Antibiotics susceptibility patterns of *Citrobacter freundii* isolated from patients with urinary tract infection in Al-Najaf governorate – Iraq

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**ABSTRACT**

*Citrobacter freundii* is one of the most causative agents of urinary tract infection in human due to their high antimicrobials resistance. Therefore, this study aimed to evaluate the ability of *Citrobacter freundii* to resist to fifteen antimicrobials. A total of 461 urine samples were collected from patients infected with urinary tract infection, females and males, age groups between 18 to 60 years old performed in Al-Najaf central hospital in Al-Najaf City, Iraq, during April to December 2018. Antimicrobials susceptibility testing was performed by disc diffusion method according to the Kirby-Bauer method onto Mueller Hinton agar surface. There were 30 isolates (6.5%) diagnosed as *Citrobacter freundii*. Antimicrobial resistance rate of the 30 isolates to Amoxicillin and Chloramphenicol were all high (25 isolates 83.4%), Ciprofloxacin, Streptomycin and Sulfonamide (24 isolates 80%). The good resistance rate was observed for Penicillin, Amoxiclav and Ceftazidime (21 isolates 70%). The moderate resistance rate was observed for Cefotaxime (19 isolates 63.3%), Ceftriaxone (20 isolates 66.7%), Gentamicin (15 isolates 50%), Tobramycin (18 isolates 60%) and Erythromycin (20 isolates 66.7%). The lowest resistance rate was observed for Tetracycline (19 isolates 63.3%). While Imipenem provided full antibacterial activity (30 isolates 100%). Of the total 30 isolates, 22 (73.3%) were multi-drug resistance, 8(26.7%) extensive-drug resistance and no isolates were pan-drug resistance. While 19 isolates (63.3%) were extended-spectrum beta-lactamase producing *Citrobacter freundii*. *Citrobacter freundii* has highly resistant against most antimicrobials and became more dangerous bacteria cause urinary tract infection.

**INTRODUCTION**

Urinary tract infection is one of the most important recurrent infections infect man and women worldwide (Mohammed *et al.*, 2016; Aljanaby 2018a) mainly caused by gram-negative bacteria such as *Escherichia coli*, *Klebsiella pneumonia* and *Citrobacter freundii* (Perslev *et al.*, 2019; Aljanaby and Aljanaby 2018b). *Citrobacter freundii* is a gram-negative bacterium, motile, oxidase negative and non-capsulated (Lakhundi *et al.*, 2017; Liu *et al.*, 2018). *Citrobacter freundii* is one of the most important gram-negative bacteria cause urinary tract infection and other diseases in human such as bacteremia (Park *et al.*, 2013; Ouyang *et al.*, 2018; Anderson *et al.*, 2018). Recently, *Citrobacter freundii* isolated from hospitals patients with urinary tract infection become more resistant to different antibiotics like beta-lactamase, 3rd generation cephalosporins and aminoglycosides (Aljanaby and Gafil 2013; Castanheira *et al.*, 2018; Akinbami *et al.*, 2018). In Iraq, there was no...
enough studies focused on *C. freundii*, and they’re resistant to different antibiotics. Therefore, the main aim of the present study is to investigate the antibiotics sensitivity pattern of *C. freundii* isolated from patients infected with urinary tract infection in Al-Najaf governorate – Iraq.

**MATERIALS AND METHODS**

**Study design and patients**

This is a cross-sectional descriptive study, performed in Al-Najaf central hospital in Al-Najaf City, Iraq, during the period from April to December 2018. A total of 461 urine samples were collected from patients infected with urinary tract infection, females and males, age groups between 18 to 60 years old.

**Eligibility criteria for patients**

Patients will be considered eligible for registration into this study if they fulfill all the inclusion criteria and none of the exclusion criteria as defined below;

- Patients (female or male) at least equal or more than 18 years old.
- All infected patients have been diagnosed by a physician and primary microscopic characters such as the presence of pus cells in urine samples.
- Patients should have sufficient capacity for informed consent.
- Patients should don’t have any other infection.
- Patients should don’t take any antibiotics for treatment.

**Samples collection, culture and bacterial identification**

Ten ml of mid-stream urine samples were collected in sterile disposable containers after cleaned the genitals (Aljanaby and Aljanaby 2018b). All urine samples were centrifuged at 2000 rpm for 3 min, immediately, the sediment incubated aerobically with brain heart infusion broth at 37°C for 24h, then streaked with a sterile loop on to blood agar and MacConkey agar (Oxoid™) plates. Colony Forming Units (CFUs) method was used for growing single and pure bacterial colony; all urine samples containing less than 105 CFUs/ml were excluded (Tan *et al.*, 2012). All emerged bacterial isolates were identified according to colony morphology and standard microbiological tests such as; colony morphology, gram stain, oxidase test, catalase test, invic test, motility test, coagulase test, growth on MacConkey agar (Oxoid™) (Mac Faddin 2000).

