Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.4, No.23, 2014

Determination the genotyping diversity between biofilm forming and collagenase producing *Pseudomonas aeruginosa* strains

Amal Aziz Kareem[1*] Shatha Salman Hassan[2]
1.Middle Technical University, Foundation of technical Education , Collage of medical and Health technology ,Unit of Researchs
2.University of Baghdad,Department of biology,Collage of science
Tel:009647801347597 E-mail:amal.ziz68@yahoo.com

Abstract

A total of 82 *Pseudomonas aeruginosa* strains isolated from four hospitals in Baghdad. The isolates were studied by repetitive element based PCR (rep-PCR) using BOX primer.**Methode**: Biofilm determination method was used to screen the 82 isolates of forming biofilm .Collagenase production assay was used to screen the 28 isolates that were strong biofilm formers Collagenase production assay was used to screen the 28 isolates that were strong biofilm formers.**Results:** collagenase production increases when bacteria switch from a planktonic to biofilm phenotype. This indicates that biofilms and collagenase are more virulent and have a greater ability to cause tissue destruction . The REP-PCR analysis using BOX-primer, showed a clusters genetic relatedness among the isolates. The isolates were grouped according to the REP-PCR in 9 different genotypes, named cluster 1 to 3 which included C1, C2 ,C3 with relatedness :8 (80%), 8 (86%) ,3 (80%) respectively . A19 and A20 both of them were not included in any cluster , they have 78% similarity .The REP-PCR analysis showed that the genotypic relatedness is consistently high between the 8 producer isolates and non producer isolates (13),showed similarity reached 86% between collagenase and biofilm producers .

Keywords: Genotyge ,rep-PCR,Collagenase,Biofilm,*Pseudomonas aeruginosa*,BOX primer

Introduction

Pseudomonas aeruginosa is an ubiquitous organism which has emerged as a major threat in the clininical and environmental habitates. This bacterium is the most frequently isolated Gram-negative organism in blood stream and wound infections, pneumonia and intra-abdominal and urogenital sepsis, and is a serious problem, infecting immunocompromised patients with cystic fibrosis (CF), severe burns, cancer, AIDS, *etc*. [(1-2)]. One of the most worrying characteristics of this bacterium is its low antibiotic susceptibility, which can be attributed to a concerted action of multidrug efflux pumps with chromosomally-encoded antibiotic resistance genes and the low permeability of the bacterial cellular envelopes [(3-4)]. *Pseudomonas aeruginosa* is wonderfully adept at forming highly organized surface associated communities encased within an exopolysaccharide and protein matrix, known as biofilms [(3)], biofilm represents a protected mode of growth that allows bacteria to withstand harsh environmental conditions. The ability of bacteria to colonize virtually any surface and form biofilms has made them a major cause of medical infections [(5)]. Biofilm structures protect cells from environmental stresses, host immune responses and antimicrobial therapy [(6)]. The first step in *P.aeuroginosa* infection is that adherence of *P.*aeuroginosa to epithilum surface is mediated by pili, flagella and Alaginate [(7)]. the biofilm formation that helped *P.*aeuroginosa to escape from host defense mechanisms and resist to antibacterial action of antibiotics [(8)] the second step include colonization of *P.*aeuroginosa and produce several extracellular virulence factors which involved pyocyanin , hemolysine, alkaline protease ,elastease, neuraminidase, and exotoxins A,S,U,Y,T responsible for extensive tissue damage ,blood stream invasion and dissemination , many of these extracellular virulence factors are controlled by cell to cell signaling system [(9)]. Collagens are the major protein constituents of the extracellular matrix and the most abundant proteins in all higher organisms. The tightly coiled triple helical collagen molecule assembles into water-insoluble fibers or sheets which are cleaved only by collagenases, and are resistant to other proteinases. Various types of collagenases, which differ in substrate specificity and molecular structure, have been identified and characterized. Bacterial collagenases differ from vertebrate collagenases in that they exhibit broader substrate specificity [(10)]. Collagenase is a zinc metalloproteinase that catalyses the hydrolysis of native collagens, requires zinc and calcium ions as enzyme cofactors for its optimum activity with the pH of 6.3-7.5.It normally target the connective tissue in muscle cells and other body organs. Collagen, an inert, rigid protein found predominantly in skin, tendon, blood vessels, ligaments and bones, a key component of the animal extracellular matrix, is made through cleavage of procollagen by collagenase [(11)]. Trains of *P. aeruginosa* can be internally divided into subgroups by classical methods such as: biotyping, serotyping, pyocin typing, phage typing and antibiotic sensitivity of tested strains.However, the discriminatory power is much lower than that obtained by molecular typing methods. DNA typing methods have been frequently used to investigate the diversity of collections of *P. aeruginosa* [(12)]. These methods include pulsed-field gel electrophoresis (PFGE) [(12-13)], ribotyping [(13-14)] restriction fragment length polymorphic DNA analysis (RFLP) [(14)] random amplified polymorphic DNA assay (RAPD) [(12-15)] , arbitrary primed PCR (AP-PCR) [(16)] amplified fragment length polymorphism (AFLP) [(12)] , and repetitive element based PCR (rep-PCR) [(13-14)]. Rep-

