Effect of Inhibitors β-Lactamase on Recovery Effectiveness of Some β-Lactam Antibioticis Against Pseudomonas Aeruginosa

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Abstract

Thirty-four samples with position *Pseudomonas aeruginosa* cultures isolated from bourns, wounds urinary tract infection and Otities media were collected from Baquba General Hospital during September-December 2010. The sensitivily of these isolates were tested against (16) antibiotics. The results showed that the highest resistances were for Amoxicillin, Ampicillin, CO-Trimoxazole and Nitroforautoin with 100%, while the lowest resistance was for Ofloxacin with 3% .The results of minimum inhibitory concentration (M.I.C) toward eleven antibiotics showed different range among isolates, some were able to resist high concentration of Ampicillin and Amoxicillin reach to 1024µg/ml, while others were inhibited by 2µg/ml of Ciprofloxacin. The isolates showed low sensitivity for combination Ampicillin-Sulbactam with 0%, while it shwed high sensitivity toward combination of Piperacillin-Tazobactam and Ceftazidime-Clavulanic acid 91.17, 100% respectively. The results of plasmid content was studied indicate that all isolates contain single large plasmid band, while the study of plasmid curing appear the plasmid loss at concentration 512 µg/ml of acridin orange.

Key wards: Antibiotics, *Pseudomonas aeruginosa*, β-Lactamase inhibitors, Plasmid curing.

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Introduction

Pseudomonas aeruginosa is widely distributed in nature and commonly presents in moist environments of hospitals. It can colonize normal humans, in whom it is a saprophyte [1]. *Pseudomonas aeruginosa* and other *Pseudomonades* are resistant to many antimicrobial agents and therefore become dominant and important when more susceptible bacteria of the normal flora are suppressed [2]. *Pseudomonas aeruginosa* which is considered important bacterial species responsible for numerous nosocomial infections causes burn and post-operative wounds infections [3].

The extensive use of third and fourth generation cephalosporins as an important component of empirical therapy in intensive care units and high risk wards, resistance to these drugs has become amajor problem all over the world [4]. Resistance has developed in bacteria by possessing extended spectrum beta – lactamase (ESBLs) capable of hydrolyzing these newer cephalosporins [5,6]. Beta – lactamase mediated resistance may be overcome by combining beta – lactam antibiotics with beta – lactamase inhibitors which bind irreversibly to the beta – lactamases and render them inactive thus sparing the beta – lactam antibiotic [7].

In 2005 Using of beta-lactamase inhibitors in combination with beta-lactam antibiotics represents an effective measure to combat a specific resistance mechanism of beta-lactamase producing organisms [7]. In 2001 Three beta-lactamase inhibitors sush as Clavulanic acid, Sulbactam and Tazobactam are in clinical use, and in combination with beta-lactam antibiotics, represent a successful strategy to combat a specific resistance mechanism [8,9,10].

The aim of study is to illustrate the comparative *invitro* activities of three beta-lactamase inhibitors such as Clavulanic acid, Sulbactam and tazobactam against Beta-lactamase producing *Pseudomonas aeruginosa* causing different infections in Baquba Hospitals.

Materials and Methods

Activation of *Pseudomonas aeruginosa*

Thirty-four *Pseudomonas aeruginosa* isolated from various clinical samples(12 from urin , 9 from ear , 6 from wound , 7 from burn) collected from Baquba General Hospital over a period of 4 months (September 2010 to December 2010) were activated by brain heart infusion medium at37 C^0, 24 hour and 120r.p.m.

Antimicrobial susceptibility test and determination of MIC

Sixteen antibiotics including, Beta lactam group, Quinolones group and aminoglycoside group were used to testing sensitivity of *Pseudomonas aeruginosa*. The minimum inhibitory concentration (MIC) was determined for each bacterial isolate by an agar dilution technique on Mueller – Hinton agar plates, the antimicrobial agents were obtained from standard laboratory powders and were used immediately after their solubilization, the agents were Ampicillin, Amoxicillin, Cephalexin, Carbencillin, Cefotaxime, Ceftriaxone, Ceftazidime, Piperacillin. Results of susceptibility testing were recorded according to the guidelines of the National Committee for Clinical Laboratory standars [11] after incubation at 37ºC for 18h. The MIC was determined by using β-lactamase inhibitors including (Clavulanic acid, Sulbactam, Tazobactam).

Plasmid profile (Plasmid DNA analysis)

Plasmid DNA of the four isolates (PU5 (urin), PE20 (ear), PW27 (wound), and PB32 (burn)) are extracted using the Pure Yield™ Plasmid Miniprep Kit (Promega U.S.A). Plasmid DNA was analyzed by electrophoresis on 0.7% agarose gel containing o.5µg of ethidium bromide per ml (12).

