Republic of Iraq
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Al-Nahrain University
College of Sciences

# A Comparative Study between the Effect of Lactobacillus acidophilus and EDTA with Antibiotics on *Pseudomonas aeruginosa* Isolates *in Vitro*

**A thesis**
**Submitted to the College of Science at AL-Nahrain University as partial fulfillment of the requirements for the degree of Master of Science in Biotechnology**

**By**

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جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة النهرين
كلية العلوم

# دراسة مقارنة بين تاثير بكتيريا *Lactobacillus acidophilus* و EDTA مع المضادات الحيوية على بكتيريا الـ *P. aeruginosa*

رسالة

مقدمة الى كلية العلوم جامعة النهرين
وهي جزء من متطلبات نيل درجة الماجستير في علوم التقانة الاحيائية

من قبل

احمد نوري فيصل العزاوي

بكلوريوس تقانة احيائية – جامعة النهرين (2002)

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# Abbreviations

| | |
|---|---|
| **ATCC** | American Type Culture Collection. |
| **EDTA** | Ethylenediamineteraactic acid. |
| **LAB** | Lactic acid bacteria. |
| **MIC** | Minimum Inhibitory Concentration. |
| **FIC** | Fractional Inhibitory Concentration. |
| **LPS** | Lipopolysacchrides. |
| **ADP** | Adenosin diphosphate. |
| **SDS** | Sodiumdodecylsulfate. |
| **SET** | Salain EDTA tris. |
| **TE** | Tris EDTA. |
| **TBE** | Tris Bourate EDTA. |
| **Syn** | Synergism. |
| **G -** | Gram Negative. |
| **G +** | Gram Positive. |
| **D.W** | Distilled water. |
| **O.D** | Optical density. |

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# Conclusions and Recommendations

## 4.1 Conclusions

1. In this study *Pseudomonas aeruginosa* isolates found different in their antibiotic resistance considerably, Amikacin, Gentamycin, Ceftazidim, Ciprofloxacin, Tetracycline and Pipracillin were the most effective.
2. The combinations of aminoglycoside and β-lactam antibiotics have the highest synergistic effect against the isolated strains of *Pseudomonas aeruginosa* in this study
3. EDTA increases the activity of antibiotic against *pseudomonas aeruginosa* especially when combined with aminoglycoside antibiotics.
4. *Lactobacillus acidophilus* filtrate had some effect against the tested *pseudomonas aeruginosa* isolates.
5. Third fold of concentrated filtrates of *Lactobacillus acidophilus* gave the highest inhibitory effect on the growth of *Pseudomonas aeruginosa* comparing to the first and second fold filtrates.

## 4.2 Recommendations

1. Extraction, purification and identification of the inhibitory substance produced by *Lactobacillus acidophilus* and evaluation of their therapeutic use.
2. Study the possibility combination effect of LAB and antibiotics against pathogenic bacteria.
3. Study the effect of *Lactobacillus acidophilus* against *Pseudomonas aeruginosa in vivo*.
4. Study the possibility of using *Lactobacillus acidophilus* substance in the treatment of burns and wounds cases in which antibiotic almost totally resisted.

# Introduction and Literature Review

## 1.1 Introduction

*Pseudomonas aeruginosa* is an important nosocomial pathogen due to its ubiquitous presence wherever there is water, the devastating effect of infection up on patients, usually is a result of an excessive immune response, and the organism's high resistance to both host defenses and antibacterial agents in general (Govan and Deretic *et al*., 1996). *P. aeruginosa* has for long been regarded as an antibiotic-resistant organism, its low permeability outer membrane preventing access of many agents to their sites of action (Nikaido *et al*., 1994). More recently the presence of constitutive and enhanceable efflux mechanisms removing a huge range of antimicrobial agents from the cell is considered as important factor of resistance, especially if coupled with enzymatic mechanisms of resistance (Nikaido and Poole, 1995).

Antimicrobial drugs may either kill microorganism or inhibit their growth. Those that inhibit growth are called bacteriostatic, these drugs depend on the normal host defense to kill or eliminate the pathogen after it's growth has been inhibited, for example sulfa drugs. Drugs that kill bacteria are bactericidal effect, these drugs are particularly useful in situation in which the normal host defenses can not be relied on to remove or destroy pathogen.

Consequently minimum inhibitory concentration are specified as that bacteriostatics effect that prevent visible growth of microbes on culture medium, application of such concentration were also important from genetic point of view as long as resistance development and spread are a function of dosage of applied antibiotics.

Combinations of antimicrobials are sometimes used to treat infections, but care must be taken when selecting the combination because some drugs will

counteract the effect of others. When the action of one drug enhances the activity of another, the combination is called synergistic. In contrast, combinations in which the activity of one interferes with the other are called antagonistic. Combination that are neither synergistic nor antagonistic are called additive.

In the last decades, microorganisms and their metabolic products were broadly used in treatment of various diseases and infections. Normal flora such as lactic acid bacteria (LAB), found in the gastrointestinal tracts can produce different types of materials; organic acids, ammonia, hydrogen peroxide, diacetyl, bacteriocins and others which be used as inhibitory means against pathogenic bacteria (Donohue and Salminen., 1996).

## Aims of Study

1. Isolation and identification of *Pseudomonas aeruginosa* isolates from patient suffering from burns and wounds.
2. Detecting the resistant isolate of *Pseudomonas aeruginosa* to be used in the combination experiment.
3. Determining the MIC of antibiotics to be use in combination.
4. Determining the effect of antibiotics combination against *Pseudomonas aeruginosa* isolates.
5. Determining the effect of EDTA in increasing the inhibition effect of antibiotics against *Pseudomonas aeruginosa* isolates.
6. Detecting the effect of LAB filtrates against *Pseudomonas aeruginosa* isolates and determining the minimum inhibitory concentration of the filtrate.

## 1.2 Genus *Pseudomonas*

*Pseudomonas* is very large and important group of aerobic, non fermentative, gram negative bacilli bacteria belonging to the family Pseudomonadaeace.

Members of this family are saprophytic found widely in soil, water, plants and animals (Mandelle *et al*., 1995; Green wood *et al*., 1997). They are straight or slightly curved rod bacteria but not helical, it`s occur singly, in pairs or in short chain, they are (0.5-1.0)µm in diameter by (1.5-5.0)µm in length, motile by one of two polar flagella, forming pili, non sporing, non capsulate, catalase test mostly positive, oxidase usually positive, gelatin positive (produce gelatinase), simon citrate positive, all members of genus *Pseudomonas* are negative in Methyl red and Vogas Proskaur, it`s metabolism is aerobic, and the G+C percent of DNA range from 58-71%(Krieg *et al*., 1984; Jawatz *et al*., 1998).

*P. aeruginosa* has very simple nutritional requirements, it`s often growing in distilled water which is evidence of it`s minimal nutritional needs. In the lab the simplest medium for growth of *P. aeruginosa* consist of acetate as carbon source and ammonium sulfate for nitrogen (Mandelle *et al*., 1995).

*P. aeruginosa* possesses the metabolic versatility, organic growth factors are not required, and it can use more than seventy five organic compounds for growth (Todar, 1997).

*P. aeruginosa* has an optimum temperature of 37°C and it`s able to grow at temperature as high as 42°C while *Pseudomonas fluorescence* can grow at 4°C, and this is use as an criterion for species differentiation, and it can grow well at pH (6.6-7.0) (Sneath *et al*;1992).

*P. aeruginosa* isolates may produce three colony types; natural isolates from soil or water typically produce a small rough colony while clinical samples in general yield one or two smooth colony types. One type has a fried-egg appearance which is large, smooth, with flat edges and an elevated appearance.

Another type, frequently obtained from respiratory and urinary tract secretions, has a mucoid appearance which related to the production of alginate slim. The smooth and mucoid colony presumed to play role in colonization and virulent (Collee *et al*., 1996; Todar, 1997).

## 1.3 Epidemiology

*P. aeruginosa* is a common inhabitant of soil, water, vegetarian and it`s part of the normal microbial flora of humans. The prevalence of colonization in non hospitalized patients or upon entry to hospitals is low, the specific colonization rate of *P. aeruginosa* are (skin 2%, nasal mucosa 3.3%, throat 6.6%, stool (3-24) %. In contrast the hospitalization may lead to greatly increased in the rate of carriage to 20% within 72 hr. of admission particularly on skin with patient have serious burns and gastrointestinal patients (Mandelle *et al*., 1995).

Within hospital *P. aeruginosa* finds numerous reservoirs: disinfectants, respiratory equipments, food, skin and taps. Furthermore it`s constantly reintroduced into the hospital environment on fruits, vegetables as well as by visitors and patient transferred from other facilities, spread occur from patient to patient on the hand of hospital persons, by direct patient contact with contaminated reservoirs and by ingestion of contaminated food and water (Todar, 1997).

The spread of *P. aeruginosa* can best be controlled by observing proper isolation procedure, aseptic technique and careful cleaning and monitoring of respirators, catheters, food and other instruments (Hector *et al*., 1993).

*P. aeruginosa* is frequently resistant to many commonly used antibiotics. Although many strains are susceptible to antibiotics such as Gentamycin, Tobramycin and Amikacin resistant forms have developed (Todar, 2004).

## 1.4 Pathogenesis

*P. aeruginosa* constitute a large group of the normal aerobic intestinal flora. Within the intestine they are generally don't cause disease and may even contribute to normal function and nutrition, but these organisms become pathogenic only when they reach tissue outside the intestinal tract, particularly the urinary tract, biliary tract, meninges, lung, eye, kidney, ear, intestine and damage or burn skin, causing inflammation at these sites only when normal host defense are inadequate, particularly in early infancy, in old age, in terminal stages of other disease, in patients with immuonodiffeciencies or nutropenic malignancies, chronically debilitated patient (Kinoshita *et al*., 1997; Bouze *et al*., 1999).

*P. aeruginosa* is able to secrete several toxic proteins that are thought to act as virulence factors and these proteins after colonization can cause extensive tissue damage and blood stream invasion (Todar *et al*., 2004).

Exotoxin A is potentially important virulence factor of P. *aeruginosa*, which has the same mechanism of diphtheria toxin. Exotoxin A is responsible for local tissue damage, bacterial invasion and immunosuppression (Wood *et al*., 1989). Exotoxin A is a major inhibiter of mammalian protein synthesis by mediating the ADP ribosylation of elongation factor 2 process (Todar, 2004).

Another potentially pathogenic extracellular enzyme produce by most isolates of *P. aeruginosa* is Exoenzyme S, which is responsible for tissue distribution and impairing the function of phagocytes in blood stream and organs to prepare for invasion by *P. aeruginosa* (Brint *et al*., 1995; Todar, 2004).

*P. aeruginosa* produce three other soluble proteins involves in invasion, cytotoxin and two haemolysins (Van-delden and Igleweski, 1998). The cytotoxin is a pore-forming protein, it was originally named leukocidin because of it`s effect on neutrophilis but it appears cytotoxic for eukaryotic cells (Todar, 2004). Of the two haemolysins, one is a phospholipase and the other is

lecithenase, they appear to act together to break down lipid and lecithin (Todar, 2004).

*P. aeruginosa* also produce two exoproduct, protease and elastase during infection, in order to facilitate the invasion and dissemination of these bacteria (Todar, 2004).

Elastase cause destruction of immunoglobulins (Igs) and other complements, and also lyses fibronectin to expose the mucosa of lung for bacterial attachment while protease cause lyses of fibrin by interfering with its formation process (Todar, 2004).

The two enzymes acts together to destroy the structure of fibrin and elastin and cause inactivation of gamma interferon (INF) and tumor necrosis factor (TNF) (Todar, 2004).

Also there is a lipopolysaccharide that is responsible for many endotoxic properties and exopolysaccharide (alginate) which is antiphagocytic (Brook *et al*., 1995).

*P. aeruginosa* don't produce the virulence factors until it respond to environmental signal which its production variant according to the stage of inflammation, the virulence factors of *P. aeruginosa* are summarized in table (1-1) (Todar, 2004)

## 1.5 Infection caused by *P. aeruginosa*

*P. aeruginosa* is now a widely recognized as an important opportunistic pathogen that produce sever infections since this pathogenic only when introduce into area devoid of normal defense, this may lead to the death of the compromised host (Sakata *et al*., 1996; Jawatz *et al*., 1998).

**Table (1-1)** Virulence factors of *P. aeruginosa* (Todar, 2004).

| Function | Determinant |
|---|---|
| **1.** Adhesion | Fimbria, alginate slim (biofilm) polysaccharide capsule (glycocalyx). |
| **2.** Invasion | Alkaline protease, elastase, haemolysin (phspholipase and lecithecin), cytotoxin (leukocidin), pyocyanin (diffusible pigment). |
| **3.** Motility/chemotaxis | Flagella. |
| **4.** Toxins | Exotoxin A, exoenzyme S, lipopolysaccharide. |
| **5.** Antiphagocytic properties | Capsule, slim layer, LPS. |
| **6.** Defense against serum bacteriocidal reaction | Slim layer, capsul, LPS, protease enzyme. |
| **7.** Defense against immunorespone | Capsul, slim layer, protease enzyme. |
| **8.** Genetic attribute | Genetic exchange by (transduction, conjugation), inheriting (natural) drug resistance. |
| **9.** Ecologic criteria | Adaptability to minimal nutritional requirements, metabolic diversity, widespread occurrence in a variety of habitat. |

### 1.5.1 Respiratory Infection

Respiratory infection caused by *P. aeruginosa* occurs almost exclusively in individuals with a compromised systemic defense mechanism. Primary pneumonia occurs in patients with chronic lung disease and congestive heart failure (Todar, 1997).

Lower respiratory tract colonization of cystic fibrosis patients by mucoid strain of *P. aeruginosa* is common and difficult if not impossible to treat (Giwercman *et al*., 1990; Marty *et al*., 1998).

### 1.5.2 Wound and Burn Infection

*P. aeruginosa* is one of the most common causes of infection in burns and wounds (Hsueh *et al*; 1998). It could cause burn sepsis through bacterial colonization of the burn site, destruction of the mechanical barrier to tissue invasion and multiple systemic immunological defects related to serious burns. *P. aeruginosa* is a major cause of death in burn patients (Tredget *et al*., 1992; Richard *et al*., 1994).

### 1.5.3 Urinary Tract Infection

Urinary tract infection (UTI) caused by *P. aeruginosa* are usually hospital acquired and related to urinary tract catheterization instrumentation or surgery (Martinez *et al*., 1999).

*P. aeruginosa* is the third leading cause of hospital acquired UTI, accounting for about 12% of all infection of this type, the bacteria appears to be among the most adherent of common urinary pathogen to the bladder uroepithelium, and the infection can occur via an descending or ascending route, in which *P. aeruginosa* invades blood stream from the urinary tract and this is the source of nearly 40% of *P. aeruginosa* (Mandelle *et al*., 1995; Todar, 2004).

### 1.5.4 Endocarditis

*P. aeruginosa* infect heart valves of intravascular drug user and prosthetic heart valves. The organism establishes itself on the endocardium by direct invasion from the blood stream (Pollack, 1998).

### 1.5.5 Eye Infection

*P. aeruginosa* can cause devastating infection in the human eye. It`s one of the most common cause of bacterial keratitis (Todar, 2004). The result can be a rapidly progressive and destructive infection that may develop to blindness (Hazlett *et al*., 1989).

### 1.5.6 Ear Infection

*P. aeruginosa* is the predominant bacterial pathogen in some case of external otitis including (swimmers ear), the bacterium is infrequently found in the normal ear but often inhabitate the external auditory canal in association with injury, maceration, inflammation, or simple wet or humid condition (Mims *et al*., 1993; Gates *et al*., 1998).

### 1.5.7 Gastrointestinal Infection

*P. aeruginosa* is an important cause of gastrointestinal infection, which represent an important portal of entry in *Pseudomonas* septicemia (Pollack, 1998). and it`s also associated with relatively diarrhea which could be referred to toxin production. Mortality remains high despite advance in therapy (Mandelle *et al*., 1995; Gallagher *et al*., 1998).

### 1.5.8 Bacteremia

*P. aeruginosa* cause bacterimia primarily in immunocompromised patients, predisposing condition include hematologic malignancies,

immunodeficiency relating to AIDS, neutropenia, diabetes mellitus and severe burns. *P. aeruginosa* bacterimia is acquired in hospitals and nursing home, it account about 25% of all hospitals acquired gram negative bactermia (Todar, 2004).

## 1.6 Resistance to Antimicrobial Agent

With increasing usage of antibiotics for treatment of disease against different microorganism, there was a respective increase in microbial antibiotic resistance (Summer, 1986).

Antibiotic resistant bacteria are also found in environments where antibiotic are not use (Kelch and Lee, 1978).

