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Molecular feature of *las*B gene of *Pseudomonas aeruginosa* isolated from different clinical sources

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Abstract

The study was carried out through a period from June to November 2017 from out-patients and in-patients of Al-Hussein Teaching Hospital in Thi-Qar province South of Iraq. A sum of 132 specimens from the hospital facility condition and different clinical samples of various patients were randomly collected and analyzed for diagnosis of *Pseudomonas aeruginosa*. These clinical specimens were included wound, burn, ear swabs, urine and sputum specimens. Every single specimen was screened to detect of *P. aeruginosa* by culturing on suitable media, and 36 isolates of *P. aeruginosa* were distinguished through biochemical tests and using API 20NE system for confirmation the isolates. All *P. aeruginosa* isolates were tested against nine antimicrobial discs *in vitro*. They revealed that having different classes and the results demonstrated that variable resistance to the anti-microbial agents. In attempting to the recognizable proof of *P. aeruginosa* having the DNA, Polymerase chain reaction (PCR) was utilized in light of particular groundwork for 16S rRNA genetic marker. The results demonstrated that PCR has observed to be quick and sensitive and particular for recognizable proof of *P. aeruginosa*. Furthermore, 16S rRNA was utilized as confirmation feature, while *las*B detected as virulence gene also to utilize as pathogenesis quality of the bacteria.

Keywords: *Pseudomonas aeruginosa*,16S rRNA gene, *las*B gene.

Introduction:

Pseudomonas aeruginosa is an opportunistic pathogen that is known to be in charge of an extensive assortment of genuine and often dangerous sicknesses. Its pathogenicity is emphatically identified with the creation of a remarkably wide number of harmful factors, which cause tissue harm, delay epithelium wound repair, extraordinarily effective of biofilm development and restricted generation resistant (Quick *et al.* 2014).

P. aeruginosa is most associated with human diseases; the bacterium is viewed as a pioneering pathogen. The bacteria is frequently in responsibility of nosocomial diseases and contaminations in immunocompromised patients, contaminations caused by the bacteria are of specific worry in patients with serious consumes, growth, cystic fibrosis and (AIDS) (Coggan and Wolfgang, 2007). The capacity of *Pseudomonas* to cause infection is additionally aggravated by an abnormal state of resistance against antibacterial agents, which make it hard to treat (Breathnach *et al.* 2012). In the previous years, entire genome sequencing has begun to be utilized to research episodes in healing facilities. Entire genome sequencing is appealing a direct result of its advanced, shareable configuration and ultra-high determination, which can segregate two disconnects varying by only a solitary transformation (Quick *et al.* 2014). *P.* aeruginosa also has countless factors, for example, exotoxin A, exoenzyme S, elastase and sialidase, which are capably managed by cell-to-cell flagging bases. The las framework has been appeared to direct the outflow of a

few harmfulness factors, for example, extracellular compounds (*las*B elastase, *las*A protease, antacid protease), optional metabolites (pyocyanin, hydrogen cyanide, pyoverdin), poisons (exotoxin A) (Rumbaugh *et al.* 1999).

Part of elastase B in have pathogen collaboration Elastase B was first recognized as an elastolytic protease and this movement is accepted to assume a key part in the lung contamination. Elastase B can corrupt human elastin (Van Der Plas *et al.* 2016). The aim of this study is to isolate *P. aeruginosa* from different clinical sources and to confirm the isolates using PA-SS specific primer with detection *las*B gene as a virulence factor.

Materials and Methods:

The study was conducted through a period from June to November 2017. The samples were collected from out-patients and in-patients of Al-Hussein Teaching Hospital in Thi-Qar province south of Iraq. A sum of 132 specimens and swabs from hospital environment and different clinical of various patients were randomly collected and inspected for identification of *P. aeruginosa*. These clinical specimens included 45 burn (34.09%), 14 wound (10.60%), 22 ear swabs (16.66%), 14 sputum (10.60%), 12 urine (9.09%) and 25 environmental specimens (18.93%).

All specimens were inoculated on blood agar and MacConkey agar and incubated for 24 hours at 37 °C under aerobic conditions. The isolates were diagnosed depending on morphological features of colonies and microscopical examination with Gram stain then biochemical tests which were used to detection *P. aeruginosa* isolates. Diagnosis of species was confirmed by API 20NE system. Susceptibility tests were performed for all bacterial isolates against nine antimicrobial agents (Oxoid, UK) from different classes have been determined using Kirby-Bauer disc diffusion method (Bauer *et al.* 1966). Inhibition zones around antibiotic discs were measured as in Clinical and Laboratory Standards Institute (CLSI 2017).

