

Qualitative and Molecular Detection of Peroxidase and Lipase Enzymes from *Pseudomonas Aeruginosa* Isolated from Different Clinical Infections and Testing Their Resistance to Certain Different Antibiotics

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Abstract

In this study, 20 samples were collected from various clinical sources from Tikrit Teaching Hospital, Ghazi Hariri Specialized Surgery Hospital, and Burn Hospital in the City of Medicine. To confirm that the isolation belongs to *Pseudomonas aeruginosa*, several tests were performed such as coloring techniques, biochemical tests, and the Vitek 2 system. The results of the biochemical tests matched the traits of *Pseudomonas aeruginosa* and the diagnosis was confirmed with the Vitek system. Peroxidase is a type of oxidation-reduction reaction enzyme, useful for accelerating many oxidation reactions that protect cells from the harmful effects of free radicals. It is found in many common sources such as plants, animals and microbes, and it has wide uses in many fields, such as industry and medicine. Lipase enzymes are enzymes belonging to the category of hydrolases. They stimulate the hydrolysis process of triglycerides into glycerol and free fatty acids. Lipase enzymes are found in various sources. The qualitative detection of each enzyme for isolates on the basis of its susceptibility to the production of enzymes on the production media of each enzyme. The results showed the emergence of decomposition zones around the colonies ranging from 28mm and 5 mm, respectively, for each enzyme, which indicated the productivity of the enzyme. The results were confirmed by molecular detection through the polymerase chain reaction, where the results showed the presence of the peroxidase gene and lybase in all tested isolates by 100%. He tested the resistance of bacteria to antibiotics. The results showed that the highest resistance was to the antibiotic Tetracyclin at 95.10% and the lowest resistance was to the antibiotic Amikacin, where resistance reached 30.18%. As for the rest of the antibiotics, resistance was in varying proportions according to each antibiotic.

Keywords: Qualitative, Molecular, *Pseudomonas aeruginosa*, Lipase, Peroxidase.

1-1: Introduction

Pseudomonas aeruginosa is one of the most important types of bacteria that cause outbreaks of hospital-acquired infection, [1] as it is often noted that it is difficult to treat infections resulting from these bacteria because they have the character of natural resistance in addition to their ability to acquire resistance to many antibiotics such as aminoclycosides, fluoroquinolones and beta-actam antagonists. [2]

Enzymes have become more important products in the food and pharmaceutical industry Peroxidase is an oxidation and reduction enzyme that accelerates reaction rates between hydrogen peroxide (H₂O₂) as an electron receptor and various types of substrate by releasing oxygen from it. Harmful

electrons such as superoxide and hydroxide radicals can be found in cells due to the presence of oxygen. Hydrogen peroxide peroxides are used as an electron receptor, catalyzing many oxidative reactions[3]. Peroxidase is an iron-containing enzyme, which catalyzes the transfer of oxygen from hydrogen peroxide to a suitable substrate, thereby oxidizing the substrate[4].

Lipases are typical enzymes produced by bacteria. It is responsible for the hydrolysis of fats and oils to release fatty acids, diglycerides, monoglycerides, and glycerol. They can also be useful in esterification, esterification exchange, amino decomposition, and lactonization processes. Thus, they are of great use at the industrial level[5]. As a result, there is a growing need to intensify the production of lipases from bacteria[6]. Microorganisms such as bacteria synthesize high-value substances such as lipase enzymes that have applications in scientific industries and innovations[5]. Enzymes are more important when they are derived from microorganisms such as bacteria, because they are more adaptive, more consistent, more synergistic, more productive, more stable, safer, logical and useful and [7] can be modified to increase yields or give other useful unique properties (in this[8] context, lipase is used in food companies, dairy production, the oil sector, medicine, pharmacy, detergents, perfumes, cosmetics, agriculture, etc[5]). Enzymes produced from sources other than microorganisms are more expensive, less productive, less modifiable, and less effective than other defects. Other methods are also less safe, producing a significant amount of contamination in the context of intensive downstream processes that also increase the cost of production [9].

