



Bacterial Isolates of Urine and their Susceptibility to Antimicrobials

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Abstract

BACKGROUND: Urinary tract infection (UTI) is a collective term used to describe the microbial invasion of any part of the urinary tract, which consists of the kidneys, ureters, bladder, and urethra. It is one of the most common bacterial infections in both sexes with a predilection in females.

OBJECTIVES: The objectives of the study were to isolate the bacteria causing UTI and the highest resistance microorganisms with resistance rates to different antibiotics.

STUDY DESIGN: This was a cross-sectional study.

METHODS: A cross-sectional study with a sample size of 1000 patients both inpatient and outpatient referred to the laboratory of Al-Kindy Teaching Hospital for urine culture and antibiotic susceptibility examination.

RESULTS: Of 1000 samples, 318 (31.8%) urine samples were found positive while 682 (68.2%) were negative. Females with bacteriuria held the highest percentage (72.3%) over males (27.7%). Most frequent pathogen was *Escherichia coli* (40.5%) followed by *Klebsiella pneumonia* (25.7%) which together accounted for 66.2% of the total uropathogens. The uropathogenic bacterial isolates showed different resistance rates to antimicrobial agents: Cefixime (74%), ticarcillin/clavulanic acid (73.87%), rifampin (68.75%), ceftriaxone (60.31%), cefepime (44.44%), ciprofloxacin (43.65%), aztreonam (33.78%), nitrofurantoin (29.61%), gentamicin (25.64%), and amikacin (7.31%) being the most effective with a susceptibility rate of (89.63%).

CONCLUSIONS: *E. coli* remains to be the most frequent bacterial uropathogen causing urinary infections. Effective drug may be in the clinical study, in microbiology is only that bacteria were most susceptible to amikacin. β -lactams have the highest resistance rates, and the most resistance antibiotic in this study is cefixime.

Edited by: Sasho Stoleski
Citation: Mahdi BM, Khudhur HB, Abdul-Hussein MM. Bacterial Isolates of Urine and their Susceptibility to Antimicrobials. Open Access Maced J Med Sci. 2020 Mar 15; 8(A):84-88. https://doi.org/10.3889/oamjms.2020.4086
Keywords: Urine; Infection; Antibiotic
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Received: 20-Nov-2019
Revised: 12-Jan-2020
Accepted: 03-Mar-2020
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Funding: This research did not receive any financial support
Competing Interests: The authors have declared that no competing interests exist
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Introduction

Urinary tract infection (UTI) is a collective term used to describe the microbial invasion of any part of the urinary tract [1]. It is one of the most common bacterial infections in both sexes, with a predilection in females [2], [3]. Antibiotics are often used to prevent and treat these bacterial infections, but some bacteria developed the ability of growing effectively in an antibiotic-rich environment, giving the rise of what's called "antibiotic resistance" [4], [5]. There are several risk factors associated with UTIs such as sex, vaginal infection, antibacterial activity of prostatic fluid, diabetes, personal hygiene, obesity, and genetic susceptibility [6]. The diagnosis is urine culture with the presence of clinical symptoms while antibiotic susceptibility can be measured by disc diffusion, dilution tests, and killing curve [7]. The most common uropathogen is *Escherichia coli*, which is responsible for 80% of community acquired and 40% of nosocomial infections include fecal Gram-negative rods, *Klebsiella*, *Proteus* spp., *Enterobacter*, *Serratia*, and *Pseudomonas aeruginosa* and Gram-positive cocci including enterococci, other organisms including *Candida* spp. [7], [8]. Knowing about that

is important to ensure high cure and low resistance rates [9], [10]. Meanwhile, antibiotic resistance is rising to dangerously high levels in all parts of the world. The causes of increased antibiotic resistance are overusing, improper prescribing, availability of few new antibiotics, and regulatory barriers [11]. Therefore, the enforcement of antibiotic supervised programs is effective to reduce the chances of bacterial resistance [12]. Without urgent action, we are heading for a post-antibiotic era, in which common infections [13]. Recently, in 2013, the Centers for Disease Control and Prevention published that about 2 million people develop infections with antibiotic-resistant pathogens each year [14]. Looking into the common pathogens and testing their antibiotic susceptibility is of great importance since there is an increase in antibiotic susceptibility in all age groups [15], [16]. Antibiotic resistance is a global and a local crisis in Iraq, affecting hundreds of lives, particularly low socioeconomic or war areas [17]. Nobel laureate Joshua Lederberg put it better nearly 19 years ago when he wrote "The future of humanity and microbes will likely evolve as ... episodes of our wits versus their genes" [18].

