CLINICAL IMPORTANCE OF BETA-LACTAMASE PRODUCED BY SOME GRAM-NEGATIVE AND GRAM-POSITIVE BACTERIA

ATHESIS

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CERTIFICATION

We certify that the preparation of this thesis titled ***Clinical Importance of β-lactamase Produced by Some Gram- negative and Gram- positive Bacteria*** was under our supervision at the University of Babylon in partial fulfillment of the requirements for the degree of Master of Science in Microbiology

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DEDICATION

TO MY

Husband, Kareem;

Lovely son,Mustafa;

Father and mother;

Sister; and brothers

With Love and Respect

Rasha

ACKNOWLEDGEMENT

I pray Allah for his help to give me power to conduct this work.

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List of Abbreviations

AMC	Ampiclox (Ampicillin+Cloxacillin)
AMO	Amoxicillin
AMP	Ampicillin
Amox.Clav	Amoxicillin+ Clavulanic acid)
CARB	Carbencillin Hydrolysing Enzyme
CEF	Cefazolin
CL	Cephalexin
CTX	Cefotaxime
CTX -1	Cefotaxime Hydrolysing Enzyme
CTZ	Ceftizoxime
EDTA	Ethylen Diamine Tetra Acetic Acid
IP	Isoelectric Point
P	Penicillin
PBPs	Penicillin Binding Proteins
PCMB	p- Chloromercuribenzoate
PIP	Pipracillin
PSE	Penicillin Sensitive Enzyme
OXA-1	Oxacillin Hydrolysing Enzyme
SA	Salicylic acid
SHV	Sulfohydryl Variable
TEM	Temonera

Abstract

Fourty pathogenic isolates of Gram-negative and Gram-positive strains have been subjected to diagnostic and differential investigations: eighteen isolates of *Klebsiella*, five of *E. coli*, five of *Ps. aeruginosa*, seven of *E. faecalis* and five of *S. aureus* and *S. epidermidis*. A number of common β-lactam antibiotics such as Penicillin, Ampicillin, Amoxicillin, Ampiclox, Pipracillin, Cephalexin, Cefazolin, Cefotaxime, Ceftazim, (Amoxicillin + clavulanic acid). have also been used. The results show that *Klebsiella* was 100% resistant to Penicillin, Ampicillin, Amoxicillin; the rest of the antibiotics show remarkable variations in respect of resistance ratio. It has also been noticed that the *E. coli* is 100% resistant to Penicillin, Pipracillin, Cephalexin and Cefotaxime; the rest of the antibiotics have resisted in lesser degrees. Besides, the Staphylococci have revealed remarkable resistance to Penicillin, Ampicillin, Cephalexin, Cefazolin (100%). As for the rest of the antibiotics, the percentage has been different and variable. It has also been found that the *E. faecalis* has resisted at a 85.7% ratio to each of penicillin and ampicillin, and at lesser degrees to the other antibiotics. The *Ps. aeruginosa* has shown 100% resistance to all antibiotics except pipracillin where the percentage has been 60%.

The (amoxicillin + clavulanic acid) has also been the choice to investigate the effect of clavulanic acid on the β-lactamase enzyme. It has been found that nine isolates (out of the forty isolates) have resisted this compound: one isolate of *Staphylococcus*, one of *Klebsiella*, two of *E. coli* and five of *Ps. aeruginosa*. Other chemical compounds such as salicylic acid and EDTA(ethylen diamin tetra-acetic acid) have been used to investigate their effects on microorganism activity including that of β-lactamase production. It has been noticed that the (Salicylic acid) increases the resistance of the bacteria to the antibiotics where the EDTA reduces their resistance since it has an effect on metallo-β-lactamase.

The rapid iodometric method has been employed to investigate the production of beta-lactamase. Twenty-one isolates have revealed positive results: 4 isolates of *Staphylococci*, one of *E. faecalis*, nine of *Klebsiella*, three of *E.coli*, and five of *Ps*. *aeruginosa*. The activity of β-lactamase enzyme has been evaluated in respect of some of the enzyme- producing isolates by using the Macroiodometric method. It has been seen that there are remarkable differences in the specific activity of this enzyme. Other environmental factors such as temperature and pH have been used to study their effects on the enzyme activity. It has been found that the optimal activity of β-lactamase for all isolates is at temperature ranging between (35 -37) C^{o}, and pH between (6.5 - 7.5).

Introduction

Resistance to antibiotics is considered one of the most common world problems economically as well as medically. The excessive wrong use of antibiotics has resulted in the appearance of new strains of bacteria characterized by their resistance to a variety of antibiotics, such as beta-lactam (Goth, 1984). The microorganism resorts to resist the activity of the antibiotics by different mechanisms, i.e., producing destructive enzymes that are able either to hydrolyze the molecules of the antibiotics or modify them in such a way as to make them inactive (Jacoby and Sutton, 1985). Among these enzymes are the Beta-lactamase which are associated with resistance to beta-lactam antibiotics and found in most bacterial species.

The clinical problems caused by beta-lactamase in certain bacteria are being recognized with increasing frequency. These problems include the rapid emergence of multiple beta-lactam resistance during therapy with many of the newer beta-lactam antibiotics. Such multiple resistant organisms are now spreading within the hospital and have become important nosocomial pathogens. This has been a particularly difficult problem for Intensive Care Units, and Burn Units where there are clusters of patients who are highly susceptible to infections microorganisms like *Enterobacter*, *Serratia*, and *Pseudomonase*

aeruginosa, which possess inducible beta-lactamases. Only through an awareness of these problems, their cause, and restriction of the use of certain newer beta-lactam antibiotics can these problems be controlled (Sanders and Sanders, 1987).

These enzymes belong to those that modify or destroy the antibacterial agent that may be produced. When present, Beta-lactamase will destroy beta-lactam antibiotics that penetrate the outer membrane. They can also inactivate the beta-lactams before they reach their target, hence protecting the cells from the action of beta-lactams. The ability or inability of beta-lactamases to confer resistance depends on their location, kinetics, quantity and their physiochemical conditions. These enzymes are produced by gram positive and gram negative bacteria. In gram positive bacteria, the Beta-lactamase are largely extracellular by contrast to those of gram negative bacteria which are larger periplasmics, although some extracellular release may occur, mediated by leakage rather than secretion (Livermore, 1995).

The commonest producers of beta-lactamases in gram-positive bacteria are: *Staphylococci* which are the only common gram-positive pathogens in which beta-lactamases cause major resistance problems*, Enterococcus*, many Bacilli, *Clostridium* and *Nocardia* (Lacey and Stokes, 1977).On the other hand, in gram-negative bacteria, the commonest producers for these enzymes are *E. coli*, *Klebsiella*, *Pseudomonas*, *Morganella*,

Providencia Stuartii, and *Proteus* species (Livermore, 1995). These enzymes have attracted the attention of many research workers due to their significance. It has not been discovered yet which among these enzymes have the ability to modify their activity due to modification in their amino acid sequence as in the case in beta-lactamase (Jacoby, 1994).Many studies have been conducted to study these enzymes, especially those related to genetic factors controlling their production. The genes encoded for these enzymes are either located on bacterial chromosomes or on the plasmids, however, these genes are also seen to be found on transposon (Bush, 1989; Bush *et al*., 1995).

Most epidemics, especially among in- patients admitted to hospital, are caused by strains producing enzymes encoded by plasmids. Most these strains are able to resist more than one antibiotic at a time (Katsanis *et al*., 1994, Urban *et al*., 1994). The risk of these enzymes has increased after mutations in genes responsible for their encoding, which could modify into extend spectrum enzyme resisting to the majority of antibiotic active against beta-lactam i.e. third generation of cephalosporins (Thomson, 1995).

Despite the importance of beta-lactamase, the available studies in Iraq are very few if not really absent. The genetic factors that control their production have not yet been modified in local isolates.

Aims of Study

This study aimed to:

1- **Study the ability of some local Gram-negative and Gram- positive bacteria to produce β-lactamase under certain circumstances by using different generation types of substrates.**
2- **Determine whether the enzyme is produced constitutively or inducibly.**
3- **Study the effect of substrate-inhibitor combination on enzyme activity.**
4- **Show the effect of temperature and pH on enzyme activity.**

CHPTER ONE

Literature Review

1.1 Literature Review

Beta-lactamase are enzymes which catalyze the hydrolysis of an amid bond in a beta-lactam ring of antibiotics belonging to the penicillin / cephalosporin family.(Moremen Lab Homepage BCMB 8010 Homepage Contact Ritesh Tandon 2004, WWW.β-lactamase/ Background. htm)

1.1.1 Beta-lactamase Discovery

In 1940, Abraham and Chain issued an article in *Nature Journal*, and stated that *bacillus coli* bacteria (currently known as *E. coli*) produced an enzyme that inhibited penicillin antibiotics, called penicillinase. The first enzyme of beta-lactamase that has the ability to destroy the beta-lactam antibiotics was discovered in 1941. Datta and Kontomichalon (1965) isolated the first enzyme that belongs to TEM-1 (Temonera) family. From *E. coli* bacteria, the two researchers succeeded in the same year in isolating another strain of *E. coli* that could produce enzymes that hydrolyze cloxacillin and oxacillin antibiotic, named OXA enzyme.

In 1971, Sykes and Richmond purified TEM-2 from *pseudomonas aeruginosa*. At the beginning of 1970's, other isoenzymes were also isolated and could be distinguished by their sensitivity to sulfohydryl reagents which were called

SHV-1 (sulfohydryl variable) (Neu, 1985). SHV-2, on the other hand, was derived from SHV-1 which was produced and purified from cefotaxime resistant *Klebsiella ozaenae* strains (Philippon, *et al*., 1989). In 1984, a strain of *Klebsiella pneumonia* was found resistant to cefotaxime antibiotics due to the production of an enzyme called TEM-3 and derived from TEM-1 (Ibid.).

1.1.2 Beta-lactamase Classifications

The first classification of beta-lactamase enzymes depending on its activity was put by Fleming, *et al*., (1963) in which the beta-lactamases were divided into two groups:

a. Penicillinase group, which hydrolyzes penicillin group antibiotics.
b. Cephalosporinase group, which hydrolyzes cephalosporin group antibiotics.

In 1973, Richmond and Sykes proposed a classification system for beta-lactamase in gram-negative bacteria. They subdivided the enzyme into five classes: Class-I enzyme is predominately a cephalosporinase, Class-II is a penicillin class, Class-III enzymes show a broad spectrum activity and are sensitive to inhibition by cloxacillin but resistant to p-chloromercuribenzoate (PCMB), Class-IV is also a broad spectrum enzyme but resistant to inhibition by cloxacillin and sensitive to (PCMB), and finally Class V enzymes, the

penicillinases, that are able to hydrolyse cloxacillin and resistant to PCMB inhibition. Sykes and Mattew (1976) proposed two major groups: Classes A and B that are subdivided into three subclasses. Class A enzymes are chromosomally mediated and subclassified into: (a) penicillinases, (b) cephalosporinases, and (c) broad spectrum beta-lactamases. Class B enzymes are determined by R-plasmid and subclassified into (a) isoxazolyl-non-hydrolysing, (b) isoxazolyl-hydrolysing, and (c) other beta-lactamases.

In 1980, a classification system was proposed by Ambler, and was extended by Jaurin and Grund Strom (1981), and Bush (1988). The system included beta-lactamases from gram-positive and gram-negative bacteria, and it was based on the amino-acid sequence of active site of the enzymes. Three classes, A, B and C exist. Classes A and C contain beta-lactamase that operate via the serine ester mechanism to disrupt the beta-lactam ring, whereas Class B contains only beta-lactamase from *Bacillus cereus* and is related to bacilli which require bivalent metal cautions such as Zn^{+2} (Weidmann, *et al.*, 1989).

Plasmid-coded beta-lactamases can be distinguished by isoelectric focusing (Mattew *et al*., 1975), and on the isoelectric point (IP), more than 30 different enzymes have been described (Mattew, *et al*., 1979; Foster, 1983; Medeiros, 1984). Bush (1989) divided the enzymes into four groups. These are A, B, C and D depending on substrate profile, inhibition profile and physical characteristics. Groups A and B contain subgroups. The

more recent classification of this enzyme was proposed by Bush, *et al*., (1995), who made some arrangement, on the 1989 classification through adding new subgroups to group B.

1.1.3 Action of Beta-lactamases

A few beta-lactamases utilize zinc ions to disrupt the beta-lactam ring, but far greater number operates via the serine ester mechanism. See Fig (1).(Livermore, 1995).

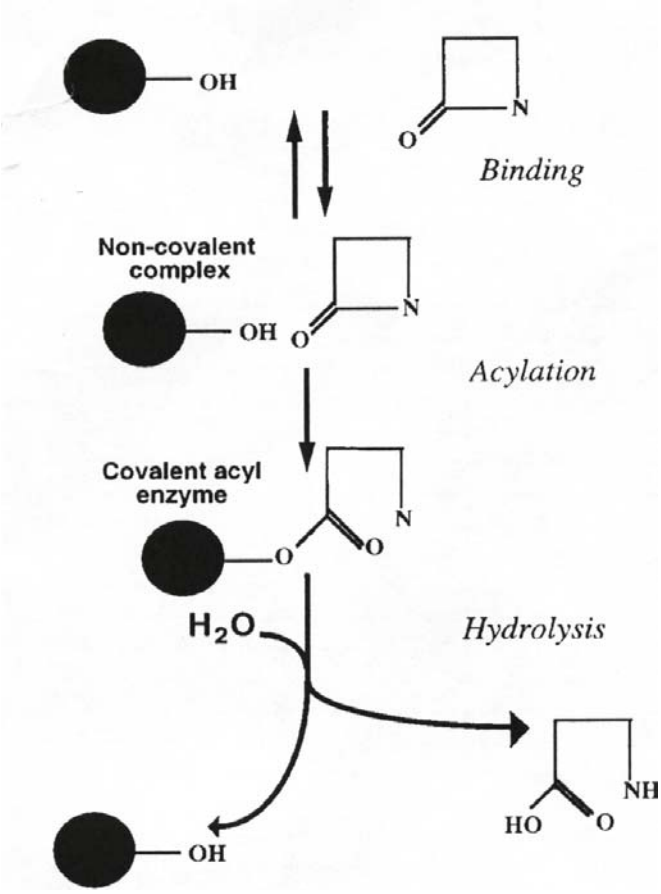

Fig. (1): Action of serine beta-lactamase. The enzyme first associates noncovalently with the antibiotics to yield the noncovalent Michaelis complex. The beta-lactam ring is then attacked by the free hydroxyl on the side chain of a serine residue at the active site of the enzyme, yielding a covalent acyl ester. Hydrolysis of the ester finally liberates active enzyme and the hydrolyzed, inactive drug. This mechanism is followed by beta-lactamases of molecular classes A, C, and D, but class B enzymes utilize antibiotics a zinc ion to attack the beta-lactam ring (Walley, 1992).

1.1.4 Resistance to Beta-lactam Antibiotics

There are three different mechanisms by which bacteria can acquire resistance to beta-lactam antibiotics.

(1). Reduction in permeability of outer membrane of gram-negative bacteria to the antibiotics. It is a simple method in which the cell tries to prevent antibiotic from reaching the (PBPs) in the cytoplasmic membrane. As a means of resistance, this is not very effective.(Spratt,1989)

(2). Beta-lactamase production. These enzymes hydrolyse a bond in beta-lactam molecules, preventing their capability of binding to the bacterial (PBPs) (Russell and Chopra, 1996). In general, Beta-lactamases greatly resemble (PBPs) in form and function, and they are typically characterized as being plasmid-determined or chromosome determined. There are two distinct ways in which beta-lactamases may have their effects.(Ibid)

(2-a) In gram-negative bacteria, the enzymes are screted into the periplasmic membrane and the PBP-harboring cytoplasmic membrane. This is an ambush strategy in which the antibiotic molecules are sequentially destroyed upon crossing the outer membrane by way of porin channels.(Medeiros,1989)

(2-b) Gram-positive bacteria employ different methods for using beta-lactamase in resistance. This enzyme is produced upon introduction of beta-lactam antibiotic. It is excreted into the medium where it can reduce beta-lactam concentration. If the concentration gets low enough, the growth of the bacteria will be permitted (Medeiros, 1989).

