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A Study of the Characteristics and Properties of *P. aeruginosa* Isolated from Different Sources and their Resistance to Antibiotics

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ABSTRACT: *Pseudomonas aeruginosa*, a common opportunistic bacterium, is a major source of human surface infections. Infections with these bacteria are difficult to cure and cause significant economic implications. Objectives The current study looked at the antibiotic resistance patterns of *P. aeruginosa* isolated from burns, wound swabs, and urinary tract infections that were referred to Iraq Hospitals' emergency health care units. From October 2022 to May 2023, 180 swabs were collected from burn and wound patients (110 burns, 67 wounds, and 3 urinary tract infections) of both sexes, (80) males and (100) females, aged 1-72 years, at Baghdad government hospitals: Ghazi Al-Hariri Hospital, Central Laboratories, Specialized Burns Hospital, and Yarmouk Hospital. Results:(180) diagnostic swabs from patients with burns and wound infections of both sexes, (80) males and (100) females, aged 1–72 years. The current study found 25(31.25) more *P. aeruginosa* positive cases in males than females, with 29(29) out of 54 positive cases. The study discovered that the age group 21-30 years had the highest rate of bacterial infection, followed by the age groups 11-20 and 1-10, with 11 (20.37%), 10 (18.51%), and 10 (18.51%), respectively. According to the current study's findings, the antibiotics Amikacin, Gentamicin, Ceftazidime, Cefepime, Netilmicin, and Tobramycin showed the highest resistance rates (81.4%, 81.4%, 79.6%, 72.2%, 74%, and 79.6%, respectively).The test findings showed that 52 isolates (96%) produced pyrocyanin and hemolysins. Bacterial colonies were white to gray with a sticky feel. All isolates exhibited β-hemolysis.Alkaline protease production All isolates exhibited a translucent halo surrounding the bacterial colonies.Lipase production All isolates exhibited a hazy halo surrounding the development zone of *P.aeruginosa* colonies. The current study discovered that the antibiotics Amikacin, Gentamicin, Ceftazidime, Cefepime, Netilmicin, and Tobramycin were the most resistant in cases of burn and wound patients, as well as urinary tract infections in Iraq.

Keywords: *P. aeruginosa*, Burn swabs, Wound swabs, Cetrimide agar, Antibiotic sensitivity

1. INTRODUCTION

Pseudomonas aeruginosa is a Gram-negative rod. *P. aeruginosa* is an obligate aerobic bacterium that grows well in a range of culture mediums. It occasionally emits a lovely, grape-like odor. Some strains are beta hemolysers. *P. aeruginosa* is isolated and diagnosed using traditional bacterial growth, culture, and morphological characteristics (1). *Pseudomonas aeruginosa* colonies are smooth and spherical, with a light green tint. Many strains of P. aeruginosa contain pyocyanin, a blue pigment that diffuses in agar. It also creates a fluorescent pigment, which, when coupled with pyocyanin,

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gives the agar its green hue. Some strains generate red pigments (pyorubin), whereas others produce brownish-black pigments (pyomelanin). (2). *P. aeruginosa* isolates. Three types of colonies exist. Small, rough colonies are usually natural isolates from soil or water, but large, smooth colonies with flat edges and elevated appearances might be clinical isolates. These clinical colonies might have a fried egg appearance(3).

Infections with *P. aeruginosa* only arise when it reaches places with no natural defenses, such as when mucosal membranes and skin are injured by direct tissue injury, as in wounds and burns; when intravenous or urinary catheters are used; or when neutropenia is common, such in cancer therapy. The bacteria adhere to and colonize mucosal membranes or skin, then penetrate locally, causing systemic problems such as bloodstream infections(2).. The pathogenicity of *P. aeruginosa* strains is linked to many virulence components, including proteases, elastases, exotoxins, and phenazine pigments, some of which are regulated by a cell density recognition process known as quorum sensing(4).

