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Resistance of Uropathogenic Staphylococci to Commonly Used Antimicrobials for Treatment of Urinary Tract Infections

Aya M. Radhi[1]*, Sareaa M. Al-Mayahie[2]

[1,2]Department of Biology, College of Science, Wasit University, IRAQ

*Corresponding Author: Aya M. Radhi

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ABSTRACT: This study addresses the pressing issue antibiotic resistance in uropathogenic staphylococci secluded from female patients with acute urinary tract infection (UTIs) in Iraq. Antibiotic resistance, driven by the overuse and misuse of antimicrobials, presents a major public health challenge worldwide. The research focuses on determining the resistance patterns of these bacteria to routinely used antibiotics and developing viable therapy choices.

Samples were taken from patients aged 18 to 40 years at Al-Hajj Jalal Hospital in Wasit Province, Iraq, over a six-month period. Urinalysis, bacterial culturing, biochemical, and molecular identification methods, including 16S rRNA gene amplification, confirmed the presence of *Staphylococcus* spp. antimicrobial susceptibility tests were performed using Kirby-Bauer's disc diffusion method with various antibiotics.

Results revealed a high prevalence of Gram-positive cocci (81.0%), predominantly *Staphylococcus* species, among UTI isolates. Alarmingly, staphylococcal isolates exhibited significant resistance to β-lactam antibiotics such as penicillin (84.4%) and cefoxitin (61.4%). Resistance to other antibiotics, namely gentamicin, norfloxacin, and trimethoprim were 37.6%, 27.5% and 34.8%, respectively. However, lower resistance rates were observed for nitrofurantoin (7.3%) and ciprofloxacin (20.1%), supporting their use as first-line therapies.

The study emphasizes the critical need for antimicrobial stewardship, localized resistance monitoring, and routine susceptibility testing to optimize UTI treatment strategies. Additionally, it advocates for further research to understand the genetic mechanisms of resistance and explore alternative therapeutic options. These findings underscore the global urgency of addressing antibiotic resistance to improve patient outcomes in managing UTIs.

Keywords: urinary tract infections, Staphylococci, antibiogram assay

1. INTRODUCTION

Antibiotic Resistance occurs when a bacterium can withstand antibiotic exposure [1]. The increasing use of antibiotics in and out of the medical field is contributing significantly to the creation of resistance in bacteria [2]. Illnesses produced by resistant bacteria are becoming increasingly prevalent, and certain illnesses have evolved [3]. Many human diseases are caused by bacterial pathogen infections, which can occur both outside and internally to the human host. A urinary tract infection (UTI) a kind of bacterial sickness that arises when germs invade the normally sterile urinary tract (UT) [4]. Urinary tract infection is most usually seen in people with morphologically and functionally normal UT and is caused by germs ascending from the urethra to the bladder. As the name says, the afflicted regions are the upper and lower urinary tracts. The illnesses are referred to as cystitis and pyelonephritis, depending on the afflicted bodily component [5]. Urinary tract infection is a common condition that affects people of all ages and genders. It can be separated into asymptomatic and symptomatic instances based on the pathophysiology of the infection [6]. The symptoms of bladder and kidney infections are different; cystitis causes painful and frequent urination, whereas pyelonephritis causes high temperatures and flank discomfort. Poor diagnosis can result in a urinary tract infection, the most prevalent hospital-acquired illness [7, 8]. It can be caused by a number of bacteria, but the Enterobacteriaceae family, which includes *Escherichia coli*, is the most commonly seen [9], because they are part of the human microbiota, they are likely

to colonize the urinary tract [10]. In recent years, *Staphylococcus* species have developed a number of virulence characteristics that keep them harmful while appealing to urinary tract epithelial cells. These variables have led to a clearer knowledge of their pathogenic involvement in UTIs, especially among the elderly, pregnant women, and those with additional risk factors for UTI [11]. Staphylococcal species have traditionally been classed as either coagulase-negative staphylococci (CoNS) or coagulase-positive *Staphylococcus aureus* [12]. *Staphylococcus saprophyticus* is the second most common cause of UTIs in sexually active young women. It stands out for its resistance to novobiocin [13]. As antibiotic-resistant bacteria develop, treating UTIs in both inpatient and outpatient settings become more difficult [14]. Doctors have limited treatment alternatives due to extensive and incorrect antibiotic usage and the selection of resistant mutant bacteria [15]. The epidemiology of UTI and antibiotic resistance trends exhibit substantial regional and temporal variation. There is little information available in Iraq on the frequency and antimicrobial resistance of uropathogenic staphylococci, thus this study was conducted to discover resistance to routinely used antibiotics in our community among uropathogenic staphylococcal isolates from female outpatients with acute UTIs.

