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Antibiotic Susceptibility Manner of the Bacteria Causes Urinary Tract Infections in Basra, South Iraq

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Abstract

Infections of the urinary tract are involved with the common infectious diseases in the population, by the yearly occurrence of 18 per 1000 people. This study carried out to determine the causative pathogenic bacteria of the urinary tract infections (UTIs) and to measure the frequency and susceptibility outline of different bacteria causing UTIs that acquired by the community in Basra city south Iraq. This study recruit102 persons (77 females with25 males) with the age range of (15-55) years old, clinically suspected for UTI. Clean midstream urine gathered from the patients in a sterile tube and tested by the use of typical procedures. Kirby-Bauer technique used to test antibiotic sensitivity of the isolated pathogenic bacteria according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Examination of centrifuged urine samples microscopically revealed 42 (31 females with 11 males) had pyuria (a significant number of pus cells) while 50 (37 females with 13 males) had scarcer pus cells. Eight urine samples (6 females and 2 males) had calcium oxalate crystals and 20 samples had numerous epithelial cells. Combi-9 (Medi-Test) used for urinalysis revealed 58 samples (44 females with 14 males) had protein, of which 37 (29 females and 8 males) with growth and 21 without growth. Gram stain besides biochemical tests used for identification of the pathogens, among the positive samples, the most popular pathogens identified as *Escherichia coli* (49.0%), *Klebsiella* species (19.6%), *Proteus* species (11.8%), *Pseudomonas aeruginosa* (10.8%), and *Staphylococcus aureus* (8.8%).Regarding to the isolated Gram-negative bacteria, all are resistant to Amikacin (AK), Rifampicin (RA), Nitrofurantoin (F), Trimethoprim + Sulfamethoxazole (SXT), Novobiocin (NV), Nalidixic Acid (NA), Chloramphenicol (C), Tetracycline (TE), Cefepime (FEP), Bacitracin (B), Erythromycin (E) and Streptomycin (S). However, some isolates were less resistant to Amoxicillin (AX), Ceftizoxime (ZOX), Cefoxitin (FOX), Gentamicin (GM), and Cefotaxime (CTX). Gram-positive isolates were resistant to all antimicrobials except Amikacin, Trimethoprim + Sulfamethoxazole, Gentamycin and Chloramphenicol. Females infected with UTIs more than males in Basrah, South Iraq. *E. coli* was the major isolate, as found in other studies.

Keywords: Urinary tract infections (UTIs), Pathogenic bacteria, Basra, Antibiotic sensitivity

INTRODUCTION

Infections of the urinary tract are involved with the common infectious diseases in the population, by the yearly occurrence of 18 per 1000 person[1]. Currently, it considered as one of the most popular diseases affecting people of all age groups from the neonate to the geriatric[2]. Furthermore, UTI found as the major ordinary cause of the hospital-acquired infections between patients[3]. Complications of UTI involve pyelonephritis that needs hospitalization and can cause permanent renal damage. It reported that UTIs are the most popular infections in women, frequently caused by pathogenic bacteria from fecal flora (mainly *Escherichia coli*) that raises the urethra to infect the bladder[4]. UTI in men is frequently complicated, because they commonly occur in old age patients in the state of abnormalities of the urinary tract and only a little of younger men affected[5]. In the USA, a developed country, there is about eight million physician visits per year for UTIs only[6]. The condition is worst in developing countries, particularly in the poor areas, clinicians frequently start the antibiotic treatment the the availability of the laboratory diagnostic test results[7].

Currently, there is an increase in the rate of antibiotic resistance among UTIs pathogens, particularly in developing countries. Studies indicated that there is an increasing rate of resistance of uropathogens to the ordinary prescribed antibiotics, reducing therapeutic potential[8,9]. Therefore, the precise investigation of UTI pathogens and reporting antibiotic susceptibility patterns is essential for the selection of suitable drugs for efficient treatment. That is why this research is essential globally. Regardless of the guidelines published for the better choice of an antimicrobial drug and length of therapy, studies showed large differences in prescribing practices[10-12].

No recent information particularly on the antibiotic susceptibility manners of urinary pathogens amongst UTIs patients in Basra. Therefore, this study carried out to determine the causative pathogenic bacteria of the urinary tract infections (UTIs) and to measure the frequency and susceptibility outline of different bacteria causing UTIs that acquired by the community in Basra city south Iraq.

