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Molecular Identification of *Pseudomonas Aeruginosa* Isolated from Operations Hall and Their Antibiotics Resistance

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Abstract: *This study aimed to determine the P.aeruginosa that causes infection by molecular technique. Bacterial isolated and identificated phenotypically and genotypically, and their antibiogram detection. One hundred clinical specimens were collected from patients suffering from different clinical infections during the period from September 2024 to January 2024 in AL-Sadder Medical city. The identification of the P.aeruginosa isolates depended on colonial morphology, microscopic examination and biochemical tests. Paeruginosa had the ability to produce capsule, biofilm , adhesion, protease, bacteriocin, haemolysin, B□-lactamase and gelatinase. Regard to molecular study 16 RNA gene appeared th at all isolates due to (100%) of P.aeruginosa isolates. To conclusion that P.aeruginosa able to produce sever pathogenicity markers which responsible for pathogenicity of P.aeruginosa infections.*

Key words *: Pseudomonas aeruginosa, skin infection, virulence factors encoding genes, PCR assay*

1. INTRODUCTION

Pseudomonas aeruginosa is a ubiquitous Gram negative bacterium and an important opportunistic pathogen in the healthcare setting.

Most of the strains of *P.aeruginosa* cultures give a characteristic fruity odor due to the production of aminoacetophenone from tryptophan . Many strains non-lactose fermenting pale coloured on MacConkey agar. oxidase positive. Nosocomial *P. aeruginosa* outbreaks are frequently reported and associated with water sources such as taps, showers, mixer valves and flow straight teners, sink traps and drains. Other potential routes of transmission include cross-infection, for example, through contaminated medical equipment such as endoscopic devices. *P. aeruginosa* also has a large number of virulence factors such as exotoxin A, exoenzyme S, elastase and sialidase which are tightly regulated by cell-to-cell signaling systems. Protein biosynthesis is inhibited by exotoxin A and virulence factor exoenzyme S is secreted by a type III section system.

The production of a biofilm mediated adherence of the bacteria to a surface.The formation of a biofilm gives the bacteria several advantages: it immobilizes the cells, it creates a reservoir of nutrients from lysed cells including DNA,., it also protects the cells from the host immune defense, antibiotics, ultraviolet radiation and oxidizing or charged biocides.

The virulence factors associated with cell structures as single polar flagellum that enables it to move through liquid media to acquire nutrients. **Alginate** is the sticky

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polysaccharide capsule that is over-expressed by mucoid variants of *P. aeruginosa* alginate appears to be a protective mechanism through host immunity and also in resistance to penetration by antibiotics .**Lipopolysaccharide** LPS provides protection against antimicrobial activity of host peptides including defenses and **Outer membrane**, (OM) which provides the bacterium with a hydrophilic surface and functions as a permeability barrier for many external agents.

P. aeruginosa maintains antibiotic resistant plasmids, and is able to transfer these genes by means of the bacterial processes of transduction and conjugation. Only a few antibiotics are effective against Pseudomonas, including some B-lactams, aminoglycosides and fluoroquinolones.The bacterium is naturally resistant to many antibiotics including tetracyclines and benzylpenicillin by mechanisms include: Membrane Permeability ,.Efflux Pumps , Enzymatic inactivation and Mutational Resistance.

The study is aimed to detect the *Pseudomonas aeruginosa* by PCR technique that causes operation hall contamination and antibiogram.

2. METHODOLOGY

-Specimens collection

A total of 10 clinical specimens were collected from various site of operation hall like earth , wall and beds during one month of the study .

-Isolation and identification of *P. aeruginosa*

All specimens were cultured on media including blood agar and MacConkey agar, incubation of for 24 hr at 37ºC, the suspected colonies of pure cultures is bacilli with atypical macroscopic appearance. They were sub cultured on blood agar plates 24 hr after incubation at 37ºC for heamolysis .Catalase test was preformed and their ability to produce pigments on MacConkey and positive for Voges-Proskauer, Then the bacteria were confirmed by PCR system .

-Molecular identification of *P. aeruginosa*.

The PCR assay was performed to detect the (16S rRNA) genes for confirmation the identification of P.aereuginosa. All primers in this study were synthesized by Bioneer Company (Korea) in; F-5 CGACG ATCCGTAACTGGTCT3 and R 5CCGGTGCTTATTCTGTTGGT3 with 203 bp. According to Sambrook et al. (8).

PCR program that apply in the thermocycler to *P. aeruginosa* with condition of initial denaturation 94°C/5 min and the cycling condition of denaturation 95°C / 1min,annealing 54°C /1min and extention 72°C /1min.The final extention was 72°C /5min.The PCR products and

the ladder marker are resolved by electrophoresis on 1.0 % agarose gel. The resolved band is indicative of the corresponding of studied genes. The molecular weight identification of resolved band is based on their correspondence to the ladder bands.