The antibiotic agents tested (Oxoid, UK) included Amikacin (AK: 30 µg), Ciprofloxacin (CIP:5 µg), Cefotaxime (CTX:30µg), Levofloxacin (LEV:5 µg), Refampicin (REF:10 µg), Ciftriaxone (CRO:30 µg), Cefixime (CFM: 5 µg), Doxycycline (DXT: 30 µg) and Gentamycin (GM:10 µg). In addition, pure colonies of the clinical and environmental isolates were subjected for DNA extraction (Qiagen, UK). PA-SS primers of 16S rRNA gene (956 bp) and *lasB* gene (284 bp) genes, which designed by (Spilker *et al.* 2004) and (Wolska and Szweda 2009) respectively were used in the study (Table 1).

Table 1: The specific primers used to confirm *P. aeruginosa* isolates and *las*B gene

Primer		DNA Sequences (5'-3')	Length bp	Product Size bp	Reference
*PA-SS	F	GGGGGATCTTCGGACCTCA	19	956	(Spilker *et al.* 2004)
	R	TCCTTAGAGTGCCCACCCG	19		
lasB	F	GGAATGAACGAAGCGTTCTCCGAC	24	284	(Wolska and Szweda 2009)
	R	TTGGCGTCGACGAACACCTCG	21		

*PA-SS: specific marker for *P. aeruginosa*; F: forward primer; R: reverse primer; bp: base pair

PCR amplification was used of targeted DNA for both genes were carried out in 20 µl reaction volumes, each of them containing 2 mM MgCl2, 50 mMTrizma (pH 8.3), 250 µM (each) deoxynucleoside triphosphates (Promega, USA). Also, Primers (0.4 µM), 1 U of *Taq* polymerase (Invitrogen, UK), and 2 µl of cell bacterial lysate were added and adjusted to 20 µl by the addition of molecular grade H_2O. Amplification of 16S rRNA gene was performed by RapidCycler thermocontroller. After an initial denaturation for 2 min at 95 °C, 25 cycles were completed, each consisting of 20 sec at 94 °C, 20 sec at the appropriate annealing temperature and 40 sec at 72 °C. A final extension of 1 min at 72 °C was applied. In the *lasB* gene the DNA was amplified using the following protocol: 94 °C for 3 min, 30 cycles of 94 °C for 30 sec, 57 °C for one min and, 72 °C for one min and 30 sec, and 72 °C for 5 min. DNA fragments were analysed by electrophoresis in a 1.5 % agarose gel at 85 volte for one hour in 1X TBE 40 mMTris–HCl (pH 8.3), 2 mM acetate and 1 mM EDTA) containing 0.05 mg/L ethidium bromide.

Results:

A total of 132 specimens and swabs from environmental and various clinical of different patients were randomly collected and examined for detection of *P. aeruginosa* bacteria. These clinical specimens included: burn swabs, wound swabs, ear swabs, sputum tests, urine of patients with urinary tract infections and environmental specimens (Table 2). Every single gathered specimen was screened for detection of *P. aeruginosa* by culturing on suitable media and 36 *P. aeruginosa* isolates were recognized by means of biochemical tests and confirmed by API 20NE system. All the *P. aeruginosa* isolates were tested against nine

antimicrobial discs *in vitro*. They revealed that having different classes and the results demonstrated that variable resistance to the anti-microbial agents as found in Table 3.

The amplification products were identified 16S rRNA and *las*B positive from their sizes in agarose gels. Overall, PA-SS primer for 16S rRNA gene of *P. aeruginosa* (956 bp) was identified in 36/36 (100%) of all clinical and environmental isolates. The results of screened for *las*B virulence gene of *P. aeruginosa* gave positive results 27/36 (75.00 %) that equal to target (284 bp) product size as found in Table 4.

