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# Serotypes and Antibiogram of *Pseudomonas Aeruginosa* Isolated from Hospitals in Yemen

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## Abstract

**Background:** *Pseudomonas aeruginosa* (*P. aeruginosa*) is the most common microorganism, which isolated from hospitalized patients for longer than one week. It is a frequent cause of nosocomial infections such as pneumonia, urinary tract infections, and bacteremia. Traditionally, *P. aeruginosa* has been typed based on its phenotypic characteristics, such as serotyping, biotyping, bacteriophage typing, pyocin typing, and antimicrobial susceptibility testing. This study was conducted in order to identify and determine the prevalence of *P. aeruginosa* isolated from clinical and non-clinical samples from three main hospitals in Hadhramout-Yemen, its incidence, to detect epidemiology and source of nosocomial infection by serotyping and antibiogram.

**Materials and Methods:** A total of 200 samples were collected from patients', staff hands and the environment from different sections: intensive care unit, neonatal intensive care unit, pediatric unit, endoscopy unit, male surgical ward, female surgical ward, male medical ward, female medical ward and delivery room. A collection of 56 isolates of *P. aeruginosa* isolated from the studied samples from the three hospitals were typed using serotyping and antibiogram. All strains of *P. aeruginosa* were serotyped by a slide agglutination test with 16 monovalent antisera.

**Results:** The prevalence of *P. aeruginosa* at the different sites was evaluated and was found to be highest in intensive care unit, and the lowest prevalence percentage was in operation theatres. About 98.2% of isolates were typed and 1.78% were not typed, serotype E (O : 11) was the most frequently encountered and serotypes C (O : 7/O : 8), D (O : 9) and M were not detected. Antimicrobial resistance pattern A1 was the more frequent, and high among serotypes O : 11. Isolates from suction apparatus tubing, respirators, staff hands, endoscope, water tap, antiseptic solutions and air condition outlet were linked to those from patients who had the same antibiogram. Patients' isolates had serotype E (O : 11) linked to all clinical (except blood) and environmental isolates, that indicated there was direct relationships between them.

**Conclusions:** Serotype method is a more precise to antibiogram and can increase the efficiency of infection control procedures by determining the patient sources of infections and the environmental sources to prevent or reduce the spread of *P. aeruginosa* infections.

***Keywords:*** *Pseudomonas Aeruginosa; Serotyping; Antibiogram*

## Introduction

*Pseudomonas aeruginosa* is an opportunistic secondary pathogen for humans capable of causing major nosocomial infections [1].

Nosocomial infection also called "hospital acquired infection" can be defined as: An infection occurring in a patient in a hospital or other health care facility in whom the infection was not present or incubating at the time of admission. This includes infections acquired in the hospital but appearing after discharge, and also occupational infections among staff of the facility [2]. The high mortality associated with this pathogen, especially in immunocompromised patients, and excessive mortality and morbidity associated with inefficient empirical therapy, leading to complications during treatment. There is a global emergence of multidrug resistant strains of *Pseudomonas* [3]. Metallo-β-lactamase (MBL) producing *P. aeruginosa* strains lead to higher severity and mortality infections [4]. The MBLs were recently highlighted as one of the most resistance mechanisms due to their ability to hydrolyzing practically all agents containing β-lactam, including carbapenems [5]. The prevalence of MBL production in *P. aeruginosa* strains, important NI causing agent, rose in many countries such as South-East Asia, Europe, Latin America and, from North America and Oceania [6].

Traditionally, *P. aeruginosa* has been typed based on its phenotypic characteristics, such as serotyping, biotyping, bacteriophage typing, pyocin typing, and antimicrobial susceptibility testing [7]. Among the above techniques, serotyping is the most frequently used procedure. The most accepted serotyping method for *P. aeruginosa* is the International Antigenic Typing Scheme (IATS) [8], which identifies 20 different serotypes based on the expression of the O-antigen moiety of the lipopolysaccharide (LPS); however, there are variations in the distribution of *P. aeruginosa* serotypes, with some serotypes being more prevalent in clinical samples [9].

Determination of antimicrobial profiles is another typing method used frequently as a supplemental epidemiological tool for strain differentiation of *P. aeruginosa*. It should be noted, however, that antibiotic susceptibility profiles are less stable than O-antigenic markers, given the resistance factors that can occur under pressure of antibiotic therapy [10].

