Molecular Detection of *Pseudomonas aeruginosa* and its Relationship with Multidrug Resistance and Transposons

Wathiq Abbas Hatite Al-Daraghi[1], Mohammed Sattar Abdulkadim Al-Badrwi[1], Haider Rida Jassim[1]

[1]*Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Iraq.*

Abstract

P. aeruginosa is currently one of the most frequently nosocomial pathogens and the infections due to this organism are often difficult to treat due to antibiotic resistance. *P. aeruginosa* is an important pathogen in hospitalized patient's causative to their morbidity and mortality due to its multiple resistance mechanisms. Therefore, as a therapeutic option becomes restricted, the search for a new agent is a priority. One hundred and fifty samples were collected from different sources, divided into two main groups: clinical (80)samples and (70) hospital environment samples as a Nosocomial, collected all from October to the December of the year 2018. All of these samples were cultured by specific and differential media, Forty (40) isolates of *P.aeruginosa* bacteria were identified by using microscopic examination, biochemical tests. The identification of 40 isolates of *P.aeruginosa* bacteria confirmed by VITEK-2 system. A molecular detection the presence of *Tnp-R* gene in *P. aeruginosa* bacteria by conventional PCR to detect the Transposons and their relationship with multiple resistance of bacteria.

Keywords: *Multidrug Resistance; P. aeruginosa; and PCR*

Introduction

Pseudomonas aeruginosa is a Gram's negative opportunistic pathogen has emerged as one of the most problematic of the nosocomial pathogens; considered multi-resistant infections in both community and hospital settings, It causes infections in cancer, burn, urinary tract, surgical wound, eye, blood, ear infection, sepsis cystic fibrosis, and (ICU) (1). Because of it's an extremely ubiquitous organism and abundantly found in soil, water, plants, humans, animals, and in a hospital setting. P. aeruginosa is a common pathogen in hospital particularly in ICU although it has the ability to colonize healthy subjects, in addition to, bacterial exposure to some antibiotic classes may potentially induce endogenous resistance-conferring mutation in bacterial genes that encode drug targets (2). It has been progressively clear that resistance expansion in P. aeruginosa is their contexts with mutations in genes encoding porins, efflux pump, penicillin-binding proteins, and chromosomal β-lactamases, all contributing to resistance to β-lactamases, carbapenems, aminoglycoside, and quinolones(3). P. aeruginosa is an important pathogen in hospitalized patient's causative to their morbidity and mortality due to its multiple resistance mechanisms. Therefore, as a therapeutic option becomes restricted, the search for a new agent is a priority (4). The pathogenicity of P. aeruginosa is largely caused by multiple bacterial virulence factors and genetic flexibility enabling it to survive in a varied environment. A number of these factors aid colonization, while others allow bacterial invasion(5). Antibiotic resistance in bacteria has reached a near-crisis point in nosocomial health care, with many bacterial isolates now multi-resistant as a result of the presence of additional DNA element. Earlier studies have shown that genes for resistance markers do occur on plasmids and they can be transferable, and most of them have demonstrated it by plasmid curing experiments alone. Resistance gene can occur on chromosomes, transferable plasmid, Transposons or jumping gene and specialized transposons called integrons that can assemble multiple resistance genes into the cassette (6, 7).

Materials and Method

- **Specimens' Collection:**

During the period extended from October to the December of the year 2018, One hundred and fifty samples, divided into two main groups: (80)clinical samples and (70) samples hospital environment as a Nosocomial, were collected from hospitals. Clinical samples included: Urine samples from Urinary Tract Infections (UTI)patients, exudate samples from wounds

of the burn units patients, stool samples, Sputum samples from Cystic Fibrosis(CF) patients and Ear Swabs. The Nosocomial samples included many Nosocomial sources: Intensive Care Unit, Operations Hall, Birth Hall, Burning Hall, Devices and medical equipment, and hospital bed rooms.

