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Original Research Article

Characterisation and Detection of Virulence Factors, Alginate and Phospholipase 'C' in Pseudomonas Aeruginosa in a Tertiary Care Hospital

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ABSTRACT

Background and Objectives*: Pseudomonas aeruginosa* is a common nosocomial pathogen. It is a major cause of multidrug resistance infections in hospitalized patients. Production of virulence factors increases the morbidity and mortality associated with Pseudomonal infections. This leads to rising costs of care resulting from prolonged hospital stay. It therefore becomes imperative to study the clinical correlation between the production of virulence factors and the infections caused by P aeruginosa. Objectives of the study were, to isolate and identify Pseudomonas aeruginosa from various clinical Samples and to detect virulence factors, Alginate & Phospholipase 'C' in these isolates.

Methods: 250 isolates of P aeruginosa obtained from various clinical samples were identified using standard procedures. Isolates were screened for the production of virulence markers, Alginate &Phospholipase-C using prescribed methods.

Results: Of the 250 P. aeruginosa included in the study, 238 were isolated from Pus/swab and 12 from urine samples. Among Pseudomonas aeruginosa isolated from the Pus/swab samples, maximum isolation was from patients presenting with Infected wound and/or gangrene (n=99) followed by patients presenting with diabetic foot ulcer (n=22). Among the Pus/swab samples most of the P aeruginosa isolates were from Surgical wards (General surgery, n=87 and Plastic surgery, n=44) followed by burns unit (8.8%). 3.36% of the Isolated Pseudomonas aeruginosa were from patients admitted in the Intensive care unit. While 50% of the isolates produced both alginate and phospholipase C (n=125). None of the strains produced phospholipase C alone. Alginate production as a lone virulence factor was observed in 10.4% of the isolates.

Keywords: *Pseudomonas aeruginosa*, Alginate, Phospholipase C.

INTRODUCTION

Pseudomonas aeruginosa is an opportunistic bacteria which is ubiquitous in nature and in moist environmental hospital sites such as sinks, toilets, mechanical ventilators, dialysis equipment. [1]

Pseudomonas aeruginosa are rod shaped, Gram negative bacteria, motile by means of one or more polar flagella. [2]

While many of them are saprophytic but some are pathogens of humans and animals. It belongs to the group called

Pseudomonads and is the major pathogen of this group. A detailed taxonomic study of this group has demonstrated that actually it includes several genera of bacteria, but now the name Pseudomonas is reserved only for species closely related to the most outstanding species, P aeruginosa.

The importance of this species derives from the widespread distribution of its strains in nature, their resistance to many antibiotics and the number of pathogenicity factors that they can possess. P.aeuginosa is one of the so called fluorescent species because they can produce pigments that fluoresce under UV light. [2]

P.aeruginosa produces infections in patients with abnormal host defenses and is a common nosocomial pathogen and a major cause of drug resistant infection in immunocompromised and hospitalized patients. [2]

The pathogenesis of P aeruginosa is multifactorial mediated by an array of virulence factors which play an important role in the pathogenecity. [1] Cell associated factors such as alginate, facilitate adherence and forms a mucoid exopolysaccharide capsule, protecting them from host phagocytic activity as well as from antibiotics. Pseudomonas infections are mostly invasive and usually occur in various stages such as bacterial adherence, colonization, invasion, dissemination and systemic disease. The significance of the different virulence factors probably depends on the infection. Alginate production and phospholipase C are likely to have special significance in respiratory infections, particularly in cystic fibrosis. [1,3]

Alginate/biofilm production by P aeruginosa is recognized as a major problem due to its severity in chronic wound infections. The complex build-up of the extracellular matrix encasing the biofilm-associated bacteria as well as the elaborate signaling mechanisms employed by the bacterium enables it to withstand the continuous stresses imposed by the immune defense and administered antibiotics resulting in a state of chronic inflammation that causes damage to the host. [4]

