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Original Research Article

Efficacy of the oprL gene in identifying hospital-isolated Pseudomonas aeruginosa

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Abstract

Background: Pseudomonas aeruginosa is one of the opportunistic species that is present in the hospital environment and causes many hospital-acquired infections such as wound infections, burns infections and UTI.

This study aims to detect the prevalence of *Pseudomonas aeruginosa* in hospital environments and testing the ability of obtained strains to produce biofilms as well as evaluating *oprL* gene as specific molecular marker to identify *Pseudomonas aeruginosa*.

Materials and Methods: (100 clinical and 100 environmental) samples were collected from Al-Diwaniyah Hospital and Burn Hospital in Diwaniyah. Samples were cultured and isolates then identified using biochemical tests, VITEK2-automated system, and molecular detection of *oprL* gene. The method of Tissue Culture Plate was used for detection of biofilm formation.

Results: The results showed that 25 *Pseudomonas aeruginosa* isolates were obtained; 13 of them were clinical isolates and 12 environmental isolates. The ability of bacteria to produce biofilms was also measured, as (23) (92%) isolates capable of producing biofilms were obtained, distributed among (8) (32%) weak, (12) (48%) moderate, and (3)(12%) strong, and only two isolates were unable to produce biofilms. We also found that the *oprL* gene was identified in 25 (100%) out of the 25 isolates of *Pseudomonas aeruginosa*.

Conclusion: The environment can be a source for certain bacterial pathogens associated with nosocomial infections, particularly *Pseudomonas aeruginosa* which can be identified rapidly by *oprL* gene as a specific molecular marker alternative to traditional methods.

Keywords: EDTA, Biocides, Pseudomonas aeruginosa, Clinical samples, Environmental samples, MIC, MBC

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1. Introduction

Pseudomonas aeruginosa is among the most important factors causing multidrug-resistant (MDR) and extensively drug-resistant (XDR) bacterial infections, which cause significant virulence and mortality when they enter the respiratory system, circulatory system, urinary tract, and central nervous system, as they cause infections in those systems into which they enter.¹

As the most common source of nosocomial infections in humans, *Pseudomonas aeruginosa* strains have become a major threat to the health of hospitalized patients with chronic diseases, weakened immune systems, and diseases.² It is difficult to treat infection with *Pseudomonas aeruginosa*, as

it has a high intrinsic resistance to many antibiotics or because of acquired resistance genes that develop over time, which occurs through genetic genes that encode chromosomal mutations or through horizontal genetic transfer of factors that cause antibiotic resistance. These are considered main reasons for bacteria to become resistant to antibiotics.^{3,4} There is very little information regarding nosocomial infections, such as *Pseudomonas aeruginosa* infection, due to the lack of quality and surveillance systems and the lack of mechanisms to control diseases and their spread.⁵ Infection occurs in the hospitals during surgical operations or during injury or burns, and the infection may be transmitted or infect medical staff in hospitals.⁶

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Pseudomonas aeruginosa affects a large percentage of patients who recover from several diseases during their stay in hospitals, such as lungs, infected corneas, and burns, as they appear as side effects that accompany recovery from other diseases.⁷ It is clear that Pseudomonas aeruginosa infection has become common and new strains with high drug resistance that have appeared frequently in hospital corridors and intensive care rooms due to their ability to survive and grow in different environmental conditions of temperature, humidity, air, and lack of nutrients.⁷

Pseudomonas aeruginosa is found in various places, especially on surfaces, such as medical equipment, devices in the patient's room, bed, and hospital floor. It produces biofilms that enable it to resist antibiotics, causing long-term infections that are difficult to treat over time.⁸

Biofilm acts as a firewall to defend bacterial cells from various factors that threaten them such as the host's immune system, antibiotics, various environmental conditions, and disinfectants. 9-11 oprL gene, has high sensitivity and specificity of Pseudomonas aeruginosa 12 and it is a peptidoglycan-related outer membrane protein, that bond the outer membrane to the main peptidoglycan. It is also consider as Lipoprotein (oprL) in charge of intrinsic resistance to antibacterial agents like antibiotics and antiseptics. 13

2. Materials and Methods

2.1. Samples collections

The samples that collected from the Teaching Hospital and the Burns department in Al-Diwaniyah Governorate for the period from July 2023 to December 2023 with a total of 200 samples were 100 Clinical samples that collected from patients with burns of different degrees. In addition 100 hospital environmental samples, as shown the Distribution of *Pseudomonas aeruginosa* isolates in **Table 1**, all of environment samples were collected by sterile tubes with tryptic soy broth (TSB). Ear and burns samples were collected in sterile cotton swab sticks with media. Patients suffering from urinary tract infections (UTI) were instructed on how to collect a sample of urine.

