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Role of Some Virulence Genes and Antibiotic Susceptibility of *Pseudomonas aeruginosa* Isolated from Different Clinical Samples

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Abstract: A total of (200) clinical specimens were taken from the patients with various age groups (3-60) years, from (Al Yarmouk Teaching Hospitals) in Baghdad from beginning of December 2021 to the end of March 2022, different specimens included wounds, burns, ear and diabetic foot swabs. All specimen were cultured on (MacConkey agar and cetrimide agar). The culture results revealed 53 isolates to the *Pseudomonas* bacteria depend on culture characteristic, while the conformation by Vitek2 system showed that 53 isolates belonged to the genus *P. aerugenosa.* Antibiotic susceptibility of the bacterial isolates against 8 types of antibiotics. isolates showed resistance to: amikacin (94.3%), gentamicin (86.7%), tobramycin (84.9%), ceftazidime (90.5%), imipenem (38.09%), meropenem (30.1%), ciprofloxacin (0%), ceftriaxone (66.03%).

Keywords: *P. aeruginosa* , Biofilm formation , Antimicrobial susceptibility

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Introduction

Pseudomonas aeruginosa is a leading cause of nosocomial infection; categorize second most common pathogen isolated from patients among the gram-negative pathogens reported to National Nosocomial Infectious Surveillance (NNIS) system. (1). The bacteria can cause a wide range of acute and chronic infectious diseases. The symptoms of these infections are generalized inflammation and sepsis. Particularly in burns patients where the skin host defenses are destroyed and patient with impaired immune defense including HIV or cancer infection who are immunosuppressed (2). *P. aeruginosa* was the second most common cause of nosocomial pneumonia, the third most common cause of nosocomial urinary tract infections, and the seventh most common cause of nosocomial bacteremia (3). *Pseudomonas aeruginosa* is a virulent agent having a tendency to develop resistance to majority of the antibiotics available for the treatment. It is a leading cause of life-threatening nosocomial infections. It's intrinsic resistance to many antimicrobial agents and development of multidrug resistance imposes severe therapeutic problem for clinicians (4). *Pseudomonas aeruginosa* infections are problematic due to its intrinsic as well as acquired resistance to many effective groups of antibiotics.Multi drug resistance *P. aeruginosa* (MDRPa) is defined as an isolate intermediate or resistant to at least three groups of antibiotics among β-lactams, carbapenems, aminoglycosides, and

Fluoroquinolones. Intrinsic MDRPa is attributed by limited permeability of outer membrane, production of inducible β- lactamase and Multidrug Efflux system (5). *Pseudomonas aeruginosa* tends to form biofilms, which are complex bacterial communities that adhere to a variety of surfaces, including metals, plastics, and medical implant materials, and tissues. Growth in biofilms promotes bacterial survival, once a biofilm is formed it is extremely difficult to destroy (6). *P. aeruginosa with a* selective advantage and facilitates survival Biofilms are formed from individual free-floating (planktonic) cells and are defined as exopolysaccharide surrounded bacteria or microcolonies growing on biotic or abiotic surfaces. Biofilms are ubiquitous in nature and are also associated with numerous chronic recurrent bacterial infection and diseases (7).

Materials and methods

A Total of (200) specimen was collected from patient specimen with various age groups (3-60), from (Al Yarmouk teaching hospitals) in Baghdad. from December 2021 to the end of March 2022, 53 isolates were collected from different specimens include wounds, burns, ear and diabetic foot swabs. All specimen were cultured on (MacConkey agar, and cetrimide agar). The culture results revealed 53 isolates to the *Pseudomonas* bacteria depend on culture characteristic, while the conformation by Vitek2 system showed that 53 isolates belonged to the genus *P. aerugenosa.* Microtiter plate methods used to detect biofilm formation.

