**ORIGINAL ARTICLE**

# Evaluation of Multi-Drug Resistance in Biofilm Forming *Pseudomonas Aeruginosa* Isolated from Urinary Catheter Tips in Patients from ICU and CCU in DHQ Teaching Hospital Gujranwala

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## ABSTRACT

**Objectives:** The objectives of this study are to identify the isolates by means of different identification tests, to assess the infection frequency of *Pseudomonas aeruginosa,* biofilm formation ability and antibiotic susceptibility of biofilm forming isolates.

**Study Design:** Cross-sectional/Observational study.

**Place and Duration:** Conducted at Microbiology Laboratory, Gujranwala Medical College / D.H.Q Teaching Hospital Gujranwala Pathology Lab from August 2020 to October 2020.

**Methodology:** A total of 250 urinary catheter tip samples were collected from patients admitted in ICU/CCU. This study includes the methodology in which the study organism was inoculated and confirmed by standard microbiological methods including culture inoculation, Gram staining, biochemical tests, biofilm formation test, antibiotic sensitivity. Qualitative method has been used to see biofilm formation in *Pseudomonas aeruginosa* isolates. 10 different CLSI recommended necessary disc of antibiotics were used to observe the sensitivity and resistance of *Pseudomonas aeruginosa* by Kirby-Bauer disc diffusion method.

**Results:** There were 170 (68%) male patients and 80 (32%) female patients. Majority of patients 160 (64%) were between 46 to 66 years of age. 72 samples were confirmed by morphological confirmation, biochemical tests and microscopic analysis with those of *Pseudomonas aeruginosa.* From 72 confirmed isolates of *P. aeruginosa,* 18 show strong biofilm formation, 26 show weak biofilm formation and 28 show no biofilm formation which is 25% ,36% and 38% respectively. The total number of biofilm forming *Pseudomonas aeruginosa* are 44 out of 72 confirmed isolates which are 61.1% respectively. Out of 44 biofilm forming *P. aeruginosa* isolates 35 shows multi drug resistance (MDR) which are 79% respectively. Out of 10 drugs that were used Polymyxin and Imipenem were found to be most sensitive against *P. aeruginosa* while Ciprofloxacin and Nitrofurantoin were found to be most resistant against *P. aeruginosa.*

**Conclusion:** This study shows that the higher percentage of extracted organism show biofilm formation and from these biofilm forming isolates maximum show multi-drug resistance. Further advances in the prevention of nosocomial infections will require new approaches to infection control. The growing evidence on the ability of *Pseudomonas aeruginosa* to form biofilms, mainly on medical devices, and recent data supporting the correlation of this behavior with the acquisition of antibiotic resistance should alert even more to the risk of this pathogen in the hospital setting.

**Keywords:** *P. aeruginosa*, Biofilm formation, Antibiotic susceptibility, Multi-drug resistance, Urinary catheter tips.

## INTRODUCTION

The adaptation of a microorganism to complicated and diverse environments is expressed in international changes in organic phenomenon profiles that can be reinforced by adapting for survival in demanding habitats (Cooper et al., 2003). To track the microbial pathology and fully perceive ways of adapting microorganisms, it is important to explore the genomic make-up of a microorganism, and research into this affects the transcriptional landscape. One of the main diversifications to be accomplished by a microorganism is the adaptation to antimicrobial compound action. In the last decade, multi-drug-resistant micro-organisms have been increasingly isolated from patient's material (Tavares et al., 2013). This poses a major danger to human health and prohibits the treatment of Gram-negative pathogens such as *P. aeruginosa* and Enteric bacteria (Boucher et al., 2009). *P. aeruginosa*, the leading and well-studied member of the *Pseudomonas* genus (DJ Brenner et al., 2005), may be extremely versatile and resilient bacteria that is able to survive in a variety of terrestrial and aquatic environments (Parsek et al., 2006, Stover et al., 2000, Green et al., 1974). It has versatile metabolic capacity that allows the use of over eighty organic compounds as energy and carbon sources (Silby et al., 2011) (Palleroni et al., 1984). Typically*, P. aeruginosa* generates aerobic metabolism assisted by energy, but can also survive and continue under anaerobic conditions using nitrate as another acceptor of lepton (Williams et al., 2007) or fermentation by essential amino acid and pyruvate (Eschbach et al., 2004). The vast and complex ordering that contains nearly 10 non-transcriptional regulators and two-component restrictive systems allow for high metabolic versatility that increases its high ecological competence

