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Original article

Prevalence, Biofilm Formation, and Antimicrobial Resistance of Uropathogens Isolated from Patients with Urinary Tract Infections in Misurata, Libya

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Abstract
Urinary tract infections (UTIs) are among the most prevalent bacterial infections, affecting all ages and ranging from uncomplicated cystitis to severe pyelonephritis. This study was conducted to determine the prevalence of uropathogens causing urinary tract infections (UTIs), their capacity for biofilm formation, and their antimicrobial resistance patterns in Misurata, Libya. A cross-sectional study was conducted at two medical centers in Misurata. A total of 40 patients clinically diagnosed with UTIs were included. Patient ages ranged from 5 to 88 years, and the majority of them were female (80%). All isolates were identified using standard microbiological techniques. Identified bacteria were subjected to biofilm detection using the Congo Red Agar (CRA) method, and antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method. Staphylococcus aureus (37.5%) and E. coli (32.5%) were the most predominant bacteria among UTI patients, followed by Coagulase-negative staphylococci (CONS) (15%), P. aeruginosa (7.5%), and Klebsiella spp. (5%) and Streptococcus spp. (2.5%). Biofilm formation on Congo Red Agar was observed for 40% of the isolates, with the highest frequency in P. aeruginosa (2/3; 66.7%), followed by S. aureus (8/15; 53.3%) and Klebsiella spp. (1/2; 50%). Antimicrobial susceptibility testing revealed variable resistance patterns, particularly to amoxicillin, nitrofurantoin, and ceftriaxone. Multidrug resistance was detected in several isolates, including E. coli, S. aureus, Pseudomonas spp., Klebsiella spp., and CONS. Although the sample size was small, the detection of pathogenic bacteria with biofilm-forming capacity and multidrug resistance represents a significant clinical concern, underscoring the urgent need for ongoing antimicrobial resistance surveillance. Further studies with larger sample sizes and molecular characterization of resistance and biofilm-associated genes will provide deeper insight into the epidemiology of uropathogens in Libya and guide effective treatment strategies for UTI management.
Keywords. *Urinary Tract Infections (UTIs), Uropathogens, Biofilm Formation, Antimicrobial Resistance (AMR), Multidrug Resistance (MDR), Misurata.*

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Introduction

Urinary tract infections (UTIs) are among the most prevalent bacterial infections, affecting all ages and ranging from uncomplicated cystitis to severe pyelonephritis [1]. It has been estimated that approximately 150 million people worldwide are affected by UTIs each year, and the estimated incidence of UTIs in the North Africa and Middle East (MENA) region in 2021 was 25,815,054 cases [2].

Studies have shown that the persistence and recurrence of UTIs are often complicated by various factors, including biofilm formation. Biofilm formation is a key factor in the persistence, recurrence, and antibiotic resistance of UTIs [1, 3-5]. Biofilms are formed when free-floating bacterial cells attach to a surface and become enclosed within a self-produced extracellular polymeric matrix. The structural and physiological characteristics of the biofilm community contribute to evading host immune responses and their resistance to antimicrobial agents [1, 5]. Furthermore, the ability to form biofilms represents a significant challenge, particularly in medical devices such as urinary catheters, which leads to persistent infections that are difficult to treat [1, 6, 7]. The National Institutes of Health (NIH) reported that biofilms are responsible for approximately 65% and 80% of all microbial infections and chronic infections, respectively [2].

In Libya, urinary tract infections (UTIs) represent a significant public health concern due to the growing burden of antimicrobial resistance and the contamination of urinary catheter devices, which serve as reservoirs for biofilm-producing pathogens [8]. Determining the prevalence of antimicrobial resistance profiles and the capacity for biofilm formation among UTI-causing pathogens is essential for guiding empirical therapy. This study aimed to determine the prevalence of bacterial pathogens causing urinary tract infections (UTIs), their capacity for biofilm formation, and their antimicrobial resistance patterns in Misurata, Libya.

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Materials and Methods

Study Design

A cross-sectional study was conducted from May 2024 to June 2024 to investigate the prevalence, biofilm-forming capacity, and antimicrobial susceptibility profiles of urinary pathogens.

Study Population

A total of 40 midstream urine samples were collected from patients attending two medical centers in Misurata. Patient ages ranged from 5 to 88 years. Informed consent was obtained from all participants prior to sample collection.

Sample Collection and Transport

Aseptically collected urine samples were immediately transported to the microbiology laboratory for processing. Samples were inoculated onto suitable culture media (MacConkey and Blood Agar) within one hour of collection and incubated at 37 °C for 24 hours.

