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Original Research Article

Comparative Study of Slime Production as a Virulence Factor In *Pseudomonas Aeruginosa* Isolates at a Tertiary Care Hospital in Navi Mumbai

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ABSTRACT

Introduction: *Pseudomonas aeruginosa* is an important nosocomial pathogen characterized by its innate resistance to multiple antimicrobial agents. Detection of slime in *Pseudomonas aeruginosa* can be useful in understanding the virulence of this organism.
Aims and objectives: Aim of the present study is to detect slime production in clinical isolates of *Pseudomonas aeruginosa* by two phenotypic methods as tube method and spectrophotometric method.
Material and method: Total isolates were identified by standard microbiological procedure and subjected to antimicrobial susceptibility testing as per CLSI guidelines. Slime production in all isolates was detected by using Tube method and Spectrophotometric method.
Results: Out of total 100 isolates of Pseudomonas aeruginosa 59% were slime positive by Tube method and 34% by Spectrophotometric method. Tube test was found to be superior test as compared to the Spectrophotometric method.
Conclusion: Tube method is more qualitative and superior method to detect slime producing P. aeruginosa isolates.

Key Words: Pseudomonas, slime production, Virulence, Biofilm.

INTRODUCTION

Pseudomonas aeruginosa is an opportunistic nosocomial pathogen, highly versatile microorganism able to tolerate low oxygen conditions. *P. aeruginosa* 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. [1]

Biofilm is a microbially derived sessile community characterized by cells that are irreversibly attached to a substratum or interface or to each other, are embedded in a matrix of extracellular polymeric substances that they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription Biofilm extracellular polymeric substances(EPS), which is also referred to as a slime, is a polymeric conglomeration generally composed of extracellular DNA, proteins, and polysaccharides.

A wide variety of medical devices like urinary catheters, prosthetic cardiac valves, intrauterine devices have been showed to be colonized by biofilm forming microorganisms. Infectious processes in

which biofilms have been implicated include urinary tract infection (UTI), middle ear infection, formation of dental plaque, gingivitis, coating contact lenses, endocarditis, infections in cystic fibrosis.

All microbes like Gram positive and Gram negative bacteria have capacity to synthesize biofilm. Bacteria commonly involved include are staphylococcus aureus, staphylococcus epidermidis, streptococcus viridians, and Pseudomonas aeruginosa, Enterococcus faecalis, Kilebsiella pneumoniae and proteus mirabilis. *Pseudomonas aeruginosa* is one of the most studied microorganisms in the context of biofilms. So it is important to detect slime production by a method which is reliable and effective that is why we aimed this study to compare slime production by Tube method and Spectrophotometric method in clinical isolates of *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

Study design: It was an observational comparative study conducted in the Department of Microbiology at a tertiary care hospital, Navi Mumbai, between September 2010 and September 2012. A total 100 clinical isolates were subjected to slime detection method. Samples were collected from pus, sputum, urinary catheter tip, blood, pleural fluid, endotracheal secretions.

Sample collection and processing: Samples were collected in sterile, wide mouthed containers and then transferred to Microbiology Laboratory for further processing. Samples were cultured onto Pseudomonas isolation agar plates (Hi-media). Colonies with appropriate colonial morphologies were classified presumptively as P. aeruginosa and they were further identified by conventional biochemical tests. Antimicrobial susceptibility testing was done by Kirby Bauer disk diffusion method as per Clinical Laboratory Standard Institute (CLSI) 2010 guidelines. P. aeruginosa was stored in 1% nutrient agar slant at 4^0Centigrade for doing further analyses. P. aeruginosa ATCC 27853 was used as a positive control for the test. Slime production was tested by the Tube method and Spectrophotometric method.

These isolates were tested for Slime production by two methods.

i. **Tube Method: -** Two to three colonies were inoculated into 5 ml of BHI broth in glass tubes. Cultures were incubated at 37^0C for 24-72 hrs and the culture contents were aspirated. Tubes stained with saffranin. The presence of a visible stained film on the wall of the tube was considered to be positive for slime production. [2]

ii. **SPECTROPHOTOMETRIC METHOD :-** This method is used to demonstrate the production of slime by P. aeruginosa as per the method described by Christensen et al [3] 18 hours cultures in Brain heart infusion broth(BHI) was standardized by McFarland's standards. 200 micro litres of standardized cultures were added to the flat bottom wells of sterilized polystyrene plate and incubated for 18 hrs at 37^0 C

Following incubation, the contents of the plate were gently aspirated. The plates washed with sterile phosphate - buffered saline four times at ph 7.2. Slime and adherent organisms fixed overnight with Bouins fixative. The fixative was removed by washing the wells three to four times with 50% ethanol. The wells were stained with Huckers crystal violet and excess stains removed by washing the plate under distilled water and then the plates were dried.

