Culture and in vitro antimicrobial susceptibility test results of these *Staphylococcus species* were analyzed retrospectively.

2. Isolation and identification of organisms

The samples were collected as clean-catch midstream urine from each patient, and all samples were cultured on 5% blood agar as well as eosin methylene blue (EMB) media and incubated at 37 °C for 24-48 h. Urine cultures were re-evaluated if two or more different types of bacteria growth were observed. Urine culture was accepted as positive if the colony count exceeded 105 CFU/ml. Bacterial isolates were identified based on Gram staining, culture characteristics, and the biochemical test result implemented on the isolates.[17] To identify Gram-positive bacteria biochemical tests such as catalase, coagulase, and novobiocin disc (5/µg) were performed.

3. Antibiotic sensitivity test

The antibiotic sensitivity test using the disc diffusion method was carried out in accordance with the European Committee on Antimicrobial Susceptibility Testing (version 8.0-12.0) instructions. The interpretation was done after overnight incubation at 37°.[17] The used antibiotics in this study were ampicillin/sulbactam, cefoxitin, ciprofloxacin, daptomycin, erythromycin, fusidic acid, gentamicin, clindamycin, chloramphenicol, linezolid, oxacillin, penicillin-G, teicoplanin, tetracycline, tigecycline, trimethoprim/ sulfamethoxazole, vancomycin. In antimicrobial susceptibility determination, *S. aureus* ATCC 25923 strains were used as quality control organisms.

4. Statistical Analysis

Attained data were statistically analyzed by The Statistical Package for Social Sciences (SPSS version 20). Categorical variables (i.e., age categories, organism isolated, sensitivity, and resistance) were presented as frequency and percentage however continuous variable (i.e., age) was described as mean ± Standard deviation (SD), binary logistic regression test was done to determine the association with statistical significance of ($p<0.05$).

RESULTS

Over 5 years, out of 4562 urine samples obtained 166 women had UTIs due to *Staphylococcus species*, other CNS is the predominant *Staphylococcus species* 110 (66.2%), Followed by *S. aureus* 42 (25.3%), and *S. saprophyticus* 14 (8.4%), as illustrated in Figure 1.

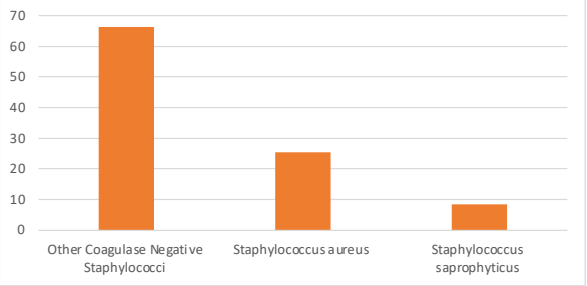

Figure 1. Distribution of Staphylococcus species isolated from urine samples

The mean age of women involved in the study was 29.4 ± 10.9 (15–49 years). A considerable number of women were between the ages of 15 to 24 years (39.8%), followed by 36 to 49 years (33.1%), and 25 to 35 years (27.1%) as shown in Figure 2. While UTIs due to *S. aureus* are more prevalent in the old age group (36-49 years), other CNS and *S. saprophyticus* UTIs were more frequent in the young age group (15-24 years) (Figure 2).

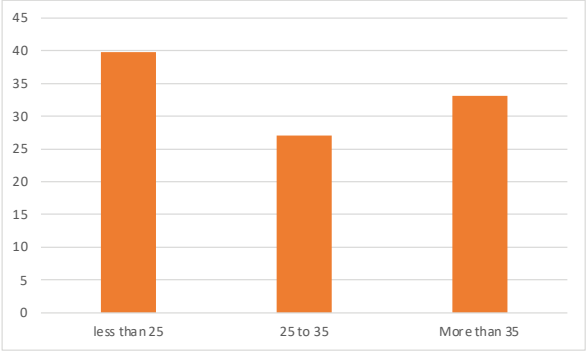

Figure 2. Distribution of age groups

An insignificant association was found between age groups and the infectious agents. The majority of samples (135) are from outpatients and an insignificant association was found between patient care and the infectious agents, as shown in Table 1.

The susceptibly pattern of Staphylococcal isolates to the used antibiotics is illustrated in Table 2. Against 17 antibiotics assayed all isolates are sensitive to tigecycline and linezolid (100%). Isolates show high susceptibility to vancomycin and chloramphenicol (99.4%), followed by daptomycin (98.2%) and gentamicin (89.8%). Most isolated *Staphylococcus species* showed high resistance to penicillin-G (64.5%) however, only (16.3%) were resistant to oxacillin, while resistance to ampicillin/sulbactam was (21.7 %). Regarding other antibiotics, resistance to erythromycin was (40.4%), fusidic acid (31.9%), clindamycin (23.5%), tetracycline (20.5%), cefoxitin (19.9%) and trimethoprim/ sulfamethoxazole was (18.7%).

