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Original Research Paper

Cross-Sectional Investigation of Pathogenic Bacteria Associated with Urinary Tract Infections in Women in AL-Najaf City, Iraq

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ABSTRACT
Background: Urinary tract infections (UTIs) are the most frequent bacterial infection in women and an important public health issue because of their recurrent nature and rising antimicrobial resistance. This research was conducted to find the bacterial causes of UTI in women, and to determine the resistance pattern including MDR, XDR and ESBL production. **Methods:** One hundred and forty urine samples were obtained from women with confirmed UTIs. Bacterial isolation and identification of the bacterial isolates was carried out as per standard microbiological procedures including Gram staining followed by biochemical characterization. Susceptibilities to antibiotics were tested by the disk diffusion method. Patterns of resistance were categorized as XDR, MDR, or non-MDR, and ESBL production in Gram negative isolates was detected phenotypically. **Results:** A total of 157 bacterial isolates were recovered from a population of 140 urine samples: the presence of Gram-positive bacteria was observed more frequently than Gram-negative ones. Most of the isolates were related to chronic UTIs, predominantly among women aged 21-30 years and more commonly in married and pregnant women. The predominant pathogens were *Enterococcus faecalis* and *Staphylococcus aureus*. The rates of resistance to commonly used antibiotics were also very high with 61.2% and 28% isolates being classified as MDR and XDR respectively. Ciprofloxacin and imipenem were most potent antimicrobial agents among the tested. **Conclusion:** This study demonstrates the prevalence of chronic UTIs with a shift toward Gram-positive uropathogens and high prevalence of antimicrobial resistance, justifying regular susceptibility testing in addition to rational use of antibiotics.
Keywords: Cross-Sectional, Investigation, UTI, Women, HMGB-1, BAFF

1. Introduction

Urinary tract infections (UTIs) form one of the most common bacterial infections in both clinical and community based healthcare service, occurring predominantly within women of all ages [1]. These are a worldwide public health problem in terms of prevalence, frequency and healthcare utilization. It has been estimated that over half of all women will experience at least one UTI episode in their lifetime, while a significant fraction may suffer from recurrent infections [2]. The reason behind a higher risk of UTIs in women is mainly due to anatomical and physiological factors (shorter urethra, proximity to the anorectal area and hormonal impact on normal urogenital flora). Other cofactors, such as sexual intercourse, pregnancy, the postmenopausal state, catheterization and poor personal hygiene as well as predisposing conditions (e.g., diabetes mellitus) play also a part in the pathogenesis of urinary tract infections [3].

Clinically, UTIs can vary from simple lower (cystitis, urethritis) to more complex and severe upper (i.e., pyelonephritis) urinary tract infections. The typical presentation of UTIs includes dysuria, increased urinary frequency, urgency, suprapubic pain and hematuria [4].

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Unless treated and managed appropriately, UTI may result in severe complications such as renal scarring, hematogeneous bacterial dissemination to the blood (bacteremia), and sepsis or urosepsis especially in high-risk population [5]. The predominant cause of UTIs is bacterial infection, and most infections are due to Gram-negative bacteria. *Escherichia coli* is frequently described as the leading uropathogen in the world, due to its inherent capacity to adhere to uroepithelial cells by means of virulence factors fimbriae and adhesins. Other significant Gram-negative pathogens are *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Enterobacter* species. Furthermore, Gram-positive bacteria including *Staphylococcus saprophyticus*, Enterococcus and Streptococcus species are also causative agents of UTI especially in patients with predisposing factors [6]. Prevalence and distribution of the pathogens can vary across geographical region, healthcare facility type, patient populations, and environmental conditions [6].

Nowadays, the increasing rate of rapid emergence and dissemination of resistance to antimicrobial agents among uropathogenic microbes are one of the threatening key problem in dealing with urinary tract infections [7]. Uncontrolled and excessive use of antibiotic with in both community and hospital settings lead to the emergence of MDR strains which restrict treatment options. Uropathogens resistant to antibiotics are correlated with increasing treatment failure, prolonged hospitalization, increased cost of healthcare and greater complications. As a result, more surveillance studies are required for the continuous monitoring of local and regional trends in bacterial cause and antimicrobial susceptibility [8].

