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Isolation of Methicillin Resistant Staphylococcus epidermidis from Young Females with Acute UTI in Wasit Province/Iraq

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ABSTRACT

Staphylococcus epidermidis is considered as a uropathogen in children with and without urinary tract abnormalities, as well as in patients with indwelling urinary catheters and other devices in the urinary tract. Whereas, the role of this bacterium in females is not well documented. So that, this project was proposed to determine the role of S. epidermidis as a uropathogen in female outpatients with acute UTI. Urine specimens were collected from pregnant and nonpregnant outpatient women (18-40 years) and surveyed for S. epidermidis during July 2023 to January 2024. The isolates were characterized phenotypically and genotypically for methicillin resistance and also tested for antimicrobial resistance. Of 137 positive bacterial cultures, S. epidermidis was isolated from 36 patients (26.2%). Statistically, the difference in distribution of S. epidermidis among pregnant (19/36: 52.7%) and nonpregnant (17/36: 47.2%) females was insignificant. mecA gene was detected in 32 (88.8%) isolates, of which 19 (52.7%) and 13 (36.1%) were cefoxitin resistant and cefoxitin sensitive, respectively. Whereas, 4 (11.1%) isolates were cefoxitin sensitive and mecA negative. Resistance percentage to penicillin was 88.8%, while resistance to trimethoprim, gentamicin, nitrofurantoin, norfloxacin, and ciprofloxacin was 33.3% 16.6%, 5.5%, 2.7%, and 2.7%, respectively.

In the study area, there is relatively high prevalence of Staphylococcus epidermidis among pregnant and nonpregnant outpatient women with acute UTI and most of these isolates are methicillin resistant and subsequently resistant to β-lactams. Hence, ciprofloxacin, norfloxacin, and nitrofurantoin are strongly recommended for empirical treatment of these cases.

Keywords: Urinary tract infection, Staphylococcus epidermidis, outpatient women, antibiogram assay.

INTRODUCTION

Across the world, the third most prevalent infection in humans is UTI which annually occurs at a rate of 100–180 million cases. Both outpatients and inpatients are affected by UTI to a significant degree. Therefore, UTIs are important factor of morbidity, particularly because of their recurring nature. Females, immunosuppressed patients and those with underlying diseases or anatomical abnormalities of the urinary tract are more vulnerable to UTIs. In addition, these infections are also linked to some lifestyle choices. If these infections are left without appropriate treatment, they may lead to complications, debilitating sequelae and a decreased quality of life [1]. In women, anatomy, sexual intercourse and the use of spermicide predisposes them to UTIs which are more common among them [2]. The uropathogens during pregnancy are the same as those that cause UTIs in nonpregnant women [3]. Furthermore, UTIs may be symptomatic or asymptomatic and serious obstetric complications and poor maternal and neonatal outcomes can occur because of them [4].

Bacteria are the most common cause of UTIs, whereas fungi and viruses are scarce etiologies of this infection. *Escherichia coli* (*E. coli*) is the most common bacteria (75–90%) in both the community and hospital acquired UTIs. Other bacterial causative agents of UTI are: *Proteus mirabilis* (*P. mirabilis),* *Staphylococcus saprophyticus* (*S. saprophyticus*), *Enterococcus faecalis* (*E. faecalis*), *Klebsiella pneumoniae* (*K. pneumoniae),* and *Pseudomonas aeruginosa* (*P. aeruginosa*) [5]. Gram-positive bacteria [coagulase-negative staphylococci (CoNS), *Staphylococcus aureus* (*S. aureus*), *Streptococcus* group B, Enterococci] are comparatively more common in some hospitalized patients [6].

Staphylococcus saprophyticus is the second most common cause of UTIs in sexually active young women [7].