(Stover et al., 2000). The ability to form biofilms on surfaces including rocks and soil, implant materials, tubes and alternative medical device is a major contributor to the spread of *P. aeruginosa* across ecological niches. Biofilm square measure surface-related microorganism populations integrated into an associated level of sugar content, which enables survival in hostile environments (Coterton et al., 1999). *P. aeruginosa* can cause severe infections in a broad range of hosts ranging from plants to amoebas, insects and vertebrates, as a result of this genomic and metabolic ability. Although healthy humans are rarely contaminated, *P. aeruginosa* ranks second to third among Gram-negative pathogens and accounts for all hospital-acquired infections (Sievert et al.,2013) (Gaynes et al., 2005).

Virtually all acute infections by *P. aeruginosa* occur in immunocompromised patients, such as those undergoing therapy with AIDS or leukopenia (Bendig et al., 1987), those with a breached tissue barrier due to serious burn wounds or those undergoing urinary catheterization or ventilation (Richard et al., 2000) (Lyczaket al., 2000). In addition to these acute infections associated with healthcare, *P. aeruginosa* colonises the respiratory organs of patients with chronically obstructive pulmonary disease (COPD), or cystic fibrosis (CF), causing significant damage to the functions of the respiratory organ. *P. aeruginosa* infections account for the majority of morbidity and mortality in CF patients (Franklin et al., 2011). The gene recessive disease CF, is caused by mutations in the chloride particle channel called CFTR, which induces pathological changes in various organs and tissues (Goan et al., 1996). A non-functional CFTR interferes with metallic element particle transport in the epithelial tissue (Banar et al. 2016), which results in inefficient mucociliary clearance and hyper-osmolarity of airway surfaces, which promotes organization of the microorganism. *P. aeruginosa's* biofilm composes of at least 3 distinct exopolysaccharides and alginates and sugar synthesis locus. Alginate in particular is made from the lungs of patients suffering with cystic fibrosis (CF) caused by *P. aeruginosa* clinical isolates. It is a linear chemical compound made of β-D-mannuronic acid and α-L-guluronic acid and plays a very significant role in structural stability. Alginate synthesis is dominated by deoxyribonucleic acid in *P. aeruginosa*. AlgD encoded with algD may be a GDP mannose dehydrogenase that catalyses the GDP mannuronic acid assembly. The algD factor mediates the management of alginate synthesis and alginate transcript and also ensures the ultimate production of GDP-mannuronic acid, the chemical shift muse molecule of the alginate synthesis (Laerty et al., 2014).

*P. aeruginosa* isolates obtained from the surroundings also contain two separate exopolysaccharides. The polysaccharides synthesis locus is a neutral polysaccharide, consisting of D-manose, D-glucose and L-rhamnose. During the formation of biofilm, the polysaccharide synthesis locus has shown that cell-cell and surface interactions are critical in initiating biofilm formation and protecting the structure of biofilm (Ma L et al., 2009).

