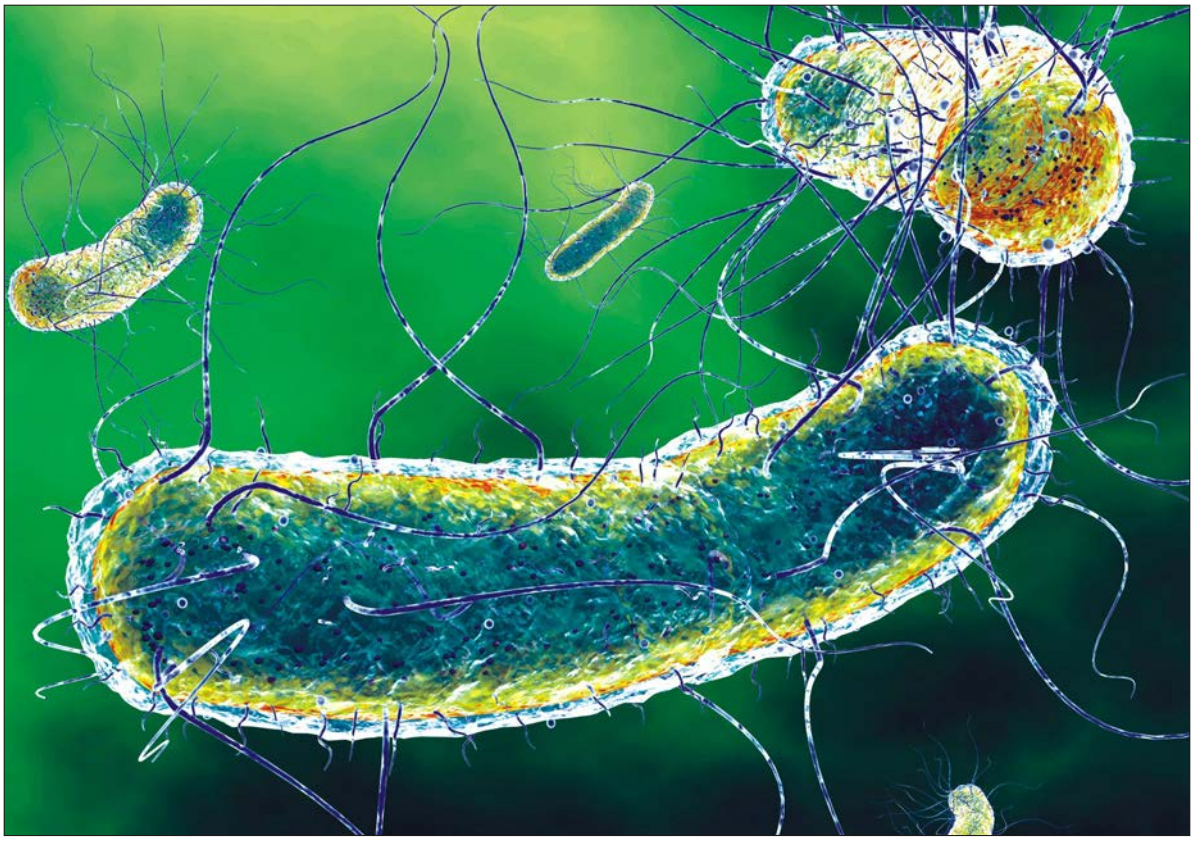

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Research Article
Antimicrobial Susceptibility Profile and Immune Response of Bacteria Isolated from Urinary Tract Infections

