Short Communication

Molecular Detection of *Psts* Antibiotic-Resistant Gene in *Pseudomonas aeruginosa* Isolates

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Abstract

Background: Otitis media is a very common infection among people of different age groups. **Objective:** The goal of the current work was to determine the existence of *PstS* gene among *Pseudomonas aeruginosa* isolates recovered from otitis media cases in Iraqi patients. **Materials and Methods:** A total of 100 ear swabs were collected from patients suffering from otitis media attending the ENT outpatient clinic at Baghdad Teaching Hospital from July 2022 to the end of December 2022. The age range was 18-75 years. Detection of morphology and biochemical testing by Vitek 2 system. The antibiotic sensitivity test of the bacteria spp. was the detection of 18 antibiotics for different types using Vitek2 system. Identify the genes for antibiotic resistance mechanisms. DNA was extracted from samples, and resistance genes *PstS* were amplified using polymerase chain reaction (PCR). **Results:** This study showed 80 out of 100 swabs revealed *Pseudomonas aeruginosa* isolates, and 71 (89%) isolates out of these 80 exhibited *PstS* genes. Age groups of the patients were 38-47, 48-57, and 58-67 years with percentages of 22.5%, 25%, and 25%, respectively. The highest resistance percentages of strains to tested antibiotics were for Ceftoriaxone, Cefpodoxime (100%), and the lowest resistant percentage was 22.5% for colistin, which determined the relationship between the presence of Psts and resistance to 18 antibiotics for different types. Colistin was the most effective antibiotic and can be considered as the drug of choice against *Pseudomonas aeruginosa*. **Conclusions:** *Pseudomonas aeruginosa* isolates recovered from patients suffering otitis media show the prevalence rate appears to be higher among the elderly and high virulence and antibiotic resistance due to the presence of *PstS* antibiotic resistance gene and ability to form biofilms, thus aggressive antimicrobial therapy is highly recommended in such conditions.

Keywords: antibiotics, *Pseudomonas aeruginosa*, *PstS* gene

Introduction

Chronic-suppurative otitis media (CSOM) is a chronic, middle ear, and mastoid cavity microbial infection characterized by discharge (otorrhea) that lasts for two weeks duration.[1] The pathogenesis of this condition is not fully understood, and the relationship between host factors, environment, and microbial infection was thought to facilitate the development of this condition. Many bacterial species have been implicated in the pathogenesis of this condition, including *Pseudomonas aeruginosa* (*P. aeruginosa*), *Staphylococcus aureus* (*S. aureus*), *Proteus* spp., *Klebsiella* spp., and *Escherichia coli* (*E. coli*).[2] *Pseudomonas aeruginosa*, however, has been demonstrated to be the most implicated pathogen related to this bacterial infection in many studies done in different parts of the world.[3] Moreover, *P. aeruginosa* has been found to be associated with progressive destruction of middle ear tissues.[4]

P. aeruginosa can avoid host immunity by reducing blood flow, secreting exotoxins and enzymes, and forming biofilms.[5,6] The misuse and abuse of antibiotics in the treatment of bacterial infections has resulted in a resistance to many antibiotics used previously and effectively in the treatment of such infections. Studying the virulence factors that have resulted in the existence of resistance strains of *P. aeruginosa* was of high

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importance, and for this reason, this study was carried out and aimed to identify the virulence factors that have been associated with the emergence of antibiotic resistance strains.

One of the important genes that is linked to the multidrug resistance exhibited by *P. aeruginosa* toward antibiotics is the *PstS* gene.[7,8] The *PstS* gene is part of a *Pst* operon, encoding for a specific phosphate-transport system, which in turn belongs to a *Pho* regulon and is controlled by *PhoB*/*PhoR* that is highly protected, found in both positive and negative gram organisms and controls the expression of multiple genes[9,10]

Materials and Methods

Collection of sample

A total of 100 ear swabs were collected from patients suffering from otitis media attending the ENT outpatient clinic at Baghdad Teaching Hospital from July 2022 till the end of December 2022. The age range was 18-75 years.

Exclusion criteria

Patients on antimicrobial treatment within the past 3 days, patients with discharging ears of less than 3 weeks duration, and immunocompromised patients with chronic illnesses such as diabetes mellitus, organ transplant, or immunosuppressive drugs.

Ethical consideration

Ethical approval for conducting this study was requested from the College of Medicine Baghdad, and patients were informed about the study before collecting specimens. Number and date for taking ethical approval (Ref. No. 026, Date 26/7/2022)

Clinical examination

A complete history was taken and physical examination was done for each patient, and aural discharge was collected through sterilized ear swab as a main clinical feature.

Bacterial isolation

Collected swabs were sent to the microbiology laboratory department for microbiological examination. The procedure involves the cultivation of blood, MacConkey's, and chocolate agar media, followed by microscopic examination and biochemical identification.

