Synergistic Effect of Potassium Clavulanate in Combination with Cefamandol and Ceftazidime on β- Lactamase, Extracted From Resistant E.coli

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Abstract

The aim of this study was to evaluate in-vitro activity of Cefamandol and Ceftazidime, in combination with potassium clavulanate against 10 uropathogenic E.coli isolated from patients with chronic complicated urinary tract infections (UTIs), these isolates were identified by the Api identification systems. The antimicrobial susceptibility tests were determined by Kirby-Bauer method and the minimum inhibitory concentrations of Cefamandol and Ceftazidime, were determined, by tube method. These isolates were resistant to Ampicillin (Amp), Amoxicillin (Amo), Carbenicillin (Cb), Ticarcillin (Tic), Amoxicillin Potassium Clavulanate (Augmentin), (AmoCA), Ticarcillin Potassium Clavulanate (Timentin) (TicCA), Cefamandol (Cfm) and Ceftazidime (Cfz), also resistant to other antibiotics, such as Tetracycline, Chloramphenicol, Trimethoprim and (50% of the isolates were resistant to Nalidixic acid and Rifampicin). Transfer of plasmids by direct conjugation experiments were performed by mating 10 strains with recipient strain E.coli K12 C 600 Rif or Nal resistant, and cell free β-lactamases were prepared and detected by macro-iodometric method. The activities of each cell free β-lactamases extract against Cfm and Cfz were determined by disks diffusion method (microbiological Masuda method) and by macro-iodometric method. The activity of β-lactamases was inhibited by the addition of Potassium Clavulanate.

Conclusion: Good effectiveness of Cfm CA and Cfz CA was obtained against resistant strains of E.coli due to complicated urinary tract infection (UTIs).

Key words: β- lactamases, Cefamandol, Ceftazidime, Timentin and Augmentin.
Introduction
Clavulanic acid is a β-lactam; structurally it differs from penicillins in two respects, the replacement of Sulfur in the penicillin thiazolidine ring with oxygen in the clavam oxazolidine ring and the absence of the side chain at position 6. Clavulanic acid a naturally occurring clavam isolated from Streptomyces clavuligerus has poor antibacterial activity but exerts a potent and irreversible inhibitory effect on β-lactamases especially penicillinases by blocking the active sites of these enzymes and is strongly synergistic with most of the β-lactamines in vitro. Due to this combination, Amoxicillin is protected from degradation and its spectrum is therefore extended to include bacteria normally resistant to amoxicillin and other β-lactam antibiotics. In the case of β-lactam resistant bacteria a bacterial enzyme, β-lactamase, cleaves the β-lactam ring and renders the antibiotic inactive, lactamases are a large and diverse group of enzymes in which four clinically relevant classes are known.

β-lactamases continue to be the leading cause of resistance to β-lactam antibiotics among Gram-negative bacteria. In recent years there has been an increased incidence and prevalence of extended-spectrum β-lactamases (ESBLs), enzymes that hydrolyse and cause resistance to oxyimino-cephalosporins and aztreonam. The majority of ESBLs are derived from the widespread broad-spectrum β-lactamases TEM-1 and SHV-1. ESBLs have become widespread throughout the world and are now found in a significant percentage of E.coli and Klebsiella pneumoniae strains in certain countries. There are also new families of ESBLs, including the cefotaximase (CTX-M) and OXA-type enzymes, cefazidimase, as well as novel unrelated β-lactamases. The stability of different cephalosporins to the most important β-lactamases was assessed and many clinical studies have shown that up to 75% of the β-lactamases responsible for β-lactam resistance in G-negative bacteria were R-plasmid mediated. Recently, new fourth generation cephalosporins, such as Cefepime, Cefpirome, Cefoselis, Cefditoren, Cefozopran, were introduced into antibacterial chemotherapy and their activities were compared with other β-lactams such as Cefazidime, Imipenem and Carbapenem, against P. aeruginosa, Enterobacteriaceae (E.coli, Klebsiella pneumoniae) and G-positive bacteria. In addition several drug combinations have been produced which contain both a β-lactam antibiotic and a β-lactamase inhibitor; the inhibitor has high affinity for β-lactamases it irreversibly binds to it, and thereby preserves the activity of the β-lactam. Currently, four penicillin inhibitor combinations are in clinical use: Amoxicillin Salbactam (Unasyn), Amoxicillin Clavulanate (Augmentin), Ticarcillin – Clavulanate (Timentin) and Pipracillin-Tazobactam (Zosyn).