P. aeruginosa is a ubiquitous bacterium that can rapidly develop resistance to a variety of broad-spectrum medicines. Multidrug resistance (MDR) P. aeruginosa is a major cause of death in burn patients and accounts for 4-60% of hospital infections over the world (5). MDR *P. aeruginosa* secretes inactivating enzymes such ESBLs and MBLs, making beta-lactams and carbapenems ineffective (6). Over the last few decades, various definitions of multidrug-resistant P. aeruginosa have been used, including bacteria resistant to antimicrobial agents from three or more classes (carbapenems, fluoroquinolones, penicillins, cephalosporins, and aminoglycosides) (7).The World Health Organization has identified carbapenem-resistant *P. aeruginosa* as one of three categories of bacteria that require the development of new antibiotics to treat infections. The evolution of various resistance mechanisms has a profound clinical impact (8, 9)

2. MATERIALS AND METHODS

2.1 Collection of Sample

From October 2022 to May 2023, 180 swabs were collected from burn and wound patients (110 burns, 67 wounds, and 3 urinary tract infections) of both sexes, (80) males and (100) females, aged 1-72 years, from Baghdad government hospitals: Ghazi Al-Hariri Hospital, Central Laboratories, Specialized Burns Hospital, and Yarmouk Hospital.

Characterization of Bacterial Isolates by Morphological Examination. Blood agar, MacConkey agar, King A agar, and Cetrimide Agar were used to study the phenotypes of *P. aeruginosa* colonies, such as colony shape, color, and size, as well as the kind of hemolysis.

2.2 **Microscopic Examination**

Gram stain was employed to detect *P. aeruginosa* in the samples. On a clean slide, a drop of normal saline solution was combined with one isolated bacterium colony. The smear was then air-dried and cemented by swiftly passing it over a Bunsen burner. The dried bacterial smear was then stained with Gram stain and viewed under oil immersion using a 100X oil loupe (10).

2.3 **Biochemical Tests**

2.3.1 The biochemical tests were performed to identify the isolated *P. aeruginosa* bacteria:

Diagnosis of *P. aeruginosa* using the VITEK-2 system

This instrument uses the same ID/AST cards, data management software and ADVANCED EXPERT SYSTEM™ (AES) as the larger VITEK 2 instrument

P. aeruginosa isolates were identified and diagnosed using both traditional methods and the VITEK-2 system. VITEK 2 is produced and developed by the French company Biomerieux and is a fully automated system that identifies and diagnoses bacteria based on biochemical tests using the diagnostic kit (ID card), where it diagnoses bacteria by performing more than 67 biochemical tests automatically, and the device also performs a sensitivity test of bacteria to antibiotics using the kit (AST card) It is based on 18 to 22 antibiotics, with each antibiotic having 3-4 concentrations since the minimum inhibitory concentration (MIC) value of the antibiotic can be determined, allowing us to select the optimal antibiotic and dose for each patient(11).

3. RESULTS AND DISCUSSION

P. aeruginosa counts and proportions vary according to sample origin, gender, and age. From October 2022 to May 2023, (180) clinical swabs of patients with burns and wound infections of both sexes, (80) males and (100) females, aged 1-72 years, were collected from Baghdad's government hospitals: Ghazi Al-Hariri Hospital, Central Laboratories, Specialized Burns Hospital, and Yarmouk Hospital. Following the final identification of the samples, (54) P. aeruginosa isolates were discovered at a rate of 32.2%, as shown in Table 1.

Table 1. *P. aeruginosa* bacteria proportions were obtained from skin swabs, burns, and urinary tract infections using selective and improved culture medium.

No.of isolations *P. aeruginosa*	(٪)	Source of isolates
(68.51) 37	(61.11)110	Burn swabs
(31.48)17	(37.22) 67	Wound swabs
(100) 54	(100)180	total

The current study included *P. aeruginosa* positive cases in male recorded that (31.25)25 more than female which recorded (29)29 from total positive cases number 54.

Table 2. Shows the percentages of *P. aeruginosa* bacteria in males and females with wound and burn infections.

Total(%)	*P.* aeruginosa Negative cases No.(%)	*P. aeruginosa* positive cases No.(%)	Sex
(100)80	(68.75)55	(31.25)25	Male
(100)100	(71)71	(29)29	Female
(100)180	126	54	Total

The current study discovered that the age group 21-30 years had the highest rate of bacterial infection, followed by the age groups 11-20 and 1-10 years, with 11 (20.37%), 10 (18.51%), and 10 (18.51%), accordingly (Table 3).

