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# Isolation of Pathogenic Gram-Negative Bacteria from Urinary Tract Infected Patients

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## Abstract

**This study investigated the susceptibility pattern of different bacteria isolated from urinary tract infection to different antibiotics. 83 uropathogen bacteria were isolated from 300 urine samples taken from patients attended to Tikrit Teaching Hospital from March, 2011 through February, 2012. The patients were males and females aged between 4 days to 95 years. Bacteria obtained from urine samples were cultured and tested for antimicrobial susceptibility to 16 kinds of antibiotics. Urine samples were cultured on different media and incubated, thereafter bacteria were isolated and purified by streaking four times on the same media; isolates were identified depending on morphological, microscopic, and biochemical characteristics. The isolated strains of bacteria were tested for their susceptibility to some antibiotics using disk diffusion method. The antagonistic activity was evaluated by observing a clear zone of inhibition growth. The results showed that the bacterial species of *Eschericia coli*, *Proteus mirabilis*, *Klebsiella pneumonia*, *Citrobacter diversus*, *Citrobacter freundii*, *Enterobacter aerogenes*, *Yersinia pestis*, *Pseudomonas aeruginosa*, *Klebsiella oxytoca* and *Hafnia alvei* were identified in 44 (53%), 18 (21.7%), 4 (4.8%), 4 (4.8%), 3 (3.6%), 3 (3.6%), 3 (3.6%), 2 (2.4%), 1 (1.2%) and 1 (1.2%), respectively, of the isolates. The results of antimicrobial susceptibility test showed that 83 (100%) isolates were resistant to Ampicillin, Rifampicin and Erythromycin. 75 (90.3%) isolates were resistant to Cefotaxime, 67 (80.7%) isolates were resistant to Tobramyci. 66 (79.5%), 65 (78.3%), 56 (67.4%) and 48 (57.8%) isolates showed susceptibility to Nalidixic acid, Tetracycline, Nitrofurantoin, Chloramphenicol, respectively. 45 (54.2%) isolates were resistant to Azithromycin, Norfloxacin and Ciprofloxacin. Meropenem, Gentamicin, Amikacin, and Imipenem show significant effect on 35 (42.1%), 32 (38.5%), 27 (32.5%) and 1 (1.2%) isolates, respectively. In conclusion, significant bacteria count isolated from urine samples is pathogenic. The most effective antibiotic in inhibiting the bacterial growth**

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**was Imipenem while Ampicillin, Rifampicin and Erythromycin showed no effect on all 83 isolates (100%).**

## Keywords

**Urinary Tract Infections, Antibiotics, Bacteria, Antimicrobial Susceptibility Test**

## 1. Introduction

Urinary tract infection (UTI) is the second most common clinical indication for empirical antimicrobial treatment in primary and secondary care, and urine samples constitute the largest single category of specimens examined in most medical microbiological laboratories. Healthcare practitioners regularly have to make decisions about prescription of antibiotics for UTI. Criteria for the diagnosis of UTI vary greatly, depending on the patient and the context [1].

UTIs, including cystitis and pyelonephritis, are the most common infectious diseases in childhood [2]. *E. coli* accounts for as much as 90% of the community-acquired and 50% of the nosocomial UTIs [3]. UTIs are responsible for more than 7 million patient visits and one million hospital admissions (due to complications) per year in the United States only. Additional diseases include neonatal meningitis responsible for about 0.25 per 1000 live births in industrialized countries and 2.66 per 1000 in developing countries [4].

Bacterial identification and susceptibility tests are important for selecting the appropriate antimicrobial agent affecting bacterial diseases [5]. Thus, the accurate diagnosis of UTI and determination of antibiotic sensitivity pattern is important for selection of appropriate drug for effective treatment. This is why this study is important worldwide. Despite published guidelines for the optimal selection of an antimicrobial agent and duration of therapy, studies demonstrate a wide variation in prescribing practices [6]-[10]. This study investigates the susceptibility of different bacterial organisms isolated from urinary tract infection to different antibiotics.