Antimicrobial susceptibility test

Antimicrobial sensitivity conducated for 53 isolates of testing of the 53 isolates of *P.aeruginosa* using 8 types of antibiotics used in the hospitals by using Kirby-Bauer disk diffusion test test were according to CLSI update 2020, In these analysis antibiotic discs we used listed in (Table 1) as follows: amikacin (30 µg), gentamicin (10 µg), tobramycin (10 µg) , ceftazidime (30 µg), imipenem (10 µg), meropenem (10 µg), ciprofloxacin (10 µg), ceftriaxone (10 µg). zones of inhibition diameter are measured by ruler and the findings are clarified by guidance according to (CLSI, 2020). The study resulted that the percentages of resistant toward these antibiotics were as follow: amikacin (94.3%), gentamicin (86.7%), tobramycin (84.9%), ceftazidime (90.5%), imipenem (38.09%), meropenem (30.1%), ciprofloxacin (0%), ceftriaxone (66.03%).

Molecular methods

DNA extraction

Genomic DNA was extracted from bacterial isolate using Purification depending on instruction of manufacturing company (Intron / Korea). Genomic DNA was isolated from bacterial growth according to the protocol of FavorPrep Blood/ Cultured Cells Genomic DNA Extraction Mini Kit extraction genomic DNA.

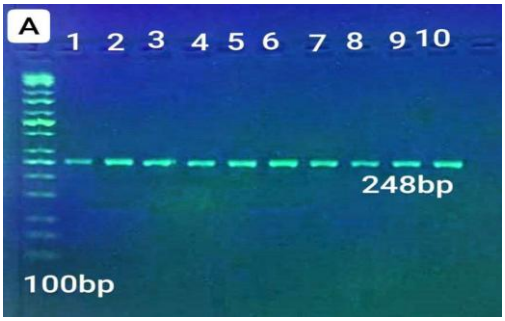
Specific primers were used for detecting the *P. aerugenosa* bacteria and some virulence genes.They were prepared according to information of supplying company (Table 1).

Table (1): The primers, sizes for detection of genes

		Primer sequence	Size of Product (bp	Ref.
algD	F	ATGCGAATCAGCATCTTTGGT	1310	**(8)**
	R	CTACCAGCAGATGCCCTCGGC		
pilA	F	ACAGCATCCAACTGAGCG	1675	**(8)**
	R	TCGAACTGATGATCGTGG		
oprI	F	*ATGAACAACGTTCTGAAATTCTCTGCT*	248	**(8)**
	R	*CTTGCGGCTGGCTTTTTCCAG*		

Results and discussion

Isolation and identification of isolates were done following their morphology in gram staining, cultural characteristics and biochemical properties. A total of 200 samples were cultured on medium MacConkey agar, and cetrimide agar.The isolates that were obtained from these media were identified according to their shape characters, which were observed .The DNA was extracted from 53 bacterial isolates that diagnosed by vitek2 system and molecular method using *oprI* gene . Total DNA was extracted by using of FavorPrep Blood/ Cultured Cells Genomic DNA Extraction Mini Kit extraction genomic DNA For identification of all 53 isolates of *P. aerugenosa* depending on use of specific primers for *oprI* gene which give the same results when compared with traditional methods and vitek2 system. Product of conventional PCR for 53 isolates detected by using gel electrophoresis as showing in Figure(1).



Figure(1) :Agarose gel electrophoresis (1.5% agarose, 75 Vol / 1.30 hour) of multiplex PCR for identification *Pseudomonas aeruginosa*, M: marker (100bp ladder), lanes (1, 2, 3,4, 5,6, 7,8,9,10) positive amplification of (A) *Opr*I gene (249bp).

