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Detection of Swarming, Swimming and twitching motilities among clinical isolates of *Pseudomonas aeruginosa*

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ABSTRACT

Pseudomonas aeruginosa is a gram negative bacteria causing wide spectrum of hospital acquired infection in immunosuppressed patients. They know to produce recalcitrant biofilm and show resistance to multiple antibiotics. Due to the production of array of virulent factors and motilities of *P.aeruginosa*, can adhere to many different surfaces and thus forms biofilms in lungs, urinary tract, kidney and also in catheters of hospitalized patients. A sum of 20 clinical isolates of *P. aeruginosa* were collected from different clinical specimens. Antibiotic sensitivity pattern was performed by disc diffusion method followed by the detection of swarming, swimming and twitching motilities by simple plate methods. All isolates showed complete resistant to all antibiotics used except imipenem. 30% of isolates were positive for swarming. While, 100% of isolates were uniformly showed positivity for the other motilities. We proposed that these motilities might play an important role in pathogenesis and establishment of infections.

KEY WORDS: *Pseudomonas aeruginosa*, swarming motility, swimming motility, twitching motility.

1. INTRODUCTION

Pseudomonas aeruginosa is a gram negative bacteria causing wide spectrum of hospital acquired infection in immunosuppressed patients. They know to produce recalcitrant biofilm and show resistance to multiple antibiotics. Due to the production of array of virulent factors and motilities of *P. aeruginosa*, can adhere to many different surfaces and thus forms biofilms in lungs, urinary tract, and kidney and also in catheters of hospitalized patients. The flagella and type 1V pilus are the two main important bacterial appendages necessary for the formation of biofilm in *P. aeruginosa*. Exhibition of motilities are playing a crucial role in the pathogenesis of P.aeruginosa and its ability to colonize various environment. It is capable of swimming in aqueous environments and twitching on dry environment. Since these motilities are important in forming biofilm and establishing infection in hospitalized patients we have taken this objective to determine these motilities in our isolates.

2. MATERIALS AND METHODS

Bacterial isolates: A total of 20 non repetitive clinical isolates of *P. aeruginosa* were collected from Saveetha Medical College, Thandalam. They were processed for a battery of standard bio chemical tests and confirmed. Isolates were preserved in semi-solid trypticase soy stock and stored at 4ºC until for that use.

Antibiotic susceptibility testing: Antibiotic susceptibility testing was determined for this isolates to routinely used antibiotics such as to piperacillin-tazobactam, cefotaxime, ceftazidime, tetracycline, cotrimoxazole, aztreonam, gentamicin and imipenem by Kirby Bauer disc diffusion method as per CLSI guidelines.

Swarming assay: Swarming plates were prepared by incorporating 8g/L of nutrient broth and 0.5% (W/V) agar supplemented with D-glucose (5g/L filter sterilized and added separately). Media was autoclaved and poured into a sterile petridishes and dried briefly. Spot inoculation of the freshly grown *P. aeruginosa* isolates were made at the centre of the plate deeply using sterile tooth picks and the plates were incubated at 37ºC for 24 hours.

Swimming assay: 0.3% of Luria Bertani (LB) agar was prepared and poured to an average depth of 3mm in sterile petri plates and dried briefly. Spot inoculation of the freshly grown *P. aeruginosa* isolates were made at the surface of the plate using sterile tooth picks and the plates were incubated at 37ºC for 24 hours.

Twitching assay: 1 % of Luria Bertani (LB) agar was prepared and poured to an average depth of 3mm in sterile petri plates and dried briefly. Spot inoculation of the freshly grown *P. aeruginosa* isolates were made using sterile tooth picks and the plates were incubated at 30ºC in an air tight container for 24 hours. 1% of coomassie brilliant blue solution was poured on the motility plate to visualize the twitching motility. It was observed by the appearance of spreading zones from the point of inoculation between the bottoms of the petri dish.

3. RESULT

Sample wise distribution of clinical isolates of *P. aeruginosa*: Of the 20 isolates of clinical isolates of *P. aeruginosa,* 9/20 (45%) isolates were from sputum, 5/20 (25%) from blood, 3/20 (15%) from urine, 3/20 (15%) from pus.

Results of antibiotic susceptibility testing: In our isolates, we have observed an increased percentage of isolates were shown to be resistant to most of the routinely used antibiotics. Only 2/20 (10%) isolates showed sensitivity to imipenem. Other than that, for all other antibiotics such as piperacillin-tazobactam, cefotaxime, ceftazidime, tetracycline, cotrimoxazole, aztrionum, gentamicin isolates showed complete reistance 20/20 (100%). The detailed resistant pattern of *P. aeruginosa* isolates were showed in table 1.

