# DETECTION OF BIOFILM GENES AMONG CLINICAL ISOLATES OF *PSEUDOMONAS AERUGINOSA* RECOVERED FROM SOME EGYPTIAN HOSPITALS.

**BY**

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## *ABSTRACT*

Biofilm formation in ***Pseudomonas aeruginosa*** (***P. aeruginosa***) is controlled by about 1% of its chromosomal genes; from which four genes were selected for prospective work. The aim of this study was to determine the biofilm formation in ***P. aeruginosa*** clinical isolates and to evaluate the role of the selected genes in biofilm formation. A total of fifty isolates were recovered from different clinical samples isolated fromsome Egyptian hospitals by isolation on cetrimideagar media and then biochemically identified as ***P. aeruginosa***. The antibiogram of the planktonic cells of all isolates was determined and showed that amikacin was the most potent antibiotic against all isolates. Quantification of biofilm formation of isolates was done by the microtiter plate method using crystal violet (CV) assay. According to the optical density (OD) readings, isolates were classified into the following categories: strong, moderate or weak biofilm producers. Screening for some selected biofilm genes as ***RhlI, PilA, PilT*** and ***PelB*** genes in some isolates using PCR, revealed the presence of these genes in both strong and weak biofilm producer isolates. These final results suggest the importance of these genes in biofilm formation and suggest the presence of other factors which may contribute in determining the degree of biofilm formation in ***P. aeruginosa***.

## *INTRODUCTION*

Biofilm may be defined as multicellular surface-associated communities of microorganisms enclosed in an extracellular matrix which exhibits a characteristic structure and is responsible for the increased resistance to both antimicrobial agents and environmental stresses (Aaron *et al*., 2002). A wide variety of medical devices have been shown to be colonized by biofilm forming microorganisms (Costerton *et al.*, 1999). The establishment of biofilm architecture follows an order of events, going from initial attachment of a single cell and ending with mushroom-shaped mature biofilm formation (Stoodley *et al.*, 2002) Chemical analysis of solid surface-associated (SSA) biofilms of *P. aeruginosa* formed in flow cells suggests that the matrix that encases the cells in such biofilms is composed of water, DNA, RNA, exopolysaccharide, ions and proteins (Whitchurch *et al.*, 2002).

*P. aeruginosa* is one of the most studiedmicroorganisms in the context of biofilms (Costerton, 2001). Biofilms are prevalent in diseases caused by *P.*

*aeruginosa*, a common nosocomialopportunistic pathogen that is mostly considered as the leading cause of morbidity and mortality incystic fibrosis patients (Tummler and Kiewitz, 1999). *P. aeruginosa* biofilms are up to 1000-fold more antibiotic tolerant than its planktonic cells (Aaron*et al*., 2002).

In most of the model systems described for the *in vitro* studyof biofilm formation and development, the quantification of biofilm biomass is done by conventional plating, which is labour-intensive and slow (Donlan and Costerton, 2002). CV staining was first described by Christensen *et al*. (1985) and has since then been modified to increase its accuracy and to enable biofilm biomass quantification in the entire well (Stepanovic *et al*., 2000). Both dead and living cells, as well as matrix are stained by CV, so it is poorly suitable to estimate killing degree of biofilm cells (Pitts *et al*., 2003).

*P. aeruginosa* has been extensively studied for genetic determinants that contribute to biofilm formation. *P. aeruginosa* DNA microarray analysis detected only 1% of genes as differentially expressed in the biofilm growth mode, with about 0.5% of the genes being repressed and 0.5% of the genes being activated (Whiteley *et al*., 2001). Recently, it was discovered that *P. aeruginosa* quorum-sensing (QS) signal molecules termed autoinducers (AI) play a significant role in the differentiation process (Davies *et al*., 1998). *P. aeruginosa* has two hierarchical QS systems known as *las* and *rhl* (Juhas *et al*., 2005; Schuster and Greenberg, 2006). It has been repeatedly reported that one *rhl*-controlled factor, the surfactant rhamnolipid, has multiple functions in biofilm development (Davey *et al*., 2003; Boles *et al*., 2005).

Twitching motility is depending on polar type IV pili, which are also known to play an essential role in mediating adherence to and colonization of mucosal surfaces (Hahn, 1997). *P. aeruginosa* cells deficient in flagellar motility show poor surface attachment, while cells deficient in type IV pili are unable to form microcolonies (O'Toole and Kolter, 1998). In *P. aeruginosa*, *PilT* is required for twitching motility. Mutations in *PilT* result in a nontwitching hyperpiliated phenotype (Whitchurch and Mattick, 1994).

The *Pel* gene cluster of *P. aeruginosa* was previously identified by (Friedman and Kolter, 2004a). They showed that a mutation in *Pel* gene cluster in the *P. aeruginosa* (PA14 strain) prevents the pellicle formation at the air–liquid interface of bacterial cultures grown in static conditions. On the basis of this observation, they could further show that the *Pel* mutants are not able to form robust and shear force-resistant biofilms on a solid surface of glass or plastic. Also they suggested that *Pel* genes are involved in the production of a putative exopolysaccharide, which holds the bacterial cells together within the mature biofilms and makes them resistant to thorough washing. Finally, they revealed that the *P. aeruginosa* PA14 matrix contains a glucose-rich structural component that is not found in the *Pel* mutant PA14.