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Abstract

Background and Objective: Urinary tract infections (UTI) forms the largest single group of hospital-acquired infections and accounts for about 35 % of total nosocomial infections. This study aimed to detect the prevalence and antibiotic resistance profile of uropathogenic bacterial infection also to determine the immune response of infected patients. **Materials and Methods:** A hundred patients with clinical symptoms of UTI were investigated, 63% females and 37% males. About 5 mL of clean-catch midstream urine of patients was collected. Bacterial isolation and antimicrobial sensitivity profiles were applied on all collected urine samples. Serum samples were also collected for measuring phagocytosis and IgG levels. **Results:** Obtained data revealed, *E. coli* was the predominant uropathogenic organism (40 isolates), followed by *K. pneumoniae* (30 isolates), *P. mirabilis* (16 isolates), *Staph. saprophytic* (10 isolates) and *Enterococcus faecalis* (4 isolates). Obtained data, *E. coli*, were highly sensitive to Co-trimoxazole, Ceftriaxone and Imipenem (95%), while *K. pneumonia* was sensitive to Imipenem 66% and Nitrofurantoin 63%. *Proteus mirabilis* was sensitive to Amikacin 25% while *S. saprophytic* and *Enterococcus faecalis* were sensitive to Amikacin 20% and 25%, respectively. ESBL-producing organisms almost highly occur in female patients (65%) than male patients (27%). Phagocytic percentage and phagocytic index were elevated in patients infected with *E. coli*, also IgG titer was higher in *E. coli* infected patients (4.47) in comparison with other infected patients. **Conclusions:** *E. coli* is the most common isolated bacteria from urinary tract infections, a patient infected with *E. coli* showed high levels of CRP, phagocytosis and IgG. Much needed information to clinicians on the prevalence of antimicrobial susceptibility testing for judicial use of drugs and proper institution of therapy. This study underlined the importance of adequate antimicrobial prescription for UTIs to avoid multidrug resistance.

Key words: Urinary tract infections, *E. coli*, *Klebsiella pneumonia*, IgG, phagocytosis

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Gram-negative enteric constitutes a serious problem in Urinary Tract Infection (UTI) in many parts of the world. UTI has become the most common hospital-acquired infection, accounting for as many as 35% of nosocomial infections and it is the second most common cause of bacteremia in hospitalized patients, resulting in significant morbidity and high medical cost[1]. UTIs are clinically complex or simply classified. Complex UTIs occur in patients with anatomical urinary tract abnormalities or renal failure, or patients using medical devices such as catheters. UTIs in this category require long-term treatment. Urinary tract infections that occur in patients who have no anatomical urinary tract abnormalities and do not use urinary instruments are considered simple[2].

The most common bacteria causing UTIs are *Escherichia coli* and *Klebsiella pneumoniae*. *Pseudomonas aeruginosa*, *Proteus* spp., *Staphylococcus saprophytic* and *Enterococcus* spp.[3]. *E.coli* is observed as the most common bacteria causing urinary tract infection in all ages and both sexes[4]. *E. coli* accounts for approximately 80% of the UTI. *Klebsiella* species account for approximately 17% of the nosocomial urinary tract infections, urinary tract infections are often treated with broad-spectrum antibiotics[5]. The incidence of urinary tract infection in pregnant women is up 8% and a bacteriuric rate to have 4-7%. Marks the bladder inflammation, which is a disturbing case, about 20% of women over the age but rarely affects men[6]. Uncomplicated urinary tract infection is predominantly caused by *E. coli*, which has increasing antimicrobial resistance. Because uncomplicated urinary tract infections are often treated empirically assessment of antibiotic resistance is difficult and current data to characterize prevalence over time are limited[7].

Various antibacterial agents are used to treat infections caused by *E. coli*, *K. pneumoniae* and *P. mirabilis*[8,9]. Nevertheless, these organisms produce the beta-lactamase enzyme which renders them resistant to most classes of antibiotics, hence referred to as ESBL-producing organisms[10]. Nowadays, carbapenem is considered the treatment of choice for such organisms[11].

