Cephalosporins Susceptibility Test in Urinary Tract Infection

Mithaq Sabeeh Al-Nassiry*, Haider Hashim Zalzala**

ABSTRACT:
BACKGROUND: This study was conducted at the Al-Kindy Teaching Hospital to determine the resistance patterns to cephalosporins of members of the family Enterobacteriaceae isolated from urinary tract infections (UTIs). A total of 270 urine specimens were collected from February, 2008 to May, 2008.

OBJECTIVE: Determination the Resistance patterns to cephalosporins in Enterobacteriaceae isolated from urinary tract infection.

METHODS: Urine specimens were processed for culture, and susceptibility testing using Kirby-Bauer method. The minimum inhibitory concentration (MIC) was determined by twofold dilution.

RESULT: Escherichia coli was found to be the most organism, followed by Klebsiella spp. The results of susceptibility of isolates under study to different cephalosporins were moderately or highly resistant to many of the test agents. The observations on the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) for the cephalosporins explained the high level of resistance to cephalothin and cefaclor, and a moderate level of resistance to cefotaxime, ceftazidime and cefixime.

CONCLUSION: The increasing MIC of cephalosporins, especially third generation, indicates decreasing susceptibility of these organisms to these types of β-lactam agents due to the production of extended spectrum β-lactamases (ESBLs).

KEY WORDS: cephalosporins, enterobacteriaceae, urinary tract infection.

INTRODUCTION: Urinary tract infections remain the most common among acquired infections. Their importance lies in the fact that a considerable proportion of population may acquire asymptomatic infections\(^1\). This state of bacteruria, particularly, in female has related to more serious subsequent infection of the upper urinary tract\(^2\).

Urinary tract infections, including cystitis and pyelonephritis are the most frequent nosocomial and community-acquired infections\(^3\). When antibacterial are selected for the empiric treatment of UTIs, knowledge of most likely causative organisms and the prevalence of resistance of pathogens to antibacterial agents are essential\(^4\). Such infections are caused by a variety of Gram-negative bacteria that ascend into the urinary tract and establish bacteruria often at levels more than or equal to 10\(^5\) CFU/ml. of urine\(^4\).

Escherichia coli dominate as the causative agent in all patient populations according to international data. Others would include Staphylococcus saprophyticus, Enterobacteiraceae group (Klebsiella spp., Proteus spp., Enterobacter spp., etc.) and Pseudomonas aeruginosa \(^4\). The clinically relevant members of the Enterobacteriaceae can be considered as two groups: Opportunistic pathogens and overt pathogens. The first group most commonly includes: Citrobacter spp., Klebsiella spp., Proteus spp., Providencia spp., and Morganella spp. Although considered opportunistic pathogens, these may produce significant virulence factors. Although E.coli is a normal bowel inhabitant, its pathogenic classification is somewhere between the overt and opportunistic pathogens. Some strains express potent toxins and cause serious gastrointestinal infections\(^5\).

The goals in the treatment of UTI is to prevent or treat systematic symptoms, to relieve symptoms, to eradicate sequestered infection, to eliminate uropathogenic bacterial strains from fecal or vaginal reservoirs, and to prevent long-term squeal at minimal cost, with the lowest rate of side effects, and with the least selection of an antibiotic.
CEPHALOSPORINS SUSCEPTIBILITY TEST IN URINARY TRACT INFECTION

resistant flora. A steady increase of resistance patterns to antimicrobials, particularly to cephalosporins has been documented.

Therefore, decided to carry out this study to provide some knowledge on the determination of resistance patterns to cephalosporins of some members of the family Enterobacteriaceae. Special emphasis has been paid to evaluate the MICs of third generation cephalosporins and decreasing susceptibility of β-lactam agents due to ESBLs production.

MATERIALS AND METHODS:
Patients seen at Al-Kindy Teaching Hospital with UTI, i.e. with urinalysis result showing more than 10 pus cells per high power field, and with no previous history of antibiotic intake, are included in the study.

Urine specimens were processed for culture, and susceptibility testing using the Kirby-Bauer method. The following antibiotic discs were used: cephaplatin, cephalixin, cefazolin, cefuroxime, cefoxitin, cefaclor, cefprozil, cefotaxime, ceftazidime, ceftriaxone, cefixime, cefditibutin and ceftizoxime, the concentrations of the discs are shown in table (1).