## 2. Materials and Methods

### 2.1. Collection of Samples

The population of the study consists of all patients who did urine tests in Tikrit Teaching Hospital during the period from 1st March, 2011 till last February, 2012. An appropriate sample was selected. In specific, 300 patients were selected from different age groups of male and female who aged between 4 days to 95 years to be the sample of study. The selected patients were suffering from different Urinary tract infections (UTIs). For each case, the information was recorded in the form prepared for this purpose included a range of data such as name, age, sex, address, type of sample, date of sample collection, type of chronic diseases, date of entry to the hospital and whether the patients smoke or not. The samples of urine were collected in a disposable sterile container. Samples were collected in the morning and transferred immediately to the unit of laboratories for culturing on Blood, Nutrient and MacConkey agar. Ethical clearance was obtained for this study from the management of Tikrit Teaching Hospital and the patients involved.

### 2.2. Isolation and Identification of Bacteria

**1. Isolation**

Urine samples were cultured on different media including Blood agar, McConkey agar and Nutrient agar and incubated at 37˚C for 24 hours. Thereafter bacteria were isolated and purified by streaking four times on the same media [11].

**2. Identification of Isolates**

**A. Microscopic examination**

The suspected colonies were stained using gram stain method, and their shapes, colors and arrangements were observed under light microscope.

**B. Biochemical tests**

All bacteriological isolates were examined and confirmed by biochemical tests according to Baron and Bergey's manual of determinative bacteriology and other references [12]-[16].

### 2.3. Antimicrobial Susceptibility Test

Antimicrobial Susceptibility Test was done by Kirby-Bauer method according to [17] which modified by World Health Organization [18] as follows:

The adjusted suspension used within 15 minutes to inoculate the plates by dipping a sterile cotton-wool swab into the suspension and removing the excess liquid by turning the swab against the side of the container above the level of the liquid. The swab was streaked evenly over the entire surface of the plate by swabbing in three directions, rotating the plate through an angle of 60˚ after each application. Finally, the swab was passed round the edge of the agar surface. The plate was allowed to dry with the lid closed before applying discs. The antimicrobial discs were placed on the inoculated plates using a pair of sterile forceps. Plates were incubated at 35˚C - 37˚C for 18 - 20 hrs. After overnight incubation, the diameter of each inhibition zone (including the diameter of the disc) was measured and recorded in mm [18]-[20]. The antibiotics used were Ampicillin Rifampicin, Erythromycin, Cefotaxime, Tobramyci, Nalidixic acid, Tetracycline, Nitrofurantoin, Chloramphenicol, Azithromycin, Norfloxacin, Ciprofloxacin, Meropenem, Gentamicin, Amikacin, and Imipenem. Stata statistical package used to analyze the data.

## 3. Results

**Table 1** and **Table 2** show the numbers of isolated and identified bacteria from urine samples. A total of 83 bacteria were isolated with *E. coli* 44 (53.0%) being the major organism. Next organism of importance is the *Proteus mirabilis* 18 (21.7%). (7) isolates of *Citrobacter* spp. were identified which is equal to (8.4%) of the total. Their number was (4) isolates of *C. diversus* and (3) of *C. freundii*.

(5) isolates of *Klebsiella* spp. were identified which is equal to (6%) of the total, (4) of them belongs to the species of *K.pneumoniae*, the other one belongs to the species of *K.oxytoca*. (2) isolates of *Pseudomonas aeruginosa* were identified which is equal to (2.4%) of the total.

The results had also showed identification of *Enterobacter aerogenes* with (3) isolates which is equal to (3.6%) of the total.

Finally, results showed identification of (3) isolates of *Yersinia pestis* which is equal to (3.6%) of the total and identification of (1) isolates of *Hafnia alvei* which is equal to (1.2%) out of the total numbers of isolates.

