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

Siham S. Shaokat*[1] , Hamoudi A. Hameed*

**Ministry of Industry and Mineral*
***Ministry of Industry and Mineral*

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}, (Amo\CA), Ticarcillin\ Potassium Clavulanate {Timentin} (Tic\CA), 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.coliK12C 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 .

خلاصة

يهدف البحث الى تقييم فعالية كل من السيفاماندول السيفتازيديم بخلط كل منهما مع حامض الكلافولانيك (ملح البوتاسيوم) ضد جراثيم اشريشيا القولون الممرضة والمشخصة والمعزولة(بطريقة نظام التوصيف الوظائفي) من ادرار عشرة مرضى مصابين بالتهابات المجاري البولية المزمنة.تم ايجاد حساسية هذه الجراثيم لمضادات الحيوية المختلفة بطريقة كيربي وكذلك الجرع المثبطة الصغرى بطريقة الوسط السائل, ووجدت بأنها مقاومة, للامبسيلين, الاموكسيسيلين, الكاربينسلين,التيكارسلين, بوتاسيوم كلافيولانيت\ اموكسيسيلين(اوكمنتين), بوتاسيوم كلافيولانيت\ تيكارسلين(تايمنتين) ,السيفاماندول, والسيفتازيديم, وكذلك للمضادات الاخرى مثل التيتراسايكلين, الكلورامفينيكول, الترايميثوبريم ,وخمسون بالمئة من العزلات كانت مقاومة لحامض النالديكسيك والريفامبسين. تم تنفيذ تجربة الاقتران المباشر البسيط لمعرفة انتقال البلازميدات الى عزلة اشريشيا القولون الحساسة للمضادات الحيوية والمقاومة للريفامسين او لحامض النالديكسيك , وكانت النتائج جميعها ايجابية مما دل على ان جميع العزلات تحمل مقاومة بلازميدية للمضادات أعلاه,ووجد بطريقة اليود في المحيط الصلب ان جميع العزلات منتجة لأنزيمات البيتا- لاكتامیز, كما تم تقييم السيفاماندول والسيفتازيديم بخلطهما مع الكلافولانيت, بطريقة اليود وبطريقة الانتشار في الوسط ألزرعي (طريقة العالم ماسودا المايكروبايولوجية) , وأعطت جميع العزلات مناطق تثبيط كاملة بعد إضافة حامض الكلافولانيك مع السيفتازيديم والسيفا ماندول, مما يجعلنانفكر باستخدام هذه الخلطات كعلاج ناجح لالتهابات المجاري البولية المزمنة, بعد اجراء الدراسات السريرية اللازمة.

[1]*corresponding author email albiatyss84@yahoo.com*
Received 11- 4-2005
Accepted 25-7-2006

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 andirreversible 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[1,2].Due to this combination, Amoxicillin is protected from degradation and its spectrum is therefore extended to include bacteria normally resistant to amoxycillin and other β - lactam antibiotics [3] . 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 [4].

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 [5,6,7,8]. There are also new families of ESBLs, including the cefotaximase(CTX-M) and OXA- type enzymes , ceftazidimase , as well as novel unrelated β- lactamases [9,10]. 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[11,12,].Recently, new fourth generation cephalosporins, such as Cefepime, Cefpirome, Cefoselis, Cefditoren, Cefozopran [13,14], were introduced into antibacterial chemotherapy and their activities were compared with other β-lactams such as Ceftazidime,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: Ampicillin Salbactam (Unasyn) , Amoxicillin Clavulanate (Augmentin), Ticarcillin – Clavulanate (Timentin) and Pipracillin-Tazobactam (Zosyn) [15,16] .Urinary tract infections (UTIs) cause a significant health problem and E.coli has been reported to be the primary pathogen in approximately 80% of cases. E.coli, express structures called adhesions, fimbriae or pili that help them bind to specific tissue [17].