The study aims to :

- 1- Isolating and diagnosing *Pseudomonas aeruginosa* .
2. Detection of bacterial resistance to certain antibiotics.
3. Qualitative detection of peroxidase and lybase enzymes
4. Molecular detection of peroxidase and lipase enzymes

Materials and methods:

2-1: Isolation and Identification of Bacteria

(20) bacterial isolates were obtained from Tikrit Teaching Hospital, Burn Hospital and Ghazi Hariri Specialized Surgery Hospital from patients with burns, wounds, urinary tract infection, and respiratory tract infection on January 3, 2025. The isolates were then tested to produce the enzymes under study.

2-1-1: Sampling

All bacterial isolates were examined for their susceptibility to Gram staining. The structure and color of the cells were [10] found using a light microscope using the oil immersion technique. Lines of *Pseudomonas aeruginosa* bacteria were placed on the surface of the agar. Some individual bacterial cells were separated and paved at distances. Due to the dilution of the original sample with its lines on consecutive quarters, and then incubated at a temperature of 37° C for 24 hours, the number of organisms decreased, and the morphology of the bacteria appeared[11].

2-2: Qualitative Detection of Enzymes

2.2.1: Qualitative detection of peroxidase enzyme

Specific activity of the peroxidase enzyme was determined as shown by [12]. Briefly, isolates in nutrient agar were inoculated. Thereafter, 30 µL of equal parts hydrogen peroxide (H₂O₂) at 0.4% (v/v) and pyrogallol at 1% in water were added to the colony and incubated at 37°C for 24 hours. The yellowish-brown colonies recorded a positive result.

2.2.2: Qualitative detection of lybase enzyme

Egg yolk agar medium was implanted with pure isolates to investigate the production of lybase enzyme by bacteria. It was observed after implantation and incubation for 24-48 hours at 37 m. The appearance of a clear area around the colonies is evidence of the production of lecithinase enzyme . The dish is then immersed in a sufficient amount of a solution saturated with copper sulfate CuSo4 for 20 minutes, then the excess is removed from the dish and the dish is dried with the incubator for 30 minutes, so the blue color shows evidence of lipolysis by the enzyme lipase[13]

2-3: Molecular detection of peroxidase and lipase gene

2-3-1: DNA extraction

The DNA of the *Pseudomonas aeruginosa* bacteria was extracted in several steps according to the method mentioned in the previous studies. In this procedure, the kit prepared by GENE AID was [14] used for this purpose.

2-3-2 : Polymerase chain reaction (PCR)

The manufacturer's (MacroGen) Korean primers were prepared in powder form at a concentration of 300 picomol / μ L and the primers were dissolved by adding 300 μ L of deionized water Nuclease free water according to the manufacturer's instructions.

Table 1: Sequence of gene initiators used in the study

Source	Band	Sequence primer		geno-	NO
Designed for study	396	F	TGAAGGACTATCCGGTGCTC	DYP	1
		R	GTTCTTCGTCGTCGGACTTG		
Designed for study	309	F	ACCGGTACGCAGAATTCAC	LIP	2
		R	CAGGTGTTTCATCCGGTAGT		

The PCR reaction was carried out using a PCR-Premix kit equipped by Bioneer and in addition to the required reaction components as shown in the following table: -

Table 2: Reaction components in test tubes

Component	Volume ul/sample
MASTER	10 μ L
Primer	0.5 μ L
Primer	0.5 μ L
Nuclease free water	6 μ L
Template	3 μ L
FINAL VOLUM	20 μ L

Mix the reaction components well and then concentrate by placing them in the centrifuge for a few seconds (Spin)

The tubes were placed in the thermoplastic device and then carried out with the following program:

The program that was used to perform the PCR interaction:

Table 3: Polymerase Chain Reaction Program

Seq.	Grade	Temperature	Time	No. of Courses
1	Primary DNA teratogenicity stage	95	5min	1 cycle
2	Secondary DNA teratogenicity stage	95	45 seconds.	35 cycles
3	Correlation of DNA	58	60 seconds.	35 cycles

4	DNA Elongation Phase	72	45min	35 cycles
5	Final DNA Elongation Phase	72	10 min	1cycle

2-4: Antibiotic susceptibility test (AST)