Genotypic identification

Amplification was carried out by polymerase chain reaction (PCR) to detect the existence of *PstS* drug-resistance gene. The description and sequence of the PCR primers are shown in Table 1. The procedure was carried out according to the manufacturer's manual. The procedure involves initial denaturation at 94°C for 4 min 30 cycles and a final extension step at 72°C for 10 min.

Table 1: Description and sequence of the PCR primers

Primer		Oligonucleotide sequence	Size of amplicons
PstS	F	GGCTTTCGAGCAGAAGTACG	606 bp
	R	ATGTAGCCGTCCTTGACCAC	

Table 2: Distribution results of the sample according to age groups/patients group

Age group (year)	Total no	Positive	Negative
		No. (%)	No. (%)
18-27	11	5	6
	-11.00%	-6.25%	-30.00%
28-37	14	12	2
	-14.00%	-15.00%	-10.00%
38-47	20	18	2
	-20.00%	-22.50%	-10.00%
48-57	23	20	3
	-23.00%	-25.00%	-15.00%
58-67	22	20	2
	-22.00%	-25.00%	-10.00%
68-77	10	5	5
	-10.00%	-6.25%	-25.00%
Total	100	80	20
P value	---	0.0001**	0.0294*

*$P \leq 0.05$,
**$P \leq 0.01$.

Amplicons were separated on 1.5% agarose gel, and stained by ethidium bromide stain.

Statistical analysis

SAS 2018 program was used to detect the effect of the factors of difference in the study coefficients. Chi-square test was used to compare percentages (0.05 and 0.01 odds) in this study.

Results

Isolation and identification of *P. aeruginosa*

Eighty out of 100 total number of swabs revealed *P. aeruginosa* isolates. The age groups of the patients were 38-47, 48-57, and 58-67 years with percentages of 22.5%, 25%, and 25%, respectively, which was found to be highly statistically significant association ($P \leq 0.01$). The results are shown in Table 2.

Detection of *Psts* gene

The PCR amplification showed that seventy-one (89%) isolates have the *PstS* gene, with a size of amplicons of about (606 bp) product on the agarose gel. The results are shown in Table 3 and Figure 1.

Occurrences of *PstS* gene and its relation with ceftoriaxone, cefpodoxime, piperacillin, ticarcillin,

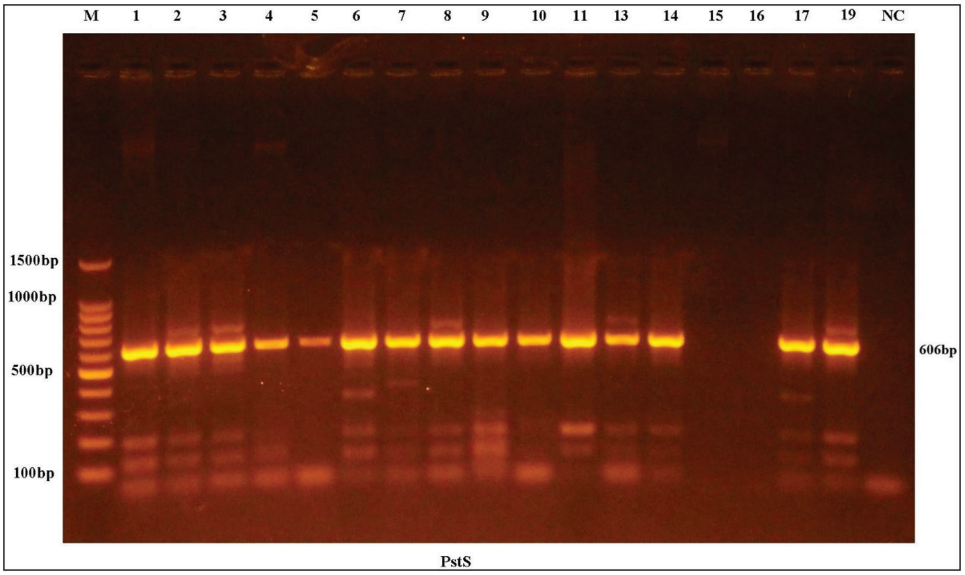

Figure 1: Amplification of the *PstS* gene. M: 100bp ladder marker. Lanes 1-19 resemble 606 bp PCR products

Table 3: Distribution of sample study

PstS gene	Number	Percentage (%)
Positive	71	89
Negative	9	11.25
Total	**80**	**100%**
X^2	-	48.05**
P value	-	0.0001

**$P \leq 0.01$

clavulanic acid, Meropenem, Imipenem, Ceftazidime, Cefepime, amikacin, gentamicin, tobramycin, ciprofloxacin, levofloxacin, norfloxacim, and ofloxacin resistance. The results showed that all antibiotic-resistant strains were also positive for PstS, as shown in Table 4.