In our study, detection of biofilm formation and studying the effect of four selected genes on biofilm formation in *P. aeruginosa* clinical isolates were done. The selected genes were *RhlI* (gene encoding for quorum sensing), *PilA* and *PilT* genes (genes encoding for twitchting motility) and *PelB* gene (one of *Pel* gene cluster which is suggested to be involved in the exopolysaccharide production in biofilm).

## *MATERIALS AND METHODS*

**Clinical isolates:**

A total of fifty isolateswere recovered from different clinical samples by isolation on cetrimideagar media (Oxoid). These isolates were collected from patients in the Department of Urology at Mansoura University Hospital, the Department of Urology at Beni-Suef University Hospital and Al-Eman medical lab, Beni suef, Egypt.

These isolates were identified depending on Gram stain, cultures characters of colonies, growth on cetrimideagar, growth on MacConkey agar, pigment production on modified tech agar medium, catalase test, oxidase test, gelatinase test, citrate utilization test, motility test, ornithine decarboxylation test and reaction on triple sugar iron agar.Isolates were cultured aerobically on tryptic soya agar (Oxoid).The identification ofthese isolates was done according to Gilardi (1971) andYabuuchi and Ohyama (1972)

**Antimicrobial susceptibility testing:**

The antimicrobial susceptibility testing of the planktonic cells of all isolates towards penicillin, oxacillin, imipenim, cefuroxime sodium, cefotaxime, ceftazidime, cefepime, amikacin, amoxicillin/clavulanic acid, erythromycin and ofloxacin were determined by the disk diffusion method using Muller Hinton agar (Oxoid) according to the clinical laboratory standards institute (CLSI) (2009). The results were interpreted as resistant,intermediate or susceptible based on the criteria listed in (CLSI) (2009).

**Biofilm quantification:**

The microtiter plate assay was used for quantification of biofilm formation of isolates. The experiments were performed in presterilized, polystyrene; flat-bottom, tissue culture-treated, 96-well microtiter plates (Corning Incorporated, Corning, N.Y.).

**Biofilm formation:**

The biofilm formation in microtiter plate was performed as previously described (Stepanovic *et al*., 2007). Briefly, the wells of a sterile 96-well flat bottomed polystyrene microtiter plate were filled with 200 µl of two tenfold dilution of the overnight bacterial culture tryptic soya broth (Oxoid) (about $1.0 \times 10^6$ CFU/ml). The negative control wells were filled with 200 µl tryptic soya broth (non-inoculated). The plates were covered with a lid and incubated aerobically at 37°C for 24 hours. After incubation, the contents of the wells of each plate were decanted into a discard container and each well was washed three times with 200 µl of phosphate-buffered saline (PBS). Following every washing step, the wells were emptied by flicking the plates.

**Biofilm quantification using crystal violet (CV) assay:**

The quantification of biofilm formation of isolates (including matrix, dead and living cells) using CV assay was based on the previously described method (Stepanovic *et al*., 2007). The prewashed attached biofilm was heat-fixed using hot air at 60° C for 15 minutes then a 150 µl of 1% CV was added and kept for 15 minutes at room temperature. The excess stain was aspirated and washed by running tap water until the washing was free from stain. The microtiter plates were air dried at room

temperature and the dye bound to the cells was re-solubilized by adding 150 µl of 95% ethanol to each well. The plates were covered and left at room temperature for at least 30 minutes without shaking. Finally, 125 µl of each well was transferred to a new plate where the OD of each well was measured at 570 nm using the microtiter plate reader (Sunrise, Tecan, Austria). Experiment was performed in triplicate and mean OD and standard deviation (SD) for each isolate were calculated. The OD values were considered as an index of bacteria adhering to surface and forming biofilms.

The cut-off OD ($OD_c$) was then calculated. The ODc was defined as three standard deviations (SD) above the mean OD of the negative control i.e ODc=mean OD of negative control+ (3x SD of negative control). Biofilm formation in between isolates was classified according to Stepanovic *et al*., (2000), as non-biofilm producer, weak, moderate or strong biofilm producer. Isolates were classified as non-biofilm producers if $OD \leq OD_C$, weak biofilm producer if $OD_C < OD \leq (2xODc)$, moderate biofilm producer if $(2 \times OD_C) < OD \leq (4 \times OD_C)$ and strong biofilm producer if $(4 \times OD_C) < OD$.

**DNA isolation and manipulation:**

The chromosomal DNA of the isolates were prepared using GenoElute DNA Bacterial Genomic DNA Kit (Cat. no NA2110) (Sigma-aldrich, USA) and was used according to the instructions of the manufacturers.Success of chromosomal DNA extraction was confirmed by running of the DNA extracts on 0.8% agarose gel (w/v) (Invitrogen) stained with ethidium bromide using Compact XS/S gel electrophoresis (Biometra, Germany) and then illuminated under Whatman U.V Spectrophotometer (Biometra, Germany) according to Sambrook *et al.*, (1989).

DNA concentration and purity was determined using the Nano drop 2000-spectrophotometer (Thermo Scientific, Wilmington, USA), as DNA concentration was determined by (ng per µl) and the DNA purity was determined by the ratio of dividing 260/280nm readings.