The sensitivity test was carried out based on the method of disk diffusion Kirby-Bauer according to what was stated in [15] the use of manufactured antibiotics of Turkish origin (Bioanalyse) with a concentration used globally as stated in the recommendations of (CLSI, 2024), which was mentioned in Table (4-2). A number of pure developing bacterial colonies at the age of (18-24) hours were transferred to a test tube containing 50 ml of physiological saline solution to obtain a bacterial suspension. The turbidity of the solution was controlled with the turbidity of McFarland's solution (0.5) and the use of a cotton swab. After that, the bacterial suspension was spread over the center of the Muller-Hinton Acre. After that, the tablets were placed on the center of the Muller-Hinton and then incubated at a temperature of 37 °C for a period of 24 hours. After the end of the preparation period, the inhibition area around the colony was measured using a measuring ruler compared to the measurement ratios mentioned in the table to determine the extent of resistance or sensitivity of the cell bacteria to the antibiotics used.

Table 4: Antibiotic tablets used in the study and manufactured by

Family Name	Concentration tablet/ μ g	Code	maintained
Cephalosporin III	30	Caz	Ceftazidime
Cephalosporin IIII	30	CPM	Cefepime
Penicillins	100 10	TZP	Piperacillin-tazobactam
Aminoglycosides	10	TM	Tobramycin
Quinolones	5	CIP	Ciprofloxacin
Carbapenems	10	IPM	Imipenem
Tetracyclines	30	TE	Tetracyclin
Monobactams	30	ATM	Aztreonam
Aminoglycosides	30	Ak	Amikacin
Penicillins	75	TIC	Ticarcillin

3. Results and discussion

3-1: Identification of Bacteria

Bacterial isolates were determined using Gram staining, morphological and biochemical tests, and finally the Vitek 2 system was applied to ensure that the isolated sample belonged to *P. aeruginosa*. The result was consistent with the previous identification of *Pseudomonas aeruginosa* [12] as shown in Table 5.

Table 5: Identification of *P. aeruginosa*

RESULT	life
+	Oxidase
+	Catalase
-	Production

-	Urease
-	Indole production
-	Lactose fermenter
-	methyl-red
+	Glucose
Forms 2-3mm round colonies, smooth with irregular surface	Colonies(morphological)
Greenish color by procaine stain and fruity odour	Color and odour
Pink rod gram (-ve) bacteria	Gram stain

3-2: Qualitative Detection of Enzymes

3-2-1: Qualitative detection of peroxidase enzyme

Specific activity of the peroxidase enzyme has been identified for organisms. The results revealed that all assessed *Pseudomonas aeruginosa* species showed peroxidase activity on dishes (Figure 1) with yellowish brown discoloration on the bacterial colony after reacting with hydrogen peroxide (H_2O_2) at 0.4% (v/v) and pyrogallol at 1% after 24 hours of incubation on nutritious agar.

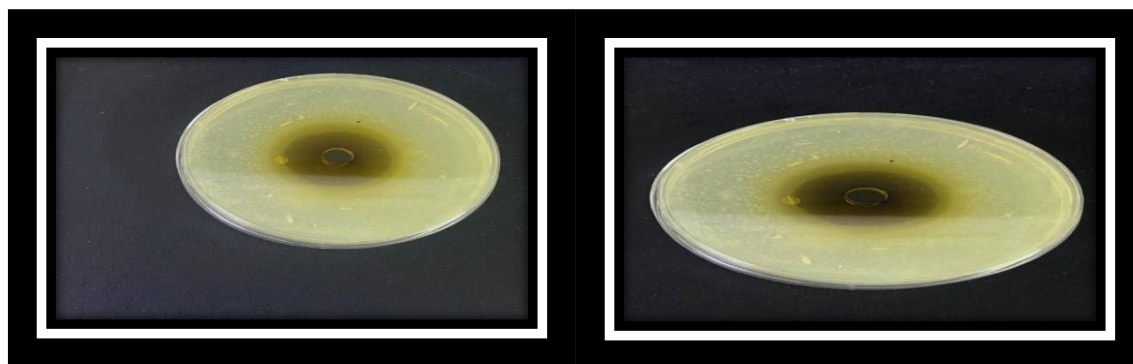


Figure 1: Peroxidase production from Sycobacter aeruginosa

This study agreed with a study he conducted [16] on the bacterium *Bacillus germinolytic*, where the results showed that all types that were tested showed activity of peroxidase on dishes, and in another study he conducted [17] revealed that all types that were tested showed activity of the enzyme peroxidase and left a decomposition zone on the enzyme production medium.