The optical density of the stained adherent films was read by an Elisa reader (MULTISCAN MS) at a wavelength of 620 nm. The measurements were repeated in duplicates and the mean OD was calculated.

OD value greater than 0.1 was considered positive for slime production. [3]

OBSERVATIONS AND RESULTS

Out of 100 *P. aeruginosa* isolates 38 were from Pus followed by Sputum (23), Urine (12), ET Tip (6), Ear swab (6), Blood (4), Catheter tip (4), Pleural fluid (2), Bronchoalveolar lavage (2), ETA spirate (1), Suction tip (1) and Tissue(1). [Table 1]

Table 1: Incidence of *P.aeruginosa* isolates in clinical samples

Sr.No	Samples	Total
1.	Pus	38
2.	Sputum	23
3.	Urine	12
4	ET Tip	6
5	Ear swab	6
6	Blood	4
7.	Catheter tip	4
8.	Pleural Fluid	2
9.	BAL	2
10.	ET Aspirate	1
11.	Suction Tip	1
12	Tissue	1
	TOTAL	100

Table 2: Distribution of slime positive P.aeruginosa isolates by both method

Method	Total no. of Pseudomonas aeruginosa isolates(100)	Chi-square Value	P value
Slime positive % by Tube Method	59 (59%)	12.562	<0.001
Slime positive % by Spectrophotometric Method	34 (34%)		
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Table 3: Production of slime in clinical samples by both methods

Sr. No.	Clinical Samples	No. of isolates tested	Tube Method			Spectrophotometric Method			Chi-square Value	P value
			Strong Positive	Weak Positive	Negative	Strong Positive	Weak Positive	Negative		
1.	Pus	38	21(55.2)	4(10.5)	13(34.2)	8(21)	2(5.2)	28()	11.982	<0.01
2.	Sputum	23	7(30.4)	3(13.4)	13(56.5)	7(30.4)	1(4.3)	15(65.2)	1.143	0.565
3.	Urine	12	7(58.3)	2(16.6)	3(25)	3(25)	2(16.6)	7(58.3)	3.20	0.202
4.	ET Tip	6	2(33.3)	1(16.6)	3(50)	2(33.33)	-	4(66.6)	1.143	0.565
5.	Ear Swab	6	2(33.3)	1(16.6)	3(50)	1(16.6)	-	5(83.3)	1.833	0.40
6.	Blood	4	1(25)	-	3(75)	1(25)	-	3(75)	0.00	1.00
7.	CT Tip	4	3(75)	-	1(25)	2(50)	-	2(50)	0.533	0.465
8.	Pleural fluid	2	1(50)	1(50)	-	1(50)	1(50)	-	0.00	1.00
9.	BAL	2	1(50)	-	1(50)	1(50)	-	1(50)	0.00	1.00
10.	ET Aspirate	1	1(100)	-	-	1(100)	-	-	0.00	1.00
11.	Suction Tip	1	1(100)	-	-	1(100)	-	-	0.00	1.00
12.	Tissue	1	-	-	1(100)	-	-	1(100)	0.00	1.00

Significant Value:P<0.05

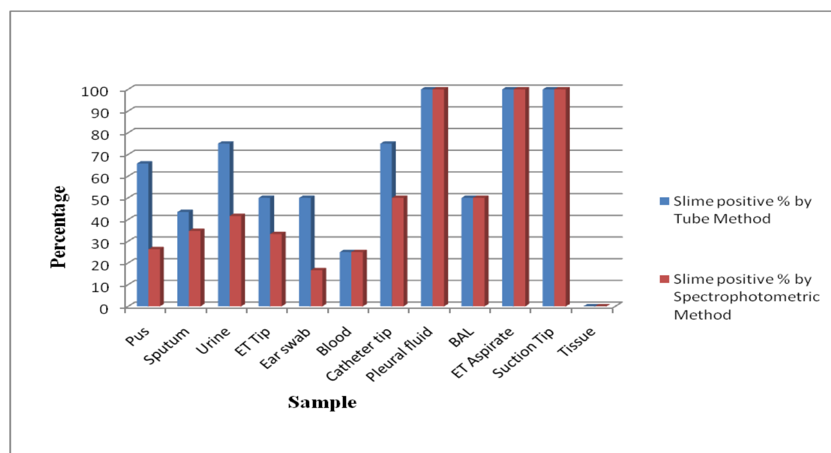

Figure 1: Slime production in clinical isolates of P. aeruginosa isolates by both method