The present study was to identify and determine the prevalence of *Pseudomonas aeruginosa* isolated from clinical and non-clinical samples from three main hospitals in Hadhramout–Yemen, its incidence, to detect epidemiology and source of nosocomial infection by serotyping and antibiogram.

## Materials and Methods

This study was conducted of Department of Medical Microbiology in the National Center for Central Public Health Laboratories of Hadhramout, Yemen, during the period from November 2013 to May 2014. The standard methods were: isolation and identification of *P. aeruginosa* from the collected samples and then we tested these bacteria by antibiotic susceptibility tests and serology tests.

### Samples Study

A total of 200 samples were collected from patients, staff hands and the environment from different sections: intensive care unit (ICU), neonatal intensive care unit (NICU), pediatric unit (PU), endoscopy unit, male surgical ward, female surgical ward, male medical ward, female medical ward and delivery room.

- One hundred forty samples were collected from patient sources, including wound and burn exudates, urine, sputum, throat swab, ear secretions, cerebral spinal fluid and blood.
- Fifteen samples were collected from staff hands at midday, by which time staff members had been in contact with patients for several hours. Some staff members refused to give samples for testing.
- Forty-five environmental samples were taken, including suction apparatus tubing, respirators (artificial ventilation tubing), air condition outlet, endoscopes, antiseptic solutions and water tap. From each we collected about 3 - 4 samples.

Information about age, sex and type of infection were obtained from medical records.

The protocol of the Isolation and identification of *P. aeruginosa* was followed as recommended by Guessas and Kihal [11].

### Antibiogram

Antibiotic susceptibility tests were carried out by the Kirby-Bauer disk diffusion technique according to Clinical Laboratory Standard Institute guidelines [12]. The following antibiotics (OXOID and HIMEDIA) were sometimes described in our country against *P. aeruginosa*: amikacin (AK 30 µg), aztreonam (AT 30 µg), ceftazidime (CAZ 30 µg), gentamicin (GEN 10 µg), imipenem (IPM 10 µg), piperacillin (PI 100 µg), piperacillin/tazobactam (PIT 100/10 µg), ciprofloxacin (CIP 5 µg), ceftriaxone (CRO 30 µg), ampicillin (AMP 10 µg), cefotaxime (CTX 30 µg), tobramycin (TOB 10 µg), chloramphenicol (C 30 µg) and erythromycin (E 15 µg). The test medium was Mueller-Hinton agar [13]. Screening for MBL-producing isolates were performed by disk potentiation test with EDTA impregnated imipenem disk as described by Yong., *et al*. [14].

## Serological test

### *Pseudomonas aeruginosa* antisera

Sixteen *P. aeruginosa* antisera (1 to 16) based on the International Antigen Typing Scheme types (IATS) [8]. The O-group was identified by slide agglutination with *P. aeruginosa Antisera* Kit (Denka Seiken Co. Ltd., Tokyo, Japan), according to Homma [15] and Liu., *et al*. [16].

The preparation of antigens and slide agglutination test were done according to Jamasbi and Proudfoot [10].

## Results

### Isolation rate *of P. aeruginosa* and patient information

During a study period, the total positive number of *P. aeruginosa* isolates was 56 (28%) out of (200) samples from three hospitals. *P. aeruginosa* was isolated from patient samples (30%), the highest isolation rate were taken from wounds and burns 60%, and the lowest percentage belongs to blood 16.6% (Table 1).

| Sources | No. of samples | No. of P. aeruginosa positive growth | Percentage of positive culture | $\chi^2$ | P-value |
|---|---|---|---|---|---|
| Patient samples | | | | | |
| Wounds and burns | 30 | 18 | 60 | 17.9 | 0.000 |
| Sputum | 30 | 8 | 26.7 | 0.03 | 0.86 |
| Urine | 27 | 6 | 22.2 | 0.52 | 0.47 |
| Throat swabs | 20 | 4 | 20 | 0.71 | 0.4 |
| Ear secretions | 16 | 3 | 18.8 | 0.74 | 0.39 |
| Cerebral spinal fluid | 11 | 2 | 18.2 | 0.56 | 0.46 |
| Blood | 6 | 1 | 16.6 | 0.39 | 0.53 |
| Total | 140 | 42 | 30 | - | - |
| Environment samples | | | | | |
| Suction apparatus tubing | 8 | 4 | 50 | 2.0 | 0.16 |
| Endoscope | 5 | 2 | 40 | 0.37 | 0.55 |
| Respirators | 8 | 2 | 25 | 0.04 | 0.85 |
| Water tap | 8 | 2 | 25 | 0.04 | 0.85 |