MATERIALS AND METHODS

Sample collection

This study carried out during the term from February to May 2018 and the patients selected during their visit to the outpatient clinic of Basra teaching hospital in Basra, Iraq. Institutional Ethical Committee accepted the research and informed consent achieved from the subjects. Patients with clinical symptoms of UTIs, with no record of antibiotic use within 14 days before their attendance were included in this study. The exclusion criteria were diabetes mellitus, renal disorders and infectious diseases. In addition, the patients on antibiotics or on corticosteroid therapy excluded. Furthermore, patients who not provide informed consent excluded from the study. Recruited patients provided with sterile containers with a broad mouth and they informed on the way of collecting a mid-stream urine sample.

From 150 patients, 102 selected according to this study inclusion criterion. About (25) males with (77) females, in the age group of (15-55) years old (mean, 34.01years). Clean mid-stream urine gathered from the patients in a sterile tube (4- 5ml) and transported directly to the lab for examination. Rules for suitable specimen gathering given to patients on a written card[13].

Urine analysis

Collected urine samples investigated for their turbidity and colour macroscopically, then all samples were centrifuged and the precipitates investigated microscopically for blood cells, epithelial cells, Schistosoma eggs, protozoa, yeasts and crystal forms.

Inoculation of primary isolation culture media

Each fresh urine sample inoculated on various media by the use of loop, media includes blood agar (Himedia, India), Nutrient agar (Merck, Germany) and McConkey agar (Merck, Germany), and then all inoculated media incubated at 37°C for one day in aerobic condition, after that the colonies number counted. Subsequently, isolated bacteria streaked four times on the identical media. Calibrated loop method used for colony count using the measured quantity of urine, inoculated to the nutrient agar medium(Merck, Germany).Colony counts that show bacterial growth equal /more than 104 CFU/ml of urine with single or two pathogens were regarded positive. Experiments were repeated if the results between

102-104 CFU/ml, and results were negative if it is less than 102 CFU/ml of urine. Colony counts yielding bacterial growth equivalent to or extra than 104CFU/ml of urine (≥100,000 colonies), single possible pathogen or for each of two possible pathogens regarded as UTI positive and a result repeated if it is 102-104 CFU/ml. Negative UTI results fewer than 102 CFU/ml of urine.

Identification of Bacteria

Gram stain besides biochemical tests used for the identification of the pathogens, based on cultural characteristics. Clean isolates of pathogenic bacteria initially recognized by colony morphology, catalase test and gram stain. Then the doubted colonies investigated under the light microscope for their colours, shapes and arrangements, in addition to gram stain analysis. All of the isolated bacteria identified using classical biochemical tests[13-15].

Antibiotic Susceptibility

Kirby-Bauer technique used to test antibiotic sensitivity of the isolated pathogenic bacteria according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. 0.1mL of 0.5 MacFarl and (about 107CFU/mL) consistent isolates seeded on Sterile Mueller-Hinton Agar plates using a sterile curved glass rod and left on the bench for about 30 minutes to dry. By the use of sterile forceps antibiotic discs placed on the plates, all plates incubated for 18 - 20h at 35 - 37°C. After that, inhibition zone diameters documented and regarded following CLSI accepted standard guidelines[16].

The subsequent antimicrobial drugs tested for both Gram-positive and Gram-negative bacteria. These are Amikacin (10μg), Amoxicillin (25μg), Rifampicin (5μg), Nitrofurantoin (300μg), Trimethoprim(1.25μg)+Sulfamethoxazole (23.75μg), Cefatizoxime (30μg), Cefoxitin (30μg), Novobiocin (30μg), Nalidixic Acid (30μg), Gentamicin (10μg), Chloramphenicol (30μg), Cefotaxime (30μg), Tetracycline (30μg), Cefepime (30μg), Bacitracin (0.4μg), Erythromycin (15μg) and Streptomycin (10μg).

RESULTS

Isolation and description of bacterial isolates

Approximately 102 urine samples (77 from females and 25 from males) with the age range of (15-55) years old (mean, 34.01 years) were collected from the outpatient clinic of Basra teaching hospital in Basra, Iraq. Examination of centrifuged urine samples microscopically revealed 42 (31 females with 11 males) had pyuria (a significant number of pus cells) while 50 (37 females with 13 males) had scarcer pus cells. Eight urine samples (6 females and 2 males) had calcium oxalate crystals and 20 samples had numerous epithelial cells.

