Role of *Pseudomonas aeruginosa* in Nosocomial Infection

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Abstract: P.aeruginosa (Pseudomonas aeruginosa) **is the most common pathogen isolated from patients who have been hospitalized longer than 1 week. The relative frequency of** *P. aeruginosa* **as a nosocomial pathogen has increased.** *P.aeruginosa* **can cause multi-systemic infections. Our study aims to assess number of nosocomial infection with** *P. aeruginosa***, and its prevention and control measures.**

Methods: **A set of 100 microbiological samples collected from different patients in three different hospitals suspected with nosocomial infection. Mechanism of action to detect the prevalence of P. aeruginosa studied by using: Culturing, staining, microscopy, Biochemical reactions, enzymatic test, and fermentation test.**

Results: **The total positive cases of the infection occurred in 7 cases, the positive nosocomial infection rate was 19.62%. The results have been shown in the three hospitals where the hospital that has a protocol to deal with infection and prevention will be much lower than its peers and this is positive to reduce the incidence of bacteria causing many diseases.**

Conclusions: **Our study showed that following the protocol of infectious diseases in the three hospitals had a positive effect on the spread of** *P. aeruginosa* **as the infection rate is less than 20% in those hospitals, knowing that they are also in different places.**

Keywords: Pseudomonas aeruginosa, **Infection control, Nosocomial infection.**

1. INTRODUCTION

Throughout the 20th century, different types of bacteria that live almost everywhere are harmful and others can be harmless. *Pseudomonas aeruginosa* has become an important cause of aerobic gram-negative infection, especially in patients with compromised host defense mechanisms. [1] P. aeruginosa, first isolated in 1882 by Gessard from green pus. The ubiquitous life-style of P. aeruginosa allows this bacterium to contribute to frequent infections in humans. It is a highly adaptable bacterium, with soil being the primary habitat; however, *P. aeruginosa* also survives in aquatic environments. [2] It is the most common pathogen isolated from patients who have been hospitalized longer than 1 week, and it is a frequent cause of nosocomial infections. *Pseudomonal* infections are complicated and can be life-threatening. It spread through improper hygiene, such as from the unclean hands of healthcare workers or via contaminated medical equipment that wasn't fully sterilized.[3] The bacterium is a difficult organism to eradicate from areas that become contaminated, such as operating rooms, hospital rooms, clinics, and medical equipment.[4] In recent years nosocomial infections caused by *P. aeruginosa* have been recognized as an acute problem in hospitals due to its intrinsic resistance to many antibiotic classes and its capacity to acquire practical resistance to all effective antibiotics.[5] the analysis of 24,179 adults with nosocomial bloodstream infections in the United States from 1995 to 2002, *P. aeruginosa* accounted for 4% of cases, and was the third leading cause of gram-negative infection.[6]

To prevent spreading *Pseudomonas* infections between patients, healthcare personnel must follow specific infection control precautions. These precautions may include strict adherence to hand hygiene and wearing gowns and gloves when they enter rooms where patients infected with *Pseudomonas* are staying. Healthcare facilities must also follow proper cleaning procedures to prevent the spread of *Pseudomonas*.

This study aims to assess number of nosocomial infection with *P. aeruginosa*, and its prevention and control measures in the hospitals.

2. METHODOLOGY

A set of 100 microbiological samples collected randomly from different patients both males and females in three different hospitals for those who admitted in the hospital more than 1 week. We applied the Culturing, staining, microscopy, Biochemical reactions, enzymatic test, and fermentation test for each sample that taken from the patients as follow:

- **Culturing technique:** we used a streak culture technique.

1. Sterilize the inoculating loop in the bunsen burner by putting the loop into the flame until it is red hot. Allow it to cool.

2. Pick an isolated colony from the agar plate culture and spread it over the first quadrant (approximately 1/4 of the plate) using close parallel streaks or Insert your loop into the tube/culture bottle and remove some inoculum. You don't need a huge chunk.

3. Immediately streak the inoculating loop very gently over a quarter of the plate using a back and forth motion.

4. Flame the loop again and allow it to cool. Going back to the edge of area 1 that you just streaked, extend the streaks into the second quarter of the plate.

5. Flame the loop again and allow it to cool. Going back to the area that you just streaked (area 2), extend the streaks into the third quarter of the plate.

6. Flame the loop again and allow it to cool. Going back to the area that you just streaked (area 3), extend the streaks into the center fourth of the plate.