3-2-2: Qualitative detection of lipase enzyme

Based on the clear establishment of lipolytic zones (Figure 2), lipase activity was determined and out of a total of 20 bacterial isolates, 20 isolates showed positive lipase activity *P. aeruginosa* showed relatively high activity in lipase production (100%). Moreover, we evaluated the association of these enzymes with wound severity, and found that the severity of infection in wounds was higher in patients whose wounds isolated bacteria producing larger amounts of lipase enzyme, as shown by wound size and depth, secretions, delayed wound healing, pain, and swelling.

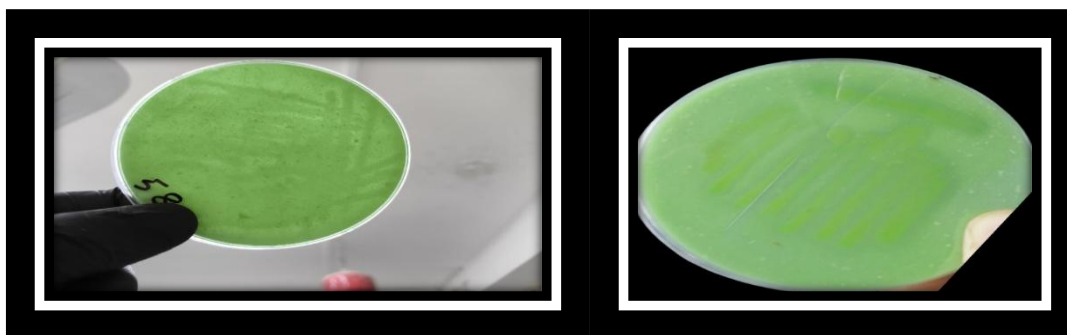


Figure 2: Lipase production from *Pseudomonas aeruginosa*.

In a [18] study he conducted, *P. aeruginosa* had a relatively high production of lipase (76.92%). The study was conducted on isolates from patients' wounds. The productivity of the enzyme is commensurate with the depth and severity of the wound, which causes delayed wound healing and retardation. In another study, he [19] was able to detect the productivity of lipid anzyme from isolated pseudomonas aeruginosa samples from the soil at a high rate. These two studies agreed with the findings of the current study.

3.2.3: Molecular detection of peroxidase enzyme

The results of the molecular detection showed the presence of the Dyp gene for the isolates used in this study, where the Dyp gene was found in all 20 *Pseudomonas aeruginosa* isolates (100%).

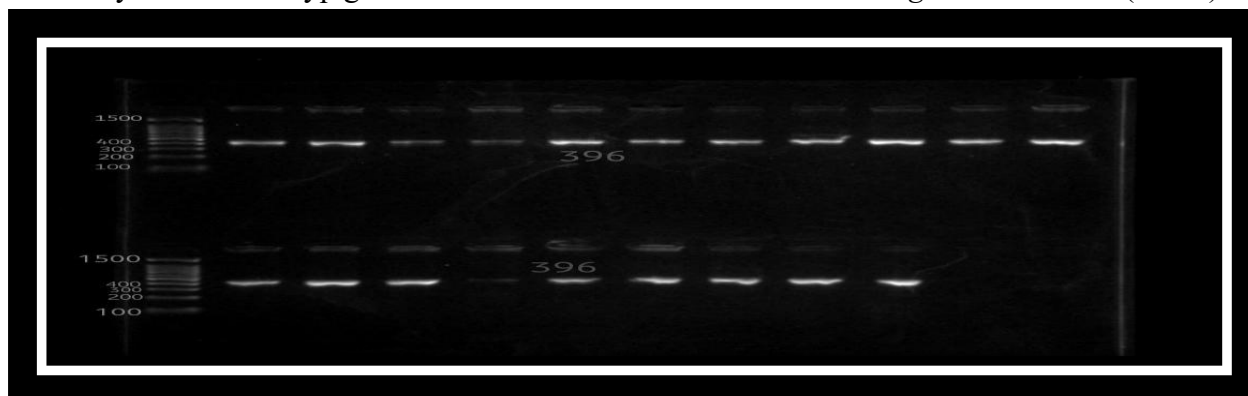


Figure 3: Electrophoresis of the DYP gene in *Pseudomonas aeruginosa*.

