# ANTIBIOTIC SUSCEPTIBILITY PATTERNOFGRAM-NEGATIVE BACTERIA ISOLATED FROM ELDERLY PATIENTS INFECTED WITH URINARY TRACT INFECTIONS AL-NAJAF CITY, IRAQ.

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**Abstract**- Background: Urinary tract infection is one of the most important diseases infect all individuals groups mainly elderly women and men worldwide. Objective: The aim of the present study was to detect the antibiotic susceptibility patterns of gram-negative bacteria isolated from elderly patientsinfected with urinary tract infection in Al-Najaf City, Iraq. Materials and methods: A total of 120 urine samples were collected from elderly patientsinfected with UTI in Al-Najaf City, Iraq. The antibiotic susceptibility testing was done according to Kirby-Bauer method. Results: One hundred and twenty sixof different Gram-Negative bacterial strains were isolated. E.coli was the most predominantbacteria followed by K. pneumoniae, P. aeruginosa, C. freundii , E. aerogenesand P. mirabilis .Most bacterial isolates were highly resistant against most antibiotics especially against amoxicillin and third generation cephalosporins. While, imipenem provided best antibacterial effect against most isolates Conclusions: This study demonstrated that most bacterial isolates from elderly patients with urinary tract infection becamehighly resistant to most antimicrobials.

## I. INTRODUCTION

Urinary tract infection is one of the most important recurrent diseases in worldwide especially in Middle East countries and the second most commonly illness in both males and females with percentage about 1/2, respectively[1,2]. Annually, about more than 150 million cases infected with UTI in worldwide[3,4,5]. Often, UTI caused by ESBL-producing Gram-Negative bacteria such as Pseudomonasaeruginosa andKlebsiella pneumoniae[6]. Almost, all these pathogens are multi-drug resistance (MDR) and become clinical problems because of limited therapeutic options[7,8]. Enterobacteriaceae and other of someGram-Negative pathogensbecame highly ESBL-producing bacteria. Extended-spectrum ß-Lactamase -encoding genes are located on bacterial DNA, plasmids or transposons that can transfer easily between two or more bacteria such as Escherichia coli , klebsiella pneumoniae , Enterobacter cloacae and others[9,10,11,12,13]. Since the 1980s, extended spectrum ß-lactam antibiotics have been widely used for treatment of different infections caused by Gram-Negative bacteria such as burns infections, wound infections and UTI[14,15]. Therefore, the main aim of the present study was to investigatethe antibiotic susceptibility patternsinGram-Negative bacteria isolated from elderly patientsinfected with UTI Al-Najaf City, Iraq.

## II. MATERIALS AND METHODS URINE SAMPLES COLLECTION

Five ml of clean and mid-stream of urine samples were collected in sterile containers (Himedia-India) from elderly patients who visitedto the laboratory of microbiology in Al-Najaf hospital in Al-Najaf City, Iraq during March to August 2017.Age range between 65-70 years old. All containers were labeled according to gender and age of each patient. Immediately, the urine samples were processed for bacterial cultivation and identification[16].

### Gram-negative bacteria: Isolation and identification

All urine samples were inoculated by sterile loop (Himedia-India) immediately on blood agar plate and MacConkey agar plate(Oxoid, UK). All agar plates were incubated aerobically at 37oC for 24h. Colony forming units(CFUs) method was used for each single and pure growing of bacterial colony;all urine samplescontaining less than 105 CFUs/ml were excluded[17]. All single and pure bacterial colonieswereidentified according to standardized bacteriological methods[16]and by cultivation on CHROM agar.

## III. ANTIBIOTIC SUSCEPTIBILITY TEST

Antibiotic susceptibility testing was done by Kirby-Bauer disc diffusion method[18]. Five to three pure and fresh bacterial colonies were suspended in nutrient broth (Oxoid,UK) and adjusted to 0.5 Mc-Ferland standard tube (1.5 × 108 CFUs/ml). By using sterile swab (Bioanalyse ,Turkey) the suspension was streaked onto the surface of Mueller Hinton agar (Oxoid,UK).By sterile forceps, all antibiotics disc were placed onto the surface of Mueller Hinton agar(Oxoid,UK)and incubated aerobically at 37oC for 24h.Clinical and Laboratory Standards Institute[17]was used as a guideline ofantibiotic susceptibility and resistance according to bacterial growth zone diameter. Twelve antibiotics were used in the current study provided from (Bioanalyse ,Turkey); Amoxicillin 25 µg (AX), Amoxicillin + Clavulanic acid 30 µg (AMC), Cefotaxime 30 µg (CTX),