Combi-9 (Medi-Test) used for urinalysis revealed 58 samples (44 females with 14 males) had protein, of which 37 (29 females and 8 males) with growth and 21 without growth. Gram stain besides biochemical tests used for identification of the pathogens, among the positive samples, the most popular pathogens identified as *Escherichia coli* (49.0%), *Klebsiella* species (19.6%), *Proteus* species (11.8%), *Pseudomonas aeruginosa* (10.8%), and *Staphylococcus aureus* (8.8%) (Table 1).

Antimicrobial susceptibility profile of urine isolates

Isolated Gram-negative bacteria were all resistant to Amikacin (AK), Rifampicin (RA), Nitrofurantoin (F), Trimethoprim + Sulfamethoxazole (SXT), Novobiocin (NV), Nalidixic

Table 1. Gender wise allocation and occurrence of pathogens producing urinary tract infections (UTIs) in Basra, south Iraq

	Name of isolates	Male (%)	Female (%)	Total (%)
Grame -ve	*Escherichia coli*	10 (40)	40 (51.9)	50 (49.0)
	Klebsiella pneumonia	7 (28)	13 (16.9)	20 (19.6)
	Proteus mirabilis	2 (8)	10 (13)	12 (11.8)
	Pseudomonas aeruginosa	2 (8)	9 (11.7)	11 (10.8)
Grame+ ve	*Staphylococcus aureus*	4 (16)	5 (6.5)	9 (8.8)
	Total	25	77	102

Acid (NA), Chloramphenicol (C), Tetracycline (TE), Cefepime (FEP), Bacitracin (B), Erythromycin (E) and Streptomycin (S). However, some isolates were less resistant to Amoxicillin (AX), Ceftizoxime (ZOX), Cefoxitin (FOX), Gentamicin (GM), and Cefotaxime (CTX).Gram-positive isolates were resistant to all antimicrobials except Amikacin, Trimethoprim + Sulfamethoxazole, Gentamycin and Chloramphenicol (Tables 2 and 3).

DISCUSSION AND CONCLUSION

Identification of the pathogens causing UTIs and their sensitivity manner is very essential in the management of UTIs cases. Our main objective was to recognize the problem of UTIs in Basra by using a simple survey approach. Information bias could not significantly affect the results of the study because the hospital staffs were not fully aware of the aims of the study. Detection bias

Table2. Antimicrobial sensitivity of the isolated bacteria from urine samples of102UTI patientsin Basra, south of Iraq

Antimicrobials	%of Susceptible (S)and resistant (R) bacterial isolates									
	E.coli		*Klebsiella pneumonia*		*Proteus mirabilis*		*Pseudomonas aeruginosa*		*Staphylococcus aureus*	
	S	R	S	R	S	R	S	R	S	R
Amikacin	83.2	16.8	32.0	68.0	84.1	15.9	36.3	63.7	68.0	32.0
Amoxicillin	42.4	57.6	17.8	82.2	10.0	90.0	13.6	86.4	32.8	67.2
Rifampicin	7.7	92.3	11.0	89.0	11.8	88.2	18.7	81.3	35.8	64.2
Nitrofurantoin	55.0	45.0	47.1	52.9	38.0	62.0	21.6	78.4	39.7	60.3
Trimethoprim + Sulfamethoxazole	48.5	51.5	33.6	66.4	44.8	55.2	10.8	89.2	82.3	17.7
Ceftizoxime	55.9	44.1	53.8	46.2	44.1	55.9	54.8	45.2	47.3	52.7
Cefoxitin	53.7	46.3	54.6	45.4	52.0	48.0	51.6	48.4	46.1	53.9
Novobiocin	25.0	75.0	27.4	72.6	41.4	58.6	31.0	69.0	35.2	64.8
Nalidixic Acid	30.7	69.3	38.5	61.5	43.5	56.5	12.8	87.2	15.5	84.5
Gentamicin	21.7	78.3	84.2	15.8	67.0	33.0	51.7	48.3	60.0	40.0
Chloramphenicol	43.2	56.8	43.0	57.0	40.2	59.8	40.3	59.7	74.2	25.8
Cefotaxime	56.2	43.8	51.8	48.2	54.0	46.0	45.0	55.0	36.2	63.8
Tetracycline	39.7	60.3	22.0	78.0	22.5	77.5	23.6	76.4	46.6	53.2
Cefepime	40.4	59.6	25.9	74.1	26.9	73.1	19.5	80.5	33.1	66.9
Bacitracin	10.8	89.2	11.9	88.1	27.6	72.4	20.7	79.3	31.0	69.0
Erythromycin	4.7	95.3	11.0	89.0	20.9	79.1	33.6	66.4	40.0	60.0
Streptomycin	7.1	92.9	9.0	91.0	20.0	80.0	30.8	69.2	23.4	76.6