-Detection of DNA content by Agarose gel electrophoresis

Gel electrophoresis was used for detection of DNA by UV transilluminator .Agarose was weighted 1g, boiled in 100ml (1X) TBE buffer, left to cool at 50oC and 5 µl of ethidium bromide is added to agarose and poured on preparing tray. Comb was removed after hardening of agarose leaving wells. 5-10 µl of DNA sample was mixed with 1-2µl of loading dye.

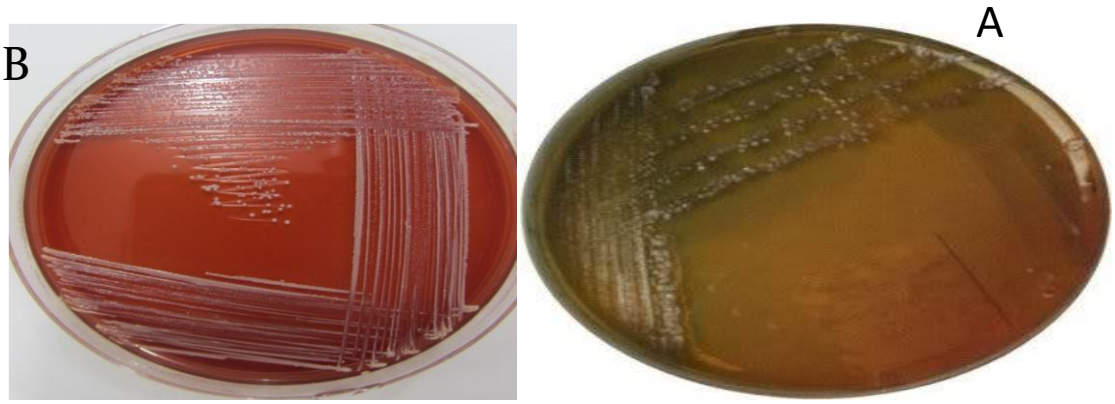
-Agarose electrophoresis

TBE (1X) buffer was added to the electrophoresis tank, tray with agarose was immersed in electrophoresis tank. Each well is loaded with 7µl of DNA sample and standard molecular weight of DNA ladder (marker) is loaded in a first well. Electrophoreses run at 80 volt/cm for 1 hr. Gel was visualized with UV transilluminator and photographed by using digital Camera.

3. RESULTS AND DISCUSSION

-Identification of bacterial isolates

The bacterial isolates obtained basis of colonial morphology and comparison with biochemical characteristics with standard description in Bergeys manual of determinative bacteriology. The microscopic examination showed *P.aeruginosa* are gram negative bacteria bacilli in gram stained films and blue-green in color on Macchonkey agar that expresses the exopigment pyocyanin (11,12.13).



A.*P.aeruginosa* isolate on Macchonkey agar medium

B. A.*P.aeruginosa* isolate on Blood agar medium

The results were compared with referral reported by Collee *et al*. (14). According to the results obtained , a total of 10 *of P.aeruginosa* isolates of studied bacteria were distributed as

follow:5 (50%)isolates from earth , 2(0%) from wall and 3(30%) from beds swabs, as in the table 1.

Type of specimens	No.of P.aeruginosa specimens
earth	5(50%)
Wall	2(20%)
bed	3(30%)
Total	10(100%)

The results of biochemical tests that recorded in table (2) were considered as a complementary of the initial identification of studied bacterial isolates.The isolates confirm to general characteristics, Regard to *P.aeruginosa* gave positive results for indol,Vogus Proskuer, simmon citrate test,oxidase,and catalase while gave negative results for methyl red test, also, the both bacterial isolates . *P.aeruginosa* gave positive results for biofilm formation as in figure 2. (15,16,17).

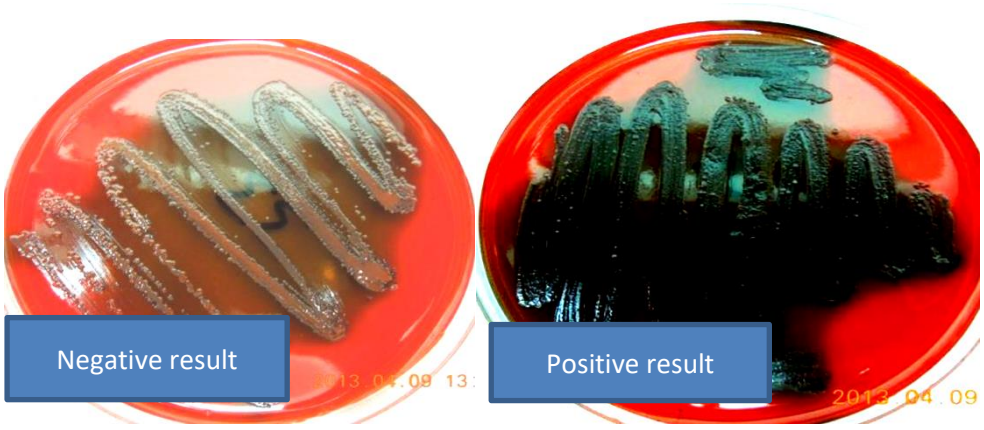


Figure- 1: Biofilm - producing isolates of *P.aeruginosa*

Table -2: Phenotypic characteristics & Carbohydrate ferment of *Pseudomonas aeruginosa* isolates.