There are different mechanisms by which microorganism might exhibit resistance to drug which are:-

**A.** Microorganism changes their cell membrane permeability to drugs, like tetracycline, aminoglycoside and chloramphenicol resistant bacteria (Martinez *et al*., 1999).

**B.** Microorganism produce enzymes destroy the active drug like staphylococcus produce β-lactemase that destroy the β-lactam ring of penicillins (Livermore *et al*., 1995).

**C.** Microorganism develops an altered structural target for drug like alteration of specific protein on 30s subunit of bacterial ribosome that serve as binding site in susceptible organisms (Brook *et al*., 1995).

**D.** Microorganism develops an altered metabolic pathway differ from that inhibited by the drug such as (sulphanamide resistant bacteria) (Brook *et al*., 1995).

**E.** Microorganism develops an altered enzyme that can still perform it's metabolic function but its much less affected by the drug (Jawetz *et al*., 1998).

### 1.6.1 Resistance to β-lactams

These antibiotics disrupt the structure of the cell wall of microorganism by either inhibiting the formation of molecule vital for the cell wall structure or prevent bonds formation within the cell wall, causing it to loss it`s strength (Todar, 2004).

β-lactams antibiotics divide into two groups the penicillin (Figure 1-1) and cephalosporin (Figure 1-2) which are susceptible to enzymatic modification and degradation (Ito *et al*., 1997). *P. aeruginosa* may exhibit reduced susceptibility to β-lactam antibiotics by number of mechanisms including, target site modification or change could occur in the affinity of β-lactam to the penicillin binding protein which are the target protein of β-lactam antibiotics, and play role in the synthesis of peptidoglycan layer, and reduce outer membrane permeability (Arakwa *et al*., 1989; Stapleton *et al*., 1995).

Other mechanisms of resistance to β-lactams are by enzymatic inactivation of β-lactam by β-lactamase enzyme which destroy the β-lactam ring and terminate the activity of antibiotic (Stapleton *et al*., 1995; Laurance *et al*., 1997).

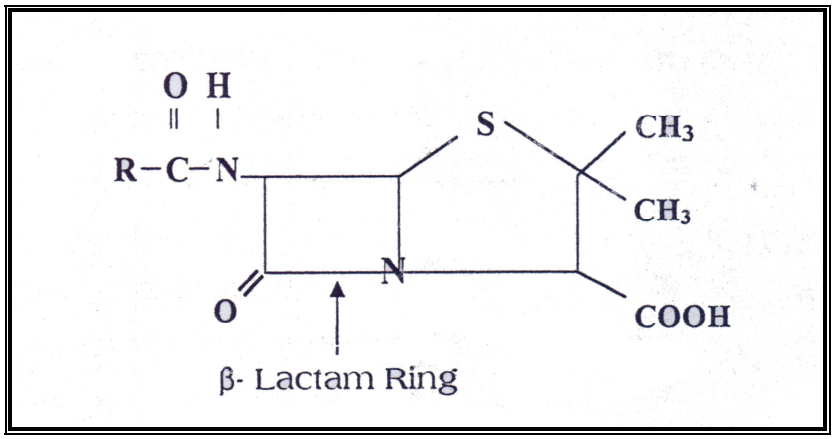


**Figure 1-1:** Structure of Penicillin (Henry *et al*, 2001).

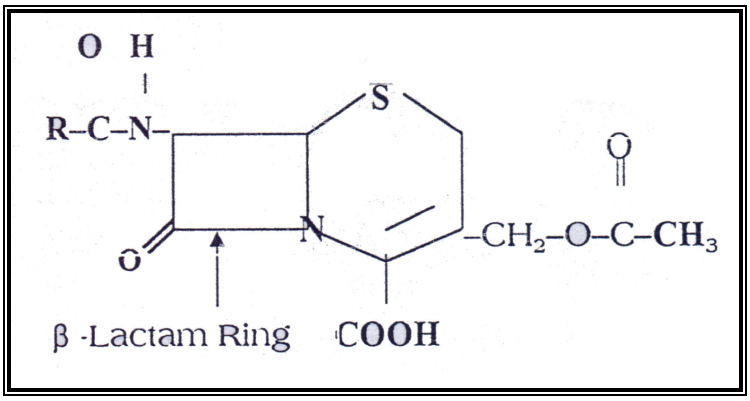


**Figure 1-2:** Structure of cephalosporin (Henry *et al*, 2001).

## 1.6.2 Resistance to Aminoglycosides

Aminoglycosides (Figure 1-3) are highly potent, broad spectrum antibiotics with many desirable properties for treatment of life threating infection (Lecleco *et al*., 1999).

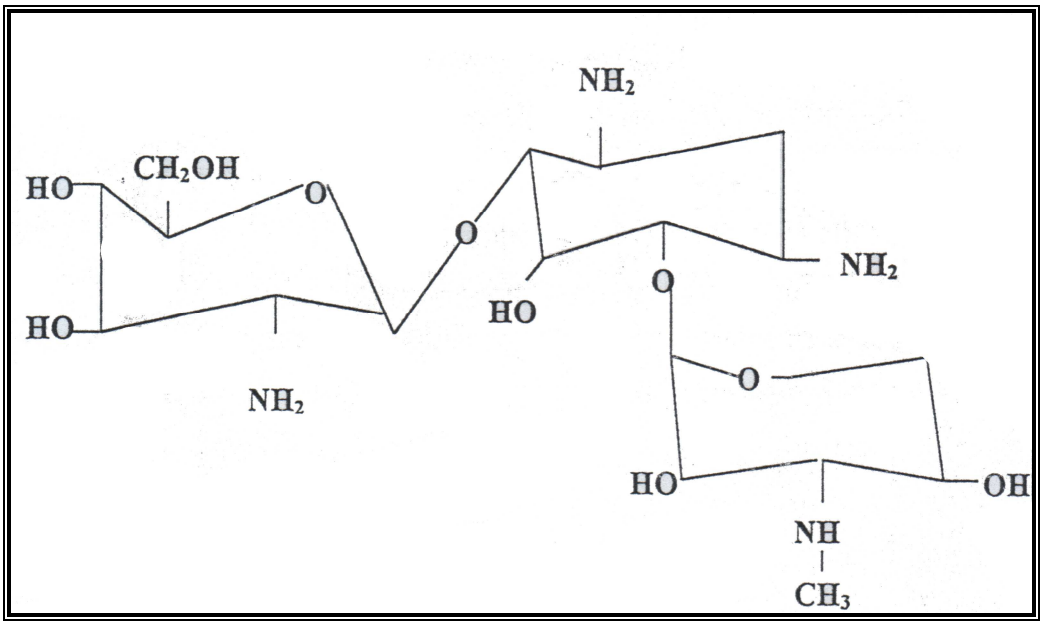


**Figure 1-3:** Structure of Gentamycin (Henry *et al*, 2001).

They acts primarily through impairing bacterial protein synthesis by binding to the 30s subunit of the ribosomes (Aires *et al*., 1999). Resistance to this group of antibiotics in *P. aeruginosa* could occur in three mechanisms:

- First production of enzyme that cause modification of aminoglycosides ring by (adenyllation, acetylation or phosphorylation), (Shaw *et al*., 1993; Miller *et al*., 1995).
- Second deletion or alteration occur in the receptor protein of 30s ribosomal subunit resulting from mutation that prevent the binding of antibiotic to the receptor (Henry *et al*., 2001).
- Third change occurs in the outer membrane composition of *P. aeruginosa* including alteration in the structure of lipopolysaccharides and change in the electron transport chain (Shannon *et al*., 1999).

## 1.6.3 Resistance to Flouroquinolones

Flouroquinolones (Figure 1-4) are extremely useful agents and an important therapeutic advance, they are relatively non toxic, well tolerated, broad spectrum agents, their excellent bioavailability permit their use for treatment of variety of serious bacterial infection such as those caused by *P. aeruginosa* (Henry *et al*., 2001).

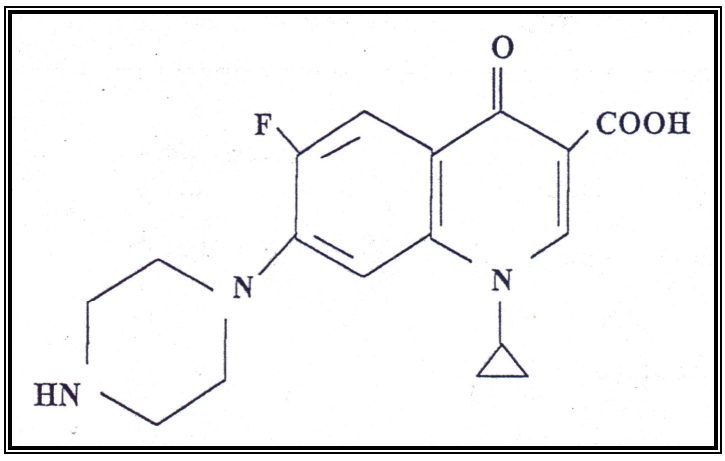


**Figure 1-4:** Structure of Ciprofloxacin (Henry *et al,* 2001).

This group of antibacterial acts on microorganisms by inhibiting the synthesis of bacterial enzyme DNA gyrase and topoisomerase IV (Martinez *et al*., 1999).

The resistance of *P. aeruginosa* for this group of antibiotics occurs by different mechanisms, the main mechanism is mutation in the target gene, those encoding DNA gyrase and topoisomerase IV (Heiby *et al*., 1989).

Another mechanism of flouroquinolone resistance is alteration in the outer membrane of *P. aeruginosa* that change the permeability to antibiotics (Young and Hancock, 1992).

## 1.6.4 Resistance to Macrolides

The macrolides antibiotics are group of closely related compounds characterized by a macrocyclic lacton ring, example of this group is erythromycin (Henry *et al*., 2001).

Antibiotic of this group inhibits microorganisms by binding to the 23s rRNA on the 50s ribosomal subunit, where they block the elongation of the growing peptide chain (Kawamura-Sato *et al*., 2000).

Resistance of microorganisms to this group of antibiotics occur by reducing permeability of the cell membrane or increasing active efflux, production of enzyme that cause hydrolyzing of the antibiotics or modification of the ribosomal binding site that prevent binding of antibiotic to the target protein and this result by chromosomal mutation (Henry *et al*., 2001).

## 1.6.5 Resistance to Other Antibiotics

Such as tetracycline and chloramphenicol:

This kind of antibiotics acts on microorganisms by inhibiting protein synthesis process (Henry *et al*., 2001).

Microorganisms resist to such antibiotic by decreasing intracellular accumulation by either impairing influx or increase efflux by an active transport protein pump, production of enzyme that cause inactivation of antibiotics or ribosomal protection due to production of protein that interfered with antibiotics that bind to ribosome (Jawetz *et al*., 1998).

## 1.7 The Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) is the lowest concentration of an antibiotic that still inhibit the growth of a particular microorganism, can be determined using tube dilution procedure. This procedure establishes the lowest concentration of an antibiotic that is effective in preventing the growth of the pathogen. It also gives an indication of the dosage of that antibiotic that should be effective in controlling the infection in the patient. Standardized microbial inocula are added to tubes containing serial dilutions of an antibiotic, and the growth of the microorganism is monitored as a change in turbidity. In this way, the break point or minimum inhibitory concentration of the antibiotic that inhibit growth of the microorganism in vitro can be determined (Baron *et al.,* 1994).

The MIC indicates the minimal concentration of the antibiotic that must be achieved at the site of infection to inhibit the growth of the microorganism being tested. By knowing the MIC and the theoretical levels of the antibiotics that may be achieved in body fluids such as blood and urine, we can select the appropriate antibiotic, the dosage schedule and the route of administration. Generally a margin of safety of ten times the MIC is desirable to ensure successful treatment of the disease (Henry *et al.,* 2001).

## 1.8 Antimicrobial Combination

Most infections in humans with normal host defenses system can be treated with a single antimicrobial agent, but there are indications for the use of combinations (usually two) of antimicrobials for treatment of infections. Because combinations may provide more broad-spectrum coverage than single antibiotics can provide, the physician is often tempted to use combinations for treatment of diseases caused by bacteria resistant to this antibiotics when it`s single, even in situations in which they are not indicated (Abramowicz *et al*.,

1998). Such inappropriate use of antimicrobial combinations may have significantly deleterious effect. When two antimicrobial agents are combined, they may have one of three types of activity against a given organism in vitro (Moellering *et al*., 1998) :- (1) an additive effect, when the activity of the drugs in combination is equal to the sum of the effect of the two antibiotics singly, (2) synergism, when the activity of the drug in combination is greater than the sum effect of the two antibiotics when measured alone, or (3) antagonism, when the activity of the drug in combination is less than the sum effect of the two antibiotics when measured alone (Ball, 1998).

## 1.9 EDTA and its Antimicrobial Effect

Salts of EDTA have long been used as antimicrobial agents, particularly against bacteria (Hague *et al*., 1974a). They have also been enhancer of other antimicrobial agents, such as: lysozyme, antibiotics, and irridation, by increasing permeability of bacterial membrane or by removal or destruction of covalently bound lipid components (Figure 1-5) (Payne, 1994).

Its activity appears to be more effective when used in combination with antibiotics with activity against gram negative than with gram positive bacteria. This is due to the differences in the cell wall structure of the two groups. Gram positive bacteria contain more phospholipids comparing with peptidoglycans in their cell walls than Gram negative (Foster *et al*., 1998).

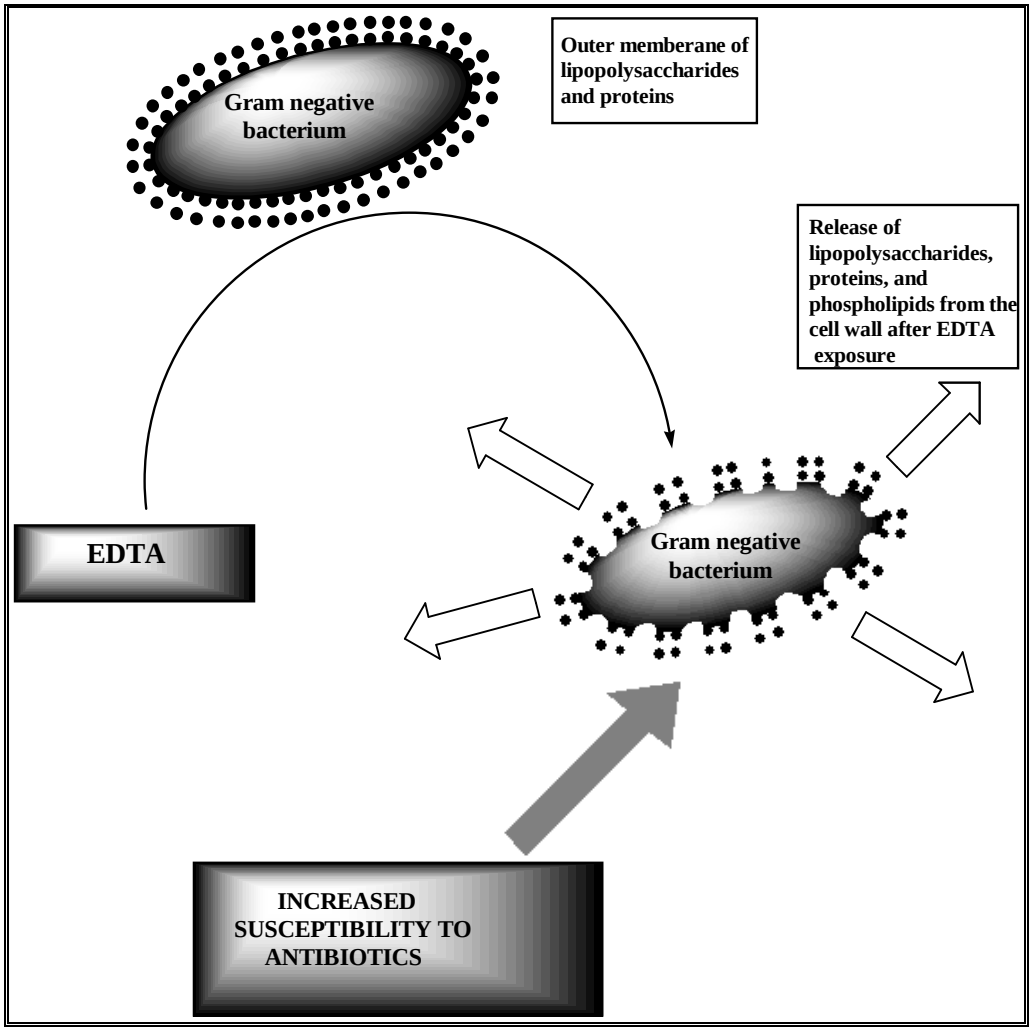


**Figure 1-5:** Schematic diagram demonstrating the action of EDTA on gram negative bacteria (Payne, 1994).

Two modes of action of EDTA has been recognized, first EDTA potentiate the effect of antibiotics by binding to the metal ions which compete with aminoglycosides for cell wall receptor that allow them into bacteria (Farco *et al*., 1997).