Some clinicians do not consider CoNS the cause of disease rather they look at them as saprophytes and are rarely be pathogenic. However, a growing number of research indicate that CoNS can cause urinary tract infections in a wide range of patients, whether or not they use urinary tract instrumentation [8]. Uropathogenicity of staphylococci is complex and linked to virulence determinants, compromising the adherence ability and biofilm production, beside antibiotic resistance. Globally, multidrug resistant (MDR) *Staphylococcus* spp. represent a public health crisis and the problem persists. Additionally, staphylococci that resistant to methicillin and vancomycin, which are considered serious pathogens, poses a major threat to public health because of their potential to cause widespread mortality in the absence of effective treatment options [9]. The ability of staphylococci that cause urinary tract infections to develop antibiotic resistance and form biofilms highlights the need for periodic regional surveillance of these infections, which constitute a serious public health problem [10].

Staphylococcus epidermidis is generally not considered a causative agent of UTIs in patients without any previous illness. This bacteria have been more commonly linked with UTIs in patients with indwelling urinary catheters and other urinary tract devices. *Staphylococcus epidermidis* has been identified as the causative bacteria of UTIs in children with any urinary tract abnormality. Urinary tract infections caused by *S. epidermidis* in a previously healthy child should not be ignored as a contaminant and further investigations are recommended to detect any urinary tract abnormalities [11]. Here in Iraq/ Wasit Province, bacteriologists in clinical medical laboratories claimed an increase isolation of *S. epidermidis* from cases of UTI especially from women (personal communication). Survey of literature in Iraq and in Wasit Province revealed no information about the role of this bacterium as a cause of UTI especially in women. Hence, this study was designed to determine the prevalence of *S. epidermidis* among pregnant and nonpregnant outpatient females with acute urinary tract infection and to characterize them at the level of methicillin and antimicrobial resistance.

MATERIALS AND METHODS

Patients

This study included pregnant and nonpregnant outpatient females (aged 18 to 40 years) clinically diagnosed with symptomatic acute UTI. Exclusion criteria included those in the menstrual phase of the menstrual cycle, those with known urinary tract abnormalities and those undergoing antimicrobial treatment within two weeks of information gathering.

Specimen Collection and Processing

Midstream urine specimens were collected from female outpatients (one specimen/ each patient) visiting gynecology and obstetrics outpatient clinics and hospitals in Al-Kut and Al-Numaniyah/ Wasit Province/Iraq during the period from July 2023 to January 2024. The urine specimens were analyzed microscopically by general urine examination and by culture on MacConkey agar, blood agar (BAP) and mannitol salt agar (MSA) plates for bacterial isolation [12]. The plates were incubated aerobically at 37°C for 24-hr. Presence of ≥ 10 pus cells/ field during general urine examination and ≥ 10^5 CFU/ml through bacterial culture was considered as positive bacterial etiology of UTI [13].

Morphological and Biochemical Identification of the Isolates

A 24-hr. old pure bacterial culture on tryptic soy agar (TSA) plates was utilized for biochemical identification of the isolates depending on standardized microbiological methods [14, 15].

PCR Speciation of the Isolates

Depending on Mutasher and Fleih [16], bacterial DNA was extracted by a boiling procedure with brief modification. A 24-hr. fresh bacterial culture on tryptic soy agar (3 loopfuls) was suspended in 1 ml of sterile 1X TE buffer (pH 8.0) instead of sterile D.W., then the bacterial suspension was boiled in an 85˚C water bath for 20 min, then placed in an ice bath for 10 min, and finally centrifuged at 10,000 rpm for 10 min. The supernatant (containing purified DNA) was distributed in quantities of 100 µl and stored at -20°C till use.

Staphylococcus epidermidis was identified genotypically according to Kim *et al*. [17], where primers targeting the *gseA* gene were used (Epi F: 5`-GGCAAATTTGTGGGTCAAGA-3` and Epi R: 5`-TGGCTAATGGTTTGTCACCA-3`). The reaction mixture was performed in 25 µl PCR master mix containing 5µl of template DNA, 1 µl of each of forward and reverse primer at concentration of 5 pmol and 18 µl sterile distilled water. Amplification conditions comprised an initial denaturation at 95°C for 5 min., followed by 30 cycles of denaturation at 95°C for 30s., annealing at 53°C for 30s. and extension at 72°C for 30s and final extension at 72°C for 7 minutes. The size of the amplified product was 194bp.