**Antimicrobial susceptibility test**

**Table 3** showed percentages of bacterial strains that were resistant to Ampicillin, Rifampicin, Erythromycin 83 (100%), Cefotaxime 75 (90.3%), Tobramycin 67 (80.7%), Nalidixic acid 66 (79.5%), Tetracycline 65 (78.3%), Nitrofurantoin 56 (67.4%), Chloramphenicol 48 (57.8%), Azithromycin, Norfloxacin and Ciprofloxacin 45 (54.2%), Meropenem 35 (42.1%), Gentamicin 32 (38.5%), Amikacin 27 (32.5%) and Imipenem 1 (1.2%).

**Table 1.** Results of biochemical tests that were applied on isolates.

| Isolates | No. | Biochemical tests | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Catalase | Oxidase | Indole | M.R | V.P | Simmon citrate | Motility test |
| *Eschericia coli* | 44 | + | + | + | + | − | − | + |
| *Proteus mirabilis* | 18 | + | − | − | + | − | + | + |
| *Klebsiella pneumoniae* | 4 | + | + | − | − | + | + | − |
| *Klebsiella oxytoca* | 1 | + | + | + | + | + | + | − |
| *Pseudomonas aeruginosa* | 2 | + | + | − | − | − | + | + |
| *Citrobacter freundii* | 3 | + | + | − | + | − | + | + |
| *Citrobacter diversus* | 4 | + | + | + | + | − | + | + |
| *Enterobacter aerogenes* | 3 | + | + | − | − | + | + | + |
| *Yersinia pestis* | 3 | + | − | − | − | − | − | − |
| *Hafnia alvei* | 1 | + | − | − | − | − | − | + |

+: positive; −: negative.

**Table 2.** Ability of isolates to fermentation of variety sugars.

| Isolates | No. | Sugars | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Glu | Lac | Suc | Mal | Sor | Xyl | Man | Ram | Raf |
| *Eschericia coli* | 44 | + | + | V | + | + | + | + | V | V |
| *Proteus mirabilis* | 18 | − | − | V | − | − | + | + | − | − |
| *Klebsiella pneumoniae* | 4 | − | + | + | + | + | + | + | + | + |
| *Klebsiella oxytoca* | 1 | + | + | + | + | + | + | + | + | + |
| *Pseudomonas aeruginosa* | 2 | − | − | − | V | − | − | − | + | + |
| *Citrobacter freundii* | 3 | + | V | V | + | + | + | + | + | V |
| *Citrobacter diversus* | 4 | + | V | V | + | + | + | + | + | − |
| *Enterobacter aerogenes* | 3 | + | + | + | + | + | + | − | + | + |
| *Yersinia pestis* | 3 | − | − | − | + | V | + | + | − | − |
| *Hafnia alvei* | 1 | − | − | V | + | − | + | + | + | − |

**Table 3.** Results of antimicrobial susceptibility test.

| No. | Antimicrobial agents tested | Conc. µg/disc | Antimicrobial class | Number of strains which were resistant to antibiotics 83 (100%) |
|---|---|---|---|---|
| 1 | Chloramphenicol (C) | 10 | Phenicols | 48 (57.8%) |
| 2 | Ampicillin (AM) | 25 | Penicillins | 83 (100%) |
| 3 | Nalidixic acid (NA) | 30 | Quinolones | 66 (79.5%) |
| 4 | Tobramycin (TOB) | 10 | Aminoglycosides | 67 (80.7%) |
| 5 | Amikacin (AK) | 10 | Aminoglycosides | 27 (32.5%) |
| 6 | Tetracycline (TE) | 10 | Tetracyclines | 65 (78.3%) |
| 7 | Ciprofloxacin (CIP) | 10 | Fluoroquinolones | 45 (54.2%) |
| 8 | Imipenem (IPM) | 10 | Carbapenemes | 1 (1.2%) |
| 9 | Cefotaxime (CTX) | 10 | Cephalosporines | 75 (90.3%) |
| 10 | Gentamicin (CN) | 10 | Aminoglycosides | 32 (38.5%) |
| 11 | Meropenem (MEM) | 10 | Carbapenemes | 35 (42.1%) |
| 12 | Rifampicin (RA) | 5 | Ansamycins | 83 (100%) |
| 13 | Azithromycin (AZM) | 15 | MLSK | 45 (54.2%) |
| 14 | Erythromycin (E) | 15 | MLSK | 83 (100%) |
| 15 | Nitrofurantoin (F) | 100 | Nitrofurans | 56 (67.4%) |
| 16 | Norfloxacin (NOR) | 10 | Fluoroquinolones | 45 (54.2%) |