Phospholipase C is another important virulence factor produced by P. aeruginosa which plays an important role in the pathogenesis of Pseudomonal disease. Phospholipase C is a heat-labile hemolysin which catalyzes the hydrolysis of phosphatidylcholine which is the main constituent of the surfactant of the lung. It destroys the pulmonary surfactant and hence plays a role in the chronic respiratory infections especially in patients with cystic fibrosis. [5]

MATERIALS AND METHODS

This study was conducted in the Department of Microbiology St John's Medical College Hospital between September 2009 and January 2011.A total of 250 isolates of Pseudomonas aeruginosa obtained from various clinical samples received at the laboratory were included in this study. The information collected was computerised and analysed by using Statistical Package for Social Science (SPSS 17th version) software.

Inclusion criteria:

1. Pseudomonas aeruginosa isolates from in-patient clinical samples received in the microbiology laboratory.

Exclusion criteria:

1)*Pseudomonas aeruginosa* isolated from out -patient clinical samples.

2)*Pseudomonas aeruginosa* isolated from urine with a colony count of <105 cfu/ml.

3)*Pseudomonas aeruginosa* isolated from pus samples without pus cells in the Gram stained direct smear.

4) *Pseudomonas aeruginosa* isolated from a single blood culture.

Sample processing:

Pseudomonas aeruginosa from various clinical samples was identified using standard procedures. [6]

Detection of virulence factors.

a) Alginate production. [7] Tube Method: 2-3 colonies were inoculated with 5ml of BHI broth in glass tubes. Cultures were incubated at 370C for 18-20 hrs and the cultures were aspirated. Tubes were stained with saffranine. The presence of visible stained film on the wall of the tube was considered to be positive for slime production. If the wall of the glass tube remains unstained, the strain was considered as a non-slime producer (Figure 1).

b) Phospholipase-C. [8] After touching several colonies from a 18-24 hour culture, the egg yolk agar was inoculated to get isolated colonies. The agar plate was incubated at 35OC for 24-48 hours. Development of a milky white opaque halo around the colony was considered as positive for the production of phospholipase C (Figure 2).

RESULTS

Specimens and isolates: Of the 250 P. aeruginosa included in the study, 238 were isolated from Pus/swab and 12 from urine samples

Patient Demographics:

The 250 clinical isolates were identified as Pseudomonas species using standard methods. They were obtained from patients, between the ages 6 years to 85 years. Majority were isolated from patients in the age range of 15-30 years (29.2%), closely followed by 41-60 years (28.8%) and 31-40 years (29.2%) (Table 1). The male (70.8%) to female (29.2%) ratio observed was 2.4 (Table 2).

The distribution of the isolates from different clinical conditions is described in Table 3. Among Pseudomonas aeruginosa isolated from the Pus/swab samples, maximum isolation was from patients presenting with infected wound and/or gangrene (n=99) followed by patients presenting with diabetic foot ulcer (n=22). However no specific diagnosis could be obtained for 32.8% of patients (n=82). 50% of the P. aeruginosa isolated from urine were associated with UTI. (Table 3).

The distribution of the isolates from different wards is shown in the table-4. Among the Pus/swab samples most of the P aeruginosa isolates were from Surgical wards (General surgery, n=87 and Plastic surgery, n=44) followed by burns unit (8.8%). 3.36% of the isolated Pseudomonas aeruginosa were from patients admitted in the Intensive care unit. 66.6% of the urine isolates of P aeruginosa were from urology ward (n=8).

Alginate and phospholipase C production was determined in the isolated P aeruginosa as a measure of virulence. 39.6% of the isolates showed no alginate or phospholipase C production (n=99). While 50% of the isolates produced both alginate and phospholipase C (n=125). None of the strains produced only phospholipase C and was present with alginate production. Alginate production as a singular virulence factor was observed in 10.4% of the isolates. (Table 5).

Table 1: Age wise distribution of the 250 patients from whom *P aeruginosa* was isolated.

