e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2022; 14(10); 606-611

Original Research Article

Colony Variants of *Pseudomonas aeruginosa*-Rising MIC Value of Imipenem Posing Threat in Treating Pseudomonas Infections

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Received: 25-08-2022 / Revised: 25-09-2022 / Accepted: 30-10-2022
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Conflict of interest: Nil

Abstract:

Background: *Pseudomonas aeruginosa* has the ability to produce about seven types of colonies. Small colony variants have been isolated from critically ill patients and are known to possess high adherence capacity. Carbapenems play a major role in treating Pseudomonas infections. As a result of increased antibiotic use, the development of resistance to carbapenem is being reported nowadays.

Objective: The objective of this study was to study the colony variants of *pseudomonas aeruginosa* isolated from various clinical specimens, correlate clinically with the patient presentation, and study the minimum inhibitory concentration of Imipenem of all these isolates in a tertiary care hospital. This study was conducted in Sri Siddhartha medical college and hospital. A prospective study was conducted for a duration of 18 months.

Methods: All the isolates presumptively identified as *pseudomonas aeruginosa* from various clinical samples received at the department of microbiology were included in the study. Variation in the type of colony morphology was noted and the MIC value of imipenem was tested on Muller-Hinton agar using E-test strips.

Results: The study revealed that small colony variants and mucoid variants isolated showed an increased resistance pattern to an anti-pseudomonal group of antibiotics. They also showed a high MIC value of imipenem.

Conclusion: Characterization of *pseudomonas aeruginosa* isolates showed that small colony and mucoid variants showed increased resistance patterns to antibiotics. Studies have also shown that there is an association between biofilm formation and colony morphology. Virulence factors are likely to contribute to biofilm formation and also contribute to antibiotic resistance.

Keywords: minimum inhibitory concentration, imipenem, pseudomonas aeruginosa, small colony variants.

Introduction

Pseudomonas aeruginosa (P. aeruginosa) is an opportunistic non-fermentative gram negative bacterium which can survive under different environmental conditions. It is usually isolated from moist and humid environment. It can colonize healthy individuals and cause variety of infections like burns wound infections, skin and soft tissue infections, ventilator associated pneumonia, cystic fibrosis, chronic obstructive pulmonary diseases, urinary tract infections. [1,2,3] Association of this isolate is seen increasingly with healthcare associated infections, chronic infections and potentially life-threatening infections. [4,5,6] P. aeruginosa has an ability to produce phenotypic variants of colony morphologically. Small colony variants have shown an association to antibiotic tolerance. They have been recently isolated from persistent or chronic infections. [7,8]

These small colony variants (SCVs) have an ability to produce biofilm formation, increased tendency to attach to surfaces, produce exopolysaccharides. [9] Mucoid colony variants of P. aeruginosa isolates are found to be less inflammatory when isolated from chronic infections. Prolonged antibiotic therapy have resulted in evolution of increased antibiotic resistant small colony variants. Morphological changes in the bacterium like changes in the lipopolysaccharide, lipid A, loss of O-antigen in quorum sensing (QS) and inactivation of lasR have resulted in emergence of small colony variants. [10] Imipenem is effective in treatment of critically ill and chronic pseudomonas infections in clinical practice. There is a need to monitor the increasing imipenem resistance. Therefore, present study was done to find MIC of Imipenem among small colony variants of Pseudomonas isolates from chronic infections and critical patients.

Materials and Methods

This study was carried for a period of 18 months. The present study was approved by institutional ethical committee of Sri Siddhartha Medical College and Hospital, Tumkur, India.

Inclusion criteria:

P. aeruginosa isolated from various clinical samples of all age groups and both gender was included in the study.

Sample collection and processing was done according to recommended standard procedures. [11,12,13]. A total of 103 isolates were included in the study.