Table 1. Association of *Staphylococcus species* with age groups and patient care.

		Microorganisms				Sig.
		S. aureus	*S. saprophyticus*	Other CNS	Total	
Age groups	15 to 24	12	5	49	66	0.107
	25 to 34	8	2	28	38	
	35 to 49	22	7	33	62	
	Total	42	14	110	166	
Patient Care	Inpatient	9	3	19	31	0.769
	Outpatient	33	11	91	135	
	Total	42	14	110	166	

Table 2. The antimicrobial susceptibly patterns of *Staphylococcus species* causing urinary tract infections

	Sensitive	Intermediate	Resistant
Ampicillin/sulbactam	75.9	2.4	21.7
Cefoxitin	80.1	0	19.9
Ciprofloxacin	83.1	1.2	15.7
Daptomycin	98.2	0	1.8
Erythromycin	59	0.6	40.4
Fusidic Acid	65.1	3	31.9
Gentamicin	89.8	0.6	9.6
Clindamycin	76.5	0	23.5
Chloramphenicol	99.4	0	0.6
Linezolid	100	0	0
Oxacillin	83.1	0.6	16.3
Penicillin-G	33.7	1.8	64.5
Teicoplanin	100	0	0
Tetracycline	79.5	0	20.5
Tigecycline	100	0	0
Trimethoprim/ Sulfamethoxazole	80.7	0.6	18.7
Vancomycin	99.4	0.6	0

As demonstrated in Table 3, the highest antibiotic resistance level was exhibited by *S. aureus* against penicillin-G which was (83.30%), followed by resistance to clindamycin and erythromycin (21.4%) for each antibiotic. While other

Table 3. The antibiotics susceptibly pattern against the different *Staphylococcus species*

		S. aureus	*S. saprophyticus*	Other CNS
Ampicillin/sulbactam	Sensitive	85.7	100	69.09
	Intermediate	4.80	0	1.81
	Resistant	9.50	0	29.09
Cefoxitin	Sensitive	90.50	100	73.63
	Resistant	9.50	0	26.36
Ciprofloxacin	Sensitive	90.50	100	78.1
	Intermediate	0	0	1.81
	Resistant	9.50	0	20.00
Daptomycin	Sensitive	97.60	100	98.18
	Resistant	2.40	0	1.81
Erythromycin	Sensitive	78.60	35.71	54.54
	Intermediate	0	0	0.90
	Resistant	21.40	64.29	44.54
Fusidic Acid	Sensitive	88.10	71.42	55.45
	Intermediate	0	0	4.54
	Resistant	11.90	28.57	40
Gentamicin	Sensitive	92.90	100	87.27
	Intermediate	0	0	0.90
	Resistant	7.10	0	11.81
Clindamycin	Sensitive	78.60	71.42	76.36
	Resistant	21.40	28.57	23.63
Chloramphenicol	Sensitive	100	100	99.09
	Resistant	0	0	0.90
Linezolid	Sensitive	100	100	100
Oxacillin	Sensitive	90.50	100	78.18
	Intermediate	0	0	0.90
	Resistant	9.50	0	20.90
Penicillin-G	Sensitive	16.70	100	31.81
	Intermediate	0	0	2.72
	Resistant	83.30	0	65.45
Teicoplanin	Sensitive	100	100	100
Tetracycline	Sensitive	92.90	100	71.81
	Resistant	7.10	0	28.18
Tigecycline	Sensitive	100	100	100
Trimethoprim/Sulfamethoxazole	Sensitive	90.50	100	74.54
	Intermediate	0	0	0.90
	Resistant	9.50	0	24.54
Vancomycin	Sensitive	100	100	99.09
	Resistant	0	0	0.90

CNS exhibited high resistance to penicillin-G (58.1%) and then erythromycin (46.8%), fusidic acid (38.7%), ampicillin/sulbactam (25.8%), tetracycline (25%) and clindamycin (24.2%), the noteworthy resistance pattern of *S. saprophyticus* was shown against erythromycin (64.2%).

DISCUSSION

The frequency of urinary infection in females is considered among the most widespread condition in clinical practice,[8] previous literature emphasizes the fact that the infection occurred predominantly in women of fertility age.[18] The commonly isolated causative agents that are involved in UTIs include; *Enterobacteria, Enterococci*, *Group B streptococci*, and *Staphylococci. S. aureus* and *S. saprophyticus* have high medical importance and are isolated frequently from clinical samples, nevertheless other CNS species demonstrated great significance over the previous years.[13,19] Our study was conducted to assess the occurrence of Staphylococcal UTIs in women of reproductive age and to inspect their patterns of antimicrobial susceptibility over the past five years.