Aim of the Study
The aim of the study is to evaluate the following combinations, Cefamandol / Clavulanate and Ceftazidime / Clavulanate for their in vitro antimicrobial activity against complicated urinary tract infections caused by β-lactamase producer E.coli.

Materials and Methods

Bacterial strains
Standard strains with plasmid – mediated beta – lactamases were used:
1-E.coli K12 (TEM-1 type β-lactamase with isoelectric point 5.4) confer plasmid (R 111) and E.colae P99 (11). 2-E.coli K12 (SHV-1 type β-lactamase Pitton (type II) Lp 7.7 (11)). 3-E.coli K12 C 600 Rif and E.coli K7 C 600 NaL Sensitive to antibiotics. 4-Clinical isolates of E.coli. 5-Pure enzyme of Med Labs. All types of antibiotics powder were obtained and kindly provided by SDI. 6-E.coli ATCC 25922 kindly provided by Medical city.

Identification of E.coli
Strains were isolated on MacConkey agar and identified by Api 20 E System (Biomerieux vitek, Inc) (18).

Antibiotic susceptibility test (Disk diffusion method)
The resistance pattern for antibiotics were determined by Bauer - Kirby (19) diffusion assay on Mueller – Hinton agar (20ml / plate) the inoculum was 104 – 105 bacteria / ml, of 6 hours cultures incubated at 37°C for 24 hours. The antibiotics used were as follow:
Amoxicillin30µg, Amoxicillin30 µg Augments (Amo20µg+CA10 µg), Carbencillin 100 µg, Ticarcillin100 µg, Timentin(Tic75µg+CA 10 µg), Cefamandol 30 µg and Cefazidime30 µg, Rifampicin 30 µg, Nanidixic acid 30 µg, Tetracycline 30 µg, Chloramphenicol 30 µg and Cotrimoxazole (Triethoprime 2.5 µg + Sulfamethaxazole 22.5 µg) Powders of Cefamandol and Cefazidime were also obtained from (Roussel, Beecham and Sepcia).
Minimum inhibitor concentration (MICs)

This test measures the concentration of an antibiotic necessary to inhibit growth of a standardized inoculum under defined conditions. Minimum inhibitory concentrations (MICs) were determined by dilution of different concentrations of antibiotics in Mueller-Hinton broth. The tubes were inoculated with a 6 hour incubation cultures, diluted, given a final concentration of inoculum (10^7 – 10^9 CFU/ml) and incubated at 37°C. The lowest concentration of antibiotic preventing growth and remaining clear (free from microbial growth) (MIC) was estimated after 18 hours of incubation.

Remaining clear (free from microbial growth) (MIC) was estimated after 18 hours of incubation. As control, fully sensitive E.coli K12 strain was tested under the same conditions. Table 1 and Table 2 shows normal MICs values and diameters of zone of inhibition according to the method recommended by the National Committee for Microbiology Laboratory Standards (FRANCE) (20).

Transfer of genetic information by direct conjugation method.

Conjugal transfer of 3GC resistant ESBL producing strains was done at 35°C-37°C in liquid medium (Brain heart infusion (B.H)) or in solid media (Trypticase Soya agar (T.S.A) or Mueller-Hinton (M.H)) using E.coli K12 C 600 Rif and E.coli K12 C 600 Nal as recipient. Equal volumes (1 mL) of culture of the donor and the recipient strain (10^6-10^9 CFU/ml) grown with agitation in tryptic soya broth were mixed and incubated statically for 18 hours at 35°C. Transconjugants were selected on M.H agar containing Nalidixic acid (150 μg/ml) or Rifampicin (300 μg/ml) to inhibit the growth of donor and Amoxicillin, Ticarcillin, and Ceftazidime to inhibit the growth of recipient strain (11).