PCR is amethod for fingerprinting bacterial genomes, which examines strain-specific patterns obtained from PCR amplification of repetitive DNA elements present within bacterial genomes. Three main sets of repetitive elements are used for typing purposes: the repetitive extragenic palindromic (REP) sequence, the enterobacterial repetitive intergenic consensus sequence (ERIC) and the BOX elements (17). The aim of this work was to determine the genetic patterns for differant *Pseudomonas aeruginosa* strains (collagenase producer and strong biofilm formers with non collagenase producers and moderate biofilm formers) by using repetitive element based PCR (rep-PCR) using BOX primer .

Material and methods

Bacterial strains. Eighty two strains of *Pseudomonas aeruginosa* were originally isolated from a variety of clinical specimens from different wards of the Hospitals, the main Hospitals in (Baghdad), and environmental isolates from hospital environment,soil , food , water between May 2012 and October 2012. The isolates were identified as *Pseudomonas aeruginosa* according to biochemical patterns in the VITEC-2 compact system . The stock cultures were stored in BHI (Brain Heart broth, LAB) containing 20% glycerol at –80°C.

Biofilm Formation Determination: BHI broth with 1% glucose was used as a medium for growth of bacteria and biofilm formation. In each test tube, containing 10mL of this medium one loopful of 18hrs fresh culture of *P. aeruginosa* was inoculated and incubated for 24 hrs at 37°C. The cell suspensions were poured out off the tubes and washed with distilled water and dried, keeping the tubes in inverted state. The walls of the tubes were stained with 1% crystal violet for 15 min and then washed with distilled water. The tubes were again dried and the viscid layer produced on the walls were interpreted as biofilm production. Ring formation only at the liquid interface should not be considered as biofilm formation, it should be a visible ring along with the film lined the wall and the bottom of the test tube. Biofilm, production was scored as negative (-), weak positive (1+), moderate positive (2+) or strong positive (3+) [18] .

Congo red agar method: BHI broth 37g, glucose 50g and agar 15g dissolve in 900 ml of D.W. then sterilized, cooled to 55 ºC, and 100 ml of congo red solution(congo red (Merck) 0.8g dissolve in 100ml D.W) was added and poured into sterilized Petri-dishes [19] .Then was inoculated with single colony of the bacteria by streaking, and incubated at 37ºC for 24 hr.; a black colonies with a dry crystalline consistency indicated biofilm formation while non-slime producers usually remain pink. The isolates that appeared as black colonies and black medium were given +++ or ++++ results and the isolates that gave red colonies without black medium were given ++ or + . The experiment was performed in triplicate.

Collagenase Detection : Mineral salt agar with collagen was used for this purpose [20]. This medium is composed g/100ml **:**Glucose 1.0g,Collagen 0.4g,K2HPO4 0.1g,KH2PO4 0.05g,MgSO4.7H2O 0.05g ,FeSO4.7H2O 0.01g,ZnSO4.7H2O 0.001g,CaCO3 0.2g Agar 1.5g,all these components were dissolved in 100ml of distilled water, pH was adjusted to 7. This medium was used for semi-quantitative screening for collagenase production.

Collagenase production: The medium was prepared according to [21] with some modification, it composed (g /00ml) :NaCl 1g,glucose 0.25g,Yeast extract 0.25g,collagen 0.5g(Bovina Achills tendon/Sigma Aldrich),all these components were dissolved in 100ml of distilled water, pH was adjusted to 7.

DNA extraction. Isolates were grown in blood agar at 37°C for 24 h and DNA was extracted using the ZymoResearch Fungal/BacterialDNA MiniPrep™ kit (USA).

