

ORIGINAL ARTICLE

Antimicrobial Susceptibility Patterns of Nosocomial *Pseudomonas aeruginosa* Strains Isolated from Clinical Specimens in Tanta University Hospitals

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ABSTRACT

Key words:

P. aeruginosa, Antibiotic susceptibility, Multi-drug resistance

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Background: *Pseudomonas aeruginosa* is an ubiquitous microbe capable of infecting practically any tissues. Pulmonary tract, skin and blood stream infections with multi-drug resistant (MDR) *P. aeruginosa* represent major causes of morbidity and mortality especially in immunocompromised patients e.g., patients with cystic fibrosis. **Objective:** To detect the susceptibility pattern of nosocomial *P. aeruginosa* isolates collected from different clinical specimens in Tanta University Hospitals. **Methodology:** Thirty isolates of *P. aeruginosa* were collected (10 from respiratory tract infection samples, 10 from burn samples and 10 from blood stream infections). Samples were introduced to macroscopic, microscopic, bacteriological examination and susceptibility testing using disc diffusion method. **Results:** There was no significant difference detected between isolates of *P. aeruginosa* in relation to gender or age, while there was a significant antibiotic resistance against the used antibiotics in blood specimens more than respiratory and burn specimens. **Conclusion:** Significant detection of MDR *P. aeruginosa* isolates ensures the importance of controlling antibiotic misuse and self-prescriptions.

INTRODUCTION

Hospital-acquired infections (HAIs) also known as healthcare associated infections (HCAIs) are nosocomial acquired infections that are typically not present at the time of the patient admission¹.

The danger of HAI varies in relation to the immune status of patients, implementation of infection control planes, and the presence of the various pathogens in the local community. From the common risk factors for HAIs are older age, immunosuppression, much stay in hospital and presence of underlying chronic illnesses, presence of healthcare facilities, usage of invasive procedures, mechanical ventilator support, indwelling devices and need for a critical care unit with high risk of HAIs².

Multidrug-resistant organisms (MDROs) are also important causes of infections in the hospitals, mainly in the ICU, with a propensity to much time of stay and mortality. They are defined as giving resistance to minimum two antibiotics from each antibiotic class or mechanism of action³.

The genus *Pseudomonas* contains more than 140 species, most of which are saprophytic. Above 25 species are able to cause infection in humans. Most species of *Pseudomonadaceae* that known to cause disease in humans cause opportunistic infections. These are *P. aeruginosa*, *P. fluorescens*, *P. cepacia*, *P. stutzeri*, *P. putida*, *P. maltophilia*, and *P. putrefaciens*⁴.

Pseudomonas aeruginosa is the most important. It is an ubiquitous bacterium as it is found much more in moist environments. Although it difficulty causes disease in healthy individuals, it is a major risk to hospitalized patients, especially those having serious underlying diseases such as cancer and burns⁵. It is a Gram-negative bacilli measuring 0.5 to 0.8 um by 1.5 to 3.0 um. Almost all strains are motile having means of a single polar flagellum⁶.

Pseudomonas aeruginosa metabolism is respiratory and never fermentative. It can grow in moist environments as it is naturally present in soil and water. It also presents in hospital environments (sinks, showers, respiratory equipment), rarely part of normal flora of healthy humans^{7,8}.

There are many factors for *P. aeruginosa* virulence. Several of these virulence factors may cause pathogenicity that increases adhesion and/or affect host cell signaling pathways that targeting the extracellular matrix. These virulence factors are lipopolysaccharides, flagellum, pili, Exotoxin A, proteases, alginate, quorum sensing, biofilm formation, type VI secretion systems and oxidant generation in the airspace⁹.

Getting rid of *P. aeruginosa* increasingly becomes hard due to its ability to overcome on antibiotics. Strains of *P. aeruginosa* utilize the intrinsic and acquired mechanisms to resist most antibiotics. In addition, adaptive mechanism of resistance is a new mechanism, which depends on biofilm formation and production of

multidrug tolerant cells. It is also responsible for recurrence of infections¹⁰.

