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DETECTION OF ALGINATE AND COMPARISON OF DRUG SUSCEPTIBILITY PATTERNS IN PSEUDOMONAS AERUGINOSA ISOLATES

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ABSTRACT

Introduction: Pseudomonas aeruginosa, an ubiquitous Gram negative motile rod has been implicated in causing multi-drug resistant nosocomial infections. It produces several virulence factors which enhance the colonization and infection of the host tissue. These include alginate,lipopolysaccharide, exotoxin A, leucocidin and enzymes such as proteases and phospholipases. This study aims to detect the virulence factor- Alginate and to correlate the alginate production with the drug susceptibility pattern. Assessment of the susceptibility patterns of the resistant isolates will guide us in charting out the infection control measures to curb the spread of virulent isolates and in turn reduce the morbidity and mortality due to Pseudomonal infections.

Aim of the study: To detect the production of Alginate in *Pseudomonas aeruginosa* isolates and determine the correlation between alginate production and drug susceptibility.

Materials and Methods: This was a cross sectional study conducted between January-March 2017, in which 75 non-repetitive clinically significant isolates of *Pseudomonas aeruginosa* were collected from various clinical samples. The antimicrobial susceptibility and production of alginate among clinical isolates were determined and the drug susceptibility patterns of alginate producing and alginate non-producing isolates were compared.

Results: Alginate production was detected among 57% of the isolates and Alginate producing *P.aeruginosa* were reported to have decreased susceptibility to Piperacillin Tazobactam, Ceftazidime and Ciprofloxacin.

Conclusion: Detection of Alginate in *P.aeruginosa* and comparison of the drug susceptibility patterns between alginate producers and non-producers are essential to identify the virulent isolates which will play a keyrole in instituting effective infection control measures.

KEYWORDS : *Pseudomonas aeruginosa,* virulence, alginate.

INTRODUCTION:

Pseudomonas aeruginosa, an opportunistic pathogen is a physiologically versatile Gram negative motile rod which thrives in warm moist conditions in the human environment, including respirators, humidifiers and disinfectants.[1] It is ubiquitous in nature and colonizes various animate and inanimate surfaces causing multi-drug resistant nosocomial infections such as urinary tract infections, pneumonia, wound and surgical site infections and infections in burns patients.

Pseudomonas aeruginosa produces several substances, called virulence factors which enhance the colonization and infection of the host tissue. These include alginate,lipopolysaccharide, exotoxin A, leucocidin and enzymes such as proteases and phospholipases.[2]

Drug resistance has been attributed to the, presence of alginate which forms a biofilm, reducing the penetration of the antibiotic. Alginate is an exopolysaccharide consisting of mannuronic and guluronic acids which gives rise to strikingly mucoid colonies and formation of bacterial microcolonies.[1] Biofilm is defined as "a structured community of bacterial cells enclosed in a self produced polymeric matrix adherent to an inert or living surface".Studies have proved that drug resistant infections caused by *P.aeruginosa* have not only increased the morbidity and mortality due to infections but also the length of hospital stay and chronic care, thus contributing to an overall increase in the cost of treating the infection.[3]

This study aims to detect the presence and distribution of virulence factor- Alginate and to correlate its production with the drug susceptibility pattern, which will help in formulating Infection Control measures to curb the spread of virulent isolates and in turn reduce the morbidity and mortality due to Pseudomonal infections.

AIM

To detect the production of Alginate in *Pseudomonas aeruginosa* isolates and determine the correlation between alginate production and drug susceptibility.

OBJECTIVES

1. To determine the antimicrobial susceptibility pattern of all the isolates
2. To study the production of Alginate in various clinical samples.
3. To compare the drug susceptibility patterns of alginate producers and non-producers.

STUDY DESIGN

Cross sectional Study

PLACE OF STUDY

Institute of Microbiology,MMC & RGGGH

STUDY PERIOD

3 months (Jan 2017-Mar 2017)

SAMPLE SIZE

75

MATERIALS AND METHODS

SAMPLE COLLECTION AND PROCESSING:

75 non-repetitive, clinically significant isolates of *P.aeruginosa* were collected from various clinical samples based on colony morphology and characteristics on Nutrient agar and MacConkey agar and standard bacteriological tests such as oxidase positivity, nonfermentative pattern on Triple sugar iron agar, oxidative utilization of glucose on Hugh and Leifson's medium and growth at 42°C[2]

ANTIBIOGRAM

The antimicrobial susceptibility pattern was determined for all the isolates by Kirby Bauer's disc diffusion method on Mueller Hinton agar in accordance with CLSI guidelines. The following antimicrobial agents were included in the panel- Piperacillin Tazobactam, Ceftazidime,Amikacin, Ciprofloxacin and Imipenem[4]

DETECTION OF ALGINATE PRODUCTION[5].

1. A loopful of the test organism was inoculated in 10 ml of trypticase soybroth with 1% glucose in test tubes and incubated overnight at 37°C
2. After incubation, tubes were decanted and washed with phosphate buffered saline (pH 7.3) and dried.
3. Tubes were then stained with crystal violet (0.1%) ,excess stain washed with deionized water and dried in inverted position.
4. Alginate formation will be seen as a thin layer covering the bottom and walls of the tube.
5. The test was performed using *Pseudomonas aeruginosa* ATCC 27853 as positive control and an in house strain as negative control.

RESULTS.

