



## Emerging biofilm producing multi-drug resistant mucoid strains of *Pseudomonas aeruginosa* in a Rural Medical College Hospital in North Kerala

Ramakrishna Pai Jakribettu, Syed Mustaq Ahamed\*, Anju M M, Safeera M I V and Ashthami V Chandran

Department of Microbiology, MES Medical College and Hospital, Perinthalmanna, Kerala

### ABSTRACT

*Pseudomonas aeruginosa* has emerged as the multi-drug pathogen in nosocomial infections causing Urinary tract infections, pneumonia, surgical site infections, burns infections. The emergence of drug resistance is due to production of biofilm and thus significantly reducing the penetration of antibiotic through the glycocalyx matrix. In this study, we have studied the antibiogram and biofilm production in both mucoid and non-mucoid strains of *Pseudomonas aeruginosa* isolated from nosocomial infections. Sample from patients suspected with Nosocomial infections were collected and processed according to standard procedure and antibiotic susceptibility was done according to CLSI guidelines and subjected for biofilm production by tissue culture plate and test tube method. A total of 64 *Pseudomonas aeruginosa* were isolated from different clinical samples. Among 64, 36 (56.25%) was isolated from sputum samples and least 2 (3.12%) was from blood samples. Among the 64 isolates of *Pseudomonas aeruginosa*, 53 (82.81%) were non-mucoid and 9 (17.19%) were mucoid strains. The Mucoid strains were more resistant to fluoroquinolones (45%), followed by  $\beta$  lactum and monobactam group (27%), and aminoglycosides (18%). Among the isolates tested strong biofilm producers were 41 (64%) and moderate biofilm producers were 23 (36%) by Tissue culture plate method. The Mucoid strains of *Pseudomonas aeruginosa* were multidrug resistant, which is a concern in the nosocomial infections. These can cause increase in morbidity and mortality due to nosocomial infection.

**Key words:** Mucoid *Pseudomonas*, Nosocomial Infections, Biofilm and resistance

### INTRODUCTION

Bacterial infections are becoming more difficult to treat, at present 70% nosocomial infection are resistant to at least one antimicrobial drug that previously was effective for causative pathogens. Microbes that are notorious for their virulence and able to develop resistance include *Staphylococcus aureus*, *Enterococcus species*, member's of *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Acinetobacter species*. Data from the U.S National health care safety net work indicated that Gram negative bacteria were responsible for more than 30% health care associated infections (HAI) [1].

*Pseudomonas aeruginosa* is an opportunistic pathogen and most commonly present serious infection and therapeutic threat within hospital environment. Infection caused by multidrug resistance *Pseudomonas aeruginosa* have been associated with significant increases in patient's morbidity and mortality, length of the hospital stay, requirement for additional medical procedure and surgery, chronic care and overall cost [2]. Although antimicrobial therapy has undoubtedly prolonged the lives of patients with cystic fibrosis [3]. *Pseudomonas aeruginosa* isolates are naturally resistant to large number of antibiotics that can be acquired during treatment as a result of treatment failure [4]. A

major reason for therapeutic failure in the treatment of the infection is its high intrinsic resistance to multiple classes of antibiotic due to low outer membrane permeability and active antibiotic efflux systems [5]. The organism contains extracellular slime, which may have originated from the capsular polysaccharide associated with the outer membrane complex, as in the capsular polysaccharide with other gram negative species [6]. As a natural consequence of intensive treatment, the bacteria have become increasingly resistant to the anti pseudomonal drugs, including an increase in the number of highly beta lactamase producing *Pseudomonas aeruginosa* isolates [7].

Mucoid and non mucoid phenotypes of *Pseudomonas aeruginosa*, with apparent differences in their antimicrobial susceptibility pattern, are frequently isolated [8]. A key aspect of *Pseudomonas aeruginosa* infections is the ability of this organism to form bio films. Bio films are distinct-matrix encased communities specialized for surface persistence and biofilm bacteria are notoriously resistant to antibiotics [9]. They may be up to 1000 times more resistant to antibiotics than their free swimming counterparts and also resist the host's killing mechanisms, such as phagocytosis [10, 11]. Chronic colonization with *Pseudomonas aeruginosa* frequently accompanies phenotypic changes, such as the exopolysaccharide alginate [12]. Once formation of bio film by such mucoid strain is established, it is rarely, if ever, eradicated, despite treatment with combination of antimicrobials with demonstrated potency *in vitro* [13].

