

# Multidrug Resistant Enteric Bacteria Isolated From Patients With Urinary Tract Infections

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## Abstract

**Background and Objectives:** Urinary tract infections (UTIs), among a wide range of microbial infections, are of a double-edged worry with health-care and economic implications. They are serious diseases that can influence various parts of the urinary tract. The aim of this study was characterization of the enteric bacteria isolated from urine of human UTIs and studying their antimicrobial sensitivity. **Materials and methods:** A total of 50 urine samples were collected from patients with UTIs of both genders. The isolates identification was done using routine diagnostic methods and confirmed by Vitek2. Antimicrobial susceptibility was done against 10 antimicrobials. **Results:** Both genders of human were found to suffer from urinary tract problems caused by bacteria. Out of 50 patients, 45 (90%) of the cases showed bacterial growth. Approximately, 30.43% of the human infections were found to be caused by members of the *Enterobacteriaceae* family. The ratio of female patients with UTIs was more than that of males, the most common bacterium isolated from human urine was *E. coli*, which constituted approximately 85.7% of the enteric bacteria isolated and 26.1% of all bacterial isolates. Other members of *Enterobacteriaceae* family were also isolated from patients enrolled in this study, such as *Citrobacter freundii*, which constituted the same incidence rate as *K. pneumoniae*. Concerning antimicrobial resistance, 11, 10, 9, and 8 out of 12 of *E. coli* isolates were resistant to Erythromycin, Vancomycin, Tetracycline, and Ceftazidime together, respectively, with a range of resistance from 91.7% to 66.7%. Low percentages of bacteria showed intermediate sensitivity to Imipenem, Gentamicin, Chloramphenicol, Vancomycin, and Erythromycin. However, 12, 11, 10, 10, 9, and 8 out of 12 isolates were susceptible (susceptibility ranged from 100% to 66.7%) to each of Cefotaxime, Chloramphenicol, Imipenem, Amikacin, Ciprofloxacin, and Gentamicin. **Conclusions:** *Escherichia coli* was the most common bacteria isolated from human

UTIs. All of the isolates were multi-drug resistant toward at least four antimicrobials. Particularly, Erythromycin and Vancomycin had no effect on the enteric bacteria at all. Imipenem might be the most effective drug against a large number of the human isolates.

**Keywords:** *Enterobacteriaceae*, urinary tract infections, antimicrobial resistance.

## Introduction

Infectious diseases constitute one of the most significant difficulties within human communities all over the world. Urinary tract infections (UTIs), among a wide range of microbial infections, are of a double-edged worry with health-care and economic implications (Jahandeh *et al.*, 2016). They are serious diseases that can influence various parts of the urinary tract (Behzadi, 2016). Approximately, seven million individuals are expected to visit clinics and hospitals due to UTIs per year at an expenditure of over one billion dollars (Gradwohl *et al.*, 2016). Microbial invasion for any portion of the urinary tract, which includes the urethra, bladder, ureters, and kidneys, is referred to as a UTI (Mahdi *et al.*, 2020). Infection of the urinary tract refers to the existence of bacteria in the urine, as well as symptoms and often signs of inflammation, such as repeated micturition, dysuria, pyuria, nycturia, fever, suprapubic pain, and hematuria (Liza *et al.*, 2006; Heising, 2010). It is estimated that UTIs can cost over \$1 billion per year (Ahmed *et al.*, 2018). Despite the UTIs constitute 1-6% of total medical consultations; these infections are still the most common among the bacterial infections (Foxman, 2003). It has been suggested that their frequency and burden must be higher than available data because they are not among mandatory diseases to be notified (Öztürk *et al.*, 2020). Women usually have a higher incidence of this infection and are more vulnerable to it due to numerous risk factors leading to infection of up to 2/3 of women with UTIs at some period during their lifetimes (Valiquette, 2001; John *et al.*, 2016). Up to 40% of women will develop UTIs at least once during their lives, and a significant number of these women will have recurrent UTIs (Gradwohl *et al.*, 2016).

Many superimposing factors interact to cause a urinary infection, such as the bacterial inoculum size, the virulence of the infecting bacteria, or factors linked to the host. Inoculation is an early step that results in a UTI, and the most popular hypothesis for inoculation is the ascending route. Perineum was found to be colonized by enteric bacteria, which then travel up the urethra and bladder (Foxman, 2002). Gram-negative pathogens, mainly from the *Enterobacteriaceae* family, <http://annalsofrscb.ro>

such as *Klebsiella pneumoniae*, *E. coli*, *Proteus mirabilis*, and *Enterobacter species*, cause the majority of UTIs (Wilson and Gaido, 2004; Griebeling, 2005; Foxman, 2010).