Bacterial identification

A bacterial load greater than 10^5 CFU/ml was considered significant bacteriuria. Bacterial colonies were identified using standard microbiological techniques such as Gram stain and biochemical analyses.

Biofilm detection test

The bacterial isolates were cultured on Congo red agar (CRA) for the detection of biofilm production. Briefly, the media was prepared by adding 0.8 g of Congo red and 36 g of sucrose to 1 L of Brain Heart Infusion broth (Oxoid). The isolates were subcultured on CRA and subsequently incubated for 24 h at 37 °C. The production of black colonies was used to differentiate biofilm from non-biofilm-producing isolates.

Antimicrobial susceptibility test

Bacterial suspension was prepared and compared to a 0.5 McFarland standard prior to inoculation on Muller-Hinton agar, and then the antibiotic discs were added and incubated for 24 hours at 37°C. The diameter of the inhibition zone around each of the antibiotic discs was measured. Results were reported as “susceptible, intermediate,” or “resistant” according to CLSI guidelines. Multidrug resistance (MDR) was defined as resistance to at least one agent in three or more antimicrobial categories [9].

Statistical analysis

Data were analysed using Microsoft Excel (version 2019). Given the small number of isolates, the analysis was limited to descriptive statistics, which are presented as frequencies and percentages. All figures were generated using Microsoft Excel.

Results and Discussion

A total of 40 midstream urine cultures were examined for bacterial growth, biofilm formation, and antimicrobial susceptibility testing. All collected samples showed bacterial growth, indicating bacteria as the main cause of UTIs. This result is consistent with other studies across diverse populations and geographic regions [2, 5, 6].

The distribution of cases according to gender revealed that out of 40 samples, 32 (80%) were females and 8 (20%) were males (Figure 1). This is in agreement with previous studies that demonstrated that UTI is more prevalent in females than in males [5, 10, 11]. Anatomical differences, such as short urethral anatomy and other risk factors—including poor genital hygiene, sexual activities, pregnancy, insufficient hydration, delayed urination, and inadequate sanitation enable bacterial colonization of the urinary tract [5, 11].

The highest proportion of UTI cases (50%) was found in the age group 26–46 years, followed by 5–25 years (32.5%), 47–67 years (15%), and 68–88 years (2.5%) (Figure 2). This distribution widely aligns with reports indicating the prevalence of UTI among adolescent and middle-aged adults, particularly women in their reproductive years [5, 11]. However, the very low frequency observed in the elderly is in contrast with other studies. This could be due to a small sample size (n = 40), sampling bias, or underdiagnosis in older adults [5, 12].

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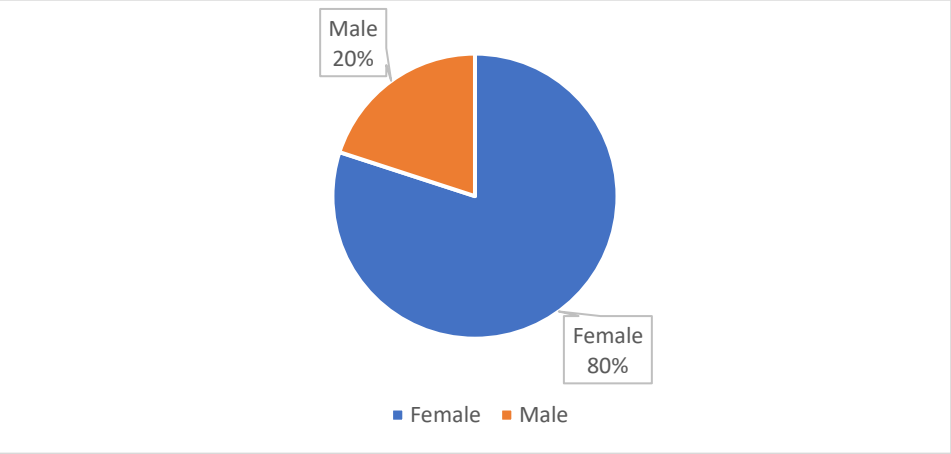