**Screening of specific sequences of the selected genes:**

Four genes contributing to biofilm formation in *P. aeruginosa* were selected in this part of study. These selected genes were *RhlI*, *PilA*, *PilT* and *PelB* genes.

To estimate their role in biofilm formation, thirteen isolates were selected to be screened for these genes. These isolates were selected according to CV assay results. Nine of these isolates were strong biofilm producers and four isolates were weak biofilm producers.

The sequences of the selected genes were taken from the GenBank sequence database of the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/). These sequences were aligned by the CLC sequence analyzer program and the Multiple Sequence Alignment (SRS) using the online program (http://srs.ebi.ac.uk/srsbin/cgi-bin/wgetz?-page+Launch+-id+12F2q1huXNL+-appl+NClustalW2+-launchFrom+top) to detect the conserved region of these genes. PCR primers of these genes were designed with the online program primer 3 using the online program (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) available by Invitrogen wereshown in table (1).

PCR reaction was performed in a total volume of 25 µl containing 12.5 µl of Taq master mix (QiaGen, Germany), 1 µl of each forward and reverse primer (1 µM) together with equivalentµlsto 250 ng of the genomic DNA and finally completed to 25 µl by nuclease free water. Amplification was performed in Gene AMP PCR System 9700 thermocycler (Applied Biosystem). The PCR reaction for these specific sequences of these genes was performed at 94°C for 3 min, followed by 30 cycles of 94°C for 30 sec, 55.5°C for 30 sec and 72°C for 1 min, with a final extension at 72°C for 5 min. Finally, the PCR products were detected by gel electrophoresis by running on 1.5% agarose (w/v) stained with ethidium bromide using 100 bp DNA ladder (QiaGen, Germany) as reference and then illuminated under U.V Spectrophotometer according to Sambrook *et al.*, (1989).

**Table (1): List of primer pairs used in the study, their sequences, their expected product size and their annealing temperature.**

| Gene | Accession number | Primer | Sequence | Reference | Product size (bp) | Annealing temperature (℃) |
|---|---|---|---|---|---|---|
| *RhlI* | FN601384.1 | Forward | 5CTCTCTGAATCGCTGGAAGG-3 | This study | 245 | 55.5 |
| | | Reverse | 5-GCGAAGACTTCCTTGAGCAG-3 | | | |
| *PilT* | NP_249086.1 | Forward | 5-CTTGGCATGGGAGAAGTGTT-3 | This study | 156 | 55.5 |
| | | Reverse | 5-GTCCTCGATGGTGAGGATGT-3 | | | |
| *PilA* | YP_006480639.1 | Forward | 5-ACTGTTGGTCGTCGTCTTCC-3 | This study | 160 | 55.5 |
| | | Reverse | 5-CCGTCCTACCAGGGTTACCT-3 | | | |
| *PelB* | YP_002439601.1 | Forward | 5-CGCCTGCTCTGGTTCTACAT-3 | This study | 190 | 55.5 |
| | | Reverse | 5-AGTCGTTGGGATTGGACTTG-3 | | | |

## *RESULTS*

### Clinical isolates identification:

By microscopical examination by Gram stain, all isolates were appeared as gram negative rods and culture characters of the colonies of all isolates were observed as opaque colonies with characteristic aromatic odor (grape-like odor of aminoacetophenone).

The growth of the isolates on modified Tech Agar medium showed that all isolates had a growth on this medium and produced different types of pigments; 33 isolates (66%) produced pyocyanin(blue- green) pigment; 11 isolates (22%) produced pyoverdine (yellow-green)pigment; 4 isolates (8%) producedpyorubin (red-brown) pigmentand the last 2 isolates (4%) appeared as non-pigmented colonies.

All isolates had a growth on cetrimide agarmedium and MacConkey agar medium. All isolates were oxidase positive and catalase positive. Regarding sugar fermentation, all isolates were non-lactose fermenters on MacConkey agar medium and non-fermentive on TSI, giving no change in the color of the medium.All isolates were motile and negative for ornithine decarboxylation on motility indole ornithine

media (MIO). All of them had a positive citrate utilization activity and positive gelatinase activity. According to these results, all the fifty isolates were identified as *P. aeruginosa* (Gilardi, 1971; Yabuuchi and Ohyama, 1972).

**Antimicrobial Susceptibility Testing:**

It was observed that all isolates were resistant to penicillin, oxacillin, erythromycin, cefuroxime sodium and amoxycillin/clavulanic acid. *P. aeruginosa* isolates showed a relatively high resistance for cefotaxime and ofloxacin of 76% and 58% respectively. A relatively low resistance of isolates was recorded for cefipime (22%), ceftazidime (16%) and imipenem (16%). While amikacin showed the highest potency against all isolates for which only 8% of isolates were resistant as shown in Fig. (1). It was also observed that only 6% of isolates were resistant for all tested antimicrobial agents.