Molecular detection of the Dyp gene by PCR is one of the most common techniques, where the polymerase chain reaction of the Dyp gene of size 396bp when comparing the apparent packages with the volumetric evidence of molecular size 1500bp known and processed by the company macrogen as in Figure 3. This study is similar to a study that the current study [20]proved that PCR amplification using the Dyp gene was an ideal method for detecting the enzyme peroxidase.

3.2.4 Molecular detection of lybase enzyme

The results of the molecular detection showed the presence of the lip gene for the isolates used in this study, where the lip gene was found in all 20 *Pseudomonas aeruginosa* isolates (100%).

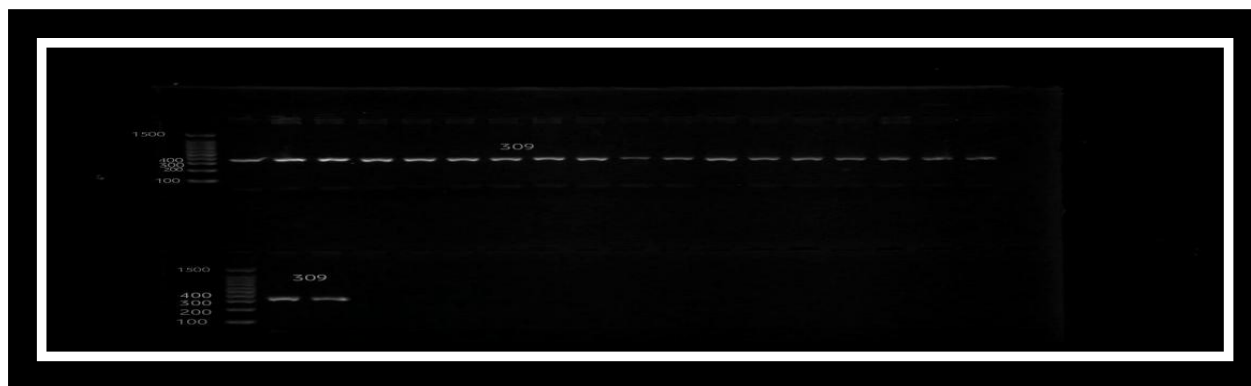


Figure 4: Electrophoresis of the lip gene in *Pseudomonas aeruginosa*

Molecular detection of the lip gene by PCR is one of the most common techniques, as it showed the polymerase chain reaction of the lip gene of size 309bp when comparing the apparent packages with the volumetric evidence of molecular size 1500bp known and processed by the company macrogen in a study that [21] enables the detection of the enzyme lipase in bacteria, which is necessary in genetic engineering in order to isolate and purify it, which is useful in the industrial and pharmaceutical fields.