Ceftriaxone 30 µg (CRO), Ceftazidine 30 µg (CAZ), Imipenem 10 µg (IMP), Gentamicin 15 µg (CN), Amiikacin 30 µg (AK), Tobromycin 10 µg (µg), Tetracycline 30 UI (TE), Ciprofloxacin 5µg (CIP) and Levofloxacin 30 µg (LIV).E.coli ATCC25922 was used as a control isolate.

## IV. RESULTS

### Total bacterial isolates

A total of 126 different Gram-Negative bacterial strains were isolated from 120 urine samples of elderly patients infected with UTI. E.coli was the most prevalence bacteria (49 isolates 38.9%) followed by K.pneumoniae (35 isolates 27.8%), P. aeruginosa (18 isolates 14.3%), C. freundii (12 isolates 12.9%), E. aerogenes (8 isolates 6.3%) and P.mirabilis (4 isolates 3.2%)(Figure1 and 2).

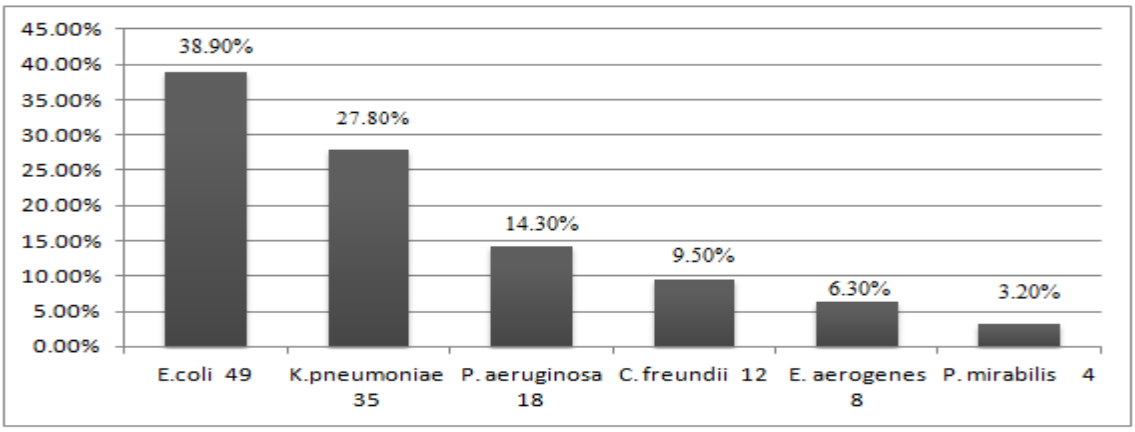



**Figure 1: Distribution of gram-negative bacteria isolated from 120 urine samples of elderly patients infected with urinary tract infection.**

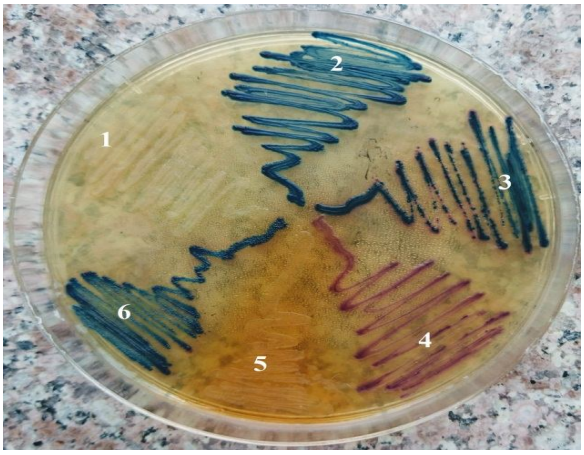



Figure 2: Six gram-negative bacteria on CHROMagar surface after 24h of incubation at 37 $^{o}$C isolated from urine of elderly patients infected with urinary tract infection. 1- P. aeruginosa 2- K.pneumoniae3- C. freundii 4- E.coli 5- Proteus 6- E. aerogenes.

