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Phonotypical & Structural comparison between *Pseudomonas aeruginosa* Isolated from keratitis and cystic fibrosis patients

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Abstract : An eighty samples containing *pseudomonas auroginosa* were taken from Diwanyia teaching hospital 50 of them were from Cystic Fibrosis patients and the other 30 were from Microbial Keratitis infected people ,all these samples have been cultured and indicated *pseudomonas auroginosa* infection, then the bacteria under taken a comparison according to colony properties , genetically and the activity of proteinase IV enzyme, the study have been identified almost *pseudomonas auroginosa* isolated from Cystic Fibrosis(CF) patients were able to form mucoid colonies ,while the colonies of Microbial Keratitis(MK) patients were nonmucoid , genetically the study fined that there were many bands at different wave lengths can be isolated from the same infection .and finally as regarded with proteinase IV(PIV) enzyme activity it was more active in Cystic Fibrosis isolates from Microbial Keratitis once, this may come from the effect of anther virulence factors.
Keyword : Microbial Keratitis, Cystic Fibrosis, ProteaseIV.

Introduction

Pseudomonas aeruginosa is the species type of the genus Pseudomonas. It is an opportunistic human pathogen[1] which can cause both acute and chronic infections as keratitis and cystic fibrosis respectively.

And it is one of the most vital species that cause infection, causing several hospitals, including inflammation of the urinary tract and respiratory infections and inflammation of the joints especially in people who suffer from HIV infections Ail[2.]In the case of cystic fibrosis, a chronic lung disease which can be fatal .[3] and caused by *Pseudomonas aeruginosa* non mucoid isolates, which later convert to be resistant mucoid isolates advantage of the antibiotics and means of defense in the body [4]*P. aeruginosa* has alsoremained the most common cause of keratitis.it is characterized by the infiltration of inflammatory cells and tissue destruction, which can lead to corneal perforation[5]. Up to this point, most instances of bacterial keratitis were connected with visual injury ,visual surface illness and earlier visual surgery[6]. But some recent studies refers that a relationship between using contacted lenses and Keratitis [7].

Although there are substantial differences between the two cases, but they share some general characteristics such as the site of injury, a mucousal tissue, which is usually has a few normal flora in the absence of injury[8].

P aeruginosa has many virulence factors which enabled the bacteria to cause both acute and chronic infections one of these virulence factors is protease or peptidases are enzymes that can hydrolyze peptide bonds

within peptides and proteins [9]. *P. aeruginosa*is fit for discharging seven unique proteases; these are elastase A(Las An), elastase B (Las B)[10]modifiedelastase alkaline protease (AP), protease IV, *pseudomonas aeruginosa* small protease (PASP)[11], and the large exoprotease (Lep A) [12]. Las A, Las B, modified elastase, and AP are metalloproteinase and might be created by just a few strains [13].Protease IV is imperative in the pathogenesis of *Pseudomonas aeruginosa* initiated microbial keratitis, yet little is known about its part in cystic fibrosis (CF) lung contamination. The destructiveness of protease IV in visual contamination has been ascribed to the demolition of host proteins, including fibrinogen and parts of the resistant framework [14]. protease IV may add to intense lung damage initiated by *P. aeruginosa* through loss of surfactant capacity [15]. this study tried to compare between *P. aeruginosa* which isolated from CF patients and anther isolated from keratitis patients phenotypically, genetically and by the activity of protease IV in both isolates.

Materials and Methods

Clinical samples

An 80 samples of *P. aeruginsa* clinical isolates were obtained between Jeanery 2013 and February 2014 from patients attending teaching Diwanyia Hospital because of keratitis and Cystic fibrosis

All isolates were grown on nutrient agar and then characterized as *P.aeruginosa* according to colony morphology, Gram's stain appearance, oxidase reaction, and growth at 41°C [16].

Isolation of Genomic DNA

Genomic DNA of the isolates was extracted according to [17]Method .

Zymography analysis

This move has been made for the purpose of comparison between the effectiveness of the protease IV enzyme in *Pseudomonas aeruginosa* bacteria that cause chronic disease and other causes acute one. Gelatin zymography strategies in light of those of [18]was Adopted.

