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Original Research Article.

Study of *Pseudomonas Aeruginosa* Clinical Isolates with Special Reference To Drug Resistance and Biofilm Formation

J. D. Andhale[1], R. N. Misra[2*]

[1]Assistant Professor, Department of Microbiology,
Ananta Institute of Medical Sciences & Research Center, Rajsamand, Rajasthan, India.
[2*]Professor & Head, Department of Microbiology,
Dr. D. Y. Patil Medical College, Hospital & Research Centre, Pune, Maharashtra, India.

ABSTRACT

Background: *Pseudomonas aeruginosa* is a Gram negative, motile rod shaped bacterium. Production of different virulence factors make the *P.aeruginosa* more virulent. About 65% hospital acquired infections are associated with biofilms.

Objectives: To study incidence, antimicrobial susceptibility pattern and biofilm formation of *P.aeruginosa* clinical isolates.

Materials and Methods: Total 400 various clinical samples from different wards received for routine culture and sensitivity tests in the microbiology laboratory were processed during January 2015 to June 2015. Blood agar, MacConkey agar and nutrient agar plates were used for culture. Confirmed *P. aeruginosa* Isolates were screened for antimicrobial susceptibility pattern and biofilm formation. Muller-Hinton agar plates were used to study antimicrobial susceptibility pattern. Biofilm formation was studied by tube method.

Results: Total 6.25% (25/400) clinical samples were positive for *P.aeruginosa*. Out of 25, 18 (72%) isolates were from pus, 5/25(20%) urine and 2/25(8%) sputum. Twenty out of 25 (20/25, 80%) isolates were from male patients and 5/25 (20%) form female patients. Higher incidence was found in 41- 60 years of age group (19/25, 76%). *P.aeruginosa* found highly resistant to ceftazidime (48%) and 100% susceptible to imipenem. Fourteen out of 25 (14/25, 56%) isolates showed biofilm production and 11/15 (73.33%) MDR *P.aeruginosa* isolates showed biofilm production.

Conclusion: Most effective drug was Imipenem. Antibiotic resistance was higher among biofilm producers. This study showed strong association between biofilm formation and drug resistance.

Key words: *Pseudomonas aeruginosa*, Biofilm Formation, Imipenem.

***Correspondence to:**

Dr. R. N. Misra,
Professor & Head,
Department of Microbiology,
Dr. D. Y. Patil Medical College,
Pune, Maharashtra, India.

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INTRODUCTION

Pseudomonas aeruginosa *(P.aeruginosa)* is a gram negative, opportunistic pathogen. It causes acute and chronic infections.[1]

Moist sites in the hospital environment are known reservoirs of *P.aeruginosa* strains. They are often multidrug resistant due to intrinsic and acquired determinants.[2] It can survive with low levels of nutrients and grow in temperatures ranging from 4 – 42°C.[3]

P.aeruginosa is one of the members of normal flora of nasopharynx and is capable of colonizing the respiratory tract.[4]

Biofilm formation, development of drug resistance and production of different virulence factors make the *P.aeruginosa* more virulent.[5] It can cause Urinary tract infections, respiratory tract infections, dermatitis, soft tissue infections, bacteremia, bone and joint infections and gastrointestinal infections. They are more common in immunocompromised patients and in cystic fibrosis patients.[6]

Because of different metabolic and virulence properties, *P.aeruginosa* is capable of surviving in different environmental conditions.[7]

Biofilm production is an important determinant of pathogenicity in infections caused by *P.aeruginosa*. Biofilm presents strong resistance to the immune system and antibiotics. *P.aeruginosa* forms biofilm that contribute to acute, chronic and persistent infections.[8] *P.aeruginosa* is one of the main pathogenic organisms responsible for drug-resistant nosocomial infections and is becoming day-by-day very common pathogen. It may colonise healthy human without causing disease. Injudicious administration

of broad spectrum antibiotics, instrumentation and intrinsic resistance of microorganisms contribute to make *P.aeruginosa* a nosocomial pathogen.[9] Keeping these facts in mind, this study was undertaken to study the prevalence, antimicrobial susceptibility pattern and detection of biofilm formation among the *P.aeruginosa* clinical isolates.