- **Bacterial Isolation:**

In this study, the identification of 150 samples, we got 40 isolates of *P. aeruginosa* was performed by incubating these clinical and nosocomial isolates on different agar media (Nutrient agar ,Blood agar ,Maconcky agar, and Cetrimide agar which are a selective media for *Pseudomonas spp.*) and the incubation at 37˚C for 24 hrs. Forbes *et al*. [8].

- **Bacterial Identification:**

Identification of *P. aeruginosa* was confirmed by microscopically examination showed that it was single cells, a rod shape, not- spore-forming, and gram-negative, these results mention that this isolates may belong to *P. aeruginosa* growth on Cetrimide agar for characterization of *P. aeruginosa* such as mucoid, smooth in shape with flat edges and elevated center, creamy green colour and have a fruity odour.

- **PCR amplification:**

DNA template of all isolates was prepared by boiling method (30 min in 100°C). The DNA of isolates was targeted for the *bla*OXA-1 gene using the primers (Z.Tavajjohi, *et al* ., Iran)[9] listed in Table 1 and for the *Tnp-R* gene using the primers (Altaliby S. and Aldraghi w. ., Iraq) listed in Table 1. A reaction mixture (25 µl) contained 2 µl of DNA, 1 µl of each primer, 12.5 µl of Master Mix 2X(Z.Tavajjohi, *et al* ., Iran) and (Altaliby S. and Aldraghi w. ., Iraq), and 8.5 µl of Nuclease Free Water. The experiment was continued according to the following program: initial denaturation at 94°C for 5 minutes, followed by 30 cycles at 94°C for 1 minute, 55°C for 1 minute, 72°C for 1 minute and a final extension at 72°C for 5 minutes. The PCR products were analyzed using gel electrophoresis (1% agarose) and stained with safe dye and visualized by Gel Doc apparatus (BioRad, USA) (Table 2).

Table (1): Primers used in this study.

Gene	Primer Sequence		Product size (bp)	References
bla OXA-1	F	5`-AGCCGTTAAAATTAAGCCC-3`	908	Z.Tavajjohi, et al ., 2011
	R	5`-CTTGATTGAAGGGTTGGGCG-3`		
Tnp-R	F	5`-TTTTGGTTATGCGCGGGTC-3`	545	Altaliby S. and Aldraghi w. .,2018
	R	5`-AGGCCCTTTCGTCTTCAAGA-3		

Table (2): Condition of PCR Reaction for *bla*OXA-1 and *Tnp-R* genes of *P.aeruginosa* .

Steps	Temperature	Time	Number of Cycle
Initial Denaturation	95°C	5 min.	1
Denaturation	95°C	30 Sec.	30
Annealing	55°C	30Sec.	
Extension	72 °C	30Sec.	
Final extension	72 °C	7 min.	1
Hold	4 °C		1

Results and Discussion

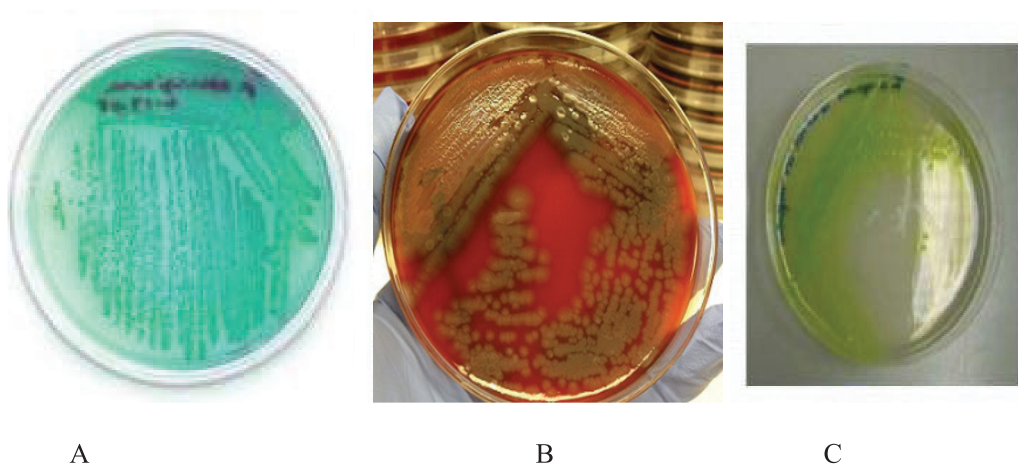
Isolation and Identification of *P. aeruginosa*:

One hundred and fifty samples clinical and Nosocomial samples were analyzed for the presence of *P. aeruginosa*, and the results of bacterial isolation and identification revealed the detection of forty (40) isolates of *P. aeruginosa*.