Second EDTA disrupt the lipopolysaccharides structure in the outer membrane of gram negative bacteria, through this disruption the membrane becomes more permeable to other agents such as antibiotics (Lambert *et al*., 2003).

## 1.10 Probiotics

The word probiotics is derived from the (Greek) and mean (for life). It was first used by Lilly and Stillwell (1965). Probiotics is a microbial dietary adjuvant that beneficially affects the host physiology by modulating mucosal and systemic immunity, as well as improving nutritional and microbial balance in the intestinal tract (Lister *et al*., 1999).

Currently probiotics preparation contain (*Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus casei, Lactobacillus fermentum, Lactobacillus lactis, Lactobacillus brevis*) are available (Tisser *et al*., 1905).

Various nutritional and therapeutic effects of lactic acid bacteria (LAB) are summarize as follow (Mckay *et al*., 1985):-

**1.** Improvement of nutritional quality of food and feed.
**2.** Metabolic stimuli of vitamins synthesis and enzyme production.
**3.** Stabilization of gut microflora and competitive exclusion of enteric pathogen.
**4.** Enhance innet host defense by production of antimicrobial substance.
**5.** Reduction of serum cholesterol by assimilation mechanism.
**6.** Decrease risk of colon cancer by detoxification of carcinogens.
**7.** Lactic acid produce by lactobacillus help enhancing the utilization of essential minerals such as calcium, phosphorus and iron.
**8.** Lactic acid produced stimulates the bowel movement and therefore relieves constipation.
**9.** Lactobacillus produces mainly vitamin B complex.
**10.** Lactobacillus controls the diarrhea due to antibiotics produced.

Further more LAB have several properties of biological importance like: lactose utilization, proteinase activity, bacteriophage defense mechanism and bacteriocin production.

## 1.11 Genus *Lactobacillus*:-

Lactobacillus is gram positive, non-spore former bacilli or coccbacilli, single, paired, chain or tetrad, catalase negative, anaerobic or microphilic and stable in the acidity and salt (Stamer, 1979).

This genus contain largest group of LAB. Hammes and Vogal (1995) mentioned that at first named by Beijernick as "bacill" in 1901. They classified by Orla-Jensen (1919) to *Thermobacterium, Streptobacterium* and *Betabacterium*.

After that new classification appeared by Kandeler and Weiss (1986) which classified LAB into three groups; obligate homofermentative, facultatively hetrofermentative, and obligatory hetrofermentative.

Lactobacilli have numerous inhibitory substances that produce through the fermentation of LAB such as: organic acids, hydrogen peroxide ($H_2O_2$), diacetyl, acetaldehyde, carbon dioxide $CO_2$, bacteriocin (Bonestroo *et al*., 1993).

## 1.12 Nutritional Requirement of Lactic Acid Bacteria

LAB needs fastidious nutritional requirements (Lidbeck *et al*., 1993), it needs broad spectrum of organic acids and inorganic acids, amino acids, vitamins (B-Plex), carbohydrates, peptides, salts and fatty acids (Stanier *et al*., 1986; Stamer, 1979; Kandeler and Weiss, 1986).

Morishita *et al*., (1981) stated that lactobacillus needs amino acids and found that absence of arginine, leucine, Isoleucine, valine, phenylalanine, tryptophane, glutamine from the medium leads to decrease the growth of *lactobacillus acidophilus, lactobacillus plantarum and lactobacillus casei*.

Narendranath *et al*., (1997) ensured that the requirements of LAB for growth are: nucleotides, amino acids, vitamin, B-12 and the biotin. They added that the addition of tween 80 and citrate to the medium caused increased and better growth, while the decrease of these growth factors from the medium like citrate, and manganese lead to decrease the growth rate.

Addition of yeast extract to the milk culture helped to stimulate the growth of LAB and protein synthesis (Smith *et al*., 1975).

## 1.13 Antimicrobial Effect of Lactic Acid Bacteria (LAB)

Several investigations have demonstrated that various species of LAB exert antagonist action against intestinal and food-born pathogens (Gibson *et al*., 1997). LABs are capable of preventing the adherence, establishment, and/or pathogenic action of specific enteropathogens (Saavedra, 1995).

The antagonist properties may be manifested by:

**a.** Decreasing the luminal pH through the production of volatile short chain fatty acids (SCFA) like acetic, lactic, propionic acid.

**b.** Rendering specific nutrients unavailable to pathogens.

**c.** Decreasing the reduction/oxidation potential of the luminal environment.

**d.** Competitive inhibition for bacterial adhesion site on intestinal epithelial surface by probiotics.

**e.** Stimulation of specific and non specific immunity against intestinal disease.

**f.** Producing $H_2O_2$ under anaerobic condition and/or producing specific inhibitory compounds like bacteriocin.

## 1.14 Using Lactic Acid Bacteria as Probiotic in Therapy

LAB making large proportion of the normal flora in the intestinal tract (gut) (Isolauri *et al*., 1991; Salminen and Deighton *et al*., 1993).

LAB strains that demonstrate a wide spectrum of antimicrobial characteristic, including acid and bile resistance, anti-microbial system (ex: bacteriocin, lactic acid, peroxide), and adhesion to various types of pathogens (Chan *et al*., 1984).

Lindgren and Dobrogosz (1990) stated that there are many mechanisms in which LAB protects the intestinal tract including; decreasing pH value,

adherence to the intestinal cell wall, production of inhibitory material (bacteriocin), production of antitoxin and ability to still life.

Salminen *et al*., (1993) suggested that the minimum concentration of LAB in the product using in the therapy should be ($1\times10^5$) CFU/ml or 1 gram, like *lactobacillus acidophilus, lactobacillus plantarum* which are widely use in the industry (food preservation) and in the therapy.

Gorbach (1990) performed several studies on the LAB to control the intestinal infection like salmonellosis and shigellosis, some types of colon cancer, and serum cholesterol level.

Probiotics are extremely safe and are not associated with any significant or deterimental side effect (Mcfarland and Elmer *et al*., 1995).

Lactobacillus therapy seems to reduce the recurrence rate of uncomplicated lower urinary tract infections in women, so it is used against urinary tract infection (Reid *et al*., 1987).

*Lactobacillus acidophilus* has a superior capability of producing lactic acid, which is antimicrobial and helps the body protection from the harmful bacteria adhering to the intestinal mucosa (Donohue and Salminen *et al*., 1996).

Winkelstin (1955) formulated "probiotic tablets" from *lactobacillus acidophilus*, there are several studies that mentioned the activity of LAB as "antigen" to the mucosal intestine layer and named (mucosal vaccine) (Mercenier, 1999).

Lactobacillus species inhibits the activities and proliferation of pathogenic bacteria by several ways such as production of lactic acid, production of antibiotics. *Lactobacillus acidophilus* produces acidophilin, *Lactobaciluus plantarum* produces lactocidin that have action in inhibiting several bacteria like *E. coli*, *Helicobacter Pylori* and *Proteus* spp. (Hirayama and Rafter, 1999).

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# Materials and Methods

## 2.1 Materials

### 2.1.1 Equipments and Apparatuses

The following equipments and apparatuses were used to perform the study:-

| Equipment | Company (Origin) |
|---|---|
| Anaerobic jar | Rodwell (England) |
| Autoclave | Gallenkamp (England) |
| Balance | Ohance (France) |
| Compound light microscope | Olympus (Japan) |
| Distillater | Gallenkamp (England) |
| Electric balance | Delta range (Switzerland) |
| Electric pH meter | Metter-Gm PH tdedo (UK) |
| Electrical oven | Memmert (Germany) |
| Glass Pasteur pipette | John poulten ltd (England) |
| Incubator | Gallenkamp (England) |
| Micropipette | Witeg (Germany) |
| Millipore filter (0.22µm) | Millipore and whatman (England) |
| Centrifuge | Hermle labortechink (Germany) |
| Refrigerator centrifuge | Harrier (UK) |
| Shake incubator | Gallenkamp (England) |
| Spectrophotometer | Aurora instruments (England) |
| Vortex | Buchi (Switzerland) |
| Water bath | Gfl (England) |

## 2.1.2 Chemicals

| Material | Company |
|---|---|
| Peptone, Glycerol, $K_2SO_4$, $MgCl_2.6H_2O$, $K_2HPO_4$, D-Glucose, Urea, EDTA, Hydrochloric acid, Methylene blue, Potassium dihydrogene phosphate, Sodium acetate hydrate, Triamonium citrate, $MgSO_4.7H_2O$, Isoamyl alcohol, | BDH (England) |
| NNNN tetramethyl-p-phenylene diamine dihydrochloride, Agar | Difco (england) |
| Phenol, Yeast extract, Hydrogen peroxide, Trypton | Fluka (Switzerland) |
| Meat extract, Tween 80, Gelatin | Oxoid (England) |
| Citramide, Sodium chloride, P-dimethyl aminobenzaldehyde, $MnSO_4.4H_2O$, Ethanol | Riedel-Dehaeny (Germany) |

## 2.1.3 Culture Media

| Media | Company |
|---|---|
| Blood agar base | Mast-diagnosis (England) |
| Brain heart infusion agar | Oxoid (England) |
| MacConkey agar | Oxoid (England) |
| Modified regosa agar (MRS) | Hi-media (Italy) |
| Modified regosa broth | Hi-media (Italy) |
| Muller Hinton agar | Mast diagnosis (England) |
| Nutrient agar | Oxoid (England) |
| Nutrient broth | Oxoid (England) |
| Urea base agar | Oxoid (England) |
| King A agar | Difco (USA) |
| Citramid agar | Biolife (Italy) |

## 2.1.4 API 20 kit (API Bio Merieux):

Api 20E kit consisted of:-

**1. Galleries**: the gallery is a plastic strip with 20 microtubes containing dehydrated reactive ingredient.

**2. Api 20E reagents:-**

- Oxidase reagent (1% tetra-methyl-p-phenyldiamine)
- Kovacs reagent (p-dimethyl aminobenzaldehyde at 4% in HCl isoamyl (alcohol).
- Voges-proskauer reagent:-
  - Vp1 (40% potassium hydroxide).
  - Vp2 (6% alpha-nephthal).
- Ferric chloride 3-4%.

## 2.1.5 Antibiotics

### 2.1.5.1 Antibiotic Powder

| Antibiotic | Source (Origin) |
|---|---|
| Amikacin | AL-Razi center for production of diagnostic kits (Iraq). |
| Ceftazidim | Gulf pharmaceutical industries (UAE) |
| Gentamicin | AL-Razi |
| Ciprofloxacin | BAL-pharma (India) |
| Tetracycline | AL-Razi |
| Pipracillin | AL-Razi |

### 2.1.5.2 Antibiotic Disks

| Antibiotics | Code | Concentration (µg)/disk | Source (Origin) |
|---|---|---|---|
| Impenem | IMP | 10 | Oxoid (England) |
| Amikacin | AK | 30 | Al-Razi (Iraq) |
| Carbenicillin | PY | 100 | Al-Razi |
| Cefotaxime | CTX | 30 | Al-Razi |
| Ceftazidime | CAZ | 30 | Oxoid |
| Ciprofloxacin | CIP | 5 | Al-Razi |
| Chloramphenicol | C | 30 | Al-Razi |
| Gentamicin | CN | 10 | Al-Razi |
| Pipracillin | PRL | 100 | Al-Razi |
| Streptomycin | S | 10 | Oxoid |
| Erythromycin | E | 15 | Oxoid |
| Tetracycline | TE | 30 | Al-Razi |
| Vancomycin | VA | 30 | Oxoid |
| Ticarcillin | TR | 85 | Al-Razi |
| Tobramycin | TOB | 10 | Al-Razi |
| Aztronam | ATM | 30 | Oxoid |
| Trimethprime + sulphamethoxazole | SXT | 25 | Al-Razi |
| Norfloxacin | NOR | 10 | Al-Razi |
| Naldixic acid | NA | 30 | Al-Razi |
| Penicillin | P | 10 | Al-Razi |

## 2.1.6 Bacterial Strains

| Strain | Supplied by |
|---|---|
| *Pseudomonas aeruginosa* ATCC 27583 | Department of Biology/ College of Science / Baghdad University |
| *Lactobacillus acidophilus* | Department of Biotechnology/ College of Science / Al-Nahrain University |

# 2.2 Methods

## 2.2.1 Solutions, Buffers and Reagents

### 2.2.1.1 Antibiotic Solutions:-

- Pipracillin, Gentamycin, Amikacin, Ceftazidime and Tetracycline were prepared as stock solution of 10 mg/ml of antibiotic powder in distilled water, sterilized by filtration and store at -20°C (Sambrook *et al.*, 1989).
- Ciprofloxacin solution were prepared as stock solution by dissolving 1g of antibiotic powder in 90ml sterile distilled water, pH adjusted to 5.0 with 1N HCl then volume completed to 100ml, obtaining a final concentration of 10 mg/ml, sterilized by filtration and stored at -20°C (Al-Yaseri, 1995).

### 2.2.1.2 EDTA Stock Solution

EDTA solution is prepared by adding 186.1g of disodium ethylene diamine tetraacetate.$2H_2O$ to 800 ml of D.W., stirring vigorously on a magnetic stirrer, pH was adjusted to 8.0 with NaOH, dispenses into aliquots and sterilized by autoclave giving a final concentration of (5mM) (Maniatis, 1982).

### 2.2.1.3 Staining Solution

It was prepared by dissolving (0.3g) of methylene blue powder in 30 ml of ethanol (Atlas, 1995).

### 2.2.1.4 Buffers

- **SDS solution**

| | |
|---|---|
| SDS | 10% |
| NaOH | 0.2N |

- **TE buffer**

| | |
|---|---|
| EDTA | 1mM |
| Tris-OH | 10mM pH was adjusted to (8.0) and sterilized by autoclaving. |

- **5X Tris-Borate-EDTA (TBE) (pH 8.0). (Maniatis *et al,* 1982).**

  Tris-base 54g

  Boric acid 27.5g

  EDTA (0.5) 20ml

  Distilled water to 1000 ml

- **SET buffer**

  NaCL 3M

  Tris.CL (pH 7.8) 0.4 M

  EDTA 20mM

- **5M NaCL**

  NaCL

  D.WSS

#### 2.2.1.5 Reagents

- **Oxidase Reagent (Baron, 1994)**

A solution of 1% N, N, N, N-tetramehtyl-p-phenylene diamine dihydrochloride was prepared in sterile distilled water when needed.

- **Catalase Reagent (Atlas, 1995)**

A solution of 3% hydrogen peroxide ($H_2O_2$) was prepared by the addition of 1 volume of $H_2O_2$ to 9 volumes of sterile distilled water; give a final concentration of 3% use for detecting the production of enzyme catalase.

### 2.2.2 Media Preparation

#### 2.2.2.1 Ready to Use Media

Nutrient agar, MacConkey agar, Brain heart infusion agar, Muller Hinton agar, Modified regosa agar, Nutrient broth and Muller Hinton broth, were prepared as recommended by manufacturing companies and autoclaved at 121°C for 15 minutes.

### 2.2.2.2 Laboratory Prepared Medium

- **Blood Agar Medium**

It was prepared by autoclaving blood agar base under 15 (Jole) pressure at 121°C for 15 minutes, cooled to 50°C, then 5% blood was added, mixed well and poured in to Petri dishes.

- **Citramide Agar (Kreig and Gerhardt, 1984)**

| | |
|---|---|
| Peptone | 20g |
| Glycerol | 10 ml |
| $MgCl_2.6H_2O$ | 1.5g |
| $K_2SO_4$ | 10g |
| Citramid | 0.3g |
| Agar | 12g |
| D.W | to 1000 ml |

pH was adjusted to 7.2-7.4 and sterilized by autoclaving.

- **King A Medium (Kreig and Gerhardt, 1984)**

| | |
|---|---|
| Peptone | 20g |
| Glycerol | 10 ml |
| $K_2SO_4$ | 10g |
| $MgCl_2.6H_2O$ | 3.5g |
| Agar | 15g |
| D.W | to 1000 ml |

pH was adjusted to 7.2-7.4 and sterilized by autoclaving.

- **Urea agar base (Christensen) (Atlas, 1995):-**

| | |
|---|---|
| Phenol | 0.012g |
| NaCl | 5g |
| $KH_2PO_4$ | 2g |
| Peptone | 1g |
| Glucose | 1g |
| Urea | 20g |
| Agar | 15g |
| D.W | to 1000 ml |

After combine ingredients in 950 ml distilled water, pH was adjusted to 7.0 and boiled to dissolve agar then sterilized by autoclave (121 °C for 15 minutes) and cooled to 50 °C. Aseptically 50 ml of 40% (w/v) of urea (sterilized by filtration) was added to give a final volume of 1000 ml.