Clinical samples were collected during the morning periods during the presence of the workers and patients, while the burn samples were collected from the patients before the sterile of the wounds in early time of morning. The Environmental swabs were taken before the sterilization and cleaning of the hospital in early time of the morning too. ¹⁴

2.2. Isolation of Pseudomonas aeruginosa

The environmental samples were taken using sterile cotton swabs and placed in sterile tubes containing 2 ml of tryptic soy broth (TSB). They were incubated at 37°C for 24 hours for activation, then they were streaked with a sterile loop into dishes containing MacConkey agar medium. Clinical

samples directly streaked by sterile loop on macConkey agar and blood agar plates, then incubated at 37°C for 24 hours. Then both environmental and clinical isolates streaked by sterile loop in cetrimide agar which is a selective medium for *Pseudomonas aeruginosa*, and then incubated at 37°C for 24 hours.¹⁵

2.3. Identification of Pseudomonas aeruginosa

Microscopic Identification Based on Gram's staining, colony phenotype on several media, blood hemolysis, and pigment production, isolates were first identified. Isolates of *Pseudomonas aeruginosa* were also confirmed by biochemical tests and the VITEK2-automated system. Further diagnosis for all isolates was performed by detecting *oprL* gene by polymerase chain reaction as follows:

The confirmed Genomic DNA was extracted using all Pseudomonas aeruginosa isolates from fresh overnight culture by using FavorPrepTM Blood/ cultures cell Genomic DNA Extraction Mini kit (Taiwan). Specific primers listed in **Table 2** were used to amplify *oprL* gene. As per instructions provided by the supplier, primers were in a lyophilised form dissolved in the nuclease-free water to make up the final concentrations up to 100 pmol/mL. A 50 µL reaction mixture was prepared to conduct PCR amplification of the oprL gene, which contains 3 µl forward and reverse primers, 14 µl nuclease-free water, 5 µl Template DNA, 25 µl Master Mix. PCR was carried out under the following conditions: denaturation at 94°C for 40 seconds, annealing at 57°C for 40 seconds, elongation at 72°C for 50seconds. Agarose gel electrophoresis with a 1% agarose gel stained with ethidium bromide was utilized to visualize the PCR products Figure 1.16

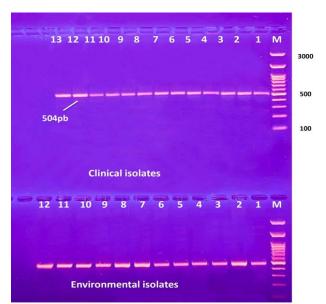


Figure 1: The PCR products of *oprL* gene by Agarose gel electrophoresis.

Table 1: The distribution of *Pseudomonas aeruginosa* isolates among environmental and clinical samples

Sample's Source	Sample's Type	No. of	No. of Pseudomonas	Percentage of
		samples	aeruginosa	Pseudomonas aeruginosa
Environmental	Patients' bed	10	2	16.7
swabs	Medical devise	14	0	0
	Waste containers	10	1	8.3
	Tables surface	9	2	16.7
	Hands of medical workers	8	0	0
	Stairs	9	1	8.3
	Doors handles	9	1	8.3
	Sinks	8	2	16.7
	Walls	9	0	0
	Floor	9	2	16.7
	Ducts	5	1	8.3
	Total	100	12	100
Clinical swabs	Wound	10	3	23.1
	Burn	30	6	46.1
	Ear swap	30	2	15.4
	UTI	30	2	15.4
	Total	100	13	100

Table 2: Oligonucleotide primers for amplification of the oprL Gene

Primers	PCR Program	Primer Nucleotide Sequence	Amplicon Size (bp)	Reference
oprL	30 cycles: 40 s 94°C,	F:5'-ATGGAAATGCTGAAATTCGGC-3'	504	16
	40 s 57°C, 50 s 72°C	R: 5'- CTTCTTCAGCTCGACGCGACG -3		

2.4. Biofilm production

The tissue culture plate method was chosen according to, ¹⁷ where several colonies from a fresh bacterial culture were inoculated with 10 ml of tryptic soy broth (TSB) supplemented with 1% glucose using a sterile loop.