The accurate isolation and identification of the uropathogenic microorganism from urine specimens is essential for the diagnosis and treatment of UTIs. Microbiologic culture remains the diagnostic gold standard for infection confirmation and identification of the causative organism. Laboratory-confirmed diagnosis permits appropriate antimicrobial therapy which is critical for patient care and prevention of resistance development. Knowledge of the contemporary bacterial profile that causes UTIs in women is crucial for making decisions on empirical treatment and for designing effective preventive strategies [9].

Cross-sectional studies are commonly used in epidemiological research to describe the frequency and distribution of diseases and their risk factors at one specific point in time. This study acquisition is particularly useful for giving a snapshot of the pathogenic bacterial uropathogens causing UTIs in a certain population. Cross-section studies allow the detection of prevalent uropathogens and comparisons to previous studies carried out in other regions or periods. These types of data are necessary for monitoring trends, determining the presence of new pathogens and guiding health policy [10].

This study was conducted to determine the prevalence and antibiotic susceptibility of nosocomial pathogens obtained from women with UTI by cross-sectional manner. More specifically, the aims are: (1) to assess the frequency of different bacterial species involved in UTI in women; (2) to update data on uropathogenic bacteria epidemiology, and (3) to add findings supporting better diagnostic orientation and clinical management. Results of this study are intended to inform prudent strategies for antimicrobial use, advance the development and implementation of infection control and prevention interventions that result in lower UTI disease burden among women populations.

2. Methodology

Isolation and Identification

One hundred and forty urine samples were collected from 140 outpatient women with urinary tract infections during the study period (June to November 2025). Cultural characteristics and biochemical tests were used to identify all growing bacterial isolates, and finally, the Vitek-2 system was used to identify all growing bacteria onto cultures media.

Antimicrobial Susceptibility Test

The susceptibility to antibiotics was tested using the Kirby-Bauer disc diffusion technique [11]. After being adjusted appropriately, five new bacterial colonies were suspended in nutritional broth (0.5 McFarland standard tubes with 1.5x108 CFU per ml). Mueller Hinton agar was streaked with suspension, coated with all antibiotic discs, and incubated aerobically for 24 hours at 37°C. Twelve antibiotics were utilised in this study: 25µg of amoxicillin, 25/10µg of amoxicillin+clavulanic acid, 30µg of cefotaxime, 30µg of ceftriaxone, 30µg of

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ceftazidime, 5µg of levofloxacin, 15µg of gentamicin, 30µg of tetracycline, 10µg of tobramycin, and 10µg of imipenem. The Clinical and Laboratory Standards Institute establishes standards for antibiotic sensitivity and resistance based on the width of the bacterial growth [12].

Phenotypic detection of Extended Spectrum Beta lactamases- producing bacteria

In accordance with the 0.5 McFerland standard tube (1.5×10^8 CFUs/ml), the bacterial suspension was adjusted. In the middle of the Mueller Hinton agar plate, surrounded by three sides of 30 µg of ceftriaxone, were 10 µg of amoxicillin and 20 µg of clavulanic acid. The plates were incubated at 37°C for 24 hours after cefotaxime (30 µg) and ceftazidime (30 µg) were positioned 15 mm from the centre of amoxicillin (10 µg) with clavulanic acid (20 µg). Any increase in the inhibition zone towards AMC disc 30µg was considered as positive for the extended spectrum beta-lactamase [13].

Statistical analysis

Data has been compared using percentages using the Graph-Pad Prism program version 8.

Ethics Consideration

The Institutional-Ethics Committees of the College of medicine at the University of Kufa and the Scientific-Committee for Research of the Health Department of Najaf both gave their approval.