In this work, we aimed to find the relation between site of infection and susceptibility pattern of nosocomial isolates of *Pseudomonas aeruginosa* isolated from various clinical specimens in Tanta University Hospitals.

METHODOLOGY

Subjects:

The research was done on 30 isolates of *P. aeruginosa*, collected from patients attending Tanta University Hospital. These were 10 isolates from respiratory tract infections, 10 from burn infection and 10 from blood stream infection. The study started from January 2020 to January 2021 and it was performed in the Department of Medical Microbiology and Immunology, Faculty of Medicine, Tanta University. All included patients had risk factors for *P. aeruginosa* infection as those on ventilator, burn, cancers or admitted for ICUs. Ethical committee of Faculty of Medicine, Tanta University approved the research plan.

Specimen collection and processing:

Different samples were aseptically collected, then transported directly to the Medical Microbiology and Immunology Department, Faculty of Medicine, Tanta University where they were subjected for routine microbiological protocol for detecting microbes.

Culture:

Respiratory and burn samples were cultured directly on nutrient, blood and MacConkey's agar plates by streak plate method that were incubated aerobically for 24 hrs at 37°C. The blood culture bottles were incubated for several days for allowing the organisms to multiply. If any growth such as turbidity, hemolysis and colonies over sediment RBC's was seen, a Gram's stain was done from the culture bottle for detecting those organisms and provided primary information about their identity.

Macroscopic examination of the colonies' morphology:

On nutrient agar plate, it was characterized by characteristically producing greenish pigment that diffused through the medium. It was also identified by its characteristic odor (fruity-grape like). On MacConkey's agar plates, it appeared as pale non lactose fermenter colonies. It appeared as large colonies with metallic sheen, mucoid, rough or pigmented (pyocyanin) and often β -hemolytic on blood agar medium.

Microscopic examination:

Gram-stained films from pure separated colonies were examined microscopically under LM using the oil immersion lens and showed Gram-negative bacilli of variable size, rod-shaped, non-sporing and non-capsulated.

Biochemical reactions:

Biochemical tests including Triple sugar iron (TSI) agar, sugar fermentation test, glucose oxidation test, oxidase strips, Simmons citrate agar, Urea agar, Motility Indole Ornithine (MIO) medium, Lysine iron decarboxylase agar and Hydrogen peroxide. The isolates of *P. aeruginosa* showed positive motility, negative ornithine decarboxylation, negative indole on MIO medium, alkaline butt, alkaline slant with no gas production at the tube of TSI agar, negative urease, positive citrate, negative LDC and positive oxidase test.

Conventional manual antibiotic susceptibility testing of *Pseudomonas aeruginosa* through disk diffusion method: Depending on the criteria set by the Clinical and Laboratory Standards Institute (CLSI)¹¹.

RESULTS

Table 1 showed no significant difference between *P. aeruginosa* isolates in relation to gender or age of the cases.

Table 1: Distribution of the studied cases according to their demographic data in each specimen type

| Demographic data | Type of specimen | | | | | | Test of sig. | P |
|------------------|-----------------------------|------|-------------------|------|------------------|------|----------------|----------------|
| | Respiratory specimen (n=10) | | Pus (n=10) | | Blood (n=10) | | | |
| | No. | % | No. | % | No. | % | | |
| Gender | | | | | | | | |
| Male | 6 | 60.0 | 4 | 40.0 | 9 | 90.0 | $\chi^2=5.376$ | $^{MC}p=0.088$ |
| Female | 4 | 40.0 | 6 | 60.0 | 1 | 10.0 | | |
| Age (years) | | | | | | | | |
| Range | 38.0 – 81.0 | | 39.0 – 61.0 | | 39.0 – 56.0 | | F=0.941 | 0.403 |
| Mean \pm SD. | 53.20 \pm 12.80 | | 48.10 \pm 7.39 | | 49.0 \pm 4.22 | | | |
| Median (IQR) | 51.50 (44.0–59.0) | | 46.50 (42.0–52.0) | | 50.0 (48.0–50.0) | | | |

Comparison between specimens according to their antimicrobial susceptibility pattern showed that Ceftazidime (CRO) was significantly resistant to blood and respiratory specimens more than pus specimens. Also, Levofloxacin (LEV) was significantly resistant in blood and respiratory specimens. Amoxicillin clavulanic acid (AMC) showed significant resistance in blood and

pus specimens more than respiratory specimens. Tobramycin (TOB) also faced significant resistance in respiratory specimens more than blood and pus specimens. Imipenem showed significant resistance in all specimen types. Blood specimens were more resistant to the used antibiotics more than respiratory specimens and pus which shown in table 2.