This study aims to isolate the bacteria causing UTI and the highest resistance microorganisms with resistance rates to different antibiotics.

Patients and Methods

A cross-sectional study consists of 1000 patients referred to Al-Kindy Teaching Hospital for urine culture and antibiotic susceptibility examination from November 2017 to April 2019. The inclusion criteria were patients with dysuria, lion pain, and frequency while the exclusion criteria were congenital renal diseases and patients with tumor of genitourinary system. Ethical approval and permissions to collect samples were obtained from the Al-Kindy College of Medicine and Al-Kindy Teaching Hospital.

Mid-stream urine samples were cultured after a general urine exam on blood and MacConkey agar media to know if there is the growth of bacteria, then incubated aerobically at 37°C for 24 h and extended up to 48 h in cases of Gran-negative. Identification of isolates was done by a standard method depending on the observation of colony characteristics and antimicrobial susceptibility test was performed by disc diffusion method using Muller-Hinton agar and choice of antibiotic disks according to the type of isolated bacteria. If there is a growth of the bacteria around disc, this means the bacteria are resistant. The growth in the inhibition zone means that there is a mixed culture of two bacterial species [19].

The results were recorded as susceptible (S), intermediate (I), and resistant (R). The following antibiotics were used: Amikacin, amoxicillin (clavulanic acid), amoxicillin, ampicillin, ampicillin-sulbactam, cefepime, cefixime, cefotaxime, ceftazidime, ceftazidime, ceftriaxone, cefuroxime, ciprofloxacin, clindamycin, erythromycin, imipenem, lincomycin, nalidixic acid, netilmicin, penicillin, streptomycin, tetracycline, ticarcillin (clavulanic acid), tobramycin, trimethoprim-sulfamethoxazole, vancomycin, aztreonam, gentamicin, piperacillin, rifampicin, ceftriaxone, nitrofurantoin, azithromycin, meropenem, trimethoprim, doxycycline, and levofloxacin.

Statistical analysis

Data were entered and analyzed using SPSS version 17.0.1 for windows (SPSS, Inc., Chicago, IL). Discrete variables were expressed as frequencies and percentages.

Results

The study included that 1000 urine samples were obtained from patients with UTI. Their mean ages were 39.5 ± 0.8 (range 6 months–88 years). The results of the urine culture showed that 318 (31.8%) of urine samples were found positive while 682 (68.2%) were negative. Females were more than males (Figure 1); of

667 females, 225 (72.3%) were found positive while among 333 males, 93 (27.92%) were positive for bacterial infection. The most frequent pathogen was *E. coli* (40.5%) followed by *Klebsiella pneumonia* (25.7%) which together accounted for 66.2% of the total uropathogens (Table 1 and Figure 2).

Of 41 antibiotics, the ten most frequently used antibiotics were nitrofurantoin, gentamicin, ciprofloxacin, amikacin, cefixime, and ceftriaxone, as demonstrated in Table 2 and Figure 3. The bacteria that showed the highest resistance rates were *E. coli* and *K. pneumonia* which are the predominant cause of UTIs. The susceptibility and resistance of *K. pneumonia* to some different antibiotics shown in Table 3 and the most common antibiotic that *K. pneumonia* sensitive to it was amikacin 47 (32%). Regarding *E. coli* was most susceptible to nitrofurantoin 85 (51.2%), as shown in Table 4.

Discussion

UTI is a common disease in females more than males which are shown in this study that females held the highest percentage (72.3%) over males (27.7%); this may be due to the distance between the anus and urethral meatus and moisture content surrounding urethra [6].

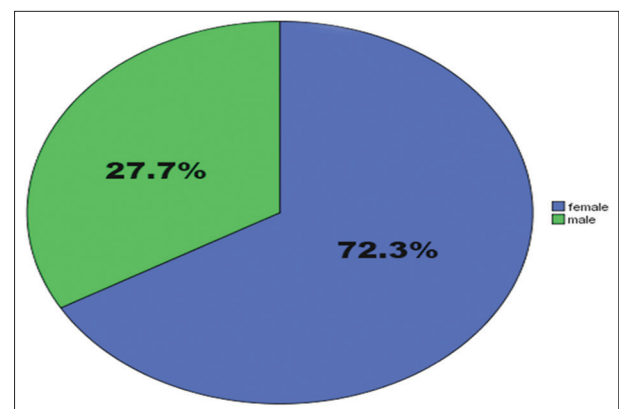


Figure 1: Sex distribution of patients

In this study, 318 (31.8%) urine samples were found positive while 682 (68.2%) were negative, high rates of negative isolates may be due to one or more of several factors that lead to diagnostic inaccuracy which include the collection of first-void urine instead of midstream urine sample [20].