Pseudomonas aeruginosa was identified using conventional methods of identification like colony morphology, pigment production, biochemical reactions. The variation in colony morphology of *Pseudomonas aeruginosa* isolates was studied and analyzed.13 MIC for imipenem was tested for all the isolates by E-test method. Colony morphology observed was correlated with the MIC value of imipenem and antibiotic sensitivity pattern observed.E-test strips of imipenem (biomeurex) were used for this purpose. E-test uses the principle of a predefined antibiotic gradient on a plastic strip to generate an MIC value. Individual antibiotic strips were placed on an inoculated Mueller-Hinton agar. After incubation, the MIC was read where the growth edge intersects the strip graduated with an MIC scale. Interpretation of MIC values was done as per manufacturer's instructions.MIC reference values were observed using CLSI guidelines. [14]

Standard strain of P. aeruginosa ATCC 27853 was used as control.

Results

Maximum isolates were from inpatients 90 (87.38%) and outpatients were 13 (12.62%). Of the 103 isolates maximum

number of isolates were from the surgery ward 45 (50%) followed by medical ward 35 (38.9%).

The risk factors associated were diabetes mellitus 25 (24.27%), COPD 12 (11.65%), surgery and catheterization 5 (4.85%) each and burns 1 (0.97%) (**Table/ Graph:1**)

All the isolates showed diffusible blue green pigment. Colony morphology was studied. Maximum isolates were type one large flat irregular colonies. Nine isolates were found to be small colony variants and mucoid colony forms. (**Table/ Graph :2**) Colony variants findings were as shown in the (**Fig :3**)

98 (95.14%) of isolates obtained showed sensitivity to imipenem. The minimum inhibitory concentration of imipenem were towards the lower end in the range of 0.25 to 1.5 µgm/ml. Five small colony variants strains showed a value of > 8 µgm/ml. One strain with a value of 3 µgm /ml was mucoid colony variant(.**Fig:4**)

Risk Factor	Burn	COPD	Diabetes Mellitus	Catheterization	Surgery	No Risk Factor	Total
Number of Isolates	1	12	25	5	5	55	103

COPD=Chronic Obstructive Pulmonary Disorder

Table 1: Risk Factors Associated with the Patients (n=103)

Type 1	Type 2	Type 3 & 4	Type 5	Type 6
77	5	-	15	3

Table 2: Culture Characters of *P. aeruginosa* (n=103)