BOX-PCR typing. BOX-PCR fingerprinting was carried out using one primer of sequence 5'-CTACGGCAAGGCGACGCTGACG-3'. Amplification was carried out with a 10 × PCR buffer (100 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 100 mM KCl, 0.5% Nonidet P40, 0.5% Tween 20) in a total reaction of 50 µl containing 2.5 mM dNTP, 20 mM MgCl2, 100 pmol of primer, 2 µl of genomic template DNA,and 1 unit of Taq DNA polymerase (Promega, USA).

BOX-PCR typing was carried out according to Dawson et al. (2002) using a PTC-100 Programmable Thermo Controller (MJ Research) according to the following procedure: initial denaturation at 94°C for 5 min followed by 35 cycles of PCR consisting of denaturation at 94°C for 1 min, annealing at 48°C for 2 min, and extension at 72°C for 2 min; in the last cycle, the extension time was 5 min. The PCR product (10 µl) was analyzed using a 2% agarose gel in the TBE buffer (5.4 g l–1 Tris, 2.75 g l–1 Boric acid, 0.37 g l–1 EDTA (pH 8.0)) and photographed under a UV light. The size of the products was analyzed using a M100–1000 bp ladder MW size marker (Promega, USA).

Results :

Screening of biofilm forming isolates Eighty two *Pseudomonas* isolates were screened for biofilm formation by two methodes included :

Qualitative methods by Congo-red agar method (CRA) Qualitative assay was applied by culturing on CRA, the non-producers form red colonies ,while the biofilm producers appear black [22] . All *Pseudomonas* isolates have the ability to produce slime layer that is very necessary for biofilm formation but in different degree as

shown in table (1) which depends on the color of the colonies pink (+), black(++),deep black (+++).Twenty nine *Pseudomonas* represented high production of biofilm and twenty seven moderate while twenty two were low producers .

Quantitative method (Micro-titer plate assay) Eighty two *Pseudomonas* were assayed for production of biofilm. The results are summarized in table (2) which includes, the statistical analysis of biofilm for all these isolates were considered as a biofilm producers but the suggested interval ranges are classified in three classes according to the mean values of each three replicates and named (weak, moderate, and strong) with respect (0.107, 0.198 and 0.289–0.386) respectively, red color represented strong biofilm producers .

Qualitative screening of collagenase producing *P.aeruginosa* : Twenty eight *P.aeruginosa* isolates that distinguished by their high biofilm production were selected and examined for collagenase production on a medium containing collagen . Only eight isolates grew on collagen medium after four days of incubation . The ability of isolates to degrade collagen in the medium differed figure (2) .*P.aeruginosa* A3 ,A5 and A7 gave high growth zone (18mm for *P.aeruginosa*A3and A5 ,16mm for A7) while the other isolates can not grow in media containing collagen.

Genotype :

BOX-PCR typing was carried out to differentiate the twenty-one *P. aeruginosa* isolates divided into two groups, the first included 8 collagenase producer and strong biofilm former and the second included 13 non collagenase producer and strong biofilm former (figure 3).

The isolates were grouped according to the BOX-PCR to 9 different genotypes being their similarity (cut off point) were greater than 80 % as it depicted in figure (4), *named clusters.* BOX-PCR fingerprinting revealed 3 main *clusters* as it is shown in table (4).

Cluster 1 (C1) members shared 80% similarity consist of 6 environmental isolates and two clinical isolates. Nevertheless, cluster 2 (C2) with similarity 86% consist of 6 clinical isolates and two environmental isolates. On the other hand, cluster 3 (C3) has 80% similarity consisted 3 clinical isolates. What's more, *P.aeruginosa* A19 and A20 both of them were not included in any cluster yet they have 78% similarity.The REP-PCR analysis showed that the genotypic relatedness is consistently high between the 8 collagenase producer isolates and the 13 non producer isolates (the similarity reached 86% for collagenase producer isolates) as shown in (figure 5).

Discussion

Eighty two *Pseudomonas* isolates were screened for biofilm formation by two methodes included : Qualitative assay was applied by culturing on CRA.The Congo red dye directly interacts with certain polysaccharides, forming colored complexes [22] . All *Pseudomonas* isolates have the ability to produce slime layer that is very necessary for biofilm formation but in different degree.Twenty nine *Pseudomonas* represented high production of biofilm and twenty seven moderate while twenty two were low producers .[23] reported that the Congo Red method is a rapid, more sensitive, and reproducible method ,furthermore the colonies remaining viable on the medium.