Uropathogens antibiotic resistance has increased and become an important problem worldwide. Prior use of antimicrobials, mainly broad-spectrum, previous hospitalization, and repeated UTIs are all risk factors for the development of resistance and the appearance of multidrug-resistant (MDR) bacteria in UTIs[12]. Gram-negative organisms exhibit resistance to antimicrobial agents through various mechanisms like target site modification, altered penicillin binding protein, poor diffusion, altered porins, active efflux mechanism and producing inactivating enzymes[13]. A sustained increase in bacterial resistance to antibiotics was detected, including ampicillin and trimethoprim rich levels. Reported increasing resistant *E. coli*, the most reported infections to the urinary system in society compounds fluoroquinolone and 36% to ciprofloxacin[14]. *E. coli* and *K. pneumoniae* produce beta-lactamase enzyme which renders them resistant to most classes of antibiotics[11]. Urinary tract infection is most frequently caused by uropathogenic *E. coli* (UPEC), infected patients had higher IgG titers to the antigens that are more prevalent across the population of UPEC isolates[15].

This study was aimed to detect the prevalence of uropathogenic infection and determine antibiotic resistance patterns of uropathogenic also detection of immune response of infected patients.

MATERIALS AND METHODS

Study design and setting: This retrospective cohort study was conducted at the Women's Hospital, Childbirth and the King Khalid Hospital in Hail, from October, 2019 to November, 2020, including patients of all age groups attending hospitals.

Study participants: Electronic search records of laboratory information system for urine samples and the results of each antibiogram was conducted. Patients whose urine culture results did not meet the definition for UTI established according to clinical practice guidelines (CPGs)[16].

Ethics approval and consent to participate: Theses study was conducted under the Research Ethics Committee Approval of Hail University. All patients enrolled in this study provided written informed consent for both participation and publication of identifying information. Ethical clearance was taken following guidelines and regulations of the Hail University, the Women's Hospital, childbirth and the King Khalid Hospital included. This study will be done in a manner that ensures the confidentiality of patients.

Data collection: Patient data was also collected in a special form designed for this study. These data included patients' demographics (gender and age), type of hospital admission (inpatient or outpatient) and urine cultures.

Sample size: In total, 100 patients with clinical symptoms of UTI were investigated. There were 63% females and 37% males, with an age range of 5-70 years. About 5 mL of clean-catch midstream urine of patients was collected in a sterile

tube and transported to the laboratory. Printed cards of guidelines for proper specimen collection were given to all participating patients[17].

Bacterial colony count of bacteria in UTI: The measured amount of urine was infused into a nutrient agar medium (Merck, Germany) using the loop method calibrated for colony count. More than 104 CFU mL^{-1} for each of a single potential pathogen or two potential pathogens interpreted as positive UTI, with repeated 102,104 CFU mL^{-1} results. Less than 102 CFU mL^{-1} was interpreted as negative for urinary tract infections[10]. Urine samples were cultured on blood agar and Mac Conky agar (Himedia, India and Merck, Germany) to isolate UTI microbial pathogens. All bacteria isolated from urine in this study were identified using conventional biochemical tests[18].

Bacterial culture: Vaccinating all urine samples on 5% sheep blood agar and McConkey agar plates using the calibration loop and incubated aerobically at 37°C. After incubation overnight, growing bacterial cultures were identified according to a standard protocol.

Antimicrobial susceptibility testing: Exposed[19] large clinically isolated *E. coli* and *K. pneumoniae* are also sensitive to antibiotics[20] on the widespread use of the CD method on Mueller-Hinton agar (million hectares). Plates using Cefoxitin 30 mg, Amikacin 30 µg, Cefuroxime 30 µg, Cefpodoxime 30 mg, Ceftriaxone 30 µg, Ceftazidime 30 µg, Cefotaxime 30 µg, Nitrofurantoin100 µg, Aztreonam 30 mh, Ofloxacin 5 µg, Cefepime 30 mg, Imipenem 10 µg, Co-trimoxazole 25 µg according to guidance from CLSI. The quality[21] using *E. coli* ATCC -25 922 and *K. pneumoniae* ATCC control 700 603 breeds.