**Antimicrobials susceptibility test**

Antimicrobials susceptibility testing was performed by disc diffusion method according to the Kirby-Bauer method onto Mueller Hinton agar (Oxoid™) surface (Bauer *et al.*, 1966). Fourteen different antimicrobial discs were used in this study provide from OxoidTM, the USA as follow: Ampicillin 10IU (AM), amoxicillin 25μg (AX), Amoxiclav 30μg (AMC), cefotaxime 30μg (CTX), ceftriaxone 30μg (CRO), ceftazidime 30μg (CAZ), gentamicin 10μg (GM), tobramycin 10μg (TM), ciprofloxacin 5μg (CIP), Streptomycin 10μg (S), erythromycin 30μg (E), tetracycline 30 μg (TE), Sulfonamide 300μg (SSS), Chloramphenicol (C) 30μg and imipenem 10μg (IMP). The diameters of inhibition zones (mm) were measured using a caliper measure each zone with the unaided eye and compared with clinical and laboratory standards institute (CLSI) guideline (CLSI 2017). Any bacterial isolate was resistance to at least three different antimicrobials classes considered as MDR, if any bacterial isolate was resistance to all antimicrobial classes except two or three antimicrobial classes considered as extensive-drug resistant (XDR) and when any bacterial isolate was resistance to all antimicrobials class considered as pan-drug resistant (PDR) (Aljanaby 2018c).

**Statistical analysis**

Fisher’s exact test was used in this study for the comparison between samples by using Graph pad prism V.10 computer software. P values less than the 0.05 level of significance were considered statistically significant (Aljanaby and Alhasnawi 2017).

**RESULTS**

**Figure 1: Numbers and percentages of total bacterial isolates from patients infected with urinary tract infection in Al-Najaf city-Iraq during the period from April to December 2018. (N=461)**

**Total bacterial isolates**

Out of 461 different bacterial isolates from patients infected with urinary tract infection, gram-negative bacteria were the most incidences with 297 isolates (64.4%) while; there were 158 isolates (34.3%) were gram-negative bacteria and 6 isolates (1.3%) with no growth (Fig.1). According to
microscopic and culture characteristics, biochemical test, Chromagar and Vitek2® system, out of 461 different bacterial isolates, there were 30 isolates (6.5%) diagnosed as *Citrobacter freundii*. On the other hand, out of a total of 297 gram-negative bacterial isolates, *Citrobacter freundii* was prevalent with the percentage of 10.10% (Fig.2 and 3).

Figure 2: Numbers and percentages of *Citrobacter freundii* isolate d from patients infected with urinary tract infection in Al-Najaf city-Iraq during the period from April to December 2018. (N=461)

Figure 3: *Citrobacter freundii* grown on to CHROM agar surface after 24h of aerobic incubation at 37°C

Patients

The results of the present study proved that out of 461 patients infected with urinary tract infection, there were 309 patients (67%) were female, and 152 patients (33%) were male. Also, the results demonstrated that out of total 297 patients infected with urinary tract infection caused by gram-negative bacteria there were 204 patients (68.7%) were female and 93 patients (31.3%) were male. While out of a total of 158 patients infected with urinary tract infection caused by gram-positive bacteria, there were 99 patients (62.7%) were female, and 59 patients (37.3%) were male. Also, the results indicated that there were 6 patients (100%) were female infected with urinary tract infection with no bacterial growth (Fig.4). According to age groups, the results demonstrated that the age group 41-50 years old was the most prevalent range among patients infected with urinary tract infection caused by *Citrobacter freundii* (12 patients 40%, 8 females and 4 male) followed by age group 31-40 years old (8 patients 26.7%, 5 females and male), 51-60 years old (7 patients 23.3%, 5 females and 2 male) and 18-30 years old (3 female patients 10%) (Table 1).