Table 1 Standard of MICs and diameters (⌀) of zone of inhibition of cephalosporins

<table>
<thead>
<tr>
<th>Cephalosporins</th>
<th>Abbreviations</th>
<th>Critical concentrations (μg/ml)</th>
<th>⌀ of Zone of Inhibition</th>
<th>Potency of Disk (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>First generation</strong></td>
<td></td>
<td>c</td>
<td>C</td>
<td>d</td>
</tr>
<tr>
<td>Cefalothin</td>
<td>Ctn</td>
<td>8</td>
<td>32</td>
<td>18</td>
</tr>
<tr>
<td>Cefaloridin</td>
<td>Cfr</td>
<td>8</td>
<td>32</td>
<td>18</td>
</tr>
<tr>
<td>Cefalexin</td>
<td>cfx</td>
<td>8</td>
<td>32</td>
<td>18</td>
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<tr>
<td><strong>Second generation</strong></td>
<td></td>
<td>c</td>
<td>C</td>
<td>d</td>
</tr>
<tr>
<td>Cefamandol</td>
<td>Cfm</td>
<td>8</td>
<td>32</td>
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<td>Cefuroxim</td>
<td>Cxm</td>
<td>8</td>
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<td>22</td>
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<td>Cefoxitin</td>
<td>Cxt</td>
<td>8</td>
<td>32</td>
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<tr>
<td><strong>Third generation</strong></td>
<td></td>
<td>c</td>
<td>C</td>
<td>d</td>
</tr>
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<td>Cefotaxime</td>
<td>Ctx</td>
<td>4</td>
<td>32</td>
<td>21</td>
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<tr>
<td>Ceftriazone</td>
<td>Cro</td>
<td>4</td>
<td>32</td>
<td>21</td>
</tr>
<tr>
<td>Cefotiam</td>
<td>Ctm</td>
<td>4</td>
<td>32</td>
<td>22</td>
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<td>Cefmenoxime</td>
<td>Cmx</td>
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<td>Ceftazidime</td>
<td>Cfz</td>
<td>4</td>
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<td>21</td>
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<tr>
<td>Ceftizoxime</td>
<td>Zox</td>
<td>4</td>
<td>32</td>
<td>21</td>
</tr>
<tr>
<td>Cefoperazone</td>
<td>Clp</td>
<td>4</td>
<td>32</td>
<td>21</td>
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<td>Cefodiazone</td>
<td>Hr221</td>
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</tr>
<tr>
<td>Moxalactam</td>
<td>Mox</td>
<td>4</td>
<td>32</td>
<td>23</td>
</tr>
</tbody>
</table>

MICs: c: Sensitive strains, C: Intermediate, C< MIC ≤ C: Resistant strains, ⌀ ≥ D: Sensitive strains, ⌀<d Resistant strains, ⌀ ≤ D: Intermediate, ⌀=diameter in mm.
Table (2) Standard values of MICs and diameters (\( \Theta \)) of zone of inhibition of Penicillins.

<table>
<thead>
<tr>
<th>Penicillins</th>
<th>Abbreviations</th>
<th>Critical concentrations in ( \mu g/ml )</th>
<th>( \Theta ) of zone of inhibition</th>
<th>Potency of disk/( \mu g/ml )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group A</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>AMPICILLIN</td>
<td>Amp</td>
<td>4</td>
<td>C</td>
<td>16</td>
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<tr>
<td>AMOXYCILLIN</td>
<td>Amo</td>
<td>4</td>
<td>C</td>
<td>16</td>
</tr>
<tr>
<td>AUGMENTIN</td>
<td>Amc</td>
<td>4</td>
<td>C</td>
<td>16</td>
</tr>
<tr>
<td>TIMENTIN</td>
<td>Tim</td>
<td>128</td>
<td></td>
<td>128</td>
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<tr>
<td><strong>CARBOXYPENICILL</strong></td>
<td></td>
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<td>CARBENICILLIN</td>
<td>Cb</td>
<td>128</td>
<td></td>
<td>128</td>
</tr>
<tr>
<td>TICARCILLIN</td>
<td>Tic</td>
<td>128</td>
<td></td>
<td>128</td>
</tr>
<tr>
<td><strong>AMIDINOPENICILLIN</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MECILLINAM</td>
<td>Mec</td>
<td>1</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td><strong>UREIDOPENICILLIN</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEZLOCILLIN</td>
<td>Mez</td>
<td>8</td>
<td></td>
<td>32</td>
</tr>
<tr>
<td>AZLOCILLIN</td>
<td>Azl</td>
<td>16</td>
<td></td>
<td>128</td>
</tr>
<tr>
<td>PIPRACILLIN</td>
<td>Pip</td>
<td>16</td>
<td></td>
<td>128</td>
</tr>
<tr>
<td><strong>MONOBACTAM</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AZHEREONAM</td>
<td>Atm</td>
<td>4</td>
<td></td>
<td>32</td>
</tr>
</tbody>
</table>

MIC\( \leq \) c: Sensitive strains, MIC\( >\) C: Resistant strains, C\( <\) MIC\( \leq \) C Intermediate, \( \Theta \)\( \geq \) D: Sensitive strains, \( \Theta <\) d Resistant strains \( \Theta \)\( <\) D \( \Theta \)=diameter (in 11).