Determination of extended-spectrum β-lactamases (ESBL): Initial ESBL screening for all common urinary tract pathogens identified isolates as potential ESBL producers showing resistance to 3rd generation cephalosporin antibiotics (30 µg) using disk diffusion methods it was done. The double-disc synergy test (DDST) was applied for the phenotypic confirmation of ESBL production. Strain ATCC 25,922 was used as a high-quality control strain for antibiotic susceptibility testing. A third-generation cephalosporin (ceftriaxone) antibiotic disc (30 µg) was placed 25 mm off from a mixed disc containing this last additionally clavulanic acid (20/10 µg). The zone of inhibition between the mixture disc and also the third-generation cephalosporin disc differed by $\geq$5 mm, the strain was identified as ESBL-producing[22].

Estimation of immunological parameters: Blood samples were collected into serum separating tubes for CRP, immunoglobulin measurement and EDTA tubes for white blood counts WBCS and phagocytosis on the sampling day. Serum samples were prepared by centrifugation (1500×g for 10 min) and stored in plain micro tubes until CRP analysis. WBCS counts were performed with an automated blood count[23].

CRP measurement: Serum CRP concentrations were measured by employing a poster CRP enzyme-linked immunosorbent assay (ELISA) kit (BD Biosciences). Serum samples were diluted (500-fold), 100 µL of the sample were added to each well and incubated for 30 min at temperature. After washing, 100 µL of detection antibody/enzyme conjugate was added and thus the plate was incubated for 30 min at temperature. After washing the plate fourfold with wash buffer, 100 µL of a 3,3',5,5'-tetramethylbenzidine substrate solution was added to each well for colour reaction and incubated for 10 min. Oxyacid (100 µL) was added to stop the reaction and absorbance was read at 450 nm within 10 min employing a microplate reader. CRP concentrations were determined per the standard curve made[24].

Measurement of phagocytosis: Phagocytosis was measured by using a direct counting procedure, blood from an individual patient was pooled on a bunch basis before calculating the whole phagocytes from the leukocyte counts. Bacteria in 0.1 ml of 0.85% NaCl were added to 0.9 mL of blood to provide an initial 10:1 ratio of bacteria to total phagocytes. Blood smears were made after mixture incubation and were stained with wright stain. The 100 phagocytes per slide were counted to detect phagocytic percentage and phagocytic index. The mean phagocytosis measured by counting the number of bacteria engulfed per phagocyte exhibiting phagocytosis[25].

Measurement IgG by using ELISA: ELISA was used for the determination of the immunoglobulin Gin patient serum. The basic procedure was the same as the one[26]. The enzyme reaction was performed to appropriate colour intensity for 100 mm, stopped with 3 M sodium hydroxide and read at 405 nm.

RESULTS

Prevalence of common uropathogens: This study involved 100 patients, out of this population, *E. coli* was the predominant uropathogenic organism (40 isolates), followed

Table 1: Prevalence of uropathogens according to patients' demographic factors

Demographic factors	Categories	*E. coli*	*Klebsiella pneumoniae*	*Proteus mirabilis*	*Staph. saprophytic*	*Enterococcus faecalis*
Age groups	Children (<12)	5 (12.5%)	6 (20%)	3 (18.8%)	2 (20%)	1 (25%)
	Adolescent (12-18)	7 (17.5%)	4 (13.3%)	9 (56.3%)	4 (40%)	2 (50%)
	Adult(19-64)	22 (55%)	18 (60%)	2 (12.5%)	3 (30%)	N/D
	Elderly (≥65)	6 (15%)	2 (6.6%)	2 (12.5%)	1 (10%)	1 (25%)
Gender	Female	25 (62%)	21 (70%)	11 (68.7%)	7 (70%)	3 (75%)
	Male	15 (37.5%)	9 (30%)	5 (31.3%)	3 (30%)	1 (25%)
Total number of isolates	100	40	30	10	4	16