| | | | | | |
|---|---|---|---|---|---|
| Air condition outlet | 8 | 1 | 12.5 | 0.99 | 0.32 |
| Antiseptic solutions | 8 | 1 | 12.5 | 0.99 | 0.32 |
| Total | 45 | 12 | 26.7 | - | - |
| Staff hand samples | 15 | 2 | 13.3 | 1.73 | 0.19 |
| Total | 200 | 56 | 28 | - | - |

***Table 1:*** *Isolation rate of Pseudomonas aeruginosa strains from samples obtained from patients, the environment and staff hands.*

The risk factors for P. aeruginosa infection were in males (57.1%), the rate was a statistically significant (P-Value = 0.91) and the age group (> 40 - 60 years) (42.8%) (P-Value = 0.51). Many of the patients were suffering from chronic diseases, such as diabetes mellitus, cancer, hypertension, cardiac disease and surgery, the highest percentage of risk was in surgery disease 47.6% (P-Value = 0.31) (Table 2).

| **Variables** | **Infected (n = 42) Sex** | | | | | | **Non-infected (n = 98) Sex** | | | | | | ***P*-value** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age [years] | M | % | F | % | Total | % | M | % | F | % | Total | % | 0.51 |
| 0 - 1 | 2 | 4.7 | - | - | 2 | 4.7 | 7 | 7.1 | 5 | 5.1 | 12 | 12.2 | |
| > 1 - 20 | 5 | 11.9 | 3 | 7.1 | 8 | 19.0 | 12 | 12.2 | 10 | 10.2 | 22 | 22.4 | |
| > 20 - 40 | 4 | 9.5 | 4 | 9.5 | 8 | 19.0 | 8 | 8.1 | 3 | 3.1 | 11 | 11.2 | |
| > 40 - 60 | 12 | 28.5 | 6 | 14.2 | 18 | 42.8 | 20 | 20.4 | 18 | 18.3 | 38 | 38.7 | |
| > 60 | 1 | 2.3 | 5 | 11.9 | 6 | 14.2 | 8 | 8.1 | 7 | 7.1 | 15 | 15.3 | |
| Total | 24 | 57.1 | 18 | 42.8 | 42 | - | 55 | 56.1 | 43 | 43.9 | 98 | - | 0.91 |
| Comorbid conditions | | | | | | | | | | | | | |
| Diabetes | 7 | 16.6 | 6 | 14.2 | 13 | 30.9 | 11 | 11.2 | 8 | 8.2 | 19 | 19.4 | 0.82 |
| Cancer | 4 | 9.5 | 6 | 14.2 | 10 | 23.8 | 6 | 6.1 | 5 | 5.1 | 11 | 11.2 | 0.51 |
| Hypertension | 8 | 19.0 | 5 | 11.9 | 13 | 30.9 | 10 | 10.2 | 9 | 9.2 | 19 | 19.4 | 0.62 |
| Cardiac disease | 5 | 11.9 | 4 | 9.5 | 9 | 21.4 | 6 | 6.1 | 3 | 3.1 | 9 | 9.2 | 0.63 |
| Surgery | 8 | 19.0 | 12 | 28.5 | 20 | 47.6 | 21 | 21.4 | 18 | 18.4 | 39 | 39.8 | 0.31 |

***Table 2:*** *Analysis of risk factors and correlation with infection Pseudomonas aeruginosa isolated from 140 clinical samples. F: Female, M: Male.*

The isolation rate of *P. aeruginosa* from environmental samples were 26.7%and the highest percentage 50% from suction apparatus tubing, with significant (P-Value = 0.16). Table 1 also, shows that 13.3% of staff hand samples were culture positive of *P. aeruginosa* (P-Value = 0.19).