Figure 1. Distribution of urinary tract infection cases according to gender

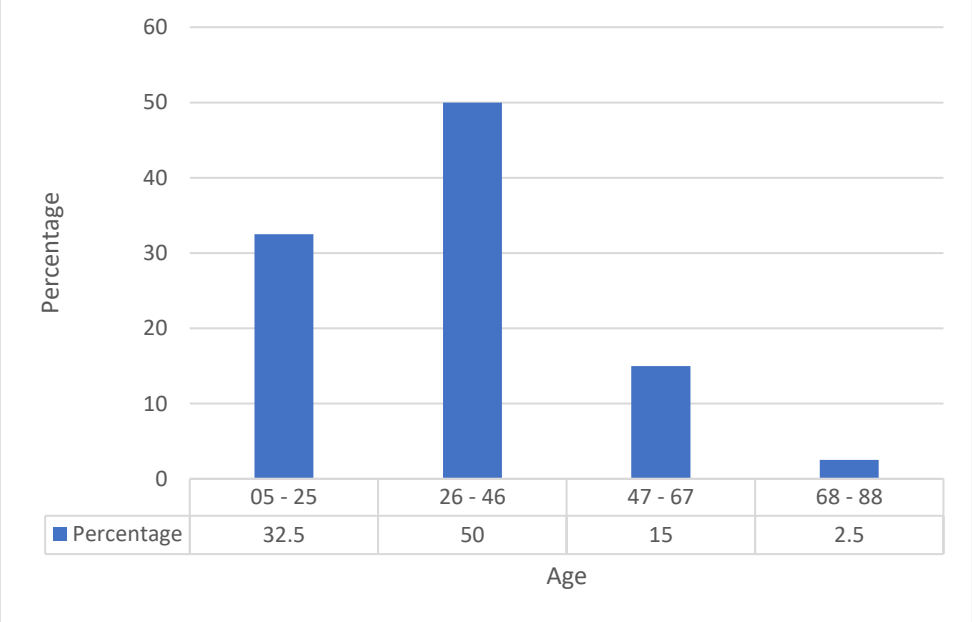

Figure 2. Distribution of urinary tract infection cases according to age groups

Analysis of urine samples from UTI patients (Figure 3) revealed that *S. aureus* (37.5%) and *E. coli* (32.5%) were the predominant bacteria. Other isolated pathogens included *coagulase-negative staphylococci* (15%), *P. aeruginosa* (7.5%), and *Klebsiella spp*. (5%), and *Streptococcus spp*. (2.5%). Similar findings have been reported in previous studies, where *E. coli* was identified as the predominant Gram-negative pathogen and *S. aureus* as the predominant Gram-positive pathogen associated with urinary tract infections [13, 14]. However, these results differ from the majority of published data, which generally identify *E. coli* as the leading cause of UTIs [5, 15, 16]. The higher prevalence of *S. aureus* in this study compared to the typically dominant *E. coli* could be attributed to the differences in the study population, epidemiological trends in Misurata in comparison to other populations, or risk factors such as hospitalization, catheterization, and other medical interventions.

Among the 40 bacterial isolates tested for biofilm formation on CRA (Figure 4), *P.aeruginosa* (2/3; 66.6%) followed by *S. aureus* (8/15; 53.3%) were the highest biofilm-forming isolates (Table 1). This is consistent with published literature describing *P. aeruginosa* and *S. aureus* as strong biofilm formers [17-19]. In *Klebsiella* spp, *CONS*, and *E. coli*, the prevalence of biofilm-forming capacity was (1/2; 50%), (2/6; 33.3%), and (3/13; 23%), respectively. None of the *Streptococcus* spp. isolates produced biofilm (0/1; 0%). These findings are consistent with other studies suggesting that biofilm-forming ability varies among pathogens [20, 21]. This variability could be attributed to factors such as differences in isolate genomes, a low number of isolates of some species, methodological cut-offs for defining "positive"

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biofilm producers, or the clinical origin of the isolates [20, 21]. In addition, the results presented in this study should be interpreted with caution due to the small number of isolates.