3.2.5: Detection of *Pseudomonas aeruginosa* antibiotic resistance

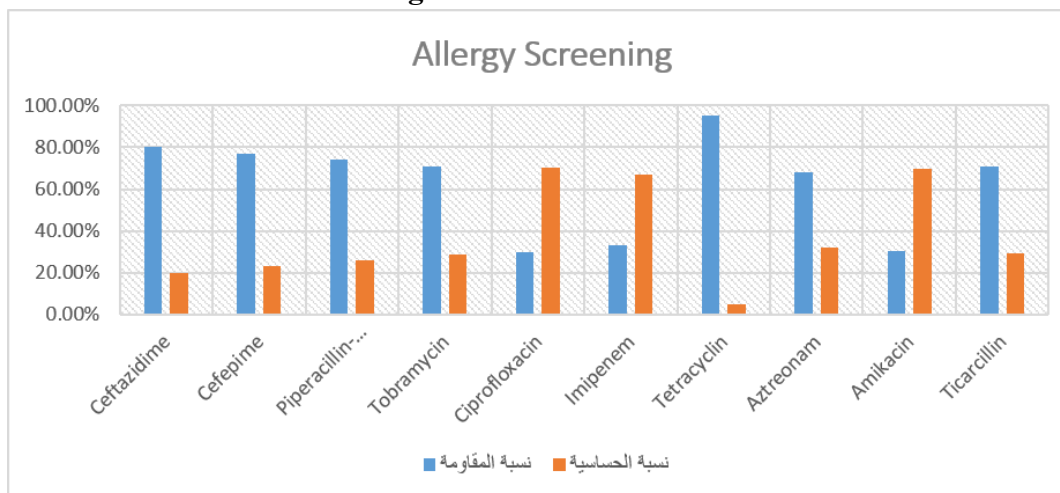


Figure 5: Antibiotic Sensitivity Test Results

This study showed high resistance by Ceftazidime 3rd generation *Pseudomonas aeruginosa* isolates of 80.40%. This result matched the findings of ceftazidime [22] resistance of concern since it is often mediated by the production of predominantly encoded ESBLs on plasmids, overproduction of beta-lactamase, or non-enzymatic mechanisms such as excessive flux or lack of permeability. The selective pressure resulting from the use of antimicrobials is a major determinant of [23] the emergence of resistant strains, while it showed resistance to cefepime by 76.91% and agreed with the [24] findings of ticarcillin, another member of the β -lactam group, which showed high resistance in our study, reaching (70.76%). This rate is close to what was found [25] in the current study. Aztreonam was used and its resistance rate reached a remarkable increase of (68.21%), as shown in (Figure 3-5). Aztrionam is a drug of the β -lactam monobactam class, characterized by excellent activity against *Pseudomonas* species, and is also effective against most organisms producing β -lactamase. This finding is consistent with a study he conducted [26] in Basra, Iraq, which found that the resistance of *P. aeruginosa* isolated to Aztrionam was 65.38%

Carbapenems showed the lowest rates of resistance: imipenem (32.98%) This study is consistent with the results of a study [27] in the city of Baghdad, Iraq, where an imipenem and meropenem resistance rate of 30% was found. But the results of a previous study [28] recorded resistance of (75%) to imipenem. Imipenem belongs to the carbapenem group of antibiotics and binds to various penicillin binding proteins. It is considered one of the most effective medicines against *Pseudomonas aeruginosa* [29]. The current study showed a high sensitivity of *Pseudomonas aeruginosa* bacteria to ciprofloxacin, reaching 70.33%. This result is consistent with a study conducted at the University of Baghdad, [30] which recorded a sensitivity of these bacteria to ciprofloxacin in burn and wound patients at a rate of 78.26%.

Pseudomonas aeruginosa has two primary mechanisms of resistance to the group of fluoroquinolones: structural modification of target enzymes (efflux pump) and [31]. *Pseudomonas aeruginosa* isolates showed low resistance to amikacin (AK), where a percentage of (30.18%) was recorded, and this result is consistent with a previous study conducted in the city of Kirkuk, Iraq, [32] which recorded a percentage of (25%). However, studies [33] in Baghdad found that *P. aeruginosa* showed a high resistance to amikacin at 79%.

The mechanism of action of aminoglycosides is inhibition of protein synthesis in bacterial cells by binding to the 30s subunit of the ribosome. As for the resistance of bacteria to the antibiotic Tobramycin, it reached 71.09%. This study agreed with the study conducted at the University of Baghdad before, [27] where the rate of resistance to the antibiotic was 76%. A high rate of resistance to Piperacillin/Tazobactam was observed at 74.33%. This was [30] in line with the resistance rate of 79.17%. As for the Tetracyclin antagonist, the resistance rate was 95.10% and agreed with his study, [25] as he indicated that the percentage of the antagonist was 100%.