Curing of plsmid DNA

Curing was conducted by using different concentrations of Acridin orange (16 , 32, 64 , 128 , 256 , 512 , 1024 , 2000 , 2500 , 3000) µg/ml (12,13).

Statistical analysis

Statistical analysis was carried out using t – test.

Results and Discussion

Determination MIC and antimicrobial susceptibility test of *Pseudomonas aeruginosa*

The sensitivily of these isolation were tested against [16] antibiotics. The results showed that high resistance of Amoxicillin, Ampicillin, CO-Trimoxazole and Nitrofourantoin with 100%. This result agrees with local studies by Al-Saffar [14]. And Abuduah *et al.* [15], who showed that resistance rates in *Pseudomonas aeruginosa* as 100%, figure (1). The resistance of Carbencillin was 93%, while *Pseudomonas aeruginosa* resists Cefotaxime, Cefriaxone and Ceftazidium with 88%, 85%, and 72% respectively**.** The results showed that *Pseudomonas aeruginosa* resists peperacillin with 73%, while resistance of aminoglycoside group including gentamicine, amikacin and tobramycin was 60%, 45% and 28% respectively. The isolates resists Norfloxacin, Ciprofloxacin and Ofloxacin

with 49%, 21%, 3% respectively. This resistance of different antibiotic due to the presence of multiple drug-resistant strains [16]. Antibiotic resistance has probably developed by the transfer of R plasmids from other drug-resistant enteric Gram-negative bacteria [17]; or because of its propensity to develop resistance during therapy [18].

The minimum inhibitory concentration (MIC) was determined for eleven antibiotics. The result showed that high resistance with 1024µg/ ml Ampicillin, Amoxicillin, Cephalexin and Carbencillin table (1), this result was agreed with [19], who found the resistance was512- 1024 µg/ml against these four antibiotics by

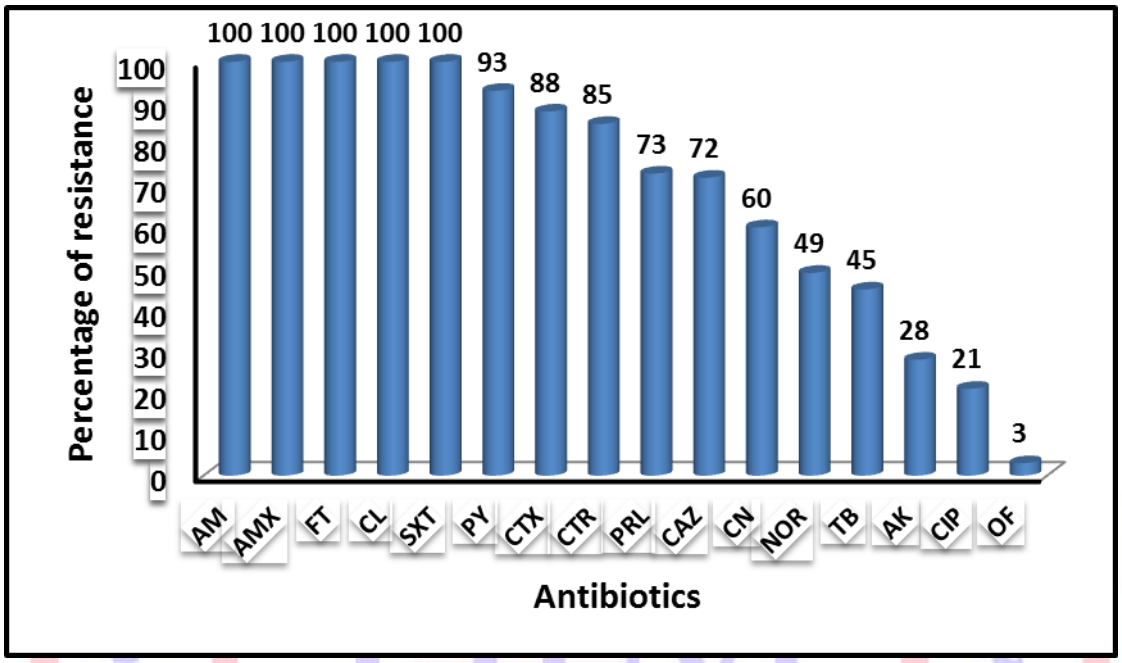

Figure (1): Percentage of antibiotics resistance.

Table (1): The minimum inhibitory concentration (MIC) of some antibiotics using against *P.aeruginosa*.