7. Flame your loop once more.

- **Gram staining:** first of all we prepared the slide smear by, Fixing material on slide with heat. After the slide is heat fixed, allow it to cool to the touch before applying stain, After that:

1. Flood air-dried, heat-fixed smear of cells for 1 minute with crystal violetstaining reagent.

2. Wash slide in a gentle and indirect stream of tap water for 2 seconds.

3. Flood slide with the mordant: Gram's iodine. Wait 1 minute.

4. Wash slide in a gentle and indirect stream of tap water for 2 seconds.

5. Flood slide with decolorizing agent (Acetone-alcohol decolorizer). Wait 10-15 seconds or add drop by drop to slide until decolorizing agent running from the slide runs clear.

6. Flood slide with counterstain, safranin. Wait 30 seconds to 1 minute.

7. Wash slide in a gentile and indirect stream of tap water until no color appears in the effluent and then blot dry with absorbent paper.

8. Observe the results of the staining procedure under oil immersion (100x) using a Bright field microscope.

- **Biochemical reactions:**

- Urease test →

1. The broth medium is inoculated with a loopful of a pure culture of the test organism; the surface of the agar slant is streaked with the test organism.

2. Leave the cap on loosely and incubate the test tube at 35 °C in ambient air for 18 to 24 hours; unless specified for longer incubation.

- MR-VP test → MR-VP broth is used for both MR Test and VP test. Only the addition of reagent differs, and both tests are carried out consecutively.

1. Inoculate two tubes containing MR-VP Broth with a pure culture of the microorganisms under investigation.

2. Incubate at 35 °C for up to 4 days.

3. Add about 5 drops of the methyl red indicator solution to the first tube (for Voges-Proskauer test, Barrit's reagent is added to another tube).

4. A positive reaction is indicated, if the colour of the medium changes to red within a few minutes.

- Citrate utilization test →

1. Inoculate simmons citrate agar lightly on the slant by touching the tip of a needle to a colony that is 18 to 24 hours old.

2. Incubate at 35°C to 37°C for 18 to 24 hours.

3. Observe the development of blue color; denoting alkalinization.

- Indole test →

1. Inoculate the tryptophan broth with broth culture or emulsify isolated colony of the test organism in tryptophan broth.

2. Incubate at 37°C for 24-28 hours in ambient air.

3. Add 0.5 ml of Kovac's reagent to the broth culture.

- **Enzymatic test:**

- Oxidase test →

1. We took a sterile cotton stick soaked with the substrate tetramethyl-p-phenylenediamine dihydrochloride.

2. Moisten the stick with a sterile distilled water.

3. Observe inoculated area of cotton for a color change to deep blue or purple within 10-30 seconds

- **Fermentation test:**

1. Prepared broth media by mixing all ingredients in 1000 mL of distilled/deionized water and heating gently to dissolve it.

2. Filled 13 x 100 mm test tubes with 4-5 ml of phenol red carbohydrate broth.

3. Insert a Durham tube to detect gas production.

4. Autoclave the prepared test media (at 121°C for 15 minutes) to sterilize. The sterilization process will also drive the broth into the inverted Durham tube.

After that, we compared our results with what the hospital is doing according to its guidelines to prevent the spread of infection.

3. RESULTS

100 samples were collected by sterile cotton swab. A sample from each patient in the three hospitals and for each sample we applied the culturing by using (MacConkey agar, Blood agar, and CLED agar), gram staining, biochemical reactions involving (Urease test, MR-VP test, Citrate utilization test, and Indole test), enzymatic tests including (Oxidase test), and Sugar fermentation test by using (Glucose, Maltose, Lactose, and Mannitol) for each sample separately.

Through our observation to the result We found some of the samples we had tested Were positive and others negative and included the presence of other types of bacteria such as streptococcus pyogenes.

The total positive cases of the infection occurred in 7 cases, the positive nosocomial infection rate was 19.62%. Of these, 40 from hospital (A), 33 from hospital (B), and 27 from hospital (C). 3 (7.5%) of 40 patients from hospital (A) and 4 (12.12%) of 33 patients from hospital (B) were detected with positive cultures of *P. aeruginosa,* also the total 27 patients in hospital (C) were clear (Table 1). We noticed that the infection rate with *P. aeruginosa* in women are more than males.