### Antibiotics susceptibility testing

As shown in table 1, the results of antibiotics susceptibility test proved thatmost bacterial isolates were highly resistant against most antibiotics especially against amoxicillin and third generation cephalosporins. While, imipenem provided best antibacterial effect against most isolates.

**Table 1: Anti-bigramof 126 gram-negative bacterial strainsisolated from urine of elderly patients infected with urinary tract infection.**

| AB | E.coli 49 (100%) | | | K.pneumoniae 35 (100%) | | | P. aeruginosa 18 (100%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | S(100%) | I(100%) | R(100%) | S(100%) | I(100%) | R(100%) | S(100%) | I(100%) | R(100%) |
| AX | 10(20.4) | 0(0.0) | 39(79.6) | 5(14.2) | 0(0.0) | 30(85.8) | 0(0.0) | 0(0.0) | 18(100) |
| AMC | 23(46.9) | 2(4) | 24(49.1) | 9(25.8) | 0(0.0) | 26(74.2) | 8(44.5) | 0(0.0) | 10(55.5) |
| CTX | 28(57.2) | 0(0.0) | 21(42.8) | 15(42.8) | 0(0.0) | 20(57.2) | 10(55.5) | 0(0.0) | 8(44.5) |
| CRO | 27(55.1) | 0(0.0) | 22(44.9) | 12(34.3) | 0(0.0) | 23(65.7) | 10(55.5) | 0(0.0) | 8(44.5) |
| CAZ | 24(48.9) | 3(6.2) | 22 (44.9) | 14(40) | 0(0.0) | 21(60) | 9(50) | 0(0.0) | 9(50) |
| IMP | 49(100) | 0(0.0) | 0(0.0) | 33(94.3) | 0(0.0) | 2(5.7) | 18(100) | 0(0.0) | 0(0.0) |
| CN | 31(63.3) | 0(0.0) | 18(36.7) | 14(40) | 2(5.7) | 19(54.3) | 11(61.3) | 0(0.0) | 7(38.7) |
| AK | 35(71.5) | 0(0.0) | 14(28.5) | 23(65.7) | 3(8.5) | 9(25.8) | 13(72.3) | 0(0.0) | 5(27.7) |
| TM | 34(69.4) | 0(0.0) | 15(30.6) | 23(65.7) | 0(0.0) | 12(34.3) | 9(50) | 0(0.0) | 9(50) |
| TE | 30(61.3) | 0(0.0) | 19(38.7) | 17(48.5) | 0(0.0) | 18(51.5) | 10(55.5) | 0(0.0) | 8(44.5) |
| CIP | 34(69.3) | 0(0.0) | 15(30.6) | 19(54.3) | 0(0.0) | 16(45.7) | 11(61.3) | 0(0.0) | 7(38.7) |
| LIV | 31(63.3) | 0(0.0) | 18(36.7) | 21(60) | 0(0.0) | 14(40) | 11(61.3) | 0(0.0) | 7(38.7) |
| AB | C. freundii 12 (100%) | | | E. aerogenes 8 (100%) | | | P. mirabilis 4 (100%) | | |
| | S(100%) | I(100%) | R(100%) | S(100%) | I(100%) | R(100%) | S(100%) | I(100%) | R(100%) |
| AX | 2(16.6) | 0(0.0) | 10(83.4) | 3(37.5) | 0(0.0) | 5(62.5) | 1(25) | 0(0.0) | 3(75) |
| AMC | 3(25) | 1(8.6) | 8(66.4) | 5(62.5) | 0(0.0) | 3(37.5) | 3(75) | 0(0.0) | 1(25) |
| CTX | 6(50) | 2(16.6) | 4(33.4) | 6(75) | 0(0.0) | 2(25) | 2(50) | 0(0.0) | 2(50) |
| CRO | 9(75) | 0(0.0) | 3(25) | 6(75) | 0(0.0) | 2(25) | 2(50) | 0(0.0) | 2(50) |
| CAZ | 9(75) | 0(0.0) | 3(25) | 5(62.5) | 0(0.0) | 3(37.5) | 2(50) | 0(0.0) | 2(50) |
| IMP | 12(100) | 0(0.0) | 0(0.0) | 8(100) | 0(0.0) | 0(0.0) | 4(100) | 0(0.0) | 0(0.0) |
| CN | 9(33.3) | 0(0.0) | 8(66.4) | 5(62.5) | 0(0.0) | 3(37.5) | 3(75) | 0(0.0) | 1(25) |
| AK | 9(75) | 1(8.4) | 2(16.6) | 8(100) | 0(0.0) | 0(0.0) | 4(100) | 0(0.0) | 0(0.0) |
| TM | 9(75) | 1(8.4) | 2(16.6) | 6(75) | 0(0.0) | 2(25) | 3(75) | 0(0.0) | 1(25) |
| TE | 8(66.6) | 0(0.0) | 4(33.4) | 5(62.5) | 0(0.0) | 2(25) | 3(75) | 0(0.0) | 1(25) |
| CIP | 8(66.6) | 0(0.0) | 4(33.4) | 5(62.5) | 0(0.0) | 3(37.5) | 3(75) | 0(0.0) | 1(25) |
| LIV | 8(66.6) | 0(0.0) | 4(33.4) | 5(62.5) | 0(0.0) | 3(37.5) | 3(75) | 0(0.0) | 2(50) |