The minimum inhibitory concentration (MIC) was determined by twofold dilution method, and a standard strain, E. coli ATCC 25922, was included in the study.

The identification of members of the family Enterobacteriaceae was performed according to the biochemical tests indicated in the scheme of Farmer and his co-workers.

RESULTS:
A total of 270 urine specimens were collected from patients admitted to Al-Kindy Teaching Hospital for the period from February, 2008 to May, 2008. From a total of 150 specimens which yielded positive growth, there were 105 isolates belonging to members of the family Enterobacteriaceae (70%). Twenty-five isolates were identified as Ps.aeruginosa (16.66%). The other isolates were as follows: 15(10%) Gram-positive cocci and 5 (3.33%) yeasts (Table 2).

Escherichia coli was the most common microorganism, representing 32 isolates (30.4%). Klebsiella spp. were found to be the second most common, making up 29% of the total isolates. Furthermore, it was found that Proteus spp. represented 21 isolates (19.9%). The Citrobacter spp. made (7.6%) of isolates. However, the Enterobacter spp. represented (3.75%). The Providencia spp., Serratia spp. and Morganella morgani accounted for (3.75%), (2.85%) and (1.9%) respectively. These results are shown in Table (1). The resistance patterns of the isolates to cephalosporines are illustrated in Table (3), resistance to cephalor was the highest.

In the present study, 39 (37%) of isolates were subjected to MIC and MBC determinations. All have given resistance to the various cephalosporins employed; Enterobactor spp. gives 100 % resistance as shown in Table (4). Table (5) gives the MICs for cephalosporins, carried out by the twofold broth dilution method, as described by Ericsson and Sheries. The MBC was determined by comparing the number of colonies present on subculture from the antibiotic-free broth after incubation, with the numbers of colonies subcultured from the antibiotic-containing tubes.
Table 1: NCCLS-performance for antimicrobial disc sensitivity test. 7th Ed. M2-A7,2000

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Disc potency/µg</th>
<th>Inhibition zone(mm)</th>
<th>MIC µg/ml</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Sensitive</td>
<td>intermediate</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>30</td>
<td>≥23</td>
<td>22-20</td>
</tr>
<tr>
<td>Cephalexin</td>
<td>30</td>
<td>≥20</td>
<td>19-17</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>60</td>
<td>≥23</td>
<td>22-20</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>60</td>
<td>≥18</td>
<td>17-15</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>30</td>
<td>≥23</td>
<td>22-20</td>
</tr>
<tr>
<td>Cefaclor</td>
<td>30</td>
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<td>19-17</td>
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<tr>
<td>Cefprozil</td>
<td>30</td>
<td>≥23</td>
<td>22-20</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>30</td>
<td>≥20</td>
<td>19-17</td>
</tr>
<tr>
<td>Ceftriaxime</td>
<td>30</td>
<td>≥23</td>
<td>22-15</td>
</tr>
<tr>
<td>Cefazidime</td>
<td>30</td>
<td>≥20</td>
<td>19-17</td>
</tr>
<tr>
<td>Cefixime</td>
<td>30</td>
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<td>19-17</td>
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<td>Cefitoxime</td>
<td>30</td>
<td>≥23</td>
<td>22-20</td>
</tr>
<tr>
<td>Cefibutin</td>
<td>30</td>
<td>≥23</td>
<td>22-20</td>
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Table 2: Bacterial species isolated from urine

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Number of cases</th>
<th>%</th>
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<tr>
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<td>E.coli</td>
<td>32</td>
<td>30.4</td>
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<tr>
<td>Klebsiella spp.</td>
<td>31</td>
<td>29</td>
</tr>
<tr>
<td>Proteus spp.</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>Citrobacter spp.</td>
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<td>7.6</td>
</tr>
<tr>
<td>Enterobacter spp.</td>
<td>4</td>
<td>3.75</td>
</tr>
<tr>
<td>Providencia spp.</td>
<td>4</td>
<td>3.75</td>
</tr>
<tr>
<td>Morganella spp.</td>
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<td>1.9</td>
</tr>
<tr>
<td>Serratia spp.</td>
<td>3</td>
<td>2.85</td>
</tr>
<tr>
<td>Pseudomonas spp.</td>
<td>25</td>
<td>16.66</td>
</tr>
<tr>
<td>Gram positive cocci</td>
<td>15</td>
<td>10</td>
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<tr>
<td>Yeast</td>
<td>5</td>
<td>3.33</td>
</tr>
<tr>
<td><strong>Total number of isolates</strong></td>
<td>150</td>
<td>100 %</td>
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Table 3: Resistance of Enterobacteriaceae isolates to cephalosporin antibiotics.