Due to increase in the resistant strains in our clinical set up, we designed a study to know the antibiotic susceptibility pattern and biofilm production in *Pseudomonas aeruginosa* isolated from different clinical samples with reference to mucoid strains.

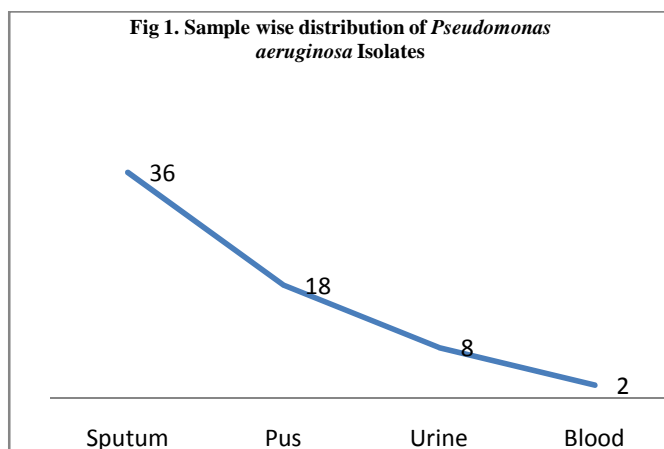
#### MATERIALS AND METHODS

This was a prospective study conducted at Clinical Microbiology Laboratory, MES Medical College, Perinthalmanna, Malappuram, Kerala, from January 2013 to June 2013.

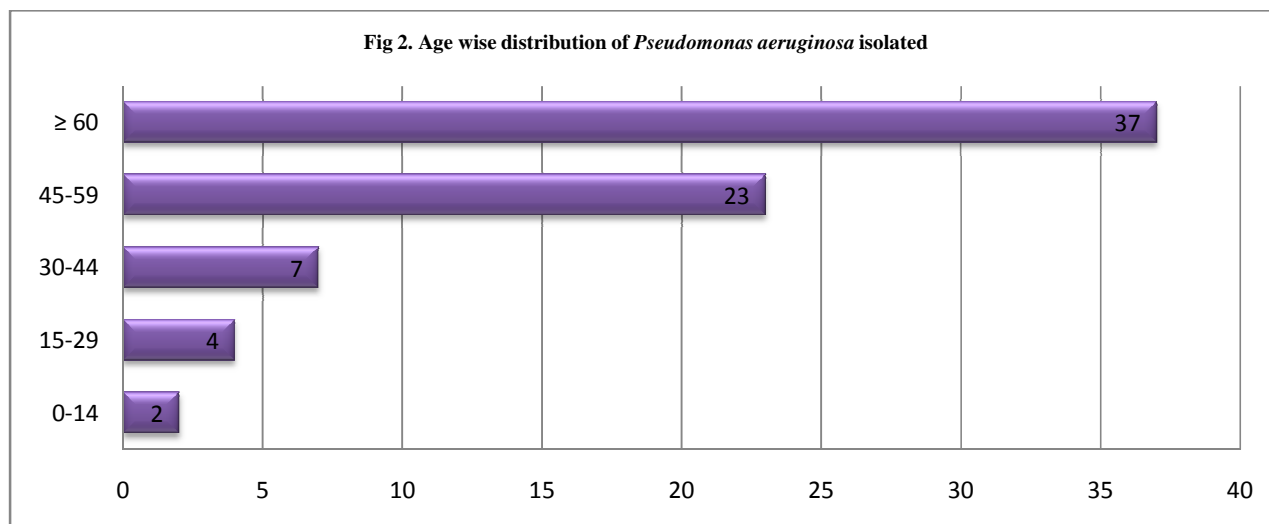
The clinical samples received in lab were processed according to standard microbiological procedure, were inoculated into 5% Sheep Blood agar and MacConkey agar and incubated overnight at 37°C. The Non lactose fermenting colonies on MacConkey's agar were identified by colony morphology, motility, positive reaction to oxidase and pyocyanin production. A total of 64 *P. aeruginosa* strains were isolated from the clinical samples. These isolates were subjected for Antimicrobial susceptibility using Kirby-Bauer disk diffusion method on Muller Hinton Agar (MHA) according to Clinical Standard Laboratory Institute. These isolates were subjected for biofilm production.