Biochemical Tests:

Some biochemical tests were performed for more validation. showed 40 isolates of *P.aeruginosa* provided a by some biochemical tests,

results showed positive results for oxidase test, catalase test, motility test, , and production of *B*-hemolysis while (40) isolations negative results to citrate utilization tests, indole production and urease production tests negative to Gram's stain and capable of growing on cetrimide agar as yellow greenish colonies (at 42˚C for 24 hrs.). (Figure 1).



A B C

Figure (1): *P. aeruginosa* colonies on (A) Cetrimide agar, (B) and Blood agar, and (C) Nutrient agar after 24 hours of incubation at 37ºC.

At the species level, *P. aeruginosa* has a wide growth temperature range, optimum growth at 37°C. Slower growth rates are seen at 4˚C. *P. aeruginosa* is distinguishable from other clinically *Pseudomonas* spp. by its capability for growth at 42˚C. They also grow well at pH range 6.6-7.0. It was tolerant of a wide variety of physical conditions, including temperature and pH[10]. Also, it was resistant to high concentrations of salts and dyes. It is typically given a positive result to the oxidase test and catalase. It does not ferment carbohydrates, but many strains oxidize glucose [11].

The identification was performed with the automated VITEK -2 system using the GN-ID cards which contains 64 biochemical tests, from (40) isolate of *P. aeruginosa* ,(40) positive result of the *P. aeruginosa* demonstrated.

Distribution of *P. aeruginosa* according to Type of Specimens

According to table (3), Out of (80) clinical samples of burns, sputum, urine, stool, ear and wound, 28(70%) isolates were positive to clinical *P. aeruginosa* and the percentage of the positive results from (70)Nosocomial samples were 12 (30%), as reported in (Table 4).

Table (3): Distribution of *Pseudomonas aeruginosa* isolates in clinical samples.

Site of samples	Numbers of sample and Percentage
burn swab	2 (5 %)
wound swab	5 (12.5%)
ear swab	11 (27.5%)
Sputum	4 (10%)
Urine	6 (15%)
Total	28 (70%)

Table (4): Distribution of *Pseudomonas aeruginosa* isolates in Nosocomial samples

Site of samples	Numbers of sample and Percentage
ICU	2 (5%)
Operations Hall	4 (10%)
Birth Hall	2 (5%)
Burn Hall	2 (5%)
Devices and medical equipment	2 (5%)
Total	12 (30%)

The low percentage was found in burn specimens which accomplished 5%. Results obtained reported that the highest percentage of *P.aeruginosa* from ear swab (27.5%) was in opposite with our results. In comparison with Nosocomial isolates of *Pseudomonas aeruginosa* the highest percentage isolation of Operations Hall (4%) where are other isolate reported low present in comparison with clinical findings.

P. aeruginosa is pathogenic only when introduced into areas devoid of normal defences, the bacterium attaches to, and colonizes the mucous membranes or skin, invades locally and produces systemic disease. *Ps. aeruginosa* infects healthy tissues rarely, but, when defences are compromised, it can infect virtually all tissues. This explains why most infections are nosocomial[12]. These infections are Pneumonia, Osteomyelitis (related to Wounds, Immunocompromised patients, Burn-wound infections, Urinary tract infections, Endocarditis, external otitis and Tissue layer infections [13].

Genomic DNA Extraction:

Using a Genomic DNA Purification Kit (Promega), Genomic DNA was extracted from (40) *P. aeruginosa* isolates that were confirmed as bands by gel electrophoresis. Quantus Fluorometer was used to detect the concentration of extracted DNA in order to detect the goodness of samples for downstream applications. all the isolates had DNA concentration between (10-40 ng/µl) by Quantus Fluorometer.