The chromosome determines beta-lactamase. Nearly all of the gram-negative bacteria that have been studied produce chromosomal beta-lactamases. Therefore, there is a great diversity in these enzymes, but, in general, their function as the plasmid-determined beta-lactamases (Sanders,1989).

(3). Modification of penicillin binding proteins (PBPs) which can be classified into three classes (Russell and Chopra, 1996).

(3-a) Altering the affinity of PBPs for beta-lactam antibiotics.

(3-b) Alteration of PBPs nature, modifies production of PBPs.

(3-c) Alteration PBP to acquire more resistant PBP through homologous recombination. Acquisition of resistance through PBP alteration can usually be expected when selection pressure is applied, and bacterium is replaced as a section of its DNA with more resistant section from another bacterium (Spratt, 1989).

1.1.5 Beta-lactamase in Gram-positive Bacteria

Beta-lactamase in gram-positive bacteria differs from those of gram-negative organisms in an important characteristic. In contrast to these enzymes from the gram-negatives, which are located in the periplasmic space, the β-lactamase of gram-positive bacteria are excreted into the medium and, therefore, destroy the antibiotics extracellularly.

1.1.5.1 Staphylococci

It is gram-positive cocci usually arranged in group-like irregular clusters. They grow readily on many types of media and produce pigments that vary from white to deep yellow. It was non motile and does not form spores (Hackborth and Chambers, 1989). The genus Staphylococcus has at least 30 spp.

There are three main spp. of clinical importance; they are *S. aureus*, *S. epidermidis* and *S. saprophyticus* (Jawetz *et al.*, 2001).

S. aureus is a major pathogen in humans, isolated from various infections. It is coagulase positive, which differentiates it from the other spp., and it usually forms grey to deep golden yellow colonies (Hackbarth and Chambers, 1989).

S. epidermidis is coagulase-negative, normal human flora, sometimes causes infection, and usually forms grey to white colonies (Kloos and Bannerman, 1994). It has been found that most strains of *Staphylococcus aureus, S. epidermidis* have the ability to resist beta-lactam antibiotics due to the production of beta-lactamase enzyme.

Staphylococci have no chromosomal β-lactamases. Chromosomally mediated resistance to β-lactam antibiotics is due to other mechanisms such as alteration of PBP (Williams, *et al.*, 1983).

Plasmid coded beta-lactamases are predominant in *Staphylococcus aureus* which are the most commonly isolated spp. not only in community but also in hospital acquired infections. About 70% of all strains show resistance to β-lactam antibiotics (Kresken and Wiedemann, 1987).

There are four immunologically distinct enzymes produced by *Staphylococcus aureus* named A, B, C and D (Richmond, 1965). These enzymes can be located on different transposons

and therefore can integrate in the bacterial chromosome (Lyon and Skurray, 1987).

Coagulase-negative Staphylococci also produce plasmid coded β-lactamase in nearly 100% of resistant strain (Rosdahl, *et al.*, 1986).

The functions and characteristics of the four types β-lactamases produced by staphylococci are similar to those mentioned by Fluit, *et. al*., (2001).

Group A: It is staphylococcal penicillinase conferring high resistance to penicillin. The extended-spectrum β-lactamases also belongs to this class and it inactivates benzylpenicillin as well as cephalosporins and/or monobactam.

Group B: Metallo β-lactamases confer resistance to carbapenems and all β-lactam classes except monobactams. They are not inhibited by clavulanic acid.

Group C: They confer resistance to all classes of β-lactams, except carbapenems (unless combined with porin changes).They are not inhibited by clavulanic acid.

Group D: They are composed of the OXA-type enzymes which can hydrolyze cloxacillin (Oxacillin). They are modestly inhibited by clavulanic acid. All these types of staphylococcal β-lactamase are produced inducibly.

Batchelor, *et. al*., (1963) studied the cellular location of staphylococcal β-lactamase and preparation of cell-free enzyme and kinetic properties of the enzyme. He found that the km with

benzypenicillin substrate varied depending on the strain of *S. aureus* used. One of the earliest indications is that different types of β-lactamase are produced by staphylococci.

Staphylococcal penicillinase are largely surface-attached and serve to reduce the external drug level.

1.1.5.2 Streptococcus

Streptococcus is gram-positive spherical bacteria that characteristically form pairs or chains during growth. They are widely distributed in nature. Some are members of the normal human flora; others are associated with important human diseases. One of colony growth characteristics is to hemolyse patterns on blood agar (α-hemolysis, β-hemolysis, or no hemolysis) (Jawetz, *et al*., 2001).

1.1.5.2.1 *Enterococcus faecalis*

They are gram-positive cocci, and react with group D antisera so they are part of the normal enteric flora. They are usually nonhemolytic and occasionally α-hemolytic. They are found capable of resisting penicillin G. due to the production of β-lactamase enzyme constitutively (Gordon, *et. al*., 1992).

Recently, plasmid-mediated β-lactamase has been isolated in strains of *Enterococcus faecalis* (Murray and Mederiski-Samraj, 1983). It is class (A) β-lactamase which confers high

resistance to benzylpenicillin. Nevertheless, this enzyme is relatively rare and has been found in only a few strains of *Enterococcus faecalis* (Murray and Patterson, 1988; Patterson, *et. al*., 1988). No chromosomal – mediated β-lactamase has been detected in enterococci (Wiedemann, *et. al*., 1989).

1.1.6 Beta-Lactamase in Gram-negative Bacteria

There are many mechanisms by which gram-negative bacteria may exhibit reduced susceptibility to β-lactam antibiotics, reducing outer membrane permeability. Other mechanisms include Target-site modification (alteration of PBPs), and efflux of the β-lactam out of the cell (Nikaid, 1994; Sawek, *et. al*., 1991). But the most common mechanism of resistance is the enzymatic inactivation of β-lactam by β-lactamase (Livermore, 1995).

Most gram-negative bacteria produce β-lactamase that hydrolyze penicillin and cephalosporin. Enterobacteriaceae and pseudomonadaceae come in advance (Jacoby and Sutton, 1985). The productions of these β-lactamases are easily detected in *Ps. aeruginosa* (Sykes and Mattew, 1976). They are so called species-specific β-lactamases (Mattew, *et. al*., 1975).

On the other hand, more than thirty different plasmid-coded β-lactamases have been isolated. The dissemination of

some of these enzymes are prevalent among *Ps. aeruginosa* and enterobateriaceae (Wiedemann, *et. al*., 1989).

1.1.6.1 Enterobacteriaceae

There are large heterogenous groups of gram-negative rods whose natural habitate in the intestinal tract of humans and animals. The family includes many genera (e.g. *Escherichia, Klebsiella, Salmonella, Shigella,* and others).

1.1.6.1.1 *E. coli*

E. coli are parts of the normal flora and incidentally cause disease; they are the most common cause of urinary tract infection. It was found that this bacterium has the ability to resist β-lactam antibiotic due to β-lactamase production.

The first β-lactamase was identified in *E. coli* prior to the release of penicillin for use in medical practice (Abraham and Chain, 1940).

Chromosomal-mediated β-lactamases (species-specific β-lactamases) of *E. coli* are mainly cephalosporinases which are not inducible, but were produced constitutively in small amounts (Weidemann, *et. al*., 1965).

The first plasmid-mediated β-lactamase in gram-negatives is TEM-1, and it was described in the early 1960's (Datta and Kontomichalou, 1985). It was originally found in a single strain

of *E. coli* isolated from blood culture from patient named Temoniera in Greece, hence the designation TEM Medeiros, (1989). Within a few years after its first isolation, the TEM-1 β-lactamase spread worldwide and is now found in many different species of Enterobacteriaceae. Up to 90% of *E. coli* is resistance to ampicillin due to the production of TEM-1 (Livermore, 1995). This enzyme is able to hydrolyze penicillin and early cephalosporin such as cephalothin and cephaloridine.

TEM-2 and TEM-3 were the first derivatives of TEM-1 type (Sougakoff, *et. al*., 1988). Over 90 additional TEM derivatives have been described.

The other plasmid-mediated β-lactamase in *E. coli* is SHV-1 which confers resistance to ampicillin. It also has CTX-M β-lactamase which hydrolyzes cefotaxime. This type includes many derivatives such as CTX-2 through CTX-10. Bradford, (2001); Seibert and Limbert, (1982) described another β-lactamase in *E-coli* which is able to hydrolyze the third generation of cephalosporins. This enzyme has an altered activity against β-lactam antibiotics *E-coli* and has also a specific enzyme that can be isolated from a single isolated of *E-coli* in patients in Vietnam (Naas, *et. al*., 1999).

1.1.6.1.2 *Klebsiella pneumonia*

It is gram-negative, rod and present in the respiratory tract and feces at about 5% of normal individuals. It can cause various infections in humans such as hemorrhagic necrotizing, consolidation of the lung-respiratory tract infection and bacteremia. It is naturally resistant to ampicillin and amoxicillin, usually by the production of SHV-1 β-lactamase encoded on the chromosom or transferable plasmid (Nugent and Hedges, 1979). It is unlike the TEM-1 type β-lactamase, since there are relatively few derivatives of SHV-1 (Bradford, 2001).

In recent years, functional group 1 β-lactamase have found their way on to plasmid and are being expressed constitutively at high level in *K. pneumonia* and *E. coli*. These enzymes were described as cephalosporinases which are not inhibited by clavulanate (Bradford, 1997). *K. pneumonia* also contain specific enzyme belonging to class A β-lactamase that are chromosomally encoded and confer resistance to ampicillin, pipracillin and, to some extent, early cephalosporins (e.g. cephalothin).TEM-1 was also found in *K. pneumonia* but is most prevalent in *E. coli*.

1.1.6.2 *Pseudomonas aeruginosa*

They are gram-negative, motile, aerobic, rods, and some of which produce water-soluble pigments. They are widely distributed in nature and, are commonly present in moist

environment in hospitals. It was found that the bacteria have the ability to resist β-lactam antibiotic due to β-lactamase production (Jawetze, *et. al.*, 2001).

Ps. aeruginosa has an inducible Ampc enzyme, some of which produce β-lactamase constitutively (derepressed mutant) (Livermore, 1995).

OXA-type β-lactamase have been found mainly in *Ps*. *aeruginosa*. It belongs to the molecular class D and confers resistance to ampicillin and cephalothin. These enzymes are characterized by their high hydrolytic activity against oxacillin and cloxacillin and the fact that they are poorly inhibited by clavulanic acid (Bush, et. al., 1995). Several of the OXA-type ESBLs have been derived from OXA -10 (OXA-11, -14, -16, and -17) (Danel, *et. al.*, 1999); (Hall, *et. al*., 1993).

There are specific enzymes of β-lactamase in *Ps*. *aeruginosa* first discovered in strains of *Ps. aeruginosa*, and isolated from patients in Turkey. They confer resistance to ceftazidime (Nordman, *et. al*., 1993).

PSE-(CARB-) enzymes are mainly distributed in *Ps*. *aeruginosa.* PSE-1, (CARB-2) have been found frequently in carbenicillin-resistant *Ps. aeruginosa* strains, whereas PSE-4 (CARB-1) and PSE-3 have not been isolated very often (Jacoby and Matthew, 1979). Recently, enzymes CARB-3 and CARB-4 have been found only in two strains of *Ps. aeruginosa* (Phillipon, *et. al*., 1986b).

1.1.7 Beta-lactamase at Molecular Level

Beta-lactamases are divided according to the molecular level into:

(a) Chromosomal beta-lactamase.

(b) Plasmid-mediated beta-lactamase.

(c) Transposon-mediated beta-lactamase.

1.1.7.1 Chromosomal beta-lactamase

Most spp. of gram-negative and gram-positive bacteria can produce chromosomal mediated beta-lactamase related to the class A beta-lactamase according to Richmond and Sykes's classification in 1973. It prefers cephalosporin antibiotics as substrate (Brown, 1975). These enzymes are divided into:

A. Constitutive beta-lactamase

These enzymes are always synthesized in the bacterial cell without need to activation by substrate. The production amount of this enzyme is between very little as in *Haemophilus* (Sykes and Mattew, 1976), and normal or high as in *Ps. aeruginosa* (Sanders *et al*., 1988). This amount is determined by enzyme activity. Other bacterial spp. that produce constitutive chromosomal enzyme are:

(a) *K. oxytoca* (Arakawa, *et al*., 1989).

(b) *Mycobacterium fortuitum* (Amicosante, *et al*., 1990).

(c) *Citrobacter diversus* (Frances Chini, *et al*., 1991).

(d) *Ps. strutzeri* (Frances Chini, *et al*., 1993).

B. Inducible beta-lactamase

This differs from the first group by need for inducer to increase its production. This enzyme was called high inducible (Sanders, *et al*., 1988). These enzymes have high affinity with cephalosporin antibiotics, and are not inhibited by normal inhibitor such as clavulanic acid, Tazobactam, Sulbactam, but are inhibited by cloxacillin, p-chloromercuribenzoate (Bush, 1989). Many spp. of gram-negative bacteria can produce these enzymes such as *Enterobacter*, *Serratia* spp., and ps. *aeruginosa* (Thomson, 1995).

1.1.7.2 Plasmid-mediated beta-lactamase

It is the most important enzyme in gram-negative bacteria, and is related to Group 2 according to Bush's classification 1995. It is a constitutive and hydrolysis penicillin and cephalosporin antibiotic inhibited by most known inhibitors (Clavulanic, Tazobactam, Sulbactam) (Bush, *et al*., 1995). Many bacterial spp. produce this enzyme such as *E. coli*, *P. mirabilis*, *Klebsiella*, *ps. aeruginosa, Acinetobacter,* and *H. influenzae* (Bush, 1989; Thomson, 1995). These enzymes are listed in Table (1).

The problem is more sophisticated when the genes encoding β-lactamase production are present on the plasmids because some of these plasmids are transmissible and can

transfer from one bacterium to another and provide the latter with the ability to produce β-lactamase enzymes. These transmissible plasmids are widely spread among gram-negative bacteria particularly those belonging to Enterobacteriaceae family (Katsanis *et. al*., 1994; Urban *et. al*., 1994).

Table (1) shows plasmid mediated beta-lactamase

I TEM-type	
Ia Penicillinase	**Ib Broad-spectrum beta-lactamase**
TEM-1	TEM-3
TEM-2	TEM-4
TLE-1	TEM-5
SHV-1	TEM-8
LCR-1	TEM-7
HMS-1	SHV-2
ROB-1	SHV-3
OHIO-1	
BRO-1	
II OXA-type (Oxacillin-hydrolysing beta-lactamase)	
OXA-1	
OXA-2	
OXA-3	
OXA-4	

OXA-5-	
OXA-6	
OXA-7	
(OXA-4)[a] (PSE-2)	
II1 CARB-(PSE) type (Carbenicillin-hydrolysing beta-lactamase)	
CARB-1 (PSE-4)	
CARB-2 (PSE-1)	
CARB-3	
CARB-4	
PSE-3	
AER-1	
SAR-1	
IV Others	
CEP-2	
NPS-1	
Staph. aureus A, B, C, D	
Cefoxitin-hydrolysing enzymes from *Bact. fragilis*	

1.1.7.3 Transposon-mediated beta-lactamase

Hedges and Jacobs in 1974 observed that Tns could transfer resistance to ampicillin from one plasmid to another or from plasmid to chromosome and vice versa.

Transposon (Tn) contains genes which can encode for the production of many type of β-lactamase enzymes that may make the bacteria reveal the resistance to more than one type of antibiotics. The most common types of Tn are those related to family which can encode for TEM-1, 2 enzymes.