The isolates these identified as *Pseudomonas aeruginosa* were subjected to tests for biofilm production by Tissue culture plate and tube method as described by Mathuret *al* [14]

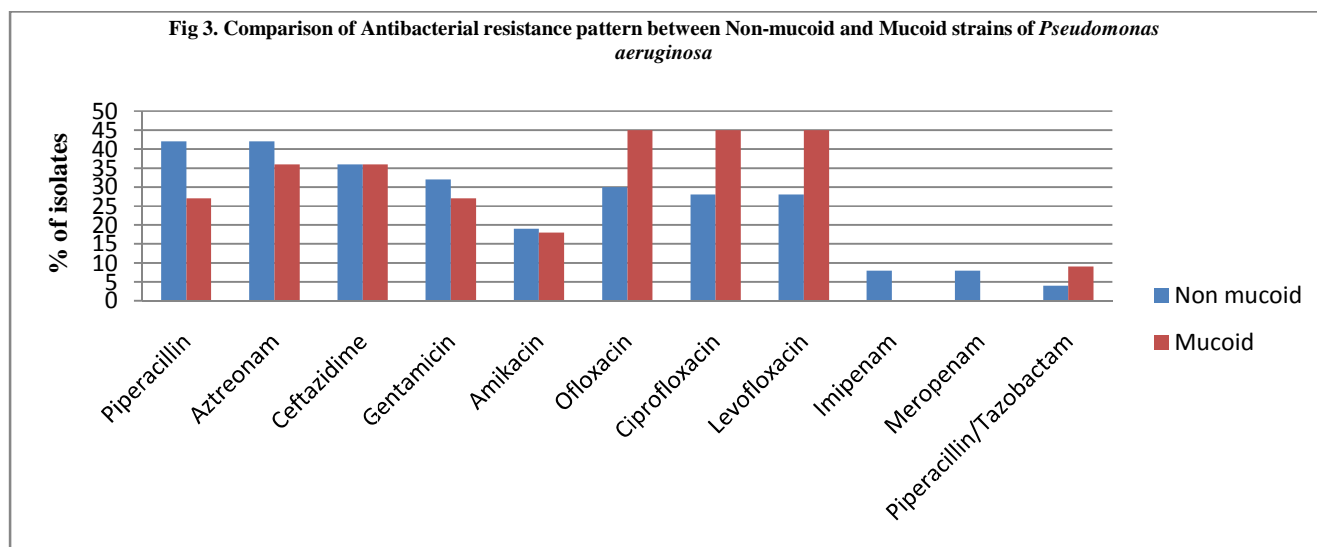
#### RESULTS



A total of 64 *Pseudomonasaeruginosa* were isolated from different clinical samples. Among 64, 36 ( 56.25%) was isolated from sputum samples and least 2 (3.12%) was from blood samples. Among the 64 isolates of *Pseudomonas aeruginosa*, 53 (82.81%) were non-mucoid and 9 (17.19%) were mucoid strains.



From the figure it is clear that the geriatric patients i.e. > 60 years have been infected the most in the study population studied, followed by the age group of 45-59 years.



The figure shows that the mucoid strains isolated from the study group were highly resistant to fluoroquinolones compared to other anti-bacterial drugs.

Table 1. Comparison of the detection of biofilm production by Tissue culture Plate method and test tube method.

	Tissue Culture Plate Method		Test Tube method	
	Strong	Moderate	Positive	Negative
<i>Pseudomonas aeruginosa</i> (n=64)	41	23	38	26
Non Muroid (n= 53)	31	21	29	24
Mucoid (n=11)	9	2	9	2

From table 1, the Tissue culture plate method has detected more strains to produce biofilm compared to the test tube method. Out of 64, TCP detected 41 compared to 38 by TT method. There was a difference among the non-mucoid strains, but both the method detected the same number of biofilm producer among the Mucoid strains.

### DISCUSSION

Biofilm producing organisms are responsible for many recalcitrant infections and are notoriously difficult to eradicate. They exhibit resistance to antibiotics by various methods like restricted penetration of antibiotic into biofilms, decreased growth rate and expression of resistance genes<sup>15</sup>. There are various methods for biofilm detection [16].