Age groups (years)	Number of Patients (n=250)
<15	3(1.2%)
15-30	73(29.2%)
31-40	58(23.2%)
41-60	72(28.8%)
>60	44(17.6%)

Table 2: Gender wise distribution of the 250 patients from whom *P aeruginosa* was isolated.

Gender	Numbers (n=250)
Male	177(70.8%)
Female	73(29.2%)

Table 3: Clinical conditions in Patients (N=250).

Diagnosis	Pus/Swab(n=238)	Urine(n=12)
No specific diagnosis(n=82)	78(32.8%)	4(33.3%)
Diabetic foot ulcer(n=22)	22(9.2%)	0
Burns(n=20)	20(8.4%)	0
Infected wound, gangrene (n=101)	99(41.6%)	2(16.6%)
Necrotising fasciitis(n=5)	5(2.1%)	0
UTI(n=6)	0	6(50%)
Pemphigus vulgaris(n=6)	6(2.5%)	0
CKD(n=2)	2(0.8%)	0
CSOM(n=5)	5(2.1%)	0
Corneal ulcer(n=1)	1(0.4%)	0
Total (n=250)	238	12

Table 4: Distribution of *P aeruginosa* isolates with respect to wards.

Wards	Pus/Swab	Urine
Medicine(n=16)	14(5.8%)	2(16.6%)
Surgery(n=87)	87(36.5 %)	0
Burns(n=21)	2 1(8.8%)	0
Plastic surgery(n=44)	44(18.48 %)	0
Dermatology(n=14)	14(5.8 %)	0
OBG(n=3)	2(.84 %)	1(8.3%)
Orthopaedics(n=12)	12(5.04 %)	0
ENT/Ophthalmology (n=9)	9(3.78 %)	
Paediatrics(n=1)	1(0.42%)	0
Vascular surgery(n=9)	9(3.78%)	0
Urology(n=9)	1(0.42 %)	8(66.6%)
MICU(n=9)	8(3.36 %)	1(8.3%)
Nephrology(n=8)	8(3.36%)	0
PMR(n=8)	8(3.36%)	0
Total (n=250)	238	12

Table 5:Alginate and Phospholipase C production in isolates (N=250)

Alginate	Phospholipase C	Isolates
-ve	-ve	99(39.6%)
+ve	-ve	26(10.4%)
-ve	+ve	0
+ve	+ve	125(50%)

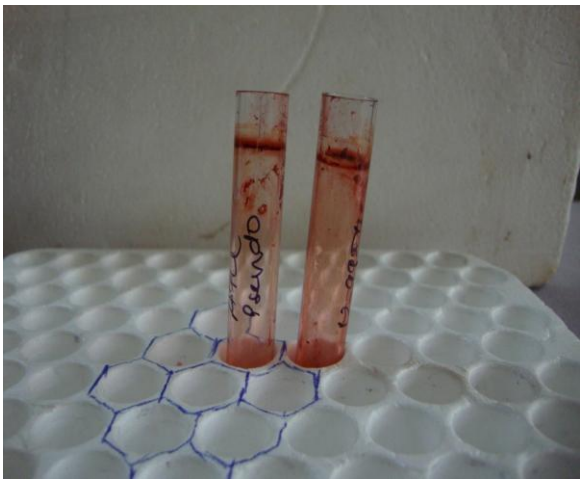

Figure 1: Alginate production-Tube method.

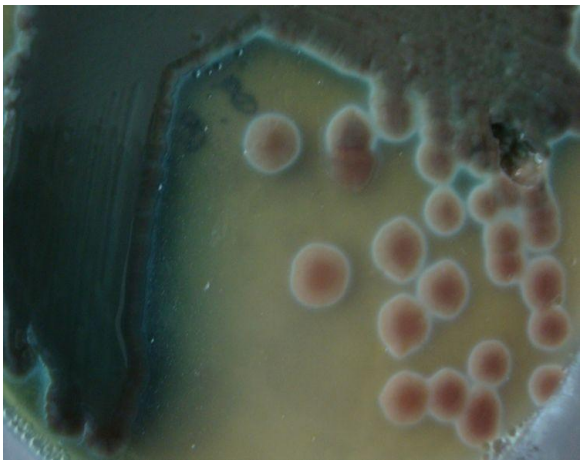

Figure 2: Phospholipase C production (opacity around the colonies) on egg yolk agar.