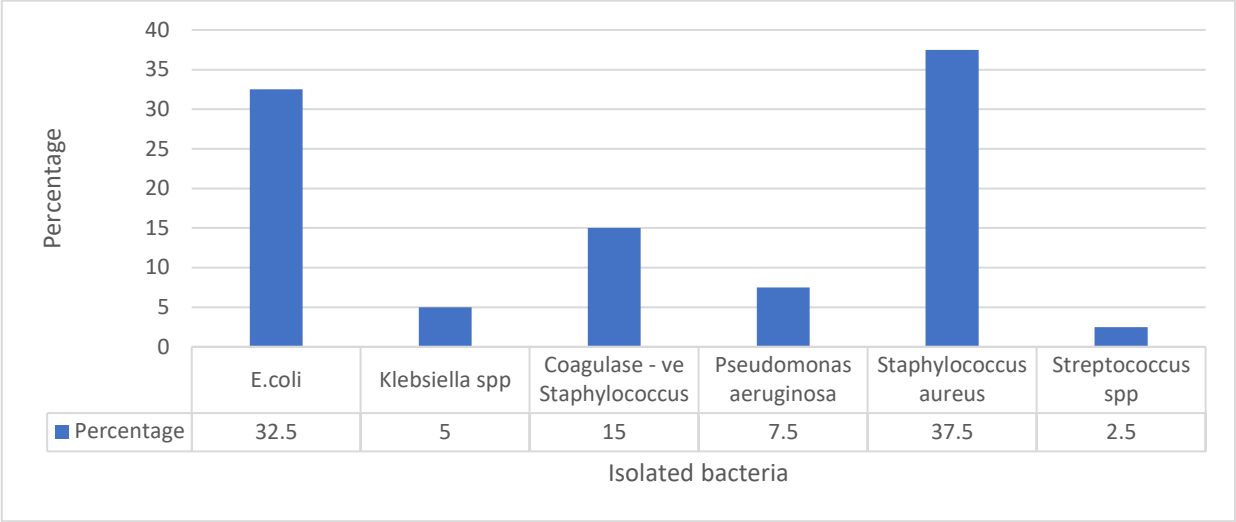

Figure 3. Percentage distribution of bacterial species isolated from urine samples of UTI patients

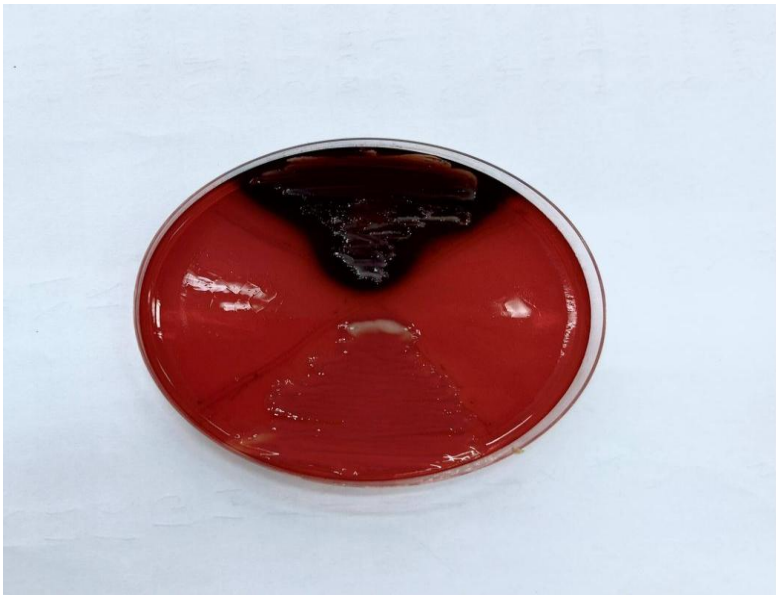

Figure 4. Detection of biofilm production using Congo red agar (CRA)

Table 1. Biofilm production by bacterial isolates from urinary tract infections (UTIs)

Bacterial species	No. of isolates	Biofilm producers n (%)	Non-producers n (%)
P. aeruginosa	3	2 (66.6%)	1 (33.3%)
S. aureus	15	8 (53.3%)	7 (46.7%)
Klebsiella spp.	2	1 (50%)	1 (50%)
Coagulase-negative staphylococci (CONS)	6	2 (33.3%)	4 (66.7%)
E. coli	13	3 (23%)	10 (77%)
Streptococcus spp.	1	0 (0%)	1 (100%)
Total	40	16 (40%)	24 (60%)
Gram-negative isolates	18	6 (50%)	12 (50%)
Gram-Positive isolates	22	10 (45.5%)	12 (54,5%)

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A total of 40 bacterial isolates, including 18 Gram-negative and 22 Gram-positive isolates, were tested for antimicrobial susceptibility according to CLSI guidelines (Table 2). Gram-negative bacteria, including *E. coli*, exhibited moderate resistance to azithromycin (30.8%), while showing lower resistance to amoxicillin and nitrofurantoin (23.1% each), ceftriaxone (7.7%), and ciprofloxacin (0%). *Klebsiella* spp. (n=2) demonstrated resistance to amoxicillin and azithromycin (100% each), and 50% resistance to nitrofurantoin, ciprofloxacin, and tetracycline. *Pseudomonas* spp. (n=3) demonstrated high resistance to amoxicillin and ceftriaxone (66.7% each) in the current study. These findings are consistent with the broader picture of high and variable resistance to older and commonly used antibiotics, such as beta-lactams [5, 14, 22, 23].