Table 1: ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF *P.AERUGINOSA* ISOLATES

	PT	CAZ	AK	CIP	IPM
P.aeruginosa (n=75)	(n=56) 75%	(n=40) 53%	(n=61) 81%	(n=40) 53%	(n=75) 100%

PT- Piperacillin Tazobactam
CAZ- Ceftazidime
AK- Amikacin
CIP- Ciprofloxacin
IPM- Imipenem

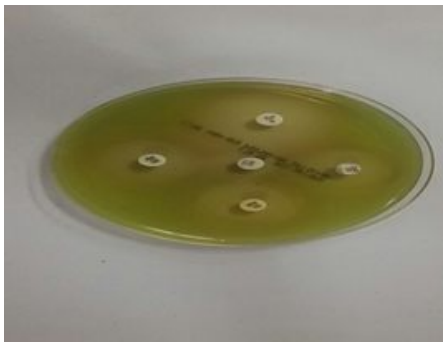

Figure 1: Antibiogram of *P.aeruginosa*

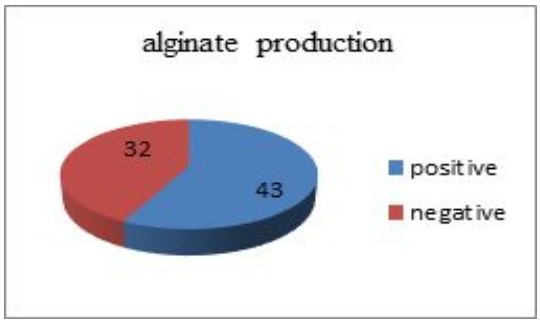

Figure 2: ALGINATE PRODUCTION

Figure 3: Alginate production by tube method

Table 2: COMPARISON OF ANTIMICROBIAL SUSCEPTIBILITY AMONG ALGINATE PRODUCERS AND NON-PRODUCERS (n=75)

	PT%	CAZ%	AK%	CIP%	IPM%
ALGINATE PRODUCERS	N=27 63%	N=14 33%	N=33 77%	N=16 37%	N=43 100%
ALGINATE NON PRODUCERS	N=29 90%	N=26 81%	N=28 88%	N=24 75%	N=32 100%

PT- Piperacillin Tazobactam, CAZ-Ceftazidime, AK- Amikacin, CIP-Ciprofloxacin, IPM-Imipenem

RESULTS

75 non-repetitive clinically significant isolates of *Pseudomonas aeruginosa* were collected from various clinical samples and tested for the production of alginate by tube method. Alginate was detected in 57% of the isolates.

All the isolates (100%) were uniformly susceptible to Imipenem whereas 81% of the isolates were susceptible to Amikacin and 75% of them to Piperacillin-Tazobactam.Alginate production was detected in 57% of the isolates.Alginate producers showed decreased susceptibility to Piperacillin -Tazobactam, Ceftazidime and Ciprofloxacin with statistically significant with p values of 0.006, 0.000 and 0.001 respectively.

DISCUSSION

In the present study 75 non-repetitive isolates of *Pseudomonas aeruginosa* were obtained from various clinical samples and examined for the presence of Alginate as a virulence marker.

Alginate production can be detected by tissue culture plate method, tube method and Congo red agar method.[6] In this study, alginate was detected by the tube method. Alginate production was detected among 57% of the isolates, (n=43)(Figure 2). Other studies have reported Alginate production among 56% of *Pseudomonas aeruginosa* isolates by the tube method, which was similar to this study[6].

A hallmark of biofilm producing bacteria such as *Pseudomonas aeruginosa* is that it can be upto 1000 times resistant to antibiotics than their free swimming counterparts.[7]In view of this, the antimicrobial susceptibility patterns of alginate producers and non-producers were compared. It was observed that 90% of the alginate non-producers were susceptible to Piperacillin Tazobactam, whereas only 63% of Alginate producing *P.aeruginosa* were susceptible. Comparing the susceptibility to Ciprofloxacin, alginate non-producers showed 75% susceptibility and alginate producers were 37% susceptible to ciprofloxacin(table 2). This was similar to the study conducted by Jeetendra Gurung et al., where 63% of the biofilm producing *P.aeruginosa* isolates were resistant to ciprofloxacin.[8] In a study conducted by Ramakrishna Pai Jakribettu et al., where the antimicrobial resistance was compared between mucoid and non mucoid strains of *Pseudomonas aeruginosa*, it was concluded that the mucoid strains were highly resistant to fluoroquinolone group of drugs.[9] Likewise a similar study from Iran showed that the tested isolates of *P.aeruginosa* were highly resistant to all the tested antibiotics such as ciprofloxacin, aztreonam, piperacillin, ceftazidime,amikacin and imipenem except gentamicin.[10]

The correlation between alginate production and drug resistance to Piperacillin-Tazobactam, Ceftazidime and Ciprofloxacin was found to be statistically significant with p values of <0.05. All the isolates were 100% susceptible to Imipenem and no difference was observed between the alginate producers and non-producers (table 1).

CONCLUSION

Pseudomonas aeruginosa has the ability to survive under minimal nutritional requirements and tolerate a variety of physical conditions, which has made it to persist in the environment and

hospital settings. Increased colonization rates have been reported in hospitalized patients, especially in those with impaired immunity. The etiology underlying the production of virulence factors is multifactorial and in a normal clinical situation the production of all virulence factors must be given due importance as they determine the outcome of the disease. Studies conducted to determine the production of virulence factors are essential as they help in identification of the virulent isolates and play a keyrole in instituting effective Infection Control measures.The alginate producing strains were less susceptible to routinely used antimicrobials such as Piperacillin-tazobactam,Ceftazidime and Ciprofloxacin. This study therefore has emphasized the importance of determining alginate production as a virulence factor and has shown a positive correlation between alginate production and drug resistance.

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