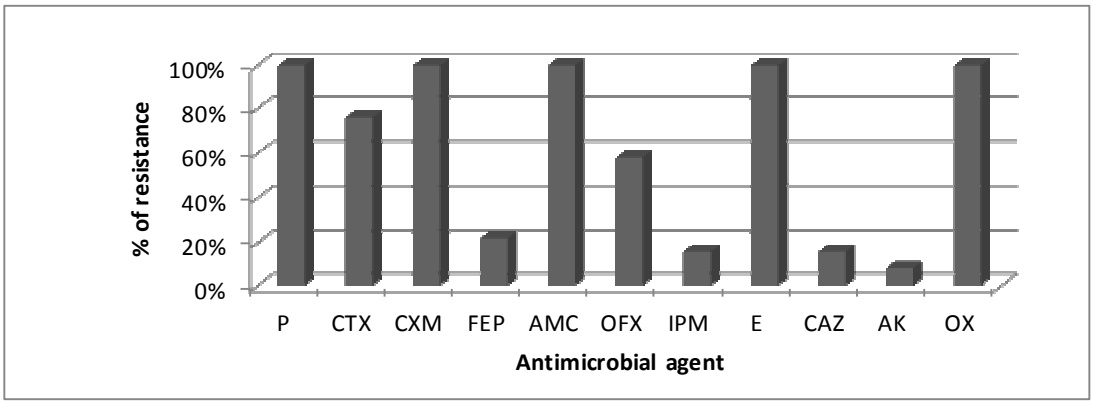


**Fig. (1): Percentage resistance of the *P. aeruginosa* isolates towards different antimicrobial agent.**

P: Penicillin; CTX: Cefotaxime; CXM: Cefuroxime Sodium; FEP: Cefipime; AMC: Amoxycillin/clavulanic acid; OFX: Ofloxacin; IMP: Imipenem; E: Erythromycin; CAZ: Ceftazidime; AK: Amikacin; OX: Oxacillin

**Biofilm quantification by CV assay:**

Using CV method, the ability of *P. aeruginosa* isolates to form biofilm was estimated. According to the OD values, biofilm production of isolates was classified into weak, moderate or strong biofilm producers. All isolates were biofilm forming; 42 isolates (84%) were strong biofilm producers; 4 isolates (8%) were moderate biofilm producers and the last 4 isolates (8%) were weak biofilm producers as shown in Fig. (2).

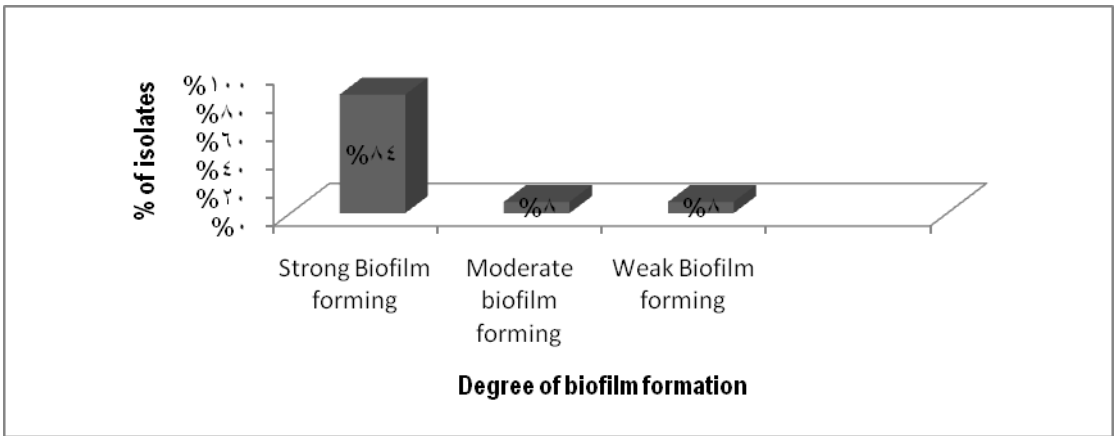


**Fig. (2): Degree of biofilm formation of *P. aeruginosa* isolates using CVassay.**

**DNA isolation and manipulation:**

Determination of DNA concentration and purity were done using the Nano drop 2000-spectrophotometer. The Nano drop spectrophotometer results indicated that DNA concentrations of the extracts were variable ranging from 31.8 ng/ µl to 256.6ng/ µl. It was also observed that the purity of DNA extracts was high ranging from 1.86 to 1.94

**PCR Screening of the selected genes:**

PCR Screening of the specific sequences of the four selected genes (*RhlI*, *PilA*, *PilT* and *PelB*) in the thirteen screened isolates revealed the presence of all of these genes in all of the screened isolates including both strong biofilm forming isolates (9 isolates) andweak biofilm forming isolates (4 isolate).

PCR screening for the specific sequences of *RhlI*gene showed a PCR product at 245 bp, as shown in Fig. (3, 4). This quorum sensing gene was detected in all screened isolates (isolates no.1, 2, 3, 9, 13, 15, 17, 30, 33, 38, 41, 45 &47) including weak and strong biofilm forming isolates.

Regarding*PilA* gene detection, a PCR product was observed at 160 bp in its PCR amplification as shown in Fig. (4, 5). For *PilT* gene a PCR product was observed at 156 bp in its PCR amplification as shown in Fig. (6, 7). Both these two genes (*PilA*&*PilT*) (twitching motility genes) were detected in all screened isolates (isolates no. 1, 2, 3, 9, 13, 15, 17, 30, 33, 38, 41, 45 & 47) including weak and strong biofilm forming isolates.

