High Prevalence of Coagulase Negative *Staphylococci* Among Outpatient Women with Acute Urinary Tract Infection

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Abstract

Urinary tract infections (UTIs) caused by coagulase-negative *staphylococci* (CoNS) represent a major public health challenge worldwide. The current research focused on the isolation and identification of CoNS' species from pregnant and nonpregnant outpatient women with acute UTI and measuring their antibiogram. From a total number of 137 positive cultures, a 111 (81.0 %) were identified as *Staphylococcus spp*., a 70.2 % of them were CoNS. Eight species of CoNS were detected by two multiplex PCR protocols. *Staphylococcus epidermidis* was the most prevalent (36/137: 26.2 %), followed by *S. haemolyticus* (26/137: 18.9 %). *S. capitis* was also isolated (1/137: 0.7 %). Whereas 10.9 % of CoNS remained unidentified. An antibiogram assay was conducted. High resistance was against penicillin (84.6 %) and cefoxitin (60.2 %). Resistance to trimethoprim, gentamicin, norfloxacin, ciprofloxacin, and nitrofurantoin were: 37.1 %, 33.3 %, 20.5 %, 16.6 %, and 8.9 %, respectively. However, there are high prevalence of CoNS as uropathogens. Nitrofurantoin, ciprofloxacin, and norfloxacin are a suitable for the treatment of UTIs caused by CoNS.

Keywords: Urinary tract infection, outpatient women, Coagulase negative *staphylococci*.

1. Introduction

Across the globe, urinary tract infections (UTIs) are one of the most common infectious diseases occurring in 150 million people annually. According to the affected part of the urinary tract, these infections are classified into urethritis, cystitis, and pyelonephritis. Bacteria are the main etiologic agents of UTI, but the contribution of other microorganisms, such as fungi and viruses, is possible but very rare [1]. Urinary tract infections are either uncomplicated (uUTIs) or complicated (cUTIs). Complicated UTIs affect patients with certain conditions that reduce immunity, such as pregnancy and acquired immunodeficiency syndrome (AIDS) and people with any urinary tract abnormality or predisposing diseases, like diabetes mellitus. uUTIs are infections that affect healthy patients without any structural or neurological abnormalities of the urinary tract [2]. UTIs can occur as symptomatic or asymptomatic infections. There are some common signs and symptoms that indicate the presence of a urinary tract infection: fever, itching, burning sensation, blister formation in the genital area, suprapubic pain and pyuria [3].

Women are more susceptible to urinary tract infections because of their anatomy; the urethra is shorter, and the urethra is relatively close to the anus. Other factors such as sexual intercourse and spermicide use are thought to affect the vaginal microflora, leading to increased incidence of potentially pathogenic Gram-negative bacteria, such as *Escherichia coli*, colonizing the genital area, such factors increase women's susceptibility to UTIs [4]. During pregnancy, women are more susceptible to UTIs due to hormonal and immunological changes that occur in normal pregnancy and partly due to the pressure of the pregnant uterus on the bladder, which leads to stagnation of urine flow [5].

Staphylococci that do not possess the coagulase enzyme are designated as coagulase-negative *staphylococci* (CoNS) which constitute a large group of Gram-positive *cocci* commonly found as microflora in the skin and mucous membranes [6]. They are opportunistic pathogens that cause a variety of diseases in patients with predisposing factors such as immunosuppression, long-term hospitalizations, premature infants, and in patients with indwelling or different implant polymer bodies [7]. The ability of CoNS to cause UTI is due to its possession of genes encoding various virulence factors associated with UTI pathogenesis. These virulence factors are biofilm formation, hemolysis of red cells, and adhesion and cell wall-anchored proteins [8]. The CoNS reported to be involved in causing UTIs include *Staphylococcus saprophyticus* (*S. saprophyticus*), *Staphylococcus epidermidis* (*S. epidermidis*), *Staphylococcus haemolyticus* (*S. haemolyticus*), *Staphylococcus hominis* (*S. hominis*), *Staphylococcus xylosus* (*S. xylosus*), *Staphylococcus simulans* (*S. simulans*), *Staphylococcus cohnii* (*S. cohnii*), *Staphylococcus auricularis* (*S. auricularis*), *and Staphylococcus lugduensis* (*S. lugduensis*) [8, 9]. Coagulase negative *staphylococci* isolated from UTIs, such as *S. epidermidis, S. hominis*, *S. haemolyticus*, and *S. warneri*, are rarely identified at the species level, making it difficult to determine their importance in UTIs. [10]. *Staphylococcus saprophyticus* is the second most prevalent cause of UTIs in sexually active young women aged from 15 to 30 years old. Other CoNS are primarily isolated from catheterized individuals and those with compromised immune systems [11]. Some doctors classify CoNS as saprophytes, and they are seldom considered the cause of sickness. Nonetheless, an increasing number of research have shown that CoNS can cause UTIs in a wide range of individuals with or without urinary tract instruments. Furthermore, when these bacteria are discovered in urine specimens, they must be tested for etiological agents [8]. Treatment of CoNS' infections has become more complex, as many isolates in hospitals show high resistance rates to multiple antibiotic drugs. They are also a reservoir for resistance genes that can be transmitted to other pathogens [7]. In Iraq, low information regarding the role of CoNS as uropathogens, especially among pregnant and nonpregnant outpatient women. Therefore, the project was proposed for isolating and identifying eight CoNS' species from outpatient women with acute UTI and assessing their sensitivity to some antimicrobials commonly used for treatment of UTIs.