Results and Discussion

Phenotypic comparison

The studied of phenotype characteristics are shown in in table 1 below which explain that 50 of 80 samples were cystic fibrosis cases, 27 of them were mucoid colonies what should be referred to M and the rest 13 were nonmucoid and referred to NM. Both M and NM colonies were isolated from the same patients .while all *p. aeruginosa*strains isolated from keratitis patients were nonmucoied.

In fact mucoid colonies consider a protection picture which forms under severe conditions (release of reactive oxygen intermediates from PMNs, high osmolarity, dehydration, nutrient limitation, presence of antibiotics).

[19]proofed that this conditions influence *P aeruginosa*to produce alginate (a negatively charged copolymer composed of a-D-mannuronic acid and guluronic acid).and alginate are capable of convert NM bacteria to M bacteria ,or the bacteria use mucoid property in order to be chronic. it also give the microorganism a protection from hosts defense and anti biotics this lead to suppose that the mucosal character is a major difference between MK and CF infection with *P.aeruginosa*.

Table1 phenotypic analysis of *P aeruginosa* isolates

source patient	Number of samples	mucoid	nonmucoid
CF	50	27	23
MK	30		30

Isolation of Genomic DNA

After isolation of genomic DNA and using UV spectrophotometer many bands at different wave lengths have been appeared not only for

P .aeruginosa which isolated from different infections ,it was also different in *P .aeruginosa* isolated from same infection with different patients as seen in fig 1 below.

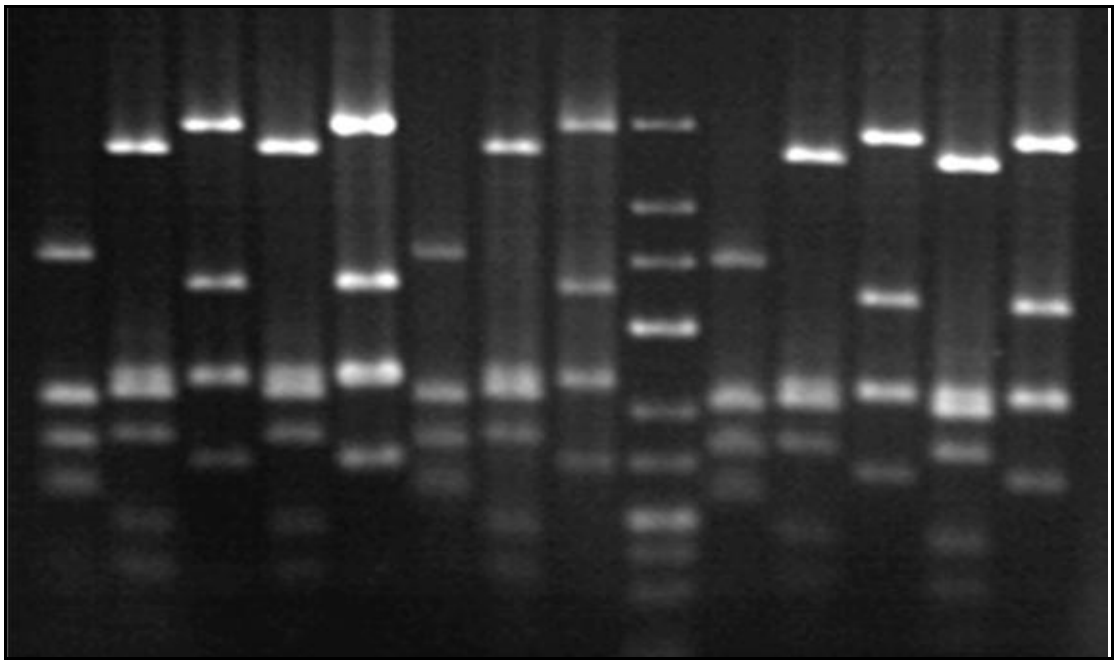

Fig 1 Isolation of genomic DNA from P.auroginosa according to

This wide genetic versatility give the microbes the capacity to invade different destinations of human body. This results agree with [19] who said the remarkable capacity of *P. aeruginosa* to adjust to a wide assortment of situations might be because of its broad hereditary adaptability that guarantees it's pathogenic potential.