## Prevalence of *Pseudomonas aeruginosa* in different sections of hospitals

The prevalence of *P. aeruginosa* at different sites was evaluated and was found to be highest in intensive care unit (ICU) (47.5%) (*P*-Value = 0.002), and the lowest prevalence percentage was (6.7%) (*P*-Value = 0.06) in operation theatres (OT) (Figure 1).

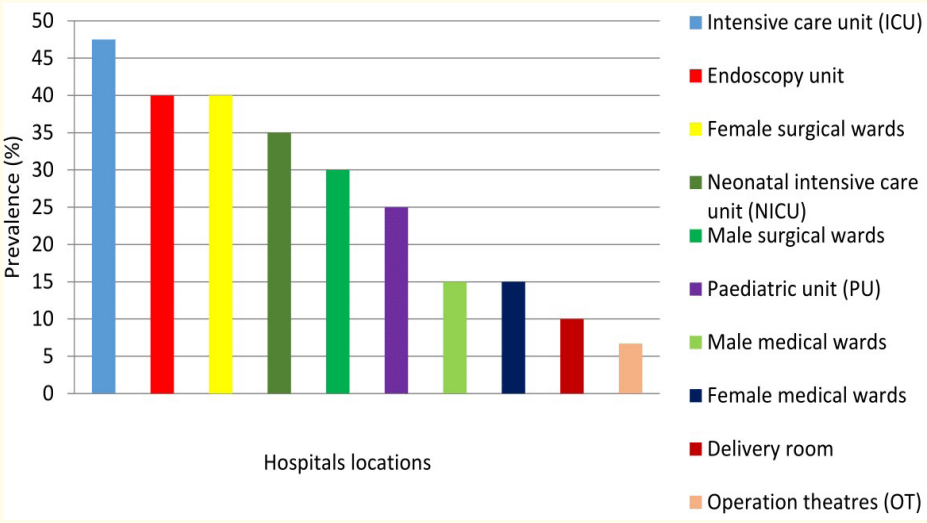


***Figure 1:*** *Prevalence of Pseudomonas aeruginosa in different locations of participating hospitals.*

## Antibiotic susceptibility

Among the 14 antibiotics used, the highest percentage of antibiotic resistance was to ceftriaxone, ampicillin, cefotaxime and chloramphenicol (100%) and low antibiotic resistance to imipenem (5.4%) (Table 3).

| Antibiotics | No. of Resistant Strains | % | No. of Sensitive Strains | % | No. of Moderate Strains | % |
|---|---|---|---|---|---|---|
| Ceftriaxone | 56 | 100 | - | - | - | - |
| Ampicillin | 56 | 100 | - | - | - | - |
| Cefotaxime | 56 | 100 | - | - | - | - |
| Chloramphenicol | 56 | 100 | - | - | - | - |
| Erythromycin | 55 | 98.2 | - | - | 1 | 1.8 |
| Tobramycin | 21 | 37.5 | 4 | 7.1 | 31 | 55.4 |
| Ceftazidime | 19 | 33.9 | 34 | 60.7 | 3 | 5.4 |
| Amikacin | 19 | 33.9 | 17 | 30.4 | 20 | 35.7 |
| Piperacillin | 15 | 26.8 | 31 | 55.4 | 10 | 17.9 |
| Aztreonam | 13 | 23.2 | 39 | 69.6 | 4 | 7.1 |
| Ciprofloxacin | 10 | 17.9 | 43 | 76.8 | 3 | 5.4 |
| Gentamicin | 10 | 17.9 | 35 | 60.7 | 11 | 19.6 |
| Piperacillin/ tazobactam | 6 | 10.7 | 45 | 80.4 | 5 | 8.9 |
| Imipenem | 3 | 5.4 | 53 | 94.6 | - | - |

***Table 3:*** *Antibiotic sensitivity tests Pseudomonas aeruginosa strains.*

### Detection of MBL-producing isolates

Screening of MBL producing by *P. aeruginosa* was percentage (5.4%) isolates, and the difference was statistically significant (*P*-value = 0.01).