In the present study the number of isolates showing strong biofilm producers were 41(64%) and moderate biofilm producers were 23(36%) by Tissue culture plate method similar to the study done by AfreenishHassenet *al*<sup>17</sup>. In their study noted that out of 110 isolates from different clinical samples tested for biofilm production, the number of biofilm producers identified by Tissue culture plate method (TCP) was 70(64.7%) and non or weak biofilm producers were 40(36.3%). The recent study has performed the Tissue culture plate method by addition of 1% glucose in trypticase soy broth. Addition of sugar helps in biofilm formation<sup>16</sup>. This was also reported by studies conducted by Mathur et al[14] and Bose et al[18].

In our study Tube method detected 59% isolates as biofilm producers and 41% non-biofilm producers, similar to Javaid et al [17]. This method correlated well with the Tissue culture plate method for identifying the strong biofilm producers, but it was hard to differentiate between moderate, weak and non-biofilm producers due to the changeability in the result detected by different observers. Hence, Tube method may not be used as a screening test to identify biofilm producing isolates[19].

In the present study mucoid strain of *Pseudomonas aeruginosa* showed 81% of strong biofilm producers and 18% moderate biofilm producers. Among the nonmucoid strains of *Pseudomonas aeruginosa* were produce 60% strong and 40% moderate biofilm producers. The study of Sylvia M et al also observed that the mucoid *Pseudomonas aeruginosa* strains were produce biofilm more than the non mucoid strains of *Pseudomonas aeruginosa*. Mucoid and non-mucoid phenotypes of *Pseudomonas aeruginosa*, with apparent differences in their antimicrobial susceptibility pattern, but the reason for the difference not clearly known [20].

In the present study mucoid and non mucoid *Pseudomonas aeruginosa* were possess different antibiotic resistance pattern. Nonmucoid *Pseudomonas* in terms of percentage of resistance was high for  $\beta$  lactum and monobactam group (42%) followed by fluoroquinolones (30%), aminoglycosides (19%) and least for the carbapenems (8%). The Mucoid strains were more resistant to fluoroquinolones (45%), followed by  $\beta$  lactum and monobactam group (27%), and aminoglycosides (18%). No mucoid strains were resistant to carbapenems. Similar results have been obtained by Lambert et al[21]. Moehario et al[22] found in his work that only antipseudomonas antibiotic showed good activity 80% was imipenem, and so was suggested as drug of choice in *Pseudomonas* infection. Fyfe et al[23] found that the mucoid strains were more resistant to penicillins, carbencillins and aminoglycosides than related non mucoid strains. Ahangarzadeh-Rezaee et al[24] reported that mucoid strains were significantly more resistant to amikacin, gentamicin, and tobramycin than non mucoid strains.

The sticky alginate slime produced by the mucoid *Pseudomonas aeruginosa* are capable of binding antibiotic molecules, thereby significantly reducing the penetration of antibiotic through the glycocalyx matrix[25]. Actually the biofilm mode of growth that alter the physiology of embedded biofilm cells, resulting in change of permeability of antibiotic across the cell envelope of these cells [26].

The present study documents that the biofilm mode of growth correlates with the antibiotic resistance pattern of the *Pseudomonas aeruginosa*. Biofilm detection values by Tissue culture plate method were associated with the resistance to Ceftazidime, Aztreonam and Piperacillin only, similar to results published by Ali Kahlid et al<sup>[17]</sup>. They observed that the resistance pattern of biofilm producers as: ampicillin 100%, ciprofloxacin 95%, aztreonam 90%, amikacin 64%, meropenem 0% [16].

**CONCLUSION**

This study reveals that the mucoid strains of *Pseudomonas aeruginosa* isolated from the rural tertiary care centre causing nosocomial infections. The mucoid strains have the capability to form biofilms and thus explain the multiple drug resistance. These multi-drug resistant increase the burden on the rural population. Higher percentage of resistance was seen with the  $\beta$  lactam and monobactam antibiotics in biofilm producing strains. We found tissue culture plate method was a reliable, accurate, cost effective procedure to detect biofilm forming microorganisms and this method can be recommended as a general screening method for detection of biofilm producing bacteria in laboratories. This article emphasizes the need for the active surveillance for the infection caused by mucoid *Pseudomonas aeruginosa* in the rural health care set-up, so as to reduce morbidity and mortality.

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