2. MATERIALS AND METHODS

Sample Collection

Urine samples were taken from outpatients with acute UTI who visited Al-Hajj Jalal hospital for Gynecology and Obstetrics in Al-Numaniyah/ Wasit Province/ Iraq, between July 2023 and January 2024. All patients were females aged 18 to 40 years. Midstream urine specimens were collected using sterile screw-capped test tubes in accordance with [16].

Urinalysis

Urine samples were examined macroscopically, taking note of their color and turbidity. The urine was centrifuged and examined the deposit under a microscope for the presence of WBCs, RBCs, casts, yeast cells, bacteria, and so on [17].

Urine Culturing

Urine samples were cultured using MacConkey agar, blood agar (BAP), and mannitol salt agar (MSA) plates and incubated overnight at 37°C. Significant growth was defined as $\geq 10^5$ CFU/mL of midstream urine [18].

3. IDENTIFICATION OF STAPHYLOCOCCAL ISOLATES

A. Biochemical Identification

To identify the bacteria, Gram staining and biochemical tests were conducted. The catalase test was performed by adding a drop of 3% hydrogen peroxide (H_2O_2) onto a glass slide, then mixing a small part of the bacterial colony with the drop using a disposable loop. The formation of bubbles within 30 seconds indicated a positive result [19], The oxidase test involved applying a drop of oxidase reagent to a small amount of bacterial colony on filter paper, occurrence of purple color within 10 seconds indicated a positive result [20].

B. Molecular Identification

For molecular identification, Mutasher and Fleih (2019) procedure was used to extract DNA by boiling method [21] with brief modification which included suspending a 24 hour-old bacterial growth (3 loopfuls) on tryptic soy agar (TSA) in 1 mL of sterile 1X TE buffer (pH 8.0) instead of sterile D.W. The cell suspension was heated to 85˚C for 20 minutes before centrifugation at 10,000 rpm for 10 minutes. The pure DNA supernatant was split into 100 µl aliquots and kept at -20ºC until required.

Identification of *Staphylococcus* genus was performed as described by [22] through the amplification of the 16S rRNA gene using the primer pair SG16P1F: (GTGATCGGCCACACTGGA) and SG16P1R: (CAACTTAATGATGGCAACTAAGC). The cycling conditions were: initial denaturation at 94˚C for 5 minutes, followed by 30 cycles of denaturation at 94˚C for 30 seconds, annealing at 60˚C for 30 seconds, extension at 72˚C for 45 seconds, and final extension at 72˚C for 5 minutes. The PCR products were electrophoresed on a 1.5% agarose gel containing ethidium bromide to stain and a 100 bp DNA ladder and assess the amount and quality of the PCR findings.

4. ANTIMICROBIAL SUSCEPTIBILITY TESTING

The examination and selection of antimicrobials was conducted according to CLSI (2023) instructions through the Kirby-Bauer disc diffusion method on Muller-Hinton agar plate (MHA). Overnight bacterial culture on TSA was suspended into sterile normal saline until reaching a turbidity comparable to that of McFarland (0.5) turbidity standard. The bacterial suspension was evenly distributed on MHA using a sterile cotton swab and allowed to desiccate. Subsequently, a set of sterile forceps were employed to place selected antibiotic discs (Table 1) onto the inoculated plates,

followed by an incubation period of 18-24 hours at 37°C. Upon completion of this incubation timeframe, the diameters of the inhibition zones were recorded and measured using a ruler in millimeters (mm).

Table 1. Antimicrobial agents used in this study

Antimicrobial class	Antibiotic	Symbol	Disc content (µg)	Company
Aminoglycosides	Gentamicin	CN	10	Liofilchem (Italy)
β-Lactams	Cefoxitin	FOX	30	
	Penicillin	P	10	
Fluroquinolones	Ciprofloxacin	CIP	30	
	Norfloxacin	NOR	10	
Nitrofurans	Nitrofurantoin	F	300	
Antifolate	Trimethoprim	TM	5	

5. RESULTS AND DISCUSSIONS

Specimens that are positive for bacterial culture on BAP and MSA and negative on MacConkey agar were further tested for Staphyloccocal identity as they were Gram-positive cocci organised in irregular clusters and were positive for catalase, negative for oxidase, consistent with the observations made by Ghayyib *et al* [23]. Confirmation of *Staphylococcus* genus identification was based on the results of PCR. The results indicated that 137 out of 318 samples had positive cultures, and among these, 111 specimens (81.0%) tested positive for *Staphylococcus* spp. (Fig 1).