Table 1: Types of isolated bacteria from urine samples

Bacteria	Frequency	Percent
<i>Escherichia coli</i>	126	40.5
<i>Klebsiella pneumonia</i>	80	25.7
<i>Staphylococcus aureus</i>	22	7.1
<i>Streptococcus faecalis</i>	21	6.8
<i>Enterobacter aerogenes</i>	18	5.8
<i>Pseudomonas aeruginosa</i>	16	5.1
<i>Enterobacter cloacae</i>	10	3.2
<i>Streptococcus pyogenes</i>	8	2.6
<i>Streptococcus agalactiae</i>	4	1.3
<i>Proteus vulgaris</i>	4	1.3
<i>Streptococcus gallolyticus</i>	2	0.6
Total	311	100.0

Table 2: Types of tested antibiotics

Antibiotic	Total uses	Resistance of all isolated bacteria %	Susceptibility of all isolated bacteria %
Nitrofurantoin	260	29.61	63.84
Gentamicin	234	25.64	64.95
Ciprofloxacin	198	43.65	54.54
Amikacin	164	7.31	89.63
Cefixime	127	74	23.62
Ceftriaxone	126	60.31	34.92
Ticarcillin (clavulanic acid)	111	73.87	20.72
Rifampin	96	68.75	14.58
Aztreonam	74	33.78	58.10
Cefepime	72	44.44	47.22

Table 3: The susceptibility and resistance of *Klebsiella pneumoniae* to some different antibiotics

Type of antibiotics	Susceptibility n (%)	Resistance n (%)
Gentamicin	35 (23)	17 (28.3)
Amikacin	47 (32)	5 (41.6)
Nitrofurantoin	39 (23.5)	22 (28.5)
Ciprofloxacin	33 (30.5)	23 (26.7)
Cefixime	7 (23.3)	29 (30.8)
Ceftriaxone	12 (27.2)	20 (26.3)

Table 4: The susceptibility and resistance of *Escherichia coli* to some different antibiotics

Type of antibiotics	Susceptibility n (%)	Resistance n (%)
Gentamicin	64 (42.1)	23 (38.3)
Amikacin	69 (46.93)	1 (8.3)
Nitrofurantoin	85 (51.2)	14 (18.1)
Ciprofloxacin	30 (27.7)	44 (51.1)
Cefixime	16 (53.3)	36 (38.2)
Ceftriaxone	23 (52.2)	37 (48.6)

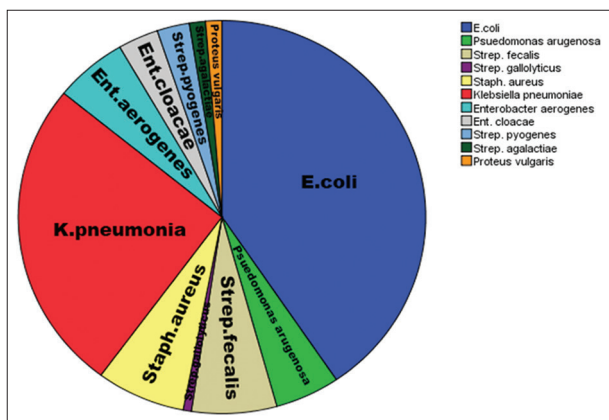


Figure 2: Frequencies of isolated bacteria from urine samples

This study results in accordance with other studies such as Kareem and Rasheed 2011 [21] study, where they obtained 125 (40.19%) bacteriuria of 311 urine samples in Al-Karkh Surgery Hospital in Baghdad. Other studies that done by Al-Barzinji *et al.* 2010 found that among 350 patients, the bacterial cultivations revealed positive results for 119 (34%) urine specimens in Hawler and Sulaimani Teaching Hospitals in Kurdistan region which is similar to this study results [22]. In contrast, Najim *et al.* 2015 recorded a result of 77.27% bacteriuria from 135 urine isolates in the intensive care unit at Baqubah General Teaching Hospital which was showed higher percentage due to sample size, procedure of collection samples, and quality of patients which they were bedridden [15].