1.1.8 Beta-lactamase Inhibitors

Beta-lactamase inhibitors are proteins designed to inhibit or destroy the effectiveness of beta-lactamase enzymes. Inhibitors generally have little antimicrobial properties themselves and so are combined with a beta-lactam antibiotic (Leiker,2000). These inhibitors (Calvulanic acid, Sulbactam, Tazobactam) function by binding to the beta-lactamase enzymes more "efficiently" than the actual beta-lactam antibiotic itself. This combination allows the antibiotic "do its job" without being degraded by the beta-lactamase enzymes. Several antibiotics/ beta-lactamase inhibitor combination exist on the market. However, only Augmentin (amoxicillin and clavulanate) is available for use in pediatric Aom. Roye *et al*., in 1989 have shown that Calvulanic acid with amoxicillin when used together

have a sufficient effect on the beta-lactamases enzymes and also on the target bacteria (Augmentin) (Leiker, 2000).

The inhibitors used against β-lactamase enzymes are very specific; however some bacterial strains can produce more than one isoenzymes, so the effect of such inhibitors becomes limited

On the other hand, some inhibitors can be modified by the some bacterial strains, such strains always search for another means of resistance to β-lactam antibiotics as that observed by *Pseudomonas aeruginosa* which can resist to all antibiotics used for treatment of infections caused by this bacterium through inducting new means for resistance to antibiotics.

CHAPTER TWO

Materials and Methods

Materials and Methods

2.1 Materials

2.1.1 Chemical Materials

Table (2) shows the chemical materials used in this study.

Table (2)

No.	Chemical materials	Sources
1	Potassium phosphate, hydrus sodium phosphate, unhydrus sodium phosphate, iodine and potassium iodine, sodium hydroxide, Alcohol, sodium potassium tartrate, sodium thiosulfate, salicylic acid,Tetra-p-paraphenylene diamine dihydrochoride, α-naphthol, methyl red.	B. D. H.
2	Acetic acid.	Al-Tharthar
	EDTA	Gainland Chemical Company U.K.
4	Ethanol (99%)	Chemika-Switzerland
5	Kovac's reagent (p-methylaminobenzaldehyde)	Fluka
6	Kliglar iron, MR-VP	Difco

2.1.2 Antibiotics Powder

Table (3) shows the antibiotics powders used in this study.

Table (3)

Antibiotics powder	Sources
A- Penicillin groups	
Penicillin G.	Oxoid
Amoxicillin.	Allepo
Ampiclox.	Aleppo
Ampicillin.	S.D.I. - Iraq
Pipracillin.	S.D.I. - Iraq
Amox-clav (Clavulanic acid+ Amoxicillin)	Hikma
B- Cephalosporin groups	
Cephalexin	S.D.I. - Iraq
Cefazolin	Aleppo
Cefotaxime (Claforan)	Franc
Ceftizoxime	Franc

2.1.3 Culture Media

Table (4) shows the culture media used in this study.

Table (4)

Culture media	Sources
Blood agar base	Oxoid
Macchonkey broth	Oxoid
Macchonkey agar	Oxoid
Muller-Hinton agar	Oxoid
Nutrient broth	Difco
Nutrient agar	Difco
Brian-hart infusion	Difco
Tryptone broth	Difco
Urea base	Difco
Kliglar iron agar	Difco

2.1.4 Instruments

Table (5) shows the instrument used in this study.

Table (5)

Instruments	Sources
Incubator	Memmert
Oven	Memmert
Autoclave	Stermite
Centrifuge	Hermle
Water bath	Gungurd
Spectrophotometer	Gungurd
Refrigerator	Gungurd
Balance	S.E.R.
Sensitive balance	S.E.R.

2.2 Methods

2.2.1 Solution and Culture Media

2.2.1.1 Solution

(a). (Clavulanic acid + Amoxicillin)

This solution is prepared by dissolving 1 g of amox-clav. in 100 ml distal water. The final concentration is 10mg/ml.

(b). Beta-lactam antibiotic solution

This solution is prepared by dissolving 500 mg of antibiotics powder (Penicillin, Ampicillin, Pipracillin, Cephalexin, Amoxicillin, Cefazolin, Cefotaxime and Ceftizoxime) in 50 ml distal water. The final concentration is 10mg/ml (Maniatis, 1982). All antibiotic solutions are sterile by filter.

(c). Penicillin G solution

This solution is prepared by the preparation of 2 stocks (A, B).

Solution A: dissolve 0.907g of KH_2PO_4 in 100 ml distal water.

Solution B: dissolve 0.946 g of Na_2HPO_4 and 1.19 g $Na_2HPO_4.2H_2O$ in 100 ml distal water.

Later, mix (87.6 ml of solution A with 12.31 of solution B, adjusted pH = 6). Then dissolve 0.5693 g of penicillin G in phosphate buffer solution pH = 6, and sterile it by filter (WHO, 1978).

(d). Iodine solution

Dissolve 2.03 g of iodine and 5.32 potassium iodine into 100 ml D. W (WHO, 1978).

(e). Phosphate buffer solution pH = 7

It is prepared by the preparation of 2 stocks (A, B).

Solution a: dissolve 31.2 g of 0.2 ml/L $Na_2HPO_4.2H_2O$ in liter distal water.

Solution b: dissolve 28.39 g of 0.2 ml/L Na_2HPO_4 in liter distal water.

Later mix 39 ml of A with 61 ml of B (Colle et al., 1996).

(f). Acetate buffer solution pH = 4

It is prepared by the preparation of 2 stocks (A, B).

Solution A: dissolve 11.55 ml of acetic acid in liter distal water.

Solution B: dissolve 16.4 g of 0.2 ml/L $C_2H_3O_2Na$ or 27.2 g $C_2H_3O_2Na.3H_2O$ in liter distal water.

Later mix 41 ml of A with 9 ml of B (Colle, *et al*., 1996).

(g). Iodine reagent

It is prepared by dissolving 0.0166M of iodine with 0.06M of potassium iodine in 1.75 M sodium acetate buffer pH = 4 (Branka Bedenic, 2002).

(h). Starch solution

It is prepared by dissolving 1 g of starch in 100 ml D. W. and putting it in water bath for 10 minutes (WHO, 1978).

(i). Methyl red reagent: 0.1gm of Methyl red was dissolved in 300 ml of 99% ethanol and then the volume was completed to 500 ml by D.W. (Macfaddin, 2000)

(j). Voges - Proskauer reagent

Reagent A) 5gm of α-naphthol was dissolved in 100 ml of 99% ethanol.

Reagent B) 40gm of KOH was dissolved in 100 ml of D.W. (Collee *et. al*., 1996).

(k). Oxidase reagent: It was prepared by dissolving of 0.1 gm of Tetra-P-paraphenylene diamine dihydrochloride in 10 ml of D.W. and stored in dark container (Baron *et. al*., 1996).

(m). Catalase reagent: It was prepared by dissolving 3 gm of H2O2 to 100 ml of D.W. and stored it in dark container (Baron *et. al*., 1996).

2.2.1.2 Culture Media

It is prepared according to the company information that supplies it, then sterile in autoclave under 121 C^o, 1.5 g for 15 minutes.

1. Blood agar: Prepare blood agar base and sterile it in autoclave then cool to reach its temp. 50 C^o, adding peapul blood until the final concentration is 5%.
2. Macchonkey broth and Macchonkey agar.
3. Muller-Hinton agar.
4. Nutrient broth and nutrient agar.

5. Brian-hart infusion.
6. Tryptone broth
7. Urea base
8. Simon citrate agar
9. Kliglar iron agar
10. MR-VP broth

2.2.2 Identification of Isolates

Forty isolates were collected from College of Medicine, and subjected to diagnostic, microscopic and biochemical differential tests.

2.2.2.1 Biochemical Tests

(a). Catalase Test: Acolony of the organism was transferred to a drop of 3% H2O2 on a microscope slid. The presence of catalase was meant that the formation of gas bubbles has occurred which indicated the positive result (Collee *et. al.,* 1996).

(b). Oxidase Test: Apiece of filter paper was saturated in a petri dish with oxidase reagent then a colony of organism was spread onto the filter paper. When the color around the smear turned from rose to purple, the oxidase test was positive (Collee *et. al.,* 1996).

(c). Coagulase Test: Several colonies of bacteria were transferred with a loop to a tube containing 0.5ml of humen plasma. The tube was covered to prevent evaporation and

incubated at 37°C overnight. The test was read by tilting the tube and observing for clot formation in the plasma. Negative test results in the plasma remained free- flowing with no evidence of a clot (Collee *et. al.,* 1996).

(d). Indol Test: A 1% solution of tryptone broth was prepared in the tubes it was sterile into the autoclave. After that the broth inoculated with bacterial colonies and it was incubated for 48-72 hours at 37°C. Testing for indole production was done by adding 6-8 drops of Kovac's Reagent (p-dimethylaminobenzaldehyde in amyl alcohol). The formation of red color ring at top of broth was positive reaction. A yellow color ring was a negative result (Macfaddin, 2000).

(e). Methyl Red Test: The test was performed on 5 ml of MR-VP broth cultured by the organism and then it was incubated for 24 hours at 37° C. After that the 6-8 drops of Methyl Red reagent were added to culture. The change of color to orange-red was a positive reaction (Collee *et. al.,* 1996).

(**f). Voges-Proskaur Test**: The test was performed on 5 ml of MR-VP broth cultured by the organism and then it was incubated for 24 hour at 37° C. After that 15 drops of 5% alpha naphthol (reagent A) were added followed by 10 drops of 40% KOH (reagent B) and shaken well allowed standing for up to 30 minutes before calling a reaction negative. The

positive culture was turning to red at the surface of the liquid, and the color was spread gradually throughout the tube (Baron et. al., 1996).

(g). Simon Citrate Test: After the sterilization of Simon Citrate slants by autoclave, inoculated the bacterial cultures and incubated it for 24-48 hours at 37^{o}C. The positive result was change of the color of media from green to blue. The unchanging of the color was negative reaction (Benson, 1998)

(h). Urease Test: The urea base agar was sterilized by autoclave. After cools it to 50^{o}C, the urea substrate was added to it and was poured in sterile tubes; then inoculated to bacterial cultures and it incubated for 24-48 hours at 37^{o}C. The positive result was a deep pink color. Failure of deep pink color to develop was a negative reaction (Benson, 1998).

(i). Kliglar Iron Agar (KIA) Test: The aim is to differentiate the Enterobacteriaceae according to carbohydrate fermentation and hydrogen sulfide production. The organism grown on KIA slant by stab and streak and then it was incubated at 37^{o}C for 24-48 hours. The changing of the color of media from orange-red to yellow was due to carbohydrate fermentation with or without gas formation at butt of slant. In addition, the formation of hydrogen sulfide was given a black color precipitation at butt (Macfaddin, 2000).

(j). Esculin Test: The organisms were grown in an Esculin slants. The dark brown color was the positive result. The unchanging of the color was a negative reaction (Capell *et.al.,* 1995).

(k). Mannitol Salt Agar: The medium was turned from pink to bright yellow when the bacteria were Mannitol fermented and the test was positive (Macfaddin, 2000).

(l). Eosin Methylene Blue (EMB) Agar: Lactose fermenting colonies were either dark or possess dark centers with transparent colorless peripheries, while organisms that did not ferment lactose remain uncolored. This purple color was due to the absorption of the eosin-methylene blue complex, which formed in the presence of acid. Certain members of the coliform group, especially *E. coli*, exhibited a greenish metallic sheen in the reflected light(Collee *et. al.,* 1996).

(m). Motility Test by Using Semisolid media: 10 ml of semisolid media was dipensed in test tube and leave to setten vertical position, inoculated with a straight wire, making a single stab down the center of the tube to about half the depth of the medium. The culture was incubated after 37^{o}C and examine at 6 hours, 1and 2 days. Non-motile bacteria had generally confined to the stab- line and given sharply defined margins with leaving the surrounding medium clearly transparent motile bacteria were typically given diffuse hazy

growths that spread through out of the medium rendering it slightly opaque (Macfaddin, 2000).

2.2.3 Sensitivity Test for Beta-lactam Antibiotics on Solid Media

Muller-Hinton agar is prepared and sterilized by autoclave and then supplemented by the required antibiotics at final concentrations as mentioned in table (6) the plates are then inoculated by the bacteria by using (picking and patching method) and incubated for 24 h at 37C^{o} (Maniatis, 1982).

Table (6-A) shows Penicillin group and their concentration as used in this study

P. μg/ml	Amp μg/ml	Amox. μg/ml	Amc. μg/ml	Pip. μg/ml	Amox-clav. μg/ml
100	100	100	100	100	100

P=pencillin, Amp=ampicillin, Amox=amoxicilin, Amc=ampiclox,Pip=pipracillin, Amox-clav=(amoxicillin+ clavulanic acid)

Table (6-B) shows Cephalosporin group and their concentration as used in this study

CL	CFZ	CTX	CFX
100	100	100	100

CL=cephalexin, CFZ= cefazolin, CTX= cefotaxime, CFX= ceftizoxim.

The same method is used to test beta-lactamase inhibitors (EDTA 100 µg/ml, salicylic acid 100 µg/ml).

2.2.4 Test for Beta-lactamase Production

The rapid Iodometric Method was used for the detection of the ability of the bacteria to produce beta-lactamase (WHO, 1978).

1. Prepare a new bacterial culture Twenty-four hours later, transfer some colonies to an Ependrof tube (3 ml capacity) which containes 100 µl of penicillin G solution and incubate this tube at 37 C^{o} for 30 minutes then add 50 µl of starch solution and mix it well with the tube content.
2. Twenty µl of iodine solution is added to this tube; the resulting colour is dark blue due to the reaction between starch and iodine.
3. The tube is finally shaking for one minute: if the colour changes from dark blue into white after less than one

minute from adding the indicator (iodine), the result is considered positive.

4. The test is repeated if the white colour appears later.

2.2.5 Beta-lactamase Assay and Enzyme Activity

A macroiodometric method was used for measuring the enzyme activity to crude extracellular beta-lactamase enzyme. This method included the following steps:

1. Preparation of crud enzyme: prepare a new bacterial culture, that is in 24 h old, the new growth is put in liquid media that contains an antimicrobial solution (cephalexin), then put the culture in centrifuge at 10000 g, for 15 minutes, then take the supernatant.
2. Cephalexin is used as substrate, prepared as 1 ml of this drug in 0.05 M phosphate buffer pH = 7.
3. Test and control flask containing 5 ml of substrate are equilibrated at 37 C^{o} in water bath before adding one ml of enzyme to each flask.
4. Following a reaction period of 30 minutes, 10 ml of iodine reagent is then added to stop the enzymatic reaction.
5. A further incubation period of 20 minutes is required for the completion of the reaction between the hydrolysis products and iodine.
6. One ml of enzyme is added to the control flask after the incubation with iodine.

7. The flasks are then removed from incubator and titrated with sodium thiosulfate (0.0166 M), using starch as indicator.

   Under these conditions, one ml of (0.0166 M) iodine reduced is equivalent to 4 μmol of substrate.

8. Beta-lactamase activity is standarized against the total protein measured by (Buriate method).

Do not use any drug in prepared crude enzyme to measure the enzyme activity for constitutive beta-lactamase (Branka Bedenic, 2002).

2.2.6 Effect of Temperature and pH on Beta-lactamase Activity

Use temperature ranging from (25 - 50) C^{o} and pH ranging from (4 - 9) to detect the Beta-lactamase activity at various conditions by using macroiodometric methods.

CHAPTER Three

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

3.1 Antimicrobial Susceptibility and β-lactamase Production

The antimicrobial susceptibility test was performed for all strains (*Staphylococcus aureus, S. epidermidis, Enterococcus faecalis, Klebsiellae, E. coli, and Pseudomonas aeruginosa*) by using the picking and patching method. (Item No. 2.2.3).

3.1.1 *Staphylococci*

Fig. (2) shows the effect of some β-lactam antibiotic on 5 isolates of *Staphylococcus aureus* and *S. epidermidis*. It has been found that all strains were resistant to penicillin, ampicillin, cephalixin and cefazolin at a rate of 100%.

On the other hand, 80% of these strains were resistant to pipracillin, ampiclox, cefotaxime, ceftizoxime; whereas 60% were resistant to amoxicillin and only 20% were resistant to amoxicillin- calvulanic acid combinations.