Antibiotic	Break point	M.I.C (µg/ml)
Ampicillin	≥ 32	512 – 1024
Amoxicillin	≥ 32	512 – 1024
Cephalexin	≥ 32	128 – 1024
Carbencillin	≥ 128	64 – 1024
Cefotaxime	≥ 32	16 – 1024
Ceftriaxone	≥ 32	16 – 1024
Ceftazidime	≥ 32	8 – 512
Piperacillin	≥ 128	32 – 512
Ciprofloxacin	≥ 4	1 – 64
Gentamicin	≥ 8	2 - 1024
Amikacin	≥ 32	4 – 256

All isolates of *Pseudomonas aeruginosa*.The lower value of resistance was toward Ciprofloxacin with 1-64 ug/ml. The results was agreed with local studies by (15), who showed that MIC value by *P.aeruginosa* was 1-16 µg/ ml.

The minimum inhibitory concentration MIC was determined by using β-lactamase

inhibitors including (Clavulanic acid, Sulbactam, Tazobactam). In this study antibiotic mixed with clavulanic acid at percentage 1:4 and use of three commercially available beta-lactam/ beta lactamase inhibitor combinations: piperacillin/ tazobactam (Tazocin), ampicillin/ sulbactam (Sulba) and amoxicillin/clavulanic acid (Augmentin). The values of (M.I.C) for β-Lactam antibiotics (Amoxacillin, Carbencillin, Cephalexin, Cefotaxime, Ceftriaxone, Ceftazidime, Pipracillin) were decreased at the presence of β-Lactamase inhibitors. Results showed that (100%) of *Pseudomonas aeruginosa* isolates were sensitive to Ampcillin – Sulbactam and Amoxicillin / Clavulanic acid with (0%, 26.47) respectively table (2) Fig (2), while these isolates showed sensitivity against (Carbenicillin / Clavulanic acid, Cephalexin/ Clavulanic acid, Cefotaxim/ Clavulanic acid and Ceftriaxone/ Clavulanic acid with (41.17,32.35,73.52,79.41)% respectively table (2) fig (3,4,5,6). The results indicate that isolates were sensitive toward Pipracillin / Clavulanic acid, Pipracillin – Tazobactam, and Ceftazidime – Clavulanic acid with (85.29 %, 91.17%) and (100%) respectively table (3) fig (7,8,9). The results were agreed with (20; 21; 22), who found that use of these combination lead to increase sensitive of *Pseudomonas aeurginosa*. These results indicate that combination have synergistic effect. This effect explain by fact that inhibitors beta lactamse enzymes is weak antibiotics and contains a ring-like-lactam antibiotics makes beta- lactamase enzymes attack this ring and leave antibiotic free[23].

Table (2): The percentage of beta-lactam / beta lactamase inhibitor combinations against Pseudomonas *aeruginosa*.

AntibioticInhibitor	Percentage of sensitive isolates (%)	Percentage of resistance isolates (%)
Ampicillin	0	100
Ampicillin / Sulbactam	0	100
Amoxicillin	0	100
Amoxicillin / Clavulanic acid	26.47	73.52
Carbenicillin	5.88	94.11
Carbenicillin / Clavulanic acid	41.17	58.82
Cephalexin	0	100
Cephalexin / Clavulanic acid	32.35	67.64
Cefotaxim	17.64	82.35
Cefotaxim / Clavulanic acid	73.52	26.47
Ceftriaxone	23.52	76.47
Ceftriaxone/ Clavulanic acid	79.41	20.58
Ceftazidime	41.17	58.82
Ceftazidime/ Clavulanic acid	100	0
Pipracillin	35.29	64.70
Pipracillin / Clavulanic acid	85.29	14.70
Pipracillin / Tozabactam	91.17	8.82