Regarding *PelB* gene detection, a PCR product was observed at 190 bp in its PCR amplification as shown in Fig. (7, 8). This gene (exopolysaccharide encoding gene) was detected in all screened isolates (isolates no.1, 2, 3, 9, 13, 15, 17, 30, 33, 38, 41, 45 &47) including weak and strong biofilm forming isolates.

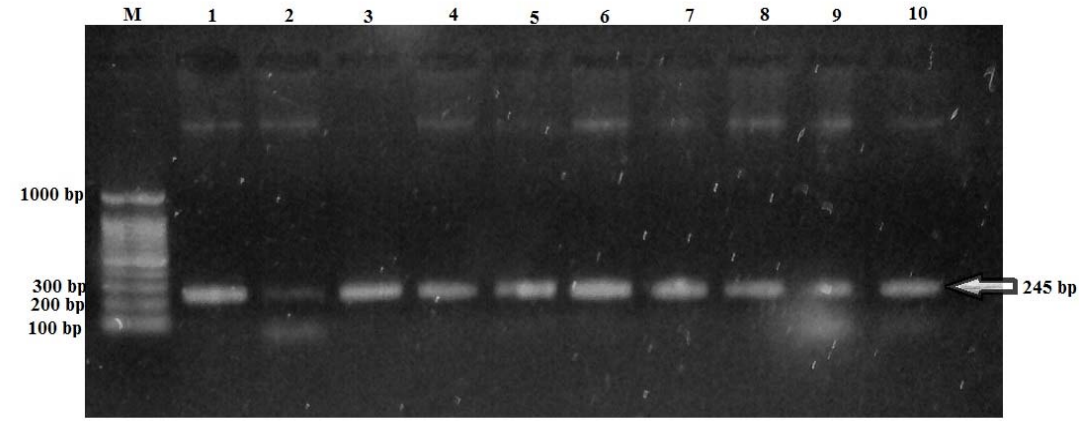


**Fig. (3): Agarose gel electrophoresis of *RhlI* gene amplicons (245 bp). Lane M was 100 bp DNA ladder. Lanes from 1 to 10 were amplicons of *RhlI* gene from isolates No. 1, 2, 3, 9, 13, 15, 17, 30, 33 and 38 respectively.**

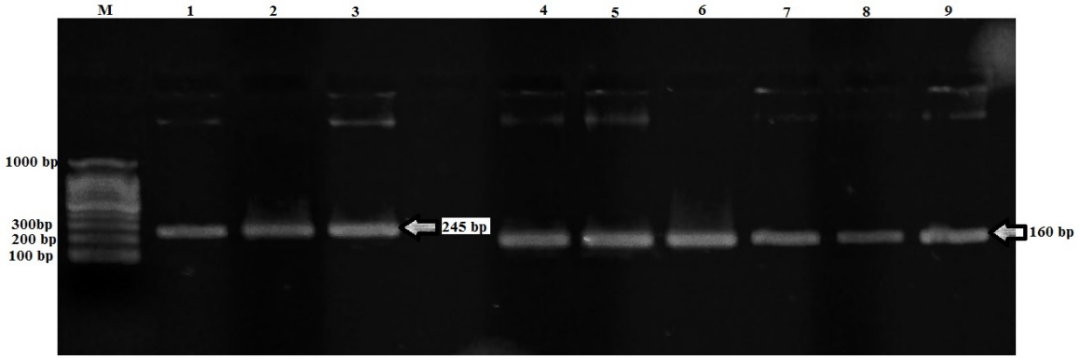


**Fig. (4): Agarose gel electrophoresis of *RhlI* gene amplicons (245 bp) and *PilA* gene amplicons (160 bp). Lane M was 100 bp DNA ladder. Lanes from 1 to 3 were amplicons of *RhlI* gene from isolates No. 41, 45, and 47 respectively. Lanes from 4 to 9 were amplicons of *PilA* gene from isolates No. 1, 2, 3, 9, 13 and 15 respectively.**

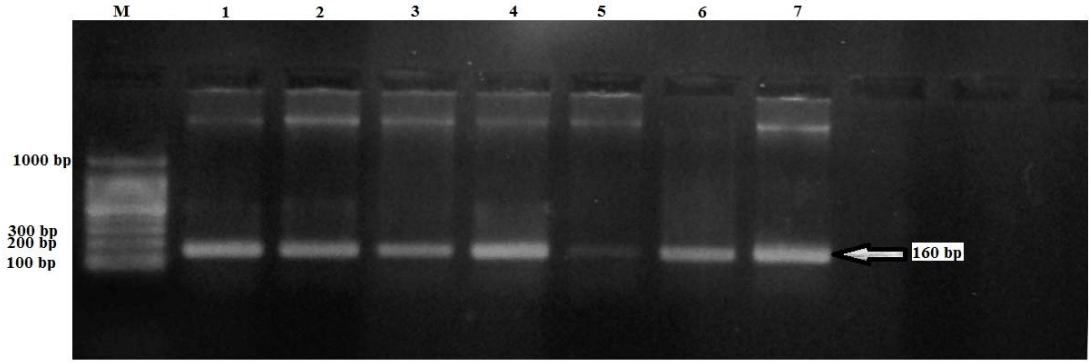


**Fig. (5): Agarose gel electrophoresis of *PilA* gene amplicons (160 bp). Lane M was 100 bp DNA ladder. Lanes from 1 to 7 were amplicons of *PilA* gene from isolates No. 17, 30, 33, 38, 41, 45 and 47 respectively.**