### Antibiogram

Table 4 showed the antibiogram of *P. aeruginosa* strains isolated from clinical, environment and staff hands samples, showing 16 ARP (antimicrobial resistance pattern) including resistance ranged from 5 to 13 antimicrobial. Resistance pattern A1 (48.2%) isolates was the more frequent.

| ARP no. | ARP (Antibiogram) | No. of strains (%) | Samples |
|---|---|---|---|
| A1 | CRO/CTX/C/E/AMP | 27 (48.2%) | Urine (3), wound and burn (7), Respirators (2), Throat swabs (4), sputum (4), staff hand, Cerebral spinal fluid, Endoscope, Suction apparatus tubing (2), Antiseptic solutions, Ear secretions |
| A2 | CRO/CTX/C/E/AMP/CAZ | 2 (3.6%) | wound and burn, staff hand |
| A3 | CRO/CTX/C/E/AMP/TOB/AK | 4 (7.1%) | Cerebral spinal fluid, Ear secretions, wound and burn, Air condition outlet |
| A4 | CRO/CTX/C/E/AMP/CAZ/PI | 2 (3.6%) | Suction apparatus tubing, Urine |
| A5 | CRO/CTX/C/E/AMP/TOB/AK/CIP/AT/CAZ | 2 (3.6%) | wound and burn, Water tap |
| A6 | CRO/CTX/C/E/AMP/TOB/AK/CIP | 2 (3.6%) | Water tap, wound and burn |
| A7 | CRO/CTX/C/E/AMP/TOB/AK/CIP/PI | 1 (1.8%) | Sputum |
| A8 | CRO/CTX/C/E/AMP/TOB/AK/GEN/PI/CAZ/AT | 4 (7.1%) | Suction apparatus tubing, wound and burn (2), Sputum |
| A9 | CRO/CTX/C/E/AMP/TOB/AK/GEN/PI/CAZ/CIP/PIT/IPM | 1 (1.8%) | Sputum |
| A10 | CRO/CTX/C/E/AMP/PI/CIP/CAZ | 2 (3.6%) | wound and burn, Blood |
| A11 | CRO/CTX/C/E/AMP/TOB/CAZ/PI/GEN | 1 (1.8%) | Sputum |
| A12 | CRO/CTX/C/E/AMP/GEN/CAZ/AT | 2 (3.6%) | wound and burn, Endoscope |
| A13 | CRO/CTX/C/E/AMP/CIP/AK/TOB/GEN/PI/PIT | 1 (1.8%) | wound and burn |
| A14 | CRO/CTX/C/E/AMP/AK/TOB/GEN | 1 (1.8%) | Urine |
| A15 | CRO/CTX/C/E/AMP/CIP/AK/TOB/PIT/AT | 1 (1.8%) | Ear secretions |
| A16 | CRO/CTX/C/E/AMP/CAZ/PI/PIT/AT/TOB | 3 (5.4%) | wound and burn (2), Urine |

***Table 4:*** *Antimicrobial resistance patterns (ARP) of Pseudomonas aeruginosa strains.*
*AK: amikacin; AT: aztreonam; CAZ: ceftazidime; GEN: gentamicin; IPM: imipenem; PI: piperacillin; PIT: piperacillin/tazobactam; CIP: ciprofloxacin; CRO: ceftriaxone; AMP: ampicillin; CTX: cefotaxime; TOB: tobramycin; C: chloramphenicol; E: erythromycin.*

The result showed, there were epidemiological relationships between suction apparatus tubing (A1, A4 and A8), respirators (A1), staff hand (A1 and A2), endoscope (A1 and A12), water tap (A5 and A6), antiseptic solutions (A1) and air condition outlet (A3) and patients who had A1, A2, A3, A4, A5, A6, A8 and A12 antibiogram, however, there was direct link between isolates from staff hands (A1 and A2) and patients.

### Distribution of *Pseudomonas aeruginosa* serotypes

About (98.2%) isolates were typed and (1.78%) isolates were not typed by the 16 monovalent antisera. The correspondence nomenclature of the serotypes A, B, C, D, E, F, G, H, I, J, K, L, M and N with the International Antigenic Typing Scheme (IATS) serotypes designation.