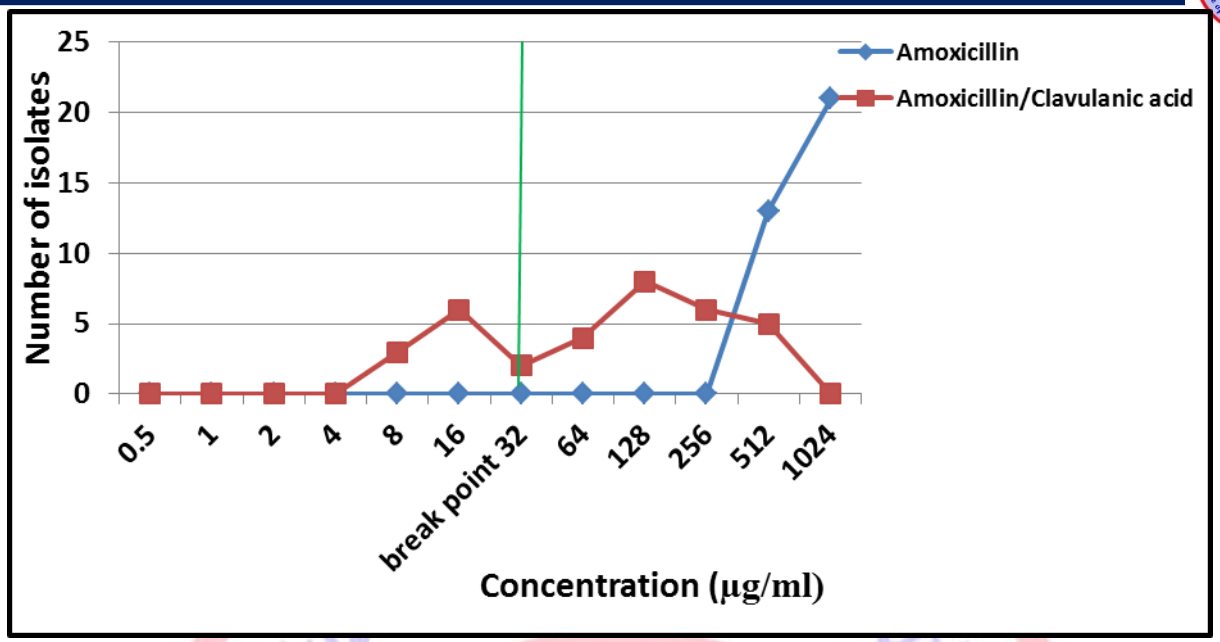

Figure (2): Synergism effect of Amoxicillin / Clavulanic acid against *Pseudomonas aeruginosa* isolates (*** * P<0.05, 0.01)**.

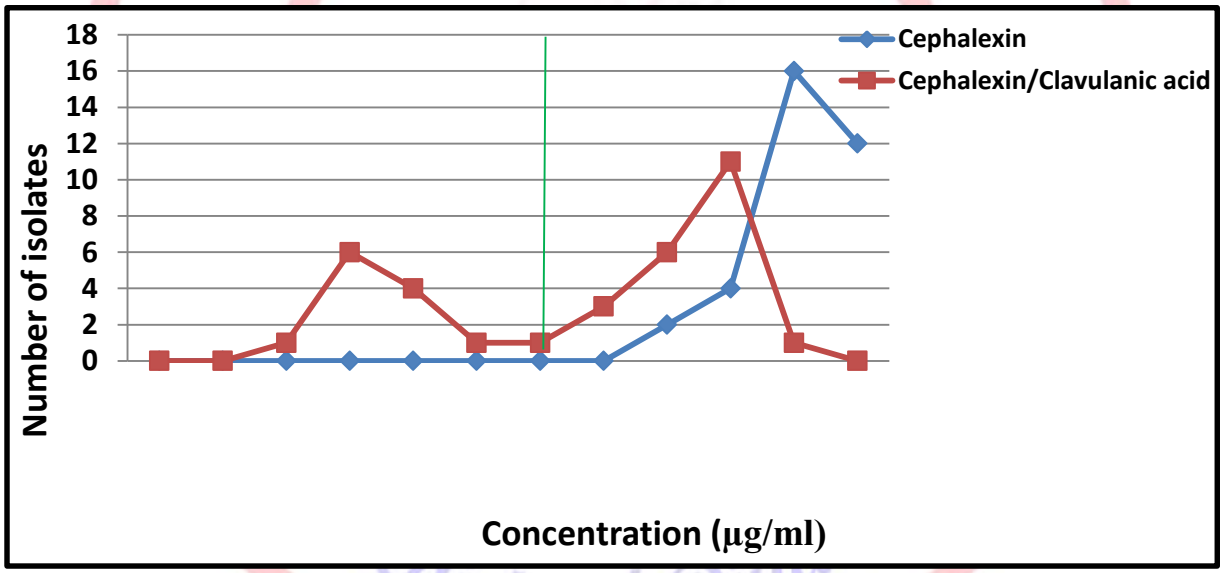

Figure (3): Synergism effect of Cephlexin / Clavulanic acid against *Pseudomonas aeruginosa* isolates (*** * P<0.05, 0.01)**.