Amikacin (10µg), Amoxicillin (25µg), Rifampicin (5µg), Nitrofurantoin (300µg), Trimethoprim (1.25µg) + Sulfamethoxazole (23.75µg), Ceftizoxime, Cefoxitin (30µg), Novobiocin (30µg), Nalidixic Acid (30µg), Gentamicin (10µg), Chloramphenicol (30µg), Cefotaxime (30µg), Tetracycline (30µg), Cefepime (30µg), Bacitracin (0.4µg), Erythromycin (15µg) and Streptomycin (10µg)

Table 3. Antimicrobial sensitivity manner for pathogens producing urinary tract infections (UTIs) in Basra, south Iraq

Isolates	AK	AX	RA	F	SXT	ZOX	FOX	NV	NA	GM	C	CTX	TE	FEP	B	E	S
E.Coli	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Klebsiella pneumonia	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R	R
proteus mirabilis	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Pseudomonas aeruginosa	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Staphylococcus aureus	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R	R

Amikacin (AK), Amoxicillin (AX), Rifampicin (RA), Nitrofurantoin (F), Trimethoprim + Sulfamethoxazole (SXT), Ceftizoxime (ZOX), Cefoxitin (FOX), Novobiocin (NV), Nalidixic Acid (NA), Gentamicin (GM), Chloramphenicol (C), Cefotaxime (CTX), Tetracycline (TE), Cefepime (FEP), Bacitracin (B), Erythromycin (E) and Streptomycin (S).

could affect the results because certain important laboratory investigations were not available. More advanced explanations sometimes need clinical and laboratory information which is not available in our hospitals regularly.

In this study, the most popular pathogenic bacteria isolated from the urine was *Escherichia coli*- 49.0%, followed by *Klebsiella pneumonia*- 19.6%, *Proteus mirabilis*- 11.8%, *Pseudomonas aeruginosa*- 10.8% and *Staphylococcus aureus*- 8.8%. The isolation rate of pathogenic bacteria from urine samples as found in other studies in other places published recently. *E. coli* prevailed among the isolated bacteria, account for 49% of the positive samples.

The majority of UTIs documented from females in this study, representing that women more than men expected to develop UTIs. These results in conformity, with the results of comparable studies.

There is a global concern about antibacterial resistance. Data on antibiotic use in the hospital concerned obviously designated the lack of antibiotic prescribing policy. The plurality of UTIs treated experiential, particularly in developing countries where patients cannot have laboratory tests done or consult a doctor. Ampiclox and cefotaxime were given to the patients frequently, because of the availability of these antibiotics in hospital pharmacy and because of their wide-ranging spectrum of activity. Second and third-generation cephalosporins are expensive drugs, and there are frequent economic restrictions. Other antibiotics that are widely used in the treatment of various infections are Erythromycin and Tetracycline, possibly because of their low cost and their good clinical efficiency. Local sensitivity manner of bacteria causing UTIs should be existing to recommend suitable antibiotics.

Isolated *E. coli* strains (48.5%) all and other species were resistant to sulfamethoxazole-trimethoprim, these study findings are consistent with earlier studies finding[17, 18]. These results are possible because of the frequent use of these antimicrobial drugs in the treatment of infections acquired from the community.

Moderate sensitivity to third-generation cephalosporins and aminoglycosides (>50%) in the majority of isolates, as well as their pharmaco kinetic characteristics, causes them to be the first line of treatment for UTI infections until microbiological test results are available.

CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION
All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

DATA AVAILABILITY
All datasets generated or analyzed during this study are included in the manuscript and/or the Supplementary Files.

ETHICS STATEMENT
Institutional Ethical Committee accepted the research and informed consent achieved from the subjects.

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