The strength of the agglutination reaction after 1 min was recorded, the reactions with a strength (at least 25% of the antigen agglutinated) were considered positive. Serotype E (O : 11) was the most frequently with percentage (41.1%) of the isolates and serotypes C (O : 7/O : 8), D (O : 9) and M were not detected (Table 5).

| Serotype | A O :3 | B O:2/ O:5/O:16 | C O:7 / O:8 | D O:9 | E O:11 | F O:4 | G O:6 | H O:10 | I O:1 | J O:15 | K O:13/ O:14 | L O:12 | M | N | NT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Wounds and burns | 1 | 2 | - | - | 7 | 1 | 0 | 1 | 1 | 0 | 3 | 0 | - | 2 | 0 |
| Sputum | 0 | 1 | - | - | 5 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | - | 0 | 0 |
| Urine | 1 | 1 | - | - | 1 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | - | 0 | 0 |
| Throat swabs | 1 | 0 | - | - | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | - | 1 | 0 |
| Ear secretions | 0 | 1 | - | - | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | - | 0 | 0 |
| Cerebral spinal fluid | 0 | 0 | - | - | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 0 | 0 |
| Blood | 0 | 0 | - | - |  | 0 | 1 | 0 | 0 | 0 | 0 | 0 | - | 0 | 0 |
| Suction apparatus tubing | 0 | 0 | - | - | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | - | 0 | 1 |
| Endoscope | 0 | 0 | - | - | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | - | 0 | 0 |
| Respirators | 0 | 0 | - | - | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | - | 0 | 0 |
| Water tap | 1 | 0 | - | - |  | 0 | 0 | 0 | 0 | 0 | 0 | 1 | - | 0 | 0 |
| Air condition outlet | 0 | 0 | - | - | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 0 | 0 |
| Antiseptic solutions | 0 | 0 | - | - | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | 0 | 0 |
| Staff hand samples | 1 | 0 | - | - | - | 0 | 0 | 0 | 0 | 1 | 0 | 0 | - | 0 | 0 |
| Total | 5 | 5 | - | - | 23 | 5 | 3 | 2 | 2 | 3 | 3 | 1 | - | 3 | 0 |
| % | 8.9 | 8.9 | - | - | 41.1 | 8.9 | 5.4 | 3.6 | 3.6 | 5.4 | 5.4 | 1.8 | - | 5.4 | 1.8 |

***Table 5:*** *Distribution the serotypes of Pseudomonas aeruginosa strains in clinical, environment and staff hands samples. NT: non-typed*

Strains with serotype E (O : 11) were found in all patients and environment samples (except blood, water tap and staff hand samples) that indicated there was direct relationships between them, probably transmission of these stains by contamination during the use of medical instruments. Moreover, serotypes B, I, K and N were prevalent only in clinical isolates but serotypes A, F, G, H, J and L were distributed in both clinical and environmental isolates or staff hand samples.

### Relationship the virulence of *Pseudomonas aeruginosa* strains between different serotypes and antibiogram

Resistance pattern A1 (48.2%) was the more frequent which resistance to (CRO/CTX/C/E/AMP). The epidemic strains belonged to serotype E O11 (41.1%) (the most prevalence of strains) were distributed in 9 different ARPs (A1, A3, A4, A6, A7, A8, A9, A10 and A12), and demonstrated antimicrobial resistance varying from 5 to 13 antibiotics. There was a statistically significant difference between antibiogram and serotype in the rate of isolation of *P. aeruginosa* (*P*-value = 0.412) and ($\chi^2$ = 1.68) (Table 6).