Table 2: Number and percentage of specimens and the bacterial growth

Sample collection	No	%	*P. aeruginosa*	
			No	%
Burn swabs	45	34.09	16	44.44
Ear swabs	22	16.60	8	22.22
Urine specimens	12	0.09	1	2.72
Sputum specimens	14	10.60	1	2.72
Wound swabs	14	10.60	3	8.33
Environmental swabs	25	18.93	7	19.44
Total	132	100	36	100

Table 3: Antibiotic susceptibility of *P. aeruginosa* isolates

Antibiotic discs	Resistant No (%)	Intermediate No (%)	Sensitive No (%)
Amikacin (AK)	9 (25.00)	6 (16.66)	21 (58.33)
Ciprofloxacin (CIP)	22(61.11)	8(22.22)	6(16.66)
Cefotaxime (CTX)	14(38.88)	10(27.77)	12(33.33)
Levofloxacin (LEV)	12(33.33)	4(11.11)	20(55.55)
Refampicin (REF)	28(77.77)	6(16.66)	2(5.55)
Ciftriaxone (CRO)	7(19.40)	8(22.22)	21(58.33)
Gentamycin (GM)	18(50.00)	10(27.77)	8(22.22)
Cefixime (CFM)	22(61.11)	8(22.22)	6(16.66)
Doxycycline (DXT)	20(55.55)	9(25.00)	7(19.40)

Table 4: Determination of 16Sr RNA and *las*B genes in *P. aeruginosa* isolates

Type of samples	No	PA-SS gene		*las*B gene	
		Positive	%	Positive	%
Burn	18	18	50.00	15	41.66
Wound	1	1	2.77	1	2.77
Urine	1	1	2.77	1	2.77
Sputum	2	2	5.55	1	2.77
Ear	4	4	11.11	2	5.55
Environment	10	10	27.77	7	19.44
Total	36	36	100	27	75.00

The *P. aeruginosa* specific primer (PA-SS) was used for amplification 16S rRNA gene at product size 956 bp to identify the bacteria in all (100%) medical and environment isolates using 1.5 % agarose gel electrophoresis. For determination of the *las*B virulence gene of *P. aeruginosa* specific primer (*las*B) was used for amplification the gene at product size 284 bp in 75 % *P. aeruginosa* isolates using 1.5 % agarose gel electrophoresis (data not shown).

Discussion:

As per the outcomes, there was a high recurrence (68.20 %) of resistance against all the used antimicrobial agents. These outcomes showed a serious antimicrobial resistance among *P. aeruginosa* which may be because of the unacceptable utilization of antimicrobial in these situations. The adequacy of antibiotic agents against *P. aeruginosa* infections, which is constrained by the species low penetrability and cell membrane, has been additionally decreased by the development of multidrug-resistant strains (Breathnach *et al.* 2012). Consequently there is an urgent requirement for novel methodologies, including effective pharmacotherapies, to treat patients with *P. aeruginosa* diseases (Kitao *et al.* 2018). This study demonstrated that the recurrence of multidrug-resistant (MRD) *P. aeruginosa* was 68.20%. This high recurrence may be because of the long staying in hospital and serious utilization of antibiotics. MDR in *P. aeruginosa* can be intervened by methods for a few components including the creation multidrug efflux frameworks, catalyst generation, or external layer protein misfortune and target transformations (Tavajjohi *et al.*, 2011). *P. aeruginosa* have capacity to cause issues for the affectability. In this way, it has turned out to be basic to create genotype-based order frameworks prepared to do precisely distinguishing in spite of any phenotypic alterations; DNA marker and using 16S rRNA specific primer utilize a fast recognizable proof of species. Among DNA marker, the PCR is exceedingly delicate particular and fast strategy which hugely upgraded the location of *P. aeruginosa* particularly when utilizing species-particular preliminary (Pearson *et al.* 2000). Specific amplification of *Pseudomonas* 16S rRNA quality by PCR has been utilized to distinguish separates *Pseudomonas* species from clinical and environmental specimens. Also it is used for genus or species determination of *P. aeruginosa* (Spilker *et al.* 2004).

In the study, the most tested isolates (75.00 %) possessed *las*B gene as virulence factor. This indicates that the *P. aeruginosa* isolates having pathogenic factor to generate different types of infections. This finding is in agreement with previous reports (Nikbin *et al.* 2012). The pathogenicity of *P. aeruginosa* is clearly multifactorial. *Las*B gene is one of the most important proteases of *P. aeruginosa* (Lomholt *et al.*, 2001). Mutation of *las*B virulence gene reduces significantly *P. aeruginosa* invasion. Prevalence of the *las*B gene in the most of environmental and clinical isolates implies the importance of *las*B factor to survival of *P. aeruginosa* in various locations (Rumbaugh *et al.*, 1999).

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