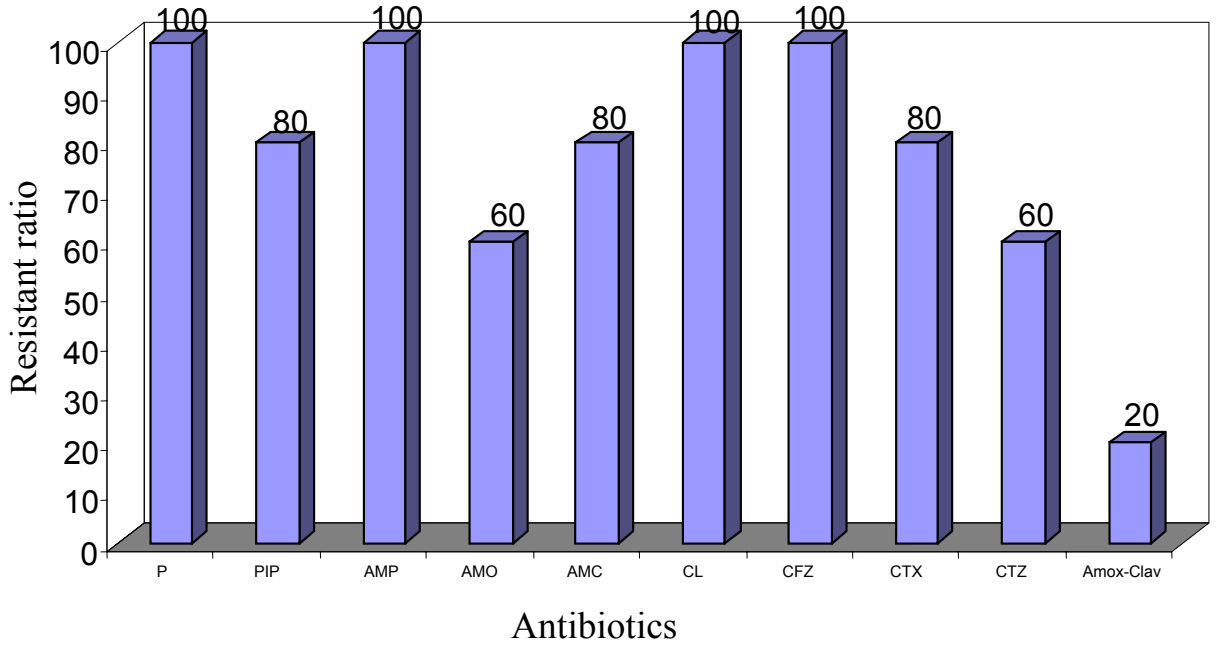

Fig. (2): The Percentage of Resistance of *Staphylococci* to β-lactam Antibiotics.

The resistant bacteria were described according to their ability to produce beta-lactamase. It was observed that four strains of *Staphylococcus* isolates had the ability to produce beta-lactamase in the presence of the antibiotic (inducer). It means that staphylococcal beta-lactamase is inducible (Jawetz *et al*., 2001) and not constitutive. It was also found that approximately 90% of *Staphylococcus aureus* produce beta-lactamase, which confers resistance to penicillin G.

In Fig. (3), it was seen that β-lactamase activity was high when cephalexin is used, but this activity diminished when cefotaxime was used instead of it. The results revealed that the enzyme had more affinity to cephalexin than cefotaxime. So, the

substrate of choice for detection β-lactamase activity produced by Staphylococci is cephalexin.

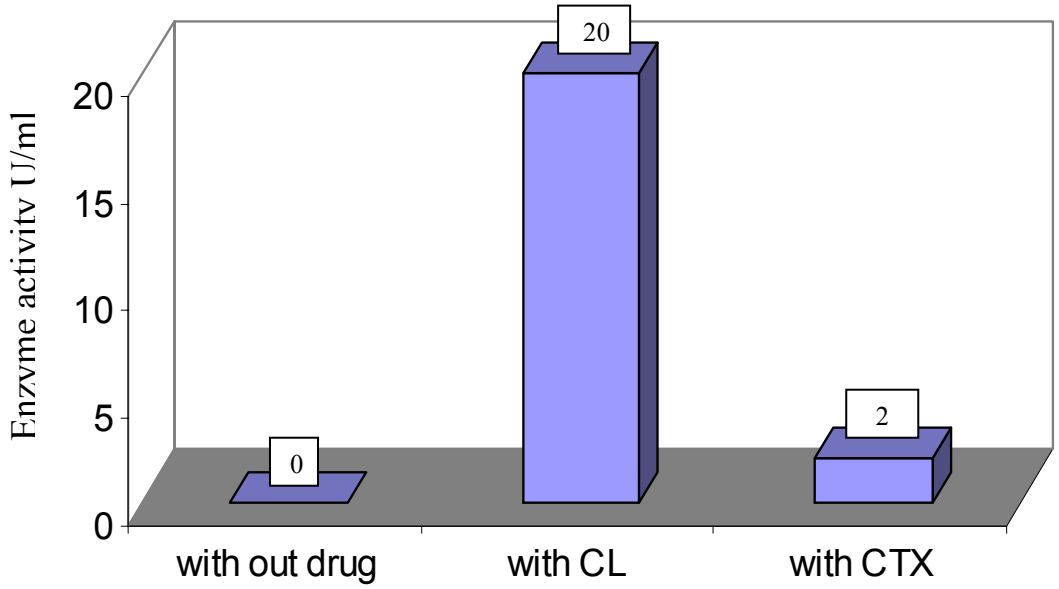

Fig. (3): β-lactamase Activity with (CL and CTX) and without Drug in *Staphylococci* Isolates

These results are similar to those observed by Livermore, (1995) who reported that *Staphylococci* were the only common gram-positive pathogens in which beta-lactamase had caused major resistance problem.

However, other results have reported that β-lactamase production is under plasmid control in the staphylococci and confer resistance to many penicillins such as penicillin G, ampicillin, pipracillin and other β-lactam antibiotics (Jawetz *et al*., 2001). Mandell and Perit, 1996; McMaus, 1997 have also reported that bacterial resistance against β-lactam antibiotics

increases at a significant rate, and there are several mechanisms of antimicrobial resistance to β-lactam antibiotics. The most important one is the production of β-lactamase enzymes, which are produced mainly by *Staphylococcus species*.

3.1.2 *Enterococcus faecalis*

The susceptibility of seven isolates of *Enterococcus faecalis* to ten β-lactam antibiotics were carried out by using the picking and patching method.

The results (shown in Fig. 4) revealed that *Enterococcus* isolates were resistant to penicillin, ampicillin at a rate of 85.7%, and to amoxicillin at a rate of 71%. These results also pointed to the remarkable susceptibility of bacterial isolates to pipracillin, cefazolin, cefotaxime but are less susceptible to ampiclox and cephalixine.

On the other hand, all the isolates of *E. faecalis* have shown the highest susceptibility to amoxicillin-clavulanic acid combinations 100%. This study has also shown that one isolate was resistant to most β-lactam antibiotics.

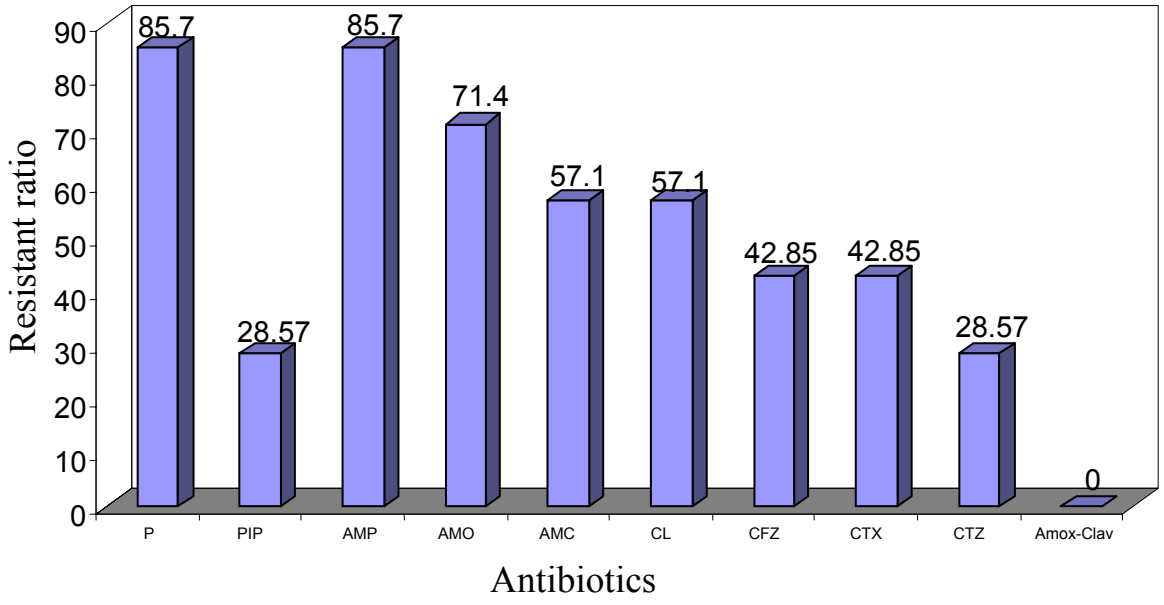

Fig. (4): The Percentage of Resistance of *Enterococcus faecalis* to β-lactam Antibiotics

Multi-resistant isolates of *E. faecalis* were ascribed to the production of constitutive enzyme which enhanced when adding cephalixine as inducer to β-lactamase, also it was found that β-lactamase activity was high when cephalexin is used, but this activity diminished when cefotaxime was used instead. The results revealed that the enzyme had more affinity to cephalexin than cefotaxime. So, the substrate of choice for detection β-lactamase activity produced by *Entreococcus faecalis* is cephalexin, Fig. (5).

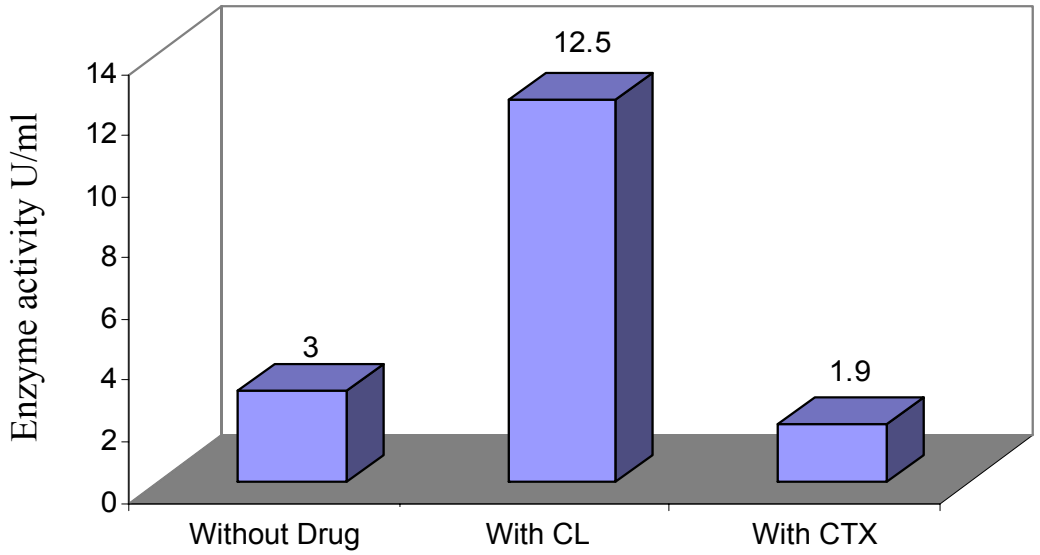

Fig. (5): β-lactamase activity with (CL and CTX) and with out drug in *E. faecalis* isolates

Furthermore, it was found that only one isolate was able to produce the enzyme by using the qualitative methods, whereas the rest failed to produce this enzyme.

These results agree with Jawetz, *et al*., 2001 who have reported that *Enterococci* may produce small amounts of β-lactamase enzyme, so they may appear to be susceptible to penicillin and ampicillin by routine susceptibility test.

In 1988, Murray and Patterson also reported that β-lactamase enzyme was relatively rare and was found in only a few strains of *E. faecalis*.

3.1.3 *Klebsiella*

The susceptibility test of *Klebsiella* to some β-lactam antibiotics was also studied. It was found that the strains were resistant to penicillin, ampicillin, amoxicillin at a rate of 100%. Some strains were also resistant to pipracillin, cefazolin and cefotaxime at a rate of (83.3%, 94.4%, 88.8%, 77.7% and 72.2%) respectively, (Fig. 6), whereas these strains appeared less resistant to cefotaxime at a rate of 50%, but the highest sensitivity rate of 94.5% was toward amoxicillin-clavulanic acid combination.

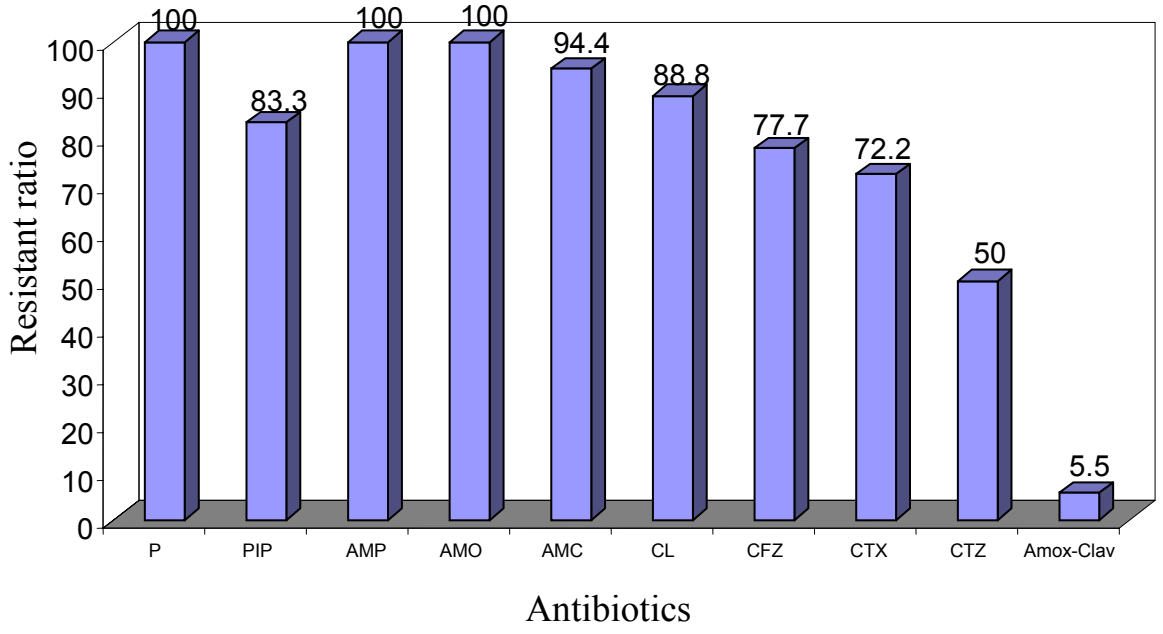

Fig. (6): The Percentage of Resistance of *Klebsiella* to β-lactam Antibiotics

The same result was found by Gupta in 1999 who found that *Klebsiella* isolates were resistant to ampicillin at a rate of 98%. Besides other results observed that *Klebsiella* strains were resistant at a highest rate to ampicillin and carbenicillin (Burman, 1992).

In 1997, Weller, *et al*., found that *Klebsiella* isolate were resistant to cefotaxime at a rate of 85%, and that indicated their ability to resist the third generation of cephalosporin.

Contrarily, Osoba, (1997) found that *Klebsiella* strains were resistant to ampicillin and pipracillin at rates of 100% and 83% respectively.

In 1991, Jacoby and Medeiros found that *K. pneumoniae* was well-known for its ability to become resistant to first line antibiotics. Bradford, *et al*., (1997) found that clinical isolates of *E. coli* and *Klebsiella* were resistant to penicillin in addition to all cephalosporins except cefepime.

In this study, only one strain of *Klebsiella* was resistant to amoxicillin-clavulanic acid combination. This may be related to these strains, having functional group-1 beta-lactamases, which are not inhibited by clavulanate (Bradford *et al*., 1997).