**Extraction of \( \beta \)-lactamase.**

Cell free beta-lactamases were prepared from strains known to be good producers of the desired enzymes, (\( \beta \)-lactamases, type TEM-1 E.coli R111 and SHV-1 E.coli453, R-plasmid mediated enzymes) and \( \beta \)-lactamase from E.cloacae P99(cephalosporinase) as reference. Crude enzymes were also prepared from test isolates of E.coli. Bacterial cultures were grown aerobically at 37°C in Brain Heart Infusion broth (Difco) over night. A 200 ml flask of the same broth was then inoculated with 2ml of the culture, incubated at 37°C for 4 hours, the cells were harvested by centrifugation, washed twice with buffer phosphate pH 7 and disrupted with ultrasound (soniprep 150HSE) at 20KHZ. To remove cell debris, the crude extracts were centrifuged; the supernatants were collected in small sterile vials under aseptic conditions (11).

**Detection of \( \beta \)-lactamase by Macro - iodometric method.**

1% of agarose and0.5% of starch were dissolved in120ml of buffer phosphate and boiled, 18 mg of penicillin G powder and0.8ml of iodine solution were added at 40°C, the medium was shaken and distributed in aliquots of 20ml in Petri dishes, 5 well were made by each plate, the enzymes were applied in each well and the zones of decolorization were observed 1-18hours at 4°C (21).

**Assessment of stability of \( \beta \)-lactams to cell-free \( \beta \)-lactamases.**

The activity of each cell free \( \beta \)-lactam extract against each \( \beta \)-lactam antibiotic was determined by the microbiological method (Masuda G., et al. 1976,modified by Labia,R. Barthelemy. M. 1979). The surface of a Muller Hinton agar was seeded with a suspension of \( \beta \)-lactam sensitive indicator E.coli ATCC 25922. Four discs containing \( \beta \)-lactams under test were placed near filter paper discs; each impregnated with 30\( \mu l \) of the enzymatic extract. The plates were incubated at 37°C for 18hours, the \( \beta \)-lactamase activity was observed like half moon zone of inhibition. Unchangeable inhibition zones demonstrate stability of the antibiotic to the enzyme.

**Inhibition by Cefamandol or Cefazidime /Clavulanate Modified iodometric method (21).**

Modified iodometric method (Labia, R., Barthelemy, M.1979), was used without incorporation of penicillin G in the medium, fives wells were made in the plate in which 10\( \mu l \) of enzyme extract , 30 \( \mu l \) of potassium clavulanate and 30\( \mu l \) of Cefamandol or Cefazidime were added. The results were noted after 4-18 hours at 4°C, absence of decolorization zone indicated positive reaction.
Ten clinical isolates were screened for \( \beta \)-lactamase inhibitor using 10\( \mu \)l potassium clavulanate in combination with 30\( \mu \)l of Ceftazidime or Cefamandol. Sensitivity discs containing Ceftazidime or Cefamandol and a filter disc incorporated with 30\( \mu \)l enzyme and 10\( \mu \)l potassium clavulanate were placed on agar plate on which a bacterial suspension of sensitive E.coli (standard) was spread the inoculum was 104 – 105 CFU / ml, of 6 hours cultures at 37\( ^{0} \)C0 for 24 hours according to the method recommended by the National committee for microbiological Laboratory standards [25].

### Results and Discussion

#### Disk agar diffusion test (Susceptibility test)

According to the results of Susceptibility test .The resistance patterns of E.coli RIII (TEM-1 \( \beta \)-lactamase) and E.coli K12 (SHV-1) type \( \beta \)-lactamase Pitton (type II) 1p 7.7 were compared with ten strains they were resistant to Ampicillin , Amoxicillin , Carbenicillin Pipracillin , Augmentin , Timentin , Cefamandol and Ceftazidime . They were also resistant to other antibiotics such as Tetracycline Chloramphenicol and Trimethoprim and (50%) were resistant to Rifampicin and Nalidixic acid . The results indicated dissemination of resistance among clinical isolates of E.coli in Iraq table 3.