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 K_{12} (TEM-1 type β- lactamase with isoelectric point 5.4) confer plasmid(R 111) and E.cloacae P99 (11). 2-E.coli K_{12} (SHV-1 type β- lactamase Pitton (type II) I.p 7.7 [11].

3-E.coli K_{12} C 600 Rif and E.coli K_{12} C 600 Nal Sensitive to antibiotics [11]. 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 37C0 for 24 hours.The antibiotics used were as follow:

Ampicillin30µg,Amoxicillin30 µg,Augmentin (Amo20µg+CA10 µg), Carbenicillin 100 µg, Ticarcillin100 µg, Timentin(Tic75µg+CA 10 µg), Cefamandol 30 µg and Ceftazidime30 µg, Rifampicin 30 µg, Nalidixic acid 30 µg, Tetracycline 30 µg, Chloramphenicol 30 µg and Cotrimoxazole (Trimethoprime 2.5 µg + Sulfamethaxazole 22.5 µg) Powders of Cefamandol and Ceftazidime were also obtained from (Roussel, Beecham and Sepacia).

Minimum inhibitor concentration (MICs)

This test measures the concentration of an antibiotic necessary to inhibit growth of a standardized inoculum under defined condition .Minimum inhibitory concentrations (MICs) were determined by dilution of different concentration of antibiotics in Mueller – Hinton broth .The tubes were inoculated with a 6 hour incubation cultures , diluted , given a final concentration of inoculum ($10^4 – 10^5$ CFU/ml) and incubated at 37C° .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. As control, fully sensitive E.coli K_{12} strain was tested under the same conditions. Table1 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- °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 K_{12} C 600 Rif and E.coli K_{12} C 600 Nal as recipient.Equal volumes (1 mL) of culture of the donor and the recipient strain (10^8-10^9 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 eftazidime to inhibit the growth of recipient strain [11].

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

cephalosporins	Abbreviations	Critical concentrations In µg/ml		∅of * Zone of Inhibition		Potency of disk/ µg/ml
First generationc		**c**	**C**	**d**	**D**	
Cefalothin	Ctn	8	32	18	12	30
Cefaloridin	Cfr	8	32	18	12	30
Cefalexin	cfx	8	32	18	12	30
Second generation						
Cefamandol	Cfm	8	32	22	15	30
Cefuroxim	Cxm	8	32	22	15	30
Cefoxitin	Cxt	8	32	22	15	30
Third generation						
Cefotaxime	Ctx	4	32	21	15	30
Ceftriaxone	Cro	4	32	21	15	30
Cefotiam	Ctm	4	32	22	15	30
Cefmenoxime	Cmx	4	32	21	15	30
Ceftazidime	Cfz	4	32	21	15	25
Ceftizoxime	Zox	4	32	21	15	30
Cefoperazone	Cfp	4	32	21	15	30
Cefodiazine	Hr221	4	32	22	15	30
Moxalactam	Mox	4	32	23	17	30

MIC≤ c: Sensitive strains, MIC>C: Resistant strains, C< MIC≤ C Intermediate, ∅≥ D: Sensitive strains, ∅<d Resistant strains d ≤∅< D ∅=diameter [in 11].

Table (2) Standard values of MICs and diameters (∅) of zone of inhibition of Penicillins.

Penicillins	Abbreviations	Critical concentrations In μg/ml		∅of * Zone of Inhibition		Potency of disk/ μg/ml
Group A		c	C	d	D	
AMPICILLIN	Amp	4	16	17	11	10
AMOXYCILLIN	Amo	4	16	21	14	25
AUGMENTIN	Amc	4	16	21	14	Amo 20+ CA 10
TIMENTIN	Tim	128	128	13	13	Tic 75+ CA 10
CARBOXYPENICILL						
CARBENICILLIN	Cb	128	128	15	15	100
TICARCILLIN	Tic	128	128	13	13	75
AMIDINOPENICILLIN						
MECILLINAM	Mec	1	8	23	17	25
UREIDOPENICILLIN						
MEZLOCILLIN	Mez	8	32	21	16	75
AZLOCILLIN	Azl	16	128	19	10	75
PIPRACILLIN	Pip	16	128	20	13	100
MONOBACTAM						
AZTHEREONAM	Atm	4	32	23	17	30

MIC≤ c: Sensitive strains, MIC>C: Resistant strains, C< MIC≤C Intermediate,
∅≥ D: Sensitive strains, ∅<d Resistant strains d ≤∅< D ∅=diameter [in 11].