<table>
<thead>
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<th>Bacterial species</th>
<th>Number of isolates</th>
<th>Number of resistance isolates</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
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<td>13</td>
<td>40.6</td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td>31</td>
<td>11</td>
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<td>Proteus spp.</td>
<td>21</td>
<td>6</td>
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<td>1</td>
<td>50</td>
</tr>
<tr>
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</tr>
<tr>
<td>Enterobacter spp.</td>
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<td>4</td>
<td>100</td>
</tr>
<tr>
<td>Providencia spp.</td>
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<td>1</td>
<td>25</td>
</tr>
<tr>
<td>Serratia spp.</td>
<td>3</td>
<td>1</td>
<td>33</td>
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<tr>
<td><strong>Total number of isolates</strong></td>
<td>105</td>
<td>39</td>
<td>37.14 %</td>
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Table 4: Isolated bacteria subjected to MIC and MBC to β-lactam agent.

<table>
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<td>Cefaclor</td>
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<td>4</td>
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<td>3</td>
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<td>80.9</td>
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<td>3</td>
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<td>80</td>
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<td>4</td>
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<td>3</td>
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<td>3</td>
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<td>76.19</td>
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<td>24</td>
<td>14</td>
<td>1</td>
<td>2</td>
<td>4</td>
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<td>2</td>
<td>73</td>
<td>69.5</td>
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<td>25</td>
<td>11</td>
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<td>2</td>
<td>4</td>
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<td>2</td>
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<td>68.5</td>
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<td>Cefoxitin</td>
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<td>12</td>
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<td>2</td>
<td>53</td>
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<td>4</td>
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<td>1</td>
<td>43</td>
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<tr>
<td><strong>Total No.</strong></td>
<td></td>
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<td>31</td>
<td>21</td>
<td>2</td>
<td>8</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>105</td>
<td>100</td>
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Table 5: MIC/MBC (µg/ml) for members of Enterobacteriaceae isolated from U

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Cephalothin</th>
<th>Cefazolin</th>
<th>Cefaclor</th>
<th>MIC/MBC(µg/ml)</th>
<th>Cefotaxime</th>
<th>Ceftazidime</th>
<th>Cefixim</th>
<th>Cetizoxime</th>
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<tbody>
<tr>
<td>E.coli</td>
<td></td>
<td></td>
<td></td>
<td>&gt;1024 / &gt;1024</td>
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<td></td>
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<tr>
<td></td>
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<td></td>
<td>64 / 128-256</td>
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<tr>
<td>Klebsella spp</td>
<td></td>
<td></td>
<td></td>
<td>&gt;1024 / &gt;1024</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
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<td>64-128</td>
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<tr>
<td>Proteus spp.</td>
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<td></td>
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<td>64 / 64-128</td>
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<td>64-128</td>
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<tr>
<td>Morganella spp.</td>
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<td>512-1024 / ≥1024</td>
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<td>Citrobacter spp.</td>
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<td>64 / 64-128</td>
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<td>64-128</td>
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<td>1024 / &gt;1024</td>
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<td>64 / 64</td>
<td>128</td>
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<td>64-128</td>
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</table>

Standard strain: E.coli ATCC 25922
2 / 4 1 / 2 2 / 4 0.5 / 1 0.5 / 1 1 / 2 0.5 / 1
DISCUSSION:

Urinary tract infection results from the ascension of faecally derived organisms and perirethral area into the bladder. They adhere to the bladder receptor sites, and if they possess the virulence factors (e.g. hemolysin, K Ag that protect bacteria from complement mediated lyases, and endotoxin) for pyelonephritis, they will ascend to the ureter reaching kidneys eliciting an inflammatory response (5).

It should be indicated, that in this work, all isolates had previously been examined for β-lactamase production by the idiomeric and nitrocefin method of Sykes and Matthew (13).