Table 2: Antibiotic susceptibility profiles of the isolated uropathogens

	E. coli (S)		*Klebsiella pneumonia* (S)		*Proteus mirabilis* (S)		*Staph. saprophytic* (S)		*Enterococcus faecalis* (S)	
Antibiotics	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
Amikacin	37	92	8	26	4	25	2	20	1	25
Ofloxacin	31	77	17	56	2	12	0	0	0	0
Nitrofurantoin	22	55	19	63	0	0	1	10	0	0
Co-trimoxazole	38	95	11	36	1	6	1	10	1	25
Cefuroxime	30	75	4	13	2	12	2	20	0	0
Cefixime	11	27	14	46	2	12	1	10	0	0
Imipenem	38	95	20	66	1	6	0	0	0	0
Aztreonam	28	70	15	50	2	12	2	20	2	50
Cefpodoxime	29	72	10	33	1	6	0	0	0	0
Ceftriaxone	38	95	9	30	1	6	1	10	0	0

N: Number and S: Sensitive

Table 3: Non-ESBL vs. ESBL producing organisms among in- and out-patients

	Non-ESBL n (%)	ESBL n (%)
Female patients (n = 63)	16 (25%)	41 (65%)
Male patients (n = 37)	25 (67%)	10 (27%)
Total (n = 100)	41 (41%)	51 (51%)

Table 4: Laboratory data related to each studied uropathogens

Parameters	*Escherichia coli*	*Klebsiella pneumoniae*	*Proteus mirabilis*
WBCs ($\times 10^3$ per mm^3)	13.28	12.64	12.84
	9.32-17.23	11.93-13.35	7.83-17.85
CRP (mg dL^{-1})	52.85	30.56	21.68
	7.56-98.13	7.34-53.78	6.93-36.43
Phagocytic (%)	85%	76%	78%
	84-86%	75-78%	77-80%
Phagocytic index (P.I.)	0.87	0.75	0.79
	0.84-0.91	0.79-0.71	0.77-0.81
IgG titer	4.47	3.18	3.92
	4.16-4.78	3.12-3.25	3.89-3.95

by *K. pneumoniae* (30 isolates), *P. mirabilis* (16 isolates), *Staph. saprophytic* (10 isolates) and *Enterococcus faecalis* (4 isolates). Regarding gender and age distributions, females had the highest incidence rates with (59%) of the total population, with adults between 19-64 years old being the predominant group (40%). The prevalence of uropathogenic bacterial was lowest in adolescents between 14 and 19 years old (7%) as shown in Table 1.

Antibiotic susceptibility profiles of the common uropathogens: Antibiotic susceptibility profile of the most common urinary pathogens based on the data obtained, *E. coli* was very sensitive to cotrimoxazole, ceftriaxone and Imipenem (95%), while *K. pneumonia* was sensitive to Imipenem 66% and Nitrofurantoin 63%. *P. mirabilis* was 25% sensitive to Amikacin, while *Staph* was more sensitive. *Staph. saprophytic* and *Enterococcus faecalis* were 20% and 25% sensitive to Amikacin, respectively in Table 2.

Distribution of ESBL-producing bacteria in patients: As expected, ESBL-producing bacteria are more common in female patients (65%) than in males (27%) and non-ESBLs are more common in males (67%) than males (67%). Is more common. Females (25%) occur in Table 3.

Immunological parameters: The revealed blood test data showed the highest median leukocyte (WBC) in patients with UTI caused by *E. coli* (13.28, 9.32-17.23). Decreased white blood cell count in the *K. pneumoniae* group (12.64, 11.93-13.35). Patients infected with *E. coli* were found to have high CRP levels (52.85) and patients infected with *Proteus mirabilis* were estimated to have low CRP levels (21.68). Phagocytosis rates and phagocytosis indexes increased in *E. coli* infected patients compared to other patients. As shown in Table 4, IgG titers were higher in patients infected with *E. coli* (4.47) than in patients infected with *K. pneumoniae* (3.18) and patients infected with *P. mirabilis* (3.92).