Extraction of β– lactamase.

Cell free beta –lactamases were prepared from strains known to be good producers of the desired enzymes, (β–lactamases, type TEM-1 E.coli R111 and SHV-1 E.coli453 , R-plasmid mediated enzymes) and β–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 370C 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°Cfor 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 β– 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 in 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 β– lactams to cell-free β– lactamases[22,23,24].

The activity of each cell free β– lactamase extract against each β– 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 β– lactam sensitive indicator E.coli ATCC 25922. Four discs containing β– lactams under test were placed near filter paper discs; each impregnated with 30μl of the enzymatic extract. The plates were incubated at 37°C for 18hours, the β– 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 Ceftazidime /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 10μl of enzyme extract , 30 μl of potassium clavulanate and 30μl of Cefamandol or Ceftazidime were added. The results were noted after 4-18 hours at 4°C, absence of decolorization zone indicated positive reaction.

Masuda microbiological method

Ten clinical isolates were screened for β–lactamase inhibitor using10µl potassium clavulanate in combination with 30 µl of Ceftazidime or Cefamandol. Sensitivity discs containing Ceftazidime or Cefamandol and a filter disc incorporated with 30µl enzyme and 10µl 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 37C0 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 K_{12} (SHV-1) type β - lactamase Pitton (type II) I.p 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

No of isolates	DIAMETERS OF ZONE OF INHIBITION/MM												
	Amp	Amo	Amc	Cb	Tic	Tim	Cfm	Cfz	T	Cm	Tm	Rif	Nal
1	0	0	4	0	0	5.5	2	3	0	0	0	16	0
2	0	0	4.5	0	0	7	3	3	0	0	0	0	18
3	0	0	3.5	0	0	8	2	5	0	0	0	18	0
4	0	0	4	0	0	8.5	0	4	0	0	0	10	0
5	0	0	5	0	0	9	6	15	0	0	0	15	0
6	0	0	5.5	0	0	9	10	15	0	0	0	0	0
7	0	0	6	0	0	7	11	11	0	0	0	0	18
8	0	0	6	0	0	6.5	5	10	0	0	0	0	19
9	0	0	6	0	0	9.5	5	11	0	0	0	0	19
10	0	0	7.5	0	0	9	7.5	13	0	0	0	10	18
E.coil[24] SHV-1	0	0	19	0	0	23	19	24	0	0	22	0	20
E.coil TEM-1	0	0	21	0	0	24	20	26	0	0	21	0	20
E.cloacae P99	0	0	4	15	13	5	6	4	-	-	0	0	0

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

Detection of β - lactamases

This test is based on the reaction of the (oic) acid of penicillin with iodine . β - lactamase hydrolyze penicillin to penicilloic acid , which in turn react with iodine , the presence of β - lactamase in a test system was shown by decolorization of starch – iodine complex . The results of detection of β - lactamases by iodometric method were positive for 10 strains compared with standard negative E.coli K_{12} C 600 Rif and positive β– lactamases R111 (TEM-1), presented in Fig 1.