3. Results and discussion

Total Urine Samples and Total Bacterial Isolates

A total 157 positive urine cultures were retrieved from 140 female subjects with UTI in this study. There were more Gram-positive bacteria (67.5%) than Gram-negative bacteria (32.5%). The majority of the isolates were isolated from chronic UTIs (88.5%) as compared to acute infections (11.5%). The prevalence of chronic infection was higher in both Gram-positive and Gram-negative bacteria, especially among the Gram-positives (Table 1). A majority of isolates were obtained from married women (80.2%) and more isolates were recovered from pregnant than non-pregnant women (Table 2).

Table 1: Total bacterial isolates from women infected with acute and chronic urinary tract infections.

Bacterial isolates	Acute infection	Chronic infection	Total (%)
Gram-negative	12 (66.6%)	39 (76.5)	51 (32.5%)
Gram-positive	6(33.3%)	106 (61%)	106 (67.5%)
Total (%)	18 (11.5%)	139 (88.5%)	**157 (100%)**

Table 2: Total bacterial isolates from married and not married women infected with urinary tract infection.

Bacterial isolates	Married		Non married	Total (%)
	Pregnant	Non Pregnant		
Gram-negative	23	20	8	**51(31.2%)**
Gram-positive	55	28	23	**106(68.8 %)**
Total	78	48	**31(19.8%)**	**157 (100%)**
Total (%)	**126(80.2%)**			

The microscopic examination (Gram's staining) isolates distribution (Table 3) revealed that there was a high percentage of bacterial isolated in the reproductive age group (21-30 years). Among these the maximum number of bacteria was obtained from women with age 21-30yrs which accounted for 96 isolates (62.4%). These includes were made up by female both positive and negative organism including; 29 Gram negative gram positive organisms respectively. The 15–20-year-old age category was next with 24 (15.6%) isolates, of which six were Gram-negative and 18 were Gram-positive bacteria. Women 31–40 years old came next as being the third most affected, with 21 isolates (13.6%) (7 Gram negative and 14 Gram positive). Decreasing isolation rates were recorded in the older age brackets: 8 isolates (5.2%) of women aged 51–60 years (equal distribution between Gram-negative and Gram-positive bacteria) and only 5 cases among subjects from 41 to 50 year old (3.2%).

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Table 3: Total bacterial isolates from women infected with urinary tract infection according to age groups.

Bacterial isolates	Age/Year					Total (%)
	15-20	21-30	31-40	41-50	51-60	
Gram-negative	6	29	7	5	4	51(32.5%)
Gram-positive	18	67	14	3	4	106(67.%)
Total (%)	**24(15.2)**	**96(61.2)**	**21(13.6)**	**8(5)**	**8(5)**	**157(100)**

Total bacterial isolates

The distribution of Gram-negative and Gram-positive bacterial isolates among women with both acute and chronic UTIs by age, marital status and pregnancy is outlined in Tables 4 and 5. In acute infections the Gram negative bacteria were isolated more commonly than in Gram positive bacteria. Most of the acute Gram negative isolates were noted in females aged 21–40 years than younger or older age. Most of these isolates were from married women and more isolates were recovered from pregnant than non-pregnant women. Acute Gram-positive bacterial infections were relatively few and had an age-related distribution.

A much higher amount of bacterial isolates were due to chronic UTIs. In chronic presentations, Gram-negative bacteria were most frequently isolated in young women (21–30 years) followed by those between 31 and 40 years of age, with a lower incidence in the older population. These isolates were obtained mostly from married (Pregnant) women. Gram-positive bacteria were highly involved in chronic infections, with the majority of isolates found among women 21–30 years old and 31–40 years old. Most of the Gram-positive isolates were collected from married women, and pregnant status was also often mentioned in those.

Table 4: Distribution of Gram-Negative bacteria isolated from women's urine infected with urinary tract infection according to type of infection.