Antimicrobials sensitivity testing

In this study, 15 different antimicrobials were used against *Citrobacter freundii*. The result proved that the antimicrobial resistance rate of the 30 isolates to Amoxicillin and Chloramphenicol were all high (25 isolates 83.4%), Ciprofloxacin, Streptomycin and Sulfonamide (24 isolates 80%). The good resistance rate was observed for Penicillin, Amoxiclav and Ceftazidime (21 isolates 63.3%), Ceftriaxone (20 isolates 66.7%), Gentamicin (15 isolates 50%), Tobramycin (18 isolates 60%) and Erythromycin (20 isolates 66.7%). The lowest resistance rate was observed for Tetracycline (19 isolates 63.3%).
While Imipenem provided full antibacterial activity (30 isolates 100%) (Table 2). Of the total 30 isolates, 22 isolates (73.3%) were multi-drug resistance: 16 isolates (72.7%) from female and 6 isolates (27.3%) from male, 8 isolates (26.7%) extensive-drug resistance: 5 isolates (62.5%) from female and 3 isolates (37.5%) from male and no isolates was pan-drug resistance (Table 3). According to modified double disc synergy test, the results indicated that there were 19 isolates (63.3%) were extended-spectrum beta-lactamase producing Citrobacter freundii 16 (84.2%) isolates from female and 3 (15.8%) isolates from male with significant increase P= 0.0419 (Table 4) (Fig.5). The antimicrobials resistance profile of 30 Citrobacter freundii isolates is given in table 5.

DISCUSSION

Urinary Tract Infection is common in most region of Iraq, and it remains the main health problem in many similar developing countries (Rödel et al., 2019). Citrobacter freundii is an opportunistic bacterium, highly adaptable and has remarkable mechanisms of survival and transmission in the host (Liu et al., 2018). This pathogen is responsible for the majority of UTIs.
compromised people, also hospital-acquired infections, are associated with higher rates of treatment failures, prolonged hospitalizations, increased costs and mortality (Ruiz et al., 2018). Antimicrobial stewardship consists of avoidance of antimicrobials when appropriate and, when antimicrobials are indicated, use of strategies to optimize the selection, dosing, route of administration, duration and timing of antimicrobial therapy to maximize clinical cure while limiting the unintended consequences of antimicrobial use, including toxicity and selection of resistant microorganisms (Chen et al. 2018). The Citrobacter freundii resistant beta-lactams by three main strategies the first one is hydrolyzing of beta-lactam ring by beta-lactamases was plasmid-borne and easy to transfer from each other (Huang et al., 2017). The second strategy is modifying the beta-lactams target (Penicillin-binding proteins), that leading to prevent...
Table 6: Antimicrobials resistance profile of 30 Citrobacter freundii isolates from patients infected with urinary tract infection in Al-Najaf City-Iraq during the period from April to December 2018 (Contd....)