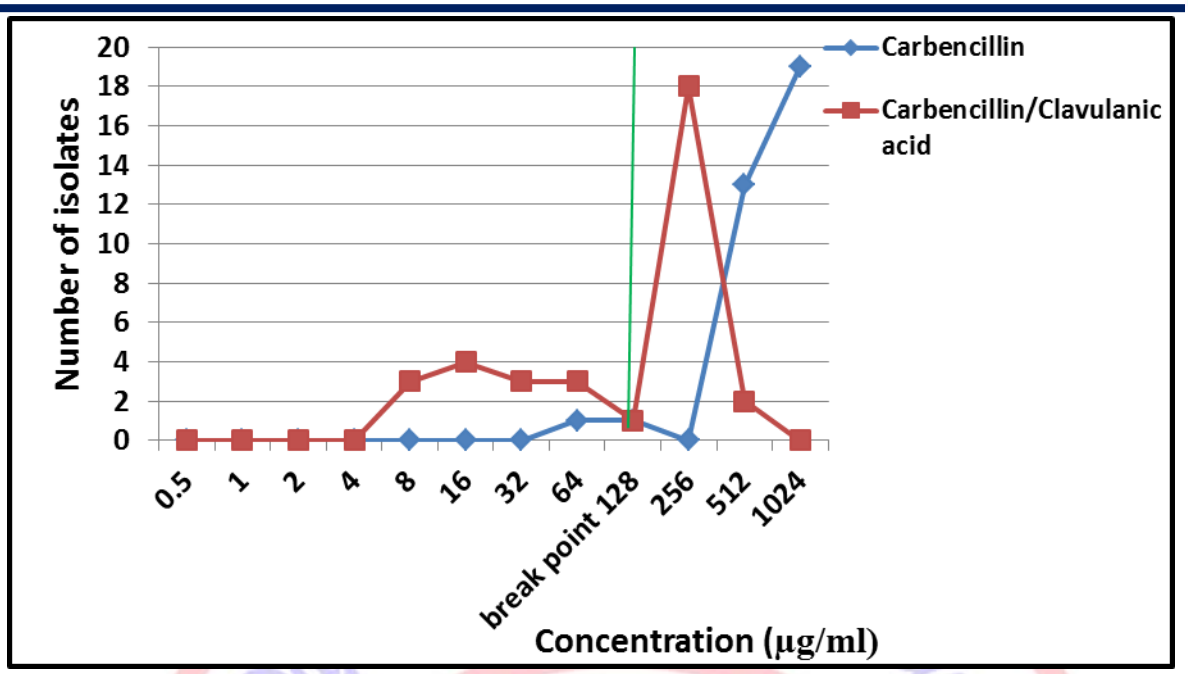

Figure (4): Synergism effect of Carbencillin / Clavulanic acid against *Pseudomona aeruginosa* isolates (*** * P<0.05, 0.01)**

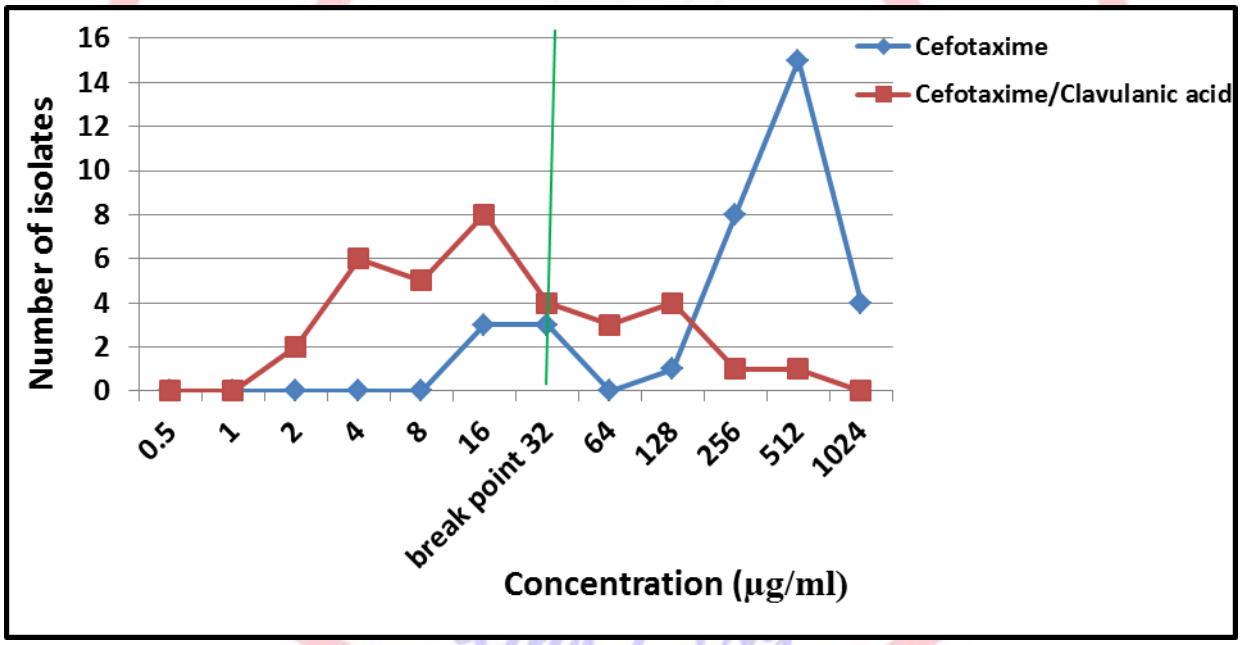

Figure (5): Synergism effect of Cefotaxime / Clavulanic acid against *Pseudomonas aeruginosa* isolates (*** * P<0.05, 0.01)**.