AIMS AND OBJECTIVES

To determine prevalence, antimicrobial susceptibility pattern and to study biofilm formation of *P.aeruginosa* clinical isolates.

MATERIALS AND METHODS

This study was conducted at the Department of Microbiology in Dr. D. Y. Patil Medical College, Hospital and Research centre, Pimpri, Pune-411018. The study was conducted during January 2015 to June 2015.

Total 400 various clinical samples received from different wards of all ages and both sexes for routine culture and sensitivity tests were processed and confirmed *P.aeruginosa* isolates were screened for antimicrobial susceptibility pattern and biofilm formation.

Isolation and Identification of *P. aeruginosa*

All clinical samples were inoculated on Blood agar, MacConkey agar and Nutrient agar plates. The inoculated plates were incubated for 24 hours at 37°C. Isolates were confirmed as *P.aeruginosa* by colony morphology, grape like odour, pigment production (Figure-1), growth at 42°C, Gram staining, motility, and biochemical tests like catalase, oxidase, nitrate reduction test and citrate utilization tests.[10]

Antimicrobial Resistance Pattern

Antimicrobial resistance pattern was studied on Muller-Hinton (MH) agar by standard disc diffusion method (Kirby-Bauer) as recommended by Clinical and Laboratory Standards Institute.[11] Bacterial inoculum was prepared by using 16-24 hours old culture. Bacterial suspension was prepared using 4-5 isolated colonies. Standard inoculum size was prepared using turbidity standards (0.5 Mcfarland=$1.5x10^8$ CFU/ml) as a reference to adjust the turbidity of bacterial suspensions so that the number of bacteria will be within a given range.[12]

Bacterial suspension was inoculated on Muller-Hinton agar plates by using sterile swab on entire surface of agar plates. Antimicrobial agents used to study resistance pattern were amikacin (30µg), ceftazidime (30µg), ceftazidime/ clavulanicacid (30/10µg), ceftazidime/ tazobactum (30/10µg), carbenicillin (100µg), ciprofloxacin (5µg), gentamicin (10 µg), imipenem (10 µg) and piperacillin (100 µg).The antibiotic discs were placed on the surface of the inoculated plates and gently pressed with sterile forcep. Plates were incubated at 37°C for 18-24 hours. (Figure-2).

The diameter of inhibition zone was measured in millimetres and isolates were scored as sensitive or resistant by comparing with values recommended on standard charts.

Pseudomonas aeruginosa ATCC 27853 was used as the quality control organism in antimicrobial susceptibility determination.

Evaluation of Biofilm Formation by Tube Method

This is a qualitative method used for detection of biofilm formation. This test was carried out as described by Christensen et al.[13] A loopful of test organisms from overnight culture plates were inoculated in 10 ml of trypticase soy broth with 1% glucose in test tubes. Inoculated tubes were incubated at 37°C for 24hrs. Then tubes were decanted, washed with phosphate buffer saline (pH 7.3). Then tubes were dried and stained with crystal violet (0.1 %). Excess stain was removed and after washing with distilled water, tubes were dried in inverted position. Then dried tubes were observed for biofilm formation. A visible thick film lined the wall and the bottom of the tube was considered biofilm positive. The amount of biofilm formed was graded and recorded as a positive and negative biofilm formation (Figure -3A and 3B).