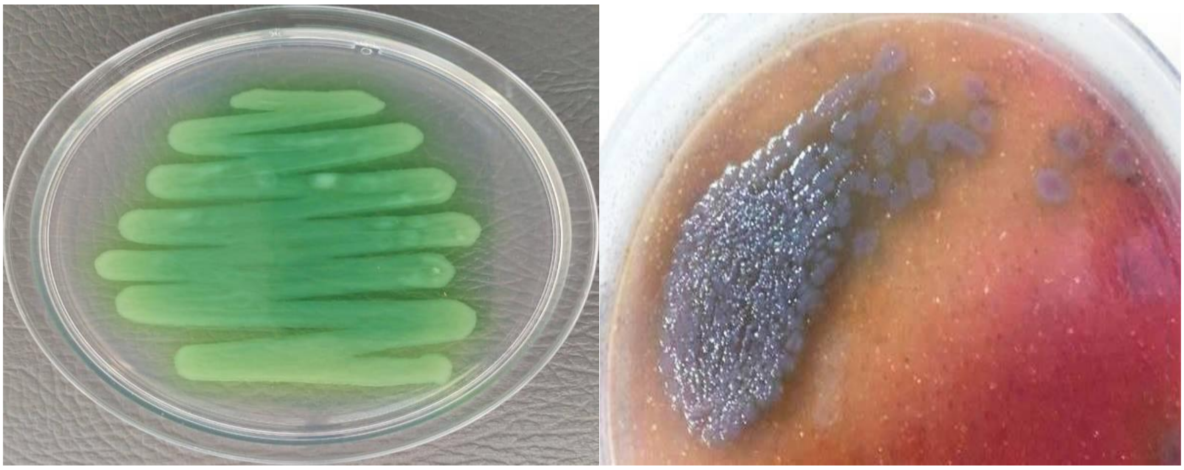
Table 3: *P. aeruginosa* prevalence by age group in wound and burn samples

Total(%)	No. and percentage of *P. aeruginosa* isolates		Age groups (years)
	Female	Male	
(18.51)10	(10.3)3	(28) 7	10-1
(18.51)10	(24.13)7	(12)3	20-11
(20.37)11	(27.58)8	(12)3	30-21
(5.55)3	(0)0	(12)3	40-31
(12.96)7	(7.4)2	(20)5	50-41
(16.66)9	(20.68)6	(12)3	60-51
(7.4)4	(10.34)3	(4)1	70-61
(100)54	(100) 29	(100)25	Total(%)

According to the results provided in Table (1), the percentage of P. aeruginosa isolation by source (burns and wounds) was 37 (68.51) and 17 (31.48), respectively, with burn patients having the highest percentage of P. aeruginosa isolation in this study (68.51%). This finding is congruent with that of the researcher (12), who discovered that P. aeruginosa is the most common bacteria in burn victims, accounting for 53%. In addition, the researcher (13) from Iraq isolated P. aeruginosa bacteria from burn patients at a rate of 48.9%, and the researcher (14) from Turkey isolated P. aeruginosa bacteria from burn and wound patients at 26.9% and 14.7%, respectively. The variation in P. aeruginosa bacteria isolation rates can be attributed to a variety of factors, including geographical differences, sample collection and separation, sample size, bacteria's ability to exist in unsuitable conditions, and the extent to which necessary procedures that limit the spread of bacteria in hospitals are implemented and applied. The current study's findings are consistent with several local, regional, and international investigations that have found that P. aeruginosa bacteria are the most commonly isolated bacteria from burn and wound injuries, accounting for more than 30% of all swabs, including the study. The bacteria were recovered by (15) in Baghdad hospitals, with an isolation rate of 52.5%, and the results were comparable to those of other researchers in Saudi Arabia (16), who found 31.6%. In India, P. aeruginosa infections in wounds and burns reached 46% (17).