Occurrences of *PstS* and its relation with ceftoriaxone, cefpodoxime, piperacillin, ticarcillin, clavulanic acid, Meropenem, Imipenem, Ceftazidime, Cefepime, amikacin, gentamicin, tobramycin, ciprofloxacin, levofloxacin, norfloxacim, ofloxacin sensitive. The results revealed that all antibiotic-sensitive strains were positive and were also PstS positive, as shown in Table 5.

Table 4: Detection of *PstS* gene and its relation with resistance of *P. aeruginosa* to antibiotics

Antibiotics	Resistance	*R* (Psts)
Ticarcillin	62 (77.5%)	40 (64.5%)
Clavulanic acid	62 (77.5%)	40 (64.5%)
Piperacillin	72 (90%)	35 (48.6%)
Cefpodoxime	80 (100%)	30 (37.5%)
Ceftazidime	36 (45%)	15 (41.6%)
Ceftoriaxone	80 (100%)	40 (50%)
Cefepime	54 (55%)	35 (64.8%)
Imipenem	45 (56.25%)	25 (55.5%)
Meropenem	45 (56.25%)	22 (48.8%)
Amikacin	62 (77.5%)	30 (48.3%)
Gentamicin	62 (77.5%)	30 (48.3%)
Tobramycin	62 (77.5%)	30 (48.3%)
Ciproflaxacin	62 (77.5%)	30 (48.3%)
Levofloxacin	72 (90%)	40 (55.5%)
Colistin	18 (22.5%)	10 (55.5%)
Moxifloxacin	72 (90%)	35 (48.6%)
Norfloxacim	72 (90%)	35 (48.6%)
Ofloxacin	71 (88.75%)	25 (35.2%)

Discussion

Pseudomonas aeruginosa are among the most important pathogens responsible for nosocomial diseases and are associated with antibiotic resistance patterns due to rapid accumulation of antimicrobial resistance mechanisms, in addition to misuse/abuse of antibiotics.[11] higher prevalence was recorded among patients in the third age group 38-47 years. These results were in agreement with the results of two recent studies[7,12] and showed a difference with other studies, where a high prevalence was recorded among patients under the age of (30) years.[13,14] The rate of infection with microbes increases with age for many reasons, including weak immune status in the elderly.[8] Molecular methods are used for detecting antimicrobial-resistant organisms directly in clinical samples to optimize therapy early in the course of infectious illnesses. Besides well-established PCR techniques for a sequence-specific molecular-biological approach. Pathogenesis of *P. aeruginosa* is due to the production of several cellular virulence and extracellular factors.[15] PstS was identified in 89% of all tested strains. PstS proteins are the cell-bound phosphate-binding elements of the ubiquitous bacterial

Table 5: Detection of *PstS* gene and its relation with sensitive of *P. aeruginosa* to antibiotics

Antibiotics	Sensitive	S (*PstS*)
Ticarcillin	18 (22.5%)	8(44.4%)
Clavulanic acid	18 (22.5%)	7(38.9%)
Piperacillin	8 (10%)	5(62.5%)
Cefpodoxime	0 (0)	0
Ceftazidime	54 (55%)	25(46.3%)
Ceftoriaxone	0 (0)	0
Cefepime	36 (45%)	15(41.7%)
Imipenem	35 (43.75%)	22(62.9%)
Meropenem	35 (43.75%)	20(57.14%)
Amikacin	18 (22.5%)	6(33.3%)
Gentamicin	18 (22.5%)	10(55.5%)
Tobramycin	18 (22.5%)	7(38.9%)
Ciproflaxacin	18 (22.5%)	5(27.8%)
Levofloxacin	8(10%)	3(37.5%)
Colistin	62 (77.5%)	40(64.5%)
Moxifloxacin	8 (10%)	2(25%)
Norfloxacim	8 (10%)	3(37.5%)
Ofloxacin	9 (11.25%)	2(22.2%)

ABC phosphate uptake mechanisms. PstS proteins are induced by phosphate deprivation in non-fermenters species of Pseudomonas.[16] The presence of this periplasmic phosphate binding protein (PstS) confers a highly virulent phenotype of MDR strains. Also the development of multi-drug resistance clinical strains might be related to the overproduction of PstS proteins[12,17] in Romania found that PstS present in almost all tested strains[12] Existence of *Pst* protein gives a high virulence phenotype of multidrug resistance to *P. aeruginosa* isolates. Existence of *Pst* gene gives a high virulence of MDR to *P. aeruginosa* insulate.[18]

Conclusion

Pseudomonas aeruginosa isolates recovered from patients with otitis media show high virulence and antibiotic resistance due to the existence of *PstS* antibiotic resistance gene and ability to form biofilms, thus aggressive antimicrobial therapy is highly recommended in such conditions.

Author contributions

Both Fatima and Maryam Kareem Ali collaborated equally during the experimental work and discussed the results. All authors have read and accepted the published version of the manuscript.

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Conflicts of interest

There were no conflicts of interest revealed by the authors.

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