Biochemical test	*result*	Biochemical test	*result*
Gram stain	_	Lactose	-
Catalase test	+	Motility	+
Oxidase test	+	Vogas-Proskaures test	+
Indole test	+	Simmon Citrate test	+
Methyl red	_		

-Molecular detection of by PCR technique:

Polymerase chain reaction technique of the *P. aeruginosa* isolates revealed one fragment with 230 bp that represented the 16S rRNA gene . The results show that all isolates of *P. aeruginosa* , carrying *16S r RNA* gene that is characteristic of *P. aeruginosa* as shown in figure(2).

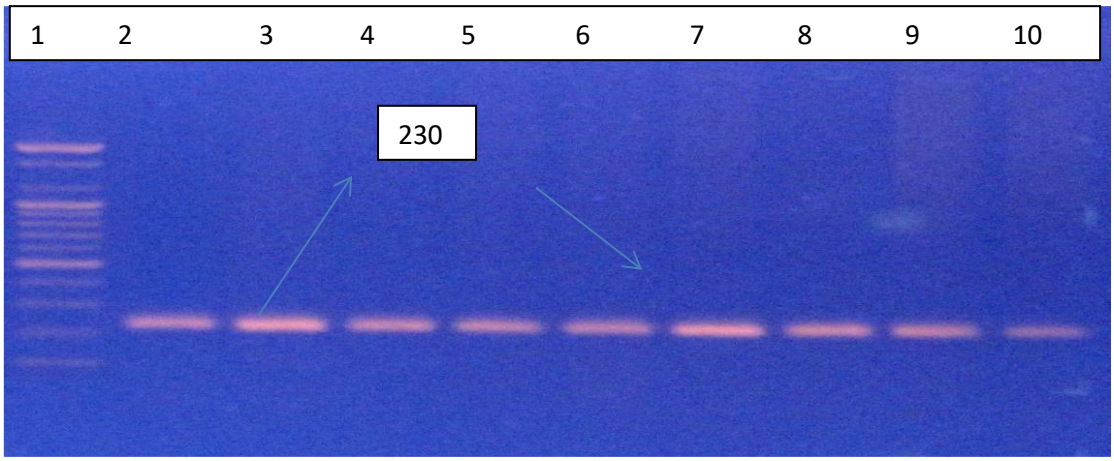


Figure 2 :PCR amplified products of *16S RNA* gene of the *P. aeruginosa* using the designed primers with expected size 230bp.

-Antibiogram for studied bacteria Isolates

The antibiotic sensitivity for antibiotics on bacterial isolates by using Kirby-Bauer disk diffusion method .The results were interpreted according to the diameter of inhibition zones and compared with inhibition zones determined by CLSI(18).

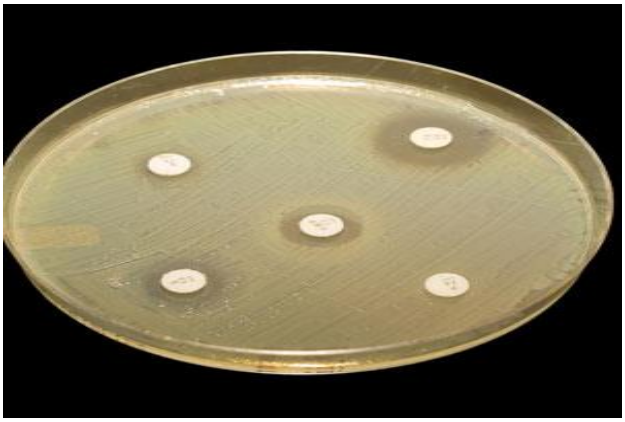


Figure shows antibiotic susceptibility to:- -P.aeruginusa on Muller-Hinton media

The results revealed that the *P.aeruginosa* isolates exhibited highly sensitivity to Amikacin 23(96%),to Ciprofloxacin 22(100%),and to Gentamycin 22(100%) respectively, while bacteria were resist to Cefotaxime as 21(96%).As in table -3.9(19.20) .

Table -3: Antibiogram results of *Pseudomonas aeruginosa* isolates

Antibiotic / *bacteria.*		Amikacin	Ciprofloxacin	Ceftriaxon	Gentamycin	Meropenem	Azithromycin	Cefotaxime
P.aeruginosa	**R**	1(4%)	0(0%)	2(8%)	0(%)	1(4%)	2(17%)	9(96%)
	S	9(96%)	10(100%	8(92%)	10(100 %	9(96%	8(83%)	1(4%)

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