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Role of *RhIR* and *RhII* Genes in Biofilm and Multidrug Resistance of Burn Isolated *Pseudomonas aeruginosa.*

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Abstract
During the period of 3 months in Baghdad Teaching hospital from March to June 2014 ,(24)isolates of *Ps. aeruginosa*were assumed from burn infection.Further identification were done by using *rpsL* gene as house keeping gene by PCR *Ps. aeruginosa*. isolates from Baghdad hospital that identified by PCR method, antibiotic sensitivity had done by Kerbey Bauer Disk method for (10)antibiotics which showed that the highest resistance (100%)(n=24) to Tetracyclin, Chloramphinicol,Ceftazidime, Cefoxitin and Ceftriaxone, while the resistance to the Cefepime(83.33%)(n=20), Pipracillin (62.5%)(n=15),the same resistance to Ciprofloxacin and Tobramycin (50%)(n=12), Azithromycin (41.66%)(n=10).Results of biofilm formation which detected by TCP Method showed that (66.66%)(n=16) of isolates had high biofilm production most of them are male isolates while the most of moderate production was in female isolates.The highest presence of quorum sensing genes was for *RhIR* gene (83.33%)(n=20) while *RhII* genewas (58.33%)(n=14).Through these results it was observed that there was not any effect of quorum sensing genes on biofilm formation or multidrug resistance while showed relationship between biofilm production and multidrug resistance of negative isolates for quorum sensing genes.

Introduction

Ps. aeruginosa is an opportunistic pathogen widely distributed in the environment and had a versatile metabolic activity. This non- fermenting Gram negative rod is responsible for about (10%) of all hospital acquired infectionsAloush,*et al*,2006;Enoch,*et al.,* 2007).Infections are opportunistic in nature and ranged from those associated with ventilator, catheter, burn and wound, to pulmonary infections in cystic fibrosis patients and keratitis in contact lens wearers (Lee,*et al*.2003;Choy*et al.,*2008).In general, the bacteria control their environmental systems and cell populations through intracellular communications to have thebest performance and response according to the demographic andthe environmental conditions (Dechoet *al*.,2010;Horswill*et al*.,2007).Pathogenic bacteria take thebest advantages of communication capability, as an example, they canovercome the host immune system barrier using the community, inthis case, they estimate the cell density using these intercellular signalsand given the concentration of signal transmitter factors, and whenthe density reaches its minimum extent in which the immune systemcannot simply cope, bacteria will release the virulence factors, thus, thehost immune system will be prevented to deliver rapid responses.Theprocess is controlled by a system in bacteria called "Quorum Sensing"(Li,& Tian,2012;Waters,& Bassler,2005).Biofilm formation has tremendous detrimental effects and has brought huge amounts ofproblems to our everyday life. In addition, biofilms show resistance to a wide range of
antibiotics such as ampicillin, streptomycin, tetracyclines, gentamicin *etc*. Also, the conventionalmethods such as antibiotics, and disinfectants to control the infections caused by biofilm formation areoften ineffective due to their special physiology and physical matrix barrier(Stewart,2002).
The aim of this study was to show the relationship between biofilm formation and multidrug resistance and determine the role of quorum sensing.

Materials and Methods

Bacterial isolates: Sample were collected from burn infections and initially characterized as *Ps. aeruginosa*. based on biochemical tests and gram staining, according to the criteria established by(Forbes *et al*.,2002),and by PCR method used *rpsL*house keeping gene primer(Alpha DNA/Canada)Table(1).The PCR amplificationwas performed to these primers in a total volume (25)µL containing (5) µl DNA template,(12.5)µL Go Taq Green Master Mix 2X (Promega/USA), (1)µL of each primer (10)pmol, then volume was completed to (25)µLbynuclease free water.

Thermal cycler was programmed as following conditions:

Initial denaturation 95c° for 5min, followed by 30 cycles each cycle included 94c° for 30 sec, 57c° for 30 sec and 72c° for 1min then final extension on 72c° for 7 min.The PCR products were separated by 1% agarose gel electrophoresis and visualized by exposure to Ultra Violate Light.

1- **Antimicrobial susbtibility test:**disk diffusion test had done for (Tetracyclin(10)µg, Chloramphinicol(10)µg,Ceftazidime(10)µg,Cefoxitin(10)µg , Ceftriaxone(30)µg, Cefepime(30)µg, Pipracillin(30)µg,Ciprofloxacin(10)µg, Tobramycin(30)µgandAzithromycin(30)µg (Bioanalyse/Turkey).According to the (**CLSI, 2013).**

Biofilm Production:was detected by Tissue culture Plate Method (TCP) as described by (Sandoe*et al.*, 2003)this is a qualitative method for biofilm detection. A loopful of tested organism inoculated in (10) ml Brain Heart Infusion broth then tubes were incubated at 37c° for 24 h.After incubation tubes were centrifugated to remove the media and the bacterial suspension was washed with normal saline, then filled the flat bottom tissue culture plates(96)wellswith (180)µLof brain heart infusion broth then (20) µL from bacterial suspension was added to the wells,the culture plate incubated at 37c° for24h .After incubation the wells were washed with normal saline by ELISA washer to remove free- floating bacteria.Then(200)µL of formaldyhide was added to the wells for (10)min. Biofilm which remained to the walls and the bottoms of the wells stained with (200) µL of 0.1% Crystal violate for (10)min,excess stain was washed with normal saline and plates were dried then (200)µL of destaining solution was added (95% Etanol) for (10) min, finally (200)µL from each well was transferred to a new microtiter plates and measured at (630)nm by microplate reader.

