Molecular Detection blaOXA50 gene of *P.aerginosae* Isolated from Otitis Media

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Abstract

Pseudomonas aerugenosa is a gram negative rod shape bacterium belonging to the family Pseudomonadaceae . *Pseudomonas aeruginosa is a major uropathogen in a hospital. It can tolerate a wide kind of physical conditions and many antibiotics via several mechanisms resistance* . The study included genetic study bla*OXA50 gene* utilizer technique PCR by specific forward and reverse primers to common bacteria that causes otitis media, 40 isolated from otitis media patients (ear swab). Primary identification was depended on Gram stain ,biochemical tests and vitek 2 system was done . The results demonstrate 30 isolate of them were *P.aerginosae*. The result appear *P.aerginosae* contain on 25(19%) *blaOXA50* gene.

Keyword: *P.aerginosae, blaOXA50 gene, Resistance antibiotic*

Introduction

Otitis media (OM) refers to a group of compound contagious and diseases in flammatory effecting the ear middle . general the Otitis media is very rife, the study appear that about 82 % of children need to experience at lower one episode via their birthday third. Otitis media have 2 kinds, chronic , acute. (A.O.M) is characterized via the fast onset of signs of infection, specially bulging and promising perforation of the tympanic membrane, fullness, erythema as well as symptoms associated with in flammation for example fever , irritability , otalgia [1] In spite of suitable therapy of the antibiotic , AOM may progress to (C.S.O.M) characterized via continual drainage from the ear middle related with perforated ear drum [1]. *Pseudomonas aeruginosa* is one of the most ecologically significant species among the genus Pseudomonas. *P. aeruginosa* is of extreme importance because of the widespread distribution of its strains in nature, its high intrinsic anti-bacterial resistance and its virulence [2]. Many antibiotic resistance mechanisms report in *P. aeruginosa* counting: 1) Reduced expression or loss of Op rD porin causing reduced antibiotic permeability 2) Over-expression of Mex AB Op r M pump which increases antibiotic efflux 3) Production of βlactams and aminoglycosides inactivating enzymes 4) Mutations of gyrases and topoisomerases which causes resistance fluoroquenolone . The mehanisms in combenation lead to multiple drug resestance [3]. β-lactamases are hydrolytic enzymes that are responsible for the resistance to β-lactam antibiotics. βlactamases have many types containing (E.S.BLs), AmpC β-lactamases, carbenicillin hydrolysing β-lactamase, Pseudomonas specific enzyme (PSE) and (M.β.Ls). ESBLs are encoded by different genes in P. aeruginosa including VEB gene. MβLs are encoded by different genes involved V.I.M and I.M.P [4].

Bacterial pumps efflux greatly included in the intrinsic resistance of Gram-negative bacteria. When overexpressed, pumps efflux can accord raise resistance to already effective antibiotics. Many pumps efflux conveyance enormous range of unrelated drugs known as multidrug resistance (MDR) pumps efflux. Four antibiotic efflux method has been reported in P. aerugenosa. Mex AB-Op r M is the efflux method that is responsible of ejaculation of quinolones and β-lactams . The evolution of isolates M.D.R.P.A through therapy report in 27% to 72% of patients by primarily suscaptible P. aerugenosa isolates. Patients by MDRPA deferent infections have to treated thru therapy combination, involving of an antipsaudomonal

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β-lactam fluoroquenolone oraminoglycoside , to some extent fluoroquinolone and aminoglycoside group, tool up convenient therapy and get better patient outcomes [2].

Methodology

Samples collection

collected (40) clinical samples from patients suffering otitis media signs through the period from) 2019 January to October, 2019) from patients present to AL-Hakem General Hospital and AL-Sadder Medical City, All the samples cultured onto MacConkey, Blood and Mannitol agar plates then protected at 37°C for 18 to 24 hr **.**

Detection *blaOXA50 gene*

DNA extraction

Genomic DNA extract via utilizer a commarcial extraction method (Genomic DNA proemega Kit).

Molecular Identification

assay the PCR ware performed to detect the blaOXA50 gene for *P.aerginosae* shown in table (2). These primers were produced via Alpha DNA Company, Caneda show of table (1). The magnified products of PCR was detectadvia agarose gel electrophoresis was visualized via staining with bromide ethidium . The result of electrophoresis was discover via utilize gel documantation method. positive results was distingueshed when the DNA band pairs base of sample unequal product base size 5. Lastly, gel ware photographed utilizer Biomaetra gel documentation method .

Table (1): Product size and Sequences of each primers.