The researcher (18) discovered that the highest risk of infection with bacteria that caused wound infection in the city of Baghdad was between the ages of 11 and 20. According to studies, the isolation rate of P. aeruginosa from clinical specimens changed between 2015 and 2020, ranging from 20% to 60%. The explanation for this substantial disparity in the level of bacterial infection could be due to the patient's immunity, as infection rises in immunocompromised patients; moreover, many cases of hospital-acquired bacterial infections may occur, and the patient is infected with Pseudomonas aeruginosa. Given that this bacteria is an opportunistic disease, the presence of antibiotic resistance complicates treatment and eradication. Another possible reason is the sample's source, the number of samples collected from patients, the geographical area, the type of wound and burn sterilization, and the number of times sterilization is performed. As a result, the level of health care must be high from the moment the patient arrives at the hospital, as the patient's stay in the hospital for several days for treatment may increase the rate of bacterial infection as a result of hospital-acquired infections, either directly after contact with patients or indirectly through the use of contaminated surgical instruments (19).

P. aeruginosa morphological traits were used to diagnose the organism. P. aeruginosa colonies on MacConkey agar appeared pale because they did not ferment lactose, as shown in Figure (1-A), which is consistent with the researcher's findings (10). Pseudomonas aerugisa colonies also appeared blue-green on pseudomoas agar, as shown in Figure (1-B), which is similar with Salfinger et al. (20).



A –MacConkey Ager B- *pseudomonas* agar.

Figure 1: *P. aeruginosa* bacteria colonies

P. aeruginosa bacterial colonies appeared greenish-yellow on Cetrimide agar, as shown in Figure (2), because the majority of these colonies produce pyocyanin, a blue-green pigment, and Pyoverdine, a greenish-yellow pigment that fluoresces when exposed to ultraviolet light. These pigments are soluble in water (21). Cetrimide agar is a selective and differential medium used to test P. aeruginosa bacteria's capacity to thrive in the presence of cetrimide (0.03%), a disinfectant that inhibits the growth of most other organisms. The findings are similar with several previous research (21, 22).

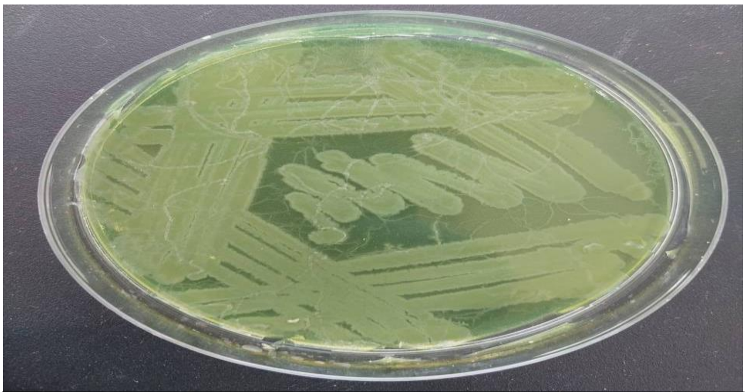


Figure 2: *P. aeruginosa* colonies on Cetrimide agar medium.

Microscopic exam[...] presence of Gram-negative rods under the [...]

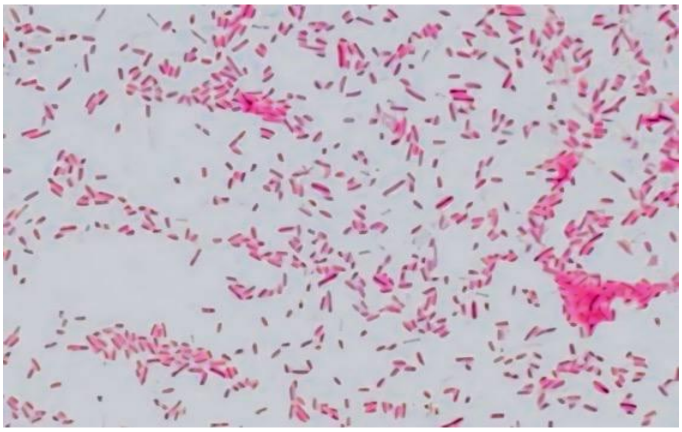


Figure 3. Microscopic examination of *P. aeruginosa* bacteria using 100X oil lens.