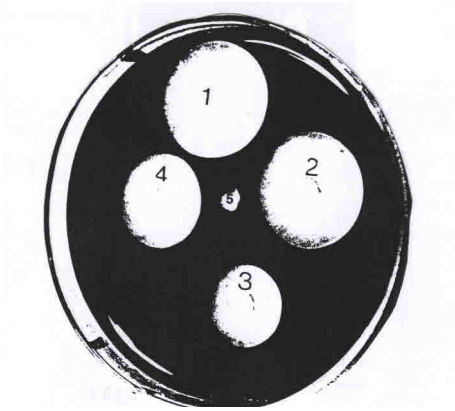


Figure (1): Iodometric Method for Detection of β -Lactamase (Enzymatic Reaction at (4 °C)No.1 , No.2 , No.3: β - Lactamase from E.coli Clinical Isolates , No .4 : TEM -1β -Lactamase as Standard , No.5: β - Lactamase negative E.coil ATCC 25922 .

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 K_{12} SHV-1 β- lactamase Pitton (type II) coded for plasmid 453 I.p 7.7, E.cloacae P99 cephalosporinase I.p 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.

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

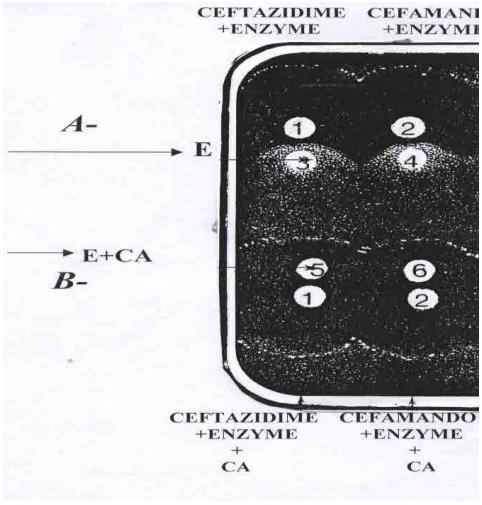
No. of Isolate		
E.coli	**Cefamandol**	**Ceftazidime**
1,2,3	512	`64
4,5,6	1024	32
7,8,9,10	2048	32
E.coli(453)[24] SHV-1(7.7)*	16	0.05
E.coli(R111) TEM-1(5.4)*	32	0.05
E.cloacae(P99)**(8.3)*		

*Isoelectric point , ** ephalosporinase

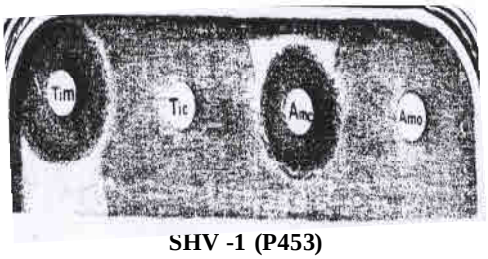
Inhibition of β– lactamases

Figure 2 shows comparisons 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 Amo\ CA, Tic\CA, Fig 5: show inhibition of enzymes by iodometric method.



Figure(2):Antibiotic – enzyme interactions, by the highly sensitive double disks technique [25] demonstrated the inhibition of β- lactamases by clavulanic acid. A and B: 1 and 2 -disks of Ceftazidime (Cfz) 30µg, and Cefamandol (Cfm) 30µg , 3 and 4 disks at a distance of 2 cm from Cfz and Cfm impregnated with (10µl) of enzyme(β- lactamase)5 and 6 disks impregnated with 10µl enzyme + 10µl Clavulanic acid (CA).



SHV -1 (P453)

Figure(3) A : Susceptibility disk diffusion method shows the activity of Augmentin (Ame) and Timentin (Tic) Against plasmid – mediated β - Lactamase produced by E.coli SHV – 1 Pitton type II(P453)[11]

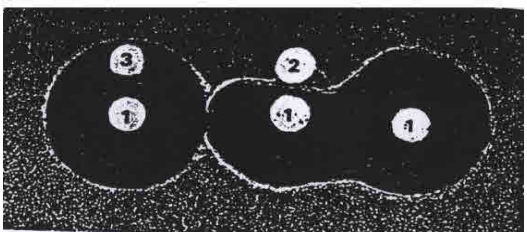


Figure (3) B : Action of inhibitor clavulanic acid (CA) on the activity of standard β-lactamase extracted from E.coli SHV-1 Pitton type 1: Ampicillin , 2: β - Lactamase 3:β-Lactamase+clavulanic acid.

