CASE STUDY
MUCOID VARIANT OF PSEUDOMONAS AERUGINOSA BUT NOT IN CYSTIC FIBROSIS LUNG

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ABSTRACT

Mucoid variant of Pseudomonas is most commonly isolated from Cystic Fibrosis patients. We have isolated a hugely mucoid variant of it from sputum of two non-cystic fibrosis patients, one aged 75 years and another aged 35 years, both having chronic lung disease, admitted at Calcutta School of Tropical Medicine, West Bengal, India. Isolation of this variant of pseudomonas from non-cystic fibrosis lung prompted us to report the cases. Both the strain further tested found to produce biofilm by the qualitative detection method, the Tube method. During the years following initial colonization, the wild-type strains uniformly mutate into mucoid variants and the mucoid matrix is believed to allow the formation of protected biofilm. Once biofilm formed, it becomes a real challenge for the physician to eradicate the organism.

INTRODUCTION

Pseudomonas aeruginosa is a well-established opportunistic pathogen. It is commonly isolated from sputum samples in hospitalized patients. However, its mucoid variant is most commonly isolated from Cystic Fibrosis patients and occasionally from patients with Chronic Obstructive Lung Disease and Bronchiectasis. In our case, both patients are having history of Chronic lung disease and suffering from recurrent lung infections for last 1 year. After a period of intermittent colonization, the organism became permanently established and was difficult to eradicate.

Case report

CASE 1: A 75 years old lady presented with frequent episodes of cough with scanty expectoration and respiratory distress for last 6 months. She had similar history 7 months back when she was diagnosed to have left sided lung collapse and bronchiectasis. She was treated for pulmonary tuberculosis 35 years back. On examination, the patient was afebrile, respiratory rate was 36 per minute on palpation, trachea and apex beat shifted to the left, vocal fremitus was decreased in the left. On auscultation, breath sound decreased in both side. CT scan lung shows left sided pleural effusion with fibrosis, collapse and bronchiectasis; Right lung was emphysematous with Basal granuloma; Mediastinum shifted to left.

CASE 2

A 32 year old female presented with chief complaint of cough with expectoration along with shortness of breath for last 12 days. She had similar history thrice for last one year. Pseudomonas aeruginosa was isolated from her sputum sample on two occasions and mucoid E.coli on one occasion. She is a known case of HIV 1 positive, on Anti-Retroviral Therapy (ART) from December, 2009. Now she is on second line ART (Abacavir, Atazanavir, and Ritonavir). She was diagnosed to have bilateral bronchiectasis. On examination, patient was afebrile. There is bilateral crepitation present on auscultation. Patient is treated with meropenem and cotrimoxazole. She was nebulized with bronchodilator.

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MATERIALS AND METHODS

Direct smear of sputum - shows plenty of Pus cells. Culture - 24 hour culture in MacConkey agar shows hugely mucoid colony resulting in merging of colonial growth. Gram stain from the growth showed gram negative rods. After 48 hours of incubation of the same plate, the hugely mucoid colony drips onto the lid of the petridish, when stored agar side up.

Motility by Hanging drop preparation: present

- Oxidase positive
- Indole negative
- Triple sugar Iron alkali/alkali
- Urease positive
- Citrate positive

Antibiotic sensitivity- Tested by the Kirby–Bauer technique according to Clinical and Laboratory Standards Institute guidelines. On inoculation in Mueller Hinton agar for sensitivity testing, the organism produced bluish green Pyocyanin pigment diffused in the medium and characteristic grape-like odour. The colony was not mucoid in nature. It is sensitive to Piperillin-tazobactum, Ciprofloxacin, Imipenem, Meropenem, and Gentamycin and resistant to Cefazidime, Cefixime, Cotrimoxazole, and Amoxicillin. The Case was therefore diagnosed to be chronic lung disease infected with Pseudomonas aeruginosa highly mucoid variant. The clinical isolates were subjected to biofilm detection method by qualitative method, the Tube method. In both the cases, it was mild positive.