Biochemical testing. The results of the biochemical tests for all *P. aeruginosa* isolates revealed, as shown in Table (4), that they were positive for the Catalase and Uerase tests due to the bacteria's ability to break down hydrogen peroxide into water and oxygen gas with the appearance of bubbles, indicating that the test is positive. The Oxidase test produced a positive result by turning the hue to purple in 30 seconds, whereas the Indole, Fox-Proskauer, and Methyl red tests all produced negative results. The citrate utilization test yielded a favorable result, showing that citrate ingestion is the sole source of carbon. It can also thrive at 42°C, which is a major distinguishing trait among Pseudomonas species. (24) These findings are congruent with those reported by Ali et al. (25). The motility test results demonstrated that bacteria can travel by growth surrounding the stabbing region.

Table 4. Results of biochemical tests for *Pseudomonas aeruginosa* bacteria

The result	Test
Gram negative rod	gram stain
+	Catalase
+	Growth at 42°C
+	Oxidase
+	Hemolysin
+	**Citrate utilization test**
-/+	Urease
-	Growth at 42°C

3.1 Antibiotic sensitivity of *P. aeruginosa* isolates:

The Improved Kirby Bauer method (Disk Diffusion technique) was used to test *P. aeruginosa* isolates' sensitivity to eleven antibiotics. The current analysis discovered all of the *P. aeruginosa* isolates shown significant resistance to the medicines evaluated (Table 5). According to the present research's results, the antibiotics with the highest resistance rates were amikacin, gentamicin, ceftazidime, cefepime, netilmicin, and tobramycin. The findings of this study are consistent with those of Al-Shwaikh and Alornaaouti (2018). P. aeruginosa isolates were extremely susceptible to ceftazidime (81%), cefotaxime (78%), piperacillin (76%), Ciprofloxacin and Tobramycin (74%), and gentamicin (72%). Resistance to amikacin and meropenem (70%), ofloxacin (66%), and imipenem (65%) has been identified. In an additional study, P. aeruginosa was discovered in 31.46 percent of burn swabs from four Baghdad hospitals, and the isolates were totally resistant to cefotaxime, cephalothin, gentamycin, and trimethoprim. Amikacin was discovered at 55%, whereas Ciprofloxacin was found at 88% (26). These percentages are similar to the resistance rates seen in *P. aeruginosa* isolates in the current investigation. Antibiotic-resistant bacteria found in hospital environments such as tap water, toilets, and hospital care staff can infect hospitalized patients' wounds and burns, causing the bacterium to spread from one patient to another.

Antibiotic resistance is caused by the coordinated activity of multi-drug efflux pumps, which include chromosomally encoded antibiotic resistance genes (e.g., mexXY) and decreased permeability of bacterial membranes.

Table 5: The percentages of resistance of *P. aeruginosa* isolates to antibiotics may be attributed

In spite of self-resistance, *P. aeruginosa* can readily acquire resistance through chromosomal mutations in chromosomally encoded genes or gene transfer of antibiotic resistance genes. *P.*

Antibiotic	Abbestraction	Resistant (No. &%)	I (No. &%)	Sensitive (No. &%)
Piperacillin	PIP	29(53.7%)	3 (5.5%)	22(40.7%)
Amikacin	AK	44(81.4%)	0 (0%)	10(18.5%)
Aztreonam	ATM	33(61.11%)	3 (5.5%)	18(33.3%)
Gentamicin	CN	44(81.4%)	3 (5.5%)	7(12.9%)
Ciprofloxacin	CIP	43(79.6%)	0 (0%)	11(20.3%)
Netilmicin	NET	40(74%)	0 (0%)	14(26%)
Ceftazidime	CAZ	43(79.6%)	0 (0%)	11(20.3%)
Tobramycin	TOB	43(79.6%)	0 (0%)	11(20.3%)
Levofloxacin	LEV	39(72.2%)	4 (7.4%)	11(20.3%)
Imipenem	IPM	15(27.7%)	0 (0%)	39(72.2%)
Cefepime	FEP	39(72.2%)	2 (3.7%)	13(24%)

aeruginosa bacteria generate modifying enzymes such as phosphotransferase and N-acetyltransferase, and their genes are transferred to plasmids or chromosomes, resulting in aminoglycoside resistance(27). Carbapenems (Imipenem), cephalosporins (e.g., Ceftazidime and Cefepime), aminoglycosides (Tobramycin and Amikacin), and fluoroquinolones (e.g., Ciprofloxacin and Levofloxacin) are used as first-line therapy until laboratory results and antibiotic sensitivity testing are completed. However, the rise of bacterial strains resistant to these medications has made it more difficult to control these bugs and discontinue the patient's therapy (28).

