Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 7, Issue, 10, pp.21750-21753, October, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

BIOFILMFORMING-*PSEUDOMONAS AERUGINOSA* ISOLATED FROM BURN PATIENTS IN BASRA CITY, SOUTH OF IRAQ

[1]Noora K. AbdulQader, [1]Waad M. Raoof and [*,2]Abdulameer A. Al-Mussawi

[1]College of Science, University of Tikrit, Iraq
[2]College of Nursing, University of Basra, Basra, Iraq

ARTICLE INFO

Article History:
Received 19th July, 2015
Received in revised form
28th August, 2015
Accepted 20th September, 2015
Published online 31st October, 2015

Key words:
Pseudomonas Aeroginosa,
Biofilm Forming,
Burn Infections,
HROMagar Media,
PCR, pslA, IcaAD.

ABSTRACT

Aims: 1- To evaluate the incidence of *Pseudomonas aeruginosa* bacterial pathogen in burn patients. 2- To determine the Biofilm producing *P. aeruginosa*
Methods: Total of 62 samples have been collected from patients who attended to the center of Burn in Al-Faihaa General Hospital in Basra City from the period 01/03/2015 to 1/6/2015. Including 36 (58%) females and 26 (42%) males, aged between 6 months – 78 years. All specimens were directly inoculated onto plated of P.P.A and incubated at 37 ºC for 24 hrs and identified by microscopic examination and biochemical tests include (Oxidase, indole, Vogas-Proskauer, urease, Methyl red, citrate utilization and Kligler) and using CHROMagar media. Ability of *P. aeroginosa* to forming biofilm tested by using Congo Red Agar and confirmed by genetic detection using *pslA*, *IcaA* and *IcaD* genes by PCR technique.
Results: The current results showed that, forty two (67.7%) positive samples were identified as *P. aeroginosa* by biochemical tests and CHROMagar medium, three samples (7.1%) were positive for biofilm forming on Congo red agar, three samples (7.1%) were positive to pslA gene, one (2.4%) positive to IcaA gene and all negative to IcaD gene.
Conclusions: We wish to emphasize that the P. aeroginosa present at high frequency among burn patients, and this give difficult to healing infections. A screening procedure using CHROMagar media give better results and faster in identifying *P. aeroginosa* than conventional methods. Gene identification was the best biofilm forming test.

Copyright © 2015 Noora K. AbdulQader et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Citation: Noora K. AbdulQader, Waad M. Raoof and Abdulameer A. Al-Mussawi, 2015. "BiofilmForming-*Pseudomonas aeruginosa* Isolated from Burn Patients in Basra City, South of Iraq", *International Journal of Current Research*, 7, (10), 21750-21753.

INTRODUCTION

Pseudomonas aeruginosa is a gram-negative, mesophilic, rod measuring 0.5 to 0.8 µm by 1.5 to 3.0 µm, optimum temperature for growth is 37ºC, aerobic, nonfermentative and obtain its energy from oxidation(Bergey's, 2001). *P. aeruginosa* produces a variety of virulence factors, these help the bacteria to adhere and invade to their host by damaging the host's immune responses and forming a barrier to antibiotics (Wilson and Dowling, 1998), include biofilms, protease enzymes, mucoid exopolysaccharide, pili, exotoxinA, lipopolysaccharide, pigments, lipase, haemolysin, histamine, exoenzyme S, and leukocidin (Schaber *et al*., 2004). The most important factor contributing to the pathogenesis of *P. aeruginosa* in causing fatal infections is its potential to form biofilms on biotic and abiotic surfaces (Karatuna and Yagci, 2010). Biofilms are complex communities of surface attached aggregates of microorganisms embedded in a self-secreted extracellular polysaccharide matrix, or slime (Stoodley *et al*., 2002). Polysaccharides are important components of the biofilm matrix, as they contribute to the overall biofilm architecture and to the resistance of biofilm-grown bacteria to certain antibacterial agents (Wozniak *et al*., 2003). At least three exopolysaccharides have been shown to be involved in biofilm formation by *P. aeruginosa,* including alginate, *Psl*, and *Pel* (Ghafoor *et al*., 2011). *Psl* is a mannose-rich polymer with an essential role in the initial steps of biofilm formation by non-mucoid *P. aeruginosa* as well as in its maintenance (Ma *et al*., 2006). *Psl* forms a helical structure around *P. aeruginosa* cells which increases the cell-to-surface and cell-to-cell interactions necessary for biofilm formation (Jackson *et al*., 2004). Synthesis of *Psl* is mediated by the *psl* gene cluster