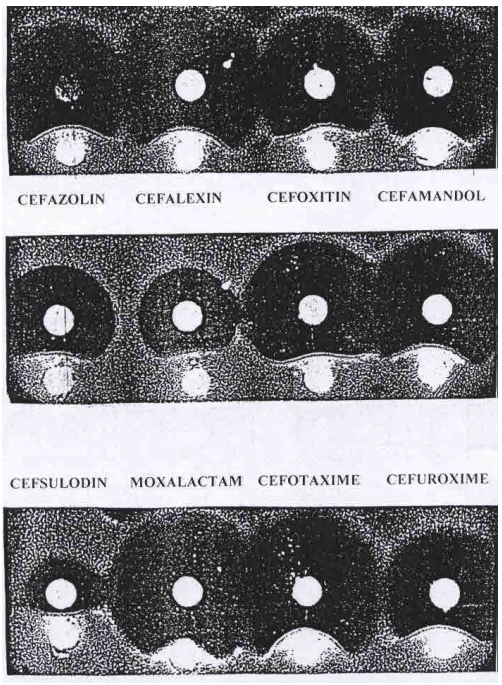


Figure (4) :Hydrolysis of Cephalosprins by Cephalosporinase (β – Lactamase) , extracted from E.cloacae P99 (According to Sirot , D,1983)

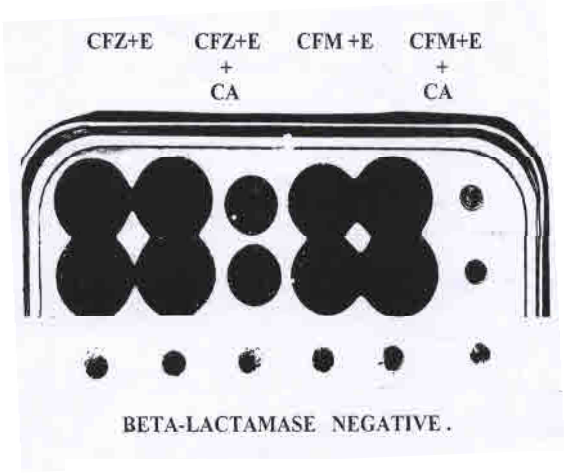
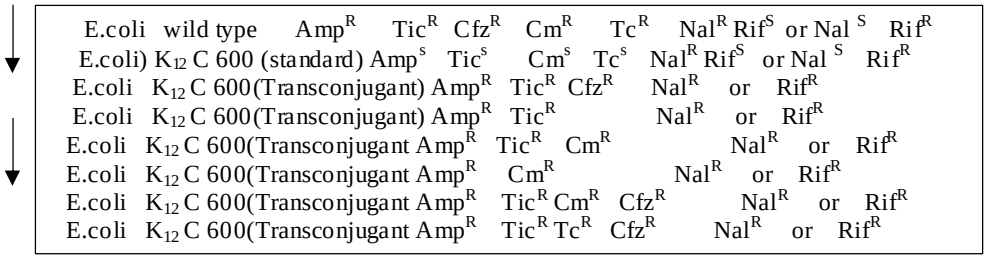


Figure (5): Inhibition of β - Lactamase of Clinical Isolates (E.coli)By Modified Iodometric Method Comparing With Standard Strain of E.coli ATCC 25922 β - Lactamase Non Producer

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 transferred resistance to Ticarcillin ,Ceftazidime, and other antibiotics after mating for 18hours, transconjugants derived from these strains produced β-lactamases, these results suggested that all strains bear plasmids and produce extended spectrum β - lactamasescapable of hydrolyzing and inactivating a wide variety of β-lactams including third generation cephalosporinspenicillins and Aztreonam , sensitive to imipenem , these result was similar to the studies of Rodrigues C , et al , Chaudhary U ,and Kurokawa ,-H et al [26,27,28] .