In 1990, Shanon, *et al*., pointed that resistance to beta-lactamase inhibitor combinations occured most commonly in gram-negative bacilli through the over production of common plasmid-mediated enzymes.

The resistant strains were ascribed to their ability to produce β-lactamase and it was seen that only nine strains were able to produce β-lactamase when penicillin G was used as substrate.

The β-lactamase produced by these nine strains of *Klebsiella* was constitutive but also enhanced when an inducer was added to the culture media. It means that *Klebsiella* strains can produce β-lactamase in the presence and absence of β-lactam antibiotic.

Although it was seen that β-lactamase activity was high when cephalexin was used, this activity diminished when cefotaxime was used instead. The results revealed that the enzyme has more affinity to cephalexin than cefotaxime. So, the substrate of choice for the detection β-lactamase activity produced by *Klebsiella pneumoniae* is cephalexin, Fig. (7).

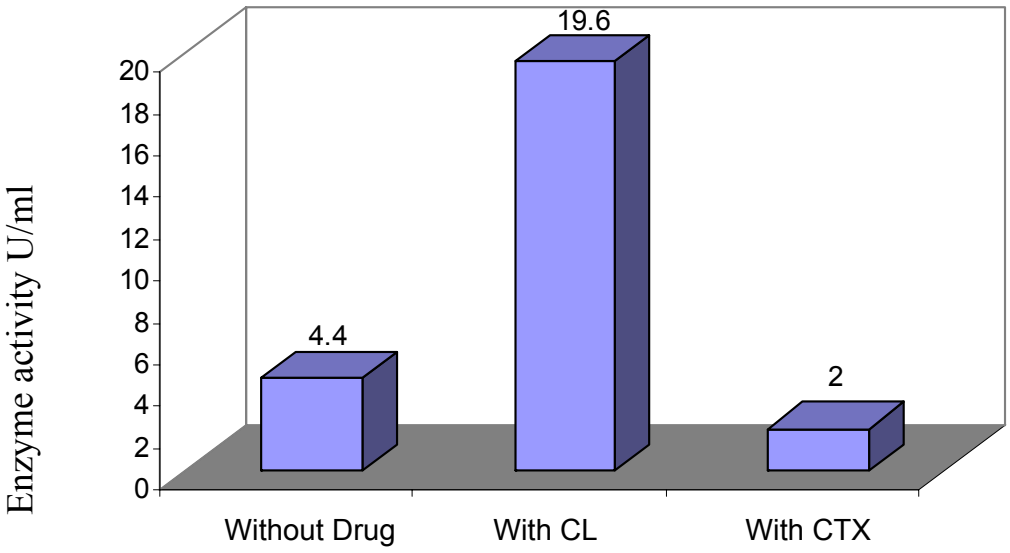

Fig. (7): β-lactamase Activity with (CL and CTX) and without Drug in *Klebsielle* Isolates

This result was correlated with such results as those of Livermore who, in 1995, reported that all β-lactamase of *Klebsiella* were constitutive and usually produced at low levels, which were sufficient to protect against ampicillin, amoxicillin and carbenicillin.

Fournier, *et al.,* 1999 reported that *Klebsiella* were naturally resistant to ampicillin and amoxicillin by the production of SHV-1 β-lactamase encoded on the chromosome or transferable plasmid.

In this study, most stains of *Klebsiella* were sensitive to amoxicillin-clavulanic acid combination at a rate 94%. However, one strain was resistant to this combination.

In 1990, Jacoby and Carrerase showed that most *Klebsiella* strain produced β-lactamase that remained susceptible to inhibition by clavulanic acid and other commercially available β-lactamase inhibitors. Thus, although this strain is resistant to β-lactam antibiotic, it is usually susceptible to amoxicillin-clavulanic acid and other β-lactam β-lactamase inhibitors combinations.

Other strains showed resistance to β-lactam antibiotics but it did not produce β-lactamase enzyme. This correlates with the results of Stapleton, *et al*., (1999) who reported that gram-negative bacteria may exhibit reduced susceptibility to β-lactam antibiotics by a number of mechanisms including the reduction

of outer membrane permeability, target site modification and efflux of the β-lactam out of the cell.

3.1.4 *Escherichia coli*

Antimicrobial susceptibility test was performed on *E. coli* isolates on Muller-Hinton agar.

The results from this study confirmed previous observations that all isolates were resistant to penicillin, pipracillin, cephalexin and cefotaxime at a rate of 100%, where as some isolates were resistant to cefazolin ceftizoxim at a rate 80. On the other hand, the resistance rate to ampicillin and ampiclox is 60%.

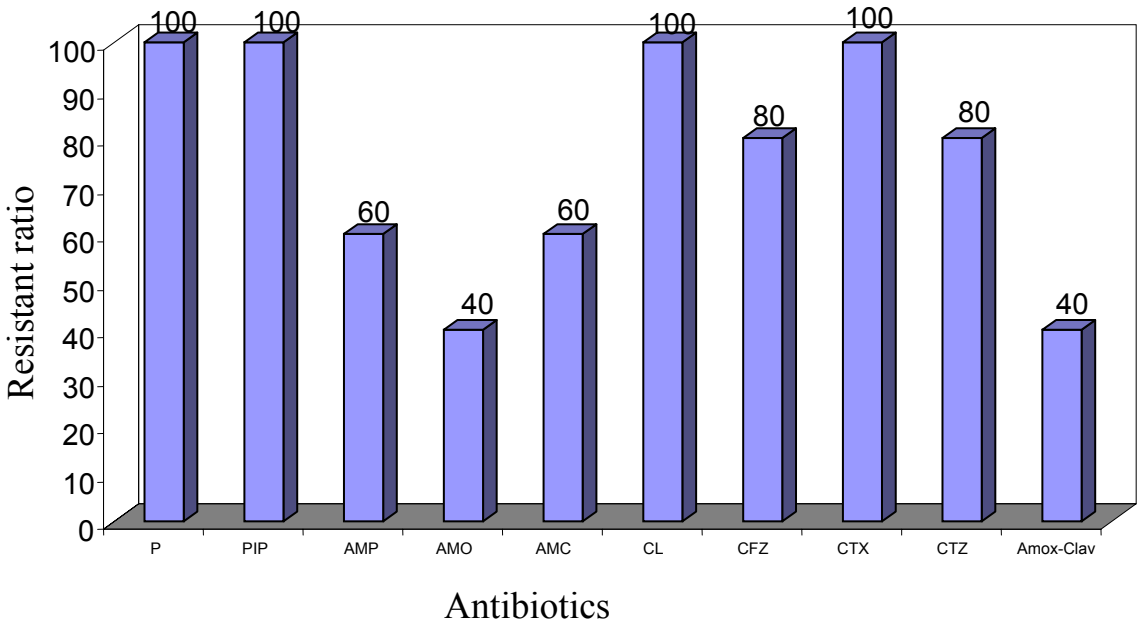

Fig. (8): Resistance Ratio of *E. coli* Isolates to β-lactam Antibiotics

In addition, this study showed that only two isolates were able to produce the β-lactamase enzyme constitutively which confers resistance to amoxicillin, amoxicillin-clavulanate combination.

The enzyme activity was enhanced when cephalixin was added to the culture media; it means that this enzyme was also inducible.

Furthermore, it was found that β-lactamase activity was high when cephalexin is used, but this activity diminishes when cefotaxime is used instead. The results revealed that the enzyme has more affinity to cephalexin than cefotaxime. So, the substrate of choice for detection β-lactamase activity produced by *E. coil* was cephalexin, Fig. (9).

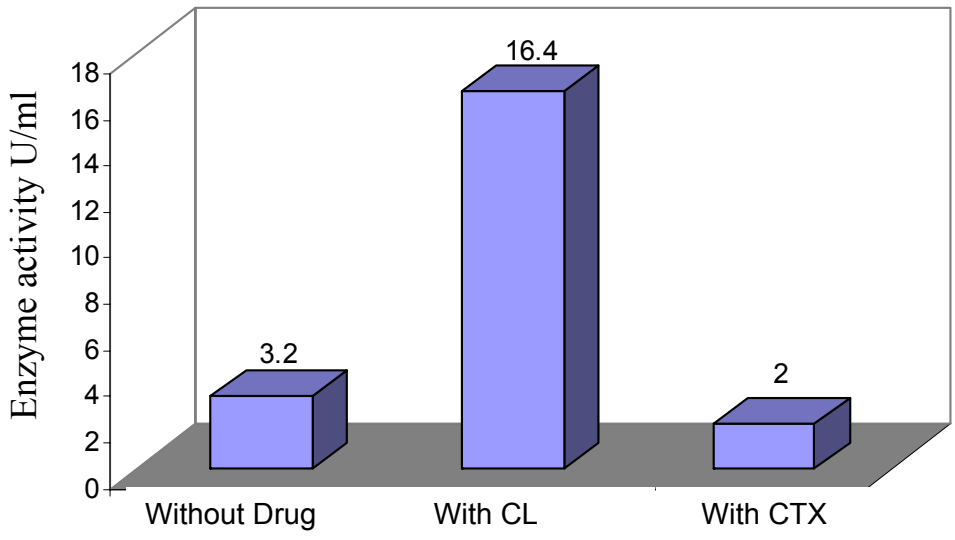

Fig. (9): β-lactamase Activity with (CL and CTX) and without Drug in *E. coli* Isolates

These results are correlated with those obtained by French *et al.*, 1996, who reported that *E. coli* was frequently resistant to ampicillin and amoxicillin by the production of TEM-1 β-lactamase. But resistant strains were usually susceptible to β-lactamase inhibitor. Although recently, there have been increasing reports of resistant strains of *E. coli* to amoxicillin-clavulanic acid combination. This resistance may be attributed to one of several mechanisms:

(a) The production of excessive amounts of TEM-1 β-lactamase that swamp the activity of clavulanic acid (French and Lings, 1988; Shannon *et al*., 1990).

(b) As shown in *Klebsiella, E. coli* strains had functional group-1 β-lactamase described as cephalosporinases which were not inhibited by clavulanic acid (Bradford, *et. al.*, 1997).

However, recent clinical isolates of *E. coli* expressed a specific β-lactamase (TEM-50) that conferred low-level resistance to cephalosporinas Sirot, *et. al.*, (1997). In this study, it has also been observed that 60% of *E. coli* isolates were not able to produce β-lactamase enzyme although they revealed resistance to some β-lactam antibiotics. This result agrees with that of Poirel, *et. al*., (1999) who attributed it to the alteration in outer membrane proteins of this bacterium. In 1999, this result was arrived by Mastumura, *et al*., 1999 who found that in *E. coli* the β-lactam antibiotic was not only hydrolyzed by

β-lactamase but the outer membrane barrier may also play an important role in resistance to β-lactam antibiotic.

3.1.5 *Pseudomonas aeruginosa*

Results of susceptibility of *Ps. aeruginosa* to β-lactam antibiotics showed that all isolates were resistant to most β-lactam antibiotics at a rate of 100%, whereas these strains showed susceptibility for pipracillin at a rate of 40%.

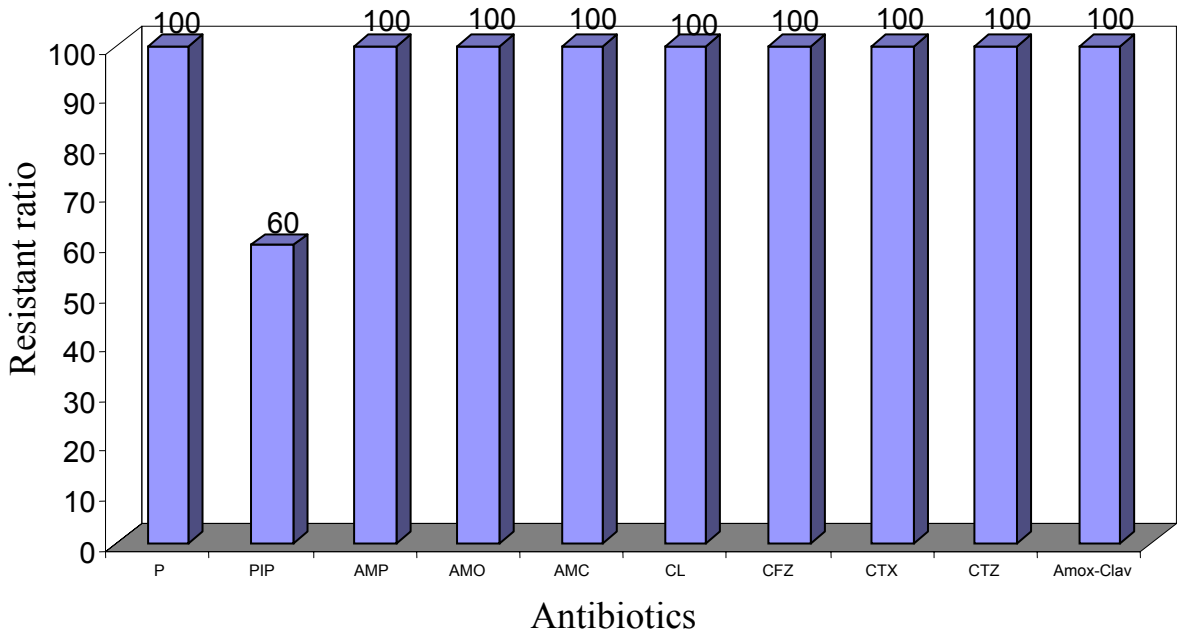

Fig. (10): Resistance Ratio of *Pseudomonas aeruginosa* Isolates to β-lactam Antibiotics

These results are correlated with those of Nakae, *et al.*, (1999), who reported that a major problem in *Ps. aeruginosa* infection was that organism exhibited natural and acquired resistance to many structurally and functionally diverse

antibiotics. This study showed that resistant strains were ascribed to their ability to produce β-lactamase and it was seen that all strains were able to produce β-lactamase when penicillin G was used as substrate, Fig (12).

The β-lactamase produced by these strains was inducible. However, some strains were able to produce such enzyme without adding the inducer.

These results are identical with those observed by Bryan, *et al.*, (1984), who reported that *Ps. aeruginosa* expressed chromosomally encoded β-lactamase in the presence of an appropriate inducer and showed elevated resistance to β-lactam antibiotics. Bonfiglio, *et. al.*, too, found in 1998 that the most frequent mechanism of resistance in *Ps. aeruginosa* was β-lactamase independent and so called intrinsic resistance. In 1992, Livermore found that *Ps. aeruginosa* had inducible β-lactamase and there were more than one isoenzyme related to β-lactamase enzyme.

In a distinct study by Cavallo, *et al.*, (2000), it was shown that the development of resistance to β-lactam in clinical isolates of *Ps. aeruginosa* was associated with the production of acquired β-lactamase, which constituted over production sephalosporinase. Gwynn and Rolinson, (1980), reported that culture of *Ps. aeruginosa* (derepressed mutant) produced β-lactamase enzyme constitutively which confers high level of resistance to β-lactam antibiotics.

On the other hand, this study has revealed that cephalixine could induce more β-lactamase enzyme than cefotaxime. It means that β-lactamase activity was high when cephalexin was used, but this activity diminished when cefotaxime was used instead. These results were ascribed to the fact that the enzyme had more affinity to cephalexin than cefotaxime. So, the substrate of choice for detection β-lactamase activity produced by *Ps. aeruginosa* is cephalexin, Fig. (11).