Location of protease IV in clinical disconnects

The exercises of protease IV in *P. aeruginosa* confines were imagined by gelatin zymography. A gelatinolytic band at an obvious sub-atomic mass of ~350 kDa was found in all examples (Fig. 3). This band has already been proposed to be protease IV[20]in spite of the fact that the catalyst has an atomic mass of 26 kda, totals under SDS decreasing circumstances and is settled at a high sub-atomic mass in the gel[21]

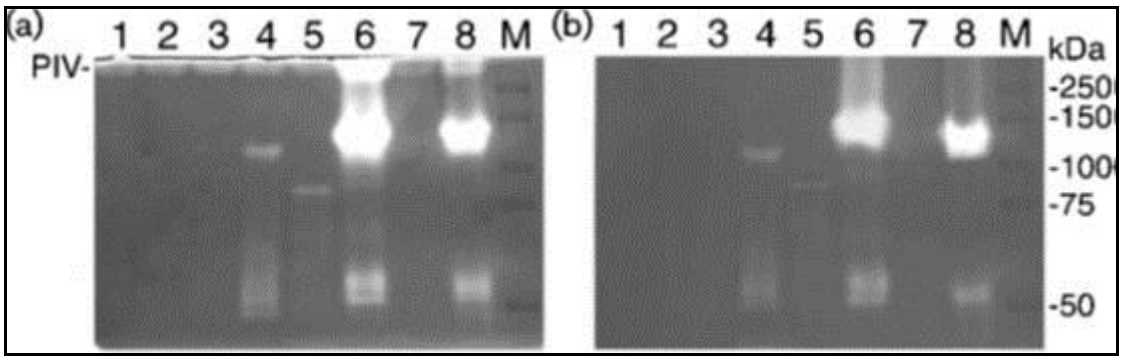

Fig. 4. Protease activity of *P. aeruginosa* strains. gelatin zymogram,(a) Afloats run innon-attendanceof the protease IV inhibitor TLCK. (b) Supernatants run in the presence of protease IV inhibitor TLCK. Lanes:(P1…..8).according to[18]

In the present study the activity of protease IV has been taken under comparison because this consider as one of the important pathogenic factor in *P. aeuroginosa* because of its Role in host-pathogen interaction as we know hemorrhage is a characteristic of *P. aeruginosa*[22]infection.which is come from the ability of Protease IV to cleave bovine fibrinogen *in vitro* .this is the last is conferred to a fibrin cloth after vascular damage. dysfunction of fibrinogen will lead to hemorrhage.

In keratitis Proteases add to pathogenesis through annihilation of connective tissue and corruption of host immunological elements[23] the present study found that the activity of protease IV in MK is less than the activity of the enzyme in CF samples . to explain this results more researches one of them the study of [24]which investigate the gene sequence of PIV gene and founded that there is relationship between the enzyme activity and the presence or absence of exotoxin S, where is the *exo*S –containing isolates are low producing PIV enzyme.

Actually its very complex to understand the mechanisms of the interaction among the virulence factors of any pathogen ,but in the present study as regarded with PIV enzyme activity it may effect on the action of anther virulence factors this need more studies and investigations .

In brief ,we have Identified that *P. aeuroginosa* isolated from Cystic Fibrosis samples are from Microbial Keratitis samples phenotypically by mucoied colonies formation in CF samples, this mucularity helps in chronic effect of the infection , and structurally both isolates possess protease IV enzyme and rather have the same action in the different cases but it's activity in MK is less than from CF samples