Quantitative method (Micro-titer plate assay) The microtiter dish assay is an important tool for the study of the early stages in biofilm formation, and has been applied primarily for the study of a wide variety of microbes .The isolate showed a different potential capacity to form biofilm under the same conditions of experimentation,The isolates from burns revealed high biofilm formation then UTI , seputum and ear infections while wounds , keratitis , soil and sewage represented less biofilm formation . Crystal violet is a basic dye known to bind to negatively charged molecules on the cell surface as well as nucleic acids and polysaccharides, and therefore gives an overall measure of the whole biofilm. It has been used as a standard technique for rapidly accessing cell attachment and biofilm formation in a range of Gram positive and Gram-negative bacteria [23-24] . The motile microbes typically adhere to the walls and/or bottoms of the wells, while the non-motile typically adhere to the bottom of the wells. *Pseudomonas* are motile organisms and form a biofilm at the air-liquid interface [25]. [26]demonstrated that *P. aeruginosa* rapidly colonizes burn wounds and formes biofilms primarily around blood vessels.

Qualitative screening of collagenase producing *P.aeruginosa* :

The ability of isolates to degrade collagen in the medium differed only eight degeraded collagen in medium other isolates could not, refer to cannot these isolates to produce collagenase or not hve the gene responsible to encode to collagenase ,in order to degraded collagen as a source of nitrogen necessary to live**.**

Genotyping

Genotyping, for discrimination of bacterial strains based on their genetic content, has recently become widely used for bacterial strain typing. The methods already used in genotyping of bacteria are quite different from each other [27] .

BOX-PCR fingerprinting is capplicable for typing of P. aeruginosa isolates and can be considered a useful complementary tool for epidemiological studies of members of this genus [28].

[29] represented that BOX-PCR is a rapid, highly discriminatory and reproducible assay that proved to be powerful surveillance tools for typing as well as characterizing clinical *P. aeruginosa* isolates. The study of [13], in which the BOX-PCR method showed a high discriminatory power.The environmental isolates arranged with clinical isolates in the same genotype clusters; which may indicate the transmission of the pathogens from hospital environment -to-patients as well as spreading of the pathogens in the environment of hospital. The pathogens may also transmit from patient-to-patient either via contaminated medical equipment, via the air of the hospital environment which may be contaminated with those bacteria or via the hands of medical staff.

The differences in the distributions of virulence factor genes in the populations strengthen the probability that some *P. aeruginosa* strains are better adapted to the specific conditions found in specific infectious sites [30].

Many extracellular virulence factors secreted by *P. aeruginosa* have been shown to be controlled by a complex regulatory circuit involving cell-to-cell signaling systems that allow the bacteria to produce these factors in a coordinated, cell-density–dependent manner. Additionally, the proteolytic potential slightly increased when biofilms are exposed to sublethal concentrations of selected antibiotics. This possibly explains the results of clinical studies that show increased severity of disease when subtherapeutic doses or inadequate duration of antibiotics are used [31].

Conclusion

All *P.aeruginosa* isolates form biofilm but at differed levels weak ,moderate and strong but few of them can produce collagenase .Afew of isolates can produce collagenase . REP-PCR showed that 21 isolates were clustered into three different genotypes and the relatedness was 86% between eight strains that was collagenase and bioflim producer.