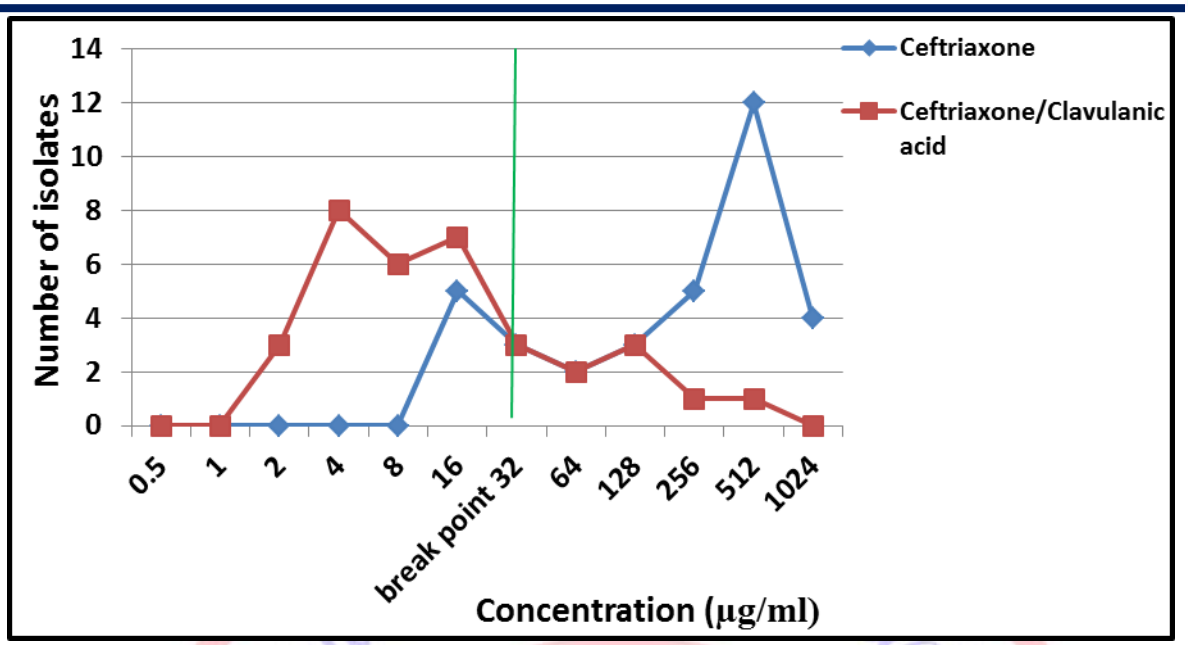

Figure (6): Synergism effect of Ceftriaxone / Clavulanic acid against *Pseudomonas aeruginosa* isolates (*** * P<0.05, 0.01)**.