Resistance of invasive microorganisms to treatment is still a major global obstacle; this health problem can annually kill thousands of people (Jim, 2014). It is attributed to increased and sometimes uncontrolled antimicrobial use in clinical and agricultural settings (Woolhouse *et al.*, 2015; Hassell *et al.*, 2019). Antimicrobial resistance refers to a microorganism's ability to tolerate their effects and grow in the presence of a chemical that would otherwise inhibit its growth or destroy it (Prestinaci *et al.*, 2015). The rates of resistance to the most commonly prescribed medications for the treatment of UTIs vary greatly across the globe (Farajnia *et al.*, 2009). Furthermore, antibiotic resistance is a major problem that hospital workers must deal with (Edelsberg *et al.*, 2014). Therefore, the current study aimed at characterization of the enteric bacteria isolated from urine of human UTIs and studying their antimicrobial sensitivity.

## **Materials and methods**

### ***Ethics***

Permission from the hospital authority and signed informed consent from the patients were collected. All human specimens were handled anonymously based on the ethical and legal standards.

### ***Samples collection***

Fifty human urine samples were collected into sterile containers from patients already diagnosed by specialist physicians to have urinary tract infections (UTIs). The patients' ages ranged from 10 to 60 years and from both genders. All of the urine samples were collected from patients who had not taken any medications that interfere with the results. The samples were gathered from Babylon city, Iraq, from private medical laboratories during 2020.

### ***Bacterial isolation and identification***

For routine bacteriological diagnosis, firstly, the urine samples were examined in the laboratory by using dipstick and under the microscope to look for the presence of pus and epithelial cells (inflammatory cells) as an indicator of possible infection. Then, a loopful of the urine sample was streaked onto MacConkey's agar, which was incubated aerobically at 37°C for 24-48 hr. Afterwards,

the plates were inspected for presence of bacterial growth, and the grown colonies were characterized in terms of their features on MacConkey's agars. The isolates were also identified based on their Gram's reaction. Subsequently, pure Gram-negative colonies were applied for the biochemical tests including: catalase, oxidase, SIM (sulfide indole motility), sugar fermentation on TSI (triple sugar iron), urease production, indole formation, gelatin liquefaction, citrate utilization, methyl red and Voges-Proskauer tests. All of the isolates were confirmed by using the automated Vitek2 system (bio-Merieux SA, France) according to the manufacturer's instructions.

### ***Antimicrobial susceptibility tests***

Disk diffusion susceptibility method was performed as described by Performance Standards for Antimicrobial Susceptibility Testing (CLSI). Ten different antimicrobial agents were used involving: Imipenem, Tetracycline, Gentamicin, Chloramphenicol, Amikacin, Ciprofloxacin, Vancomycin, Cefotaxime, Ceftazidime, and Erythromycin.

## **Results**

### ***Identification of the bacterial isolates***

The most common bacterium isolated from human urine collected in this study was *E. coli*, which constituted approximately 85.7% of the enteric bacteria isolated and 26.1% of all bacterial isolates. Other members of *Enterobacteriaceae* family were also isolated from the patients enrolled in this study, such as *Citrobacter freundii*, which constituted the same incidence rate as *K. pneumoniae*. All of the samples were detected by the traditional biochemical test and confirmed by the Vitek2 system.

### ***Antimicrobial Susceptibility tests***

There were 11, 10, 9, and 8 out of 12 *E. coli* isolates resistant to Erythromycin, Vancomycin, Tetracycline, and Ceftazidime, respectively, with a range of resistance from 91.7% to 66.7%. However, 12, 11, 10, 10, 9, and 8 out of 12 isolates were susceptible to each of Cefotaxime, Chloramphenicol, Imipenem, Amikacin, Ciprofloxacin, and Gentamicin, respectively (Figure 1). Additionally, *K. pneumoniae* isolate showed multi-drug resistance to five antimicrobials including: Amikacin, Erythromycin, Vancomycin, Cefotaxime, and Ceftazidime (100% resistance). Furthermore, the isolate revealed 100% intermediate sensitivity to each of Imipenem and Chloramphenicol. However, Tetracycline, Gentamicin, and Ciprofloxacin were more effective

against the isolate (100% susceptibility) (Figure 2). Interestingly, *C. freundii* was 100% resistant to all antimicrobials tested, and no susceptibility at all was reported to any drug (Figure 3). Table 1 and table 2 reveals the antimicrobial sensitivity findings of all the enteric bacteria isolated.