Gram-positive bacteria included 22 isolates. *S. aureus* (n=15) demonstrated high resistance to nitrofurantoin (60%) and ceftriaxone (53.3%), while imipenem remained relatively effective (13.3% resistance). CONS isolates (n=6) exhibited high resistance to nitrofurantoin (66.7%) but moderate resistance to levofloxacin (33.3%), while *Streptococcus* (n=1) showed resistance only to amikacin. Similarly, other Libyan studies reported high resistance rates, particularly to penicillin and cephalosporins [14, 23]. In addition, resistance to ceftriaxone (53.3%) is lower than the reported rate in previous Libyan studies, where ceftriaxone resistance reached 90% indicating that resistance remains a major concern across the country [14]. Furthermore, resistance of most isolates to nitrofurantoin suggests that nitrofurantoin resistance among uropathogens may be emerging as a concern in the present study compared to earlier Libyan data [14, 23]. Resistance to nitrofurantoin, a widely prescribed first-line oral agent for UTIs, may compromise its effectiveness for the treatment of UTIs.

Multidrug resistance (MDR), defined internationally as resistance to ≥3 antibiotic classes, was detected in 32.5% (13/40) of all uropathogens isolated in this study [9]. Among the Gram-negative isolates, *Escherichia coli* showed MDR in approximately 23% (3/13) of cases, *Klebsiella spp.* in 50% (1/2), and *Pseudomonas spp.* in 33% (1/3) of isolates. For the Gram-positive group, *S. aureus* showed MDR in 40% (6/15) of isolates, while *CONS* exhibited MDR in 33% (2/6). The presence of these MDR pathogens highlights growing national and regional concerns regarding antimicrobial resistance in uropathogens. Furthermore, among the MDR isolates, 76.9% (10/13) had biofilm-forming capacity as shown on Congo Red agar. This finding is consistent with previous studies, where a strong association between biofilm formation and multidrug resistance was observed [5, 19, 20, 24].

Conclusion

The study provided insight into the bacterial etiology of UTIs, their prevalence, capacity for biofilm formation, and their antimicrobial resistance patterns in Misurata, Libya. *S. aureus* and *E. coli* were the most commonly isolated pathogens, followed by CoNS, *P. aeruginosa*, *Klebsiella* spp., and *Streptococcus* spp. The detection of biofilm production among clinically significant isolates underscores the challenge of persistent and recurrent infections. In addition, the observed resistance to commonly prescribed antibiotics raises serious concerns regarding their therapeutic effectiveness. These findings highlight the urgent need for establishing antimicrobial stewardship programs and reinforce the importance of continuous monitoring of resistance patterns to improve patient outcomes. Future studies with larger sample sizes and molecular characterization of resistance and biofilm-associated genes will provide deeper insights into the epidemiology of uropathogens in Libya.

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Table 2. Antimicrobial resistance rates (%) among Gram-negative and Gram-positive bacterial isolates

Antibiotic	**Antibiotic Class**	***E. coli* (n=13)**			***Klebsiella spp.* (n=2)**			***Pseudomonas aeruginosa* (n=3)**			***S. aureus* (n=15)**			***CONS* (n=6)**			***Streptococcus spp.* (n=1)**		
		S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Amoxicillin	β-lactam, Penicillin class	69.2	7.7	23.1	0	0	100	0	33.3	66.7	40	20	40	50	16.7	33.3	100	0	0
Nitrofurantoin	Nitrofuran	61.5	15.4	23.1	50	0	50	33.3	66.7	0	20	20	60	33.3	0	66.7	100	0	0
Ceftriaxone	3rd generation Cephalosporin	84.6	7.7	7.7	--	--	--	33.3	0	66.7	26.7	20	53.3	83.3	16.7	0	100	0	0
Azithromycin	Macrolide	69.2	0	30.8	0	0	100	33.3	66.7	0	20	33.3	46.7	0	100	0	100	0	0
Levofloxacin	Fluoroquinolone	53.8	30.8	15.4	--	--	--	33.3	33.3	33.4	60	20	20	16.7	50	33.3	100	0	0
Ciprofloxacin	Fluoroquinolone	84.6	15.4	0	50	0	50	100	0	0	--	--	--	--	--	--	--	--	--
Imipenem	Carbapenem	--	--	--	100	0	0	--	--	--	60	26.7	13.3	--	--	--	--	--	--
Tetracycline	Tetracycline	--	--	--	50	0	50	--	--	--	--	--	--	--	--	--	--	--	--
Amikacin	Aminoglycoside	--	--	--	--	--	--	--	--	--	--	--	--	66.7	0	33.3	0	0	100

Note: "--" indicates that the antibiotic was not tested for the corresponding isolate. S = sensitive, I = intermediate, R = resistant

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Conflicts of Interest

All authors declare no conflict of interest.

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