## MATERIALS AND METHODS

All the research work was conducted in Microbiology Laboratory, Gujranwala Medical College / D.H.Q Teaching Hospital Gujranwala Pathology Lab**.** A total of 250 urinary catheter tip samples were collected from patients admitted in ICU/CCU of D.H.Q Teaching Hospital Gujranwala in a time span of 3 months from August 2020 – October 2020. Samples were preserved into sterile specimen containers and were taken in cold packages under aseptic conditions for quality assessment of microorganisms within an hour of collection. Samples were assessed for Total plate count**.** Samples were homogenized with Butterfield's phosphate buffer (pH 7.2). Every sample was mixed with 90 ml of Butterfield's phosphate buffer. 1 ml aliquot volumes were transferred to Petri dishes with plate count agar and mixed with medium. After inoculation of samples, they were incubated at 37°C for 48 hours and the colonies became visible inside and on the surface of medium after 48 hours. Colonies were counted by using colony counter (Gallenkamp, England). Counts were expressed as colony forming unit per gram of homogenate sample (cfu g-1). Various morphological attributes of the colonies were pragmatic and recorded. Separate colonies were isolated and purified by numerous sub-culturing. Pure culture was preserved on slants at 4°C for further tests. The bacterial isolates were identified based on standard microbiological methods. Cultural characteristics, Gram staining and biochemical tests (Citrate test, Oxidase test) were carried out as preliminary tests as given in Monica Cheesbrough-2nd edition (Part2) 2006.

## RESULTS

Total 250 clinical samples were taken from ICU and CCU of DHQ Teaching hospital Gujranwala. Samples from patients of both gender and age groups were collected for the study. Data is present in table 1.

Table 1: Total number of samples collected and their gender wise distribution

| Category | No. of Samples |
|---|---|
| Male | 170 |
| Female | 80 |
| Total | 250 |

During the period of three months from August 2020 to October 2020 samples were collected, distinguished between males and females (170 and 80**)** with percentage of incidences as 68% and 32% respectively.

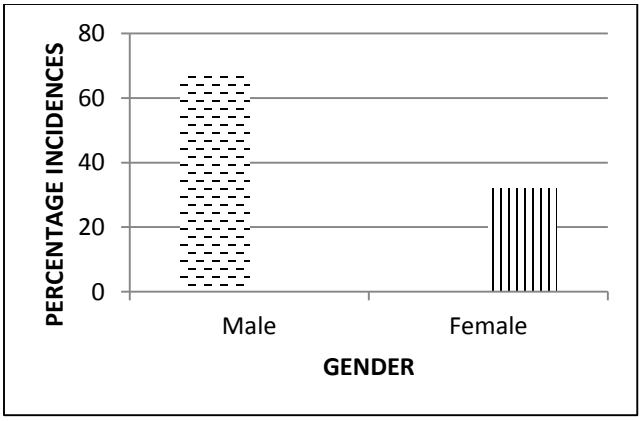


Figure 1: Indicated percentage of male and female patients

Table 2: Age wise distribution of patients

| Category | No. of samples |
|---|---|
| 25-45 years | 90 |
| 46-66 years | 160 |

It represents collection of total number of samples from age groups of 25 to 45 years and 46 to 66 years which were 90 and 160 respectively.

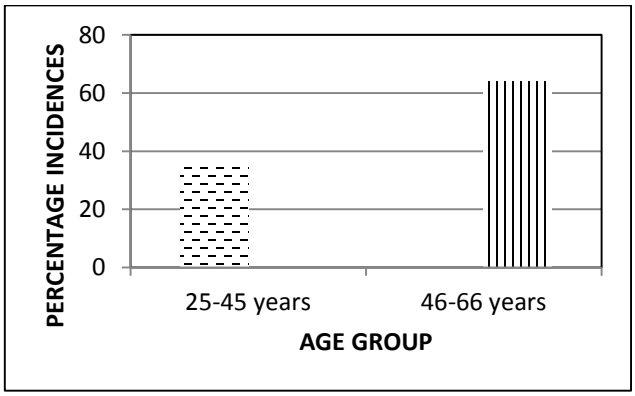


Figure 2: Age wise distribution of patients according to frequency

Figure 2 represents frequency distribution of patients according to age which were divided into two groups i.e.; 25 to 45 years in which low percentage (36%) of patients was shown and higher percentage (64%) was shown in 46-66 years of patients.