3.2 Morphology detection of virulence factors

3.2 1 Pigments production

The test results indicated that 52 isolates (96%) generated Pyocyanin, which is consistent with the results of (29) who had 81.5% of their isolates produce Pyocyanin, implying that Pyocyanin is produced by more than 85% of *P.aeruginosa* strains. Our data indicate that Pyocyanin production is quite common in antibiotic-resistant P. aeruginosa isolates. The inhibitors had sizes ranging from 5 to 88 mm, showing a major function in *P. aeruginosa* protection, virulence, and antibiotic resistance. Fan *et al*. 2019 found the role of pyocyanin in antibacterial activity and its contribution to resistance to the antibiotic Ciprofloxacin. Pyocyanin production is genetically governed by chromosomes, and a decrease in production might be attributed to the presence of genetic mutations that alter the production characteristic. Alternatively, it might be owing to a shortage of stimulants in manufacturing (30). Vrenna *et al*. (31) discovered that pyocyanin is becoming increasingly prevalent in hospitalized patients with a range of serious illnesses, including pneumonia and cystic fibrosis, and that clinical isolate pus has more pyocyanin than its surroundings

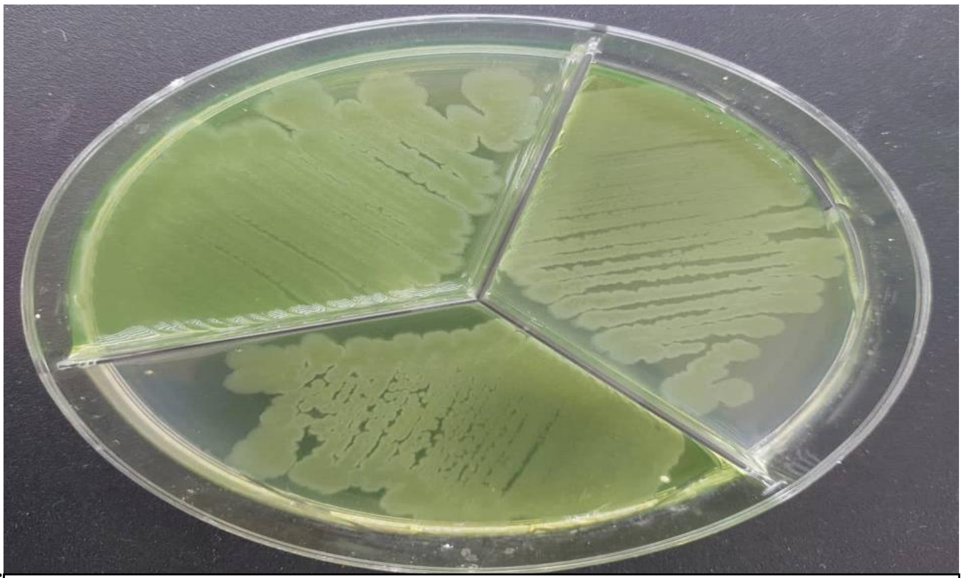


Figure 4. **Production of pigments by *P.aeruginosa***

3.3 Hemolysin production

The bacterial colonies gave a white to gray color and had a sticky texture and all isolates showed β-hemolysis as shown in Figure (4-8), which showed evidence of hemolysin production (32).