Total 51 Gram-Negative bacterial isolates			Age / year					Total (%)
			15-20	21-30	31-40	41-50	51-60	
Acute infection (12)	Married 10	Pregnant 4	1	1	2	0	0	4
		Non pregnant 6	0	2	2	0	2	6
	Non married 2		0	1	1	0	0	2
Total 12			**1**	**4**	**5**	**0**	**2**	**12 (23.5%)**
Chronic infection (39)			15-20	21-30	31-40	41-50	51-60	Total
	Married 31	Pregnant 19	0	17	2	0	0	19
		Non pregnant 12	3	5	1	1	2	12
	Non married 8		2	3	0	3	0	8
Total 39			**5**	**25**	**3**	**4**	**2**	**39 (76.5%)**
Total 51 (100%)			**6**	**29**	**8**	**4**	**4**	**51 (100%)**

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Table 5: Distribution of Gram-Positive bacteria isolated from women's urine infected with urinary tract infection according to type of infection.

Total 106 Gram-Positive bacterial isolates			Age / year					Total (%)
			15-20	21-30	31-40	41-50	51-60	
Acute infection (6)	Married 3	Pregnant 1	0	0	1	0	0	1
		Non pregnant 2	0	2	0	0	0	2
	Non married 3		0	2	1	0	0	3
Total 6			**0**	**4**	**2**	**0**	**0**	**6 (5.7%)**
Chronic infection (100)	Married 80		15-20	21-30	31-40	41-50	51-60	Total
		Pregnant 54	6	42	6	0	0	54
		Non pregnant 26	4	9	6	2	5	26
	Non married 20		8	11	1	0	0	20
Total 100			**18**	**62**	**13**	**2**	**5**	**100 (94.3%)**
Total 100 (100%)			**18**	**66**	**15**	**2**	**5**	**106 (100%)**

The prevalence of the various bacterial species amongst the 157 isolates was as follows: 41 *Enterococcus faecalis* (26.1%),40 *Staphylococcus aureus* (25.5%), 25 *Escherichia coli* (15.9%), 25 *Staphylococcus haemolyticus* (15.9%) and 23 *Klebsiella pneumoniae* (14.7%), *Proteus mirabilis* and *Pseudomonas aeruginosa* found in only one isolate (0.6%) encountered less frequently signatures. Two types, a single pattern and mixed pattern of growth were recognized. Pure growth was observed in 87 isolates (55.4%) and mixed growth in 70 isolates (44.6%). In single-growth isolates, Gram-positive bacteria had the upper hand (65 strains), all of them referring to chronic infections, and Gram-negative were 22 (including acute and chronic cases). Gram-positive (41 isolates) were more abundant than Gram-negative bacteria (29 isolates) in mixed-growth cultures, mainly isolated from chronic infections. Acute infections accounted for relatively fewer of both the Gram-negative and Gram-positive isolates in mixed flora (Table 6).

Table 6: Total bacterial isolates from women's urine infected with acute and chronic urinary tract infection.

Gram-negative bacteria	Single isolates		
	Acute infection	Chronic Infection	Total
E.coli	3	7	10
K.pneumoniae	4	8	12
P.mirabilis	0	0	0
P.aeruginosa	0	0	0
Total	7	15	**22**
Gram-positive bacteria	Single isolates		
	Acute infection	Chronic Infection	Total
E.faecalis	0	24	24
S.aureus	0	22	22
S.heamolyticus	0	19	19
Total	0	65	**65**
Total (%)	Total single isolates		87(55.42%)
Gram-negative bacteria	Mix isolates		
	Acute infection	Chronic infection	Total
E.coli	4	11	15
K.pneumoniae	1	10	11
P.mirabilis	0	2	2
P.aeruginosa	0	1	1
Total	5	24	**29**
Gram-positive bacteria	Mix isolates		
	Acute infection	Chronic infection	Total
E.faecalis	2	15	17
S.aureus	2	16	18
S.heamolyticus	2	4	6
Total	6	35	**41**
Total (%)	Total mix isolates		70(44.58%)