2. Materials and methods

2.1 Urine Specimens

Urine specimens were obtained from pregnant and nonpregnant outpatient women with acute UTI attending Al-Hajj Jalal hospital for Gynecology and Obstetrics in Al-Numaniyah / Wasit Province / Iraq and outpatient clinics of Obstetrics, Gynecology and Pediatrics in Al-Kut / Wasit Province / Iraq , from July 2023 to January 2024. After collection of urine specimens into sterile screw capped test tubes, they were spread immediately on MacConkey agar, blood agar (BAP) and mannitol salt agar (MSA) plates (Liofilchem / Italy) for bacterial isolation. Cultural, morphological and biochemical tests were employed for preliminary identification of the isolates as *Staphylococcus* genus [12, 13].

2.2 PCR-based Identification of the Isolates

2.2.1 DNA extraction

According to a boiling method that invented by Mutasher and Fleih [14], a one-day old bacterial growth (3 loopfuls) on TSA was suspended in 1 mL of sterile 1X TE buffer (pH 8.0). The cell suspension was boiled in water bath at 85 ˚C for 20 minutes, left in ice bath for 10 minutes, centrifuged at 10,000 rpm for 10 minutes and then the supernatant containing purified DNA was dispensed in 100 µl aliquots and stored at -20 ˚C till use.

2.2.2 Determining Identity of Coagulase negative *Staphylococcus spp*.

Isolates that grew on MSA, and were catalase positive and oxidase and coagulase negative were further identified to species level by two multiplex-PCR protocols designed for detection of eight species of CoNS. The first protocol included primers for detection of: *S. xylosus, S. pasteuri, S. warneri and S. haemolyticus*. While, in the second protocol: *S. caprae, S. epidermidis, S. capitis and S. saprophyticus*, were surveyed. Primers' sequences and amplification conditions were carried out according to Kim et al. [15] with modification which is the use of primers at concentration of 5 pmol instead of 50 nM.

2.3 Antimicrobial Susceptibility of the Isolates

Standard disk diffusion method was applied according to Clinical and Laboratory Standards Institute (CLSI) guidelines [16] on Mueller–Hinton agar (Liofilchem/ Italy) using the following antibiotic disks: gentamicin (10 µg); cefoxitin (30 µg); penicillin (10 U); ciprofloxacin (30 µg); norfloxacin (10 µg); nitrofurantoin (300 µg) and trimethoprim (5 µg).

2.4 Statistical Analysis

Chi square was applied to determine the differences in the distribution of the studied determinants (SPSS software, version 2.1, IBM, NC, USA). Statistical significance was indicated at A P value of ≤ 0.05.

3. Results

3.1 Identity of Coagulase Negative *Staphylococcus spp*.

In this work 137 specimens were positive for bacterial culture with ≥ 105 CFU/mL which is indicative of UTI causation by bacteria. One hundred and eleven isolates (111/137: 81.0 %) were preliminarily suspected to be *staphylococci* by their: growth on mannitol salt agar with and without mannitol fermentation, morphology on Gram-stained smears being Gram-positive *cocci* in irregular clusters, and enzymatic reactions where they were positive for catalase and negative for oxidase tests. Utilising coagulase test, 78 (70.2 %) of these 111 *staphylococcal* isolates were coagulase negative. Based on spices-specific PCR (figure 1) *S. epidermidis* was predominant (36/137: 26.2 %), followed by *S. haemolyticus* (26/137: 18.9 %), in addition to *S. capitis* (1/137: 0.7

%). While the remaining 15 isolates (10.9 %) were not identified as one of the species selected in this study as listed in (table 1).