Detection of *RhII*and *RhRI* genes by PCR: Genotype detection of these genes by specific primers Table(1).under following conditions:Initial denaturation 94 c°for 5 min,35 cycles included: denaturation at 94c° for 30 sec, primer annealing with DNA template at 50 c° for30 sec, extension at 72c°for30 sec, and final extension at 72c° for10 min, these condition for the two genes .

Table(1):Sequences and product size of specific primers

Gene		Sequence of primers (5 ’-3’)	Product sizebp	reference
rpsL	**F**	**GCAAGCGCATGGTCGACAAGA**	210	Xavier *et al.,* 2010
	R	**CGCTGTGCTCTTGCAGGTTGTGA**		
RhlR	**F**	**CAATGAGGAATGACGGAGGC**	370	Sandoe*et al.*,2003
	R	**GCTTCAGATGAGGCCCAGC**		
RhlI	**F**	**CTTGGTCATGATCGAATTGCTC**	625	Sandoe*et al.,* 2003
	R	**ACGGCTGACGACCTCACAC**		

Results and Discussion

A total of *Ps. aeruginosa* isolates were recovered from burn infections (n=24) which identified by PCR method according to house keeping gene *rpsL***Table 2.**as described in (Xavier *et al.,* 2010).

The resistance patterns of *Ps.aeruginosa* isolates to (10)antibiotics were shown in **Table3**. The highest resistance was observed to Tetracyclin, Chloramphinicol,Ceftazidime, Cefoxitin and Ceftriaxone which was (100%),followed by Cefepime(83.33%) and Pipracillin (62.5%),while the isolates show the same resistance to theCiprofloxacin and Tobramycin(50%) and Azithromycin (41.66%).(Senturk*et al.,*2012)Observed that most of burn *Ps.aeruginosa* isolates showed high resistance rates tocefepime (98%) followed by piperacillinceftazidime (91%) andceftriaxon(87%)All isolates were totally resistantto tetracycline .MDR *P. aeruginosa* develops resistance by various mechanisms like multidrugresistance efflux pumps, production ofβ-lactamases, aminoglycoside modifyingenzymes, and decrease outer membranepermeability (Mahmoud*et al.,*2013).

TCP Method showed that (66.66%)of isolates had high biofilm production most of them are male isolates while the most of moderate production was in female isolates Table2.The association between the potential to form strong biofilms by *P. aeruginosa* and antibiotic resistance has also been shownwhich(Lambert, 2002).

The highest presence of quorum sensing genes was for *RhIR* gene (83.33%)while*RhII* gene was (58.33%).Bacteria are known as one of the simplest and the most primitivelife forms, which have the single-cell life and their reproduction,feeding and communication mechanisms are identified as basic andsimple patterns, however, the complex and interesting mechanisms areprovided by studies in recent decades that control bacterial behaviorsimilar to what occurs in multicellular organisms. This mechanism is controlled by quorum sensing

system that enables intraspeciesand interspecies communication(Drenkard,&Ausubel,,2002;Rutherford,&Bassler,2012).In current study was observed that negative isolates (4) showed resistance for all antibiotics Table2. So it due to think that was no role of Quorum sensing in antibiotic resistance while it was observed high biofilm producer isolates showed resistance for all antibiotics.

Table2:TCP Method and PCR Results.

Male or female	**TCP method**	rpsL	RhIR	RhII
Female	modrate	+	+	-
Female	modrate	+	+	-
Female	modrate	+	+	+
Male	modrate	+	+	+
Male	high	+	+	+
Male	high	+	+	+
Male	high	+	+	+
Male	high	+	+	+
Female	modrate	+	+	+
Female	modrate	+	+	+
Female	high	+	+	+
Female	modrate	+	+	-
Male	high	+	+	+
Female	high	+	+	-
Female	high	+	+	-
Female	high	+	-	-
Female	modrate	+	+	+
Male	high	+	+	-
Male	high	+	-	-
Male	high	+	-	-
Female	high	+	-	-
Male	high	+	+	+
Female	high	+	+	+
Male	high	+	+	+
Male	high	+	-	-

Table3:Antibiotic sensitivity test

TE	CIP	C	CAZ	FOX	TOB	PRL	FEP	CRO	AZM
R	S	R	R	R	R	R	R	R	R
R	R	R	R	R	R	R	R	R	R
R	S	R	R	R	R	R	R	R	I
R	S	R	R	R	R	R	R	R	S
R	R	R	R	R	S	R	R	R	S
R	R	R	R	R	S	R	R	R	S
R	R	R	R	R	S	R	R	R	S
R	R	R	R	R	S	R	R	R	S
R	S	R	R	R	S	S	S	R	R
R	S	R	R	R	R	R	R	R	R
R	S	R	R	R	S	S	S	R	S
R	S	R	R	R	S	S	S	R	R
R	S	R	R	R	S	S	R	R	S
R	R	R	R	R	R	S	R	R	S
R	R	R	R	R	R	S	R	R	S
R	R	R	R	R	R	R	R	R	R
R	S	R	R	R	S	R	R	R	R
R	R	R	R	R	R	R	R	R	S
R	R	R	R	R	R	R	R	R	R
R	R	R	R	R	R	R	R	R	I
R	R	R	R	R	R	R	R	R	R
R	S	R	R	R	S	S	S	R	S
R	S	R	R	R	S	S	R	R	R
R	S	R	R	R	S	S	R	R	S

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