Primer type	Primer (5'-3')		Product size(bp)	Reference
blaOXA50	F	3′-GAAAGGCACCTTCGTCCTCTAC-5′	400	6
	R	5′-CAGAAAGTGGGTCTGTTCCATC-3′		

Table (2): PCR conditions of *blaOXA50 gene detection*

Name of Gene	Temperature (°C) / Time					Cycles Number
	Initial Denaturation	Cycling Conditions			Final Extension	
		Denaturation	Annealing	Extension		
blaOXA50	94 Cº for 5 min.	94 Cº for 45 Sec	53Cº/62 for 1 min	72 Cº for 1min.	72Cº for 7min.	30 cycle

Results and discussion

Pseudomonas aeruginosa Isolation and Identification

Identification the primarily of specimens bacterial relied of some criteria which like biochemical tests , cultural, morphology . concurrence the last complete with the automated vitek-2 compact method utilizer GNID cards include 64 tests biochemical and 1 negative control. accentuation of aeruginos Pseudomonas showed utilizer P.C.R method . deferent physiological, biochemical and morphological, tests was made to identify isolates bacterial .

Results appeared the Pseudomonas aeruginosa contemned 30 isolates (30%), and another isolates bacterial was ,Proteus, Kleabisella pneumoneae, S.aureus and Enterobecter aearogenes . Bacterial isolates was specified give to the cultural, biochemical and microscopical physical appearance that approval [7]. aeruginosa Pseudomonas was products pyocyenin and

granting positive strongly oxidase , grant indole (Indole test is utilized locate the capability of an organism to split tryptophan amino acid to form compound indole , methyl red (test reveal the product the acid sufficient through the form of glucose, the result concur [8].

Isolates Pseudomonas aeruginosa give tests biochemical ; result

positive oxidase , and capable toward used citrate by way of sole source of carbon, in Kliegler Fe agar give alkaline slant and not alteration the bottom, negative H2S without production gas payable to fact the aerobic strictly [9].

Detection of the *blaOXA50*gene

All isolates was investigated to discover genes blaOX50 utilizer technique PCR with specified forward and invert primers. results appear in fig. (1) present the study to *blaOXA50* gene tested isolates represanted 25(19%) in bacterial isolated , all the resistant carbepenem isolates P. aeruginosa was found to harbor the gene blaOXA50 . Oxacilleinase is ambler kind D β-lactamases by hydrolytic action against the peniciillins , spectrum extended cephelosporins, aztreonam and methicillin . determination β-lactamases of group B and group D, like OXA and genes IMP, at the same order . was found to harbor the gene blaOXA50.

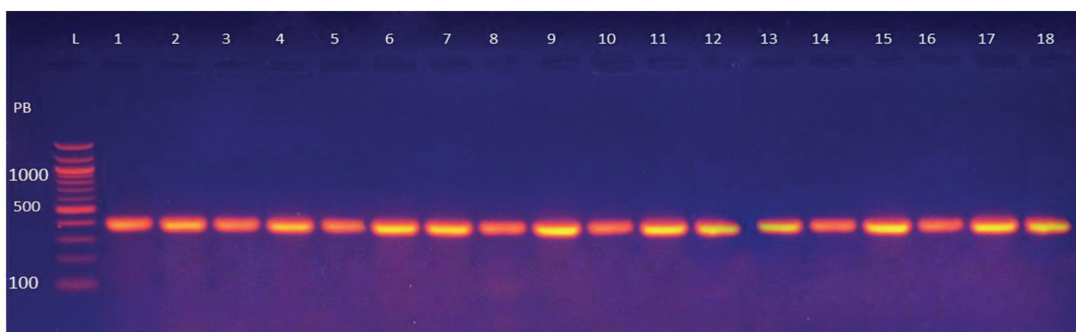

Figure (1): PCR amplification produces of P.aerginosae isolates that amplified with blaOXA50 primers gene with product 130 bp.Lane (L), Lanes (1 to18) appear results positive with blaOXA50 gene, DNA molecular size marker (100-bp ladder) .

The meteallo-β-lactamase (MβL) from group B. encodes the carbapenemases besides oxacillinase is report the first obtained MβL in 1994 Japan , genes encoding IMP-kind enzymes need diffusion fast between species Pseudomonas . MβLs are especially encoded via integron-borne genes and confer resistance against all β-lactams, excluding for the monobeactams [10].

The inclusion of plasmids in resistance antibiotic have been formerly reported via deferent studies . the technique PCR was exercised to DNA plasmid the tested MDRPA isolates in order to determine the genes involved in their expressed antimicrobial phenotypes resistance . β-Lactamases utmost rife , utmost imperative mechanism of resistance to antibiotics β-lactam the able of hydrolyzing the members of group β-lactam antibiotics like monobactams , cephelosporins , peniciillins, and carbepenems. The β-lactamases might mediated plasmid or chromosomally intercede . β-Lactamases may possibly divided in to four groups (A, B, C, and D) give to the similarities sequence [11].

The mechanisms catalytic , has been established two group ; the group B enzymes are metaello-β-lactamases that demand Zn of the activity, the group A, C, and D β-lactameses include groups serine at active site . Oxecillinases are Ambler group D β-lactamases with against penicillins that active hydrolytic , spectrum extended methicillin , cephalosporins, aztreonam [12].

Ethical Clearance: The ethical approval belong environmental and health ministries in Iraq

Conflict of Interest: Non.

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