References

1. Driscoll JA, Brody S.L, Kollef MH. (2007) The epidemiology, pathogenesis and treatment of *Pseudomonas aeruginosa* infections. Drugs 67: 351–368.
2. Page M.G.P.; and Heim J. (2009) Prospects for the next anti-Pseudomonas *drug. Curr. Opin. Pharmacol.* 9:558-565.
3. Masadeh MM, Mhaidat NM, Alzoubi KH, Hussein EI, Al-Trad Esra'a I.(2013):In vitro determination of the antibiotic susceptibility of biofilm-forming *Pseudomonas aeruginosa* and *Staphylococcus aureus*: possible role of proteolytic activity and membrane lipopolysaccharide. Infection and Drug Resistance .6:27–32
4. Pires D, Sillankorva , Faustino SA and Azeredo J .(2011) Use of newly isolated phages for control of Pseudomonas aeruginosa PAO1and ATCC 10145 biofilms. *Research in Microbiology* 162 :798-806
5. Freire-Moran L, Aronsson B, Manz C, Gysson IC, So,et al. (2011) Critical shortage of new antibiotics in development against multidrug-resistant bacteria--Time to react is now. *Drug Resistance Updates* 14:118-124.
6. Mathias MS, Stefano Di F, Ute R.mling and Susanne H.ussler .(2010) A 96-well-plate|[ndash]|based optical method for the quantitative and qualitative evaluation of *Pseudomonas aeruginosa* biofilm formation and its application to susceptibility testing .*Nature Protocols* 5:1460–1469.
7. Cotar A, Chifiriuc M, Dinu S, Bucur M, Iordache C, Banu O.et al.(2010) Screening of Molecular Virulence Markers in Staphylococcus aureusand Paeudomonas aeuroginosa Strains Isolated from Clinical Infections. *Int.J.Mol.Sci.*11:5273-5291 .
8. Prasad SV, Ballal M. and Shivananda PG. (2009) Slime production a virulence marker in Pseudomonas aeuroginosastrains isolated from clinical and environment specimens : A comparative study of two methods. *Indian.J. Pathol. Microbiol.* 52.
9. Nishi and Akinobu Okabe, Junzaburo M, Seiichi K, Osamu M, Chang-Min J,(1998) A Study of the Collagen-binding Domain of Collagenase a 116-kDa *Clostridium histolyticum .J. Biol. Chem.* 273:3643-3648.
10. Lodish HA, Berk SL, Zipursky, et al.(2000) *Molecular Cell Biology*. 4th edition, New York, W.H. Freeman.
11. Speert, D.P. (2002). Molecular epidemiology of *Pseudomonas aeruginosa*. *Front. Biosci.* 1: 354-361.
12. Speijer H, Savelkoul PHM, Bonten MJ, Stobberingh EE, Tjhie JHT. (1999) Application of different genotyping methods for *Pseudomonas aeruginosa* in a setting of endemicity in an intensive care unit. *J. Clin. Microbiol.* 37: 3654-3661.
13. Syrmis MW, O´Carrol MR, Sloots TP, Coulter C, Wainwright CE, et al (2004) Rapid genotyping of *Pseudomonas aeruginosa* isolates harboured by adult and paediatric patients with *cystic fibrosis* using repetitive-element-based PCR assays. *J. Med. Microbiol.*53:1089-1096.