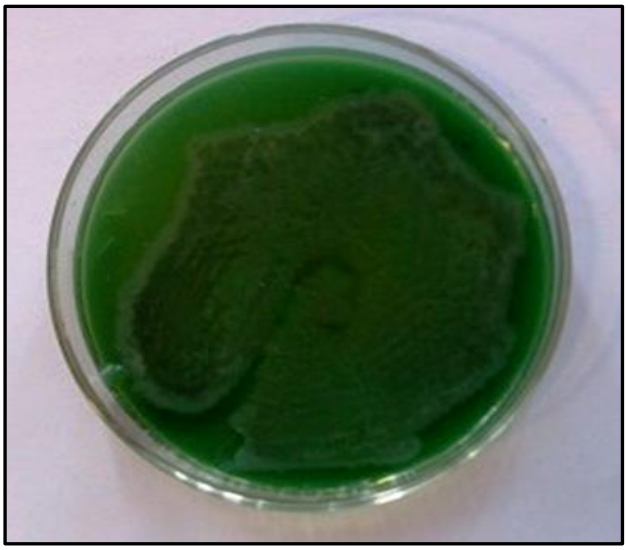

Figure 1: Growth of *P.aeruginosa* on nutrient agar.

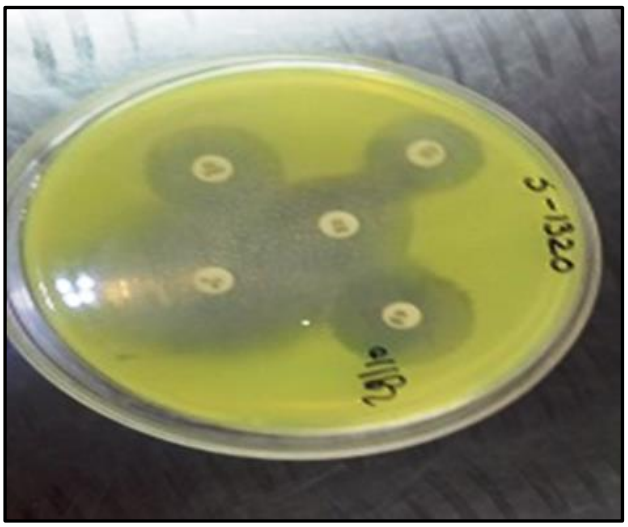

Figure 2: Antibiotic susceptibility test.

RESULTS

Out of 400 various clinical samples, 25 (6.25%) were positive for *P.aeruginosa.* Out of 25, 20 (80%) were from male patients and 5 (20%) were female patients (table 1).

Higher rate (19/25, 76%) of *P.aeruginosa* was found in 41-60 years of age group (table 2)

Out of 25 *P.aeruginosa* isolates, 18 (72%) were from pus followed by urine 5 (20%) and sputum 2 (8%) (table 3).

All Isolates were sensitive to imipenem (25/25, 100%) and antibiotic resistance was higher in ceftazidime (12/25, 48%). A total of 60% (15/25) *P. aeruginosa* were Multidrug resistance.

Out of total 25, 14 (56%) *P.aeruginosa* showed biofilm formation. Out of 15 MDRPA, 11 (73.33%) showed biofilm formation.

Figure 3A: Positive biofilm formation.

Figure 3B: Negative biofilm formation

Table 1: Gender-wise distribution of clinical isolates of *P.aeruginosa* (n=25).

Gender	n	%
Male	20	80%
Female	05	20%
Total	25	100%

Table 2: Prevalence of *P.aeruginosa* based on age.

Age in years	n	%
0-20	01	04%
21-40	05	20%
41-60	19	76%
Total	25	100%

Table 3: Distribution of specimens of *P.aeruginosa* clinical isolates.