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Sex</th>
<th>Age group</th>
<th>No (%)</th>
<th>R</th>
<th>ESBL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>51-60</td>
<td>12(80)</td>
<td>MDR</td>
<td>Positive</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>18-30</td>
<td>9(60)</td>
<td>MDR</td>
<td>Positive</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>41-50</td>
<td>13(86.6)</td>
<td>XDR</td>
<td>Positive</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>31-40</td>
<td>14(93.3)</td>
<td>XDR</td>
<td>Positive</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>51-60</td>
<td>14(93.3)</td>
<td>XDR</td>
<td>Positive</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>41-50</td>
<td>14(93.3)</td>
<td>XDR</td>
<td>Positive</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>51-60</td>
<td>14(93.3)</td>
<td>XDR</td>
<td>Positive</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>41-50</td>
<td>14(93.3)</td>
<td>XDR</td>
<td>Positive</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>41-50</td>
<td>12(80)</td>
<td>MDR</td>
<td>Positive</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>41-50</td>
<td>12(80)</td>
<td>MDR</td>
<td>Positive</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>31-40</td>
<td>12(80)</td>
<td>MDR</td>
<td>Positive</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>31-40</td>
<td>12(80)</td>
<td>MDR</td>
<td>Positive</td>
</tr>
<tr>
<td>13</td>
<td>M</td>
<td>31-40</td>
<td>14(93.3)</td>
<td>XDR</td>
<td>Positive</td>
</tr>
<tr>
<td>14</td>
<td>F</td>
<td>41-50</td>
<td>12(80)</td>
<td>MDR</td>
<td>Positive</td>
</tr>
<tr>
<td>15</td>
<td>F</td>
<td>18-30</td>
<td>12(80)</td>
<td>MDR</td>
<td>Positive</td>
</tr>
<tr>
<td>16</td>
<td>F</td>
<td>18-30</td>
<td>11(73.3)</td>
<td>MDR</td>
<td>Positive</td>
</tr>
<tr>
<td>17</td>
<td>F</td>
<td>41-50</td>
<td>12(80)</td>
<td>MDR</td>
<td>Positive</td>
</tr>
<tr>
<td>18</td>
<td>F</td>
<td>31-40</td>
<td>11(73.3)</td>
<td>MDR</td>
<td>Positive</td>
</tr>
<tr>
<td>19</td>
<td>F</td>
<td>41-50</td>
<td>13(86.6)</td>
<td>XDR</td>
<td>Positive</td>
</tr>
<tr>
<td>20</td>
<td>F</td>
<td>31-40</td>
<td>10(66.6)</td>
<td>MDR</td>
<td>Negative</td>
</tr>
<tr>
<td>21</td>
<td>F</td>
<td>31-40</td>
<td>6(40)</td>
<td>MDR</td>
<td>Negative</td>
</tr>
<tr>
<td>22</td>
<td>F</td>
<td>31-40</td>
<td>4(26.6)</td>
<td>MDR</td>
<td>Negative</td>
</tr>
<tr>
<td>23</td>
<td>F</td>
<td>51-60</td>
<td>3(20)</td>
<td>MDR</td>
<td>Negative</td>
</tr>
<tr>
<td>24</td>
<td>F</td>
<td>51-60</td>
<td>4(26.6)</td>
<td>MDR</td>
<td>Negative</td>
</tr>
<tr>
<td>25</td>
<td>F</td>
<td>41-50</td>
<td>4(26.6)</td>
<td>MDR</td>
<td>Negative</td>
</tr>
<tr>
<td>26</td>
<td>F</td>
<td>41-50</td>
<td>4(26.6)</td>
<td>MDR</td>
<td>Negative</td>
</tr>
<tr>
<td>27</td>
<td>M</td>
<td>41-50</td>
<td>4(26.6)</td>
<td>MDR</td>
<td>Negative</td>
</tr>
<tr>
<td>28</td>
<td>M</td>
<td>41-50</td>
<td>4(26.6)</td>
<td>MDR</td>
<td>Negative</td>
</tr>
<tr>
<td>29</td>
<td>M</td>
<td>51-60</td>
<td>4(26.6)</td>
<td>MDR</td>
<td>Negative</td>
</tr>
<tr>
<td>30</td>
<td>M</td>
<td>51-60</td>
<td>4(26.6)</td>
<td>MDR</td>
<td>Negative</td>
</tr>
</tbody>
</table>


Antibiotics from binding to it. The third one is lowering the outer membrane permeability of antibiotics inside the cell and through it out of the cell by many mechanisms like efflux pumps (Juhas et al., 2019). Citrobacter freundii phenotypically having the ability to resist aminoglycosides antibiotics due to many mechanisms like reducing uptake or decreasing cell permeability by a transport defect or membrane impermeable this mechanism is chromosomally mediated and results in cross-reactivity to all aminoglycosides (Brahmi et al., 2015). The most of isolates in this study showed an obvious resistance to chloramphenicol antibiotic. The target modifying may also contribute to chloramphenicol resistance, the efflux pump that is throwing away this antibiotic, causing decreasing of antibiotic concentration inside the bacterial cell (Anisimova and Yarullina, 2018). The bacterial cell that has an ability to resist the tetracycline antibiotic by many mechanisms such as hydrolyzing enzymes encoding by tet(X) genes, efflux pumps (A-E) types (Leski et al., 2016). The bacterial cell can be resisting the sulfonamides due to the presence of an R-plasmid that determining sulfonamide-resistant di-hydropeteroate synthase (DHPS), DHPS enzymes by this mechanism is uncommon since they bind to multiple sites on both ribosomal subunits and high-level resistance cannot be selected by a single step (Feng et al., 2015). The other mechanism is uncommon since they bind to multiple sites on both ribosomal subunits and high-level resistance cannot be selected by a single step (Feng et al., 2015).
which hydrolyzing the sulfonamide antibiotics, the high resistance rate to this drug it’s may be due the property of plasmid-bearing that lead to the spreading of resistant (Lee et al., 2016). In this study showed the lower susceptibility rate to ciprofloxacin. The ciprofloxacin antibiotic determined as effective fluoroquinolone antibiotic against Enterobacteriaceae members but the resistance toward it is increasing, the resistance is due to many reasons like mutation in genes that encoding for gyrase enzymes alone or with mutation in genes that encoding to topoisomerase IV, another reason is excessive activity of efflux pumps, the mutations happing in regions called quinolone resistance-determining regions (responsible for quinolones resistance). In conclusion: *Citrobacter freundii* became is one of the most drugs resistance dangerous pathogens caused urinary tract infection in Iraq.

REFERENCES


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