14. Dawson SL, Fry JC, Dancer BN. (2002). A comparative evaluation of five typing techniques for determining the diversity of fluorescent pseudomonads. *J. Microbiol. Meth.* 50: 9-22.
15. Liu Y, Davin-Regli A, Bosi C, Charrel RN, Bollet C. (1996) Epidemiological investigation of *Pseudomonas aeruginosa* nosocomial bacteraemia isolates by PCR-based DNA fingerprinting analysis. *J. Med.Microbiol.* 45: 359-365.
16. Czekajło-Kołodziej U, Giedrys-Kalemba, S, M_drala D. (2006) Phenotypic and genotypic characteristics of *Pseudomonas aeruginosa* strains isolated from hospitals in the north-west region of Poland. *Pol. J.Microbiol.* 2:103-112.
17. Olive MD, Bean P. (1999) Principles and applications of methods for DNA-based typing of microbial organisms. *J. Clin. Microbiol.* 37:1661-1669.
18. Basu S, D. Chakraborty SK, Dey and S. Das. (2011) Biological Characteristics of Nosocomial *Candida tropicalis* Isolated from Different Clinical Materials of Critically Ill Patients at ICU. *Int. J. Microbiol. Research*, 2: 112-119.
19. Freeman DJ, Falkner FR, Keane CT. (1989): New method for detecting slime production by coagulase-negative staphylococci. *J Clin Pathol,* 42:872-874.
20. UstyuzhaninaSV, YarovenkoVL, VoinarskiiI.N. (1985) Synthesis of protease and α-amaylase by washed celles of *Aspergillus oryzae* 251-90.*Appl.Biochem.Microbiol.*22:55-58.
21. Bholay AD, More SY, Patil VB, and Patil N. (2012) Bacterial Extracellular Alkaline Proteases and its Industrial Applications, International Research *Journal of Biological Sciences.* 1:1-5.
22. Jain A. And Agarwal A. (2009) Biofilm production, a marker of pathogenic potential of colonizing and commensal staphylococci. *J Microbiol Methods*,76:88-92.
23. Pfaller MA.; Davenport D.; Bale M.; Barret M.; Koontz F.;and Massanari R. (1988): The development of the quantitative micro-test for detecting the slime production by the coagulase negative Staphylococci Eur J Clin Microbiol Infect Dis. 7:30-33.
24. Djordjevic D, Wiedmann M. and McLandsborough LA (2002) Microtiter plate assay for assessment of Listeria monocytogenes biofilm formation. *Appl. Environ. Microbiol.*68:2950-2958.
25. O'Toole G.A. (2011) Microtiter dish biofilm formation *assay.J.Vis..Exp.* 47:2437.
26. Al-Dahmoshi H. and Oleiwi M. (2013) Genotypic and Phenotypic Investigation of Alginate Biofilm Formation among *Pseudomonas aeruginosa* Isolated from Burn Victims in Babylon, Iraq. *Science J. of Microbiology*. 20: 8
27. Yıldırım İH, Yıldırım SC, Koçak N. (2011) Molecular methods for bacterial genotyping and analyzed gene regions. *J Microbiol Infect Dis.*1: 42-46.
28. Wolska K, Kot B, Jakubczak A, Rymuza K.(2011) BOX-PCR is an adequate tool for typing of clinical *Pseudomonas aeruginosa* isolates. Folia Histochemicaet Cytobiologica. 49(4) :734–738.
29. Wolska K, Kot B, Jakubczak A.(2012) Phenotypic and genotypic diversity of *Pseudomonas aeruginosa* strains isolated fromhospitals in siedlce (Poland). *Brazilian Journal of Microbiology*. 274-282.
30. Lanotte P, Mereghetti L, Dartiguelongue N, Rastegar-Lari A, Gouden A. and Quentin R. (2004): Genetic features of *Pseudomonas aeruginosa* isolates cystic fibrosis patients compared with those of isolates from other origins. *J Med Microbiol* .53: 73-81
31. Frei E, Hodgkiss-Harlow K, Rossi PJ, Edmiston CE, and Bandyk DF.(2011) Microbial pathogenesis of bacterial biofilms: a causative factor of vascular surgical site infection. *Vasc Endovascular Surg.* 45:688–696.

Table (1): Number and degree of colonies color of biofilm producer isolates on CRA

No. of isolates	CRA +	CRA ++	CRA +++
82	26	27	29

+pink colonies,++black colonies,+++deep black colonies

Table (2) : Summary of statistical analysis of biofilm formation by *Pseudomonas aeuroginosa* isolates

Classes	No.	Mean	Std. Dev.	Std. Error	95% Confidence Interval for Mean		Min.	Max.
					Lower Bound	Upper Bound		
weak	29	0.168	0.021	0.004	0.160	0.176	0.107	0.193
moderate	25	0.242	0.032	0.006	0.229	0.255	0.198	0.282
strong	28	0.336	0.026	0.009	0.314	0.358	0.301	0.380

Table (4) :Genotyping clusters and source of isolates with their description depending on collagenase and biofilm production

Cluster	Isolates	Source of isolates	Description
C1	A9,A10 A11,A12 A13,A14 A15 A21	Environment Environment Environment Wound Burn	All of these isolates are moderate biofilm former and non-collagenas producer.
C2	A1,A2 A3,A4 A5,A6 A7,A8	Burn Environment Burn Burn	All of them strong biofilm former and collagenase producer.
C3	A16,A18 A17	Wound Burn	This cluster is non collagenase producer with moderate biofilm former.

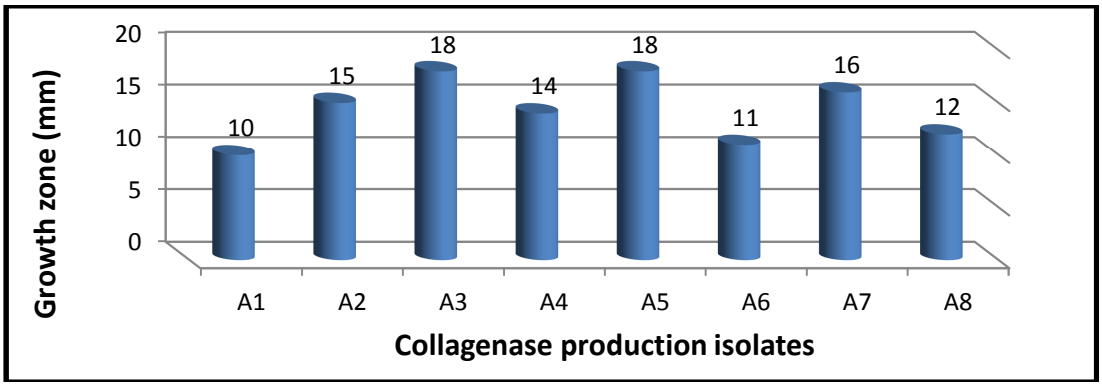

Fig.2: Growth zones (mm) of *P.aeruginosa* isolates on medium with 0.4 % collagen after 4 days incubation at 37°C.