Detection of *Methicillin Resistance*

mecA-mediated methicillin resistance of the isolates was surveyed phenotypically by cefoxitin disc diffusion according to CLSI [18] and genotypically based on method reported by Mehrotra et al. [19]. This was performed in a 25 µL PCR master mix containing 5µL of template DNA, 1µL of each of mecA-specific primers (GMECAR-F:***5***`-

ACTGCTATCCACCCTCAAAC-***3***`and GMECAR-R:***5***`-CTGGTGAAGTTGTAATCTGG-***3***`) at concentration of 20 pmol and 18 µL sterile Distilled water. In order, PCR amplification conditions were: (1) initial denaturation for 5 min. a
t 94°C, (2) 35 cycles of denaturation at 94°C for 2 min., annealing at 57°C for 2 min. and extension at 72°C for 1 min. and (3) final extension at 72°C for 7 min. Occurrence of a 163bp band gave rise to positive mecA gene.

Antimicrobial Resistance Test

Antimicrobials specific for uropathogenic staphylococci were selected and tested by disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) [18] on Mueller-Hinton agar. These antimicrobials included: penicillin (10U); cefoxitin (30µg); ciprofloxacin (30µg); norfloxacin (10µg); trimethoprim (5 µg); gentamicin (10µg); and nitrofurantoin (300µg).

Statistical Analysis

Chi square (SPSS software, version 2.1, IBM, NC, USA) was employed to analyze the significance of differences in distribution of studied determinants. Statistical significance was considered at a P value of ≤ 0.05.

RESULTS AND DISCUSSION

The percentage of positive bacterial urine culture was 43.0% (137/318), of which 73 (53.2%) were from pregnant and 64 (46.7%) were from nonpregnant females. Phenotypically, isolates that were Gram-positive cocci arranged as grapelike clusters, and were negative for both oxidase and coagulase and positive for catalase tests, were further identified to species level by *S. epidermidis* species-specific PCR protocol (Fig.1). *Staphylococcus epidermidis* was isolated from 36 patients (26.2%): 19 (52.7%) and 17 (47.2%) were from pregnant and nonpregnant females, respectively. On the other hand, the incidence rate of first episode UTI (FUTI) and recurrent UTI (RUTI) caused by *S. epidermidis* was 29.1% (7/24), and 25.6% (29/113), respectively. Statistically, the differences in prevalence of *S. epidermidis* among pregnant and nonpregnant patients and in cases of FUTI and RUTI were insignificant. This means that the nature of the *S. epidermidis* helps them to cause UTIs, whether for the first time or recurrent, in both pregnant and non-pregnant women.

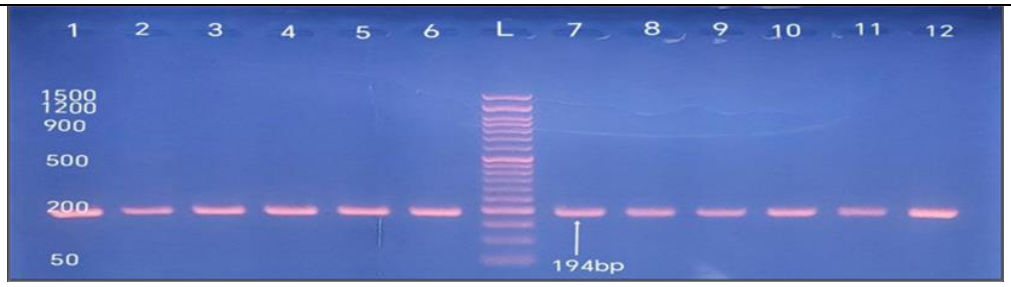

Fig. 1: Agarose gel (2%) electrophoresis (1volt/5cm) of PCR products for detection of *S. epidermidis* gene *gseA* (194 bp). Lane L: DNA Ladder (50 bp); all other lanes (1-12): positive for *S. epidermidis*.