The test is conducted in three replicates and an average of three values is taken. 12,18

3. Results

3.1. Isolation of Pseudomonas aeruginosa

Out of 200 environmental and clinical samples, 25 isolates were obtained, 13 clinical isolates (13%) and 12 environmental isolates (12%).(**Table 1**)

3.2. Identification of Pseudomonas aeruginosa

After isolating the bacteria, they were cultured on several media, including MacConkey, blood, and Cetrimide agar in order to examine the morphological properties of the colonies. On the MacConkey agar, the colonies appeared small in a pale color colonies because they can't ferment the lactose sugar, mucous in composition, smooth, some of them secreted a capsule that appeared thicker and more viscous as an expression of the resistant type of *Pseudomonas aeruginosa*. In blood agar colonies appeared as β -hemolysis surrounded by a transparent ring, indicating the decomposition of blood cells due to their production of the

enzyme Hemolysin. While on cetrimide agar isolates showed growth of mucoid, smooth in shape with flat edges and elevated center, have fruity odor and yellow to green colonies. (Figure 2)

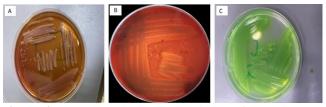


Figure 2: Morphological Characteristics of *Pseudomonas aeruginosa* isolates on A/ MacConkey agar B/ Blood agar, C/ Cetrimide agar

Table 3: The biochemical tests of *Pseudomonas aeruginosa*

No	The Test		The result
1	Catalase test		+
2	Oxidase Test		+
3	Indole Test Medium		-
6	KIA	H ₂ S production	-
		Lactose	-
		Sucrose	-
		Gas	-

The results of the biochemical tests needed to identify bacterial isolates are shown in **Table 3**. From the observation of the results of the biochemical tests of these isolates, they

gave a positive results to the tests of catalase and oxidase, and all isolates were characterized by their inability to ferment sucrose and lactose. Isolates were further confirmed using the Vitek2 Compact with a 99% percent probability ratio.

Polymerase chain reaction analysis was done to detect *oprL* gene in all the obtained (25) *Pseudomonas aeruginosa* isolates. As shown in **Table 4** the *oprL* gene was detected in all *Pseudomonas aeruginosa* isolates in this study. 13 (100%) clinical isolates and 12 (100%) environment isolates.

3.3. Biofilm formation

The results showed that only 23 isolates (with percentage 92%) from the total 25 isolates of *Pseudomonas aeruginosa* isolates formed biofilms, 2 isolates/25 (with percentage 8%) were not biofilms forming. 8 isolates (with percentage 32%) were weak, 12 of isolates (48% percentage) were intermediate, and 3 of isolates (12%) were strong biofilms producer, as shown in **Figure 3,4**.

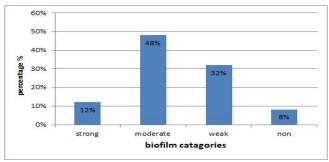


Figure 3: Biofilm categories of *Pseudomonas aeruginosa* isolates.

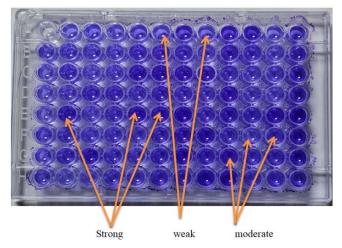


Figure 4: The variation in the intensity of biofilm production in the *Pseudomonas aeruginosa* isolates under study using the Microtiter Plate method, as dark purple pits indicate highly biofilm-forming isolates.

Table 4: The *oprL* gene distribution between clinical and environmental strains of *Pseudomonas aeruginosa*

oprL gene	Clinical	Environment	Total
Positive, n(%)	13(100)%	12(100)%	25(100%)
Negative, n(%)	0(0)%	0 (0)%	0(0%)

4. Discussion

Different clinical samples were collected from wounds, burns, otitis media, and urinary tract. 13 clinical isolates of the *Pseudomonas aeruginosa* were gotten out of 100 samples, which constituted a percentage of 13%. Similar studies have been done by other researchers, such as, 19 who found that the percentage of clinical isolates was 25.9%, while 20 found that the percentage of clinical isolates was 18%, As for 21 who found that the percentage for clinical isolates reached 10%, while 22 obtained a percentage that reached 13.3%, which is a percentage close to the results of our study, as 30 obtained (12.6%). While 4 found the isolate of *Pseudomonas aeruginosa* was (17%).