| ARP no. | Antibiogram (ARP) | No. of strains (%) | Serotypes | $\chi^2$ | *P*-value |
|---|---|---|---|---|---|
| A1 | CRO/CTX/C/E/AMP | 27(48.2%) | E (12), A (2), H (2), I, F (3), B (3) N (2), J | 1.68 | 0.412 |
| A2 | CRO/CTX/C/E/AMP/CAZ | 2 (3.6%) | K, A | | |
| A3 | CRO/CTX/C/E/AMP/TOB/AK | 4 (7.1%) | E (3), B (1) | | |
| A4 | CRO/CTX/C/E/AMP/CAZ/PI | 2 (3.6%) | E (1), A (1) | | |
| A5 | CRO/CTX/C/E/AMP/TOB/AK/CIP/AT/CAZ | 2 (3.6%) | K, A | | |
| A6 | CRO/CTX/C/E/AMP/TOB/AK/CIP | 2 (3.6%) | L (1), E (1) | | |
| A7 | CRO/CTX/C/E/AMP/TOB/AK/CIP/PI | 1 (1.8%) | E | | |
| A8 | CRO/CTX/C/E/AMP/TOB/AK/GEN/PI/CAZ/AT | 4 (7.1%) | F (1), E (2), K (1) | | |
| A9 | CRO/CTX/C/E/AMP/TOB/AK/GEN/PI/CAZ/CIP/PIT/IPM | 1 (1.8%) | E | | |
| A10 | CRO/CTX/C/E/AMP/PI/CIP/CAZ | 2 (3.6%) | E (1), G (1) | | |
| A11 | CRO/CTX/C/E/AMP/TOB/CAZ/PI/GEN | 1 (1.8%) | J | | |
| A12 | CRO/CTX/C/E/AMP/GEN/CAZ/AT | 2 (3.6%) | E (1), G (1) | | |
| A13 | CRO/CTX/C/E/AMP/CIP/AK/TOB/GEN/PI/PIT | 1 (1.8%) | B | | |
| A14 | CRO/CTX/C/E/AMP/AK/TOB/GEN | 1 (1.8%) | F | | |
| A15 | CRO/CTX/C/E/AMP/CIP/AK/TOB/PIT/AT | 1 (1.8%) | J | | |
| A16 | CRO/CTX/C/E/AMP/CAZ/PI/PIT/AT/TOB | 3 (5.4%) | N (1), I (1), G (1) | | |

***Table 6:*** *Distribution of serotypes in relation to ARP of Pseudomonas aeruginosa strains.*
*ARP: Antimicrobial resistance pattern*

## Discussion

*Pseudomonas aeruginosa* is the most important causes of infection in hospitals worldwide, including Yemen. Previous studies performed in various cities of hospitals in many cities [17].

A total of 56 strains of *P. aeruginosa* were isolated from clinical specimens from patients and non-clinical samples, out of which (30%) strains were from clinical patient specimens. This is in agreement with rate reported by Mansour., *et al*. [18] in Egypt and Saudi Arabia (32.8%) and (30.0%) respectively.

On the other hand, the highest percentage of *P. aeruginosa* was from wounds and burns (60%) (*P*-Value = 0.000), similar results were obtained in Yemen [19].

The high rate of *P. aeruginosa* from wounds and burn this may be due to the ability of *P. aeruginosa* to colonize in damaged skin of burned patients, where the first defense barrier against bacteria is lost. In addition, *P. aeruginosa* and other bacteria penetrate the skin and cause systemic infections due to their toxins and enzymes that helped them to penetrate and invasive [20].

The analysis showed that the risk factors for *P. aeruginosa* infection were in male with *P. aeruginosa* (57.1%) (*P*-Value = 0.91), the age group (> 40 - 60) years (42.8%) (*P*-value = 0.51), this is in agreement with rates previous studies [10,21], and surgery disease (47.6%) (*P*-values = 0.31) [21].

In this study, the percentage of hospital environment samples were 26.7% strains and 13.3% (*P*-Value = 0.19) of strains belonged staff hand samples. Similar results were obtained in Egypt and Saudi Arabia [18] that shown the rate of isolation from environment samples were 25.0% and 23.3% respectively, and from staff hand samples were 10.0% and 6.7 %, respectively. The isolation rate of *P. aeruginosa* from environmental samples was 26.7%, which is slightly higher than a previous study in Egypt (19.5%) by Gad., *et al.* [22]. The higher rate of isolation from staff hands could be due to lack of compliance of health care workers to hand-washing practices.

The highest percentage of *P. aeruginosa* isolates was (50%) (*P*-Value = 0.16) from suction apparatus tubing, this may be explained by the failure of sterilization of suction apparatus tubing, this is similarly that found in Egypt and Saudi Arabia (57.1%) and (42.9%), respectively [18]. However, these findings are different from previous study in which the number of suction tubing infection was lower than other infection [3].