References

1. Palleroni NJ. Introduction to the family pseudomonadaceae. In: Balows HG, Truper M, Dworkin W, Harder A, Schliefer K, editors. The Prokaryotes. 2nd edition. Springer-Verlag, New York, 1992;p:3071– 85.
2. Aloush V, Navon-Venezia S, Seigman-Igra Y, Cabili S, Carmeli Y. Multidrug-resistant Pseudomonas aeruginosa: risk factors and clinical impact. Antimicrob Agents Chemother. 2006;50(1):43–8.
3. Pier, G. B. 1985. Pulmonary disease associated with *Pseudomonas aeruginosa* in cystic fibrosis: current status of the hostbacterium interaction. J. Infect. Dis. 151:575-580
4. Yoon SS, Coakley R, La GW *et al.* Anaerobic killing of mucoid *Pseudomonas aeruginosa*by acidified nitrite derivatives under cystic fibrosis airway conditions. J Clin Invest 2006;116:436-46.
5. Rattanatam T, Heng WJ, Rapuano CJ, Laibson PR, Cohen EJ. Trends in contact lens-related corneal ulcers. Cornea 2001; 20:290-4. [PMID: 11322418].
6. Yoon SS, Coakley R, La GW *et al.* Anaerobic killing of mucoid *Pseudomonas aeruginosa*by acidified nitrite derivatives under cystic fibrosis airway conditions. J Clin Invest 2006;116:436-46.
7. Mereghetti L, Marquet-van der Mee N, Loulergue J et al. *Pseudomonas aeruginosa* from cystic fi brosis patients: study using whole cell RAPD and antibiotic susceptibility. Path Biol (Paris) 1998; 46:319-24.
8. Van Delden C, and Iglewski BH. Cell-to-cell signaling and *Pseudomonas aeruginosa* infections. Emerg Infect Dis 1998; 4:551-60.
9. Choy MH, Stapleton F, Willcox MD, Zhu H. Comparison of virulence factors in related keratitis. J Med Microbiol. 2008;57:1539–46.
10. Rattanatam T, Heng WJ, Rapuano CJ, Laibson PR, Cohen EJ. Trends in contact lens-related corneal ulcers. Cornea 2001; 20:290-4. [PMID: 11322418].
11. M. D. P. Willcox, H. Zhu, T. C. R. ConibearE. B. H. Hume, M. Givskov S. Kjelleberg5 and S. A. Rice Role of quorum sensing by *Pseudomonasaeruginosa*in microbial keratitis and cystic fibrosis. CorrespondenceS. A. Rice Scott.Rice@unsw.edu.au Microbiology (2008), 154, 2184–2194.
12. Fonseca AP, Correia P, Sousa JC, Tenreiro R. Association patterns of *Pseudomonas aeruginosa*clinical isolates as revealed by virulence traits, antibiotic resistance, serotype and genotype. FEMS Immunol Med Microbiol. 2007;51:505-16.
13. Sambrook J. Molecular cloning: A laboratory manual. 3rd ed. New York, NY, Cold Spring Harbor Laboratory, 2001.

14. Versalovic J, Koeuth T, Lupski JR. Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. Nucleic AcidsRes. 1991;19:6823-31.
15. Dawson SL, Fry JC, Dancer BN. A comparative evaluation of five typing techniques for determining the diversity of fluorescent pseudomonads. J Microbiol Methods. 2002;50:9-22.
16. Pavlícek A, Hrdá S, Flegr J. Free-Tree—freeware program for construction of phylogenetic trees on the basis of distance data and bootstrap/jackknife analysis of the tree robustness. Application in the RAPD analysis of genus Frenkelia. Folia Biol (Praha). 1999;45:97–99.
17. Page RD. TreeView: an application to display phylogenetic trees on personal computers. ComputApplBiosci. 1996;12:357–58.
18. Fuentefria DB, Ferreira AE, Corção G. Antibioticresistant*Pseudomonas aeruginosa* from hospital wastewater and superficial water: are they geneticallyrelated? J Environ Manage. 2011;92:250-5.
19. Twining, S. S., Kirschner, S. E., Mahnke, L. A. & Frank, D. W. (1993). Effect of *Pseudomonas aeruginosa*elastase, alkaline protease, and exotoxin A on corneal proteinases and proteins. Invest Ophthalmol Vis Sci 34, 2699–2712.
20. Engel, L. S., Hobden, J. A., Moreau, J. M., Callegan, M. C., Hill, J. M. &O'Callaghan, R. J. (1997). Pseudomonas deficient in protease IV has significantly reduced corneal virulence. Invest Ophthalmol Vis Sci38,1535–1542.
21. Leber, T. M. &Balkwill, F. R. (1997). Zymography: a single-step staining method for quantitation of proteolytic activity on substrategels. Anal Biochem 249, 24–28.
22. Elliott BW, Cohen C. Isolation and characterization of a lysine-specific protease from Pseudomonas aeruginosa. *Journal ofBiological Chemistry*. 1986; 261:11259-11265.
23. Engel, L. S., Hill, J. M., Moreau, J. M., Green, L. C., Hobden, J. A. &O'Callaghan, R. J. (1998). *Pseudomonas aeruginosa* protease IVproduces corneal damage and contributes to bacterial virulence.Invest Ophthalmol Vis Sci 39, 662–665.

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