Specimen	n	%
Pus/wound swab	18	72%
Urine	05	20%
Sputum	02	8%
Total	25	100%

Table 4: Antimicrobial susceptibility pattern

Antibiotic	Sensitivity (%)	Resistance (%)
Amikacin (30mcg)	19 (76%)	6 (24%)
Ceftazidine + Clavulinic acid (30 + 10mcg)	16 (64%)	9 (36%
Ceftazidime + tazobactum (30 mcg + 10 mcg)	14 (56%)	11(44%)
Ceftazidime (30 mcg)	13 (52%)	12 (48%
Carbenicillin (100mcg)	14 (56%)	11 (44%)
Ciprofloxacin (5mcg)	17 (68%)	8 (32%)
Gentamicin (10mcg)	18 (72%)	7 (28%)
Imipenem (10mcg)	25 (100%)	0 (0%)
Piperacillin (100mcg)	16 (64%)	9 (36%)

DISCUSSION

The aim and objectives of this study were to determine prevalence, antimicrobial susceptibility pattern and to study biofilm formation of *P.aeruginosa* clinical isolates. The researcher processed total 400 various clinical samples received in Microbiology laboratory during January 2015 to June 2015.

Out of 400 clinical samples processed, 25 (6.25%) were positive for *P. aeruginosa.* In India, other researchers in similar studies have reported 2.76%, 8.2% prevalence of *P.aeruginosa* clinical isolate respectively.[14,15]

Prevalence of *P.aeruginosa* in pus was found to be 72 % (18/25) followed by 20% prevalence of the organism in urine. The higher prevalence (50.7%, 55.3%) of this pathogen in pus was also reported by other researchers respectively.[16,17]

The higher prevalence of *P.aeruginosa* isolates was observed in male patients (80%) than female (20%). Similarly, in other studies also the researchers reported the higher prevalence of *P.aeruginosa* in male than in female patients.[18] This shows that the male patients are more exposed to this pathogen than the female patients due to their occupational risks.

Higher prevalence of *P.aeruginosa* was found in 41-60 years age group (19/25, 76%) both in male and female patients. The other studies have also reported similar finding in India.[19]

P.aeruginosa clinical isolates were most sensitive to imipenem (100%) followed by Amikacin (76%) and least sensitive to ceftazidime (52%). These results are in agreement with other similar studies where researchers found that the pathogen was 90% sensitive to imipenem.[20] Thus imipenem is the most effective antibiotic and there is no resistance to this antibiotic; therefore this antibiotic should be used very judiciously so that least or no drug resistance develop in this pathogen. Out of 25 *P.aeruginosa* clinical isolates, 60% (15/25) showed MDR pattern. The higher MDR in this pathogen suggests that there is urgent need to implement the antibiotics stewardship program so that no further increase in the drug resistance appears in this pathogen. Further research is needed to find out the best antibiotic combinations to minimise the emergence and transmission of drug resistance in *P. aeruginosa.* In this study, 14/25 (56%) *P.aeruginosa* isolates showed biofilm production. This finding is in agreement with the 53.3%.[21] A total of 73.33% (11/15) MDR *P.aeruginosa* showed biofilm formation. The high proportion of biofilm markers among MDR *P.aeruginosa* strains suggest that the biofilm production play

some significant roles in showing drug resistant in agreement with same results shown in different studies.[22,23] This also suggests that there is strong association between biofilm formation and drug resistance. The association of biofilm production with MDR *P. aeruginosa* should be further investigated. Many times, the biofilm producing strains showing the drug resistance, clinically, is not true drug resistance but due to poor penetration of the drug in the biofilm showing the drug resistance in this pathogen.

CONCLUSION

Most effective drug was imipenem. Antibiotic resistance was higher among the biofilm forming strains of *P.aeruginosa*. Biofilm formation in MDR *P. aeruginosa* clinical isolate was recorded as 73.33%. This study showed strong association between biofilm formation and drug resistance. There should be continuous surveillance of drug susceptibility testing among *P. aeruginosa* strains in hospital setup. Further research is needed to find out the best antibiotic combinations to minimise the emergence and transmission of drug resistance in *P. aeruginosa.*

ETHICAL STATEMENT

The present study was approved by Institutional Ethical Committee of Dr. D. Y. Patil Medical College, Hospital and Research Center, Pimpri, Pune-411018.

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