Corresponding author: Abdulameer A. Al-Mussawi
College of Nursing, University of Basra, Basra, Iraq

(*pslA-pslO*) (Overhage *et al*., 2005), and *pslA* has been reported to be the first and most important gene necessary for *Psl* synthesis (Matsukawa and Greenberg, 2004). The current study was designed to shed some light on: Isolation and identification of *P. aeruginosa* isolated from burn infection. To deterind the biofilm formation by *P. aeruginosa*.

MATERIALS AND METHODS

Isolation of *P. Aeruginosa*

Sixty two (62) samples were collected from patients admitted in burn center in Al-Fayhaa General Hospital in Basra city during the period from 01 March to 30 June 2015. All samples were from both genders (26males and 36 females) aged between 6 months – 78 year. All specimens were collected with disposable cotton swabs. A loopfull from each inoculated nutrient broth was transferred and cultivated onto the surface of pseudosel, blood and nutrient agars. All of the inoculated plates were incubated at 37°C for 24-48 hours.

Identification of the Isolates

The suspected colonies were examined for their colonial morphology, microscopical examination, Gram staining, catalase test, oxidase test and biochemical test (MacFaddin, 2000).

Chromogenic screening medium for *P. aeruginosa*

Samples identified as a by biochemical tests have been inoculated on CHROMagar*Pseudomonas* (CHROMagar™, Paris, France).

Biofilm Detection Methods

Biofilm detection was carried out by the following methods:

a. Congo Red Agar (CRA) Method

The medium composed of Brain heart infusion broth (37 gm/l), sucrose (5gm/l),agar number 1 (10 gm/l) and Congo red dye (0.8 gm/l). Congo red stain was prepared as concentrated aqueous solution and autoclaved at 121 C for 15 minutes. Then it was added to autoclaved Brain heart infusion agar with sucrose at 55 C. Plates were inoculated with test organism and incubated at 37 C for 24 to 48 hours aerobically. Black colonies with a dry crystalline consistency indicated biofilm production; weak producers usually remained pink, though occasional darkening at the center of colonies was observed (Freeman *et al*., 1989).

b. Genetic Techniques

Genomic DNA was extracted by the genomic DNA purification Kit supplemented by the manufacturing company (Promega-USA). PCR amplification of the pslA gene was carried out using the following primers: pslA-F, 5’-CACTGGACGTCTACTCCGACGATAT-3’; pslA-R, 5’-GTTTCTTGATCTTGTGCAGGGTGTC-3’ (Bioneer, Korea), generating an amplification product of 1119 bp. The reaction mixture (25 µL) contained 5 µlDNA template, 1 µl Forward primer, 1 µlReverse primer, 12.5 µlMaster mix and 5.5 µl Nuclease free water. Amplifications were performed in a thermal cycler (Peqlab, Germany) using the following program: an initial incubation at 94°C for 10 minutes, followed by 30 cycles of one minute denaturation at 94°C, 30 seconds annealing at 55°C and one minute extension at 72°C followed by 10 minutes at 72°C. The amplification products were separated on 1% agarose gels, stained with the medium bromide (Fisher, USA).

RESULTS

A total of 62 samples from examined for detection of *P. aeruginosa*. The result showed that 42 samples were positive for *P. aeruginosa*, with a percentage of (67.7%). On CHROMagar *Pseudomonas* medium, *P. aeruginosa* appeared as blue-green colonies (Fig.1).

Fig. 1. Blue-green colonies of *P. aeuroginosa* on CHROMagar *Pseudomonas*

Fig. 2. Congo Red Agar (CRA) assay for bacterial isolates of *P. aeuroginosa* isolated from burn. Positive is black colonies with dry crystalline (slime producers).

Results growth of 42 isolates of *P. aeuroginosa* from burn on CAR agar was 3(7.14%) of 42 isolates gave black colonies with a dry crystalline as positive results (slime producers) as a figure (2). The results of genetic techniquesshowed 3(7.14%) gave positive isolates of *P. aeruginosa* while 39 (92.85%) gave negative isolates of *P. aeruginosa* as a Figure (3).