DISCUSSION

In the present study which includes P.aeruginosa from 250 clinical isolates, we have examined for the presence of Alginate and Phospholipase-C as a virulence marker.

Alginate production was observed among 60.4% (n=151) of the P aeruginosa isolates. Alginate production was observed in 60.1% of the isolates from Pus and 66.7% of the isolates from urine showing no significant difference in rate of isolation from both the samples. In a study conducted at Manipal, a similar rate of 68% of Pseudomonas aeruginosa strains produced alginate. [7] A study from Russia showed alginate production among 34.2% of the Pseudomonas aeruginosa isolates obtained from urine, [9] while in the present study alginate production was detected in 66% of the urinary isolates. Alginate production was

observed more among the P aeruginosa isolates obtained from patients suffering from burns (95%), closely followed by diabetic foot ulcer (86%). It was observed that 100% of the P aeruginosa isolates from physical medicine and rehabilitation ward produced alginate, however the number of isolates (n=8) was small. Marginally higher production of alginate was observed among isolates from medicine wards (62.5%) compared to general surgery wards (40.5%). In another study alginate production was found among 75% of Pseudomonas aerginosa obtained from MICU. [10] In this study 55.5% of MICU isolates of Pseudomonas aerginosa demonstrated the production of alginate.

Alginate/biofilm production by P aeruginosa is recognized as a major problem due to its severity in chronic wound infections. The complex build-up of the extracellular matrix encasing the biofilm-associated bacteria as well as the elaborate signaling mechanisms employed by the bacterium enables it to withstand the continuous stresses imposed by the immune defense and administered antibiotics resulting in a state of chronic inflammation that causes damage to the host. [4]

Phospholipase C is another important virulence factor produced by P. aeruginosa which plays a important role in the pathogenesis of Pseudomonal disease. Phospholipase C is a heat-labile hemolysin which catalyzes the hydrolysis of phosphatidylcholine which is the main constituent of the surfactant of the lung. It destroys the pulmonary surfactant and hence plays a role in the chronic respiratory infections especially in patients with cystic fibrosis. [5]

In our study, among the 250 P aeruginosa clinical isolates, 125(50%) produced Phospholipase C. Among the clinical conditions more Phospholipase producers were detected from P aeruginosa isolated from burns (85%) closely followed by isolates from diabetic foot ulcer (72.3%).A Similar pattern to alginate was also noted in case of phospholipase C detection with reference to isolates from the various wards. The similarity occurred, as in all the strains, phospholipase C production was associated with alginate production. None of the strains produced phospholipase C alone as a virulence marker. Woods et al had previously observed elevated level of phospholipase C production in urine isolates. [11] However we have not observed any significant difference in phospholipase C production in pus and urine isolates, both at 50%. Similar to our observation, Hamood et al had suggested production of high level of phospholipase C is important in all types of infections. [12]

In the present study, number of urinary isolates was small and studies involving larger isolates have to be done, to draw any meaningful conclusions.

CONCLUSION

In conclusion virulence factor production was associated with serious morbid conditions such as burn wounds and diabetic foot ulcer. Large number of isolates from critical wards such as MICU produced Alginate and Phospholipase C, which is a dangerous trend, because isolates producing virulence factors are more virulent when compared to the isolates not producing them. Proper infection control measures should be put in place to curb the spread of virulent isolates which will inturn reduce morbidity and mortality due to Pseudomonal infections. More studies are required to validate the association of virulence factors and pathogenesis of Pseudomonal infections.

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