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Antibiotics resistance

Isolates of gram-negative bacteria (*E. coli*, *K. pneumoniae*, *P. aeruginosa* and *P. mirabilis*) had a higher resistance to most of the antibiotics used, but those from acute infections were more resistant than those from chronic infections. These isolates showed high resistance to amoxicillin, amoxicillin–clavulanic acid and penicillin, moderate resistance to tetracycline and cefotaxime and high sensitivity to ciprofloxacin. Imipenem was the best antimicrobial, having broadest activity against Gram-negative isolates. Among the isolates of chronic infection, only 2 (11.1%) *E. coli* showed resistance to imipenem and no resistant *K. pneumoniae* and *P. aeruginosa* isolate were observed**.**

This observation was also observed with Gram-positive bacteria (*Enterococcus faecalis*, *Staphylococcus aureus* and *Staphylococcus haemolyticus*). These isolates were resistant to ampicillin, azithromycin and amoxicillinin compared to susceptible ciprofloxacin.

MDR and ESBL- producing gram negative bacteria

The study on antibiotic susceptibility showed that resistance of bacterial isolates in women with UTI was very high. Among 157 isolates analyzed, 96 (61.2%) and 44 (28%) were detected as multidrug-resistant (MDR) and extensive drug-resistant (XDR), respectively, only 17(10.8%) of which non-MDR strain with most MDR and XDR strains derived from chronic infections. The highest percentage of XDR phenotype was recorded amongst E. coli in Gram negative organisms, on the contrary K. pneumoniae was predominant MDR. Isolates of P. mirabilis and P. aeruginosa that were MDR or XDR were relatively few in number. The proportion of Gram-positives also displayed high resistance levels and MDR prevailed in Enterococcus faecalis, 33 Staphylococcus aureus and Staphylococcus haemolyticus whereas XDR phenotypes were observed among these species (Table 7). In all, resistance was significantly more prevalent in chronic versus acute urinary tract infections.

Table 8 shows the frequency of ESBL-producing Gram-negative bacteria according to species, growth and type of infection. Of the 48 Gram-negatives studied, only 3 were ESBL-producers. All ESBL-producers were K. pneumoniae, accounting for 13% of all isolates identified as K. pneumoniae (Figure 1). Two ESBL-positive isolates were observed among single-growth cultures from acute infections and one isolate among mixed-growth culture of a chronic infection. Escherichia coli, Proteus mirabilis, and Pseudomonas aeruginosa was ESBL negative; thus indicating a narrow dissemination of the ESBL producing strain in this study.

Table 7: Type of resistance of bacterial isolates from women infected with acute and chronic urinary tract infection

	MDR			Non MDR			XDR		
Bacterial isolates No. (%)	Acute infection	Chronic infection	Total (%)	Acute infection	Chronic infection	Total (%)	Acute infection	Chronic infection	Total (%)
E.coli 25	1	5	6 (24 %)	0	0	0 (0%)	7	12	19 (76 %)
K.pneumoniae 23	5	17	22 (95. 7%)	0	1	1 (4.3%)	0	0	0 (0 %)
P.mirabilis 2	0	1	1 (50 %)	0	0	0(0%)	0	1	1 (50 %)
P.aeruginosa 1	0	1	1 (10 0%)	0	0	0 (0%)	0	0	0 (0 %)
E.faecalis 41	1	25	26 (63. 4%)	0	7	7 (17.1 %)	1	7	8 (19 .5 %)
S.aureus 40	1	22	23 (57. 5%)	1	6	7 (17.5 %)	0	10	10 (25 %)
S.haemolyticus 25	2	15	17 (68 %)	0	2	2 (8%)	0	6	6 (24 %)
Total 157 (100%) isolates	**10**	**86**	**96 (61.2%)**	**1**	**16**	**17 (10.8%)**	**8**	**36**	**44 (28 %)**

Table 8: Total ESBL- producing gram negative bacteria in this study

Bacterial isolates	Single growth		Mix growth		Total (%)
	Acute	Chronic	Acute	Chronic	
E.coli 25	0	0	0	0	0(0)
K.pneumoniae 23	2	0	0	1	3(13%)
P.mirabilis 2	0	0	0	0	0(0)
P.aeruginosa 1	0	0	0	0	0(0)