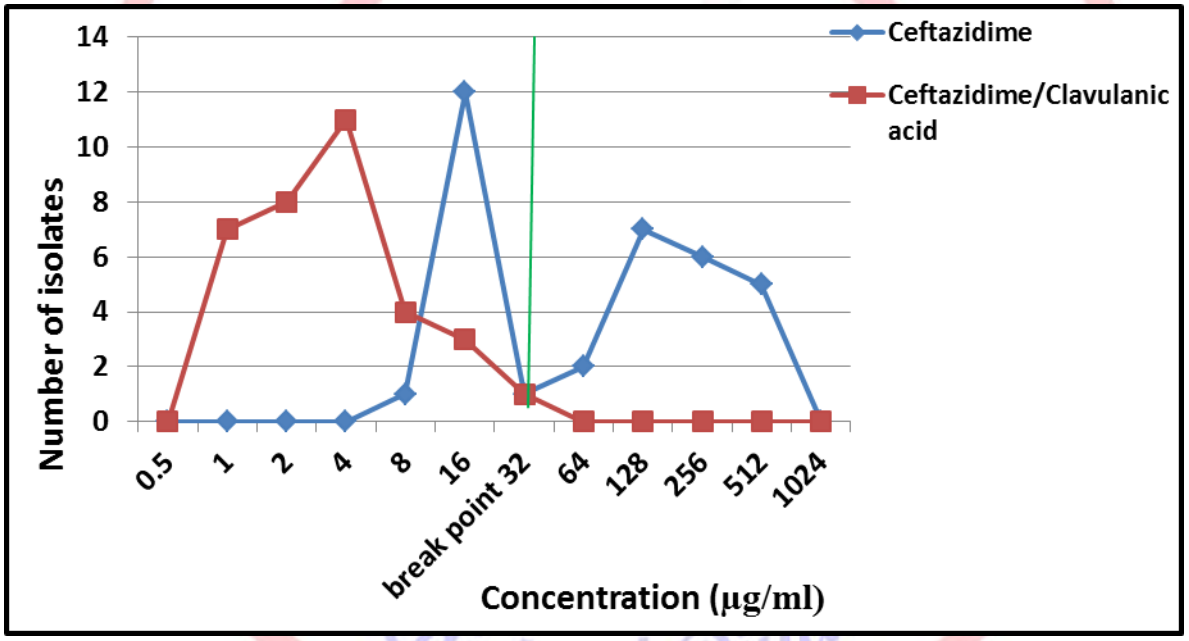

Figure (7): Synergism effect of Ceftazidime/ Clavulanic acid against *Pseudomonas aeruginosa* isolates (*** * P<0.05, 0.01)**.

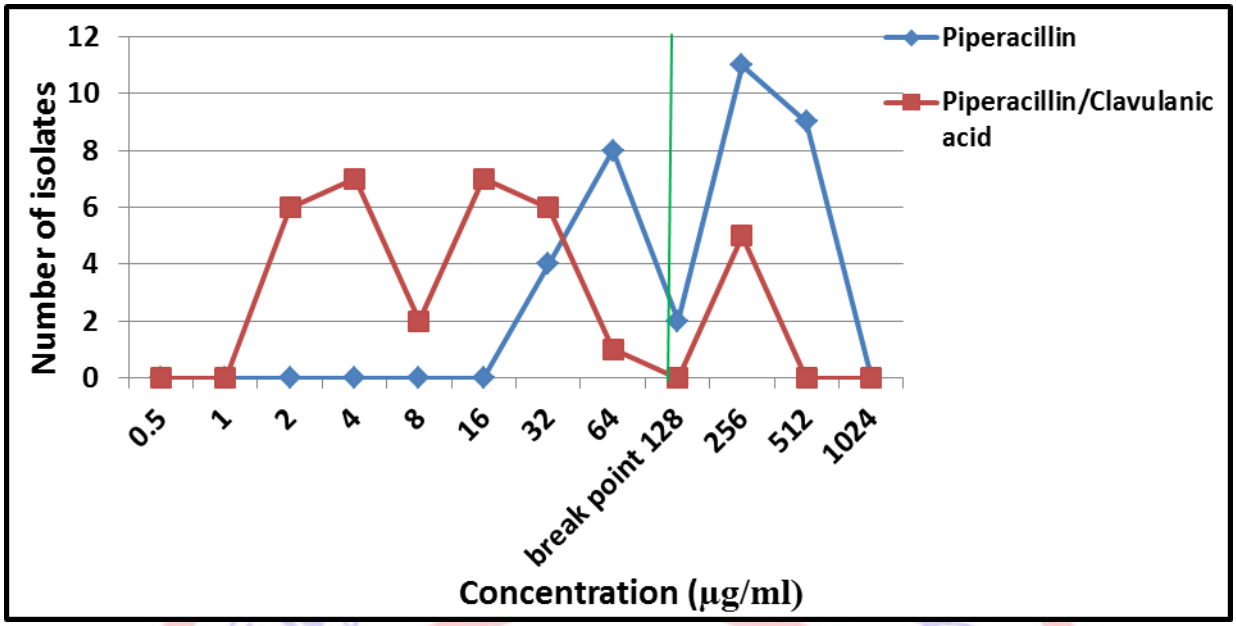

Figure (8): Synergism effect of Piperacillin / Clavulanic acid against *Pseudomonas aeruginosa* isolates (*** * P<0.05, 0.01)**.