DISCUSSION

This study focused on assessing the epidemiology of UTI among patients attending hospitals in the Hail region over and evaluating the profiles of antimicrobial susceptibility to the common uropathogens, *E. coli*, *K. pneumoniae*, *P. mirabilis*, *Enterococcus faecalis* and *Staph saprophytic*. Current results showed that females had a higher prevalence of UTIs owing to anatomical and physical factors, particularly their short urethras and the small distance between the urinary system and the genital/intestinal system[27]. Data revealed that *E. coli* was the most commonly detected isolate (40%) followed by *K. pneumoniae* (30%), *P. mirabilis* (16%), *Staph. saprophytic* (10%) and *Enterococcus faecalis* (4%), representing the mostly reported uropathogens as shown in other publications[27,28]. The current study is consistent with results from a previous study revealing that *E.coli* has the highest incidence (60.53%) followed by *K. pneumoniae*[28]. Extensive use of beta-lactam antibiotics in hospitals and communities has created major problems leading to increased morbidity, mortality and health care cost[29].

The prime step before initiating the antimicrobial therapy of infected individuals is performing antimicrobial susceptibility testing for clinical isolates to avoid indiscriminate usage of antibiotics on a trial and error basis. In the present study. All the isolates were further subjected to antimicrobial susceptibility testing as per CLSI guidelines and it revealed that both *E . coli* and *K. pneumoniae* were highly sensitive to Imipenem which is following other studies[30,31]. Imipenem is the most active agent against Gram-negative isolates, which correlates well with this study. In the present study, *E. coli* and *K. pneumonia* were 64.91% and 61.1% resistant to Cefpodoxime, respectively. Another study[32] observed that, all *E. coli* and *K. pneumoniae* isolates were uniformly resistant to Cefpodoxime which is following present study. In the present study, *E. coli* and *K. pneumoniae* were 72 and 10% sensitive to cefotaxime respectively. Another study[33] reported 66.41% sensitivity to *E. coli* and 72.3% in *K. pneumonia*, antimicrobial resistance often leads to therapeutic failure of empirical therapy.

Revealed data of blood laboratory analysis showed the highest median White Blood Cells (WBCs) levels in those with UTI caused by *E. coli* (13.28, 9.32-17.23). As for the lower WBC count in the *K. pneumoniae* group (12.64, 11.93-13.35), an explanation could be that WBCs have been reduced in the first few days post-infection and only increase after seven days of infection[34]. Urease-producing urinary tract pathogens, such as *P. mirabilis*, often convert acidic urine into an alkaline state that can lyse white blood cells, resulting in a decrease in white blood cell count during infection[35,36].

Laboratory data revealed that median WBC and C-reactive protein (CRP) levels were higher in patients with UTI caused by ESBL-producing organisms in comparison with UTI caused by non-ESBL producing organism patients. Phagocytes play an essential role in the host`s defence against uropathogenic bacteria which are extracellular pathogens. The obtained data revealed that Phagocytic percentage and phagocytic index were elevated in patients infected with *E. coli* in comparison with other patients. Few researcher[37] mentioned that phagocytosis was significantly lower in patients than healthy controls, especially in patients with chronic pyelonephritis. The results link reduced phagocytosis by blood phagocytes with recurrent urinary tract infection.

Obtained results revealed that IgG titre was higher in *E. coli* infected patients (4.47) in comparison with *K. pneumoniae* infected (3.18) and patients infected with *P. mirabilis* (3.92). IgG was increased in serum and urine rapidly as an early response to bacterial infection at five days post infections and reached total maximum by weeks four to eight, then decline but remained detectable over 24 weeks[38].

CONCLUSION

It is concluded that the most common isolated bacteria from urinary tract infections was *E. coli*. Obtained data provided much-needed information to clinicians on the prevalence of antimicrobial susceptibility testing for judicial use of drugs and proper institution of therapy.

SIGNIFICANCE STATEMENT

These results seem helpful in providing useful guidelines to the clinicians in choosing an effective antibiotic in cases

with UTI and also initiating therapy in antimicrobial-resistant strains for determining the immune response of patients against UTI.

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