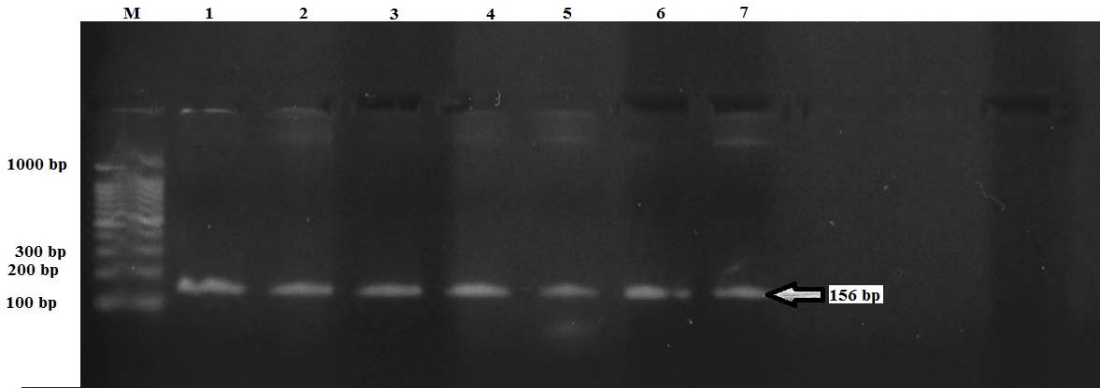


**Fig. (6): Agarose gel electrophoresis of *PilT* gene amplicons (156 bp). Lane M was 100 bp DNA ladder. Lanes from 1 to 7 were amplicons of *PilT* gene from isolates No. 17, 30, 33, 38, 41, 45 and 47 respectively.**

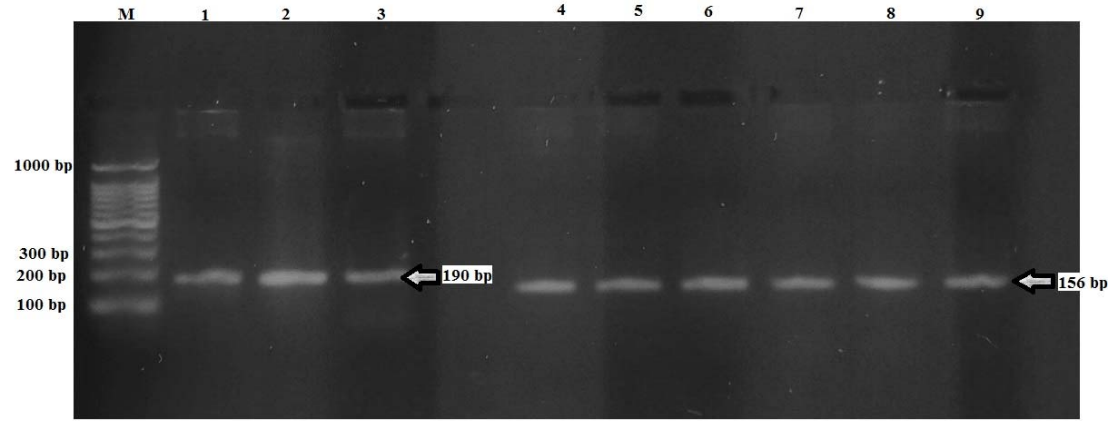


**Fig. (7): Agarose gel electrophoresis of *PelB* gene amplicons (190 bp) and *PilT* gene amplicons (156 bp). Lane M was 100 bp DNA ladder. Lanes from 1 to 3 were amplicons of *PelB* gene from isolates No. 41, 45, and 47 respectively. Lanes from 4 to 9 were amplicons of *PilT* gene from isolates No. 1, 2, 3, 9, 13 and 15 respectively.**

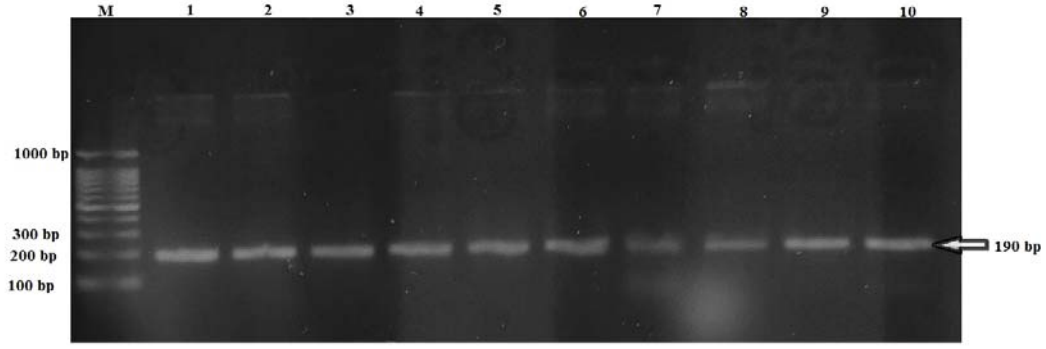


**Fig. (8): Agarose gel electrophoresis of *PelB* gene amplicons (190 bp). Lane M was 100 bp DNA ladder. Lanes from 1 to 10 were amplicons of *PelB* gene from isolates No.1, 2, 3, 9, 13, 15, 17, 30, 33 and 38 respectively.**