### Table (3) Sensitivity Tests of Strains Determined by Disk Diffusion Test

<table>
<thead>
<tr>
<th>No of isolates</th>
<th>DIAMETERS OF ZONE OF INHIBITION/MM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amp</td>
<td>Amo</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
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<tr>
<td>4</td>
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<tr>
<td>5</td>
<td>0</td>
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<tr>
<td>6</td>
<td>0</td>
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<tr>
<td>7</td>
<td>0</td>
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<tr>
<td>8</td>
<td>0</td>
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<tr>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>E.coli TEM-1 SHV-1</td>
<td>0</td>
</tr>
<tr>
<td>E.coli TEM-1</td>
<td>0</td>
</tr>
<tr>
<td>E.cloacae p99</td>
<td>0</td>
</tr>
</tbody>
</table>

Abbreviations : Amp:Ampicillin ; Amo:Amoxicillin ; Amc:Amoxiclave ; Cb:Carbenicillin ; Tic: Icarcillin ; Tim :Timentin ; Cfm: Cefamandol ; Cfx:Ceftazidime ; Tc :Tetracyclin ; Cm:Chloramphenicol ; Tm:Trimethoprim ; Rif: Rifampicin ; Nal: Nalidixic acid ; (see table1 )

#### Detection of \( \beta \)-lactamases

This test is based on the reaction of the (oic ) acid of penicillin with iodine ; \( \beta \)-lactamase hydrolyze penicillin to penicilloic acid , which in turn react with iodine , the presence of \( \beta \)-lactamase in a test system was shown by decolorization of starch – iodine complex . The results of detection of \( \beta \)-lactamases by iodometric method were positive for 10 strains compared with standard negative E.coli K12 C 600 Rif and positive \( \beta \)-lactamases R111 (TEM-1), presented in Fig 1.
Cefamandol, Ceftazidime /Clavulanate Combination

Table (4) Minimum Inhibitory Concentrations Of Four Antibiotics Towards Ten Uropathogenic E.coli Comparing with Standard Strains

<table>
<thead>
<tr>
<th>No. of Isolate</th>
<th>E.coli</th>
<th>Cefamandol</th>
<th>Ceftazidime</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2,3</td>
<td></td>
<td>512</td>
<td>64</td>
</tr>
<tr>
<td>4,5,6</td>
<td></td>
<td>1024</td>
<td>32</td>
</tr>
<tr>
<td>7,8,9,10</td>
<td></td>
<td>2048</td>
<td>32</td>
</tr>
<tr>
<td>E.coli(453)<strong>SHV-1(7.7)</strong></td>
<td>16</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>E.coli(R111)TEM-1(5.4)**</td>
<td>32</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>E.cloacae(P99)<strong>(8.3)</strong></td>
<td></td>
<td>**</td>
<td></td>
</tr>
</tbody>
</table>

*Isoelectric point, **cephalosporinase

Inhibition of β-lactamases

Figure 2 shows a comparison between antibiotic-enzyme interactions, by the highly sensitive double disk technique which demonstrated hydrolysis of Ceftazidime and Cefamandol by β-lactamase-producing E.coli. The enzymes obtained from 10 strains hydrolyzed, Cefamandol and Ceftazidime, but were highly stable to all β-lactamases tested when combined with potassium clavulanate.

Enzymes extracted from E.coli standards harboring plasmid R111 TEM-1 or SHV-1 harboring plasmid R 453 β-lactamases were inhibited with potassium clavulanate when combined with Amoxicillin or Ticarcillin (Fig 3 A, B) however β-lactamase of E.cloacae was not affected by Augmentin and inhibited by Ticarcillin and hydrolyzed all cephalosporins represented in Fig 4. In contrast β-lactamase under test were highly resistant to Amox/CA, Tic/CA, Fig 5: show inhibition of enzymes by iodometric method.

Minimum inhibitory concentrations

The inhibition of beta-lactamase production by potassium clavulanate has been demonstrated with many strains of bacteria, this effect potentiates the action of many beta lactams, such as Ampicillin, Amoxicillin, Carbenicillin and Ticarcillin. Many clinical reports of combination of Amoxicillin with Clavulanic acid (Augmentin) have been encouraging, in urinary tract infections due to β-lactamase-producing organisms type TEM and SHV, whilst Amoxicillin alone had no effect, the addition of Clavulanic acid (as salt) dramatically change the half moon inhibition zone to complete inhibition zone (3,4,11).

