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Detection of virulence genes of pseudomonas aeruginosa that isolated from environmental of hospital

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Abstract

hundred and fifty samples have been collected during February 2022 till April 2022, from wounds, burns, urinary tract and otitis media cultured directly on blood and macConkey agar after that tested with biochemical test to identify the bacteria then detect the virulence genes by molecular test, 11 isolates were identified as *P. aeruginosa* that from them eight isolates were from burn infections and wounds infection, and three isolates were from urinary tract infections and otitis media. The isolates were identified by culturing on MacConkey agar and blood agar, biochemical tests including oxidase test and catalase test.. Detection of some virulence genes of *P. aeruginosa* which included *las B, alg D, pvd A* was performed. our results showed that all strains have the (*las B, alg D, pvd A*) virulence genes except 2 strains were negative for pvdA 1281 bp.

Key word; virulence genes, *Pseudomonas aeruginosa* and Basrah hospital

Introduction

Pseudomonas aeruginosa is a main source of nosocomial contamination, classify second most normal microorganism isolated from patients among the gram negative microorganisms answered to Public Nosocomial Irresistible Observation (NNIS) framework. (1,2). *P. aeruginosa* is the second most normal reason for nosocomial pneumonia, the third most normal reason for nosocomial urinary tract diseases, and the seventh most

normal reason for nosocomial bacteremia (3,4). *P. aeruginosa* is a clinically huge astute microorganism which seldom causes infection in sound invulnerable capable people, the rise of multidrug resistant strains in *P. aeruginosa* confines has expanded overall (5,6).

Multidrug resistant (MDR) *P. aeruginosa* is hard to destroy because of raised characteristic obstruction (chromosomally encoded opposition) and its capacity to gain protection from various antimicrobials (7,8). This nosocomial microbe has different mechanisms of protection against antimicrobials, like as broad spectrum ß-lactamases and metallo-ß-lactamases (MBL), through the change of penicillin-binding proteins (PBP), porin transformations, plasmid enzymatic alteration, DNA-gyrase transformations, and dynamic siphons (9,10).

The clinical issue results from this life form are its capacity to oppose practically all antibacterial specialists, prompting prevail over it when the touchy organic entities are stifled by these specialists (11). There are restricted quantities of antimicrobial specialists the counter pseudomonal including Penicillins, Cephalosporin, Carbapenems, Aminoglycosides and Fluoroquinolons with dependable action against it (12,13).

Additionally, the restoratively significance of this organic entity might be lies in its capacity to create an assortment of poisons, extracellular proteins including elastases, proteases and hemolysins (14,15).

Materials and Methods

Patients

This study included (150) patients who were referred to teaching Basrah hospital during a period of four months from February 2022 to April 2022. The patient's age ranged from 6-65 years.

3.4.2 Specimen collection:

Totally (150) swab samples were collected, of which, 100 specimens were collected from burns and wounds infections and 50 samples were collected from urine and otitis media. Each swab was taken carefully from the site of infection and placed in transported media to maintain the swab wet until taken to laboratory.

Each specimen was inoculated on blood agar and MacConkey agar. All plates were incubated aerobically in incubator at 37ºC for 48 hours

3.5 Identification of *P. aeruginosa* isolates

3.5.1 Colonial morphology and microscopic examination

The grown colonies on the Mueller-Hinton agar characterized by diffusible pigments and sweet-grape odor were selected for further diagnostic tests. The results of the following tests regarding diagnosis at *P. aeruginosa* were according to (MacFadden, 2000).

Cultural characteristics include; colonial morphology (smooth mucoid), grape odor, its color and diffusible pigments on Mueller-Hinton agar (bluish green or yellowish green) and inability to ferment lactose on MacConkey agar (15).

Microscopic examination includes the examination of shape (rods), gram–stain reaction, arrangement of cells with each other, motility and capsule presence.

PCR reaction for virulence genes

3.7.3.1. The primers used in PCR reaction

The primers (Alph DNA company /USA/Canada) were lyophilized, they dissolved in the free ddH_2O to give a final concentration of 100 ρmol/µl as stock solution and keep a stock at -20°C to prepare 10 ρmol/µl concentration as work primer.

2. PCR reaction for *alg*D gene

The PCR reaction for *alg*D was done according to Mitov et al (2010).

Table 3.8. Presents details of the used primers.

Table 3.8. Primers used for screening of *alg* D gene among *P. aeruginosa* isolates.				
Primer	Sequence	Tm (°C)	GC (%)	Product size
F	5'- ATGCGAATCAGCATCTTTGGT - 3'	60	66	1301bp
R	5'- CTACCAGCAGATGCCCTCGGC - 3’	70	76	

3. PCR reaction for *las*B gene

The PCR reaction for *las* B was done according to Sonbol et al (2015) Table 3.12. Presents details of the used primers.