## *DISCUSSION*

*P. aeruginosa* was reported to be one of the main causes of nosocomial infections. It is well-known that *P. aeruginosa* exhibits an intrinsic resistance to several antimicrobial agents (Ibukun *et al.,* 2007) and has emerged as biofilm-forming, dominant pulmonary pathogen (Ishida *et al*., 1998). Biofilms were found to grow on materials found universally in the hospital environment like stainless steel, polyethylene and Teflon (Donlan and Costerton, 2002). Concerning *P. aeruginosa* biofilm, it was observed that cell-to-cell signaling is necessary for mature biofilm development (Davies *et al*., 1998) and that twitching motility mediated by type IV pili is necessary for the formation of microcolonies of the biofilm (O'Toole and Kolter, 1998).

In the present study, the most potent antimicrobial agent against all the 50 *P. aeruginosa* isolates was amikacin(8% resistance) followed by both imipenim and ceftazidime (16% resistance). Similar results were obtained by (De Macedo and Santos, 2005), as imipenem and amikacin were the most useful antibiotics against *P. aeruginosa*. In another study done by (Gad *et al.,* 2007), clinical isolates of *P. aeruginosa*, amikacin was the most effective drug (8% resistance).

In our study, all isolates were biofilm producers by microtiter plate method using CV assay; 42 isolates (84%) were strong biofilm producers; 4 isolates (8%) were moderate biofilm producers and 4 isolates (8%) were weak biofilm producers. Similar results were obtained in another study in Egypt as all isolates were biofilm producers using CV assay; 16 isolates (80%) were strong biofilm producers; 2 isolates (10%) were moderate biofilm producers and 2 isolates (10%) were weak biofilm producers (Hisham *et al.*, 2012).

In the present study, *RhlI* gene was detected in all strong biofilm producer isolates suggesting that *RhlI* gene might be involved in biofilm formation. This suggestion is confirmed by a previous study in Switzerland, which revealed that biofilm formation was reduced by 70% in *RhlI* mutant strain of *P. aeruginosa* PAO1 comparing to its parent strain (Favre-Bonté *et al.*, 2003), indicating the role of *RhlI* gene in biofilm formation. Also in another study in USA, they showed that if QS-inhibitory compounds or surface-associated AI analogs could shut down *RhlI* and *LasI* expression, then all the other genes in the QS cascade would be shut down also, including those involved in biofilm formation (De Kievit *et al.*, 2001), that strengthens *RhlI* role in biofilm formation.

In our study, *PilA* and *PilT* genes (twitching motility genes) were detected in all strong biofilm producer isolates suggesting that these genes had a role in biofilm formation of *P. aeruginosa*. In agreement with our suggestion, (Klausen *et al.,* 2003) revealed that wild-type and *PilA* mutant strains of *P. aeruginosa* PAO1 formed similar young biofilm with small microcolonies initially, but finally formed very different mature biofilms suggesting that type IV pili have role in the later stages of biofilm formation. In another study by (Chiang and Burrows, 2003), they demonstrated that twitching motility mediated by *PilT* is necessary for the establishment of normal biofilm morphology and that in its absence there is an increase in the accumulation of cell mass.

It was shown previously that *P. aeruginosa* produces a third exopolysaccharide, Pel,which was shown to be important for the static biofilm formation by various strains. Pel exopolysaccharide production requires a single operon containing seven genes (*pelA* to *pelG*) (Friedman *et al.*, 2007).

In our study, PCR have detected *PelB* gene in all strong biofilm forming *P. aeruginosa* isolates. This result made us believe that this gene is one of those genes involved in biofilm development. Previously published data showed that *PelB* mutation resulted in dramatic decrease of pellicle formation compared to the parent strain (Friedman *et al.*, 2007).

However in another study in USA by (Colvin*et al.*, 2011), they showed that *PelB* gene has a significant influence on later biofilm formation stages and this influence was strain dependent, as they found that *PelB* mutant strain of *P. aeruginosa* PA14 has significant decrease in biofilm biomass compared to the parent *P. aeruginosa* PA14 strain. Using an arabinose-controlled expression plasmid, pMJT-1, this biofilm defect was complemented. In contrast to *P. aeruginosa* PA14, no difference was seen between parent *P. aeruginosa* PAO1 and *PelB* mutant strain of *P. aeruginosa* PAO1.

However, in the present study, it was found that the four selected genes were detected in both strong biofilm producer isolates and weak biofilm producer isolates suggesting that these genes are contributing in biofilm formation in *P. aeruginosa* as they were detected in the strong biofilm producers isolates, table (2). Also, we suggest that there are other factors contributing to biofilm formation in *P. aeruginosa* as these genes were detected also in weak biofilm producer isolates. These factors may be strain difference, culture conditions, rate of expression of these genes, presence of other genes needed for biofilm formation or other factors affecting biofilm formation.