Our investigations indicated resistant phenotype of Augmentin and Timentin; the diameters of zone of inhibition ranged from (3.5mm-7.5mm) for Amc and (5.5mm-9.5mm) for Tim, while The standard diameters zones of inhibition were for Amc(14-21mm) and for Tim is(13mm). The critical normal MICs of Augmentin and Timentin were (4-16 μg/ml), (128 μg/ml) respectively.

The minimum inhibitory concentrations were studied for ten clinical isolates of E.coli in comparison with standard resistant strains, TEM-1 β-lactamase coded for plasmid R111, E.coli K12 SHV-1 β-lactamase Pitton (type II) coded for plasmid 453 Lp 7.7, E.cloacae P99 cephalosporinase Lp 8.3 (France) and E.coli ATCC 25922 (Medical city hospital) sensitive strains as references, the MICs of Cfm, Cfz, were very high, the range of MICs for Cfm was 512 - 2048 μg/ml for Cfz 64 - 32 μg/ml. These results are indicated in Table 4.

Figure (1): Iodometric Method for Detection of β-Lactamase (Enzymatic Reaction at (4°C) No.1, No.2, No.3: β-Lactamase from E.coli Clinical Isolates, No.4 : TEM-1 β-Lactamase as Standard, No.5: β-Lactamase negative E.coli ATCC 25922.
It was found that copies of the genes for ampicillin, ticarcillin, tetracycline, and chloramphenicol resistance could be transferred by direct conjugation method from donor cell to recipient cell. The results were presented as follow:

**Direct conjugation method**

It was found that copies of the genes for ampicillin, ticarcillin, tetracycline, and chloramphenicol resistance could be transferred by direct conjugation method from donor cell to recipient cell. The results were presented as follow:
Ten strains transfered resistance to Ticarcillin, Cefatizidime, and other antibiotics after mating for 18 hours, transconjugants derived from these strains produced β-lactamases, these results suggested that all strains bear plasmids and produce extended spectrum β-lactamases capable of hydrolyzing and inactivating a wide variety of β-lactams including the third generation cephalosporins pencillins and Aztreonam, sensitive to imipenem. These results were similar to the studies of Rodrigues C. et al., Chaudhary U. and Kurokawa, H et al. (9,27,28).

Conclusions
The clinical isolates in this study were very resistant to Augmentin, Cefamandol and Cefatizidime comparing with standard TEM-1 and SHV-1 (pasmidic pencillins) E. cloacae P99 is very resistant to Cefalothin, Cefamandol, Cefotaxime, Cefatizidime standard strain which produce β-lactamase (ESBLs) enzymes that hydrolyze and cause resistance to oxyimino – cephalosporins and aztreoname. The majority of ESBLs are derived from the widespread broad – spectrum β-lactamases TEM-1 and SHV-1. ESBLs have become widespread throughout the world and are now found in a significant percentage of E. coli and Klebsiella pneumoniae strains in certain countries (6,7,8,10). The increasing emergence cephalosporins resistant E. coli has lead to concern about the use of various combination therapy. A good in vitro response was observed in our clinical uropathogenic E. coli when Cfm and Cfz, were mixed with different concentration of potassium Clavulanate as inhibitor they were effective and safe for the treatment of UTIs caused by β-lactamases (Cefatizidimase) producing complicated strains (22,23,24,28).

<table>
<thead>
<tr>
<th>E.coli wild type</th>
<th>Amp&lt;sup&gt;R&lt;/sup&gt;</th>
<th>Tic&lt;sup&gt;R&lt;/sup&gt;</th>
<th>Cfz&lt;sup&gt;R&lt;/sup&gt;</th>
<th>Cm&lt;sup&gt;R&lt;/sup&gt;</th>
<th>Tc&lt;sup&gt;R&lt;/sup&gt;</th>
<th>NaI&lt;sup&gt;R&lt;/sup&gt;</th>
<th>Rif&lt;sup&gt;R&lt;/sup&gt;</th>
<th>or NaS&lt;sup&gt;+&lt;/sup&gt;</th>
<th>Rif&lt;sup&gt;R&lt;/sup&gt;</th>
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<td>Tic&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Cm&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Tc&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NaI&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Tic&lt;sup&gt;R&lt;/sup&gt;</td>
<td>Cfz&lt;sup&gt;R&lt;/sup&gt;</td>
<td>Cm&lt;sup&gt;R&lt;/sup&gt;</td>
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