In the current study the contribution of *S. epidermidis* in causing UTIs was higher than that in a study conducted In Iraq [20] in which they showed that *S. epidermidis* accounted for 9.8% of UTIs. The prevalence of *S. epidermidis* as a cause of UTI may be attributed to their abundance as commensal bacteria on human skin, and can therefore easily spread by hands, or transmitted by living or nonliving objects. Also, *S. epidermidis* itself behaves as opportunistic pathogen especially when patients are immunocompromised or using biomedical implants which provide the opportunity to colonize surfaces and form biofilms [21, 22]. Biofilm production by *S. epidermidis* is a major contributor in its pathogenicity [7]. As a whole, 32 (88.8%) of *S. epidermidis* isolates had *mecA* gene and hence were methicillin-resistant (Fig.2). All of cefoxitin resistant isolates (19/36: 52.7%) harbored *mecA* gene and 13 (36.1%) of cefoxitin sensitive isolates were also *mecA* positive. Whereas, 4 (11.1%) isolates were sensitive to cefoxitin and negative for *mecA* gene (Table. 2). *Staphylococcus epidermidis* has emerged as a pathogen that is resistant to many antibiotics including methicillin [23].

However, this study's result approximately agreed with others. Najar-Peerayeh *et al*. [24] reported that 92.2% of *S. epidermidis* isolates harbored *mecA* gene. Also, Rohde *et al*. [25] reported that 87.5% of *S. epidermidis* isolates carried the *mecA* gene. This gene, encodes a penicillin-binding protein (PBP2A) with low affinity for β-lactam antibiotics, is associated with resistance to methicillin, thus identifying the isolate as a methicillin-resistant *S. epidermidis* (MRSE) [26].

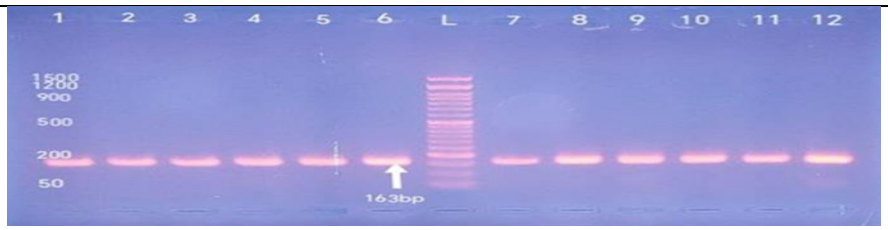

Fig. 2: Agarose gel (2%) electrophoresis (1volt/5 cm) of PCR amplified products for detection of *mecA* (163bp). Lane L: DNA Ladder (50 bp); lanes from 1-12 positive results for *mec*A gene.

Table. 2: Patterns of cefoxitin (FOX) resistance and *mec*A possession in 36 *S. epidermidis* isolates from outpatient women with acute UTI.

Patients (No.)		**No. (%) of isolates**					
		FOX-R	***mec*A+**	**FOX-R *mec*A+**	**FOX-R *mec*A-**	**FOX-S *mec*A+**	**FOX-S mecA-**
Pregnant women	FUTI (n=3)	2 (66.6)	3 (100.0)	2 (66.6)	0	1 (33.3)	0
	RUTI (n=16)	10 (62.5)	15 (93.7)	10 (62.5)	0	5 (31.2)	1 (6.2)
	Total (n=19)	12 (63.1)	18 (94.7)	12 (63.1)	0	6 (31.5)	1 (5.2)
Non-pregnant women	FUTI (n=4)	2 (50.0)	4 (100.0)	2 (50.0)	0	2 (50.0)	0
	RUTI (n=13)	5 (38.4)	10 (76.9)	5 (38.4)	0	5 (38.4)	3 (23.0)
	Total (n=17)	7 (41.1)	14 (82.3)	7 (41.1)	0	7 (41.1)	3 (17.6)
Total (n=36)		19 (52.7)	32 (88.8)	19 (52.7)	0	13 (36.1)	4 (11.1)

FOX-R: cefoxitin resistant; FOX-S: cefoxitin sensitive; *mec*A+: mecA-positive; *mec*A-: mecA-negative.

