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Antimicrobial Resistance Patterns of Staphylococcus saprophyticus Isolates Causing Urinary Tract Infections in Gorgan, North of Iran

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ABSTRACT

Background and objectives: Urinary tract infection (UTI) is one of the most common bacterial infections. *Staphylococcus saprophyticus* is a common Gram-positive bacterium that causes uncomplicated UTIs in women. The present study aimed to study the drug resistance pattern and phenotypic and genotypic variation of *S. saprophyticus* isolates from women with UTI in Gorgan, northern Iran.

Methods: This study was performed from May 2018 to September 2020. During this time, 35 *S. saprophyticus* strains were isolated from patients with UTI. The antimicrobial patterns of the isolates were determined by a conventional method. Phenotypic criteria such as pigment production, mannitol fermentation, urease production, and *16SrRNA* gene valuation were studied.

Results: All isolates were sensitive to nitrofurantoin, gentamicin, and linezolid. *S. saprophyticus* isolates showed the highest level of resistance to penicillin (85.7%) and erythromycin (51.4%). A variation was detected among *S. saprophyticus* isolates in terms of pigment production i.e. about 51.4% showed yellow pigment in Muller Hinton agar, and 62.9% of the isolates were able to ferment mannitol sugar. Of 11 isolates that were sequenced for the *16SrRNA* gene, only two isolates showed different patterns.

Conclusion: Nitrofurantoin and trimethoprim-sulfamethoxazole are the antibiotics of choice for the treatment of UTI caused by *S. saprophyticus* in the study area. Due to the phenotypic and genotypic differences among *S. saprophyticus* isolates, typing of *S. saprophyticus* at the subspecies level is recommended.

Keywords: Staphylococcus saprophyticus, Urinary tract infection, Drug Resistance, Microbial.

INTRODUCTION

Urinary tract infection (UTI) is the most frequent bacterial infection following respiratory tract infections, affecting 150 million people around the world every year (1). Gram-negative bacteria, such as *Escherichia coli*, are the main causative agents of UTIs, accounting for up to 80% of community-acquired uncomplicated UTIs, followed by *Klebsiella* spp., *Enterobacter*, and *Proteus* species. *Staphylococcus aureus*, *Enterococcus* spp., and coagulase-negative staphylococci (CoNS) are the most common Gram-positive etiologic agents of UTI (2, 3). *Staphylococcus saprophyticus* is the most common CoNS and has two subspecies: saprophyticus and bovis. The saprophyticus subspecies is the major cause of uncomplicated UTIs, following uropathogenic *E.coli* (UPEC) (2). Novobiocin resistance is a laboratory trait that distinguishes this subspecies from others (4). In addition to UTIs in young women, *S. saprophyticus* has been reported in other infections such as sepsis, acute pyelonephritis, and endocarditis (3).

As a prevalent cause of UTI, the pathogenesis of *S. saprophyticus* and its virulence factors, exclusively those distinguishing this organism from other CoNS, have not been extensively studied. In addition, epidemiological research about this uropathogenic organism is very limited. The pathogenicity of *S. saprophyticus* is related to some surface proteins including fibronectin binding autolysin, surface-associated lipase, uro-adherence factor, collagen-binding serine-aspartate-repeat protein, and two cytoplasmic enzymes i.e. D-serine deaminase and urease that have been characterized as necessary for the pathogenesis and attachment to host tissues (5-7).

Today, with the rise of resistance to common drugs, it is essential to study the antibiotic resistance pattern for each known pathogenic bacterium. Recent studies have demonstrated an increase in antibiotic resistance rate in bacteria that cause UTI, particularly *E. coli* and *S. saprophyticus* (3, 8). For this reason, the search for new therapeutic modalities such as nanoparticles, bacteriophages, or plant extracts has been considered (9).

In some countries, including Iran, the pathogenicity of *S. saprophyticus* is less studied. The present study aimed to investigate the pattern of antibiotic resistance among clinical isolates of *S. saprophyticus* and the possible phenotype and molecular diversity of this bacterium from urine samples in Gorgan, northern Iran.

MATERIALS AND METHODS

This study was performed from May 2018 to September 2020. During this period, clinical isolates of *S. saprophyticus* were collected from patients with UTI who had been referred to laboratories and hospitals in Gorgan, Iran. Reconfirmation was performed for all isolates using biochemical tests such as catalase, coagulase, urease production, growth on mannitol salt agar medium, and novobiocin resistance test (3). Patient's demographic information, such as sex and age, was recorded.

For definitive identification of the isolates, the following primers were used to amplify the *16SrRNA* gene: 16S forward 5′CATGCAAGTCGAGCGAACAG3′ and 16S reverse 5′TGCGGAAGATTCCCTACTGC3′. The primers were synthesized by Metabion Co., Germany. Amplification was performed according to the following conditions: initial denaturation at 95 °C for 10 minutes (1 cycle), denaturation at 94 °C for 45 seconds, annealing at 58 °C for 30 seconds, extension at 72 °C for 45 seconds (35 cycles), and one final elongation step at 72 °C for 5 minutes (10). Some PCR products were sequenced by Macrogen Co. (South Korea), and the sequences were deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank/).