The result of β-lactamase production of the present study was very high, in contrast to what was reported in other countries, because they employed molecular techniques, and isoelectric focusing for detection of β-lactamase production. These techniques are of higher sensitivity and specificity than the conventional ones (e.g. iodometric, acidimetric and nitrocefin method) (12).

As shown in Table 3, The isolates shows moderate to high resistance to the first and second generation cephalosporin antibiotics. This reflected the heavy exposure to antibiotic pressure and long duration of therapy (13,14).

Furthermore, the majority of the isolates were shown to possess moderate resistance to the major types of the third generation cephalosporins. Resistance to cefotaxime, ceftazidime and ceftriaxone was (69.5%), (68.5%) and (70.4%) respectively. This results is in agreement with a study done by Mauqein et al (15), they found that all enterobacteriaceae were intermediately susceptible or resistant to cefotaxime. On the other hand, 25 from 32 isolates of E.coli (78.12%) and 25 from 31 isolates of K.spp. (77.41%) are resistant to ceftazidime and 24 from 32 (75%) of E.coli isolates are resistant to cefotaxime and 24 from 31 of K. spp. isolates (80.64%) are resistant to cefotaxime. This is in keeping with the finding of Okesola et al and Kumar et al where they found that the resistance of K. spp. to cefotaxime was 69.3% and 85% respectively and Kumar et al found that E.coli is more resistant to ceftazidime than cefotaxime (16,17), but both of the above studies found that the resistance of K. spp. to ceftazidime is much less than the result in this study (45.2% and 37% as compared to 80.64% in this study), this is explained by the fact that the regional variants of resistance to antibiotics may be explained in part by different local antibiotic practices due to excessive &/or inappropriate antibiotic use especially for broad spectrum antibiotic that is prescribed empirically (18,19).

The resistance pattern of E.coli and K. spp. to third generation cephalosporin especially ceftaxime and ceftazidime and the increasing MIC of cephalosporins (as shown in table 4) indicates decreasing the susceptibility of the organisms under study to these types of β-lactam agents due to the production of extended spectrum β-lactamases (ESBLs) (such as TEM or SHV types ESBLs) that is most commonly produced by these two bacteria (20,21).

The result of MIC and MBC for the ESBL-producing isolates reveals that it is affected by the level of activity of antimicrobial agent and the inoculum size of the organism (22). The inoculum size of β-lactamase-producing isolates should be 10^4-10^5 CFU/ml. as recommended by the National Committee for Clinical Laboratory Standards guidelines (23, 24, 25).

The percentage of β-lactamase (ESBL) production by enterobacteriaceae varies according to different geographical areas (26). A study in Richmond, Virginia, reported 1.5% of isolates produced ESBLs. TEM-12 and TEM-26 seem most common in the USA. In Europe, ESBLs occur in 20-25% of Klebsiella spp, from patient in intensive care Unit., although they have been found in up to 30-40% in France. TEM-3 seems to be most common in France. This fact can explain why the resistance pattern of K. spp. to ceftazidime in this study is higher than in other studies.

Extended-spectrum β-lactamases can be difficult to detect because they have different levels of activity against cephalosporins, thus, the choice of antimicrobial agent to test is critical. For example, one enzyme may actively hydrolyze ceftazidime, resulting in ceftazidime MICs of 256 μg/ml., but may have poor activity on cefotaxime, producing MICs of only 4 μg/ml (27).

CONCLUSION:

1. The most common members of Enterobacteriaceae isolated from patients with UTI were E.coli, following by Klebsiella spp., Proteus spp., Citobacter spp., Enterobacter spp., Providencia spp., Serratia spp., and Morganella morganii.

2. The results of susceptibility testing of isolates, under study, to different β-lactam agents have shown that many isolates of the members of the family Enterobacteriaceae were moderately to highly resistant to many of the tested agent.
3. The MICs and MBCs of vast majority isolates of Enterobacteriaceae recovered from UTI were higher for cephalothin and cefaclor than for cefotaxime, ceftazidime, and cefixim; and than cefazolin and cefotioxime.

4. Poor permeability of bacteria by the drug, lack of penicillin binding protein for a specific drug, and degradation of drug by beta lactamases are the most important causes that lead to increase antibiotic resistance.

REFERENCES:
