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Antibiotic Susptipility Profile and Virulence Factors Profile of Pseudomonas Aeruginosa Isolated from Otitis Media

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Abstract

The current study aimed to find Antibiotic susptipility profile and virulence factors profile of pseudomonas aeruginosa isolated from otitis media, for this purpose 200 ear swabs collected from patients suffering from otitis media whom attending to the ENT consulting clinic at Tikrit Teaching Hospital and the private outpatient clinic in Tikrit city. Bacterial isolation and identification were done , virulence factors profile detected by classical and genetic methods, and Antibiotic susptipility were done.

The result shows that out of 200 ear sample collected from otitis media, pseudomonas aeruginosa isolated in rate of 38.6% (68 out of 200) Protease, lecithinase, and denase were detected in percentages of 69.1%, 66.1%, and 54.4%, respectively. AprA, Ple and PlcH were detected in rate of 100%, 93.3% and 86.6%, respectively. Also the current study reveal that Highly resistant of pseudomonas aeruginosa to antibiotic

Introduction

pseudomonas aeruginosa is gram positive bacilli non-spore-forming and non capsulated , arranged as singly or in pairs or short chains, with dimensions (1.5-3.0) µm in length and (0.5-0.7) µm in diameter, mobile with one polar flagellum, growing in a wide range of temperatures (18-42°C) and The optimum temperature is 37°C (Căpățînă et al.,2022; Mai-Prochnow et al.,2016). Most of its strains produce dyes that are widespread in the culture media, including the pyocyanin (blue-green) , the yellow-green fluorescence, red pyorubin and the black pyomelanin. pseudomonas aeruginosa positive to oxidase and catalase tests, consumption of citrate, gelatin hydrolsis, and a negative test for MR and VR, indole production (Brook,2001; Quinn et al.,1998).

It have groups of virulence factors include Cell-related virulence factors (Lipopolysacharide, Exogenous lipopolysaccharide (Alginate), Outer membrane proteins, Flagella, Cilia and pili), Factors that are secreted outside the cell include:Exotoxin A, Exo enzymes (Protease, Elastase, and Alkaline Phospholipase c,) and Pyocyanin (Zhivaki, & Kagan,2022; Qin et al.,2022).

Materials and methods :

- 200 ear swabs were collected from patients suffering from otitis media, the Patients attending the ENT consulting clinic at Tikrit Teaching Hospital and the private outpatient clinic in Tikrit city in period from September 2021 until the end of April 2022.
- Bacterial isolation and identification: each swab put in tube contain nutrient broth, then transport to bacteriology laboratory, then sub culturing on nutrient agar, MacConkey agar and blood agar and cultivation on 37C for 48hours, then sub culturing in same agar and cultivation condition. Gram stain and group of biochemical tests were done as to (Quinn et al.,1998).
- Detection of virulence factors by classical methods which inculde

a- Protease production : conducted according to (Cruickshanke et al.,1975)

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b- Lecithinase production: : conducted according to (Cruickshanke et al.,1975)

c- DNAas: conducted according to (Cruickshanke et al.,1975)

d- Hemolysin production: conducted according to (Cruickshanke et al.,1975)

e- Urease production: conducted according to (Cruickshanke et al.,1975)

- Detection of virulence factors by PCR test:

a- DNA extraction: by using a ready-made kit (G-spin™ Total DNA Extraction) and according to manufacturer instructions.

b- Primer: primers us in current study as in table (1)

Table (1): primers us in current study

Primer	Sequence	Size of DNA product	Annealing temp	Annealing time (second)
aprA	F: GTCGACCAGGCGGCGGAGCAGATA R: GCCGAGGCCGCCGTAGAGGATGTC	800	60	60
Pel	F: CTGGAACAGCCAGGTAATG R: CGGCTTGACCTTGAGTTT	700	55	60
Plc	F: GCACGTGGTCATCCTGATGC R: CCAGTAGGCGTCGACGTAC	300	56	60

c- Components of the PCR master mix for one sample as in table (2)

Table (2): mixture compound and amount

Compound	Amount (microliter)
DNA Template	2.5
2X Master Mix	12.5
For word primer	1
Reverence primer	1
DNAse free water	8
Total	25

d- Thermocyclar program: as in table (3).

Table (3): thermocyclar program

Step	Temperature	Time (mints)	Cycles
First Denaturation step	94	4	1
Denaturation step	94	1	30
Primer-annealing step:	According to primers type		
DNA extension step	72	1	
Final DNA extension step	72	10	1
End Temperature	4	--------	------

Antibiotic susceptibility test: conducted by disc diffusion methods and according to (Atlas et al.,1995).

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Results and discussion

Out of 200 ear sample collected from otitis media, pseudomonas aeruginosa isolated in rate of 38.6% (68 out of 200)

pseudomonas aeruginosa appear as pigmented colony on nutrient agar (figure 1), and give positive results on oxidase and catalase tests, and negative results on VP and MR test.

The isolation rate recorded in current study is more than rate recorded by (Ferede et al ,2001) which are 23%, and less than rate recorded by (Gul et al., 2006) which are 43%, this may by due to geographic difference, study date and age of patients

Figure (1): pseudomonas aeruginosa grown in nutrient agar and produce pigment

virulence factor detection by classical methods shows that Protease, lecithinase, and denase were detected in percentages of 69.1%, 66.1%, and 54.4%, respectively as from table (4)

Table (4): virulence factor detected by classical methods

Virulence factors	Number of positive isolates	Rate
Heamolysin	46	%67.6
Protease	47	%69.1
Lecithinase	45	%66.1
Dnase	37	%54.4
Urease	26	%38.2

When using genetic methods, the detection of virulence genes AprA, Ple and PlcH was 100%, 93.3% and 86.6%, respectively as in table (5) and figure (2,3,4).

Table (5) Virulence factors of Pseudomonas aeruginosa bacteria detected by genetic methods

Gene	Rate of positive isolates
AprA	%100
Ple	%93.3
PlcH	%86.6

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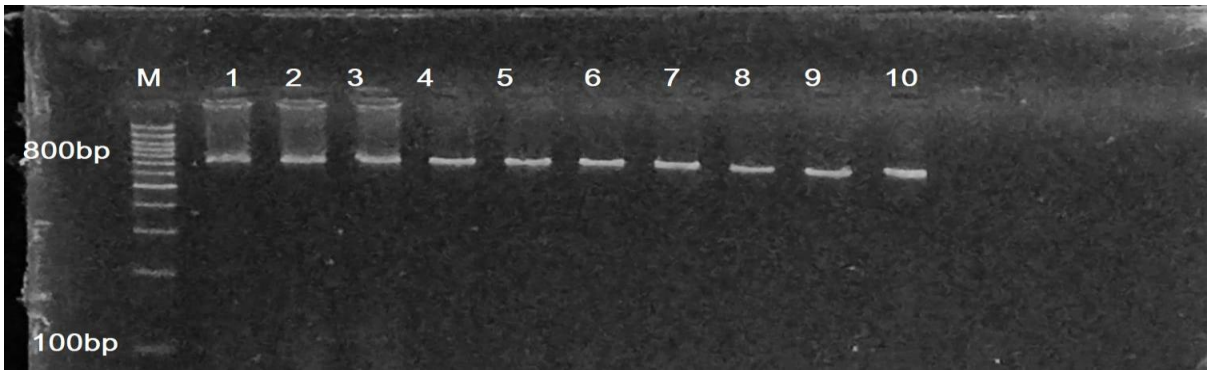

Figure (2) Results of electrophoresis of Pseudomonas aeruginosa for the detection of aprA gene. M : DNA marker. pore 1-10 indicate positive results. It gave DNA regions of 800 pb

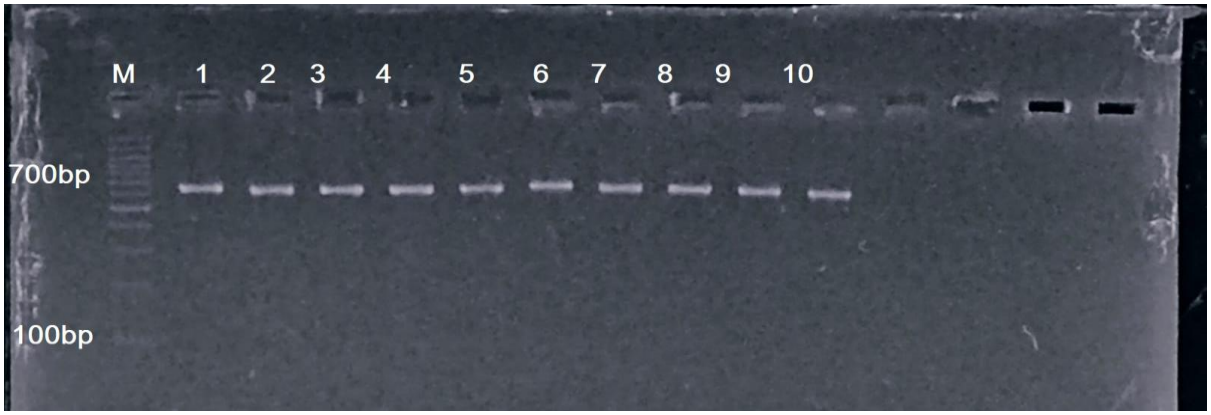

Figure (3) Results of electrophoresis of Pseudomonas aeruginosa for the detection of aprA gene, M: DNA marker. Pores 1-10 indicate positive results. It gave DNA regions of 800 pb.

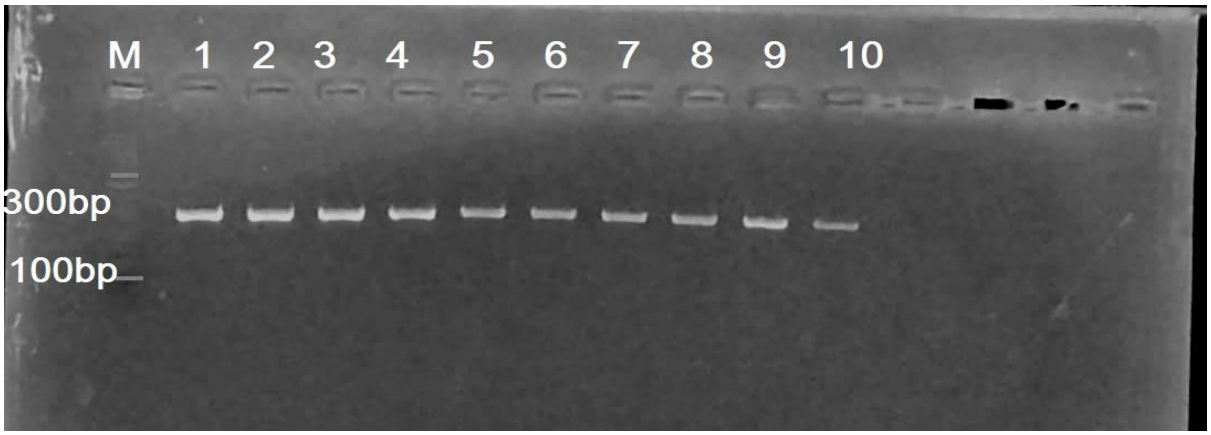

Figure (4) Results of electrophoresis of Pseudomonas aeruginosa for the detection of plcH gene, M: DNA marker. Pores 1-10 indicate positive results. It gave DNA regions of 300 pb.

AprA is one of the genes encoding exogenous enzymes. The apr gene encodes for the production of the protease enzyme of Pseudomonas. Through the study of mutant genes, it was found that bacteria that do not produce protease are less virulent than strains that produce this enzyme (Pillar et al. 2000). The incidence rate of this gene detection in current study was 100% matched the results of Bradbury et al. (2009) who indicated that all Pseudomonas aeruginosa isolates were apr gene producers.

Alkaline phosphatase (encoded by the gene AprA) plays an important role in the pathogenesis of Pseudomonas aeruginosa, as it reduces TLR5 stimulation and inhibits complement activation.

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While PlcH acts as genes encoding important proteins that play an important role in bacterial pathogenicity and as a catalyst for the production of proinflammatory mediators, the enzymatic activity of PlcH on the production of PC or SM phosphorylcholine (ChoP), which phosphorylates choline (Wood, 2018; Casabona, 2013).

From table (6) and figure (5,6) showed antibiotic resistant test

Table (6)Antibiotic susceptibility test

Antibiotic	Resistant	Intermeted	Senstive
Amoxicillin	53 (%77.9)	2 (%2.9)	13 (%19.1)
Tetracycline	56 (%82.3)	3 (%4.4)	9 (%13.2)
Chloramphenicol	35 (%51.4)	6 (%8.8)	27 (%39.7)
Gentamicin	12 (%17.6)	3 (%4.4)	53 (%77.9)
Impenem	6 (%8.8)	1 (1.4)	61 (%89.7)
Vancomycin	40 (%58.8)	0 (%0)	28 (%41.1)
Erythromycin	38 (%55.8)	3 (%4.4)	17 (%25)
Azithromycin	27 (%18.3)	6 (%8.8)	35 (%.77)
Ceftazidime	17 (%25)	9 (%13.2)	42 (%61.7)
Ciprofloxacin	10 (%14.7)	2 (%2.9)	56 (%82.3)
Trimethoprim	7 (%10.2)	3 (%4.4)	58 (%85.2)
Cefotaxime	20 (%29.4)	9 (%13.2)	39 (%57.3)
Ampicillin	41 (%60.2)	3 (%4.4)	24 (%35.2)

Figure (5): Antibiotic susceptibility test

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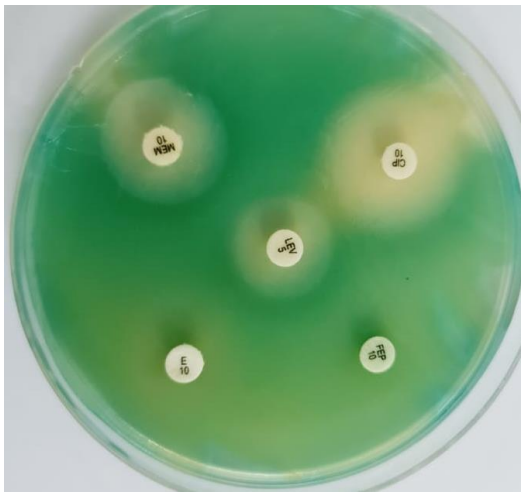

Figure (5): Antibiotic susceptibility test

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