Table 1:

	Pseudomonas aeruginosa	**Another organism**	**Non**	**Total**
Male	2	7	48	57
Female	5	4	34	43
Total	7	11	82	100

The positive results we have found based on our tests are as follows:

➢ *Culturing method showed:*

- MacConkey agar ➔ non-lactose fermentation (Image 1).

- CLED agar ➔ green colonies with typical matted surface and rough periphery (Image 2).
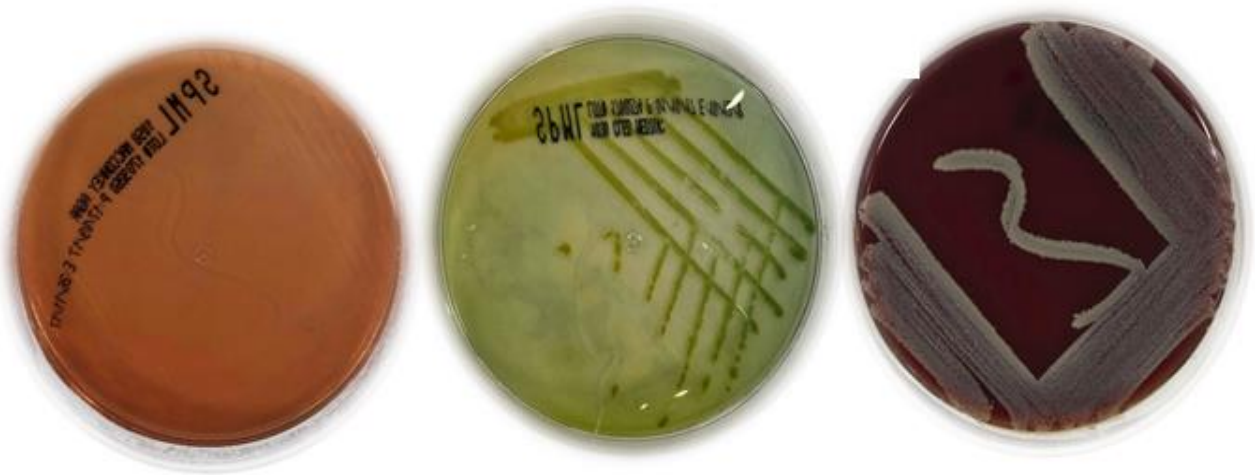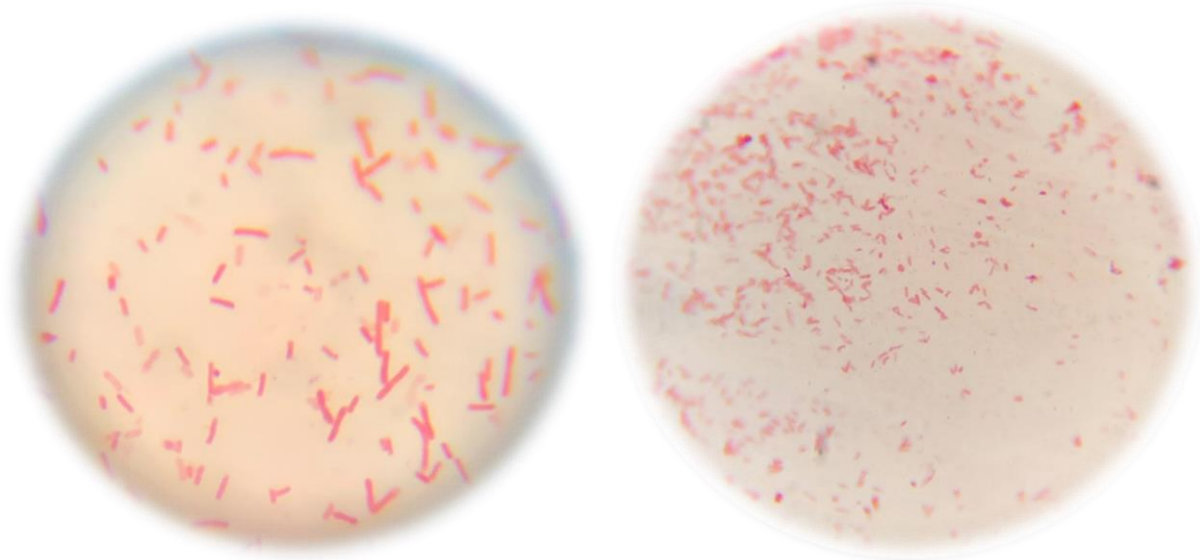
- Blood agar ➔ beta-hemolysis (Image 3).