This study revealed a prevalence of 3.6% UTIs due to Staphylococcus species in women of reproductive age. The highest rate of infection was in the age group between 15 to 24 years (40%) which is inconsistent with studies conducted by Simon-Oke et al.[7] The infection with different Staphylococcus species was age-independent and variation caused by the difference in the population and sampling pattern of the study. Owing to the high number of samples isolated for outpatients, all *Staphylococcal species* showed high incidence in the outpatient group, and the infection by the species is unassociated with the patient care condition

In our study, the clinically significant isolated *Staphylococcal species* are other CNS (66.2%), *S. aureus* (25.3%), and S. saprophyticus (8.4%) were isolated from urine samples, the isolation pattern is dissimilar with other studies conducted in Nigeria,[7] Ethiopia,[10] and Iran.[16] However, it is consistent to some extent, especially in the aspect of S. aureus prevalence in the study conducted by Nahab M. et al..[20] and Mohydin M. et al..[21] *S. saprophyticus* incidence is close to the study conducted by Abate D. et al..[1] Those differences and similarities in the type and distribution of *Staphylococcal species* are accredited to the different ecological circumstances, population factors, and practices such as healthcare, socioeconomic conditions, and hygiene habits in each topographical area.

In this study, *Staphylococcus isolates* were susceptible to several antibiotics including linezolid, vancomycin (100%), and daptomycin (98.2%), the finding is consistent with the reported results by Osman O. et al..[20] Moreover, the isolates were sensitive to tigecycline (100%), chloramphenicol (99.4%), and gentamicin (89.8%), these can be explained by the fact that Turkey follows a countrywide antimicrobial control campaign on antibiotic usage which promoting rational use of medicines and thereby limiting antimicrobial resistance.

Staphylococcus species demonstrated variable resistance rates to β lactam antibiotics. While resistance to penicillin-G was (64.5%), unlike the results of a study conducted in Turkey,[22] ampicillin/sulbactam and cefoxitin resistance were (21.7%) and (19.9%) respectively, dissimilar to a study done by Abate D. et al..[1] This resistance may occur due to the ability of *Staphylococcus species* to colonize the surface and form biofilms which may lead to the exchange of resistant genes. Regarding oxacillin resistance in our study, it was remarkably low (16.3%) in comparison with other studies[16,19]. Other CNS displayed the highest oxacillin resistance (20.90%) among Staphylococcus species as they harbor the mecA gene responsible for methicillin resistance.

The isolates showed resistance to erythromycin and clindamycin, which is in line with the study carried out by Omidifar N. et al.,[16] the resistance may be due to the msrA gene which is accountable for the efflux machinery in

staphylococci and becomes stimulated after introduction to a macrolide.

While *Staphylococcal species* show low or almost no resistance to ciprofloxacin, tetracycline, fusidic acid, and trimethoprim/sulfamethoxazole, other CNS demonstrated higher resistance. However, this resistance is lower compared to the study done by Osman O. et al..[22] Some previously mentioned antibiotics are used empirically to treat UTIs, which may lead to resistance development.

CONCLUSION

In conclusion, the study highlights that *Staphylococcus species* was a significant uropathogenic, especially in women of reproductive age. These pathogens cause biofilm infections and develop decreased susceptibility to antibiotic drugs, due to the exchange of resistant genes between the members of the biofilm population. Their detection is crucial to accomplish a suitable antimicrobial treatment. Although the study results revealed a high level of susceptibility to antibiotics used, there is clear evidence of penicillin-G resistance which may suggest the production of penicillin-binding protein 2a, with low predilection for the β-lactam group. Therefore, further studies must be conducted with advanced techniques to ensure the detection of resistance genes. UTI influence the quality of life among infected women and have life-threatening consequences as infection may initiate renal damage. Therefore, the study recommends that the frequency and antibiotic susceptibility pattern of pathogens causing UTI such as *Staphylococcus species* should be monitored regularly, keeping in mind the risky patient group mentioned in our study.

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Ethical Approval

Necmettin Erbakan University, the Ethics Committee for Non-Medical and Medical Devices Research-20 January 2023 (Decision no. 2023/4166)

Peer-review

Externally and internally peer-reviewed.

Author Contributions

Concept: M.D., Design: M.D., S.R.G, S.A.A.I., Data collection or Processing: S.R.G, Analysis or interpretation: M.D., S.A.A.I., S.R.G, Literature Search: S.A.A.I., Writing: S.A.A.I.

Conflict of Interest

The authors declare that they have no conflict of interest.

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