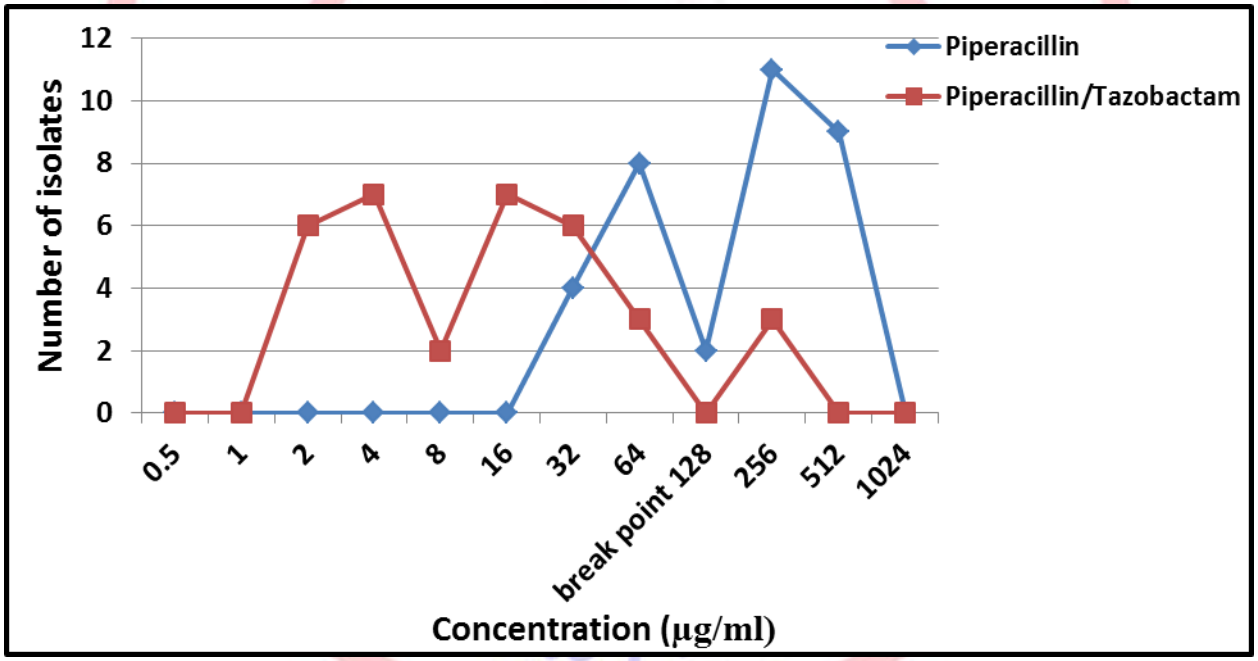

Figure (9): Synergism effect of Piperacillin / Tazobactam against *Pseudomonas aeruginosa* isolates (*** * P<0.05, 0.01)**.

***Pseudomonas aeruginosa* plasmid profile**

The plasmid –DNA content for four isolates was detected, findings showed that isolates have one (large) plasmid band table (4) figure (10). This result was agreed with (24), who showed that *Pseudomonas aeruginosa* contain one mega plasmid.

Table (4): Plasmid content of *Pseudomonas aeruginosa* isolated from different clinical sources.

Number of isolate	Site of infection	Number of Plasmid band
PU5	Urin	1
PE20	Otities media	1
PW27	Wound	1
PB32	Burn	1

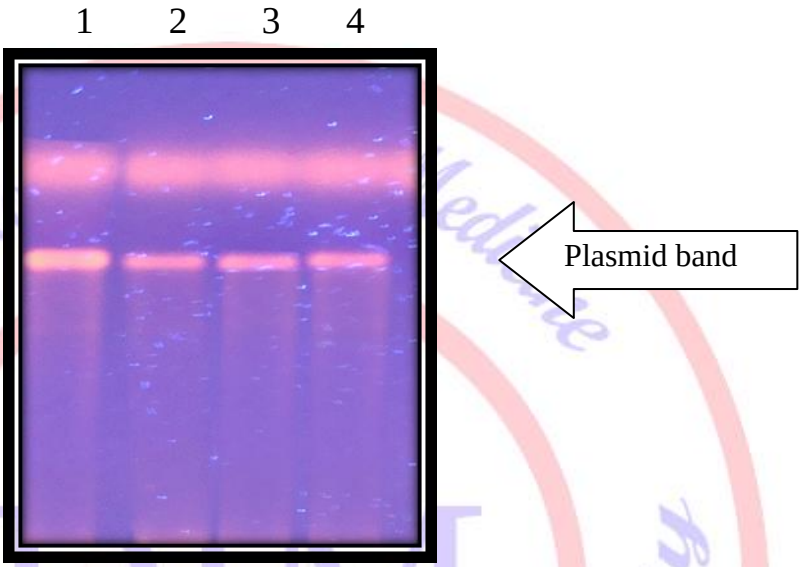

Figure (10): Agarose gel electrophoresis of plasmids from *Pseudomonas aeruginosa*.
(1) Plasmid content of PU5 isolate (2) Plasmid content of PE20 isolate
(3) Plasmid content of PW27 isolate (4) Plasmid content of PB32 isolate

Plasmids curing

Acridin orange was used in order to cure plasmids of *Pseudomonas aeruginosa*. The result showed the best concentration was 512 ug/ml, which able to cure plasmids from all isolates. The results was agreed (partially) with (21), who found the best concentration was 1024 figure (11).

Figure (11): Losing of plasmid band from curing *Pseudomonas aeruginosa* isolates.

Conclusions

The study shows that the combination of β-lactams / β-lactamase inhibitors is highly effective in treatment of *Pseudomonas aeruginosa* infections. Ceftazidime/ Clavulanic acid has the best activity against nosocomial *Pseudomonas aeruginosa* followed by Piperacillin/Tazobactam.

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