Susceptibility of *S. saprophyticus* isolates to trimethoprim-sulfamethoxazole, tetracycline, ciprofloxacin, nitrofurantoin, gentamicin, chloramphenicol, erythromycin, linezolid, penicillin, clindamycin, and levofloxacin was tested using the disc diffusion method (Kirby Bauer). The results were interpreted according to the clinical and laboratory standards institute guidelines (2019). The antibiotic discs were purchased from Rosco, Denmark.

Statistical analysis of data was carried out in SPSS (version 18.0).

RESULTS

In this study, 35 isolates were confirmed based on culture, biochemical tests, and PCR amplification. All isolates had novobiocin resistance and intense urease activity. Twenty-two isolates (62.9%) were able to ferment mannitol sugar, and about 51.4% of the

isolates showed yellow pigment in Muller Hinton Agar (Merck, Germany) after 24 hours of incubation.

All *S. saprophyticus* isolates were from women with UTI aged 4 to 65 years. Most *S. saprophyticus* isolates (68.6%) were collected in the summer season. Six, two, and three isolates were collected in the autumn, winter, and spring seasons, respectively. Only three *S. saprophyticus* isolates were collected from hospitalized patients.

After the PCR amplification, a 327 bp fragment was detected for all *S.saprophyticucs* isolates. The PCR products for 11 cases isolates were sequenced and read by the Chromas software to confirm the amplified gene. The sequences were deposited in GenBank with the following accession numbers: MW453014, MW453015, MW453016, MW453017, MW453018, MW453019, MW453020, MW453021, MW453022, MW453023, and MW453024. The sequencing results demonstrated two closely divergent clades of 16srRNAs (MW453023, MW453024), which have different sequencing patterns (Figure 1).

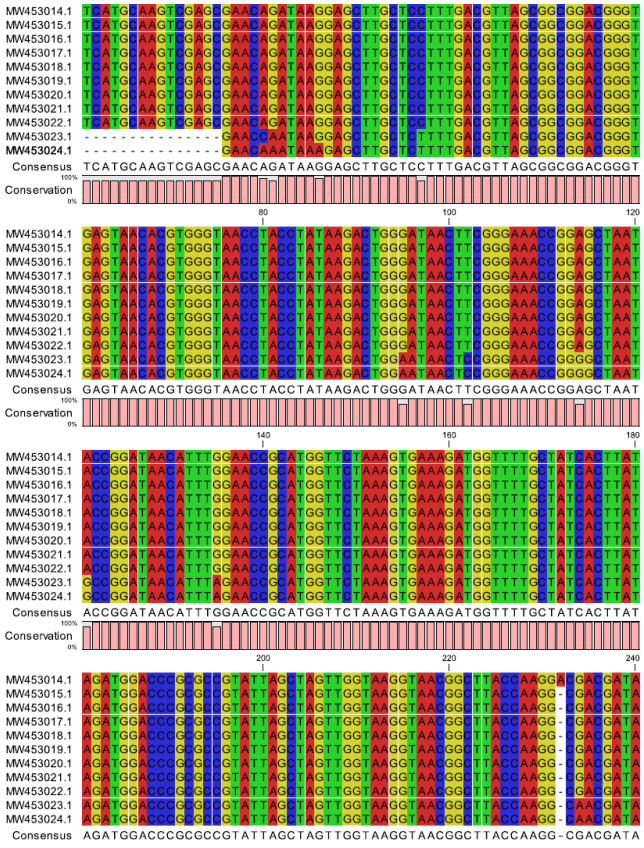

Figure 1- The sequencing results of 11 *S. saprophyticus* isolates from patients with UTI in Gorgan, northern Iran

All isolates were sensitive to gentamicin, nitrofurantoin, and, linezolid. The highest sensitivity was observed against cotrimoxazole, levofloxacin (94.3%), and ciprofloxacin (91.4%). The highest level of resistance was related to penicillin. Moreover, 9 isolates were resistant to multiple antibiotics (Table 1).

Table 1- Results of antimicrobial susceptibility testing

Antibiotics	Susceptible Number (%)	Resistant Number (%)
Nitrofurantoin	35(100%)	0
Linezolid	35(100%)	0
Tetracycline	32(91.4%)	3(8.6%)
Clindamycin	26(74.3%)	9(25.7%)
Penicillin	5(14.3%)	30(85.7%)
Ciprofloxacin	32(91.4%)	3(8.6%)
Levofloxacin	33(94.3%)	2(5.7%)
Erythromycin	17(48.6%)	18(51.4%)
Chloramphenicol	27(77.1%)	8(22.9%)
Gentamicin	35(100%)	0
Trimethoprim-sulfamethoxazole	33(94.3%)	2(5.7%)

DISCUSSIONS

S. saprophyticus is the second most common cause of uncomplicated UTI in young women. The clinical symptoms range from asymptomatic bacteriuria to pyelonephritis, perirenal abscesses, acute cystitis, and sepsis. Infection with this bacterium rarely results in hospitalization. As expected, the majority of cases (91.3%) in our study were outpatients; however, a study in Brazil reported the opposite (11).