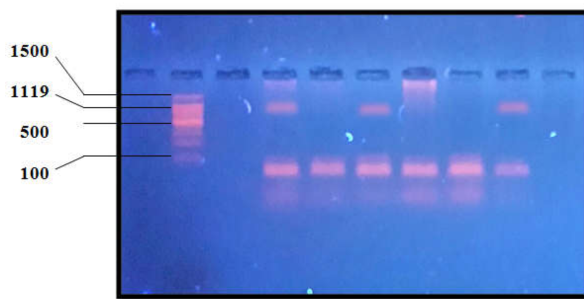

Figure 3. Agarose (1%)gel electrophoresis showed the PCR product of gene for*P. aeruginosa* isolates: Lane 1: DNA ladder (marker) 1500-100bp.Lane 13, 18, 24 : 1119-bp (*PslA*) band of biofilm producing in *P. aeruginosa* isolated from burn

DISCUSSION

In the present study, CHROMagar *Pseudomonas* was evaluated for the first time in Iraq as a direct isolation medium for burn clinical specimens. Forty two (67.7%) positive samples were identified as *P. aeroginosa* by biochemical tests and confirmed by CHROMagar medium. Identification of clinical bacterial samples by using CHROMagar medium give easily and rapid results by color and morphology characteristics to differentiate between the bacterial colonies. *P. aeruginosa* is capable of causing chronic infections mostly due to its potential to form biofilms (Matsukawa and Greenberg, 2004). The hallmarks of a mature biofilm include production of an extracellular matrix and increased resistance to antibiotics (Wozniak *et al*., 2003). Our study results agree with (Rewatkar*et al.,* 2012) that reported that the isolates of bacteria *P. aeroginosa* gave growth on Congo Red Agar, while disagree with this study (Hou *et al.,* 2012) reported isolates of bacteria *P. aeroginosa* gave negative results (0%) on Congo Red Agar.

Congo Red Agar was simple and reliable to determine whether an isolate has the potential for biofilm production or not (Jain and Agarwal, 2009). In study of (Hou *et al.,* 2012) found that (31.03%) of *P. aeruginosa* isolates gave positive, while (Heydari and Eftekhar, 2015) reported that 43.5% isolates of *P. aeruginosa* gave positive results (these isolates were positive for biofilm formation) and reported 14% isolates of *P. aeruginosa* gave positive (these isolates were negative for biofilm formation). The presence of the *pslA* gene was shown as a good predictor of biofilm formation in non-mucoid isolates of *P. aeruginosa* in a number of studies (Ma *et al.,* 2006) and (Overhage *et al.,* 2005) and (Hou *et al.,* 2012). Compared to our current results with researches results shows the gene ratio was varied from search to another but agree with terms of the presence of the gene *PslA* in bacteria, which means that the expression of the *pslA* gene is influenced by other factors, or that the *pslA* gene is unnecessary for the formation of biofilms in bacterial strains taken from patients with infections.

In addition, there are reports that the *pel* gene clusters and *alg* gene clusters are also associated with biofilm formation (Franklin *et al.,* 2011). When compared between the CRA test results and PCR (*PslA* gene). We found that the *pslA* gene was present in the biofilm-negative isolates, Hou *et al.* (2012) found that *pslA* gene was present in 31% of biofilm-negative of *P. aeruginosa* isolates, and agree with (Heydari and Eftekhar, 2015) also observed that 14.2% of the biofilm-negative isolates harbored the pslA gene. This may suggests that gene presence does not necessarily result in its expression and biofilm formation is regulated by a complicated network of factors in addition to the pslA gene, where Ma *et al*. (2009) reported formation and development of biofilm depending on several components like exopolysaccharides (*Pel, and alginate)* in addition to *Psl* genes. In spite of the results that we have obtained on Congo red agar method these results don't recommend the CRA method as a suitable method for detection of biofilm formation, these were in agreement with (Knobloch *et al*., 2002) did not recommend the CRA method for biofilm detection in their study.

REFERENCES

Bergey's Manual of Systematic Bacteriology. 2001. Ed. Krieg, N. R. and Holt, J.G. Williams and Wilkins Publishers, Baltimore. Vol. 2.