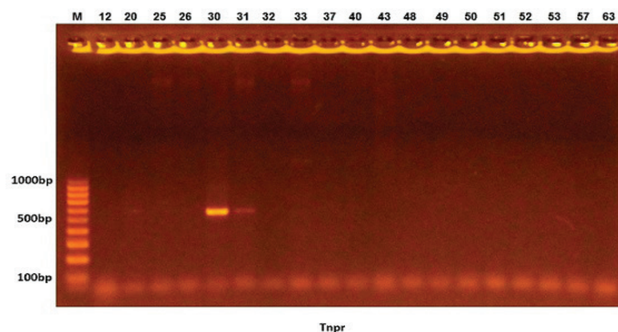
Molecular Detection of *P. aeruginosa* and co-strains by $bla_{oxa1\ like}$ gene:

The result of PCR analysis concerning of the found the blaoxa-1 in (10) positive isolates, showed that studies P. aeruginosa possess the blaOXA-1 like gene from 10(25 %) isolates positive showed 4 (40%) from clinical isolates and 6 (60 %) from nosocomial.

The ESBL*bla*oxa-1 of *P. aeruginosa* isolates exhibited co-resistance against most of the antibiotics tested. This is consistent with most of the recent findings [14]. The *bla*OXA-1 ESBLs provide *P. aeruginosa* with an additional powerful resistance mechanism with potentially serious clinical implications, including limitation of the therapeutic options. ESBLs manufacturing organisms create distinctive challenges to clinical microbiologists, clinicians, infection control professionals and scientists engaged in finding new antibacterial agents [15].The development and spread of ESBLs are most likely caused by the overuse of expanded- spectrum Ciprofloxacin in the hospital setting. Proper infection management practices and barriers are essential to stop spreading and outbreaks of ESBL-producing microorganism [16].

Molecular Detection of Transposons of *P. aeruginosa*:

The result of PCR analysis concerning of the found the *Tnp-R* gene in (2) (12%) positive isolates from 10 *bla*oxa1 positive *P. aeruginosa* isolates ,which identified the presence of Transposon in this bacteria as reported in figure(2).



Figure(2): Agarose gel electrophoresis (1% agarose, 100Vol / mAmp for 75 min.) of PCR amplification products (Tnp-R gene) at 545bp for P. aeruginosa. lanse 30and 31 show positive results for P. aeruginosa; ladder 100 bp DNA marker.

Two strains of nosocomial isolates of *P. aeruginosa* exactly show bands for *Tnp-R* gene which represent the nosocomial sample number (30) and (31) were collected from the hospital's operation hall and birth rooms respectively.

Strains of bacteria resistant to antibiotics, particularly those that are multi-drug resistant, are an increasingly major health care problem around the world. [17]. This is achieved through the cooperative activities of mobile genetic elements able to move within or between DNA molecules, which include insertion sequences, transposons, and gene cassettes/integrons, and those that are able to transfer between bacterial cells, such as plasmids and conjugative elements. Together these types of mobile genetic elements play a central role in facilitating horizontal genetic exchange and therefore promote the acquisition and spread of antibiotic resistance genes in both Gram-negative and Gram-positive bacteria, focusing on the group of organisms (*S. aureus , K. pneumonia , A. baumannii , P. aeruginosa , Enterobacter spp., and Escherichia coli*), which have become the most problematic hospital pathogens [18].

Whereas one or two classes are left in presence of Integron and/or Transposons, extensive drug resistance **(XDR)**. **MDR/XDR** has appeared in *P. aeruginosa*, *Acinetobacter baumannii*, *E. coli*, and *K. pneumonia,* producing extended-spectrum β-lactamases (ESBL), vancomycin-resistant *enterococci, Enterococcus faecium* (VRE), MRSA, vancomycin-resistant *Staphylococcus aureus* VRSA, *Salmonella enterica* serovar *Typhimurium*, *Shigella dysenteriae*, and *Burkholderia* [19-21].

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

Funding: Self-funding

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