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# Isolation and Identification of *Pseudomonas. aeruginosa* from Nosocomial infections patients in Al Diwaniyah Province

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## Abstract

*Pseudomonas aeruginosa* is an opportunistic clinical pathogen, and it's found in different organisms such as soil, plants and water and considered common pathogenic infections that causes nosocomial infections Present study included collecting about 316 random samples in different ways from January 2022 to February 2023 from Al Diwaniyah teaching hospitals the combination process was randomly investigated for *P. aeruginosa* bacteria specimens were inculcated on different culture media like Blood-agar, MacConkey-agar then Muller Hinton-agar to find specimen of *P. aeruginosa* based on their phenotypic features, for the identification and resistance to antibiotics study biochemical tests and Api 20 E system were used. 316 samples, 33(25.58%) *P. aeruginosa* were isolated from ear, 16(21.33%) from burn, 8 (16%) from wound, 6 (14.28%) from urine and 2(10%) from sputum. In males 27(19.56%) isolate and 38(21.34%) in female. *P. aeruginosa* biochemical examinations showed that all specimens produce catalase, oxidase, then growth at (42°C) and most of the specimens non able to yield (H**2**S), reduce nitrates and hemolysis β-hemolytic. While they gave negative results for Methyl-red, Voges Proskauer and Indole test as diagnosed with Api 20 E system to further confirm the isolation yield for *P. aeruginosa* bacteria. Majority of isolates 55(84.61%) showed in results had Ceftriaxone resistance, furthermore other specimen showed different antibiotics sensitivity response. Isolation proportion of *P. aeruginosa* from ear is more than the isolation rate from other body sites in patients. Nearly every *P. aeruginosa* specimen showed resistance to most antibiotics in present study, particularly Ceftriaxone where resistance rate was 55 (84.61%).

**Keywords:** Agar; Api 20 E system; isolation; *pseudomonas aeruginosa* and resistance to antibiotics

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## 1. Introduction

*Pseudomonas. aeruginosa* is opportunistic pathogenic infections, causes different of health care connected infections like (pneumonia, sepsis, wounds, and (UTI) urinary tract infections [1]. It has been of specific important since it is the chief reason of (mortality and morbidity) in cystic fibrosis (CF) patient and one of the important hospitals acquired infections pathogen and resistant to most of antibiotics [2]. This Gram-negative bacteria structure contains (0.5 to 0.8 µm) by (1.5to 3 µm) rod shaped, and uniflagellar for enlistment adapting from almost gram-negative bacteria, *Pseudomonas aeruginosa* is oxidase (+). Furthermore, it nonfermented lactose. *Pseudomonas. aeruginosa* in soil, plant, water and on skin of animals. Naturally it's found swimming in water like a plankton or like a biofilm, bacteria bunches having the same phenotype and biochemical features [3]. Exclusively *Pseudomonas. Aeruginosa* live in different temperatures and infrequent nutrition. The bacterium has the ability to grow in distilled water as showed in previous studies charitable *Pseudomonas. aeruginosa* benefit in adapting to altering environment [4]. *Pseudomonas aeruginosa* is opportunistic pathogen needs a deficiency of immunity to infect its host [5]. And this explained why *Pseudomonas. aeruginosa* nosocomial risk of death and sepsis especially ventilation machines, malignances and burns patients [6],[7].

## 2. Materials and Method

### 2.1 Samples Collection

The present study included collecting about 316 random samples in different ways from January 2022 to February 2023 from Al Diwaniyah teaching hospitals the combination process was randomly investigated for *P. aeruginosa* bacteria.

### 2.2 Identification of Bacterial Isolates

#### 2.2.1 Morphologic Examination:

The main objective of the collection of samples was to isolate *P. aeruginosa* bacteria based on the phenotypic characteristics of the colonies growing on a pale MacConkey agar medium due to its inability to ferment the lactose sugar in the middle and has a grape-like odor Their ability to degrade blood was also demonstrated when transplanted into the blood agar medium.65 bacterial isolates were isolated and tests were conducted.

#### 2.2.2 Microscope examination:

These include the examination of shape, gram stain reaction, arrangement of cells with each other, motility and capsule presence.