Table 3.12. Used primers for *las* B gene amplification.				
Primer	**Sequence**	**Tm (°C)**	**GC (%)**	**Product size**

F	**5'- GGAATGAACGAAGCGTTCTC - 3'**	**60**	**68**	**300 bp**
R	**5'- GGTCCAGTAGTAGCGGTTGG – 3'**	**64**	**72**	

The reaction mixture was prepared as described for *alg* D gene amplification. Same PCR program was also used except that 50°C was used as annealing temperature.

Statistical analysis

was exhibited by expending Chi-square ($\chi 2$) examination to control the numerical variations between various collections by consuming **an** application statistical stand for social science (SPSS 19). The opportunity of ($P \leq 0.05$) was restrained to be statistically important.

RESULTS AND DISCUSSION

Isolation and identification of *P. aeruginosa*

In this study a total of (150) samples were collected, of which 100 (66.66%) samples were obtained from patients suffering from burns and wound and 50(33.33%) samples were collected from urinary tract infections (UTIs) and otitis media. Then, the samples were subjected to different types of media to isolate *P. aeruginosa.* From the 150 clinical samples only 11(7.33%) isolates were belonged to *P. aeruginosa* and others were belonging to the other genera. These isolates were obtained from burn and wounds 8(5.3%) and 3 isolates were obtained from urinary tract infections (UTIs) and otitis media. These samples were subjected to media for primary isolation of *P. aeruginosa* which appears circular mucoid smooth, and then

cultured on blood agar, most isolates produced β-hemolysis on blood agar and other do not produce hemolysis. All isolates grew on MacConkey agar, but do not ferment lactose, and the isolates grew on the Mueller- Hinton agar which produces the diagnostic pigment, that varied from yellowish-green to bluish-green and also the isolates emit a grape-like odor.

In present study, some biochemical tests were carried out, and the results compared with standard result documented by (101), which is shown in the table (4.1). All isolates were Gram negative rods and positive for oxidase, catalase, able to grow at 42^{o}C and did non-ferment any sugar.

Table 4.1. Biochemical tests for identification of *P. aeruginosa*

Tests	**Result**	**Tests**	**Result**	**Tests**	**Result**
Catalase test	+	Indole test	-	H_2S	-
Oxidase test	+	Kligler's iron agar	K/K	Motility	+
Gelatin Liquefaction	+	Methyl-red	-	Simmon's citrate	+
Growth at 42°c	+	Voges-Proskauer	-	ß-hemolysis	+/-
Gram-stain	- rods	Pigments production	+		

All isolates of *P. aeruginosa* produce different diffusible pigments (pyocynine, fluorescein) as with other when grow on Mueller-Hinton agar and they produce grape-like odor.

Molecular detection of virulence genes

The bacterial DNA extracted from 11 isolates of *P. aeruginosa* which grown in nutrient agar medium, according to the protocol of the supplying company. Purity and concentration of DNA was confirmed with Nanodrop spectrophotometer system and electrophoresis in agarose gel (Figure 4.3).

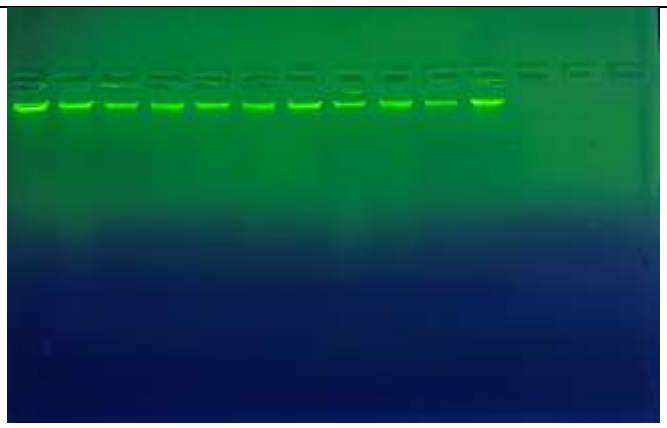

Figure 4.3. Gel electrophoresis of genomic DNA extracted from isolates, 1% agarose at 5 vol /cm for 30 min.

In this study the B *las*, D *alg* and A*pvd* virulence genes of *P. aeruginosa* were investigated for detection of pathogenesis of this bacteria. Our results showed that all strains possess the B *las*, D *alg* and A*pvd* virulence genes except 3 strains that was postive for A*pvd* 1281 bp. Table 4.2.

Present the results of molecular detection of bacteria.

Table 4.2. PCR results of virulence genes of P. aeruginosa isolates

Strains No.		1	2	3	4	5	6	7	8	9	10	11
PCR Result	*alg*D (1310bp)	+	+	+	+	+	+	+	+	+	+	+
	*las*B (300bp)	+	+	+	+	+	+	+	+	+	+	+
	*pvd*A (1281bp)	+	-	-	-	+	-	-	-	-	-	+

The gel electrophoresis of B *las*, D *alg* and A*pvd* genes has been presented in figures 4.4 till figure 4.6.