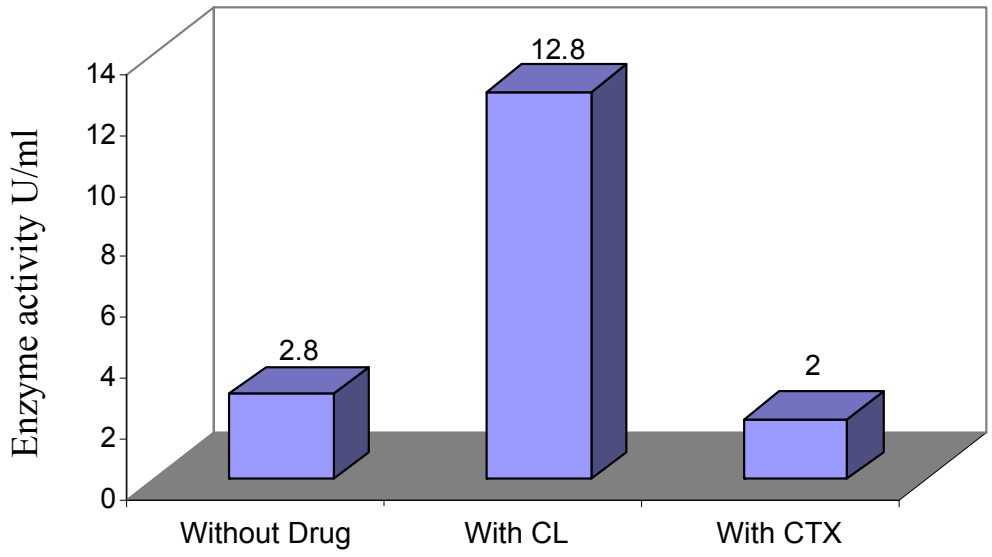

Fig. (11): β-lactamase Activity with (CL and CTX) and without Drug in *Ps. aeurginosa* Isolates

This observation was witnessed by Livermore, (1995), who observed that ampicillin and narrow spectrum cephalosporins were liable to hydrolysis by β-lactamase produced by *Ps. aeruginosa* and induced this enzyme strongly, destroying their own activity; whereas ureidopenicillins and

extended-spectrum cephalosporin were liable but induced weakly. This study showed that *Ps. aeruginosa* strains were resistant to β-lactamase inhibitors. These results correlated with those of Maiti, *et al.*, (1998), who reported that β-lactamase enzyme were inactivated by inhibitors such as clavulanic acid. However, not all pseudomonad β-lactamase were affected, because some inhibitor-resistant *Ps. aeruginosa* have recently been described (Chaibi, *et al.*, 1999).

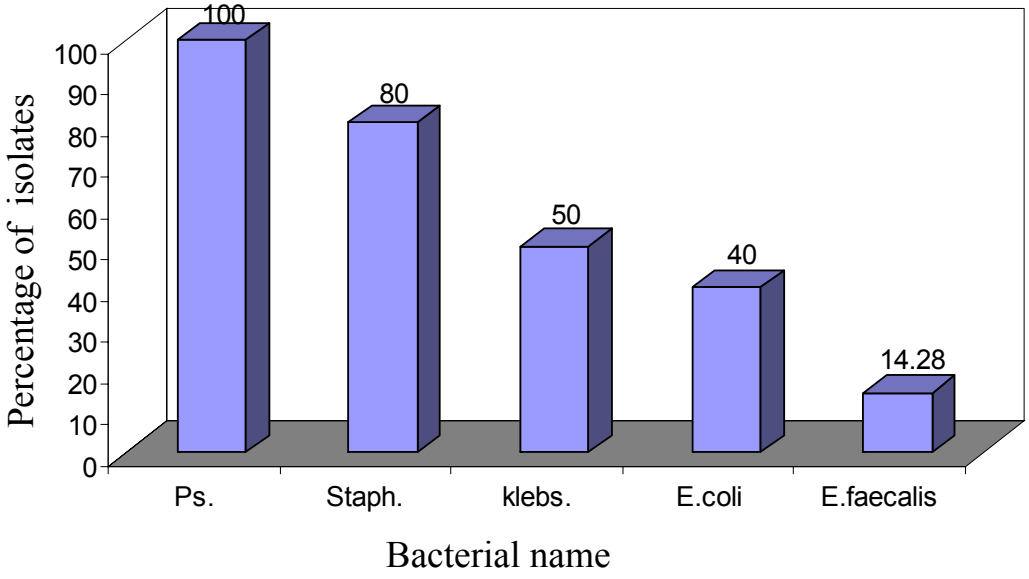

Fig. (12): Percentage of Isolates Produced β-lactamase in Some Gram-positive and Gram-negative Bacteria

Note: This figure shows the percentage of isolates that produced β-lactamase in *Ps. aeruginosa* 100%, *Staphylococcus* 80%, *Klebsiella* 50%, *E. coli* 40%, and *E. faecalis* 14.28%.

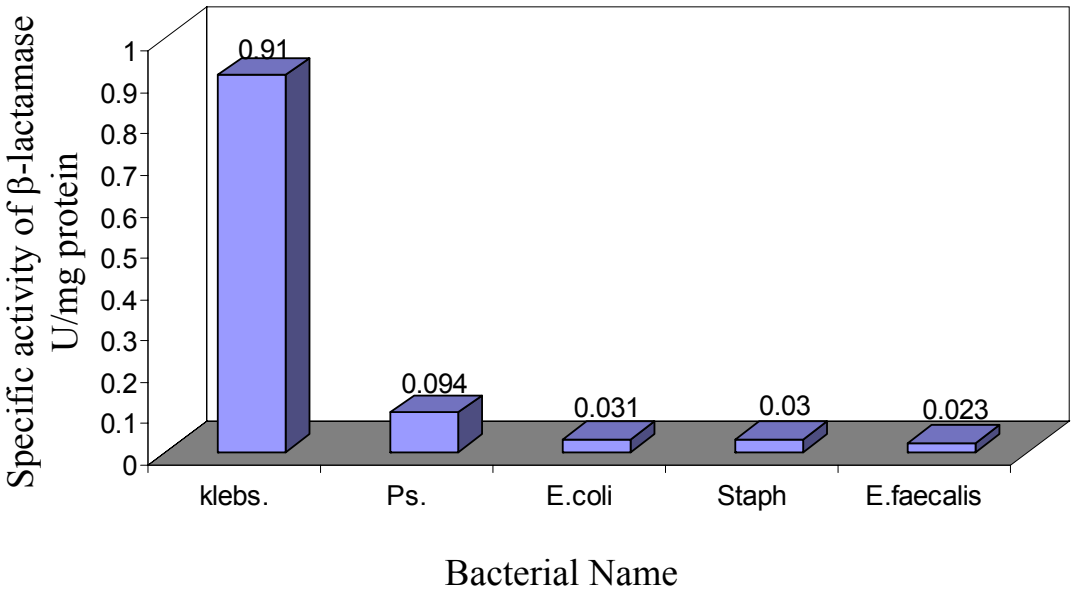

Fig. (13): Specific Activity of β-lactamase in Some Gram-positive and Gram-negative Bacteria

Note: This figure shows the specific activity of β-lactamase in some gram-positive and gram-negative bacteria. It was found that the specific activity of β-lactamase for *Staphylococci* = 0.03, *E. coli* = 0.031, *Klebsiella* = 0.91, *Pseudomonas aeruginosa* =0.094 and E. faecalis =0.023, and this belongs to the content of external protein produced by these bacteria. Therefore, the external protein produced by *Klebsiella* is very little by contrast with other bacteria.

3.2 Effect of Salicylic Acid and EDTA on Bacterial Behavior & Enzyme Activity

The effect of some compounds (salicylic acid, and EDTA) on some bacterial behavior against β-lactam antibiotics was studied.

The results showed that the use of salicylic acid significantly increased bacterial resistance to cephalosporin. This result occurs because of the effect of salicylic acid on the outer membrane permeability (Cohen, *et al*., 1993). In 1989, Faulds, *et al*., reported that the resistance of gram-negative bacteria to β-lactam antibiotics significantly would increase in the presence of (Salicylic acid). Besides, they observed that (Salicylic acid) might increase the resistance to, and decrease the outer membrane permeability of, cephalosporin in a virulent pathogen. The rate of β-lactam hydrolysis by β-lactamase was reduced in the presence of (Salicylic acid).

The results give the evidence that (SA) cannot inhibit or reduce the production of inducible β-lactamase because β-lactam antibiotics cannot reach the target as a result of change in the outer membrane permeability.

Furthermore, EDTA was also used to show its effect on the bacterial behavior against β-lactam antibiotics. It was found

that EDTA could effect the growth of bacteria on β-lactamase production particularly Metallo-β-lactamase. The effect of EDTA on the production of Metallo-β-lactamase was attributed to the ability of EDTA to block the gene that produces Metallo-β-lactamase which confers resistance to broad spectrum cephalosporin (Marumo, *et. al*., 1995 : Goto, *et. al*., 1997). In 2000, Arakawa, *et al*., reported that EDTA inhibited Metallo-β-lactamase produced by some gram-negative bacteria such as *E. coli*, *Ps. aeruginosa*, *K. pneumonia*. They also showed that EDTA (Ethylene diamine tetra acetic acid) created growth inhibitory zone but its appearance and re-productivity were relatively poor in several strains even when thick EDTA solution was used.

It was shown that the enzyme is not produced when (Salicylic acid) is used, this may be related with that the enzyme is carried on plasmid and (Salicylic acid) can effect on the plasmid content through causation plasmid curing (Al-Saeed, 1997).

On the other hand, the presence of EDTA causes inhibition of the enzyme activity through pulling the metal ions present in the active side because EDTA is known as chelating agent (Arakawa, *et.al*.2000)

Table(6): Effect of salicylic acid and EDTA on bacterial resistance and β-lactamase production

Bacterial NO.	No.Of isolates that resist CL.	No.Of isolates that resist CL.+SA	No.Of isolates that resist CL.+EDTA
Staph. aureus (5)	5	4	3
E.faecalis (7)	4	5	3
K. pneumonia (18)	16	18	14
E.coli (5)	5	5	3
PS. aeruginosa (5)	5	5	3
Total (40)	35 (88%)	37 (93%)	26(10.4%)

3.3 Effect of Temperature on Enzyme Activity

The effect of temperature on β-lactamase activity produced by *S. aureus, E. faecalis, E. coli, K. pneumonia, Ps. aeruginosa* was studied.

It was observed that the effect of temperature on enzyme activity was very clear at degrees more than 37C^{o} or less than that. The results in Fig. (14-A) indicate that the optimal temperature for β-lactamases activity produced by *S. aureus* is 37C^{o} in which the enzyme activity reaches 20 U/ml. The enzyme remains active at room temperature 25 C^{o}, and in elevated temperature 50C^{o} at a rate of (16 and 12) U/ml.

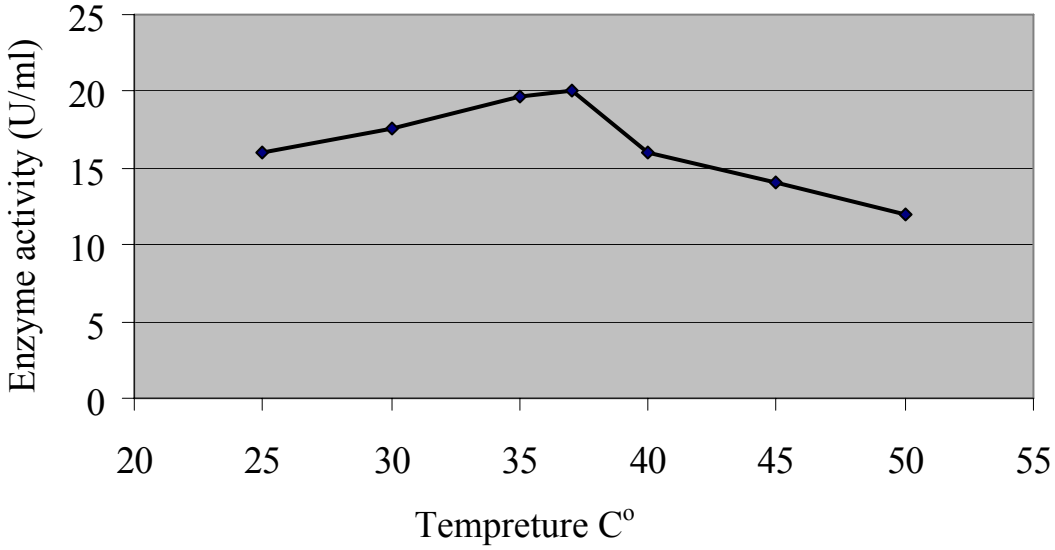

Fig. (14-A): The Effect of Temperature on β-lactamase Activity in *Staphylococcus aureus*

On the other hand, the β-lactamase produced by *E. Faecalis* records maximum activity at temperature 37C° at a rate of 12.5 U/ml Fig. (14-B). The enzyme remains relatively active at room temperature 25C° and in elevated temperature 50C° (at a rate of 8 and 8.5 U/ml respectively).

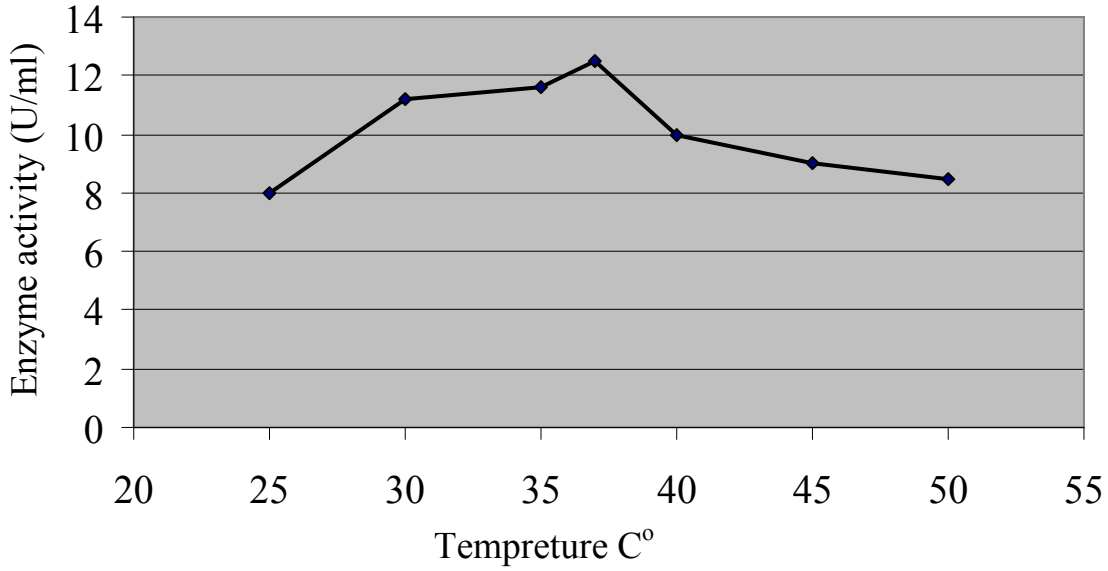

Fig. (14-B): The Effect of Temperature on β-lactamase Activity in *E. faecalis*

Furthermore, the enzyme produced by *K. pneumonia* records high activity 19.6U/ml at temperature 37C^{o} Fig. (14-C). The enzyme remains active at temperature 25C^{o} but lesser in value 14 U/ml than that recorded in temperature 37C^{o}. But the activity begins to decrease at high temperature when the activity becomes 5 U/ml at 50C^{o}.