Table 2: Distribution of studied cases according to their antibiotic susceptibility pattern

| Antibiotic susceptibility pattern | Type of specimen | | | | | | | | | P ₁ | P ₂ | P ₃ |
|-----------------------------------|-----------------------------|----|----|------------|----|----|--------------|----|----|----------------|----------------|----------------|
| | Respiratory specimen (n=10) | | | Pus (n=10) | | | Blood (n=10) | | | | | |
| | S | I | R | S | I | R | S | I | R | | | |
| Aztreonam (ATM) | 4 | 3 | 3 | 4 | 2 | 4 | 5 | 2 | 3 | FE p=1.000 | FE p=1.000 | FE p=1.000 |
| Tazobactam (TZP) | 1 | 8 | 1 | 4 | 2 | 4 | 2 | 6 | 2 | FE p=0.303 | FE p=1.000 | FE p=0.628 |
| Piperacillin (PRL) | 2 | 6 | 2 | 2 | 2 | 6 | 1 | 8 | 1 | FE p=0.170 | FE p=1.000 | FE p=0.057 |
| Azithromycin (AZT) | 2 | 7 | 1 | 6 | 4 | 0 | 5 | 1 | 4 | FE p=1.000 | FE p=0.303 | FE p=0.087 |
| Ciprofloxacin (CFP) | 3 | 0 | 7 | 7 | 0 | 3 | 6 | 2 | 2 | 0.074 | FE p=0.070 | FE p=1.000 |
| Ceftazidime (CRO) | 4 | 0 | 6 | 6 | 3 | 1 | 0 | 3 | 7 | FE p=0.057 | FE p=1.000 | FE p=0.020* |
| Levofloxacin (LEV) | 1 | 2 | 7 | 5 | 4 | 1 | 1 | 0 | 9 | FE p=0.020* | FE p=0.582 | <0.001* |
| Imipenem (IPM) | 1 | 0 | 9 | 2 | 0 | 8 | 4 | 1 | 5 | FE p=1.000 | FE p=0.141 | FE p=0.350 |
| Amoxicillin clavulanic acid (AMC) | 7 | 2 | 1 | 1 | 3 | 6 | 0 | 0 | 10 | FE p=0.057 | FE p<0.001* | FE p=0.087 |
| Tobramycin (TOB) | 0 | 1 | 9 | 1 | 5 | 4 | 6 | 2 | 2 | FE p=0.057 | FE p=0.005* | FE p=0.628 |
| Amikacin (AK) | 4 | 5 | 1 | 6 | 3 | 1 | 1 | 3 | 6 | FE p=1.000 | FE p=0.057 | FE p=0.057 |
| Total (n=110) | 29 | 34 | 47 | 44 | 28 | 38 | 31 | 28 | 51 | 0.213 | 0.587 | 0.074 |

DISCUSSION

Getting rid of *P. aeruginosa* infections has become harder due to its high ability to resist different antibiotics. MDR strains of *P. aeruginosa* have high standard of intrinsic and acquired mechanisms of resistance to resist much antibiotics.

Our results showed that no significant difference between *P. aeruginosa* isolates in correlation with gender or age was found. Similar to our results, Khattab et al.,¹² reported no significant difference in *P. aeruginosa* isolates according to gender or age. They explained that by infection caused by *P. aeruginosa* is more prevalent in immunocompromised and hospitalized patients regardless age or gender factors. In addition, Abdelrahman and Ahmed¹³ found the same results and reported that *P. aeruginosa* prevalence depends on other various factors like nature of geographical locations, degree of contamination, immune status of patients, virulence of strains and degree of implementation of measures of infection control in hospitals. On contrary to our results, Chand et al.,¹⁴ found that the highest prevalence of *P. aeruginosa* isolates was found in male patients with substantial number of these isolates found in age group 60–75. The

possible reasons for this might be having relation to the sample of studied population as their study included community acquired infections (CAI) from different geographical locations, where males have routine outdoor work making them more exposed to infection, while the higher age group is explained by being frequently applicable for infection from the physical surroundings due to weaker immune system.