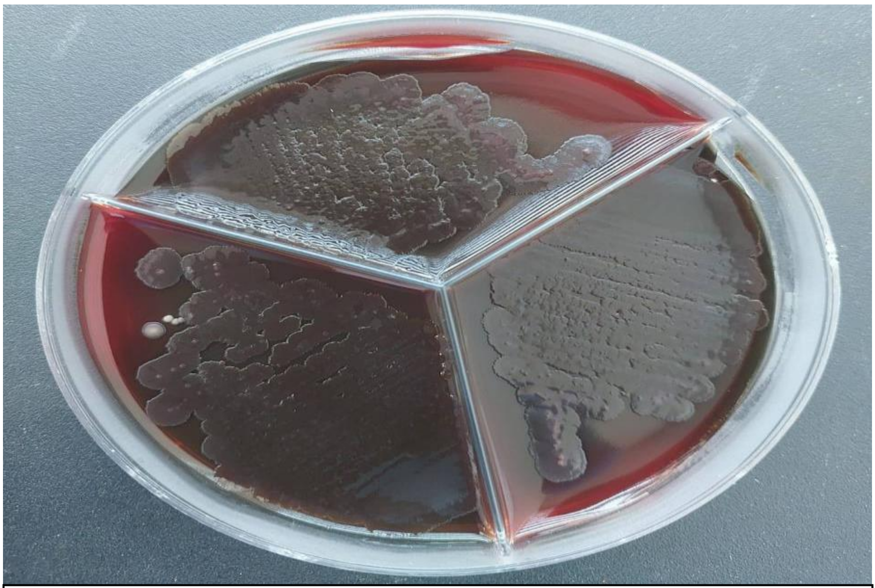


Figure 5: Hemolysin production by *P. aeruginosa*

3.4 Alkaline Protease production

All isolates produced a translucent halo around the bacterial colonies, as shown in Figure 4-9, indicating the synthesis of Alkaline Protease. (32) Alkaline Protease causes lung tissue deterioration by destroying the structure of proteins such as collagen and proteoglycan, which play important roles in burn infections, interfering with fibrin formation, and inactivating Tumor Necrosis Factor (TNF) (33).

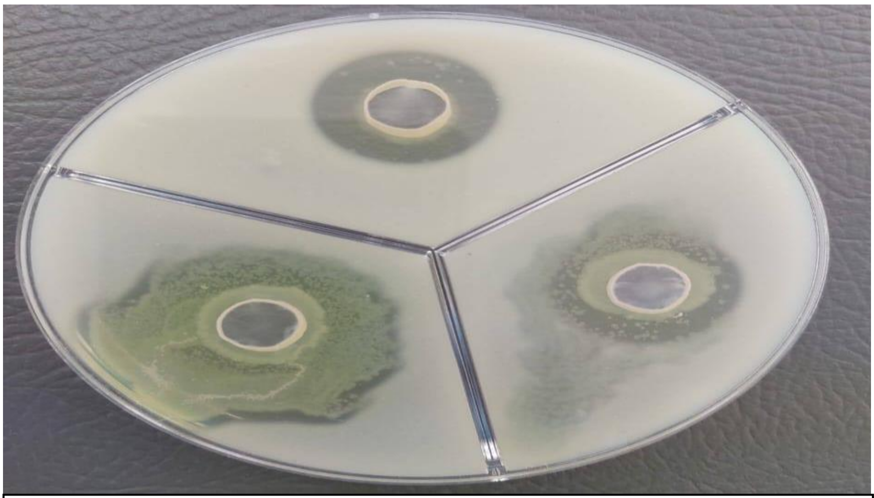


Figure 6. Production of alkaline protease by *P. aeruginosa*

3.5 Phospholipase C Production

The colonies formed black precipitate in the growth zone, indicating the generation of phospholipase C (34), which promotes bacterial colonization in afflicted tissues by hydrolyzing phospholipids.

3.6 Lipase production

All isolates exhibited a hazy halo around the development zone of *P.aeruginosa* colonies (35), where lipase promotes phospholipid analysis.

4. CONCLUSION

All *P. aeruginosa* bacteria isolated from burns and wounds have varying resistance to antibiotics, the highest resistance was to Amikacin followed by Gentamicin and Ceftazidime and the highest sensitivity was to Imipenem. The result of the antibiotic sensitivity test showed that the most effective compound against *P. aeruginosa* is Imipenem. The current study discovered that the age group 21-30 years had the highest rate of bacterial infection, *P. aeruginosa* positive cases in male recorded that more than female.

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