From 250 samples, 72 samples were confirmed by morphological confirmation, biochemical tests and microscopic analysis with those of *P. aeruginosa*. The gender wise distribution of positive samples is described in table below.

Table 3: Total number of positive strains according to gender

| Category | No. of samples |
|---|---|
| Male | 42 |
| Female | 30 |
| Total | 72 |

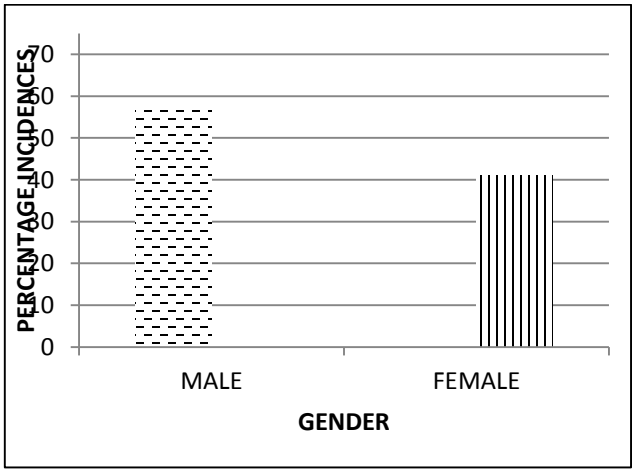


Figure 3: Percentage of positive strains according to gender which were 58% for male and 41% for female respectively

On the basis of qualitative analysis different results are shown by biofilm forming isolates. According to the results of biofilm formation ability, the isolates were distinguished into three categories i.e., strong biofilm formers, weak biofilm formers and no biofilm formers. From 72 confirmed isolates of *P. aeruginosa* 18 shows strong biofilm formation, 26 show weak biofilm formation and 28 show no biofilm formation.

Table 4: Biofilm forming ability of *Pseudomonas aeruginosa* isolates

| Categories | No. of samples |
|---|---|
| Strong | 18 |
| Weak | 26 |
| None | 28 |

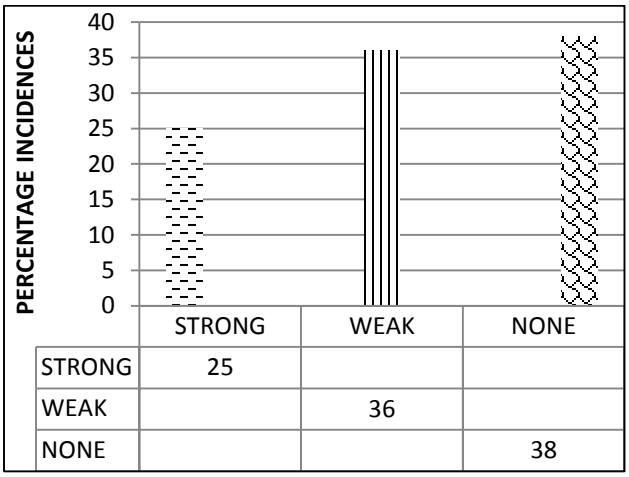


Figure 4: Categories of biofilm forming *Pseudomonas aeruginosa*

Figure 4 show percentage of incidence of strong, weak and no biofilm formers as 25%, 36% and 38% respectively. Thus, the total number of biofilm forming *Pseudomonas aeruginosa* are 44 out of 72 confirmed isolates which are 61.1% respectively.

Table 5: Total No. of biofilm forming *Pseudomonas aeruginosa* isolates found sensitive and resistant against drugs used

| Antibiotic Used | Concentration | Sensitive | Resistant |
|---|---|---|---|
| Aztreonam (ATM) | 30ug | 19 | 25 |
| Ciprofloxacin (CIP) | 5ug | 5 | 39 |
| Nitrofurantoin (NIT) | 300ug | 7 | 37 |
| Gentamicin (GEN) | 10ug | 25 | 19 |
| Amikacin (AK) | 30ug | 28 | 16 |
| Tazocin (TZP) | 110ug | 21 | 23 |
| Fosfomycin (FOS) | 200ug | 12 | 32 |
| Imipenem (IMP) | 10ug | 33 | 11 |
| Sulzone (SCF) | 20ug | 17 | 27 |
| Polymyxin (PB) | 300ug | 34 | 10 |