S. saprophyticus is sensitive to commonly-prescribed antibiotics for UTIs, and this organism is usually susceptible to trimethoprim-sulfamethoxazole (12). As the pattern of antibiotic resistance changes over time, the infection may continue to spread despite the use of antimicrobials. Therefore, studying the pattern of antibiotic resistance in UTI-causing microorganisms can help to predict and control the rise in resistance rates in the future (13). In the present study, all isolates were sensitive to nitrofurantoin, which is in line with the findings of a study conducted in Kermanshah, Iran (14). Due to its mild side effects and very low bacterial resistance rate, nitrofurantoin has been recommended by the Infectious Diseases Society of America as the first-line treatment for uncomplicated UTIs (15).

All isolates were also sensitive to gentamicin. In previous studies, the sensitivity to gentamicin among *S. saprophyticus* ranged from 63.3% to 86.21% (3, 16). Gentamicin is used for the treatment of complicated infections but not UTI because *S. saprophyticus* infection occurs as uncomplicated cystitis (17). In our study, all isolates of *S. saprophyticus* were sensitive to linezolid, which is in agreement with the findings of previous studies (8, 18, 19). Linezolid is used for the treatment of infections caused by methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *enterococci*, as well as complex skin and soft tissue infections. Thus, this antibiotic is prescribed as the last-line treatment for complex infections and is not recommended for the treatment of UTIs (20).

The sensitivity of *S. saprophyticus* isolates to trimethoprim-sulfamethoxazole in different regions of Iran varies widely. In northern Iran, sensitivity to trimethoprim-sulfamethoxazole is very high (94.3%); therefore, it is recommended as the second-line treatment for uncomplicated UTI. However, studies conducted in other parts of Iran reported sensitivity rates of 30-70% for this drug (21-23). One of the fluoroquinolones that are mostly prescribed for UTIs is ciprofloxacin due to oral and intravenous use and its rapid excretion from the body. In this study, 91.4% of the isolates were susceptible to ciprofloxacin.

Due to the indiscriminate use of antibiotics, the prevalence of multi-drug resistant (MDR) strains is increasing, which is a major health challenge in the world. In previous studies in Iran, 100%, and 58% of *S. saprophyticus* isolates were MDR, respectively (8, 24). In the present study, 25.7% of the isolates were MDR. This requires finding novel and alternative therapies for the treatment of UTIs caused by MDR strains (25).

The phenotype and genotype diversity of *S. saprophyticus* has received less attention in recent years, and there is not much information about its typing. In our study, the isolates differed in the fermentation of mannitol sugar and pigment production. Therefore, further studies on this bacterium's phenotype and genotype diversity seem necessary.

Although all *S. saprophyticus* isolates were mannitol negative in previous reports, and all isolates showed a similar growth pattern on mannitol salt agar, we found that only 13 out of 35 isolates were mannitol negative (8).

On the other hand, we found some differences among the isolates regarding pigmentation. By examining the *16SrRNA* sequence of 11 isolates, we found that the sequence of two isolates was different from the others, which allows *16SrRNA*-based typing. Sequencing results showed that the two isolates (MW43023 and MW43024= *S. saprophyticus122* and *S. saprophyticus124*) differed from the other isolates at eight nucleotide sites.

The two isolates also differed in terms of mannitol fermentation. This indicates that there is some dissimilarity between *S. saprophyticus* isolates, which needs more attention for classification at the subspecies level.

S. saprophyticus is a CoNS that mainly colonize young sexually active women (26). Approximately half of the young women have this bacterium in their urethra (2). Moreover, UTI caused by *S. saprophyticus* occurs

predominantly in young females and women of reproductive age. Shortness of the urethra in women is a key factor that predisposes women to UTIs (27). All *S. saprophyticus* cases in this study were isolated from women, which is in line with the findings of previous studies in Bangladesh and Iran (3, 23). In previous studies, a seasonal distribution pattern of *S. saprophyticus* mainly in the summer and autumn seasons has been reported (11, 28). We found that most *S. saprophyticus* were isolated from the patients in the summer. However, we found no apparent reason for this seasonal distribution.

CONCLUSION

Nitrofurantoin and trimethoprim-sulfamethoxazole are the antibiotics of choice for the treatment of UTI caused by *S. saprophyticus* in the study area. Due to the phenotypic and genotypic differences among *S. saprophyticus* isolates, typing of *S. saprophyticus* at the subspecies level is recommended.

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Ethics approvals and consent to participate

Not applicable.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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