Franklin, M.J., Nivens, D.E., Weadge, J.T. and Howell, P.L. 2011. Biosynthesis of the *Pseudomonas aeruginosa* extracellular polysaccharides, alginate, pel, and psl. Front Microbiol., 2: 167.

Freeman, J., Falkiner, F. R. and Keane, C. T. 1989. New method for detecting slime production by coagulase negative staphylococci. *J Clin Pathol*., 42:872-874.

Ghafoor, A., Hay, I.D. and Rehm, B.H. 2011. Role of exopolysaccharides in *Pseudomonas aeruginosa* biofilm formation and architecture. *Appl Environ Microbiol.,* 77(15):5238–46.

Heydari, S. and Eftekhar, F. 2015. Biofilm Formation and β-Lactamase Production in Burn Isolates of *Pseudomonas aeruginosa*. *Jundishapur J Microbiol*., 8(3): e15514.

Hou, W., Sun, X., Wang, Z. and Zhang, Y. 2012. Biofilm-forming capacity of *Staphylococcus epidermidis, Staphylococcus aureus,* and *Pseudomonas aeruginosa* from ocular infections. *Invest Ophthalmol Vis Sci.,* 53(9): 5624–5631.

Jackson, K.D., Starkey, M., Kremer, S., Parsek, M.R. and Wozniak, D.J. 2004. Identification of *psl*, a locus encoding a potential exopolysaccharide that is essential for *Pseudomonas aeruginosa* PAO1 biofilm formation. *J. Bacteriol.,* 186: 4466–4475.

Jain A and Agarwal A. 2009. Biofilm production, a marker of pathogenic potential of colonizing and commensal staphylococci. *J Microbiol Methods*, 76 :88–92.

Karatuna, O. and Yagci, A. 2010. Analysis of quorum sensing-dependent virulence factor production and its relationship with antimicrobial susceptibility in *Pseudomonas aeruginosa* respiratory isolates. *Clin Microbiol Infect.,* 16(12):1770–1775.

Knobloch, J.K., Horsetkotte, M.A., Rohde, H. and Mack, D. 2002. Evaluation of different detection methods of biolfilm formation in *Staphylococcus aureus*. *Med Microbial Immunol.,* 191(2):101-106.

Ma, L., Jackson, K.D., Landry, R.M., Parsek, M.R., Wozniak, D.J. 2006. Analysis of *Pseudomonas aeruginosa* conditional psl variants reveals roles for the psl polysaccharide in adhesion and maintaining biofilm structure postattachment. *J Bacteriol.,* 188(23):8213–8221.

MacFaddin, J. F. 2000. Biochemical Tests for Identification of Medical Bacteria. 3rd ed. Lippincott Williams and Wilkns. USA.

Matsukawa, M., Greenberg, E.P. 2004. Putative exopolysaccharide synthesis genes influence *Pseudomonas aeruginosa* biofilm development. *J. Bacteriol.,* 186(14): 4449–4456.

Overhage, J., Schemionek, M., Webb, J. S. and Rehm, B. H. 2005. Expression of the psl operon in *Pseudomonas aeruginosa* PAO1 biofilms pslA performs an essential function in biofilm formation. *Appl Environ Microb*., 71:4407–4413.

Rewatkar, A. R. and Wadher, B. J. 2013. *Staphylococcus aureus* and *Pseudomonas aeruginosa*- Biofilm formation Methods. *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*, 8: 36-40.

Schaber, J. A., Carty, N. L., MacDonald, N. A., Graham, E. D., Cheluvappa, R., Griswold, J. A. and Hamood, A. N. 2004. Analysis of quorum sensing-dificient isolates of *Pseudomonas aeruginosa*. *J. Med. Microbiol*., 53: 841-853.

Stoodley, P., Sauer K., Davies D. G., and Costerton, J. W. 2002. Biofilms as complex differentiated communities. *Annu. Rev. Microbiol*., 56:187–209.

Wilson, R. and Dowling, R. 1998. *Pseudomonas aeruginosa* and other related species. *Thorax*, 53: 231-219.

Wozniak, D.J., Wyckoff, T.J., Starkey, M., Keyser, R., Azadi, P., O'Toole , G.A. andParsek M. R. 2003. Alginate is not a significant component of the extracellular polysaccharide matrix of PA14 and PAO1 *Pseudomonas aeruginosa* biofilms. *Proc Natl Acad Sci U S A.* 100(13):7907–7912.

****