#### 2.2.3 Biochemical Tests:

Biochemical tests including Catalase test, oxidase test, hemolysin production test, urease test, motility test, sugar fermentation & IMVIC tests, methyl red test, Voges Proskauer test H2S produce test and Citrate test, gelatin liquefication and Indole production test then Api 20 E diagnosis

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system were conducted to diagnose isolated *P. aeruginosa*.

### 2.2.4 Api 20 E diagnosis system

After obtaining the results of the biochemical tests applicable to *P. aeruginosa* bacteria, the Api 20 E strips were used to diagnose this type in the final form. This tape contains a tube of specific 20 biochemical tests by BioMerieux advices.

### 2.2.5 Antibiotic Sensitivity Test (AST):

Bacterial isolates antibiotic susceptibility was studied by Kirby–Bauer standardized single disk [8], [9].

## 3. Results and Discussion

The study included collecting about 316 random samples in different ways from January 2022 to February 2023 from Al Diwaniyah teaching hospitals the combination process was randomly investigated for *P. aeruginosa* bacteria as show in **Table 1**.

*Table 1. Percentage and number of general bacterial isolates according to isolation site.*

| Sample site | Total No. | Type of isolate | | |
|---|---|---|---|---|
| | | *P. aeruginosa* | Other bacteria | No growth |
| **Ear** | 129(40.82) | 33(25.58) | 40(31.01) | 56(43.41) |
| **Burn** | 75(23.73) | 16(21.33) | 24(32) | 35(46.66) |
| **Wound** | 50(15.82) | 8(16) | 13(26) | 29(58) |
| **Urine** | 42(13.29) | 6(14.28) | 9(21.42) | 27(64.28) |
| **Sputum** | 20(6.32) | 2(10) | 5(25) | 13(65) |
| **Total** | 316(100) | 65(20.56) | 91(28.79) | 160 (50.63) |
| **$X^2$** | 137.63 | | 9.970 | |
| **P value** | 0 | | 0.267 | |

The present study isolates include both sexes male and female as show in **Table 2**, and ranging of age from 4-68 as in (**Fig. 1**)

*Table 2. Percentage and number of general bacterial isolates according to the gender.*

| Gender | Total No. | Type of isolate | | |
|---|---|---|---|---|
| | | *P. aeruginosa* | Other bacteria | No growth |
| **Male** | 138 | 27(19.56) | 46(33.33) | 65(47.1) |
| **Female** | 178 | 38(21.34) | 45(25.28) | 95(53.37) |
| **Total** | 316 | 65(20.56) | 91(28.79) | 160(50.63) |
| **$X^2$** | 10.127 | | 2.474 | |
| **P value** | 0.001 | | 0.290 | |

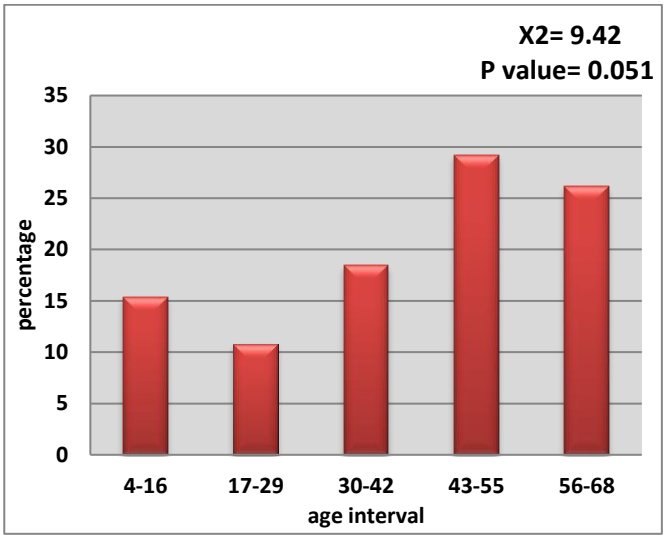


*Fig. 1. Distribution of patients with P. aeruginosa infection according to the age.*

Microscopical test presented that bacterially isolate small bacilli, gram-negative and lacking spore formation ability, using selective-media (*Pseudomonas* isolation agar) for initial isolation, it was found that the colony is mucoid, round and smooth [10].