Antimicrobial susceptibility test

This study showed high resistant toward amikacin 94.3%, gentamicin 86.7%, tobramycin 84.9% which agree with another study (9) in Babel city whom found that the resistance of *P. aeruginosa* isolated from burns and wounds to gentamicin and amikacin, was 70%, 93.4%, Tobramycin 83.4%, respectively. Also, this study nearly coincided with (10) in Baquba city, which found that *P. aeruginosa* isolates had high resistance to amikacin, tobramycin, and gentamicin 60 %, 73.3 %, and 60 %, respectively. The current study showed that there was a relatively high resistance expressed by the *P. aeruginosa* isolates for the ceftazidime (90.5%) this high resistance rates were agree with (11) in Ramadi city, which showed resistant rate to ceftazidime 94.1%. And resistant rate of *P.*

aeruginosa for imipenem was 38.09% and meropenem was 30.1% which agree with (12,15) who mentioned that both of imipenem and meropenem have the same resistant percent 34.95% respectively . While resistant rate of ceftriaxone was 66.03% match with another study in Al-Nasiriya, (13) reported, that among 65 isolates of *P. aeruginosa*, resistance rate ceftriaxone was 76.9%. The lowest resistant rate was found with ciprofloxacin with consternation 10 mg was (0 %) which can be the first choice of therapy for these infections.

Detection of some virulence genes (*AlgD* and *PilA*) in *Pseudomonas.*1-conventional polymerase chain reaction for detection of *P.aeruginosa*.

Conventional PCR amplification were performed for *pseudomonas* in order to consolidate the presence of *AlgD* and *PilA* genes coding for different virulence factor using specific primers for each one genes, the product of PCR detect using gel electrophoresis as show in Figure (2).

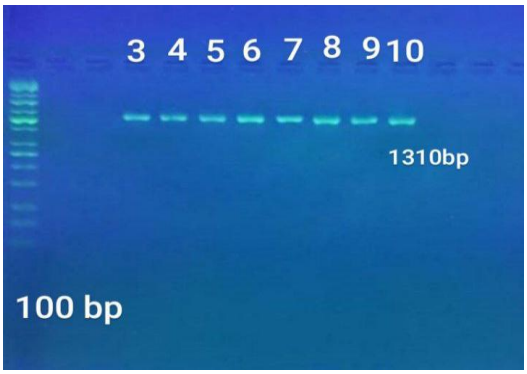


Figure (2): Agarose gel electrophoresis (1.5% agarose, 75 Vol / 1.30 hour) of conventional PCR amplification products of *P. aeruginosa AlgD* gene (1310bp). M: marker (100 bp ladder) lanes (3,4, 5,6, 7,8,9,10) .

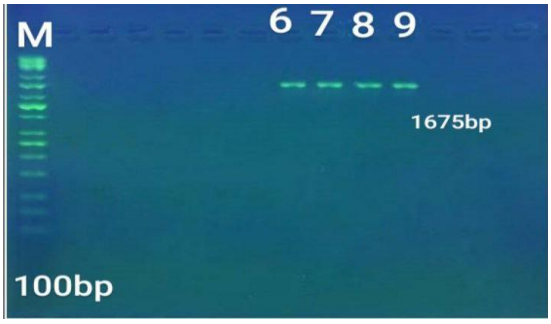


Figure (3): Agarose gel electrophoresis (1.5% agarose, 75 Vol / 1.30 hour) of conventional PCR amplification products of *P. aeruginosa PilA* gene (1675bp). M: marker (100 bp ladder) lanes (7,8,9).

Table (2): The number and percentage of *AlgD* and *PilA* genes in *P.*aeruginosa

Genes	Total	percentage
AlgD	47	90.47%
PilA	20	37.7%

Total 53 isolates were carried different kind of genes, the results are compatible with Brazilian study (8). (*AlgD* 75.7% , *PilA* 14.1%) .And agree with another in Baghdad The prevalence average of studied genes in the burns isolates was*; (alg*D 93.5%) from wounds isolates was (*alg*D81.5%) from otitis isolates was; (*alg*D 14.3%) and from diabetic foot ulcers isolates was (*alg*D 70.2%) (14).

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