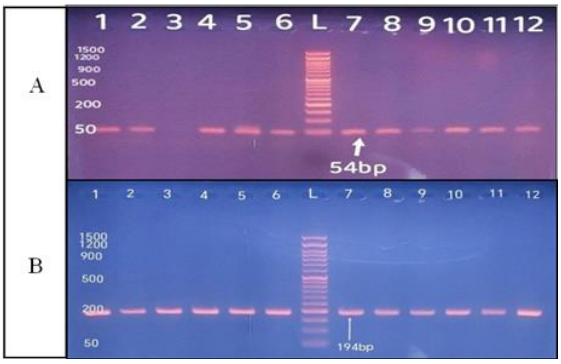

Figure 1: Agarose gel electrophoresis of PCR products for detection of CONs' species. A. First PCR protocol results. Lane L: DNA Ladder (50 bp); Lanes (1 ,2,4-12); positive for S. haemolyticus (54 bp); Lane 3 is negative for all target species. B. Second PCR protocol results. Lane L: DNA Ladder (50 bp); Lanes (1-12): positive for S. epidermidis (194 bp) only.

Table 1: Isolation percent of CoNS from outpatient women with acute UTI.

Patients number		*S. epidermidis*	*S. haemolyticus*	*S. capitis*	Unidentified	Total
Pregnant women	FUTI (n=11)	3 (27.2)	3 (27.2)	0	1 (9.0)	7 (63.6)
	RUTI (n=62)	16 (25.8)	12 (19.3)	0	8 (12.9)	36 (58.0)
	Total (n=73)	19 (26.0)	15 (20.5)	0	9 (12.3)	43 (58.9)
Non-pregnant women	FUTI (n=13)	4 (30.7)	1 (7.6)	1 (7.6)	1 (7.6)	7 (53.8)
	RUTI (n=51)	13 (25.4)	10 (19.6)	0	5 (9.8)	28 (54.9)
	Total (n=64)	17 (26.5)	11 (17.1)	1 (1.5)	6 (9.3)	35 (54.6)
Total (n=137)	Total (n=137)	36 (26.2)	26 (18.9)	1 (0.7)	15 (10.9)	78 (56.9)

FUTI: first time urinary tract infection; RUTI: recurrent urinary tract infection.

3.2 Antimicrobial Susceptibility Patterns of the Isolates

Resistance to penicillin (84.6 %) and cefoxitin (60.2 %) was the highest among this study included CoNS' isolates. Furthermore, most of these isolates were susceptible to nitrofurantoin, ciprofloxacin, norfloxacin, gentamicin, and trimethoprim (8.9 %, 16.6 %, 20.5 %, 33.3 %, and 37.1 % respectively) (table 2).

Table 2: Antimicrobial resistance of CoNS' isolates from outpatient women with acute UTI.

Antimicrobial agent	No. (%) of isolates		
	R	I	S
Penicillin	66/78 (84.6)	0	12/78 (15.3)
Cefoxitin	47/78 (60.2)	0	31/78 (39.7)
Trimethoprim	29/78 (37.1)	1/78 (1.2)	45/78 (57.6)
Gentamicin	26/78 (33.3)	1/78 (1.2)	50/78 (64.1)
Norfloxacin	16/78 (20.5)	4/78 (5.1)	57/78 (73.0)
Ciprofloxacin	13/78 (16.6)	1/78 (1.2)	64/78 (82.0)
Nitrofurantoin	7/78 (8.9)	3/78 (3.8)	68/78 (87.1)

4. Discussion

Both Gram-negative and Gram-positive bacteria are involved in etiology of UTIs which are dominant infections in hospitals and communities. In this study, 81.0 % (111/137) of uropathogens belonged to *Staphylococcus spp*. Among these *staphylococcal* isolates, CoNS represented 70.2 % (78/111). Cons were responsible for 56.9 % (78/137) of UTIs among pregnant and nonpregnant outpatient women with acute UTI. This is not consistent with what stated in a study performed in Benin [17] that they reported among the isolated *staphylococcus spp*., 73.07 % were coagulase positive *staphylococci* and 26.93 % were coagulase negative *staphylococci*. The difference in the incidence of bacterial etiologies of UTIs may be attributed to differences in socioeconomic conditions [18]. Also, in the current study, *S. epidermidis* (26.2 %), was isolated with higher frequency compared to other CoNS, followed by *S. haemolyticus* (18.9 %). This result disagreed with a study conducted in Tanzania [8] where they isolated CoNS from patients with UTI symptoms and revealed that the *S. hemolyticus* was the most common isolate among CoNS (40.5 %), followed by *S. epidermidis* (36.9 %), and other species such as *S. saprophyticus*, *S. hominis*, *S. warneri*, *S. cohnii*, *S. lugdunensis*, and *S. simulans* were also identified (7.7 %, 6.2 %, 3.1 %, 1.5 %, 1.5 %, and 1.5 %, respectively). Furthermore, in a study performed on Iraqi patients [9] *S. epidermidis* (9.8 %) preceded *S. haemolyticus* (8.9 %) in causing UTIs. The differences in uropathogens' prevalence from previous study may be due to conditions related to the different geographical environment that promote or prevent their growth.