Conclusions

The clinical isolates in this study were very resistant to Augmentin , Cefamandol and Ceftazidime comparing with standard TEM – 1 and SHV – 1 (pasmidic pencillinases) , E. clocae P99 is very resistant to Cefalothin , Cefamandol , Cefotaxime , Ceftazidime 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 – spectrumβ-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 [6,7,9,10] . The increasing emergence cephalosporins resistant E.coli has leaded 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 (Ceftazidimase) producing complicated strains [22,23,24,28] .

REFERENCES

1. Reading C and Cole .M. Clavulanic acid : A beta – lactamase inhibiting from Streptomyces clavuligerus . Antimicrob . Agents and chemotherapy . 1977, 11 , N5, 852 – 857 .

2. Jensen , S- E ; Paradkar ,A-S ;Mosher , R – H ;Anders ; - C ;Beatty , -P –H ; Brumlik , - M – j ; Griffin , - A ; Barton , - B. Five additional genes are involved in clavulanic acid biosynthesis in Streptomyces clavuligerus . Antimicrob – Agents – Chemother 2004 Jan ; 48(1) : 192 – 202 .

3. Dumon L.,Adriaens P., Anne J.,Eyssen H.Effect of Clavulanic acid on the minimum inhibitory concentration of benzylpenicillin , ampicillin , carbenicillin ,or cefalothin against clinical isolates resistant to beata – lactam antibiotics.Antimicrob .Agents and Chemother .,1979 ,N2,315-317 .

4. Bush , K ., Jacoby ,G ., A and . Medeiros .A.,A . A functional classification scheme for beat – lactamases and its correlation with molecular structure . Antimicrobial agents chemotherapy 1995 p1211 -1233 .

5. PoeschI, -P-W ;Eckel , - D ; PoeschI, - E .Post operative prophylactic antibiotic treatment in third molar surgery – a nessity ? J-Oral – Maxillofac – surg .2004 Jan ; 62(1) :3-8; discussion 9IS .

6. Bradford , - P-A.Extended – spectrum beta - lactamases in the 21st century :characterization , epidemiology , and detection of this important resistance threat .Clin – Microbiol – Rev .2001 ; 14(4):933-51 .

7. Sturenburg ,-E; Mack , -D Extended – spectrum beta-lactamases :implications for the clinical microbiology laboratory therapy , and infection control .J-infect 2003 Nov;47(4):273 – 95 .

8. Bell ,- J – M , Turnidge , - J – D ; Jones , - R – N Prevalence of extended – spectrum beta-lactamase – producing Enterobacter cloacae in the Asia Pacific region :results from the sentry Antimicrobial Surveillance Program ,1998 to 2001,Antimicrob – Agents – Chemother .2003 Dec ; 47(12):3989 -93 .

9. Edelstein , - m ; Pimkin , - M ;Palagin , -I ; Edelstein ,-I ;Stratchounski,-L prevalence and molecular epidemiology of CTX-M extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae in Russian hospitals. Antimicrob-Agents-Chemother. 2003 Dec; 47(12): 3724-32 .

10. Bauernfeind, A., H. Grimm, and S.Schweighart. Anew-plasmidic cefotaximase in clinical isolate of E.coli. Infection. 1990 18: 294-298

11. Siham., S.S, Joly,B, . Phillipon ,A. , Sirot,D., Cluzel.R. Resistance al'a carbenicilline des bacilles a Gram- negatife, frequance, determinisme biochemique et genetique, 1985 Pathol ,Biol., 33, 825-829.

12. El-Sukhon,-S-N; Faiza-Boukhatem,-Z. Activity of combinations of ceftazidime, imipenem and pefloxacin against Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa. Int-J-Antimicrob-Agents. 2003 Dec; 22(6): 613-7

13. Bantar, C ; Di-Cniara-M;Nicola,-F; Relloso ,S,S, Smayevsky,Comparative in vitro bactericidal activity between cefepime and ceftazidime, alone and associated with amikacin, against carbapenem-resistant Pseudomonas aeruginosa strains. J. Diagn. Microbiol-infec. Dis. 2000; 37(1), 41-44.