- **Modified Regosa Broth Medium (MRS) (DeMan, 1960)**

| | |
|---|---|
| Peptone | 10g |
| Meat extracts | 10g |
| Yeast extracts | 5g |
| Glucose | 20g |
| Tween 80 | 1 ml |
| $K_2HPO_4$ | 2g |
| Sodium acetate hydrate | 5g |
| Triammonium citrate | 2g |
| $MgSO_4.7H_2O$ | 0.2g |
| $MnSO_4.4H_2O$ | 0.05G |
| D.W | to 1000 ml |

pH was adjusted to 6.0 and sterilized by autoclaving. This medium used for growing lactic acid bacteria (*Lactobacillus spp*.).

## 2.2.3 Sterilization

### 2.2.3.1 Moist Heat Sterilization

Media and solution were sterilized by autoclaving under 15 (Jole) pressure at 121 °C for 15 minutes.

### 2.2.3.2 Oven Sterilization

Oven was used to sterilize glassware at 160-180 °C for 3-2 hrs.

### 2.2.3.3 Membrane Sterilization (Filtration)

Millipore filter was used to sterilize antibiotics solution and the filtration of growth of *Lactobacillus acidophilus* by using (0.22µm) in diameter Millipore filters.

## 2.2.4 Sample Collection

The swap samples from burns and wounds were collected in a sterile tubes containing nutrient broth from patients of AL-Yarmooq, Baghdad and Al-Kadhumia Medical Hospitals during the period from 1/3/2005 to 15/5/2005.

A total of 150 samples were collected and transported to the laboratory within two hrs of collection.

## 2.2.5 Bacterial Isolation

Burns and wounds samples were cultured by spreading on MacConkeys and blood agar plates. Plates were incubated overnight at 37°C.

After the incubation, non fermentative colonies which appeared pale on MacConkeys agar were selected and streaked on selective media (Citramid agar and king A agar) and incubated at 37°C for 24 hrs to test the pigmentation related to *P. aeruginosa*. These colonies were subcultured on brain heart infusion agar to obtain pure culture for further diagnosis (Jawetz *et al*., 1980).

## 2.2.6 Maintenance of Bacterial Isolate:-

Maintenance of bacterial isolate was performed according to (Maniatis *et al*., 1982) and as follow:-

### 2.2.6.1 Short Term Storage

Isolates of bacteria were maintained for few weeks on the surface of MacConkeys agar plates. The plates were tightly wrapped in parafilm and stored at 4°C.

### 2.2.6.2 Medium Term Storage

Isolates of bacteria were maintained in stab culture for period of few months. Such cultures were prepared in small screw-capped bottles containing 2-3 ml of agar medium and stored at 4°C.

### 2.2.6.3 Long Term Storage

Isolates were stored for long period in a medium containing 15% glycerol at low temperature without significant loss of viability. This was done by adding 1.5ml of sterilized glycerol to an exponential growth of bacteria in small screw-capped bottle with final volume of 10 ml and stored at -20°C.

## 2.2.7 Identification of *P. aeruginosa*

### 2.2.7.1 Microscopic Examination

A loopfull of *P. aeruginosa* isolate was fixed on a microscopic slide, then stained by gram stain to examine cell shape, grouping, gram reaction (positive or negative) and non spore forming (Atlas *et al*; 1995).

### 2.2.6.2 Biochemical Tests for Characterization of Bacterial Isolates

- **Oxidase Test (Atlas *et al*., 1995)**

This test was done by using a moisten filter paper with few drops of a freshly prepared solution of N,N,N,N-tetramethyl-p-phenylene diamine dihydrochloride. Then aseptically picked up a clump of cells from slant growth with a sterile wooden stick and smear them on the moistened paper. The development of a violet or purple color within 10 seconds indicates a positive result.

- **Catalase test (Atlas *et al.*, 1995)**

This test was performed by adding drops of $H_2O_2$ to colonies grown on nutrient agar plates. The production of gas bubbles indicate positive result.

- **Urease test (Atlas *et al*., 1995)**

Urease activity was detected by inoculating the surface of Christensen urea agar slants with bacterial growth and incubating at 37°C for 24 hrs. After incubation, the appearance of a red-violet color indicates a positive results .

- **Pyocyanin Pigment Production Test (Baron and Finegold *et al*., 1994)**

Bacterial growth on Citramid agar and king A agar was examined under UV light, blue green pigments indicates the presence of pyocyanin.

- **Haemolysin Test (Cruichshank *et al*., 1975)**

Haemolysin activity was detected by using blood agar medium. A loopfull of bacterial growth was streaked on blood agar and incubated at 37C° for 24 hrs. the appearance of clear haemolytic zones surrounding the growth indicates a positive results.

- **Growth at 42°C**

Tubes containing nutrient broth were inoculated with 1% of *P. aeruginosa* and incubated at 42°C for 24 hrs, positive result obtaining by the growth of *P. aeruginosa*.

- **Growth at 4°C**

Tubes containing nutrient broth were inoculated with 1% of *P. aeruginosa* and incubated at 4°C for 24 hrs, positive result obtained by giving no growth of *P. aeruginosa*.

### 2.2.7.3 Api 20E Identification of *P. aeruginosa* Isolates:-

Identification of the isolates was carried out by sub-culturing representative colonies from MacConkey Agar plates on API20E microtubes systems. This system is designed for the performance of 20 standard biochemical tests from a single colony on plate medium. Each test in this system is performed within a sterile plastic microtube which contains the appropriate substrates and is affixed to an impermeable plastic strip (gallery). Each gallery contains 20 microtubes.

The biochemical tests included in this system are the following:

l - Beta- galactosidase test ONPG.

2- Arginine dihydrolase test ADH.

3- Lysine decarboxylase test LDH.

4- Ornithine decarbxylase test ODC.

5- Citrate utilization test CIT.

6- Hydrogen sulphide test PLS.

7- Urease test URE.

8- Tryptophane deaminase test TDA.

9- Indole test IND.

10-Voges-Proskauer test VP.

11 - Gelatin Liquefaction test GEL.

12- Glucose Fermentation test FLU.

13- Mannitol Fermentation test MAN.

14- Inositol Fermentation test INO.

15- Sorbitol Fermentation test SOR.

16- Rhamnose Fennentation test RHA.

17- Sucrose Fermentation test SAL.

18- Melibiose Fennentation test MEL.

19- Amygdalin Fermentation test AMY.

20- Arabinose FenTientation test ARA.

2 l - Oxidase test OX1.

**-Preparation of the Galleries:**

Five ml of tap water dispensed in to the incubation tray to provide a humid atmosphere during incubation.

**-Preparation of Bacterial Suspension:**

By using a flamed loop, a well isolated colony from plating medium was picked. The inoculum was emulsified in 5 ml suspending medium (sterile distilled water) by rubbing against the side of the tube and mixed thoroughly with the water.

**-Inoculation of the Galleries:**

With a sterile Pasteur pipette, the twenty microtubes were inoculated. According to the manufactures instructions both the tube and couple section of CIT, VP and GEL microtubes were filled. After inoculation couple section of the ADH, LDC, ODC, $H_2S$ and URE microtube were completely filled with sterile mineral oil.

**-Incubation of the Galleries:**

After inoculation, the plastic lid was placed on the tray and the galleries were incubated for 18 to 24 hrs. at 37°C.

**-Reading of the Galleries**

All the reactions not requiring reagents were recorded first, then the following reagents were added to the corresponding microtubes:-

1- One drop of 3.4% ferric chloride to the TDA micortube.

2- One drop of kavoc's reagent to the IND micortube.

3- One drop of voges- proskauer reagent to VP micortube.

4-One drop of the oxidase reagent to either $H_2S$ or ONPG micortube.

The biochemical reactions performed by the API 20 E and their interpretations are listed in table (2-1).

**Table 2-1**: Interpretation of reactions performed by API 20E.

| Microtube | Positive | Negative |
|---|---|---|
| ONPG | Yellow | Colorless |
| ADH | Red/Orange | Yellow |
| LDC | Orange | Yellow |
| ODC | Red / Orange | Yellow |
| CIT | Blue-Green | Pale green / Yellow |
| $H_2S$ | Black deposit | Colorless / Grayish |
| URE | Red / Orange | Yellow |
| TDA | Dark brown | Yellow |
| IND | Red Ring | Yellow Ring |
| VP | Pink / Red | Colorless |
| GEL | Diffusion of Black Pigment | No diffusion |
| GLU | Yellow | Blue / Blue green |
| MAN | Yellow | Blue / Blue green |
| INO | Yellow | Blue / Blue green |
| SOR | Yellow | Blue / Blue green |
| RHA | Yellow | Blue / Blue green |
| SAC | Yellow | Blue / Blue green |
| MEL | Yellow | Blue / Blue green |
| AMY | Yellow | Blue / Blue green |
| ARA | Yellow | Blue / Blue green |
| OX | Violet / Dark purple | Colorless / Light purple |

**-Identification of the Isolates:**

Identification of the isolate using the analytical profile index (Numerical coding) for rapid identification at species and biotype level were done as supplied by the manufacturer.

For using the index, the biochemical profiles obtained have to be transformed into a numerical profile and to compare it with those listed in the index by transform all 21 biochemical results into a seven- figure numerical profile (seven-digit number) by placing them into groups of three and consigning a specific value for each of the positive as follows:

| **Group 1** | | | **Group 2** | | | **Group 3** | | | **Group 4** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ONPG | ADH | LDC | ODC | CIT | $H_2S$ | URE | TDA | IND | VP | GEL | GLU |
| 1 | 2 | 4 | 1 | 2 | 4 | 1 | 2 | 4 | 1 | 2 | 4 |
| **Group 5** | | | **Group 6** | | | **Group 7** | | |
| MAN | LNO | SOR | RHA | SAC | MEL | AMY | ARA | OXI |
| 1 | 2 | 4 | 1 | 2 | 4 | 1 | 2 | 4 |

Each positive reaction is given a value equal to l, 2 or 4 according to the position of the test in its group. The sum of these three values was given the corresponding figure. Thus, the figure can have a value from 0 to 7 (zero for negative reaction) the seven digits numerical profile is then looked up in the index and the identification is determined.

## 2.2.8 Antibiotic Sensitivity Test (Baron and Finegold *et al*., 1994)

### 2.2.8.1 Disk Diffusion Test:-

Ten ml of nutrient broth was inoculated with the bacterial isolate, the culture was incubated at 37°C to mid log phase ($OD_{600}$ about 0.35) giving $1\times10^8$ cell/ml. 0.1ml of incubated broth transferred to Muller Hinton agar plates. A sterile cotton swab was used to streak the inocula on plate's surface in 3 different planes (by rotating the plate approximately 60° each time to obtain an even distribution of the inocula). The inoculated plates were then placed at room temperature for 10 minutes to allow absorption of excess moisture. With sterile forceps the selected antibiotic disks were placed on the inoculated plates and incubate the plates at 37°C for 18 hrs. in inverted position.

After incubation, the diameter of inhibition zone was noted and measured in mm, results were determined according to the national committee for laboratory standard (NCCLs, 2001).

## 2.2.11 Minimum Inhibitory Concentration (MIC) Test:-

Minimum inhibitory concentration (MIC) was determined using tubes dilution method in which each tube contains 10 ml nutrient broth, a stock solution of antibiotic is prepared and sterilized by filtration, then antibiotic was added to the first tube to give final concentration of 1024 µg/ml and final volume of 10 ml, then a serial dilution is made by transferring 1 ml from the first tube and added to 9 ml of the next tube and so on until the last tube, then each tube is inoculated with 0.1 ml of previously prepared overnight culture of *P. aeruginosa*, then the tubes was incubated at 37°C for 24 hrs, the result was determined by the turbidity of the tubes and after grow it on Petri dishes.

## 2.2.10 Determining the Minimum Inhibitory Concentration for Antibiotic Combination

This test was used to determine the effect of antibiotics combination on pathogenic bacteria (*P.aeruginosa*)**.** The minimum inhibitory concentration of combined antibiotics was made in tube containing sterile nutrient broth giving a final concentration of 10 ml. A serial dilution for the combined antibiotic is made by taking 1ml from the tube containing combined antibiotics and added to the second tube containing 9 ml and so on until reach a dilution of ($10^{-6}$)/ml. Each tube inoculated with 0.1 ml of previously prepared overnight culture of *P.aeruginosa* and incubated at 37°C for 24 hrs, the result was determined depending on the turbidity of the tube, then the combination weather it's synergestics, additives, antagonistics, or indifference depending on the fractional inhibitory concentration (FIC) was determine as follow: (≤0.5) synergism, (0.5– <1) additive, (1– <2) antagonism, (≥2) indifference, and calculated using the following equation (Koneman *et al.,* 1992):

$$FIC = \frac{\text{MIC for antibiotic in combination}}{\text{MIC for antibiotic alone}}$$

## 2.2.11 Determining the Effect of EDTA in Combination with Antibiotics against *P. aeruginosa*

Different molarities of EDTA solution is prepared starting from (5mM), then the lowest molarity of EDTA that cause inhibition against *P. aeruginosa* was determined using well diffusion assay, depending on the inhibition zone around the well.

Antibiotic disks were soaked in EDTA solution(Yong., *et al.* 2002), and disk diffusion assay were used to determine the inhibition zone of antibiotic disk (with and without EDTA) against *P. aeruginosa* according to the national committee for clinical laboratory standards (NCCLs 2001), the inhibition zone were measured in (mm).

## 2.2.12 Determining the Inhibitory Effect of *Lactobacillus acidophilus*

### 2.2.12.1 On Solid Medium (MRS Agar)

A culture of LAB previously grown in MRS broth was streaked on MRS agar, and then incubated under anaerobic conditions at 37°C for 24 hrs (Silva; 1987). After incubation a corck poorer (5mm) was used to withdraw disc of LAB growth and put on surface of the nutrient agar that was inoculated (before) with 0.1ml of *P. aeruginosa*. After incubation at 37°C for 24 hrs the inhibition zone around the disc was estimated in (mm).

Same procedure was repeated by using different incubation times of LAB (18, 24, and 48 hrs) to determine the optimum incubation time that gives greater inhibition effect.

### 2.2.12.2 In Liquid Medium (MRS Broth)

MRS broth was inoculated by 1% of LAB culture, then incubated anaerobically at 37°C for different periods of time (18, 24, and 48 hrs) (Lewus *et al*., 1991; Schillinger and Luck, *et al*., 1991). After incubation the culture was centrifuged at 6000 rpm for 15 minutes, the filtrate was obtained. After that adjusting the pH of the filtrate to 6.5 by using (1ml) NaOH, it was filtered through Millipore filter unit (0.22µm),then well diffusion method that mentioned by (Vignolo *et al*., 1993) was used; when nutrient agar plates which was inoculated with 0.1ml of each *P. aeruginosa* by a spreader. Then (5mm) wells were made by a corck porer. Each well were filled with the LAB filtrate and then incubated at 37C° for (18, 24, and 48 hrs). The inhibition zone around the well was measured by (mm) and compared with that of the control which contained MRS broth without bacteria (Vignolo *et al*., 1993). The filtrate was concentrated by freeze-dryer and the well diffusion assay was repeated to detect the effect of each concentrated filtrate against the pathogenic bacteria, control was containing concentrated MRS broth without LAB.

## 2.2.13 Determining of Minimum Inhibitory Concentration for Concentrated Filtrate of *Lactobacillus acidophilus*

Different concentrations of each concentrated filtrate were made in tubes containing sterile nutrient broth. The concentrations were (1/9, 2/8, 3/7, 4/6, 5/5, 6/4, 7/3, 8/2, and 9/2) giving final volume of 10 ml in each tubes.

Then each concentration was inoculated by adding 0.1ml culture previously grown of *Pseudomonas aeruginosa* in nutrient agar and incubated at 37°C for 24 hrs. after incubation minimum inhibitory concentration was determined as the lower concentration of the filtrate that inhibit growth of *P. aeruginosa* isolate in the tubes.

## 2.2.14 Extraction of Plasmid DNA

DNA extraction was done by salting out method which described by Kieser (1995), and as follow:-

- Culture of *Pseudomonas aeruginosa* grown in brain heart infusion broth was pelleted from 20 ml by centrifugation at 6000 rpm for 15 min.
- The pellet washed with 3 ml of SET buffer and resuspend the cells with 1.6 ml SET buffer, then freshly prepared lysozyme (final concentration 1mg/ml) was added and incubated at 37°C for 30 min.
- One ml of 10% SDS was added, mixed by inversion, and then incubated at room temperature for 15 min.
- 2 ml of 5 M NaCl was added, mixed thoroughly by inversion.
- An equal volume of chloroform was added, mixed by inversion for 15 min then centrifuge (6000 rpm. at 4°C) for 20 min.
- The aqueous phase (upper) was transformed to another sterile tube, and 0.6 volume of isopropanol was added mixed by inversion, and kept at room temperature for 5 min.
- Centrifuge at 13000 rpm for 15 min at 4°C.
- The isopropanol was discarded and the precipitated DNA dissolved in 100 µl TE buffer and stored at -20°C.