The high prevalence of CoNS among women with UTIs could be attributed to the possession of these bacteria of virulence determinants related with causation of UTI such as formation of biofilms, adhesins, cell wall-anchored proteins, and hemolysins [8]. The predominance of *S. epidermidis* and *S. haemolyticus* 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 non-living objects and these species have antibiotic-resistance genes, and variety of cell wall-anchored surface proteins, including those that promote biofilms formation and bind to fibrinogen, fibronectin, and collagen [8, 18]. Biofilms formation on the surfaces of host cells and indwelling medical instruments is a main contributor to pathogenicity [19].

Although the difference was not significant, isolation of CoNS from pregnant women (58.9 %) was more than that from non-pregnant women (54.6 %). Ureteral dilatation and urinary stasis because of mechanical and hormonal changes of pregnancy are factors that makes the pregnant women more prone to uropathogens. Also increased amino acids, vitamins, and other nutrients in the urine of pregnant women in addition, most of pregnant women suffer from increasing of glycosuria, these factors encourage the bacteria to remain in the urinary tract of pregnant women and lead to the occurrence of disease [5, 20].

On the other hand, the difference was not significant regarding the distribution of CoNS in cases of FUTI and RUTI in both pregnant (63.6 vs. 58.0 %, respectively) and nonpregnant (53.8 % vs. 54.9 %, respectively) patients. Consequently, that 56.6 % of UTIs caused by CoNS in pregnant and nonpregnant women were recurrent. Several factors have been thought to contribute to women's recurrent UTIs, such as inappropriate behaviour of personal hygiene, as well as certain medical conditions [21]. The increased use of inappropriate antibiotic regimens may be a source of development of resistant bacterial strains, which resulting partial eradication of the uropathogens with the recurrence of illness [22].

In the current study, CoNS showed high resistance against penicillin and cefoxitin reaching 84.6 % and 60.2 % respectively. This result is almost like study performed in Benin of UTIs patients, where the resistance rate of CoNS to penicillin was 100 % [17]. A study that does not agree with our results in terms of the resistance of CoNS to cefoxitin, as it was very low at a rate of 18.2 % [23]. High level rate of resistance to *β*-lactam antimicrobials may be due to the frequent use of these antimicrobial agents in hospitals of Wasit Province / Iraq. However, *Staphylococci* have two strategies for resistance to *β*-lactam antibiotics: the first one is mediated by *blaZ* gene which encodes β-lactamases, while the second one included production of a modified target penicillin binding-protein, PBP2a, which is encoded by the *mecA* gene [24]. The prevalence of the *blaZ* and *mecA* genes were 49.2 % and 40 %

respectively, among CoNS isolates causing UTI [8]. The possession of these resistance genes by the CoNS explains why they are resistant. Whereas this study included CoNS were sensitive to nitrofurantoin (87.1 %), ciprofloxacin (82.0 %), norfloxacin (73.0 %), gentamicin (64.1 %), and trimethoprim (57.6 %). This is consistent with a study in which CoNS were isolated from pregnant women, and they were sensitive to nitrofurantoin (72.7 %) and gentamicin (72.7 %), ciprofloxacin (63.6 %) and trimethoprim–sulfamethoxazole (54.5 %) [23]. In another study, sensitivity of CoNS to ciprofloxacin and gentamicin was 71.4 % [17]. The low rate of resistance to these antibiotics (nitrofurantoin, ciprofloxacin, norfloxacin, gentamicin and Trimethoprim) this may be due to the infrequent use of such antibiotics to treat infections caused by CoNS in Iraq.

5. Conclusion

This study results indicated high distribution of CoNS among pregnant and nonpregnant outpatient women with acute UTI, either the infection was FUTI or RUTI. Also, most of these isolates were resistant to antibiotics commonly prescribed for the treatment of various infections; therefore, antibiotic susceptibility testing is recommended before prescribing treatment to prevent development of antimicrobial resistance. Nitrofurantoin and ciprofloxacin maintained strong activity, making them suitable choices for antimicrobial treatment of UTIs caused by CoNS when other options are unavailable.

6. References

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