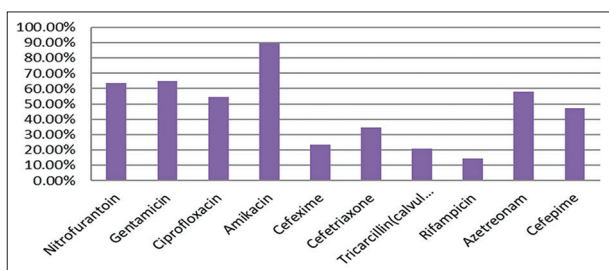


Figure 3: Types of used antibiotics and their susceptibility

This study demonstrated that *E. coli* (40.5%) was the most frequent uropathogen in this study, which is similar to other researches [21], [22], [23], [24]. The second most frequent pathogen in this study was *K. pneumoniae* (25.7%) which is equivalent to a study done at Al-Yarmouk Teaching Hospital 2010 (30.7%) [24], in contrary to another study where *Proteus* spp. was reported as the second most common bacteria which are due to methods used in isolation the bacteria in addition to previous causes mentioned above [25].

Regarding antibiotic susceptibility, nitrofurantoin is a urinary antiseptic that is use in UTI that inhibits various enzymes and damages bacterial DNA [26]. In this study, 29.61% of all isolated bacteria were resistant to nitrofurantoin and *E. coli* had a higher sensitivity to nitrofurantoin 85 (51.2%) (Table 4) comparing the result with another study done at Al-Kindy Teaching Hospital in 2016 by Nashtar that found nitrofurantoin resistance was 20% [27]. Higher resistant rate was obtained by Alhamdany 2015 that found 41.3% antibiotic resistance [25]. This due to the type of his sample was diabetic patients who suffered from recurrent UTI. On the other hand, Ghaima *et al.* 2018 obtained nearly similar to the result of this study (29.8%) [28]. The mechanism of this resistance is due to mutation [29].

The resistance rates of amikacin and gentamicin were 13.2% and 22.6%, respectively [27]. In this study, *K. pneumoniae* showed the higher sensitivity to amikacin 47 (32%) (Table 3). The cause of this decrease in the usage of amikacin which reduced its resistance. In other studies, it was found that *E. coli* did not produce any resistance against amikacin but 29% resistance to gentamicin [30]. Other studies done in Karbala by Al-Awwad and Mohsen 2018 showed 1.8% resistant to amikacin and 38.2% resistant to gentamicin [31]. Resistance to aminoglycosides developed when a bacterium changes its inner membrane permeability [29].

In this study, some β -lactam antibiotics showed high resistance rates such as cefixime 74%, ticarcillin (clavulanic acid) 73.87%, and ceftriaxone 60.31% (Table 2). Al-Samarrai study in Kirkuk 2016 showed a slightly higher resistance in cefixime 79.9% [32]. However, Al-Naqshbandi *et al.* 2019 study showed even greater resistance when it is just ticarcillin by itself with Gram-positive bacteria being fully resistant [33]. Furthermore, aztreonam showed greater resistance in Hussein *et al.* 2019 study in Duhok with 60.91% resistance to aztreonam [23], could possibly indicate a more resistance in northern Iraq because of the increased antibiotic prescribing.

In this study, 43.65% of the bacteria were resistant to ciprofloxacin (Table 2). This study results fall just

between Nashtar 2016 study with 51.1% resistance and Hussain *et al.* 2019 study with 33% resistance in [27], [30]. Meanwhile, Ghaima *et al.* 2018 study showed 49.4% resistance [28]. This resistance is caused by a point mutation in the bacterial DNA gyrase subunits [29].

In this study, rifampin had a high resistance of 68.75%, Al-Naqshbandi *et al.* 2019 study in Erbil showed a similar result of 64.10% [33].

To take a broader aspect, this study was compared to another study that took place in Kulasekharam, India, 2017; the most common organism isolated overall was *E. coli* (35.5%), females (68.63%) were more affected than males in this study. The most sensitive antibiotics to almost all organisms were nitrofurantoin and amikacin [34]. While the results differ from a study in Ankara, Turkey 2018, where the most resistant antibiotic was ampicillin, the least resistant was amikacin. This difference may be due to the type of antibiotics frequently prescribed and the sampling population [35], [36], [37], [38].

The increased resistance rates in this study are quite worrisome, which may reflect the extensive use and misuse of prescribed antibiotics by patients and healthcare and high prescribers of antibiotics in general practice [39], [40]. Duration of the treatment and patients' compliance. This overuse could lead to multidrug-resistant strains, with the potential of dissemination within a specific region.

Limitation of the study

This study was limited by the availability and use of antibiotics in antibiotic susceptibility tests.

Conclusions

E. coli remains to be the most frequent bacterial uropathogen causing urinary infections. Effective drug may be in a clinical study; in microbiology is only that bacteria were most susceptible to amikacin. β -lactams have the highest resistance rates, and the most resistance antibiotic in this study is cefixime.

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