MLSK: Macrolides, Lincosamides, Streptogramins, Ketolides.

## 4. Discussion

Urinary tract infections (UTIs) are serious infections worldwide [21]. The reasons for the enumerated resistance spread of antibiotics in the hospitals may be traced to the random and illogical use of these antibiotics by the temporary and permanent patients of the hospital [22] [23].

The active way to prevent the enumerated resistance of the drug by pathogens is the logical use of antibiotics

in addition to being restricted to the specialized physician orders which supplies with the best benefit of antibiotics, in addition to the financial expenditures [24].

Most of the studies state that there is a resistance of gram negative bacteria especially members of Enterobacteriaceae to antibiotics in their different kinds especially *β*-lactams antibiotics [25]. This increases the importance of these bacteria and the infections they cause are often available at the hospitals with the patients who are inhibited immunologically. *β*-lactamases are regarded as one of the important and most common among members of this family for being able to move between the different species through plasmids that carry encoded genes of the enzymes. Moreover, the increased amount of these enzymes in quantity and quality had expanded and complicated the problem [26] [27].

We found that *E. coli* is the main causative organism in urinary tract infections. This result is consistent with most of the previous studies [28]-[35]. The percentage of *Proteus mirabilis* conforms to what [31] [32] [36] [37] had come up. The reason behind spreading of *Proteus mirabilis* is because it has many pathogenicity factors like cilia that help it in adhesion, flagella that helps it to move, in addition to its outer membrane and capsule [38].

The percentage of *Citrobacter* spp. exceeds that [39] had come up with which was (3.7%). It also exceeds the percentage that [40] had found. They had reported the existence of *C. diversus* with a percentage of (4.8%) out of 517 samples that were collected from different parts of the body. These samples were expected to be infected in four different countries in Europe, Asia and two Americans.

The percentage of *Klebsiella* spp. is lower than that [31] [32] [37] had got, and higher than [39] had found, his percentage was (3.7%).

The results of *Pseudomonas aeruginosa* are consistent with what [41] had found when he isolated this species from different infections and injuries. This result also conforms to what [31] had come up, he got a percentage of (2.4%).

The results regarding *Enterobacter aerogenes* are close to those [37] [39] had come up with. The reason behind spreading of this species is its opportunistic normal existence in intestine and having many virulence factors like adhesion factors represented by cilia, in which the germs ability to adhere on cell's surfaces is regarded a necessary step to a successful colonization and then causing disease, in addition to having endotoxins represented by lipopolysaccharide that plays a big role to protect germs from phagocytosis [42].

The results of susceptibility test are consistent with [34] [43] [44] who found that bacteria isolates were most resistant to Ampicillin and have different resistance levels to different antibiotics. Overall, our results indicated significant pathogenic bacteria counts in urine samples. Imipenem was the most effective antibiotic in inhibiting the bacterial growth. These results have important clinical implications. Thus, these antibiotics do best in case of Urinary tract infections.

## 5. Conclusion

This study has examined the effect of sixteen different antibiotics on different bacteria isolated from urinary tract infections. The results indicated the dominance of *E. coli* isolates with a percentage of (44%), followed by *Proteus mirabilis* with a percentage of (18%). Ampicillin, Erythromycin and Rifampicin could not inhabit bacterial growth in all isolates. Most of the isolates (98.8%) were sensitive to Imipenem. These results indicate that Imipenem is the best antibiotic to be used in case of urinary tract infections.

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