AB: Antibiotics, S: Sensitive, I: Intermediate, R: Resistance, AX : Amoxicillin25 µg , AMC: Amoxicillin + Clavulanic acid 20+10µg, CTX: Cefotaxime 30µg, CRO: Ceftriaxone 30µg, CAZ: Ceftazidime 30µg, IMP: Imipenem 10µg, CN:Gentamicin 15µg, AK: Amikacin 30µg , TM: Tobramycin 10µg, TE: Tetracycline 30UI , CIP: Ciprofloxacin 5µg, LIV:levofloxacin.

## DISCUSSION

Urinary tract infection (UTI) is one of the most dangerous diseasesthat infect bothgender; males and femalesespecially in age groups between 60 to 70 years old. The main causative agents responsible for UTI are Gram-Negative bacteria mostly, E.coli and k.pneumoniae with percentage about (60-40)% and (35-25) %, respectively, followed by C. freundii , E.aerogens and P.mirabilis[19,20]. Klebsiella penumoniaeand virulence strains of E.coli are the most important uoropathogenic bacteria cause UTI (2). They are among the most common MDR bacteria causing recurrent UTI[21].Recently, K. penumoniae ,E.coli, C. freundii and most Gram-Negative bacteria have shown they are ability to acquire plasmid encoding for ESBL genes such as Bla-TEM , Bla-SHV and Bla-CTX-Mand became high resistance to different antibiotics and wide-spectrum to 3rd generation cephalosporins in hospitals and in community[5,13,22].About more than 200 different types of ESBLs have been discovered worldwide, most these genes are found in Enterobacteriaceae family epically in K. penumoniae[23]. TEM and SHV are the most ESBLs were discovered[24,25]. Extended spectrum beta lactamasesare enzymes not able to hydrolyze carbapenem and cephamycins but able to hydrolyze 3rd and 4th generation cephalosporins, theses enzymes became represent a public health concern due to the high incidence in some members of Enterobacteriaceae family such as E.coli and K.pneumoniae in the both hospitals and community[2,5]. Some high virulence strains of Gram-Negative bacteria such asE.coli , K.pneumoniae and C. freundii are associated with UTI, if untreated in fast time ,these strains become capable of adhesion and colonization in the urinary tract of human and migrate to the bladder to cause cystitis and acute pyelonephritis and may be cause kidney damage leading to chronic kidney disease. However, bacterial resistance has originated fast due to the production of special enzymes called extended-spectrum β-lactamases (ESBLs)[16]. These enzymes are derived from some genes such as Bla-TEM , Bla-SHV and Bla-CTX-M for the narrower spectrum ß-lactamases by mutations that alter the amino acid configuration around the enzyme active site[17] .They are typically encoded by plasmids that can be exchanged between bacterial species[18]. It is also reported that the predominant types of ESBLs in K. pneumoniae , p. aeruginosa and E.coli and some others type of Gram-Negative bacteria isolated from urine of elderly patients with UTI are TEM type followed by SHV and CTX-M. Usually, infections caused by ESBL-producing bacteria are associated with increased morbidity and mortality which entails enhanced healthcare costs[26,27,28].

## ACKNOWLEDGEMENT

The authors thankful to staff of the laboratory of microbiology in Al-Najaf hospital to provide all urine samples.

Conflict of Interests
There was no any conflict of interests in this work.

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