On comparing the susceptibility pattern of *P. aeruginosa* collected from different specimens, there was significant variance in antibiotic susceptibility results from one isolate to another. This may be explained by difference in types of specimens, possible different serotypes of *P. aeruginosa*, different biofilm formation ability, selective pressure of certain antibiotics used for course of treatment in different departments and virulence factors related to different infection sites^{15,16,17,18,19}. The results were that ceftazidime and levofloxacin faced significant resistance in blood and respiratory specimens more than pus specimens. Also, amoxicillin clavulanic acid was significantly resistant in blood specimens more than respiratory and pus specimens while imipenem showed significant resistance in all specimen types. In general, *P. aeruginosa* obtained from blood specimens was more resistant to the used antibiotics. It may be illustrated by

the blood stream infections are the severest forms of infection usually accompanied by very weak immune status and highly virulent strains. While the least significant resistance seen in wound (pus) specimens could be explained by the usual use of topical antibiotics for treatment of burn infections rather than systemic ones rendering them less exposed to resistant strains.

In agreement with our results, Ameen et al.²⁰ revealed the presence of high degree of resistance against imipenem and explained it by gene mutations in conjunction in addition to production of enzymes like AmpC or acquisition of new resistance genes caused this high resistance against strong beta-lactam drug like imipenem. This was also supported by the study done by Gierhart and Chukwuma²¹ in which all strains exhibited significant resistance to imipenem. Moreover, Palavutitotai et al.²², reported the presence of high degree of resistance in *P. aeruginosa* isolates and recommended colistin as the only active agent for combating infections caused by what they called extensively drug resistant *Pseudomonas aeruginosa* (XDR-PA) and they also recommended treatment of the infections resulted by XDR pathogens by combining several antibiotics with colistin to exert a synergistic effect or to decrease the possible toxicities related to higher doses of each drug according to a well-established antibiotic policy. Also, Kireççi and Kareem²³ reported that most of their isolates had significant susceptibility to imipenem and meropenem. They explained their results by different sample type, which was from CAI as that faced significant resistance to imipenem was significantly more in strains from NI than strains collected from CAI, because this antimicrobial agent is used mostly in treatment of NI. Hamed et al.,²⁴ found that antibiotic resistance was very high in the urine specimens and very high in males more than females.

However, we can always explain the high antibiotic resistance that was seen in various isolates to different antimicrobial antibiotics by the random misuse of antibiotics leading to production of various types of enzymes like carbapenamase, ampCillin-lactamases and quorum sensing modification of different target site. Furthermore, the main cause of the prevalence of MDR is prescribing the antibiotics without performing routine susceptibility test, which may be due to lack of laboratory facilities in the most of the healthcare centers especially in less developed countries.

Several surveys from developed and developing countries confirmed the direct relation between the irrational antibiotics use and the spread of resistant strains. To reduce this problem, it is very useful to implement proper strategy for infection control for example, good hygiene for hand and judicious use of antimicrobial antibiotics^{25,26,27,28}.

CONCLUSION

Infection by *Pseudomonas aeruginosa* is a major health hazard, especially in immune-deficient patients. It has variety of virulence factors that help to its pathogenesis and multi-drug resistance. Significant detection of multi-drug resistance between isolates of *P. aeruginosa* ensures the important role of controlling antibiotic misuse and self-prescriptions.

Recommendations

Future searches should be recommended about target different resistance mechanisms and genetic diversity of strains of *P. aeruginosa* to know more about *P. aeruginosa* virulence. We also recommend routine performance of antimicrobial sensitivity testing before prescribing antibiotics in order to reduce the possibility of high spread of MDR strains. For reducing the danger of spread of multi-drug resistant strains, we recommend to increase awareness among physicians and public about the rational antibiotics usage.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

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