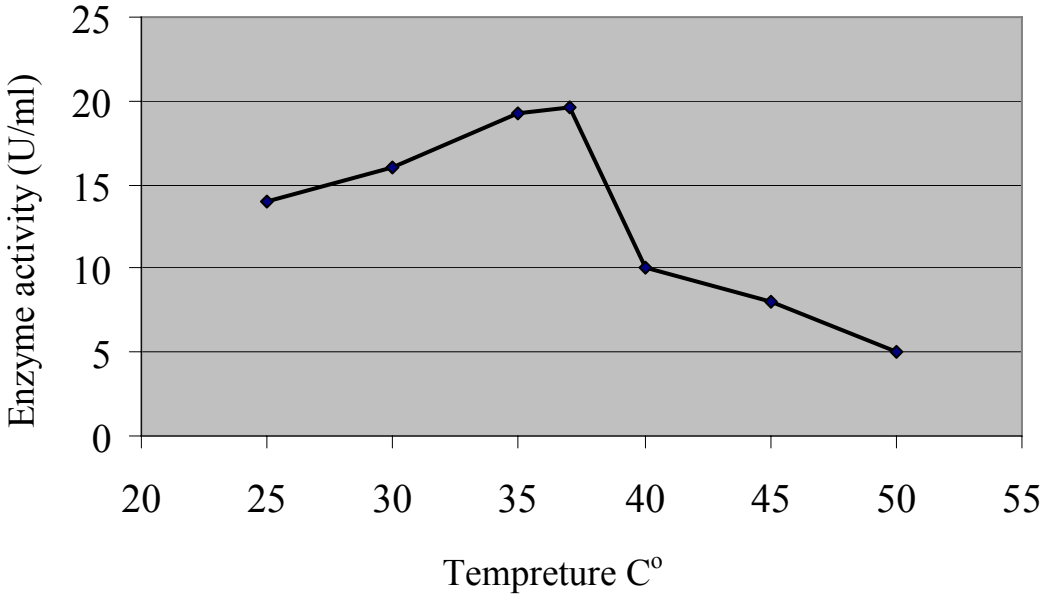

Fig. (14-C): The Effect of Temperature on β-lactamase Activity in *K. pneumonia*

Fig. (14-D) shows that in *E. coli* β-lactamase records high activity 16.4U/ml at temperature 37C^{o}. On the other hand, above 37C^{o} , the activity begins to decrease until it reaches 3 U/ml at temperature 50C^{o}. The enzyme is completely inhibited at 55C^{o}. This study has also proved that the enzyme remains active at

room temperature but the value of activity is lesser than that in temp

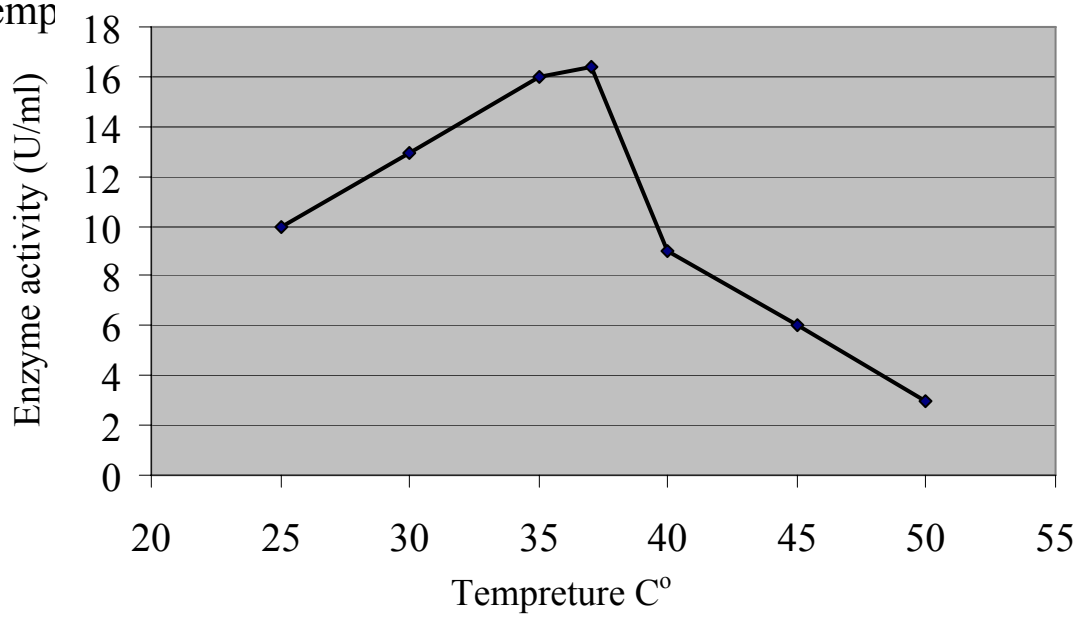

Fig. (14-D): The Effect of Temperature on β-lactamase Activity in *E. Coli*

Besides, the optimal temperature for β-lactamase produced by *Ps. aeruginosa* ranges from 35C° to 37C°, but the highest activity is 12.8 U/ml at temperature 37C° Fig. (14-E). The enzyme remains active at room temperature 8 U/ml but lesser than in 37C°. On the other hand, the activity begins to decrease above 40C°, and the enzyme activity becomes 4 U/ml at 50C°.

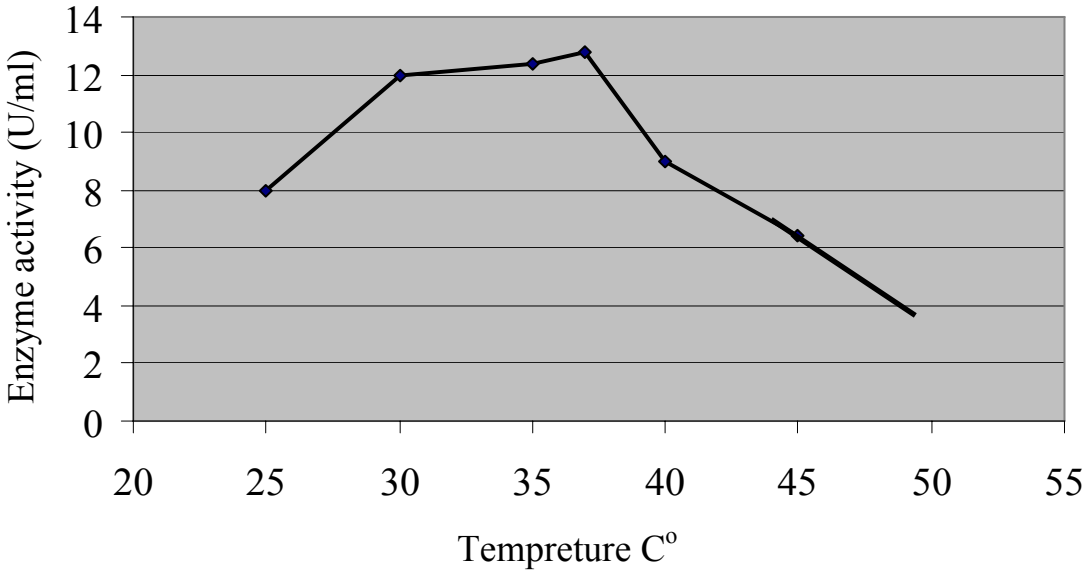

Fig. (14-E): The Effect of Temperature on β-lactamase Activity in *Ps. aeruginosa*

These results demonstrate that the optimal temperature for β-lactamase activity is 37C°, and any increase in the body temperature will affect the β-lactamase activity.

These results agree with Saino, *et al*., (1982) who have found that the optimum temperature for β-lactamase activity produced by *Ps. maltophilia* is 37C°. Although Davis, *et al*., (1974) have observed that β-lactamase produced by *B. cereus* loses its activity completely when kept at 50C° for 10 min., Castillo, *et al*., (1998) have found that the optimum temperature for β-lactamase produced by *N. gonorrhae* is 37C°. They have also pointed that β-lactamase is completely inactivated at 60C°, whereas the enzyme remains active at low temperature. These results, as well, are correlated with those observed by Vanhove, *et al*., (1997) who have found that the mutant TEM-1

β-lactamase from *E. coli* was inactivated to a large extent within a few minutes at 55C^{o}.

Huang and Palzkill (1997) have shown that TEM-1 β-lactamase has M691 substitution which serves as the nucleophile for the attack on the lactam ring and demonstrated that ampicillin hydrolysis by M691 enzyme decreases steadily from 45C^{o} to 65C^{o}.

3.4 Effect of pH on Enzyme Activity

The effect of pH ranging from (4 - 9) on β-lactamase activity produced by (*S. aureus, E. faecalis, E. coli*, *K. pneumonia, Ps. aeruginosa*) was studied.

Fig. (15) showed β-lactamase activity in various values of pH. In Fig. (15-A), it was found that β-lactamase produced by *S. aureus* was highly active in pH (7.0), in which the enzyme activity reaches 20 U/ml, but this ratio decreased in pH values below or above (7.0).

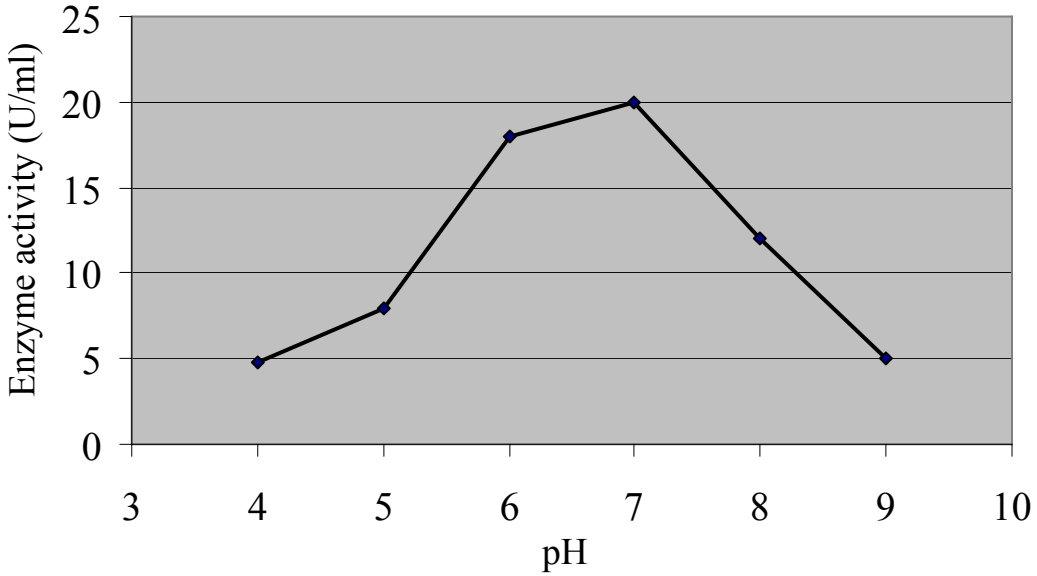

Fig. (15-A): The Effect of pH on β-lactamase Activity in *Staphylococcus aureus*

On the other hand, the optimum pH for β-lactamase produced by *E. faecalis* has also (7.0), Fig. (15-B), in which the activity reaches the high activity 12.5 U/ml, but the enzyme becomes partially active at a rate of 2.8 U/ml in pH (4.0) and 4 U/ml at pH (9.0).

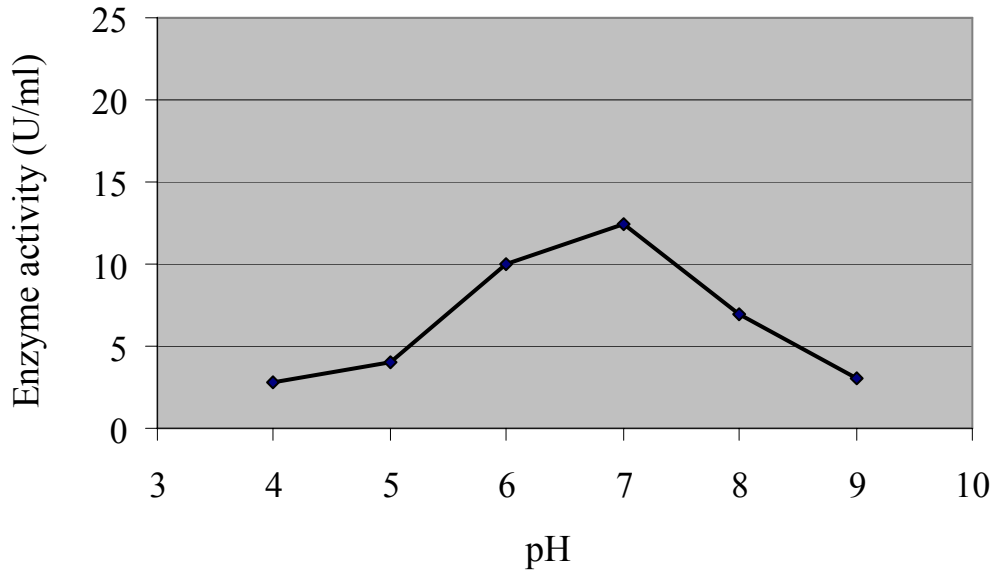

Fig. (15-B): The Effect of pH on β-lactamase Activity in *E. faecalis*

Furthermore, Fig. (15-C) showed the maximum activity 19.6 U/ml for β-lactamase produced by *K. pneumonia* at pH value (7.0). This activity diminished at alkaline pH (8.0-9.0) to (9 and 2) U/ml respectively, and the activity also decreased to 2.5 U/ml at pH value (4.0).

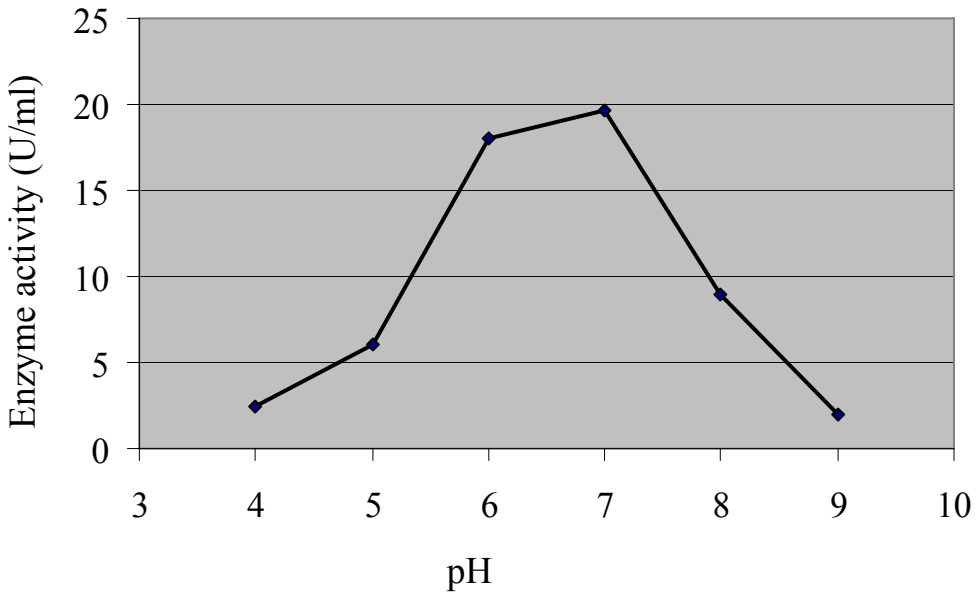

Fig. (15-C): The Effect of pH on β-lactamase Activity in *K. pneumonia*

In Fig.(15-D), the pH values (6.0 - 7.0) are optimum for β-lactamase activity produced by *E. coli* in which the activity reaches (16 and 16.4) U/ml respectively, but the activity rate decreases at high pH value (9.0) to 3 U/ml, also at low pH (4.0), where the activity becomes 4 U/ml.

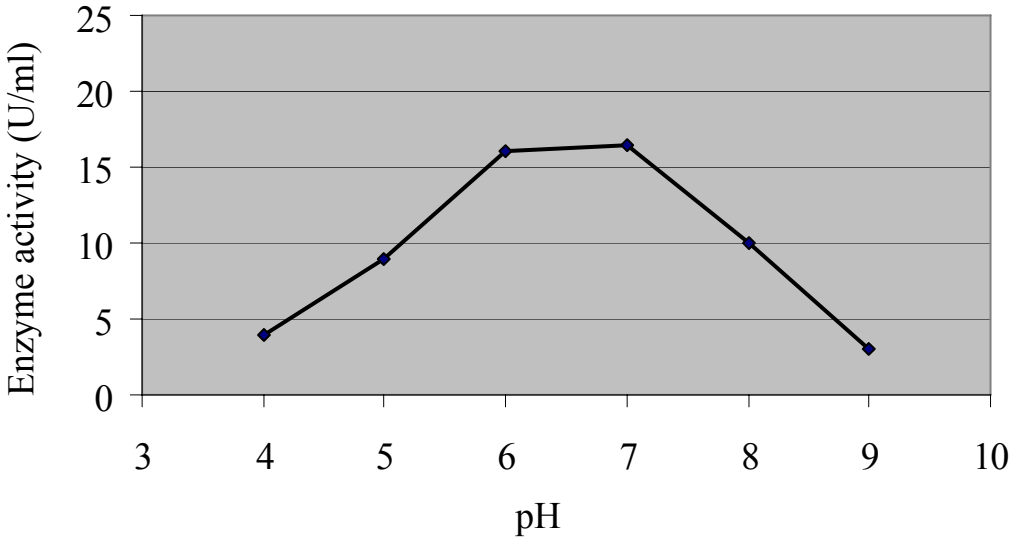

Fig. (15-D): The Effect of pH on β-lactamase Activity in *E. coli*

Besides, the β-lactamase produced by *Ps. aeruginosa* has the optimal activity at pH ranging from (6.5 - 7.5), Fig. (15-E) where the activity becomes (12.4 and 12.8) U/ml respectively, but this activity is reduced to 3.5 U/ml in pH (4.0) and to 4 U/ml in pH (9.0).