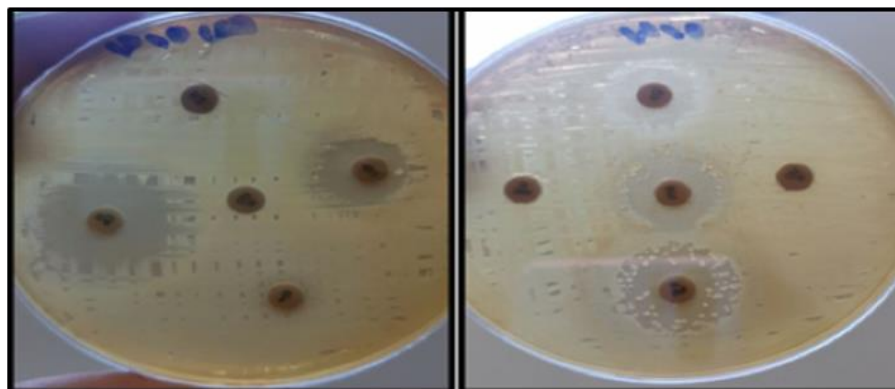
**Table 1.** Antimicrobial susceptibility of the E.coli isolates

Antimicrobial agent	E.coli (N =12)		
	R (%)	I (%)	S (%)
Imipenem	1 (8.3)	1 (8.3)	10 (83.3)
Tetracycline	9 (75)	0	3 (25)
Gentamicin	2 (16.6)	2 (16.6)	8 (66.6)
Chloramphenicol	0	1(8.3)	11 (91.6)
Amikacin	2 (16.6)	0	10 (83.3)
Ciprofloxacin	3 (25)	0	9 (75)
Vancomycin	10 (83.3)	2 (16.6)	0
Cefotaxime	0	0	12 (100)
Ceftazidime	8(66.6)	0	4(33.3)
Erythromycin	11(91.9)	1(8.3)	0

R: Resistant, I: Intermediate, S: Susceptible

**Table 2** Antimicrobial susceptibility of the klebsiella pneumonia and Citrobacter isolates

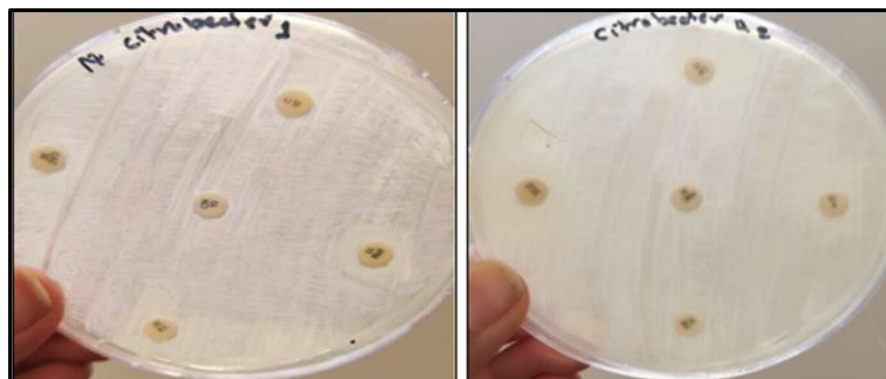
Antimicrobial agent	pneumonia (N =1)			Citrobacter. F
	R (%)	I (%)	S (%)	R(%)
Imipenem	0	1 (100)	0	100
Tetracycline	0	0	1 (100)	100
Gentamicin	0	0	1 (100)	100
Chloramphenicol	0	1 (100)	0	100
Amikacin	1 (100)	0	0	100
Ciprofloxacin	0	0	1 (100)	100
Erythromycin	1 (100)	0	0	100
Vancomycin	1 (100)	0	0	100
Cefotaxime	1 (100)	0	0	100
Ceftazidime	1 (100)	0	0	100



**Figure 1:** Disk diffusion antimicrobial susceptibility test for an *E. coli* isolate tested against 10 antimicrobials.



**Figure 2:** Disk diffusion antimicrobial susceptibility test shows *K. pneumoniae* isolate tested against 10 antimicrobials.