Out of 59isolates, 47 were strong slime producers and 12 were weak slime producers by Tube method.28 isolates were strong slime producers by Spectrophotometric

Out of 100 isolates 59 were positive for slime production by Tube method and 34 were slime positive by Spectrophotometric method. Out of 100 isolates 25 *P.aeruginosa* isolates were negative by both methods.

Slime production by Tube method was maximum from UTI (75%), followed by Pus samples (65.78%), RTI (51.21%) and Systemic infection (25%).

Slime production by Spectrophotometric method was maximum from UTI (43.75%), followed by RTI (39.02%), Pus samples (26.31%) and Systemic infection (25%).

Statistical analysis: Data collected ,compiled, tabulated and analysis was carried out for comparison between two methods by using Chi- Square Test and significance level was set at 95% and p<0.05 was considered significant.

DISCUSSION

Pseudomonas aeruginosa is a common nosocomial pathogen, notorious for its multidrug resistance and life threatening infections in critically ill patients. It exhibits resistance to antibiotics into biofilms by various methods like restricted penetration of antibiotics, decreased growth rate and expression of resistance genes.

Evaluation of the prevalence of slime production among P. aeruginosa strains isolated from clinical samples is important as it helps in deciding the pathogenicity and to assess its diagnostic value as a virulence marker. Such type of investigation has already been reported in Staphylococci. [3,4,5,6]

The slime production may be one of the reasons why P. aeruginosa can thrive for longer period in the hospital environment, acting as a potent source of nosocomial infections. In the present study, Samples were collected from various sites and analysed for slime production by Tube and Spectrophotometric method. It was found that Tube method showed 59% positive isolates for slime production which was statistically significant (p < 0.01) compared to Spectrophotometric method (34%).

Our findings are similar with study done by S. Vishnu Prasad et al [2] it was observed by them that 68 isolates produced slime by tube method and 40 isolates by spectrophotometric method. Similarly, Ruzicka et al noted that out of 147 isolates of S. epidermidis, Tube method detected slime production in 79(53.7%) and Congo red agar detected in 64(43.5%) isolates. They showed that tube method is better than Congo red agar method for slime production.

However our results are not in agreement with Asim I Shaikh et al [7] who reported percentage of biofilm positive by microplate method was more than Standard Tube Test, also it was found that P. aeruginosa isolates from 100 clinical specimen for slime production showed 75, 50, 56.7% respectively by Congo red agar, Standard Tube Test and microplate method. In this study, out of 59 isolates, 47 were strong slime producers and 41 were slime non producers by Tube method. Out of 34 isolates, 28 were strong slime producers and 6 were weak slime producers by Spectrophotometric method. [Table 3] In another study,

In this study, slime production by Tube method was maximum from UTI (75%), followed by Pus samples (65.78%), RTI (51.21%) and Systemic infection (25%).

Slime production by Spectrophotometric method was maximum from UTI (43.75%), followed by RTI (39.02%), Pus samples (26.31%) and Systemic infection (25%). These observations are in concordance with S.

Vishnu Prasad et al [2] where they found that systemic isolates produces less slime compared to urinary wound, respiratory isolates.

Biological and technical factors may have contributed to the observed differences in the results as the tube method was performed in the glass tubes and the spectrophotometric in polystyrene microtiter plate. Technical factors influencing slime production depend on the type of medium, atmosphere of incubation and the nature of the solid surface. [8]

In present study spectrophotometric method used to differentiate slime producer and non-slime producers which was based on OD measurements was found to be less sensitive as compared with tube method, because of technical factors involved.

CONCLUSION

In conclusion slime production was found to be the factor of virulence in identification of P. aeruginosa infection. Amongst the two methods Tube method is more superior and reliable method to detect slime producing P. aeruginosa isolates. In addition, the tube test can be preferred due to the ease of interpretation and performance for the detection of slime production in P. aeruginosa.

Acronyms:

CLSI: Clinical& Laboratory Standards Institute
EPS: Extracellular polymeric substance.
ET: Endotracheal tube
MDR: Multidrug resistance.
RTI: Respiratory tract infection
UTI: Urinary tract infection

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