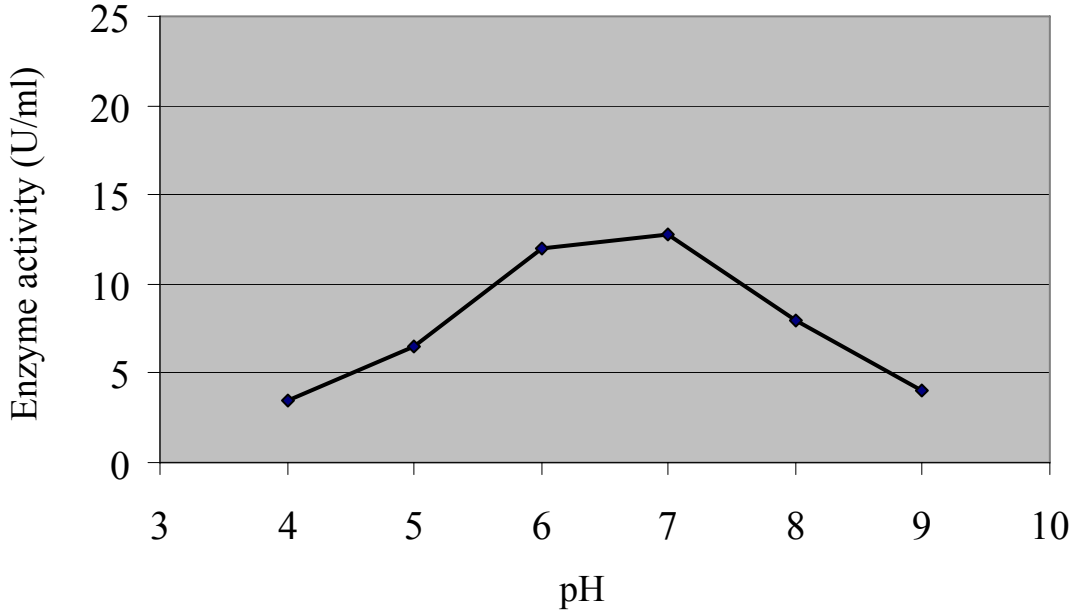

Fig. (15-E): The Effect of pH on β-lactamase Activity in *Ps. aeruginosa*

These results demonstrate that the optimum pH for β-lactamase activity is between (6.0 - 7.0). These results are identical with those observed by Livermore and Corkill, (1992) who have found that pH values are between (6.0 - 7.0), where the optimal pH for β-lactamase activity is in *E. coli*. A similar behavour has also been reported by Castillo, *et. al*., (1998) who have found that pH values (7.0 - 7.2), are optimum for β-lactamase activity in *N. gonorrhae* (β-lactamase TEM-1). Although similar results have been reported by Laraki, *et al.,* (1999), they have found that metallo-β-lactamase produced by *Ps. aeruginosa, E. coli* and *K. pneumonia* exhibit a maximum activity at pH (6.5 and 7.5).

These results indicate that all isolates produces β-lactamase at pH values ranging from (6.0 - 7.0) and the interpretation of these results may be related to the fact that the maximum pH value for bacterial growth is at pH (7.0). The enzyme stability is situated at the same pH value because most metallo-β-lactamase enzyme need zinc moieties one active and stable in pH ranging from (6.5 - 7.5) as mentioned by Laraki, *et al*., (1999). A similar behavour has been shown by Crowder, *et al*., (1998), who have observed that pH (7.0) is optimum for metallo-β-lactamase in *B. fragils*.

Chapter Four

Conclusions AND Recommendations

4.1 Conclusions

1. Most isolates investigated in this study were resistant to beta-lactam antibiotics such as penicillin, ampicillin, amoxicillin, ampiclox, cephalexin and cefotaxime.
2. Not all the resistive isolates can produce beta-lactamase enzyme.
3. Beta-lactamase activity was high when cephalexin was used as substrate when compared to cefotaxime.
4. The optimal temperature for beta-lactamase activity ranged from (35 - 37) C^{o}.
5. The optimal pH for enzyme activity was (6.5 - 7).
6. It was observed that salicylic acid increased bacterial resistance to antibiotics, where EDTA would effect directly on enzyme activity.

4.2 Recommendations

1. Further studies should be carried out for the purification of beta-lactamase enzyme.
2. Detection of the genetic elements containing the gene responsible for enzyme synthesis.
3. Investigation on novel inhibitors to arrest the enzyme productivity and activity.

TABLES

Table (7-A)

The effect of β-lactam antibiotics on *staphylococci* and β-lactamase production

Isolate No.	P	PIP	AMP	AMO	AMC	Amox + Clav	β-lactamase production by iodometric method (the substrate penicillin G)
S. aureus							
1	+	+	+	+	+	-	+
2	+	+	+	+	+	-	+
3	+	+	+	-	+	-	+
S. epidermidis							
4	+	+	+	+	+	+	+
5	+	-	+	-	-	-	-

P.: Penicillin, PIP.: Pipracillin AMP.: Ampicillin, AMO.: Amoxicillin,

AMC: (Ampicillin + Cloxacillin), Amox + Clav: (Amoxicillin + Calvulanic acid)

+ = Resistant, - = Sensitive.

Table (7-B)

Isolate No.	CL	CFZ	CTX	CTZ	β-lactamase production by iodometric method (the substrate Cephalexin)
S. aureus					
1	+	+	+	+	+
2	+	+	+	+	+
3	+	+	+	+	+
S. epidermidis					
4	+	+	+	-	+
5	+	+	-	-	-

CL: Cephalexin, CFZ: Cefazolin, CTX: Cefotaxime, CTZ: Ceftizoxime

Table (8-A)

The effect of β-lactam antibiotics on *Enterococcus faecalis* isolates and β-lactamase production

Isolate No.	P	PIP	AMP	AMO	AMC	Amox + Clav	β-lactamase production by iodometric method (the substrate penicillin G)
1	+	-	+	+	+	-	-
2	+	-	+	+	+	-	-
3	+	+	+	+	+	-	+
4	+	-	+	-	+	-	-
5	+	-	-	-	-	-	-
6	+	-	+	+	+	-	-
7	-	+	+	+	+	-	-

P.: Penicillin, PIP.: Pipracillin AMP.: Ampicillin, AMO.: Amoxicillin,

AMC: (Ampicillin + Cloxacillin), Amox + Clav: (Amoxicillin + Calvulanic acid)

+ = Resistant, - = Sensitive.

Table (8-B)

Isolate No.	CL	CFZ	CTX	CTZ	β-lactamase production by iodometric method (the substrate Cephalexin)
1	+	+	+	-	-
2	+	+	-	+	-
3	+	+	+	+	+
4	-	-	-	-	-
5	-	-	-	-	-
6	-	-	+	-	-
7	+	-	-	-	-

CL: Cephalexin, CFZ: Cefazolin, CTX: Cefotaxime, CTZ: Ceftizoxime

Table (9-A)

The effect of β-lactam antibiotics on *Klebsiella* isolates and β-lactamase production

Isolate No.	P	PIP	AMP	AMO	AMC	Amox + Clav	β-lactamase production by iodometric method (the substrate penicillin G)
1	+	+	+	+	+	-	+
2	+	+	+	+	+	-	+
3	+	-	+	+	+	-	+
4	+	+	+	+	+	+	+
5	+	+	+	+	+	-	+
6	+	-	+	+	+	-	-
7	+	+	+	+	-	-	-
8	+	+	+	+	+	-	+
9	+	+	+	+	+	-	-
10	+	+	+	+	+	-	-
11	+	+	+	+	+	-	+
12	+	+	+	+	+	-	+
13	+	+	+	+	+	-	-
14	+	+	+	+	+	-	-
15	+	+	+	+	+	-	+
16	+	+	+	+	+	-	-
17	+	+	+	+	+	-	-
18	+	-	+	+	+	-	-

P.: Penicillin, PIP.: Pipracillin AMP.: Ampicillin, AMO.: Amoxicillin,

AMC: (Ampicillin + Cloxacillin), Amox + Clav: (Amoxicillin + Calvulanic acid)

+ = Resistant, - = Sensitive.

Table (9-B)

Isolate No.	CL	CFZ	CTX	CTZ	β-lactamase production by iodometric method (the substrate Cephalexin)
1	+	+	+	+	+
2	+	+	+	+	+
3	+	+	+	-	+
4	+	+	+	+	+
5	+	-	-	+	+
6	-	-	-	-	-
7	+	+	-	-	-
8	+	-	+	-	+
9	+	+	+	-	-
10	+	+	+	-	-
11	+	-	-	+	+
12	+	+	+	+	+
13	+	+	+	-	-
14	+	+	+	+	-
15	+	+	+	+	+
16	+	+	+	+	-
17	+	+	+	-	-
18	-	-	-	-	-

CL: Cephalexin, CFZ: Cefazolin, CTX: Cefotaxime, CTZ: Ceftizoxime

Table (10-A)

The effect of β-lactam antibiotics on *E. coli* isolates and β-lactamase production

Isolate No.	P	PIP	AMP	AMO	AMC	Amox + Clav	β-lactamase production by iodometric method (the substrate penicillin G)
1	+	+	+	+	+	-	-
2	+	+	+	+	+	+	+
3	+	+	-	-	+	-	-
4	+	+	+	+	+	+	+
5	+	+	-	-	+	-	-

P.: Penicillin, PIP.: Pipracillin AMP.: Ampicillin, AMO.: Amoxicillin,

AMC: (Ampicillin + Cloxacillin), Amox + Clav: (Amoxicillin + Calvulanic acid)

+ = Resistant, - = Sensitive.

Table (10-B)

Isolate No.	CL	CFZ	CTX	CTZ	β-lactamase production by iodometric method (the substrate Cephalexin)
1	+	+	+	-	-
2	+	+	+	+	+
3	+	+	+	+	-
4	+	+	+	+	+
5	+	+	+	+	-

CL: Cephalexin, CFZ: Cefazolin, CTX: Cefotaxime, CTZ: Ceftizoxime

Table (11-A)

The effect of β-lactam antibiotics on *Pseudomonas aeruginosa* and β-lactamase production

Isolate No.	P	PIP	AMP	AMO	AMC	Amox + Clav	β-lactamase production by iodometric method (the substrate penicillin G)
1	+	+	+	+	+	+	+
2	+	-	+	+	+	+	+
3	+	+	+	+	+	+	+
4	+	-	+	+	+	+	+
5	+	+	+	+	+	+	+

P.: Penicillin, PIP.: Pipracillin AMP.: Ampicillin, AMO.: Amoxicillin,

AMC: (Ampicillin + Cloxacillin), Amox + Clav: (Amoxicillin + Calvulanic acid)

+ = Resistant, - = Sensitive.

Table (11-B)

Isolate No.	CL	CFZ	CTX	CTZ	β-lactamase production by iodometric method (the substrate Cephalexin)
1	+	+	+	+	+
2	+	+	+	+	+
3	+	+	+	+	+
4	+	+	+	+	+
5	+	+	+	+	+

CL: Cephalexin, CFZ: Cefazolin, CTX: Cefotaxime, CTZ: Ceftizoxime

الأهمية السريرية لإنزيم البيتالاكتاميز المنتج من قبل بعض البكتريا السالبة والبكتريا الموجبة لصبغة غرام

أطروحة

مقدمة إلى مجلس كلية الطب / جامعة بابل

كجزء من متطلبات

نيل شهادة الماجستير في الأحياء المجهرية الطبية

من قبل

رشـا عبد المـهدي فليح

1426 هـ **2005 م**

الخلاصـة

أخضعت في هذه الدراسة (40) عزلة مرضية مـن البكتريـا الـسالبة والموجبة لصبغة غرام ، وقد تم إخضاعها جميعا للفحوصات التأكيدية والتفريقية ، وكان من بين هذه العزلات (18) عزلة من بكتريا الالتهابات الرئوية ، و (5) عزلات من بكتريا القولون ، و (5) عزلات مـن بكتريـا الحـروق ، و (7) عزلات من البكتريا المعوية ، و (5) عزلات من بكتريا المكورات العنقوديـة . كما استخدم عدد من مضادات البيتالاكتام شـائعة الاسـتخدام ، مثـل البنـسلين والامبسلين والاموكسيسلين والامبكلوكس والسيفالكسين والسيفازولين والببراسـلين وغيرها .

أظهرت النتائج إن بكتريا الالتهابات الرئوية كانت مقاومة لكل من مـضاد البنسلين والامبسلين والاموكسيسلين بنسبة 100% ، أما بقيـة المـضادات فقـد اظهرت اختلافات واضحة في نسب المقاومة . وقد لوحظ إن بكتريا القولون كانت مقاومة لكل من مضاد البنسلين والببراسلين والسيفالكسين والسيفاتاكـسيم بنـسبة 100% . أما بقية المضادات فقد كانت المقاومة بنسب اقل . كذلك أظهرت بكتريا المكورات العنقودية مقاومة واضحة لكـل مـن مـضاد البنـسلين والامبـسلين والسيفالكسين والسيفازولين بنسبة 100% . أما بقية المضادات ، فقد كانت كـذلك مقاومة ولكن بنسب متفاوتة . كمـا وجـد إن أعلـى نـسبة مقاومـة للبكتريـا المعوية هي 85.7% لكل من مضاد البنسلين والامبسلين وبنـسب اقـل لبقيـة المضادات . أما بكتريا الحروق ، فقد أظهرت مقاومة عالية بنسبة 100% لجميع المضادات ما عدا الببراسلين ، حيث كانت نسبة المقاومة 60%.

كما تم في هذه الدراسـة اختيـار مركـب (الاموكسيـسلين + حـامض الكلافيولانك) لدراسة تأثير حامض الكلافيولانك على إنزيم البيتالاكتاميز . وقـد لوحظ إن هناك (9) عزلات من بين (4 0) عزلة مقاومـة لهـذا المركـب ، موزعة ما بين عزلة واحدة من بكتريا المكورات العنقودية وعزلة مـن بكتريـا الالتهابات الرئوية وعزلتين من بكتريا القولون و (5) عـزلات مـن بكتريـا الحروق . كما استخدمت بغض المركبات الكيميائيـة مثـل حـامض السالـسليك

ومركب الاثلين داي امين تترااسيتك أسيد لدراسة تأثيرهما على سلوك الكائن المجهري ، بما فيه إنتاج إنزيم البيتالاكتاميز . ووجد إن حامض السالسيلك يزيد من مقاومة البكتريا للمضادات الحيوية ، بينما يقلل مركب EDTA مقاومة البكتريا للمضادات ، إذ يؤثر على Metallo-β-lactamase .

لقد استخدمت طريقة اليود القياسية السريعة للتحري عن إنتاج إنزيم البيتالاكتاميز ، وقد أعطت (21) عزلة نتيجة موجبة ، موزعة ما بين: (4) عزلات من بكتريا المكورات العنقودية وعزلة من البكتريا المعوية و (9) عزلات من بكتريا الالتهابات الرئوية و (3) عزلات من بكتريا القولون و (5) عزلات من بكتريا الحروق . كما تم تقدير فعالية الإنزيم لبعض العزلات المنتجة له باستخدام طريقة Macroiodometric ، وقد لوحظ إن هناك اختلافات واضحة في الفعالية النوعية لهذا الإنزيم .

كما تم استخدام بعض المؤثرات البيئية مثل درجة الحرارة والحامضية ، لدراسة تأثيرهما على فعالية الإنزيم . وقد وجد إن أعلى كفاءة لإنزيم البيتالاكتاميز لجميع العزلات هي بدرجة حرارة ما بين (35 - 37) م ، و حامضية ما بين (6.5 - 7.5) .

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