Image 1: MacConkey agar shows NLF.   **Image 2: CLED shows green colonies**   **Image 3: Blood agar shows B-hemolysis.**

➢ *Gram staining Showed:*

- Slim, Gram-negative rods (Image 4.1 – 4.2).

A photomicrograph of Pseudomonas aeruginosa. 4.1.   **A photomicrograph of Pseudomonas aeruginosa. 4.2.**

Table 2:

Organism	Biochemical reactions				
	Urease	MR	VP	Citrate	Indole
Pseudomonas aeruginosa	_	_		+	_

➤ *Biochemical reactions showed:* (Table 2 – Image 5)*.*

Image 5: Biochemical reactions showed all the tests are negative except citrate utilization is positive.

➤ *Enzymatic test:*

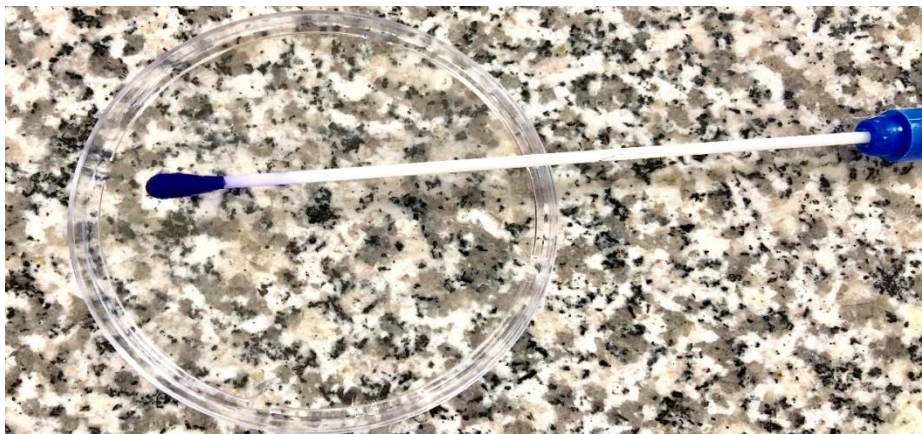- Oxidase test → positive oxidase test (Image 6).

Image 6: Oxidase test positive for *P. aeruginosa*.

Table 3:

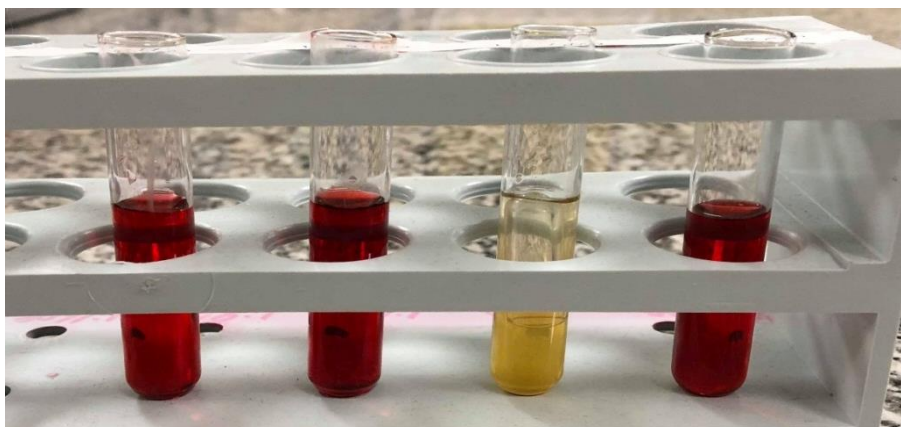| Organism	Fermentation of			
	Glucose	Lactose	Mannitol	Maltose
Pseudomonas aeruginosa	_	_	+	_

➤ *Sugar fermentation*: (Table 3)

Image 7: Sugar fermentation test of Mannitol is positive (yellow color) for *P. aeruginosa*.