In this work, the highest incidence of *P. aeruginosa* (47.5%) (*P*-value = 0.002) was found in ICU, this connection by result found by Lu., *et al*. [23]. According to the National Nosocomial Infection Surveillance, *P. aeruginosa* dominated in intensive care units among patients with hospital-acquired pneumonia [24]. The source of *P. aeruginosa* in ICUs can be either endogenous or exogenous; isolates that have unique genotypes are considered as possibly endogenous while those of the same genotype with either patient or environmental samples are considered as possibly exogenous [25]. Ventilator-associated pneumonia (VAP) caused by *P. aeruginosa* has the poorest outcome of all ICU infections. Overall mortality due to *P. aeruginosa* has been shown to be as high as 70% [26], and directly attributable mortality rates are approximately 40% [23].

The highest resistance was recorded to ceftriaxone, ampicillin, cefotaxime and chloramphenicol (100%), and the most effective antibiotic used was Imipenem (94.6%) [27]. However, these findings are different from previous study carried out by Adeyemi., *et al.* [28]. The differences in antibiotic susceptibility in different regions could be attributed to the differences in patient population, the duration of hospitalization, cross-infection, and the dose and types of antibiotics [29]. *Pseudomonas aeruginosa* strains isolated from intensive care units (ICUs) tend to show higher resistance [30].

As regards screening for MBLs production in imipenem-resistant strains, 5.4% were found to be MBL producers, which is similar to studies conducted by Mendiratta., *et al*. [31].

The 16 different ARPs observed in the strains isolated in this study presented resistance to antimicrobials characterizing the analyzed strains as multi-drug resistant, similarly to Loureiro., *et al*. [32].

*P. aeruginosa* isolated from environmental sites and staff hands in our study, had direct epidemiological relationships with patients who had the same antibiogram [18]. Among the strains tested, 98.2% isolates were typed and 1.78% isolates were not typed by the 16 monovalent antisera. Serotype E (O : 11) was the most prevalent serotype (41.1%) isolates and serotypes C, D, and M (O : 7/O : 8, O : 9 and Serotype M) were not detected [32].

The strains with serotype E (O : 11) were found in all patients and environment samples, that indicated transmission of these stains by contamination during the use of medical instruments, probably from horizontal transmission from patient to patient or from the hands

of health care workers. Based on these findings, that concluded the environment was an important source of *P. aeruginosa* infections in these three hospitals.

In our study, the distribution of serotypes according to antimicrobial resistance profile (ARP) showed the epidemic strains belonged to serotype E (O : 11) were distributed in 9 different ARPs, and demonstrated antimicrobial resistance varying from 5 to 13 antibiotics. This fact reflects the importance of controlling the emergence of strains with this serotype in participating hospitals. The significant difference between antibiogram and serotype was *P*-value = 0.412 and $\chi^2$ = 1.68. Loureiro., *et al*. [33] reported that the strains belonged to serotype O:11 were distributed in 14 different ARPs.

Multidrug-resistant strains were prevalent among serotype O:11 and the nontypeable strains [10]. Multidrug resistance is a common feature of many serotypes of *P. aeruginosa*, particularly serotype O:11 [1]. The prevalence of *P. aeruginosa* serotype varies from one hospital to another and from one country to another, O6 and O11 are often the most prevalent serotypes reported in previous studies [34].

In this connection, several authors related that *P. aeruginosa* serotype O11 has been recognized as an important hospital problem in recent years, principally in epidemic situations, because this microorganism presents multi-drug resistance with different resistance phenotypes [35].

It should be noted that the predominant serotype (O : 11) showed the highest number of MDR strains [10]. The high frequency of drug-resistant strains within this serotype has been observed by other investigators [36]. A significant decline in susceptibility of *P. aeruginosa* to β-lactams, aminoglycosides, and quinolones has been observed in many countries, including the United States [37]. It appears that the frequency and rate of resistance to individual antibiotics are different in different regions [38].

## Conclusion

The frequent phenotypic by antibiogram typing changes could complicate the epidemiological studies of *P. aeruginosa* compared with serotype that more stability. Therefore, serotype method is a more precise to antibiogram and can increase the efficiency of infection control procedures by determining the patient sources of infections and the environmental sources to prevent or reduce the spread of *P. aeruginosa* infections.

## Conflict of Interest

The authors have not declared any conflict of interest.

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