### 2.2.15 Agarose Gel Electrophoresis (Maniatis *et al*., 1982)

Agarose gel (0.7%) were run horizontally in tris-borate-EDTA (TBE 5x). Samples of DNA were mixed with 1/10 volume of the loading buffer and added to the wells on the gel. Generally, gel was run for 2-3 hr at 5 v/cm and the gel buffer added up to the level of horizontal gel surface.

Agarose gel were stained with ethidium bromide by immersing them in distilled water containing the dye at a final concentration of 0.5 μg/ml for 30-45 min. DNA bands was visualized by UV illumination at 302 nm at a UV transilluminator. Gels were destained in distilled water for 30-60 min to get rid of background before photographs were taken.

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# Results and Discussion

## 3.1 Isolation and Identification of *P. aeruginosa*

### 3.1.1 Isolation

One hundred and fifty burns and wounds samples were collected from patients in Al-Kadhumia, Baghdad and Al-Yarmook Hospital during the period from 1-3-2005 to 15-5-2005 (Table 3-1).

**Table 3-1:** Distribution of *P. aeruginosa* samples according to source of isolation.

| Isolation source | Number of samples | *P. aeruginosa* |
|---|---|---|
| **Burns** | **63** | **21** |
| **Wounds** | **87** | **29** |
| **Total** | **150** | |

### 3.1.2 Identification

The identification and characterization of the isolates were carried out according to the cultural, morphological and biochemical tests. One hundred (66.7%) of the isolates indicate the presence of different species include *P. fluorescence* (20%), *E. coli* sp. (27.7%), *Bacillus* sp. (11%), *Proteus* sp. (6%), *Klebsiallas* sp. (2%) and Only fifty (33.3%) isolates were identify as *P. aeruginosa*, in which colonies appears pale on MacConkey agar, Haemolytic activity on blood agar, non-fluorescent bluish pigment Pyocyanin (blue to green) on king A agar and capable to grow on media containing 0.03% Cetrimide agar, also they have ability to grow at 42°C but not at 4°C and at optimum pH of 7.4 – 7.6.

Cultures showed a characteristic; a sweet grape-like odor and production of Pyocyanin pigment were represented as suspected *P. aeruginosa*.

*P. aeruginosa* has different colonies types according to the medium and source of infection from dwarf colonies to large mucoid colonies. Moreover, all isolates gave positive result to Catalase and Oxidase tests, but they differ in Haemolysin, Pyocyanin and Urease production test, table (3-2) summarized the result of these biochemical tests performed in this study (Collee *et al*., 1996; Green wood *et al*., 1997; and Jawetz *et al*., 1998).

**Table 3-2:** Biochemical tests of *Pseudomonas* sp. isolated from burns and wounds.

| Test / Isolate | Oxidase | catalase | urease | haemolysin | Pyocyanin |
|---|---|---|---|---|---|
| **1** | + | + | + | **β** | - |
| **2** | + | + | + | **β** | - |
| **3** | + | + | + | **β** | - |
| **4** | + | + | + | **β** | - |
| **5** | + | + | - | **No** | - |
| **6** | + | + | - | **β** | - |
| **7** | + | + | + | **No** | - |
| **8** | + | + | - | **β** | + |
| **9** | + | + | + | **β** | + |
| **10** | + | + | - | **No** | - |
| **11** | + | + | + | **β** | + |
| **12** | + | + | + | **β** | + |
| **13** | + | + | - | **β** | + |
| **14** | + | + | + | **No** | - |
| **15** | + | + | - | **β** | + |
| **16** | + | + | - | **No** | - |
| **17** | + | + | + | **β** | + |
| **18** | + | + | + | **β** | + |
| **19** | + | + | + | **No** | + |
| **20** | + | + | - | **No** | + |
| **21** | + | + | - | **No** | - |
| **22** | + | + | + | **β** | + |
| **23** | + | + | + | **No** | + |
| **24** | + | + | + | **No** | + |

| | | | | | |
|---|---|---|---|---|---|
| **25** | + | + | - | **β** | - |
| **26** | + | + | + | **β** | + |
| **27** | + | + | + | **β** | - |
| **28** | + | + | + | **No** | - |
| **29** | + | + | - | **No** | - |
| **30** | + | + | - | **No** | + |
| **31** | + | + | - | **α** | + |
| **32** | + | + | - | **β** | + |
| **33** | + | + | + | **β** | + |
| **34** | + | + | + | **No** | + |
| **35** | + | + | + | **No** | + |
| **36** | + | + | + | **No** | + |
| **37** | + | + | + | **β** | - |
| **38** | + | + | + | **β** | - |
| **39** | + | + | + | **α** | + |
| **40** | + | + | - | **α** | - |
| **41** | + | + | - | **β** | - |
| **42** | + | + | - | **β** | + |
| **43** | + | + | + | **β** | + |
| **44** | + | + | + | **No** | + |
| **45** | + | + | + | **β** | + |
| **46** | + | + | - | **β** | + |
| **47** | + | + | + | **β** | - |
| **48** | + | + | - | **β** | - |
| **49** | + | + | + | **No** | - |
| **50** | + | + | - | **α** | - |

**(-) negative result (+) positive result (α) α-Haemolysin (β) β-Haemolysin (NO) no-Haemolysin**

Further identification of the isolates was done using Api 20E system, as in figure (3-1). All isolates of *P. aeruginosa* gave the same result using this diagnostic kit. These biochemical tests were confirmed by the diagnostic key mentioned earlier (Mandelle *et al*., 1995; Collee *et al*., 1996).

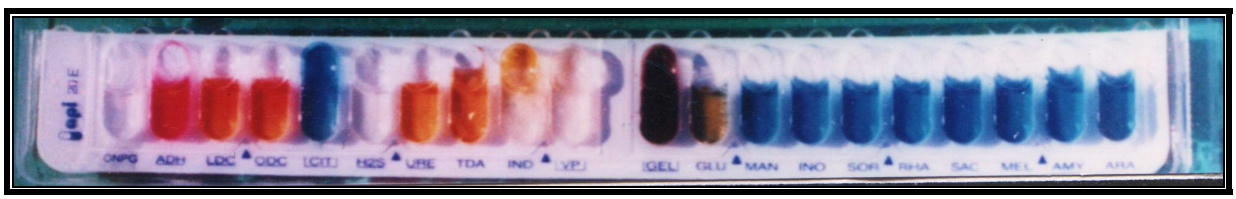


**Figure 3-1**: Identification of *Pseudomonas aeruginosa* isolates.

## 3.2 Antibiotic Sensitivity

Testing of resistance in microorganism is important for categorizing their behavior in accordance to kinds of antibiotics as well as their medical application concerning their effectiveness in treatment of disease and distribution of resistance among isolates for designing pattern of resistance, serve as a picture for following transfer of genetic elements among species and hence spread of resistance.

For screening purposes, the test is required for separation of huge number of isolates. Accordingly, disk method is useful to do so. The test is fast and simple, however it suffer from lower ness of accuracy as long as zone of inhibition widely affected by medium composition and interference of some ions with spread of antibiotics through the medium (Baron *et al*., 1994).

For research studies, minimum inhibitory concentration (MICs) is useful to specify minimum concentration of antibiotic showing inhibition of growth, such concentration could also be useful for medical purposes. Accordingly, for testing antibiotic sensitivity, the two methods were used wherever it is needed.

### 3.2.1 Disk diffusion Test

The emergence of prevalence of antibiotic resistance strains is considers as a major therapeutic problem that can be explain by several hypothesis such as, the influence of excessive and /or inappropriate antibiotic use (Sotto *et al*., 2001).

Standard disk diffusion assay was used to detect the sensitivity of pathogenic bacteria and results obtained were compared with those of (NCCLs, 2001).

Table (3-3) show that antibiotic sensitivity among *P. aeruginosa* isolate varied according to the nature of the isolate and antibiotic. The percentage of resistant isolates to each antibiotic is shown in figure (3-4)

**Table 3-3:** Results of antibiogram test by using 20 antibiotics against 50 isolates of *P. aeruginosa*.

| Isolates | Antibiotics | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AK | TR | GN | C | PY | NOR | P | TOB | PRL | SXT | CIP | NA | S | TE | E | VA | CTX | CAZ | IPM | ATM |
| A1 | S | R | S | R | R | R | R | R | R | S | R | R | R | R | R | R | S | S | S | R |
| A2 | S | S | R | R | R | S | R | R | R | R | S | R | R | R | R | R | S | S | S | R |
| A3 | S | S | R | S | S | S | R | R | S | R | S | S | R | R | R | R | S | S | S | R |
| A4 | S | S | S | S | S | S | R | S | S | R | S | S | R | S | R | R | S | R | S | R |
| A5 | S | S | R | R | R | S | R | R | R | R | S | R | R | R | R | R | S | R | S | S |
| A6 | S | S | S | R | S | S | R | S | S | R | S | S | R | R | R | R | S | S | S | S |
| A7 | S | S | S | R | R | S | R | S | S | R | S | R | R | R | R | R | R | R | S | S |
| A8 | S | S | S | R | R | S | R | S | S | R | S | R | S | S | R | R | R | R | S | S |
| A9 | S | S | S | R | R | S | R | R | R | R | S | R | R | R | R | R | R | R | S | S |
| A10 | S | R | S | R | R | R | R | R | R | R | R | R | R | R | R | R | S | R | S | R |
| A11 | S | R | R | R | R | R | R | R | S | R | R | R | R | R | R | S | R | R | S | R |
| A12 | S | S | S | S | R | R | S | S | S | S | S | R | R | S | R | R | R | R | S | S |
| A13 | S | S | S | R | R | R | R | S | S | R | S | R | R | S | R | R | R | R | S | S |
| A14 | S | S | S | R | S | R | R | S | S | R | S | R | R | S | R | R | R | S | S | S |
| A15 | S | R | S | R | S | S | S | S | S | S | S | R | R | R | R | R | R | R | S | S |
| A16 | S | S | S | R | S | R | S | S | S | S | S | R | R | R | R | R | R | R | S | S |
| A17 | S | S | S | R | S | S | R | S | S | R | S | R | S | S | R | R | R | R | S | S |
| A18 | S | S | S | R | R | S | R | R | S | R | R | R | R | R | R | R | R | R | S | S |
| A19 | S | S | R | R | R | R | R | R | S | R | R | R | R | S | R | R | R | R | S | S |
| A20 | S | S | R | R | R | S | R | R | R | R | R | R | R | R | R | R | S | S | S | R |
| A21 | S | S | S | S | S | S | R | R | R | R | R | R | R | R | R | R | S | R | S | R |

| Continued table (3-3) | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Antibiotics | | | | | | | | | | | | | | | | | | |
| A22 | S | S | S | R | R | S | R | S | S | R | S | R | R | S | R | R | R | R | S | S |
| A23 | S | S | S | R | R | S | R | R | R | R | R | R | R | R | R | R | R | R | S | R |
| A24 | S | R | R | R | R | R | R | S | S | R | S | S | R | S | R | R | S | R | S | R |
| A25 | S | S | S | S | S | S | R | S | S | R | S | S | R | S | R | R | S | R | S | R |
| A26 | S | S | S | R | R | S | S | S | S | S | S | S | S | S | S | S | R | R | S | R |
| A27 | S | S | S | S | S | S | R | S | S | S | S | R | R | R | R | R | S | R | S | R |
| A28 | S | S | S | S | S | S | R | S | S | S | S | S | R | R | R | R | R | R | S | R |
| A29 | S | S | S | S | S | S | R | S | R | R | S | S | R | R | R | R | R | R | S | R |
| A30 | S | S | S | R | S | S | R | S | R | S | R | R | R | R | R | R | R | R | S | R |
| A31 | S | S | R | R | S | S | R | R | S | R | S | R | S | S | R | R | R | R | S | S |
| A32 | S | S | R | R | R | S | R | S | S | S | S | R | R | R | R | S | S | S | S | S |
| A33 | S | S | S | R | S | S | R | R | S | S | R | R | R | R | R | R | S | S | S | S |
| A34 | S | S | S | S | S | S | R | S | R | R | S | S | R | R | R | R | S | R | S | S |
| A35 | S | S | S | S | S | S | R | S | S | R | R | R | R | R | R | R | S | S | S | S |
| A36 | S | S | R | R | S | S | R | S | R | S | S | S | R | R | R | R | R | R | S | S |
| A37 | S | S | S | R | S | S | R | S | R | R | S | R | R | S | R | R | R | S | S | S |
| A38 | S | S | R | R | S | S | R | S | S | R | S | S | R | R | R | R | S | S | S | R |
| A39 | S | S | S | S | S | S | S | R | S | S | S | S | S | S | S | S | R | R | S | R |
| A40 | S | R | R | R | S | S | S | R | S | R | S | S | R | R | R | R | R | S | S | S |
| A41 | S | S | S | R | R | S | R | S | S | R | S | R | S | S | R | R | R | R | S | S |
| A42 | S | R | S | R | S | S | S | S | S | R | S | R | R | S | R | R | R | R | S | S |
| A43 | S | R | S | R | S | R | R | R | S | R | S | S | R | S | R | S | S | R | S | S |

| Continued table (3-3) | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Antibiotics | | | | | | | | | | | | | | | | | | | |
| A44 | S | S | S | S | S | S | R | R | S | R | S | R | R | R | R | R | S | S | S | S |
| A45 | S | S | S | S | S | S | R | R | S | R | R | R | R | R | R | S | R | R | S | S |
| A46 | S | S | R | S | S | S | S | R | R | R | S | R | S | S | S | R | R | R | S | S |
| A47 | S | S | S | R | S | S | R | S | S | S | S | S | R | R | R | R | R | R | S | R |
| A48 | S | S | S | R | R | S | S | S | S | R | R | R | R | R | R | R | S | R | S | R |
| A49 | S | S | R | S | S | S | R | S | S | R | S | R | S | R | S | S | S | R | S | R |
| A50 | S | S | R | R | S | R | R | S | S | R | S | S | R | R | R | S | R | S | S | S |
| SS | S | S | S | S | S | S | R | S | S | R | S | R | R | R | R | S | S | R | S | S |

S: Sensitive; R: Resistance; SS: Standard strain; Ak: Amikacin; Tr: Tobramycin; Gn: Gentamicin; C: Chloromphenicol; Py: Carbencillin; Nor: Norfloxacin; P: Penicillin; Tob: Tobramycin; Prl: Pipracillin; Sxt: Triomethoprime; Cip: Ciprofloxacin; Na: Naldixic acid; S: Streptomycin; Te: Tetracycline; E: Erythromycin; Va: vancomycin; Ctx: Cefotaxime; Caz: Ceftazidime; Ipm: Imipenim; Atm: Aztronam.