4. DISCUSSION

A total of 2,241 P. aeruginosa isolates were evaluated between 2012 and 2017. Sensitivity and resistance of the isolates to the antipseudomonal antimicrobials colistin and tigecycline were stable. The sensitivity and resistance to other antipseudomonal antimicrobials improved after 2014, after the AMS and ICPs were implemented in 2013. The use of alcohol-based hand gel significantly increased from 0.6 to 10.9 L per 1,000 patient-days (PD) during the study period (P=0.005). The incidence rates of extensively drug-resistant (XDR) and MDR P. aeruginosa showed a sustained decrease from 2013 (4.9 and 22%) to 2017 (1 and 15%), respectively. The yearly consumption of antimicrobial agents also showed a sustained and significant decrease from 45 defined daily doses (DDDs) per 1,000 PD to 38.15 DDDs per 1,000 PD (P=0.04). A significant correlation was found between the incidence rate of MDR P. aeruginosa and the consumption of antimicrobial agents (P=0.01). Monitoring of P. aeruginosa, AMS, and comprehensive ICPs could be one of the best and effective methods to prevent the development of resistance in P. aeruginosa.[7]

During the active surveillance program, enhanced infection control measures led to a steeper downwards trend in incidence, prevalence, and colonization pressure for CRGNB compared to the infection control measures sub-period. The linear trend was for carbapenem-resistant Klebsiella pneumoniae (CRKP) and Pseudomonas aeruginosa (CRPA) infections to decrease from 19.6 to 8.1 infections per 1000 bed-days (IBD) (P = 0.001) and from 5.1 to 1.79 IBD (P = 0.043), respectively. By contrast, carbapenem-resistant Acinetobacter baumannii infections increased from 5.2 to 15.3 IBD (P = 0.001). Enhanced infection control measures including enhanced hand hygiene, active surveillance combined with contact precautions, education, audits and feedback policies and interventions could reduce CRKP and CRPA in endemic areas.[8]

P. aeruginosa continues to be a serious problem worldwide as a cause of respiratory tract infections in selected populations. Microbiologic diagnosis remains difficult and plagued with pitfalls. The application of modern PK/PD concepts should help to optimize antibiotic therapy of this increasingly difficult to treat infection, particularly at the respiratory tract level and with an increasing prevalence of resistance to all antipseudomonal agents. Inhaled antibiotics, particularly tobramycin, are increasingly used for the prevention and treatment of P. aeruginosa infection in CF patients.[9]

P aeruginosa infections in our hospital mainly affected the patients hospitalized in intensive care units and those having catheterization, burn, and/or chronic illness. Amikacin was the best antibiotic as far as bacterial resistance was considered. Although lack of major PFGE type confirmed no P aeruginosa outbreak, typing results showed that cross transmission and treatment failure are the 2 main problems, which should be consider together to prevent this bacterial infection in medical centers.[10]

A strain of P. aeruginosa was isolated from a culture obtained from the suture, and the patient was therefore diagnosed with suture-related conjunctivitis caused by P. aeruginosa. The conjunctivitis was cured by the application of an antimicrobial ophthalmic solution and removal of the suture. We used PFGE to survey of the indoor and outdoor environments around the patient's house and office in order to elucidate the route of transmission of the infection. Three strains of P. aeruginosa were isolated from the patient's indoor environment, and the isolate obtained from the patient's bathroom was identical to that from the suture. The case highlights the fact that an indoor environmental strain of P. aeruginosa can cause ocular infections.[11]

Image 8

Our study indicates that the number of *P. aeruginosa* cases spread depends on the environment surrounding the patient, hospital staff, water, etc. The results have been shown in the three hospitals where the hospital that has a protocol to deal with infection and prevention will be much lower than its peers and this is positive to reduce the incidence of bacteria causing many diseases. But what about those hospitals that do not follow the protocol? I think it will get worse and the injury rates will be greater, and this will cost both effort and money.
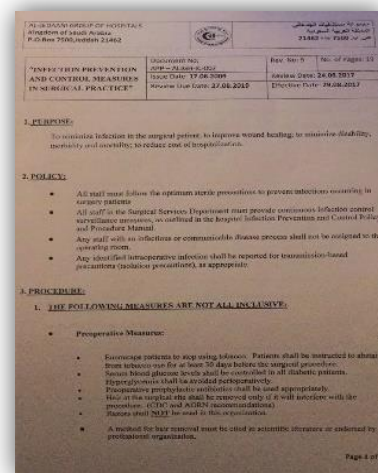
5. CONCLUSION

Our study showed that following the protocol of infectious diseases (Image 8) in the three hospitals had a positive effect on the spread of *P. aeruginosa* as the infection rate is less than 20% in those hospitals, knowing that they are also in different places.

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