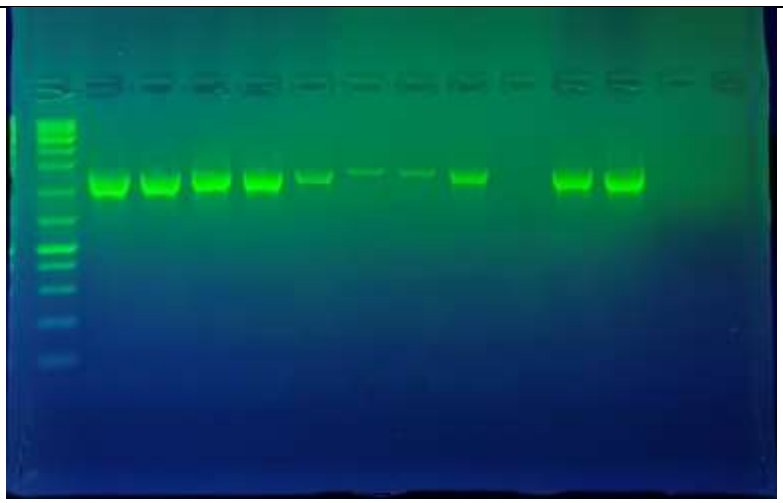

Figure 4.4. Electrophoresis of *algD* PCR product (1310 bp). 1% agarose. N: ladder (1000 plus) bp.

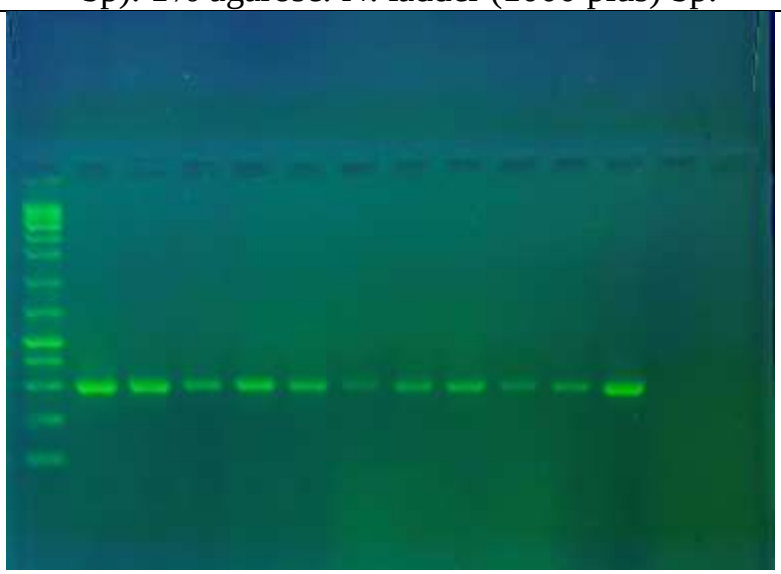

Figure 4.5. Electrophoresis of *lasB* PCR product (1310 bp). 1% agarose. N: ladder (1000 plus) bp.

Our results same that studied by (16) who found that among the (2800) isolates of *P. aeruginosa* 420(15.00%) isolates from burn infections, but the results were not agreed with the results obtained by (17) who found that among the 65 isolates of this bacterium 5(7.7%) burn were found

This result was resembling with the result reported by Philip et al., who found that *P. aeruginosa* present in wound infection in rate (5.76%),

but did not resemble the result obtained by (18) who found that from 108 isolates of *P. aeruginosa* only 99(92%) were from wound infection.

The result obtained in this study showed that 4 isolates of *P. aeruginosa* were from patients with otitis media and UTI. The isolation percentage of *P. aeruginosa* was reached to (11%) if compared with other causative agents (aerobic and anaerobic bacteria) isolates from otitis media. *P. aeruginosa* was the most common organism isolated from mild to severe form of otitis externa and chronic supportive otitis media, different types of bacteria can reach to the middle ear because they can persistent or recurrent discharge through a chronic perforation of the tympanic membrane due to perforated tympanic membrane, bacteria can gain entry into the middle ear via the external ear canal. Infection of middle ear mucosa subsequently results in ear discharge (19). Molecular typing methods are often necessary, to trace epidemic strains and to detect outbreaks or cross-transmission in the hospital setting also to characterize long-term colonizing isolates with atypical phenotypes, as have been observed in cystic fibrosis patients (20,21). Molecular diagnostics minimizes the requirement for cultivation, which reduce the time required for morphology and biochemistry diagnosis (22). The quality and quantity of nucleic acids can be maintained for a prolonged period with appropriate storage temperature and specimen preservation (23).

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