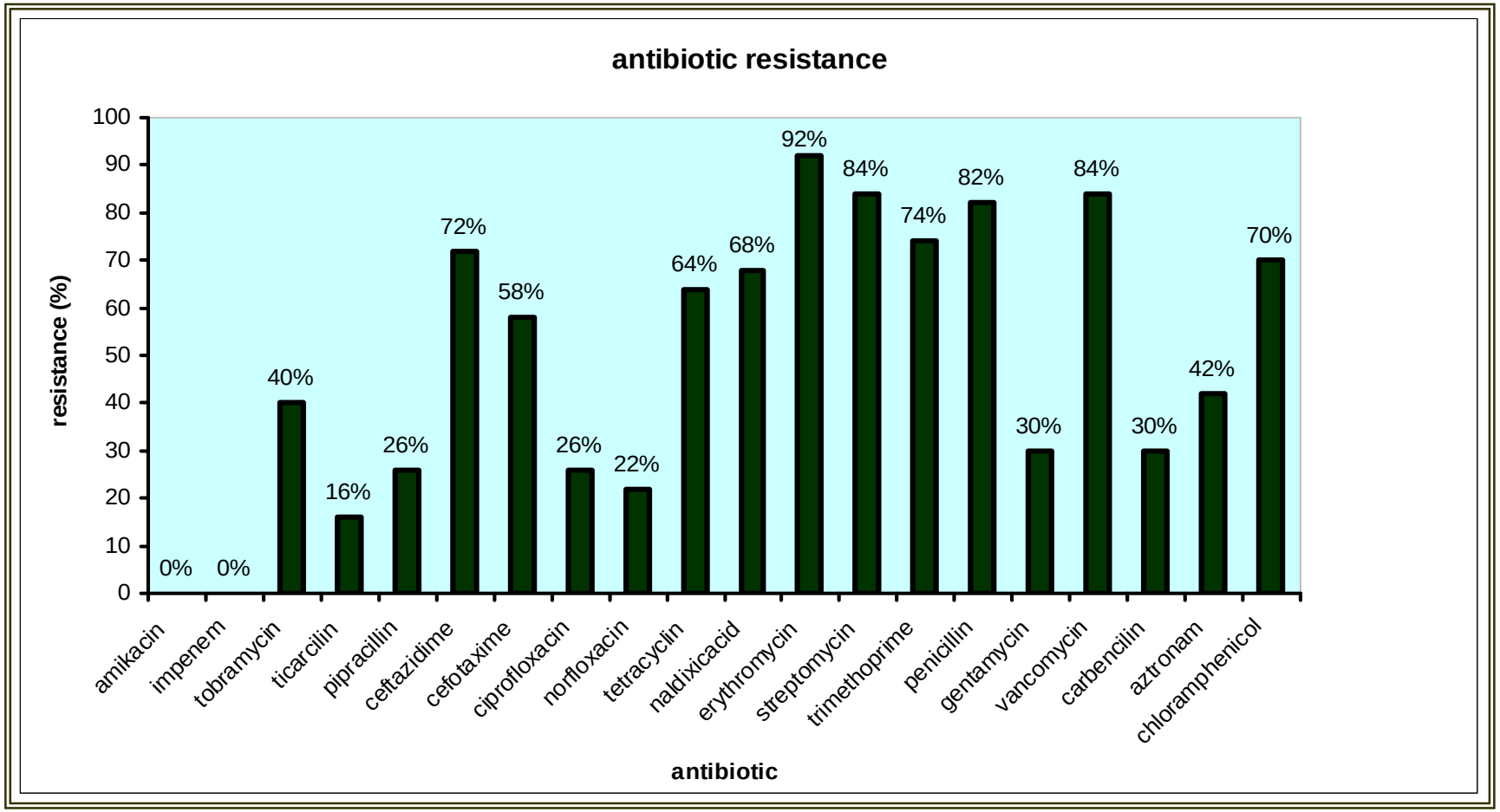


**Figure (3-2)** Percentage of resistant *P. aeruginosa* isolates to antibiotics

The results show that all isolates of *P. aeruginosa* were sensitive to amikacin and Imipenem (100%), this result may be related to the lower randomly use of this antibiotic by patient, this result was in agreement with that of Startchounski *et al*., (1998) who found in a study in Russia that resistance percentage of the isolate to Amikacin was (1%).

Of the isolate obtained 92% were found resistant to erythromycin and this similar to that found by Kandeler *et al*., (1986) who found that 90% of isolate resistant to erythromycin and this may be due to the common use of this antibiotic which lead to increase microbial resistance to this antibiotic. Eighty-four percent of isolates were found resistant to Vancomycin and this result was near to that obtained by Brumfitt and Hamilton (1998) which found that (90%) of isolate resistant to Vancomycin.

Relatively high resistant (84%) to the streptomycin was recorded in this study. This result was in agreement with Neu *et al*., (1985) which found that (76%) of the isolates resistant to streptomycin. Resistant of *P. aeruginosa* isolates to gentamycin was found to be as low as (30%) and this result was in agreement with those of Egorve, (1985) and Brumfitt Hamilton (1998) which found that resistant to gentamycin (32%).

The results of this study also shows that the resistance percentage to Cefotaxim, Ceftazidime and Penicillin were (58%, 72% and 82%) respectively, and this may be due to the ability of this isolates to produce β-lactamase enzyme which break the β-lactam ring and this results in agreement with that found by Mccloskey (1968) and Rice *et al*., (1990).

Laurence *et al*., (1997) show that Trimethoprim antibiotic is a broad spectrum antibiotic effecting both G - and G + bacteria and the result of this study shows that 74% of the isolates were resistant to this antibiotic and that is near to that found by Cormican *et al*., (1998); and Goettsch (2000) who found that (73%) of isolate were resistant to Trimethoprim.

*P. aeruginosa* isolates were found sensitive tot Fluroquinolones antibiotics like ciprofloxacin in which (26%) of the isolates was found resistant to this antibiotic and this because quinolones antibiotics act principally by inhibiting bacterial DNA Gyrase, so preventing the supercoilling of the DNA a process that is necessary it compacting chromosome into the bacterial cell.

This result was close to that obtained by Kohler *et al*., (1997) and Jalal (2000) which they found that (90%) of *P. aeruginosa* sensitive to Ciprofloxacin, also this result was in agreement with that of Shawar *et al*., (1999) who indicate lower than 63% of isolate susceptible to ciprofloxacin, this may belong to the wide use of ciprofloxacin as therapeutic agent for treatment of disease caused by *P. aeruginosa* which leading to be low level of percentage susceptibility for this antibiotic.

Resistance to tetracycline was found to be (64%) and this was may be due to the resistance gene which carried by plasmid (Merlin *et al*., 1988). Pellegrino *et al*., (2002) found that resistance of *P. aeruginosa* isolates to tetracycline was (89%), and these differences may be due to the abuse of antibiotic in treatment lead to elevate resistance (Rice *et al*., 1992).

Resistance to Carbencillin and Pipracillin were found (30% and 26%) respectively this results was in agreement with that of Laurence *et al* 1997, who mention that both of these antibiotics were effective against *P. aeruginosa* while Bujdakova *et al*., (1998) found that 92% of isolate resistant to Carbencillin and 86% were resistant to Pipracillin which disagree with this study, and this may due to the ability of *P. aeruginosa* to develop resistance to these antibiotics through the production of β-lactamase enzyme which break the β-lactam ring of Carbenicillin and Pipracillin (Henry *et al.*, 2001; Rice *et al*., 1992).

Resistance to Aztrunam and Ticarcillin were found as 42% and 16% respectively and this results was in agreement with that of Neu *et al*., (1985), while resistance to Tobramycin (aminoglycoside) were found to be as low as

(40%) and this result was in agreement with that of Egorove, 1985 which found that 37% of the isolate were resistant to Tobramycin.

Of the tested isolate, 68% showed resistance to Naldixic acid, these results disagreed with that found by Kurokawa *et al*., (2000) which found that 36% of isolates were resistant to Naldixic acid, and this may due to the development of resistance gene carried on a plasmid (Martinez *et al.*, 1998).

Norfloxacin also shows low resistance to *P. aeruginosa* isolates which found to be (22%) of isolate and this agreed with that of Shawar *et al*., (1999).

Although the use of Chloramphenicol prohibited (in developed countries) because of its side effects of causing irreversible aplastic anemia, (70%) of isolates were found resistant to Chloramphenicol and this relatively high percentage may be due to the common use of this antibiotic in treatment of patients in Iraq and other developing country and this come in agreement with that of Scott *et al*. (1999).

The result in table (3-3) showed that isolates no. (A1, A2, A5, A9, A10, A11, A18, A19, A20, A23) have the highest level of resistance so that they were selected to study the effect of antibiotics combination and inhibitory effect of LAB against *P. aeruginosa*.

## 3.2.2 Minimum Inhibitory Concentration (MIC)

Ten isolates which have the highest level of resistance were tested to determine the MIC of amikacin and gentamycin which represent Aminoglycoside by, Pipracillin and Ceftazidime which represent β-lactam, and Ciprofloxacin which represent Flouroquinolons and finally Tetracycline as in the table (3-4).

The breakpoints were applied following NCCLs 2001 recommendation. When resistance level were calculated, ``MIC`` in both the intermediate and resistant range (as define by the NCCLs 2001) considered as non susceptible in

this study. MIC was defined as the lowest drug concentration in microgram per milliter that inhibited the visible growth of the bacteria (Kinoshita *et al*., 1997).

**Table 3-4**: MIC value for six antibiotics (µg/ml) tested against *P. aeruginosa* isolates.

| *P. aeruginosa* Isolates | MIC of tested Antibiotics µg/ml | | | | | |
|---|---|---|---|---|---|---|
| | **AK** | **CN** | **CIP** | **CAZ** | **PRL** | **TE** |
| **A1** | 4 | 8 | | 4 | | |
| **A2** | 0.5 | | 8 | 2 | | |
| **A5** | 0.5 | | 8 | | | |
| **A9** | 1 | 1 | 2 | | | |
| **A10** | 2 | 8 | | | | |
| **A11** | 4 | | | | 32 | |
| **A18** | 0.12 | 0.5 | | | 64 | |
| **A19** | 0.5 | | | | 32 | 4 |
| **A20** | 2 | | | 4 | | |
| **A23** | 0.5 | 0.5 | | | | |

**(AK) Amikacin, (CN) Gentamycin, (CIP) Ciprofloxacin, (CAZ) Ceftazidime e, (PRL) Pipracillin, (TE) Tetracyclin.**

MICs were determined using serial dilution method in test tubes and then detect the growth on Petri dishes. From results in table (3-4) concluded that amikacin have the extended spectrum of antibacterial activity, such result agreed with that obtained by Muller *et al*., (1999).

Amikacin remain the first choice with lowest MIC for *P. aeruginosa* which rang from (0.12- 4) µg/ml, therefore it is more preferred in the therapy than other antibiotics and this result agree with that of Bonfiglios (1998) who

found that MIC of Amikacin against *P. aeruginosa* (2 µg/ml). *P. aeruginosa* has an outer membrane with a low permeability through which antimicrobial agents diffuse very slowly. Active efflux systems that decrease the intracellular concentration of the agents have been assumed to be especially effective mechanism of antibiotic resistance in these bacteria, therefore, in addition to altered targets, these mechanisms should be contribute to the determination of the ultimate levels of aminoglycoside resistance. While other antibiotics in this study have a higher MIC and as follow: Ceftazidime was (2, 4 and 4) µg/ml, Pipracillin was (32, 23 and 64) µg/ml and this result was agree with that found by Rolston *et al*., 1992, while Gentamycin have MIC range from (0.5-8) µg/ml, and this may be due to the ability of *P. aeruginosa* isolate to produce β-lactamase which break the β-lactam ring in the structure of antibiotic, for Ciprofloxacin the MIC was (2, 8, and 8) µg/ml and this agree with that of Craig (2000), while Tetracycline was (4) µg/ml and this may be due to the resistance gene carried on bacterial plasmid (Merlin *et al*., 1988).

## 3.3 Antibiotics Combination

The combination between antibiotics for each isolate in this study was based on the selection of antibiotics that have lowest MIC to be use for combination.

Checker board assay was used to determine the effect of the antibiotics in combination by measuring the fractional inhibitory concentration (FIC) for the combination.

The value of the (FIC) express the kind of the relation between the combined antibiotics, so when the (FIC) value is (≤0.5) the relation is synergism, while if the (FIC) value is (0.5–1) the relation is addition, if the (FIC) value is (1– <2) the relation is antagonism, and if the (FIC) value is (≥2) the relation is indifference (Perea *et al*., 1988) (Figure 3-6).

Table (3-5) show the MIC value for antibiotics (amikacin, gentamycin, ceftazidime, ciprofloxacin, tetracycline, pipracillin), before and after combination against *P. aeruginosa* isolate to determine the effect of antibiotics combination on these isolates, MIC values of antibiotics in combination were found lower than MIC values of single antibiotic when Amikacin and Ceftazidime combination tested against isolate no. (1, 2 and 20) of *P. aeruginosa*, and the same effect were noticed when Amikacin and Pipracillin tested against isolate no. (11) (Figure 3-3), revealing a synergistic effect of these combinations this result is similar to that shown by Hollander *et al*. (1998).

**Table 3-5:** Antibiotic combination.

| *P. aeruginosa* Isolates | Antibiotics combination | MIC of first antibiotic alone (µg/ml) | MIC of first antibiotic in combination (µg/ml) | MIC of second antibiotic alone (µg/ml) | MIC of second antibiotic in combination (µg/ml) | FIC | Results |
|---|---|---|---|---|---|---|---|
| A1 | AK+CAZ | 4 | 0.25 | 4 | 0.25 | 0.125 | Syn |
| A2 | AK+CAZ | 0.5 | 0.03 | 2 | 0.125 | 0.125 | Syn |
| A5 | AK+CIP | 0.5 | 0.12 | 8 | 2 | 0.49 | Syn |
| A9 | AK+CN | 1 | 0.12 | 1 | 0.12 | 0.24 | Syn |
| A10 | AK+CN | 2 | 0.25 | 8 | 1 | 0.25 | Syn |
| A11 | AK+PRL | 4 | 0.25 | 32 | 2 | 0.125 | Syn |
| A18 | AK+CN | 0.12 | 0.03 | 0.5 | 0.12 | 0.49 | Syn |
| A19 | AK+TE | 0.5 | 0.12 | 4 | 1 | 0.49 | Syn |
| A20 | AK+CAZ | 2 | 0.125 | 4 | 0.25 | 0.125 | Syn |
| A23 | AK+CN | 0.5 | 0.06 | 0.5 | 0.06 | 0.24 | Syn |

**FIC: Fractional Inhibitory Concentration; Ak: Amikacin; Caz: Ceftazidime; Cip: Ciprofloxacin; CN: Gentamycin; TE: Tetracycline; Prl: Pipracillin; Syn: Synergism**

The synergistic effect noticed also for combination of Amikacin with Gentamicin when its effect tested on isolate no. (9, 10, 18 and 23) of *P. aeruginosa* (Figure 3-4) and for combination of Amikacin with ciprofloxacin when tested on isolate no. (5) and combination of Amikacin with tetracycline when tested on isolate no. (19) (Figure 3-5) and this result similar to that shown by Hollander *et al*., (1998).

Table (3-5) showed that (FIC) values for combination of Amikacin with Ceftazidime and Pipracillin in the isolate no.(1, 2, 11, and 20) is very low in comparison with those (FIC) values of combination of Amikacin with Gentamycin, Ciprofloxacin and Tetracycline in the isolate no.(5, 9, 11, 18, 19, and 23), and this indicate that combination of aminoglycoside with β-lactam antibiotic was more effective than combination of aminoglycoside with other group of antibiotics, and this because aminoglycoside antibiotics exert their effects on protein synthesis of bacterium while β-lactam antibiotics exert their effect on bacterium cell wall and this lead to complete destroying of bacteria (Mcgrath *et al*., 1992; Carmeli *et al*., 1999; Henry, 2001).

Also combinations of aminoglycoside with β-lactams antibiotics decrease the toxicity of aminoglycoside, (Stone, 1986) found that aminoglycoside antibiotics cause defect in kidney in (5%) of the patient when they treated with Gentamycin.

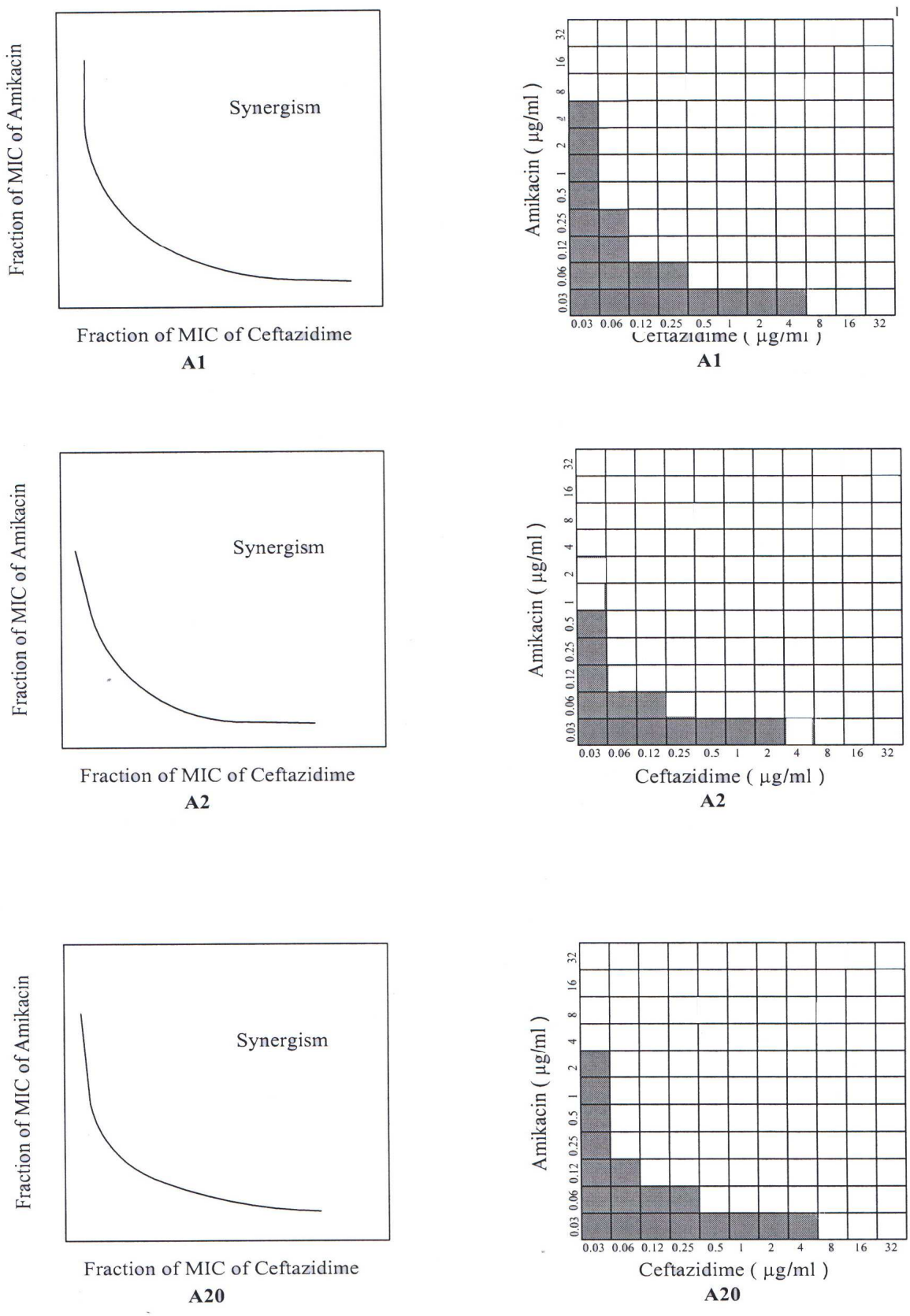


Figure (3-3) Synergism effect of amikacin with ceftazidim against *Pseudomonas aeruginosa* isolates (A1, A2 and A20).