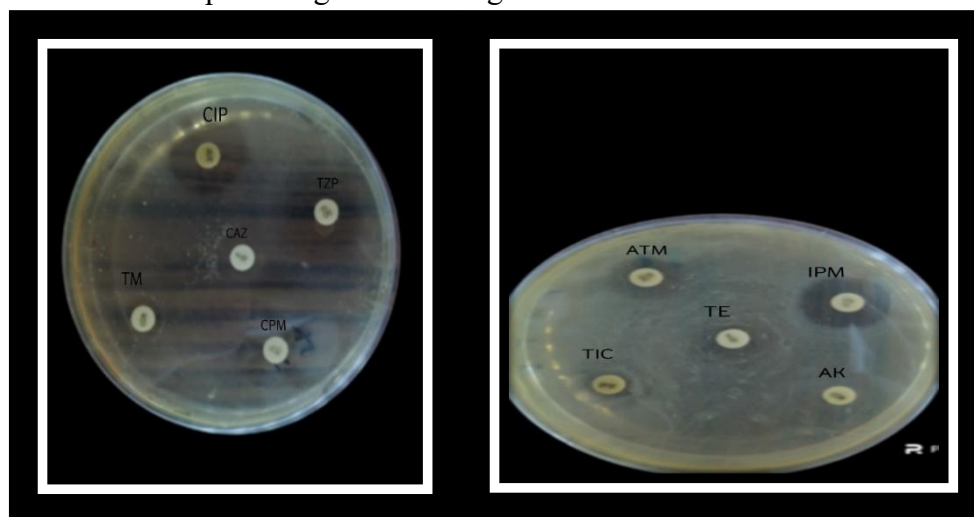


Figure 6: Sensitivity Test Results

Discussion

The findings of this study highlight the significant enzymatic and antibiotic resistance characteristics of *Pseudomonas aeruginosa* isolated from diverse clinical infections. All 20 isolates demonstrated strong enzymatic activity for both peroxidase and lipase, confirmed qualitatively and molecularly through PCR detection of the *Dyp* and *lip* genes, respectively. These results underscore the genetic

capability and phenotypic expression of these enzymes in *P. aeruginosa*, corroborating previous studies that reported similar enzymatic activity in both clinical and environmental isolates [16–21].

The universal expression of the *Dyp* gene (100%) aligns with findings in other bacterial species where peroxidase plays a critical role in oxidative stress protection and pathogenicity. The high prevalence suggests a conserved mechanism in clinical strains, potentially enhancing their survival in hostile host environments. Likewise, the detection of lipase activity in all isolates and its association with wound severity confirms the virulence role of this enzyme, supporting literature indicating its contribution to tissue degradation and delayed healing in infected patients [18,19].

Antibiotic susceptibility results revealed alarming resistance rates among the isolates. The highest resistance was observed against tetracycline (95.10%), consistent with findings from similar regional studies [25], followed by piperacillin-tazobactam (74.33%), ticarcillin (70.76%), and tobramycin (71.09%). These resistance levels reflect a concerning trend of multidrug resistance (MDR) among *P. aeruginosa*, likely driven by selective antibiotic pressure and horizontal gene transfer mechanisms. Notably, resistance to ceftazidime (80.40%) and cefepime (76.91%) points to β -lactamase-mediated resistance and potentially ESBL production, as indicated in prior work [22,23].

Conversely, lower resistance rates were observed for imipenem (32.98%) and amikacin (30.18%), suggesting these antibiotics remain relatively effective treatment options. This is in line with findings from studies in Baghdad and Kirkuk [27,32]. Nevertheless, the emergence of resistance even to carbapenems and aminoglycosides is worrisome and emphasizes the need for ongoing surveillance and prudent antibiotic stewardship.

The comprehensive detection of both virulence factors (enzymes) and resistance profiles supports the hypothesis that *P. aeruginosa* strains from clinical infections possess robust mechanisms that contribute to their pathogenicity and treatment resistance. These findings not only reinforce the clinical importance of monitoring enzyme production but also the necessity of tailoring antibiotic therapy based on local resistance patterns.

Future research should explore the regulatory pathways controlling enzyme gene expression and investigate the potential for inhibiting these enzymes as a strategy to attenuate bacterial virulence. Moreover, genomic sequencing could help identify specific resistance genes and mobile elements contributing to MDR phenotypes in these isolates.

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