The antimicrobial susceptibility pattern against 44 biofilm forming *P. aeruginosa* isolates were divided into two groups i.e.; sensitive and resistant. Out of 44 biofilm forming *P. aeruginosa* 35 shows multi-drug resistance (MDR) which are 79% respectively. Out of 10 drugs that were used Polymyxin and Imipenem were found to be most sensitive against *P. aeruginosa* while Ciprofloxacin and Nitrofurantoin were found to be most resistant against *P. aeruginosa.*

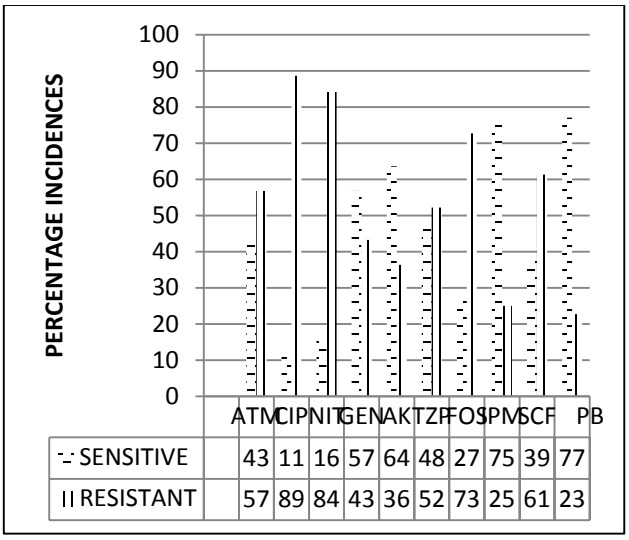


Figure 5: Comparison of antibiotics on basis of their susceptibility pattern

## DISCUSSION

(ES Snitkin et al., 2012) have researched the hospital and population acquired Gram-negative pathogens infections. They have shown that the danger posed by the emerging threat of multi-drug-resistance and highly virulent clinical isolates is drastic. One of the most troubling examples is *Pseudomonas aeruginosa* because infections with this microbe is reach alarming rates in the clinical community. They also listed carbapenem-resistant Enterobacteriaceae (including *P. aeruginosa*) as an imminent threat to public health that needs "urgent and aggressive action." Moreover, extended spectrum beta-lactamase (ESBL) generating Enterobacteriaceae and multi-drug resistant *P. aeruginosa* have been identified as seriously-threatened (second-highest) microorganisms requiring a 'prompt, long-term intervention' to ensure that these problems do not evolve in the future.

In this analysis, the prevalence of the said pathogen has increased to 10 folds since 2012. The hospital acquired infections were increased as the patients had 19% in the last decade and 28.8% were positive samples of *P. aeruginosa* in the present study. This indicates a substantial rise in the incidence of infection relative to previous research.

(A Vatopoulos, DL Monnet et al., 2012) addressed the troubling rise in multi-drug resistant infections that pose a grave threat to human health, the world economy, and society as a whole. In order to battle infections successfully, a clear understanding of the molecular processes of bacterial adaptation to infection-related environments is necessary, particularly given the decreasing efficacy of proven antibiotics for bacterial infections and the urgent need for new treatment strategies and objectives. Over the last 10 years there have been many studies on the outbreak spread of multi-drug resistant *Enterobacteriaceae*, with most concern on carbapenemase producing species. In Gram-negative bacteria, the key driver of beta-lactam resistance is the presence of beta-lactamase enzymes that are able to fasten and hydrolyze the lactam ring. Other methods such as increased efflux expression, decreased membrane permeability or target alteration through mutations in penicillin binding proteins are present but the main and most common cause of resistance to these drugs is beta-lactamases.