**Figure 3:** Disk diffusion antimicrobial susceptibility test shows multidrug resistant *C. freundii* tested against 10 antimicrobials.

### **Discussion**

Urinary tract infection (UTI) is one of the most prevalent diseases with diverse etiological agents. It is common in most regions of Iraq and remains a major health problem in many developing countries (Troeger *et al.*, 2017). In the current study, the ratio of female patients with UTI was more than that of males, this is consistent with other researchers (Hadi *et al.*, 2014; Ahmed *et al.*, 2019; Mahdi *et al.*, 2020). However, in other studies, no significant difference was observed between bacterial infections of female and male urinary system infections (Normohammadzadeh *et al.*, 2003; Hajikolaie *et al.*, 2015). By contrast to the present study, statistically significant prevalence of UTI was noticed in male more than female (Fatihu and Addo, 1991).

The most common bacterium isolated from human urine collected in this study was *E. coli*, which constituted approximately 85.7% of the enteric bacteria isolated and 26.1% of all bacterial isolates. This result is in agreement with other studies (Faisal *et al.*, 2013; Hussein *et al.*, 2017; Abdulrahman and Salih, 2018; Ghaima *et al.*, 2018; Mahdi *et al.*, 2020), in which these studies were performed in different regions in Iraq, and found that *E. coli* was the major causative agent of UTI in Iraq. In addition, it is known that the same organism was reported to be the major cause of UTI worldwide (Córdoba *et al.*, 2017; Holguin and Fernandez, 2019). However, other authors reported very low isolation rate of *E. coli*, and the most prevalent Gram-negative pathogens were *Klebsiella* spp. (Ngwai *et al.*, 2010; Hammoudi and Atyia, 2013). In contrast, the current study revealed very low incidence rate (2.17%) of *K. pneumoniae* among other isolates.

Other members of *Enterobacteriaceae* family were also isolated from UTIs of patients enrolled in this study, such as *C. freundii*, which constituted the same incidence rate as *K. pneumoniae*. It has been found that *C. freundii* is the third most common organism causing UTI in hospitalized patients after *E. coli* and *Klebsiella* species (Metri *et al.*, 2013; Salih *et al.*, 2016).

In comparison with other studies, *E. coli* was also found to be resistant to Ceftazidime, Erythromycin and Tetracycline (Kazemnia *et al.*, 2014; Hussein *et al.*, 2017; Jahani *et al.*, 2017). However, the isolates of this bacterium were 100% sensitive to Ceftazidime in the study of Yassin *et al.*, (2017). Regarding Imipenem and Cefotaxime, their findings in the current study are similar to other reports, including that of Ghaima *et al.*, (2018) in Baghdad, Hadi *et al.*, (2014) in Basra, and another international study of Dromigny *et al.*, (2005). Concerning *K. Pneumoniae* isolated in this study, it showed Erythromycin resistance, this is consistent with other studies (Ogunshe *et al.*, 2010; Osman *et al.*, 2014). While resistance of the current isolate to Ceftazidime converses Hakonsholm *et al.*, (2020) who noticed no inhibitory effect by Ceftazidime, it coincides with

Kareem and Rasheed (2011). Similarly, another study performed in Baghdad showed 100% resistance to Cefotaxime (Ahmed *et al.*, 2020). The sensitivity of the isolate to Gentamicin and Tetracycline was confirmed by other studies (Kazemnia *et al.*, 2014; Chaudhary *et al.*, 2020). Nevertheless, other studies carried out in Iraq showed resistance of *K. pneumoniae* in particular to Tetracycline (Khalaf and Al-Hasso, 2005; Hammoudi and Atyia, 2013).

Furthermore, *C. freundii*, was resistant (100%) to all antimicrobials tested, and no susceptibility at all was reported to any drug. This bacterium was also found, in a study done in China, to be resistant to multiple antibacterial agents (Li *et al.*, 2018). Likewise, another study performed in Al-Najaf city, Iraq, 50% of *C. freundii* isolated from UTI patients demonstrated extensive-drug resistance (Tuwaij, 2016).

## Conclusion

*Escherichia coli* was the most common bacteria isolated from human urinary tract infections. Approximately, all *E. coli* isolates showed a degree of similarity in their antimicrobial susceptibility profile. All of the isolates of this study were multi-drug resistant toward at least four antimicrobials. Particularly, Erythromycin and Vancomycin had no effect on the enteric bacteria at all. On the other hand, Imipenem might be the most effective drug against a large number of the isolates.

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