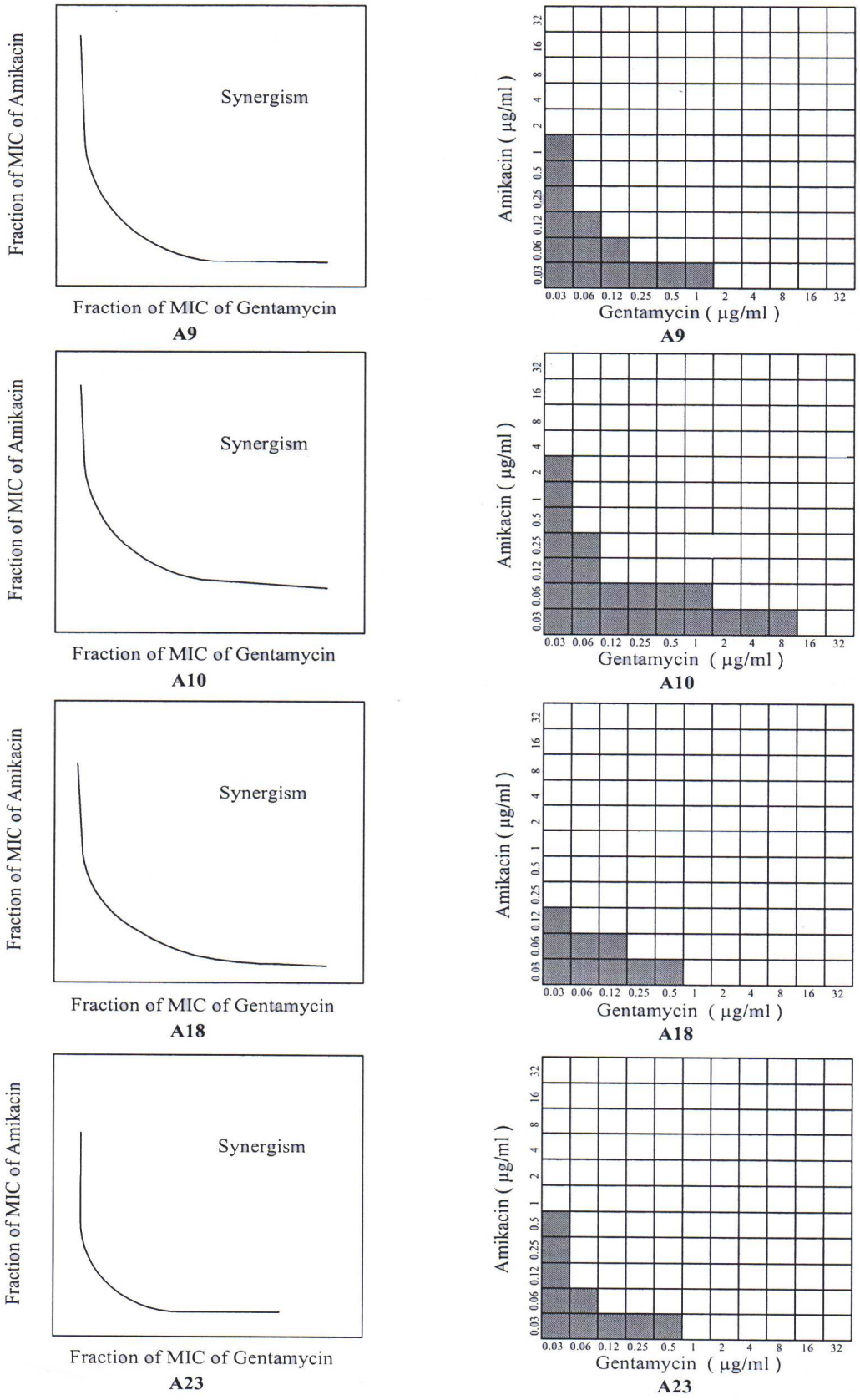


Figure (3-4) Synergism effect of amikacin with gentamicin against *Pseudomonas aeruginosa* isolates (A9, A10, A18 and A23).

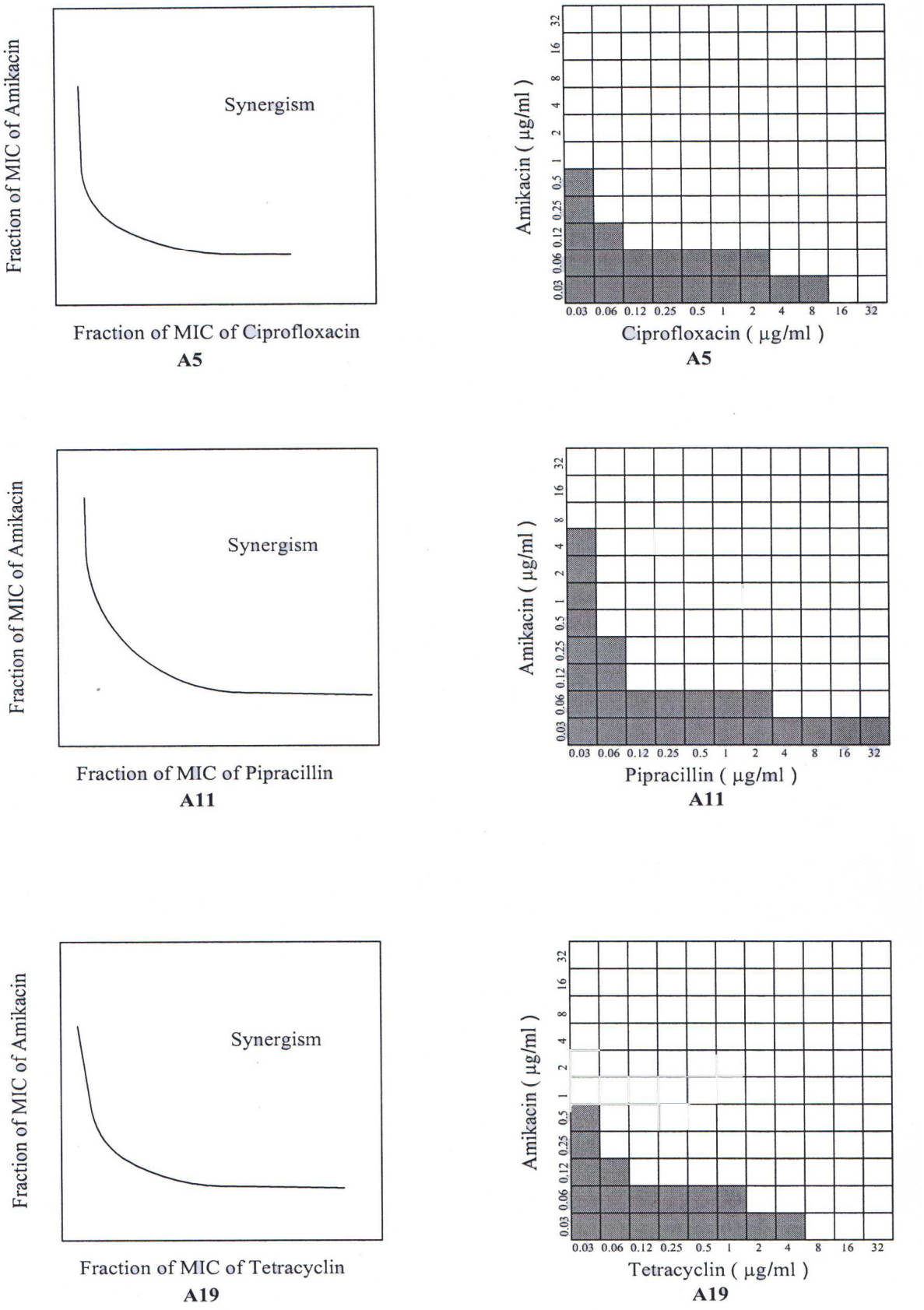


Figure (3-5) Synergism effect of amikacin with ciprofloxacin, tetracycline and pipracillin against *Pseudomonas aeruginosa* isolates (A5, A19 and A11) respectively.

## 3.4 The Effect of Combination of EDTA-Antibiotic on *P. aeruginosa*

EDTA has a more complex inhibition-concentration profile. Synergism between EDTA and other antimicrobials agents have been widely reported against *P. aeruginosa* (Lambert *et al*., 2003). Table (3-6) show the synergism effect of EDTA with other antibiotics of different groups in which the inhibition zone is increase after adding the EDTA to the antibiotics.

From results obtained in table (3-6) was found EDTA have an synergism effect on aminoglycoside antibiotics (Amikacin and Gentamycin) against *P. aeruginosa*, and this results come in agreement with that obtained by Spark (1994) who found that EDTA enhance the activity of aminoglycoside antibiotics by binding to the metal ions which compete with aminoglycoside antibiotics for cell wall receptor that allow antibiotics to enter the bacterial cell.

Gotthelf 2003 has shown that EDTA capable of reducing the MIC of ciprofloxacin against *P. aeruginosa* and this result was in agreement with the result of current study as shown in the table (3-6), and this because EDTA cause destruction of the outer membrane of the bacterial cell and become permeable to antibiotics which enter the bacterial cell and exert their effect.

While the effect of EDTA on β-lactam antibiotics which is (ceftazidime and pipracillin) and on tetracycline also gives synergism effect, and this result was found similar to that obtained by (Vaara, 1992) who reported that EDTA combination with penicillin and tetracycline cause reduction of MIC.

**Table 3-6:** Combination effect of EDTA with antibiotics on *p. aeruginosa* isolates using disk diffusion assay**.**

| Isolates | Antibiotics | Without EDTA (mm) | With EDTA (mm) |
|---|---|---|---|
| **A1** | AK | 22 | 24 |
| | CZC | 19 | 22 |
| **A2** | AK | 23 | 25 |
| | CZC | 20 | 24 |
| **A5** | AK | 24 | 27 |
| | CIP | 18 | 20 |
| **A9** | AK | 21 | 24 |
| | CN | 20 | 22 |
| **A10** | AK | 22 | 25 |
| | CN | 19 | 24 |
| **A11** | AK | 23 | 25 |
| | PRL | 20 | 24 |
| **A18** | AK | 20 | 23 |
| | CN | 18 | 20 |
| **A19** | AK | 20 | 22 |
| | TE | 17 | 19 |
| **A20** | AK | 20 | 22 |
| | CZC | 20 | 23 |
| **A23** | AK | 23 | 25 |
| | CN | 18 | 21 |

**Ak: Amikacin; Caz: Ceftazidime**; **Cip: Ciprofloxacin; CN: Gentamycin; TE: Tetracycline; Prl: Pipracillin.**

# 3.5 Inhibitory Effect of (*Lactobacillus acidophilus*)

## 3.5.1 On Solid Medium

The ability of *Lactebacillus acidophils* to inhibit pathogenic bacteria was tested by culturing the isolate on MRS agar medium. In this approach, Al Kassab and Al Khafaji (1992) mention that MRS agar medium is the best medium that gives reasonable results to test the production of inhibitory agents by LAB cultured aerobically.

Results show that *lactobacillus acidophilus* has no inhibitory effect on *P. aeruginosa* isolates when cultured together on solid medium and in the three incubation periods. This result may indicate that *lactobacillus* had no effect on *P. aeruginosa* isolates on solid medium table (3-7), and this may due to the type of pathogenic bacteria, types of inhibitory substances, it's quantity and it's ability for diffusion in the medium (Egorve *et al.,* 1985).

### 3.5.2 In Liquid Medium

Well diffusion assay had been used to determine the inhibition activity of *lactobacillus acidophilus* filtrate grew in three incubation period (18, 24, and 48 hrs) against *P. aeruginosa* isolates.

*Lactobacillus acidophilus* filtrate had no effect on *P. aeruginosa* isolates table (3-7), and this may because of the concentration of the acidophilin and other enzymes in the liquid medium (Barefoot, 1983), also the result may be related to the high resistance of *P. aeruginosa* isolates which related to the gene carried on bacterial plasmid (Merlin *et al*., 1988).

**Table 3-7:** Effect of *Lactobacillus acidophilus* on P.aeruginosa isolates on solid and in liquid medium in different incubation periods using well diffusion assay

| Time (hr) / Samples | effect of Lactobacillus acidophilus on solid medium | | | effect of Lactobacillus acidophilus in Liquid medium | | |
|---|---|---|---|---|---|---|
| | **18** | **24** | **48** | **18** | **24** | **48** |
| A1 | **R** | **R** | **R** | **R** | **R** | **R** |
| A2 | **R** | **R** | **R** | **R** | **R** | **R** |
| A5 | **R** | **R** | **R** | **R** | **R** | **R** |
| A9 | **R** | **R** | **R** | **R** | **R** | **R** |
| A10 | **R** | **R** | **R** | **R** | **R** | **R** |
| A11 | **R** | **R** | **R** | **R** | **R** | **R** |
| A18 | **R** | **R** | **R** | **R** | **R** | **R** |
| A19 | **R** | **R** | **R** | **R** | **R** | **R** |
| A20 | **R** | **R** | **R** | **R** | **R** | **R** |
| A23 | **R** | **R** | **R** | **R** | **R** | **R** |

**(R)= Resistant**

The investigation also include the inhibitory effects of the concentrated filtrate of *Lactobacillus acidophilus* on *P. aeruginosa* isolates table (3-8), *Lactobacillus acidophilus* filtrates was concentrated three folds by using freeze drying and test the inhibitory effects of each fold alone against *P. aeruginosa* isolates for the three incubation periods was done.

The inhibitory effect of concentrated filtrate of *Lactobacillus acidophilus* was increased by increasing the concentration, in the first fold of concentration *Lactobacillus acidophilus* filtrate inhibit *P. aeruginosa* isolates slightly when incubated for 24 hrs and 48 hrs while incubation for 18 hrs showed no detectable effect. In the second fold of concentration of filtrate, the inhibition zone was larger than that in the first fold and for 24 hrs and 48 hrs incubation while there were no detectable effect after 18 hrs of incubation.

While in the third fold of concentration of *Lactobacillus acidophilus* filtrate the inhibition was higher also in the 24 hrs and 48 hrs of incubation, while no effect was noticed after 18 hrs of incubation.

The inhibition zones after 24 hrs and 48 hrs of incubation have almost the same diameter, therefore 24 hrs of incubation may be considered the minimum incubation period that showed the highest inhibitory effect for *P. aeruginosa* isolates, that effect may be due to increased concentration of the inhibitory substance produced by *Lactobacillus acidophilus* such as acidophilin in the medium (Figure 3-6).