Varying levels of *S. epidermidis* isolates' susceptibility and resistance to tested antibiotics were revealed (Table 2).

Table. 2: Antimicrobial resistance of 36 *S. epidermidis* isolates from outpatient women with acute UTI.

Patients (No.)	**No. (%) of isolates**							
		P	**FOX**	**TM**	**CN**	**F**	**NOR**	**CIP**
Pregnant women (n=19)	R	16 (44.4%)	12 (33.3%)	8 (22.2%)	5 (13.8%)	1 (2.7%)	0	0
	I	0	0	1 (2.7%)	0	1 (2.7%)	0	0
	S	3 (8.3%)	7 (19.4%)	10 (27.7%)	14 (38.8%)	17 (47.2%)	19 (52.7%)	19 (52.7%)
Non-pregnant women (n=17)	R	16 (44.4%)	7 (19.4%)	4 (11.1%)	1 (2.7%)	1 (2.7%)	1 (2.7%)	1 (2.7%)
	I	0	0	0	0	0	0	0
	S	1 (2.7%)	10 (27.7%)	13 (36.1%)	16 (44.4%)	16 (44.4%)	16 (44.4%)	16 (44.4%)

Total (n=36)	R	32 (88.8%)	19 (52.7%)	12 (33.3%)	6 (16.6%)	2 (5.5%)	1 (2.7%)	1 (2.7%)
	I	0	0	1 (2.7)	0	1 (2.7%)	0	0
	S	4 (11.1%)	17 (47.2%)	23 (63.8%)	30 (83.3%)	33 (91.6%)	35 (97.2%)	35 (97.2%)

R: resistant; I: intermediate; S: sensitive; P: Penicillin; FOX: Cefoxitin; TM: Trimethoprim; CN: Gentamicin; F: Nitrofurantoin; NOR: Norfloxacin; CIP: Ciprofloxacin.

The resistance rates of 36 *S. epidermidis* isolates to penicillin (88.8%) and cefoxitin (52.7%) were higher than other antimicrobials used in this work. In Sulaimaniyah / Iraq, Anwar *et al*. [27] reported that the resistance of *S. epidermidis* to penicillin and cefoxitin had reached 100% and 67.3% respectively. The *mecA* gene confers resistance against β-lactamase-stable penicillin and almost all other β-lactam antibiotics [28]. The high prevalence of *mecA* among the isolates of *S. epidermidis* in this study, may explain the high resistance of these isolates to penicillin and cefoxitin. Resistance to trimethoprim was 33.3% (12/36) and to gentamicin was 16.6% (6/36). A study performed in Duhok Province/ Iraq, Naqid *et al.* [29] reported that resistance rate to trimethoprim in combination sulphamethoxazole was 22.2% and to gentamicin was 11.1%. The lowest resistance rate observed in the current study was to nitrofurantoin (5.5%) and ciprofloxacin and norfloxacin (2.7%, each). These results are in line with Wojtyczka *et al.* [30] who found that *S. epidermidis* were highly susceptible to ciprofloxacin (100%). Also, in Sulaimaniyah / Iraq, Anwar *et al*. [27] reported that the resistance rate of *S. epidermidis* to nitrofurantoin had reached 2.0%. The low rate of resistance to these antibiotics (ciprofloxacin, norfloxacin, and nitrofurantoin) may be due to the infrequent use of these antibiotics to treat infections caused by *S. epidermidis* strains or CoNS in Iraq.

CONCLUSION

There is high incidence of *S. epidermidis* as a uropathogen among pregnant and nonpregnant outpatient women with acute FUTI and RUTI with comparable distribution among these patients. β-lactams should no longer be used for treatment of women with acute UTI in the study area as most of these *S. epidermidis* isolates were methicillin resistant. Whereas, ciprofloxacin, norfloxacin and nitrofurantoin have good activity against *S. epidermidis* uropathogenic isolates and are strongly recommended for empirical treatment.

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