Biochemical examinations of *Pseudomonas. aeruginosa* **Table 3**. see all specimen yield catalase, oxidase and growth at (42 °C) and most of specimen don't produce H**2**S and have the ability of nitrates reduction, hemolysis β-hemolytic, results shown in **Table 3** imply they gave negative results for (Methyl red), (Vogues-Proskauer) and indole test but they gave (positive tests) for another test registered in **Table 3**.

*Table 3. Morphology, physiology and biochemical results of Pseudomonas. aeruginosa*

| Tests | | | Results |
|---|---|---|---|
| **Microscope** | | Gram- stain | negative, bacilli |
| **Growth** | Selective media | Mac-Conkey agar | Non lactose fermentation |
| | enriched media | Blood agar | β-hemolysis |
| | | Grow at (37°C) | negative |
| | | Grow at (42°C) | positive |
| **Biochemical** | | Catalase test | positive |
| | | Oxidase test | positive |
| | | Urease test | positive |
| | | Indole test | negative |
| | | Methyl Red | negative |
| | | Vogues-Proskauer | negative |
| | | Citrate Utilization test | positive |
| | | Klingler iron - agar | k/k slant and bottom |

k: alkaline

The findings in **Table 4** see the resistant of *Pseudomonas. aeruginosa* toward 12 antibiotics agent via use (disk diffusion technique) as in (**Fig. 2**) The results were understood by diameter of inhibitory regions and comparison with inhibitory regions determine through CLSI (2019).

*Table 4. Antibiotic Susceptibility for P. aeruginosa isolates.*

| Antibiotic | Sensitivity test result | | |
|---|---|---|---|
| | Resistant | moderate | Sensitive |
| **Imipenem** | 5(7.69) | 0(0) | 60(92.3) |
| **Meropenem** | 4(6.15) | 2(3.07) | 59(90.76) |
| **Amikacin** | 10(15.38) | 3(4.61) | 52(80) |
| **Azithromycin** | 13(20) | 4(6.15) | 48(73.84) |
| **Gentamicin** | 44(67.69) | 5(7.69) | 16(24.61) |
| **Norfloxacin** | 26(40) | 6(9.23) | 33(50.76) |
| **Ceftriaxone** | 55(84.61) | 7(10.76) | 3(4.61) |
| **Cefotaxime** | 50(76.92) | 13(20) | 2(3.07) |
| **Ciprofloxacin** | 24(36.92) | 9(13.84) | 32(49.23) |
| **Levofloxacin** | 32(49.23) | 6(9.23) | 27(41.53) |
| **Ofloxacin** | 39(60) | 8(12.3) | 18(27.69) |
| **Nitrofurantoin** | 54(83.07) | 7(10.76) | 4(6.15) |
| **$X^2$** | | 327.23 | |
| **P value** | | 0* | |

$X^2$: chi square value, S: significant difference (p <0.01)

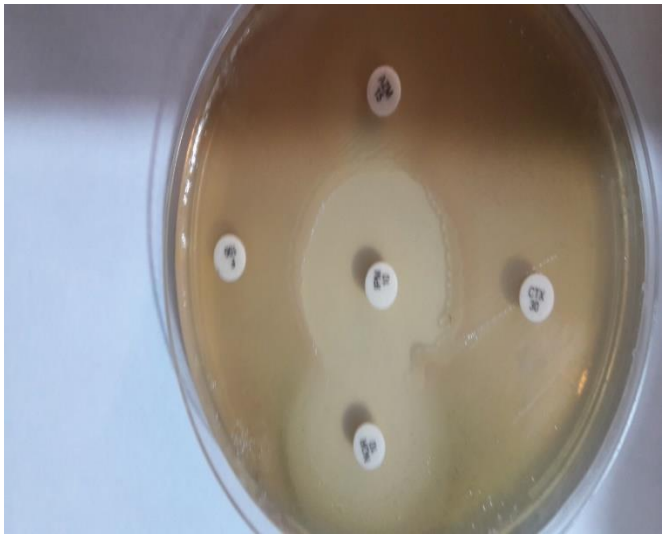

*Fig. 2. Antibiotic susceptibility for P. aeruginosa isolates.*

In this study, Total isolates 316, 65(20.56) cultures positive *P. aeruginosa* clinical isolate (ear, burn, wound, urine and sputum are 25.58, 21.33, 16, 14.28, 10 and 20.56%) and 91(28.79) other bacteria which were positive, while 160 (50.63) non growth, and P value (0.267). Almost similar reported, where they showed 313 patients

colonized with *P. aeruginosa* 60 clinical cultures positive, while 167 (50.63) non growth and P value 0.261 [11]. But this study contradiction with another study which showed that 261 patients colonized with *P. aeruginosa* 96(52%) clinical cultures positive and 167 (52%) *P. aeruginosa* negative and P value 0.78 [12].