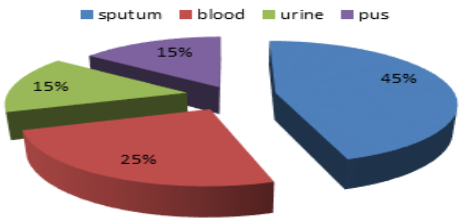


Figure.1. Sample wise distribution of clinical isolates of *P. aeruginosa*

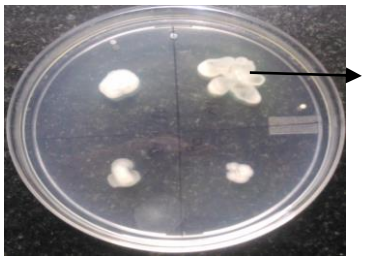
Table.1. Results of antibiotic susceptibility pattern of *P. aeruginosa*

Antibiotics	Sensitivity (20) (%)	Intermediate (20) (%)	Resistant (20) (%)
piperacillin-tazobactam	0(0)	0(0)	20(100)
Cefotaxime	0(0)	0(0)	20(100)
Ceftazidime	0(0)	0(0)	20(100)
Tetracycline	0(0)	0(0)	20(100)
Cotrimoxazole	0(0)	0(0)	20(100)
Aztreonam	0(0)	0(0)	20(100)
Gentamicin	0(0)	0(0)	20(100)
Imipenem	2 (10)	1 (5)	17 (85)

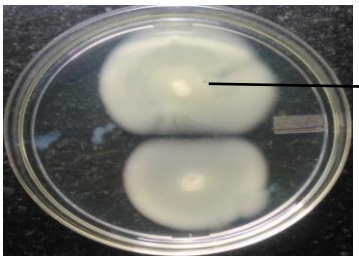
Results of motility assays: 6/20 (30%) of P.aeruginosa isolates were shown to produce swarming motility. Whereas, all isolates 20/20 (100 %) were uniformly showed positivity for swimming and twitching motilities. The detailed results of such motilities was depicted in table 2.

Table.2. Results of Swarming, swimming and twitching motilities of *P. aeruginosa*.

P. aeruginosa isolates	Swarming motility	Swimming motility	Twitching motility
1	+	+	+
2	-	+	+
3	-	+	+
4	-	+	+
5	-	+	+
6	-	+	+
7	-	+	+
8	-	+	+
9	-	+	+
10	-	+	+
11	+	+	+
12	+	+	+
13	+	+	+
14	-	+	+
15	+	+	+
16	+	+	+
17	-	+	+
18	-	+	+
19	-	+	+
20	-	+	+
Total	6/20 (30%)	20/20 (100%)	20/20 (100%)



Swarming motility of *P. aeruginosa*



Swimming motility of *P. aeruginosa*

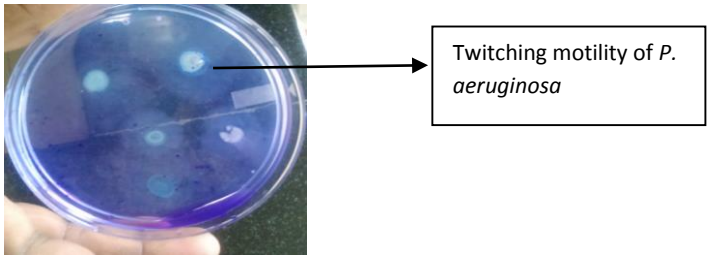


Figure.2. Pictures of Swarming, swimming and twitching motilities of *P.aeruginosa*

4. DISCUSSION AND CONCLUSION

Study conducted by O'May (2011), performed that the motilities of *P. aeruginosa* has been inhibited by cranberry proanthocyanidins and other tannin containing materials. In his study they have observed a complete positivity in forming these motilities by *P.aeruginosa* and they have arrested these by adding the above mentioned products. However, we have not done this in our study.

In contrast, we have seen only 30% of isolates were positive for swarming motility and for other motilities all isolates were uniformly showed positivity. *P.aeruginosa* is very important bacterial pathogen in causing multitude of infections in debilitated individuals. There are plenty of virulence factors are associated with these infections, we have taken only the expression of such motilities from our clinical isolates. As we found that most of our isolates were producing motilities, they might play a crucial role in attachment and forming biofilm in biotic as well as abiotic surfaces. Further studies have been performed in order to rule out the actual mechanism being associated between the expression of motilities and the disease.

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