14. Kamidono, S et al. Acomparative study on the clinical utility of cefozopran and cefpirome against complicated urinary tract infections. Jap.J. Antibiot.2000; 53(6): 430-450 .

15. Roland, -R-K; Mendes'-R-E; Silbert, -S; Bolsoni,- A-P;Sader, -H-S. In vitro antimicrobial activity of pipracillin\tazobactam in comparison with other broad-spectrum β-lactams. Braz-J-Infect-Dis. 2000; 4(5): 226-35.

16. Lister, P-D. β-lactamase inhibitor combinations with extended-spectrum penicillins factors influencing antibacterial activity against Enterobacteriacceae and Pseudomonas aerugionsa. Pharmacotherapy. 2000; 20(9 pt 2) 213S-218S; 224 S -228 S

17. Increasing prevalence of antimicrobial resistance among Enterobacteriaceae uropathogens in Dakar, Senegal: a multicenter study. Dromigny, -J-A; Ndoye, -B; Macondo, -E-A; Nabeth, -P; Siby, -T; Perrier-Gros-Claude, -J-D. Diagn-Microbiol-Infect-Dis. 2003 Dec; 47(4): 595-600

18. Cowan, S.T, Manual for identification of medical bacteria 1977. P106-Cambridge university press. Cambridge New York.

19. Baur A. W., Kirby, W. M.M., Sherris K.C. and Turck, M.Antibiotic susceptibility testing by a standardized single disc method.,Amer. J. clin.path. 1966, 45,493-496

20. Chabbert, Y.A. L antibiogramme. Ed De La Tourelle 1966, 56-87.

21. Labia, R., Barthelemy.M. L enzymogram des β-lactamases .A daptation en gel .La method iodometrique, Ann. Macrobiol., 1979, 130B, 236-240.

22. 22-- Ma, -Y; Li, -J-Y; Yao, -L; Zhang, -L; Hu,-C-Q; Jin,-S-H Zhonghua-Yi-Xue-Za-Zhi. Antimicrobial resistance of Escherichia coli isolates collected from inpatients and outpatients] 2003 Jun 25; 83(12): 1046-8

23. Tonelli,-F; Mazzei,-T; Novelli,-A; Mazzoni,-P; Ficari,-F
Amoxicillin/clavulanic acid versus cefotaxime for antimicrobial prophylaxis in abdominal surgery: a randomized trial . J-Chemother. 2002 Aug; 14(4): 366-72

24. Siham-SS. Marc O. Sirot-D- Joly-B. and Cluzel –R Spread of SHV-1 beta-lactamases in E.coli isolated from fecal samples in Africa.1987. Antimicro . agent and Chemother.vol 31 (6)943-945

25. Masuda G., Tomioka s and Haregawa M., Detection of β-lactamases production by Gram-negative bacteria.The journal of Antibiotics, 1976,vol 29,n6,662-664.

26. Kurokawa,-H; Shibata,-N; Doi,-Y; Shibayama,-K; Kamachi,-K; Yagi,-T; Arakawa,-Y-A new TEM-derived extended-spectrum beta-lactamase (TEM-91) with an R164C substitution at the omega-loop confers ceftazidime resistance. Antimicrob-Agents-Chemother. 2003 Sep; 47(9): 2981-3.

27. Rodrigues C, Joshi P, Jani SH, Alphonse M, Radhakrishnan R,Mehta A. Detection of beta-lactamases in nosocoial G-ve clinical isolates. Indian J.of Medical Microbiology 2004(22) 4,247-250

28. Chaudhary U, Aggarwal R. Extended spectrum beta-lactamases(ESBL)- An emerging threat to clinical therapeutic. Review article ,2004 (22) 75-80.