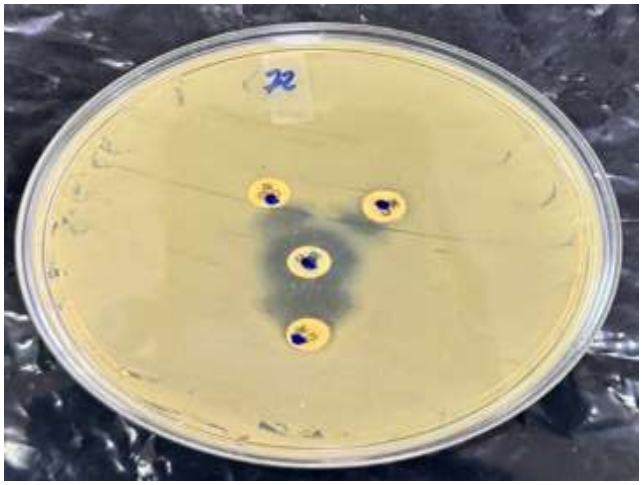

Fig1: Positive result of ESBL-Producing *K.pneumoniae* isolated from women infected with chronic UTI**.**

Results in the present study give an insight into bacteriology and antimicrobial resistance pattern in urinary tract infection among females. The prevalence of Gram-positive over Gram-negative organisms is in contrast with most studies that incriminate Gram negative bacteria as the common opportunistic invaders but reports of high occurrence of *enterococcus faecalis* and s*taphylococci* species noted here underscore the increasing significance of these organisms as etiological agents in UTI, especially when chronicity is involved [14, 15]. Higher number of isolates than urine samples also suggests the existence of mixed infections, thus complicating diagnosis and treatment [16].

UTIs chronic in nature predominated among the isolates indicating abiding or repeated infections in the studied group [17]. A similar trend was more conspicuous for G+ bacteria, which presumably have little involvement in acute casing. Distribution of age indicated that women aged 21–30 years were the most commonly affected, which is consistent with a higher degree of exposure to behavioral–physiological–reproductive risk factors during this life period [18]. Moreover, higher isolation rates in married and pregnant women further confirm the contribution of sexual activity and pregnancy-related anatomical and hormonal changes to UTI risk [19]**.**

The results of antimicrobial susceptibility testing showed an alarming high rate of resistance. The majority of isolates were defined as MDR, and a notable number of them as XDR (*E. coli* and *K. pneumoniae* in particular). Resistance was generally more frequent in chronic infections, probably due to previously antibiotic exposure and selection [20]. Meanwhile ciprofloxacin and imipenem were both highly effective (in spite of their reduced efficacy rate that has been reported by some studies), indicating the potential therapeutic value thereof [21]**.**

The identification of ESBL producers restricted to *K. pneumoniae*, despite few in number, is clinically relevant. The production of ESBL further limits therapeutic choices and emphasizes the importance for close monitoring [22]. Taken together, these results emphasize the changing epidemiology of UTI, level of AMR burden and need for targeted antibiotic stewardship and SUS testing to facilitate effective management [23]**.**

CONCLUSIONS

Overall, chronic UTIs among women are mainly caused by Gram-positive bacteria and the rates of resistance are high. The high prevalence of MDR and XDR isolates, as well as the isolation of ESBL-producing strains, underlines the need for regular microbiological diagnostic procedures including determination of antimicrobial susceptibility patterns and strict antibiotic stewardship to ensure adequate therapy and prevent spread of resistant microorganisms.

Ethics

The Institutional-Ethics Committees of the College of medicine at the University of Kufa and the Scientific-Committee for Research of the Health Department of Najaf both gave their approval.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions
AL-essa collected participants' data, analyzed and interpreted the patient data and performed the laboratory workup. Aljanaby is major contributors in writing the manuscript. All authors read and approved the final manuscript.

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