**Table (2): Degree of the biofilm formation and the presence of some biofilm genes in some *Ps. aeruginosa* isolates.**

| Isolate | *RhlI gene* | *PilA gene* | *PilT gene* | *PelB gene* | Degree of biofilm formation |
|---|---|---|---|---|---|
| 1 | +ve | +ve | +ve | +ve | Weak |
| 2 | +ve | +ve | +ve | +ve | Weak |
| +ve | +ve | +ve | | +ve | Weak |
| +ve | +ve | +ve | | +ve | Weak |
| 13 | +ve | +ve | +ve | +ve | Strong |
| 15 | +ve | +ve | +ve | +ve | Strong |
| 17 | +ve | +ve | +ve | +ve | Strong |
| 30 | +ve | +ve | +ve | +ve | Strong |
| 33 | +ve | +ve | +ve | +ve | Strong |
| 38 | +ve | +ve | +ve | +ve | Strong |
| 41 | +ve | +ve | +ve | +ve | Strong |
| 45 | +ve | +ve | +ve | +ve | Strong |
| 47 | +ve | +ve | +ve | +ve | Strong |

In a previous study in USA by (Schaber*et al.*, 2007), a clinical isolate of *P. aeruginosa* showed a considerable reduction in twitching motility when using *PilA*-deficient mutant of *P. aeruginosa* PAO1 as negative control. They suggested that this reduction is due to the reduced production of the *PilA* protein by the correlation between the biofilm phenotype of the clinical isolate and its lack of twitching motility using complementation analysis. However, using plasmid p8566, failed to complement the defect in twitching motility however, this plasmid was carrying the intact *PilA* gene. So, they suggested that there was another possible cause of the inefficient spread of the mature biofilm which could be its lack of swarming motility. Their suggestion strengthened our suggestion of the presence of different factors affecting biofilm formation in *P. aeruginosa.*

Disruption of *P.aeruginosa* quorum-sensing regulatory cascade has been shown to interfere with normal biofilm development and architecture (Davey *et al.*, 2003; Irie *et al.*, 2005). However, others have reported no difference between biofilms defective in the Las quorum-sensing system and wild-type biofilms (Kirisits *et al.*, 2005). We may explain this inconsistency between both cases by the presence of other factors affecting biofilm formation in addition to quorum-sensing system. These factors may be strain difference, culture condition or other factors affecting expression of quorum-sensing genes. This possible explanation of the role of quorum-sensing system in biofilm formation may help in the explanation of the detection of the selected genes in weak biofilm producer isolates. So we suggest that these isolates may need other factors or genes beside these genes to form high biofilm.

## *ACKNOWLEDGEMENT*

We are grateful to Dr.Eman El-gebaly and Dr.Ahmed Osama for their great support in specimens collection and continuous help during our study.

This work was supported by Faculty of Pharmacy, Beni Suef University, Egypt.

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# الكشف عن بعض الجينات المسئولة عن تكوين الغشاء الحيوي بين العزلات السريرية للسودوموناس ايروجينوزالمفصولة من بعض المستشفيات المصرية.

**للسادة الدكاترة**

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يتحكم حوالي 1% من الجينات الكروموسومية في تكوين الغشاء الحيوي في بكتريا السودوموناس ايروجينوزا ، وتم إختيار اربعة جينات من هذه الجينات لدراستهم. الهدف من هذه الدراسة كان قياس مدي قدرة عينات السودوموناس ايروجينوزا علي تكوين الغشاء الحيوي ، و التحقق من مدي تأثير الجينات المختارة علي قدرتها علي تكوين هذا الغشاء الحيوي. تم تجميع 50 عينة من عينات طبية مختلفة في مصر وذلك عن طريق فصلها علي وسط السيترميد ، ثم تم التعرف عليها كسودوموناس ايروجينوزوا بالاختبارات الكيمائية الحيوية. وبدراسة مدى مقاومة الخلايا الميكروبية لبعض المضادات الحيوية ، قد وجد أن مركب الاميكاسين له أقل معدل للمقاومة بين كل العينات. وبدراسة كمية الغشاء الحيوي التي تكونها العينات بطريقة المايكرو تايتر بليت باستخدام اختبار الكريستال فيلوت ، تم تصنيف قدرة العينات الميكروبية من حيث قدرتها علي تكوين الغشاء الحيوي الي : عينات مكونة للغشاء الحيوي بدرجات عالية ومتوسطة وضعيفة. وبعمل مسح لوجود الجينات المختارة المسئولة عن تكوين الغشاء الحيوي(*RhlI*, *PilA*, *PilT* and *PelB*) في بعض العينات عن طريق تقنية تفاعل البلمرة المتسلسل ، تم ملاحظة وجود هذه الجينات الاربعة في كل العينات المفحوصة سواء كانت لها قدرة عالية او ضعيفة علي تكوين الغشاء الحيوي. ومن خلال هذه النتائج النهائية للدراسة نقترح ان هذه الجينات مهمة في تكوين الغشاء الحيوي ، ونقترح ايضا وجود عوامل اخري تساهم في تكوين الغشاء الحيوي في السودومنس ايروجينوزوا .