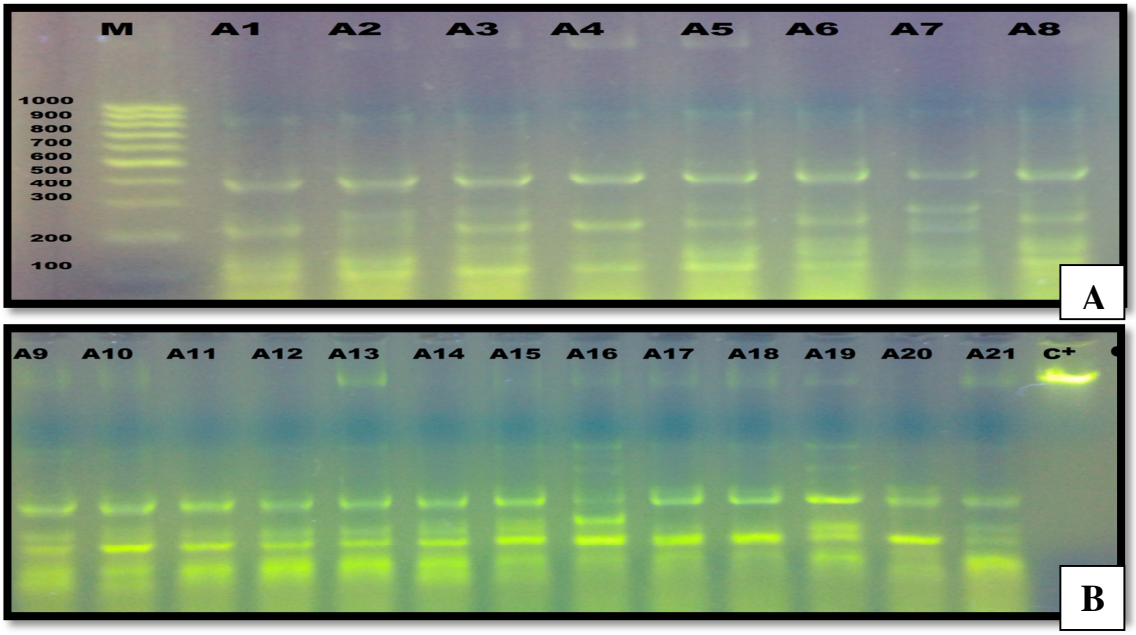

Figure (4) : BOX-PCR fingerprinting of *P. aeruginosa* isolates. A- Lanes 1-8 *P.aeruginosa* isolates of strong biofilm and collagenase producers. B – Lanes 9 to 21 - *P. aeruginosa* isolates of moderate biofilm formation and non-collagenase producers. Lane M: Molecular weight marker (MW100-1000bp) Lane C: Control positive. Lanes 1 to 8 - *P. aeruginosa* isolates of high biofilm formation and collagenase production