For the environmental samples, also noticed that the environmental isolates amounted to 12/100 (12%), while²⁵ found 34%,²⁶ obtained a percentage of 19%, and²¹ a percentage of 8%. The reason for the difference in percentages is due to the geographical location of the hospital in relation to the city, as it is preferable to be located on the outskirts of the city in terms of the entry of monsoon winds, in addition to the amount of visitors and the percentage of crowding, to the type of sterilizers used to disinfect the floors and walls, and the percentage of ventilation, as ventilation is important to reduce number of bacteria in the atmosphere.

With regard to clinical isolates, the number of isolates acquired from burns appeared at 46.1%, which is the highest percentage among the remaining isolates, while isolates from wounds amounted to 23.1%. The percentage of wounds in our study is consistent with²⁷ at 28% and,²¹ who obtained a percentage of 29%. It is also close to what²⁴ obtained with a percentage of 35.71%,¹⁹ with a percentage of 24%, while²⁸ obtained on range of wound isolate as 40%. As for burns, the percentage is close to what was obtained by with a percentage of 58.5%, while²¹ obtained a percentage of 19.35,³⁰ obtained a percentage of 22%,²⁰ obtained a percentage of 13.3%, while³¹ obtained a high percentage of 68.6%, and²⁸ get of burn isolate range as 30%.

The difference in infection percentages is due to the virulence factors specific to each bacterial strain, in addition to the fact that *Pseudomonas aeruginosa* infection spreads in damaged tissues resulting from burns. It is also indicated that *Pseudomonas aeruginosa* is considered as risk of burns infections by which may lead to death.³² In present study, the ratio of *Pseudomonas aeruginosa* isolates in urine reached to (15.4%), while,³⁰ found a rate of 6.9%, and¹⁹ found a rate of 17%. Furthermore, in Otitis, also we found the percentage of *Pseudomonas aeruginosa* isolates reached 15.4%. These results are consistent with what was found by,²⁰ where the percentage of isolates was 13.51%.

Regarding Biofilm formation, our results are consistent with what was found by³³ where the percentage of isolates capable of producing biofilms was estimated at 96%. It also agrees with his finding of 4% of isolates that were unable to

produce biofilms.³¹ found that 100% of his isolates capable of producing biofilms reached. The percentage of isolates with weak biofilm production reached 4%, while 22% is the percentage of medium isolates, and the percentage of strong isolates reached 74%. This study is also consistent with the finding of³⁴ where the percentage of isolates capable of producing biofilms reached 95.5%, and, 35 who obtained 95% of biofilm producing isolates, as strong, medium, and weak percentages of 63%, 23%, and 8%, respectively, and for those that did not produce biofilm accounted for 5%. The difference in the strength of the biofilm is due to various reasons, such as the variance in the capacity of bacterial isolates to produce living membranes, and perhaps to the initial number of cells that succeeded in attaching to the holes of the microplate. The ability to form biofilms may prevent the passage of sterilizers and treatments to reach their target in the bacterial cell.³⁶

Also the oprL gene was identified in all 25 clinical and Environment isolates in this study $Pseudomonas\ aeruginosa$. These results confirm that all our isolates are $Pseudomonas\ aeruginosa$. Our findings are agreement with those documented by 37 and 38 which they found all of them isolates contain of oprL gene. The protest of genes related to virulence- is vital for valuation of the possible pathogenicity of $Pseudomonas\ aeruginosa$.

The virulence gene, *oprL*, is the major constituents of outer membrane lipoproteins of *Pseudomonas aeruginosa* which are also used as markers for the identification of *Pseudomonas aeruginosa*-associated infections.¹⁸

5. Conclusion

The possibility of contacting with a bacterial pathogen is high in hospital environments and the acquired hospital infections are noteworthy providers to diseases and death.

The detection of *oprL* gene by PCR consider as accurate, speedy method for detection of *P.aeroginosa*, because it is specific virulence gene in *P.aeroginosa*. The source of the samples is crucial for understanding the transmission routes and origin of these pathogenic microbes, such as *Pseudomonas aeruginosa*. Environmental samples are the original habitat for pathogenic bacteria.

Through genetic mutations, plasmids, or transformation, they can transform into pathogens that infect humans and animals.

The importance of studying environmental samples is to determine the extent of contamination of the area with these pathogenic bacteria, the rates of their transmission to humans, and the incidence of infection.

It is also important to determine their species to determine the appropriate biocides and disinfectants to eliminate them.

6. Recommendation

This test, the identification of *Pseudomonas aeruginosa* by *oprL* gene can abbreviated many traditional tests that may involve some doubts, high cost and long time.

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