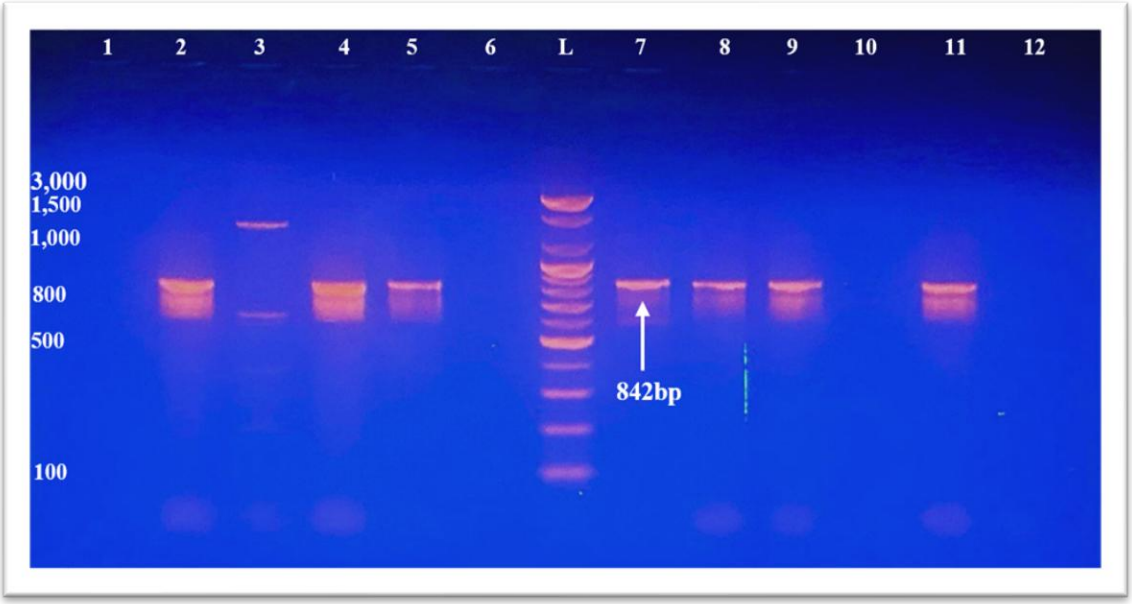

Figure (1-1): Ethidium bromide-stained agarose gel of PCR amplified Products for detection of *Staphylococcus*: 16S rRNA (842bp). Lane (L): DNA Ladder (100bp);
lanes (2,4,5,7,8,9, and 11) positive results for 16S rRNA;
lanes (1,3,6,10, and 12) negative results for 16S rRNA.

Urinary tract infection is one of the most frequent illnesses affects both the general public and hospitalized patients. Staphylococci pathogenesis in the urinary system is complex and associated with virulence factors such as bacterial adhesion, biofilm formation and resistance to antibiotics. The global public health danger posed by multidrug-resistant (MDR) *Staphylococcus* is an ongoing concern [24]. Hospital acquired UTI increases treatment cost and both morbidity and mortality rates [25]. Gram-positive cocci are a leading cause of urinary tract infections (UTIs) in both poor and high-income nations, especially in older patients with co-morbidities, pregnant women, and catheterized patients [26]. The prevalence rate of Gram-positive cocci in this study was 81.0%, this result agreed with Bachai (2018) who found the Gram-positive isolates were 57.1% in Iraq. Kamel & Ali (2024) found 5.7% were Gram-positive bacteria in Babylon. In Iran, Hegazy *et al*, (2018) demonstrated that 82.42% of isolates were Gram-negative bacteria, these results disagreed with the present study.

6. ANTIBIOTIC SUSCEPTIBILITY OF *STAPHYLOCOCCUS* SPP.

Staphylococcal isolates obtained in this study, exhibited varying levels of susceptibility and resistance to tested antimicrobials (Table 2).