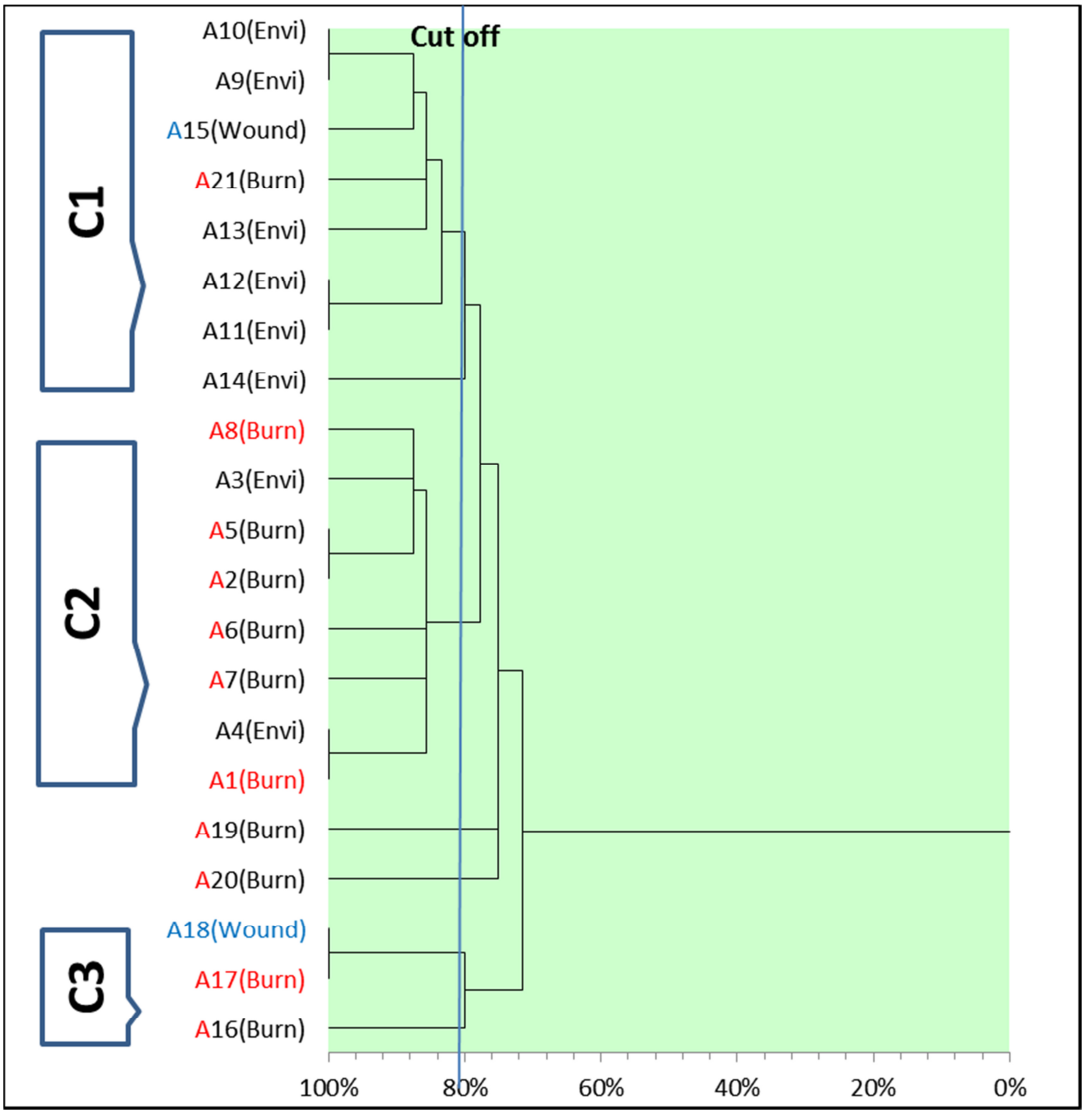

Figure (5): Dendrogram (cluster analysis) using BOX-PCR fingerprint patterns of 21 clinical and environmental *P.aeruginosa* isolates, red color referred to isolates from burns, wound insolates have blue color, while black color referred to environmental isolates.

The IISTE is a pioneer in the Open-Access hosting service and academic event management. The aim of the firm is Accelerating Global Knowledge Sharing.

More information about the firm can be found on the homepage:
http://www.iiste.org

CALL FOR JOURNAL PAPERS

There are more than 30 peer-reviewed academic journals hosted under the hosting platform.

Prospective authors of journals can find the submission instruction on the following page: http://www.iiste.org/journals/ All the journals articles are available online to the readers all over the world without financial, legal, or technical barriers other than those inseparable from gaining access to the internet itself. Paper version of the journals is also available upon request of readers and authors.

MORE RESOURCES

Book publication information: http://www.iiste.org/book/

Academic conference: http://www.iiste.org/conference/upcoming-conferences-call-for-paper/

IISTE Knowledge Sharing Partners

EBSCO, Index Copernicus, Ulrich's Periodicals Directory, JournalTOCS, PKP Open Archives Harvester, Bielefeld Academic Search Engine, Elektronische Zeitschriftenbibliothek EZB, Open J-Gate, OCLC WorldCat, Universe Digtial Library , NewJour, Google Scholar