DISCUSSION

Pseudomonas aeruginosa is a well-established opportunistic pathogen. It is found in most moist environments. Clinical and experimental observations suggest that P. aeruginosa infection often occurs concomitantly with host defense compromise, mucosal trauma, physiologic derangement, and antibiotic-mediated suppression of normal flora (Reuben Ramphal, 2012) P. aeruginosa causes infections at almost all sites in the body, but shows a rather strong predilection for the lungs. It is a nonfastidious, motile, gram-negative rod that grows on most common laboratory media, including blood and MacConkey agars. It is easily identified in the laboratory on primary-isolation agar plates by pigment production that confers bluish-green appearance (pyocyanin) and a characteristic grape-like smell of aminocetophenone (Govan, 2012). Chronic infection due to P. aeruginosa occurs mainly in
the lungs in the setting of structural pulmonary diseases. The classic example is CF; others include bronchiectasis and chronic relapsing panbronchiolitis. Hallmarks of these illnesses are altered mucociliary clearance leading to mucus stasis and mucus accumulation in the lungs. There is probably a common factor that selects for P. aeruginosa colonization in these lung diseases—perhaps the adhesiveness of P. aeruginosa for mucus, a phenomenon that is not noted for most other common gram-negative bacteria, and/or the ability of P. aeruginosa to evade host defenses in mucus. After a period of intermittent colonization, the organism becomes permanently established and is difficult to eradicate. During the years following initial colonization, the wild-type strains uniformly mutate into mucoid variants (Li et al., 2005). The mucoid matrix is believed to allow the formation of protected biofilm microcolonies (Fegan et al., 1990), (Pier et al., 2001). The biofilm can be defined as sessile communities of microbial cells irreversibly attached to a surface or interface or to each other which are embedded in a self-produced matrix of extracellular polymeric biomolecules and are physiologically different from planktonic cells with respect to growth rate and gene transcription. During colonization, microbial cells communicate via quorum sensing through auto inducers. In case of pseudomonas aeruginosa, there are two auto inducers, namely N-(butyryl)-L-Homoserine Lactone and N-(3-oxo-dodecanoyl)-L-Homoserine lactone. The five distinct stages of Biofilm formation are Reversible attachment, Irreversible attachment, Maturation 1, Maturation 2, Dispersion.

This exopolysaccharides promotes cohesive forces, increases absorption of nutrients and heavy metals, sequesters microbial products and other microbes, protects immobilized cells from environmental changes and host immune system and more importantly provides a medium for intercellular communication (via Quorum sensing) and transfer of genetic materials. Sequestration of other microbes that are not antagonistic with the original biofilm micro-organisms will result in heterogenous/mixed biofilm. Biofilm provide increased resistance to opsonization, phagocytosis, and digestion (Pier et al., 2001). Furthermore, resistance to various antibiotics occurs both by intrinsic and extrinsic mechanism. Exopolysaccharides retard the diffusion of antimicrobials chemically or by decreasing its transport. Biofilm associated organisms, particularly organisms at the base are metabolically more inactive and have reduced growth rates, so antibiotics are slowly taken into the cells and cannot impart their effect. Environmental factor also cause resistance/tolerance. Extrinsinc resistance to antibiotics occurs by acquisition of resistant plasmids by enhanced conjugation rates in the biofilm (Govan and Deretic, 1996). Alginate production allows for persistent infection and ultimately establishes the poor prognosis for the patients (Govan and Harris, 1986), (May et al., 1991). The mucoid phenotype is a result of several genes, including algD that encodes the enzyme guanosine diphosphate mannose dehydrogenase and catalyzes the last step in alginate precursor synthesis (Govan and Deretic, 1996), (Berry et al., 1989). It is thought that all wild-type P aeruginosa strains are capable of synthesizing alginate but that conversion to an overtly mucoid phenotype depends on appropriate host environmental pressures (Govan and Deretic, 1996). The mucoid phenotype is not observed outside the human host. It has long been known that mucoid P aeruginos strains may spontaneously convert to nonmucoid forms in culture, which is also seen in our case, indicating that they are the same organism (Bergan and Hoiby, 1975; Cetin et al., 1965). Studies of serologic group, phage type, and pyocyanin type confirm this direct relationship and mutations controlling the switch between wild-type and mucoid strains have been identified (Boucher et al., 1996). Conversion back to a nonmucoid phenotype is now thought to be due to new suppressor mutations, rather than reversal of the original mutations (Govan and Deretic, 1996).

**Conclusion**

We isolate Pseudomonas aeruginosa very commonly in our laboratory from specimen of sputum. However in these two cases, after 24 hours incubation of the inoculated sputum in MacConkey agar, we got an extremely mucoid growth like the albumin of a raw egg which on further incubation for 48 hours drips onto the lid of the petridish, when stored agar side up. Though cystic fibrosis is a childhood disease, rarely it may present in the adulthood. So in both the cases it had been ruled out by normal sweat chloride test. So isolation of such a mucoid variant of Pseudomonas aeruginosa from the above two non-cystic fibrosis cases can only be explained by the formation of biofilm in their diseased lung. The isolates were also further tested positive to produce biofilm by Tube method. In this era, molecular diagnosis is the confirmatory tool but in our case, the bluish-green pigment production (pyocyanin) and a characteristic grape-like smell of aminoacetophenone confirmed our diagnosis of Pseudomonas aeruginosa.

**REFERENCES**


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