(CY Effah et al., 2020) has researched the prevalence of *P. aeruginosa* drug resistance that included amikacin (40.8%), [95% CI 31.9-50.4], aztreonam (73.3%) [95% CI 59.9–83.4], ceftazidime (75.7%) [95% CI 65.4–83.6], ciprofloxacin (59.8%)[95% CI 48.6–70.1], colistin (2.9%), [95% CI 1.8–4.4] cefotaxime (79.2%) [95% CI 68.0—87.2], cefepime (72.6%) [95% CI 57.7–83.8] TEM (39.5%) [95% CI 15.4–70.1], SHV-11 (41.8%) [95% CI 16.2–72.6%], and KPC-2 (14.6%) [95% CI 6.0–31.4] were some resistance mediated genes found in this study.

In the analysis we carried out with 10 drugs for biofilm forming *P. aeruginosa* the following pattern of resistance was observed: Aztreonam (56%), Ciprofloxacin (88%), Nitrofurantoin (84%), Gentamycin (43%), Amikacin (36%), Tazocin (52%), Fosfomycin (72%), Imipenem (25%), Sulzone (61%), Polymyxin (22 %).

In addition to this extensive review, the work we have carried out in this regard has demonstrated an improved multi-drug tolerance of *P. aeruginosa*. In *P. aeruginosa*, it can be concluded that antimicrobial resistant is a clear and current danger and requires close monitoring to resolve this threat. It is very important to track and report improvements in antimicrobial resistant isolates for public health departments.

(Campisano A, Schröder M, et al., 2006) carried out a cohort study and found that *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were the cause for the majority of infections associated with various medical devices, including urinary and intravascular catheters, accompanied by a high percentage of biofilms that contain (approximately 90%) bacteria. Biofilm isolates which caused infections were resistant to multiple drugs. The pslD feature that is part of the *Pseudomonas aeruginosa* psl operon was studied in this research. Exopolysaccharide biosynthesis and biofilm formation are involved in the psl operon. An isogenic marker-free deletion mutant pslD of *P. aeruginosa* PAO1 was produced that was inadequate for the formation of differentiated biofilms. Only the expression of the area coding the pslD gene restored the wild-type phenotype. A fusion of C-terminals, hexahistidine tags allowed PslD to be identified. Translational fusions of LacZ and PhoA with PslD suggested that PslD is a secreted protein necessary for the formation of biofilms presumably through its role in the export of exopolysaccharide. These studies showed that almost 35% of the samples taken contain biofilm formation.

According to this report, out of 72 samples showing positive results for microbe under study 44 of those samples showed formation of biofilm which is 61.1%. It is concluded in this study that out of 44 biofilm formers, 35 show multidrug resistance (MDR) explaining the increase in *Pseudomonas aeruginosa* antibiotic resistance due to biofilm formation. The study of these virulence factors and new control mechanisms may be an effective way to counteract nosocomial infections in *Pseudomonas aeruginosa*. In particular, the biofilm growth mode makes bacteria more resistant to antibiotic therapy by up to 1,000 times. To date, some associations between antibiotic

resistance and the biofilm forming capability of *Pseudomonas aeruginosa* strains have been demonstrated.

## CONCLUSION

It is concluded from this study that unnecessary catheterization along with prolonged admission in hospital can lead to urinary tract infection. Most organisms causing urinary tract infection in hospitalized environment are Gram Negative microbes and it is observed that *Pseudomonas aeruginosa* is one of the leading organism causing urinary tract infection. It is also seen in this comprehensive study that most of the biofilm forming *Pseudomonas aeruginosa* are multi drug resistant (MDR). The unnecessary and inadequate use of antibiotics leads to the prevalence of MDR *Pseudomonas aeruginosa* in the hospital settings. Ciprofloxacin and Nitrofurantoin were found to be the most resistant in most patients, hence they are ineffective in the treatment of patients suffering from urinary tract infection caused by *Pseudomonas aeruginosa,* therefore these drugs should be avoided.

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