Nigatu and Gashe (1994) reported that *Lactobacillus acidophilus* isolated from fermented food, have inhibitory effect on gram negative bacteria such as *P. aeruginosa, E. coli, Proteus, and Salmonella.*

**Table 3-8:** Effect of concentrated filtrate of *Lactobacillus acidophilus* on *P. aeruginosa* isolates at different period of incubation using well diffusion assay.

| Sample | First fold of concentrated filtrate | | | Second fold of concentrated filtrate | | | Third fold of concentrated filtrate | | |
|---|---|---|---|---|---|---|---|---|---|
| | Inhibition zone (mm) | | | Inhibition zone (mm) | | | Inhibition zone (mm) | | |
| | 18 hrs | 24 hrs | 48 hrs | 18 hrs | 24 hrs | 48 hrs | 18 hrs | 24 hrs | 48 hrs |
| A1 | R | R | R | R | 8 | 9 | R | 18 | 19 |
| A2 | R | R | R | R | 10 | 10 | R | 18 | 19 |
| A5 | R | R | R | R | 9 | 9 | R | 20 | 20 |
| A9 | R | R | R | R | 10 | 10 | R | 22 | 22 |
| A10 | R | R | R | R | 10 | 10 | R | 19 | 19 |
| A11 | R | R | R | R | 9 | 9 | R | 18 | 18 |
| A18 | R | R | R | R | 8 | 9 | R | 19 | 19 |
| A19 | R | R | R | R | 10 | 10 | R | 19 | 20 |
| A20 | R | R | R | R | 8 | 8 | R | 18 | 18 |
| A23 | R | R | R | R | 10 | 10 | R | 20 | 20 |

(**R) = Resistant (no inhibition zone)**

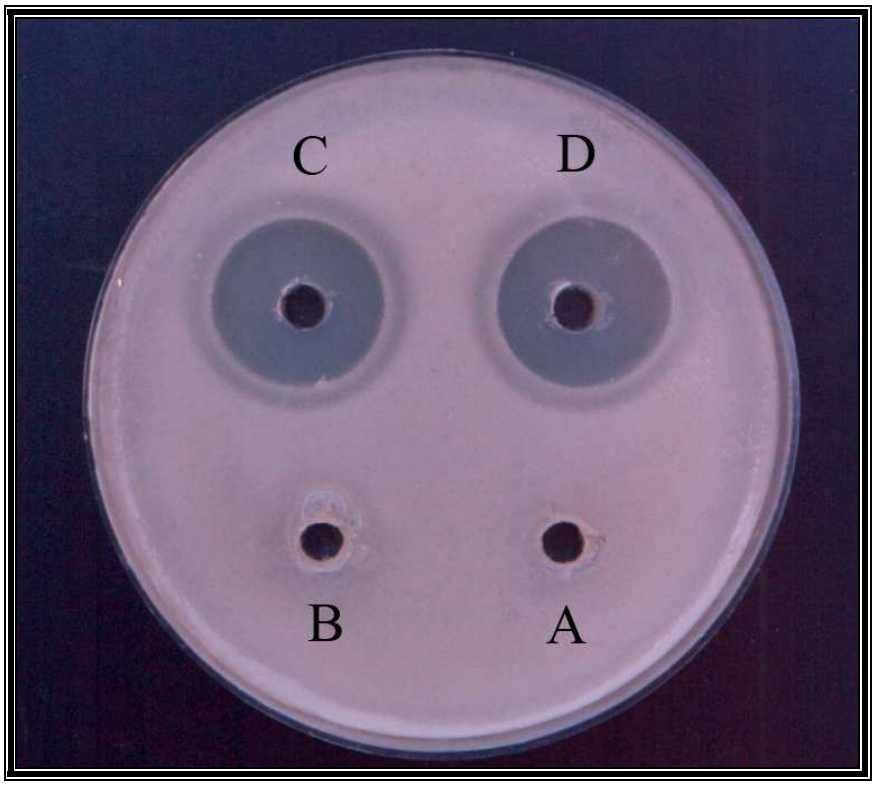


Figure (3-7).The inhibitory effect of third fold concentrated filtrate of *Lactobacillus acidophilus* against *Pseudomonas aeruginosa* isolate (2).

**A.** Control (Concentrated medium alone).

**B.** Third fold concentrated filtrate of *Lactobacillus acidophilus* after 18 hrs of incubation.

**C.** Third fold concentrated filtrate of *Lactobacillus acidophilus* after 24 hrs of incubation.

**D.** Third fold concentrated filtrate of *Lactobacillus acidophilus* after 48 hrs of incubation.

## 3.6 Minimum Inhibitory Concentration of *Lactobacillus acidophilus* Filtrates against *P. aeruginosa* isolates.

To determine the MICs of the filtrates of *Lactobacillus acidophilus* which inhibit or minimize growth of *P. aeruginosa* isolates, serial dilutions were prepared from the three-fold filtrates of *Lactobacillus acidophilus*. Table (3-9) shows that dilution 1:9, 2:8 and 3:7 (filtrate: medium) had no effect on *P. aeruginosa* isolates, while dilution 4:6 inhibit isolates (1, 2, 11, and 19), 5:5 inhibit isolates (5, 10, and 20), 6:4 inhibit isolates (9, and 23) and 7:3 inhibit isolate (18) Minimize the growth of pathogenic bacteria and produce inhibition zone.

Depending on just mentioned finding, dilution of (4/6) filtrate: medium in the isolate no. (1, 2, 11, and 19) may consider the MIC of *Lactobacillus acidophilus* filtrate against growth of tested *P. aeruginosa* isolates.

**Table 3-9:** Minimum Inhibitory Concentrations (MIC) of *Lactobacillus acidophilus* against *P. aeruginosa* isolates.

| MIC / Isolate | 1:9 | 2:8 | 3:7 | 4:6 | 5:5 | 6:4 | 7:3 | 8:2 | 9:1 |
|---|---|---|---|---|---|---|---|---|---|
| **A1** | + | + | + | - | - | - | - | - | - |
| **A2** | + | + | + | - | - | - | - | - | - |
| **A5** | + | + | + | + | - | - | - | - | - |
| **A9** | + | + | + | + | + | - | - | - | - |
| **A10** | + | + | + | + | - | - | - | - | - |
| **A11** | + | + | + | - | - | - | - | - | - |
| **A18** | + | + | + | + | + | + | - | - | - |
| **A19** | + | + | + | - | - | - | - | - | - |
| **A20** | + | + | + | + | - | - | - | - | - |
| **A23** | + | + | + | + | + | - | - | - | - |

**(+) = Growth**
**(-) = No growth**

## 3.7 Isolation of Plasmid DNA

In order to determine the plasmid profile of the efficient isolate which is A2 and A18 salting out method by Kieser, (1995) gave the best results.

Results in figure (3-8) indicate that selected isolates no. (A2, A18) containing small plasmid DNA bands and all of these plasmid bands approximately in the same size comparing with each other and with pBR322 plasmid (4.363 Kb) which not shown in the Figure. Thus the bacterial isolates, tested in this study may be containing another plasmids dose not detected (may be because of it's large size).

*P. aeruginosa* is currently one of the most frequent nosocomial pathogen and the infections due to this organism are often difficult to treat due to antibiotic resistance (Emori and Gaynes, 1993). The mechanisms of resistance to antibiotics include reduced cell wall permeability, production of chromosomal and plasmid mediated β-Lactamases, Aminoglycoside-modifying enzymes (Livermore *et al*., 1989) and an active multi drug efflux mechanism (Li *et al*., 1994).

There are many studies dealing with the isolation of antibiotics resistance plasmid from *P. aeruginosa*, Shahid and Malik (2003) found in their study in India that *P. aeruginosa* isolated from patient suffering from burn contain plasmid coding for resistance of β-lactam antibiotics and Amikacin.

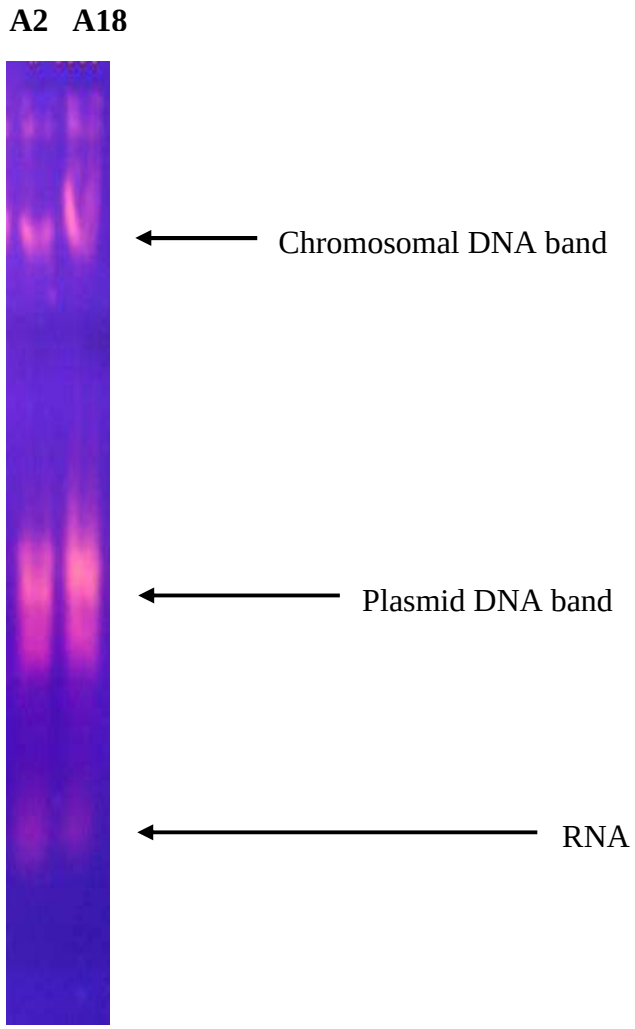


Figure (3-8) Gel electrophoresis of isolated plasmid from the bacterial isolates of *Pseudomonas aeruginosa* A2 and A18 migrated on agarose gel (0.7%) in TBE buffer at 5V/cm for 2½ hrs.

## Summary

- A total of (150) samples were collected from clinical specimens including (Burns and Wounds) belongs to patients of both sexes and of different ages hospitalized in Al-Kadhumia, Baghdad and AL-Yarmook hospitals in Baghdad during the period from 1-3-2005 to 15-5-2005. Only 50 (33.3%) isolates were identified as *Pseudomonas aeruginosa* by using Morphological, Physiological and Biochemical tests and hundred (66.7%) isolates indicated the presence of different genera includes *P. fluorescence* (20%), *E. coli* (27.7%), *Bacillus* (11%), *Proteus* (6%), *and Klebsialla* (2%).

- Susceptibility of isolates to (20) antibiotics was also tested using disk diffusion assay. Imipenem and Amikacin were to be the most effective antibiotics with no resistance at all while the other antibiotic were less effective.

- Ten isolates were selected according to their pattern of resistance as those showing multi-drug resistance and tested to specify their minimum inhibitory concentration for (Amikacin, Gentamicin, Ceftazidime, Pipracillin and tetracycline). Amikacin was found having the lowest MIC comparing with others.

- This study also include *in-vitro* effects of various combinations of five types antibiotics (Amikacin, Gentamicin, Ceftazidime Piperacillin and Tetracyclin, ) against the ten *Pseudomonas aeruginosa* isolates that were found sensitive to each of these antibiotics. Among combinations,

the combination of ß-lactam antibiotics with amikacin was found to be the most effective combination that inhibits the growth of *Pseudomonas aeruginosa*. In which the MIC of Ceftazidime and Pipracillin alone when the isolates no. (1, 2, 20 and 11) were tested was (4, 2, 4 and 23 μg/ml) respectivly while MIC of Amikacin alone in the same isolates was (4, 0.5, 2 and 2 μg/ml), after combination the MIC of Ceftazidime and Pipracillin for these isolates became (0.25, 0.125, 0.25 and 2 μg/ml) respectively while the MIC of Amikacin become (0.25, 0.125, 0.03 and 0.25 μg/ml) respectively.

- The effect of EDTA when it was combined with antibiotics also tested against the ten isolates of *P. aeruginosa* using disk diffusion assay after soaking the antibiotics disk in EDTA. Results showed that EDTA increases the effect of antibiotic against *P. aeruginosa* isolates especially when it was combined with aminoglycoside antibiotics.

- Another part of the study includes the using of *Lactobacillus acidophilus* (LAB) as probiotic to inhibit the growth of *Pseudomonas aeruginosa*. Minimum inhibitory concentration (MICs) was determined for the three-fold concentrated filtrate of (LAB) against *P. aeruginosa*. Results showed that (4/6) (filtrate**:** medium) considered as the MIC of LAB filtrate that inhibit the growth of *P. aeruginosa*

- The plasmid profile for the (2) most resistant isolates was studied. The result showed that these isolates contain a small plasmid DNA bands approximately in the same size.

# الخلاصة


- جمعت (١٥٠) عينة من مسحات سريرية شملت الجروح والحروق لمرضى بمختلف الاعمار و من كلا الجنسين المتواجدين في مستشفى الكاظمية وبغداد و اليرموك ببغداد في الفترة من شهر شباط و لغاية شهر ايار ٢٠٠٥. من مائة مسحة ايجابية للنمو البكتيري تبين انه فقط (٥٠) عزلة من المسحات هي *P. aeruginosa* و *بنسبة* (33.3%) اعتمادا على شكلها و الاختبارات الفسلجية والكيميائية اما بقية العزلات فقد احتوت على *P. Fluorescence* بنسبة (20%) و *E. coli* بنسبة (27.7%) و *Bacillus بنسبة (11%) و Proteus* بنسبة (6%) و *Klebcialla* بنسبة (2%) .
- اختبرت حساسية هذه العزلات لمضادات حياتية مختلفة باسنخدام طريقة الاقراص و قد اظهرت النتائج بان المضادات Amikacin وImipenem هما الاكثر فعالية اذ لم تظهر أية عزلة مقاومة لهما في حين كانت المضادات الاخرى أقل فعالية.
- اختبرت (١٠) عزلات اعتمادا على تعدد مقاومتها للمضادات الحياتية ثم حددت التراكيز المثبطةالدنيا MIC للمضادات Amikacinو Gentamicinو Ceftazidimeو Pipracillinو Tetracyclin فأظهرت النتائج بأن المضاد (Amikacin) يثبط نموها بالتركيز الاقل مقارنة بالمضادات الاخرى.
- تم دراسة تأثير مزج المضادات الحياتية الخمسة Amikacinو Gentamicinو Ceftazidime Pipracillinو Tetracyclin ضد عزلات البكتريا العشرة و التي كانت حساسة لها و اظهرت النتائج بان مزج المضادات الحيوية من مجموعة β -lactam مع الـ Amikacin كان اكثر فعالية في تثبيط النمو مقارنة بالمزج مع مضادات اخرى حيث كان التركيز المثبط الادنى للمضادينCeftazidime وPipracillin ) للعزلات (١و٢و٢٠و١١) هو (٤و٢و٤و٣٢) مايكروغرام/مل على التوالي في حين كان التركيز المثبط الادنى للـAmikacin لنفس العينات هو (٤و٠,٥و٢و٤) مايكروغرام/مل على التوالي و بعد المزج اصبح التركيز المثبط الادنى للمضادين Ceftazidimeو Pipracillin لهذه العينات (٠,٢٥و ٠,١٢٥و٠,٢٥و٢) مايكروغرام/مل على التوالي و التركيز المثبط الادنى للمضاد Amikacin (٠,٢٥و٠,٠٣و ٠,١٢٥و٠,٢٥)مايكروغرام/مل على التوالي.
- تم اختبار تاثير الـ EDTA التآزري مع المضادات الحياتية ضد عزلات الـ*aeruginosa* . *P* العشر باستخدام طريقة اقراص المضادات الحياتية بعد نقعها في الـEDTA و النتائج اظهرت بان الـ EDTA يزيد من فعالية المضادات الحيوية و خاصة من مجموعة الـ Aminoglycoside .
- تضمن الجزء الاخر من الدراسة استخدام بكتريا *Lactobacillus* في تثبيط نموبكتريا الـ *P.aeruginosa* وقد اجري اختبار التركيز المثبط الادنى للتركيز الثالث لرواشح بكتيريا حامض اللاكتيك و اظهرت النتائج بان التركيز(٦١٤)( راشح / الوسط الزرعي) يعتبر التركيز المثبط الادنى لراشح بكتيريا اللاكتيك التي تثبط نمو بكتيريا الـ *P. aeruginosa*.
- شملت الدراسة ايضا دراسة النمط البلازميدي للعينات وقد تم اختيار اكفأ عينتين وهما (A٢، A١٨) و اظهرت النتائج ان هذه العينتين تحتويان على بلازميدات صغيرة و متساوية بالحجم تقريبا.

بسم الله الرحمن الرحيم

فبدأ بأوعيتهم قبل وعاء أخيه ثم
استخرجها من وعاء أخيه كذلك كدنا
ليوسف ما كان ليأخذ أخاه في دين الملك
إلا إن يشاء الله نرفع درجات من نشاء
و فوق كل ذي علم عليم

صدق الله العظيم

سورة يوسف الآية (٧٦)