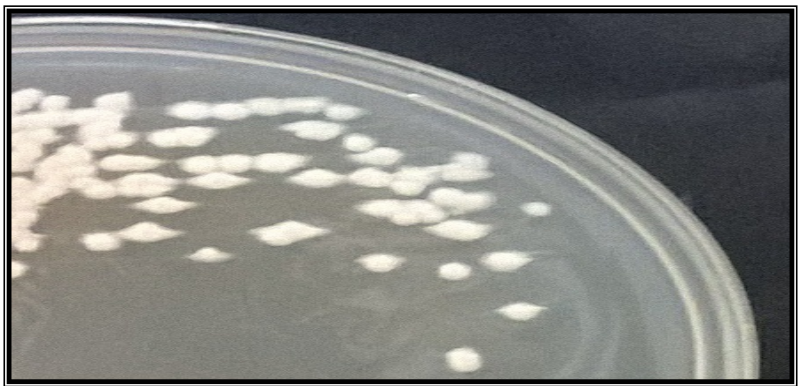


Fig 3.1: Type 1 Large irregular flat colonies

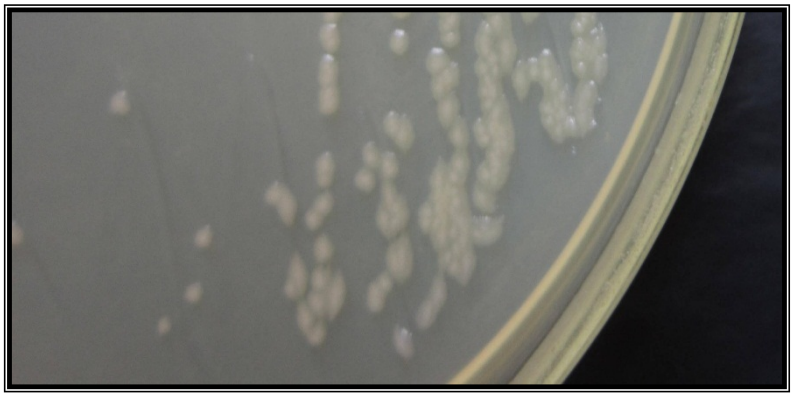


Fig 3.2: Type 2 Small smooth dome shaped colonies

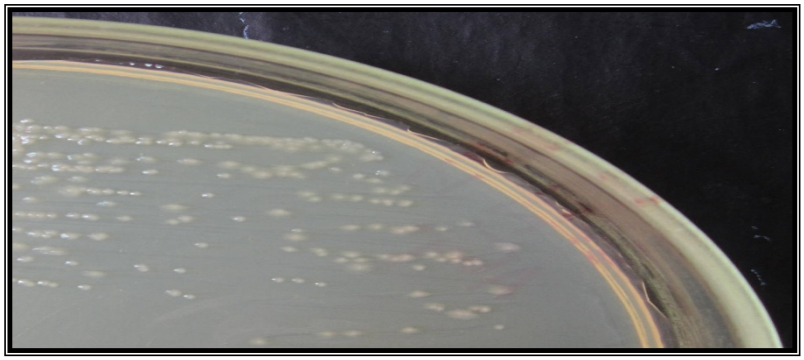
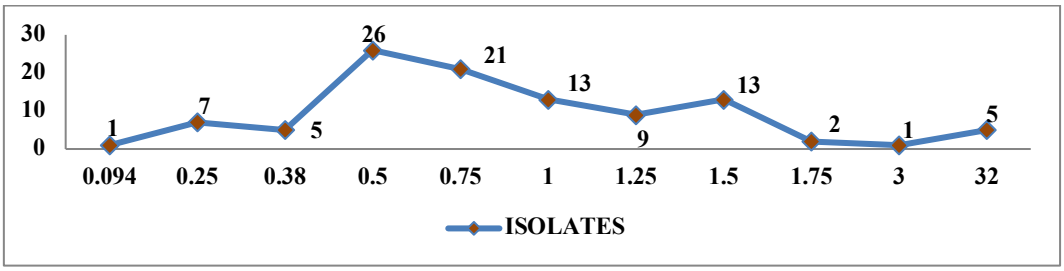


Fig 3.3: Type 6 Dwarf colonies

Fig 3: Growth of *Pseudomonas aeruginosa* on Nutrient Agar

Fig 4: Minimum inhibitory concentration of imipenem using E-test strips



Graph 1: Line Diagram Showing Minimum Inhibitory Concentration (MIC) Value of Imipenem in Different Isolates (n=103)

Discussion

Pseudomonas aeruginosa (P. aeruginosa) is the most common opportunistic pathogen of all Pseudomonas species. In normal, healthy hosts, infection is usually associated with events that disrupt or by pass protection provided by the epidermis e.g. burns, puncture wounds, use of contaminated needles by IV drug abusers, eye trauma with contaminated contact lenses. The result is infection of the skin, bone, heart or eye. P. aeruginosa is a notable cause of nosocomial infections of the respiratory and urinary tracts, wounds, blood stream and even the central nervous system. In an immunocompromised patient, such infections are severe and frequently life threatening. Studies done by Mekonnen H et al., and Raman et al. have found an association of isolated P aeruginosa to underlying risk factors like diabetes, chronic pulmonary respiratory disorders, tuberculosis similar to our study. [15,16] Brzozowski Met al., study on characterization have shown that resistant strain variation was observed from different

ward similar to our study. [17] Haynes W C. in his study on characterization of Pseudomonas has shown that this bacterium produces six different types of colony morphology on culture. [18] Variation in colony morphology from smooth to mucoid colonies was seen similar to study done by Jacome PRLA, et al. [19] mucoid colonies produced could be due to the production of alginate by some of P. aeruginosa isolates. Proctar in his study has shown that small colony variants in bacterium have shown to possess increased virulence factors thus contribute to increased resistance pattern to antiobiotics. [20] Small colony variants *pseudomonas aeruginosa* shows increased ability to form biofilms, also frequently resistant to multiple antibiotics as seen in our study was observed in study by Thomas J Evans also [21]. Changes in colony types observed in P aeruginosa isolates could be due to variation in virulence factors. [22]

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