Table 2. The susceptibility of the isolated *Staphylococcus* spp. to different classes of antimicrobials.

Criteria	Antimicrobial agent						
	P	FOX	CN	TM	NOR	CIP	F
Resistant	92 (84.4%)	67 (61.4%)	41 (37.6%)	38 (34.8%)	30 (27.5%)	22 (20.1%)	8 (7.3%)
Intermediate	0 (0%)	0 (0%)	1 (0.9 %)	3 (2.7%)	5 (4.5%)	2 (1.8%)	4 (3.6%)
Sensitive	17 (15.5%)	42 (38.5%)	67 (61.4%)	68 (62.3%)	74 (67.8%)	85 (77.9%)	97 (88.9%)

The findings delineated in Table 2, revealed that *Staphylococcus* spp. exhibited high resistance against β-lactam antibiotics (84.4% to penicillin and 61.4% to cefoxitin). These results agreed with a study performed by Aniba *et al*. (2023) who reported that *S. saprophyticus, S. aureus, S. epidermidis* and *S. haemolyticus* showed significant penicillin resistance: 100%, 83.33%, 81.25%, and 64.28%, respectively, whereas 44.44% of *S. aureus* isolates were cefoxitin resistant [27]. This is consistent with the worldwide situation of β-lactam antibiotic resistance, which originates from the selection pressure caused by incorrect and aggressive use of β-lactam antibiotics in healthcare institutions and self-medication.

Varying degrees of resistance were observed towards other antibiotics: 37.6% of the isolates showed resistance to gentamicin, 27.5% resistant to norfloxacin, and 20.1% were resistant to ciprofloxacin (CIP). This study's results agreed with Ghadiri *et al*. (2012) who reported that 28.5% of coagulase-negative staphylococci and 45% of *S. aureus* were resistant to gentamicin. However, our results disagreed with Hussein *et al*. (2017) in Duhok, who found 0% of staphylococcal isolates were resistant to gentamicin, 50% were resistant to norfloxacin, and 100% were resistant to ciprofloxacin. In which these antibiotic mechanisms include: disrupting protein synthesis, which leads to bacterial cell death by binding to the bacterial 30S ribosomal subunit by gentamicin [28]. However, norfloxacin and ciprofloxacin inhibit bacterial DNA replication, resulting in bacterial cell death. Ciprofloxacin is routinely used to treat urinary tract infections, including pyelonephritis and multidrug-resistant bacteria [29]. Nitrofurantoin and trimethoprim were suggested as first-line treatments for UTI [30]. In this study 7.3% of the isolates were resistant to nitrofurantoin. The present study's findings are consistent with previous research by Girma and Aemiro (2022), who discovered that 0% of *S. aureus* and coagulase-negative staphylococci were resistant to nitrofurantoin. In this study 34.8% of isolates were resistant to trimethoprim. In Babylon, Alhusayni *et al.* (2022) found 100% and 0% of *S. aureus* and *S. saprophyticus* respectively, were resistant to trimethoprim. Nitrofurantoin inhibits bacterial cell wall synthesis and other cellular processes, including nucleic acid and protein synthesis. This broad spectrum of action allows nitrofurantoin to target many pathogens causing UTIs. Importantly, nitrofurantoin is highly concentrated in the urine, which makes it particularly effective for UTIs [31]. Trimethoprim works by inhibiting bacterial folic acid synthesis, a critical component for DNA synthesis, thus preventing bacterial growth [32].

7. CONCLUSION

The findings of this study highlight the critical issue of antibiotic resistance among *Staphylococcus* spp. from UTI patients. The high resistance rates to β-lactam antibiotics, including penicillin and cefoxitin, reflect the consequences of excessive and inappropriate antibiotic use in healthcare settings. On the other hand, the lower resistance rates observed against nitrofurantoin and ciprofloxacin emphasize their potential as first-line therapeutic agents for UTIs. This study underscores the importance of routine antimicrobial susceptibility testing and molecular identification techniques to ensure accurate pathogen identification and effective treatment regimens. The increasing resistance to commonly prescribed antibiotics necessitates a judicious approach to antibiotic prescription and use. Implementing localized resistance monitoring and promoting awareness about antibiotic stewardship are essential steps to mitigate the spread of resistant pathogens and enhance patient outcomes in managing UTIs. Further research is recommended to explore alternative therapeutic options and understand the genetic mechanisms underlying resistance in *Staphylococcus* spp.

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