In the current study percentage of *P. aeruginosa* isolates according to the gender 41.53% in male and 58.46% in female, *P. aeruginosa* were recovered from females 57.5% and males 40.51% this results likeness with result by [13]. Reported *P. aeruginosa* from 69% males only and the present study disagree with because the present include males and females' percentage of *P. aeruginosa* isolates [14]. In present study, Total isolates 316, 65(20.56%) cultures were positive *P. aeruginosa* clinically isolated by biochemical method. Almost similar reported, showed that biochemical positive 25% among culture growth [15].

*P. aeruginosa* resistance to antibiotic is shown in **Table 4** all isolates of *P. aeruginosa* revealed the highly rate of resistance to Ceftriaxone , Nitrofurantoin, Cefotaxime, (in 84.61%, 83.07%, 76.92%, respectively ),and moderately resistance to Gentamicin(67.69%) and Ofloxacin (60%).Regard to other antibiotic the results was relatively lower resistance to Levofloxacin49.23%, Norfloxacin40%, Ciprofloxacin 36.92%, Azithromycin 20%, Amikacin15.38%, Imipenem7.69% and Meropenem 6.15%.These results were in likeness with the results obtained by [16].

Another result showed that meropenem and Imipenem had a 100% sensitivity follow through piperacillin and amikacin with all-out susceptibility others such as (gentamicin, ciprofloxacin, levofloxacin and aztreonam) were find toward be justly active and a decent amount of specimens were intermediate resistance toward ceftriaxone the rates of resistant toward (aztreonam, cefotaxime and ceftazidime) remained 11.76%, 82.35% and 5.88% correspondingly. Total resistant remained observe against (penicillin, ampicillin, cefixime and cefpodoxime) the result is semi similar to this result [17]. and also result by [18]. *Pseudomonas*. *aeruginosa* displayed very tall resistant toward Fosfomycin (85.7%). A alike resistant design was showed with Ciprofloxacin 70.4 % Levofloxacin 66.7%, Ceftazidime (61.5%), Piperacillin 57.1%, Imipenem (55.6%, Piperacillin and Tazobactam (28.6%;50.0%), tobramycin (52.0%), gentamicin (44.4%), and meropenem 50.0% high of *P*seudomonas. *aeruginosa* and (MDR) *P*seudomonas *aeruginosa* was find for Cefepime (50.0%), and amikacin (27.3%).

Concerning to Norfloxacin and Ciprofloxacin, the result resembles the result obtained by [19], which indicate that the fluroquinolone stays additional effected on *Pseudomonas aeruginosa*, but then are in contradiction by the results obtain through [20]. That found all isolates are resistant to Fluoroquinolones.

A result by a study [21]. Found that Carbapenem i.e. imipenem is most effective antibiotic and its resistance rate was detected as 24% only and thus contradict with this result.

A report proposed a phenotypical difference model anywhere *Pseudomonas aeruginosa* transform among antibiotics susceptible and antibiotics resistant stages in connotation with biofilm creation [22],[23].

This considered a high-level resistance, most importantly to the clinically used Tobramycin and specifically Gentamicin [24].

## 4. Conclusion

Isolation proportion of *P. aeruginosa* from ear is more than the isolation rate from other body sites in patients. Nearly every *P. aeruginosa* specimen showed resistance to most antibiotics in present study, particularly Ceftriaxone where resistance rate was 55 (84.61%). Too, Ceftriaxone remained exceedingly effected antibiotic in contradiction of *P*seudomonas. aeruginosa with the resistant degree of 55 (84.61%) follow, Nitrofurantoin 54(83.07%) and Cefotaxime 50(76.92%).

**Ethical Clearance:** This paper was approved ethically by research ethical committee of both MOH and MOHESER in Iraq.

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