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Studying the Incidence of Pseudomonas Species as A Causative Agent of Otitis in Iraqi Patients

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Abstract

Otitis can affect the inner or outer parts of the ear. Some times it is difficult in a rapid response to the treatment. The condition is classified according to occured suddenly for a short time (acute) or repeatedly over a long period of time (chronic).The types of treatments and sustainability were depended in most cases on bacterial infections.

23 samples of Iraqi patients infected with otitis collected from Yarmook hospital. The aural exudates screened to determine whether the infection was external or interior ear on MacConkey agar, and the negative Gram bacteria has been selected due to its higher connectivity with human diseases.

The growth colonies diagnosed by assay of biochemical reaction and we genetically confirmed the availability of *Pseudomonas aeruginosa* by PCR. The diversity of bacterial infection showed the dominancy of *Pseudomonas spp.* (60%) *and Pseudomonas aeruginosa* (17%) in it compared to other bacteria such as *Proteus Vulgaris* (13%), *Morganella morganii* (4%) and *Klebsiella spp. (4%).*

Key words: *Pseudomonas, Otitis, Azurin,*

لخلاصة:

ان التهاب الاذن قد يكون داخلي او خارجي يصعب شفاءة وعلاجة بصورة سريعة ويتوقف ذلك على مدى ونوع وظروف الاصابة اي عندما تكون الاصابة حديثة تكون استجابتها للعلاج اسرع واكثر فاعلية من الاصابة القديمة وهذا يعتمد ايضا على نوع البكتريا المسببة للالتهاب، ولمعرفة اغلب البكتريا المسببة لالتهاب الاذن تم جمع عينات من مرضى مصابون بالتهابات الاذن من مستشفى اليرموك من حزيران 2013 وحتى ايلول من نفس العام تم زرع وتنمية المسحات مباشرة على اطباق المكاونكي لتنمية البكتريا لسالبة لصبغة كرام فقط وذلك لانها مقاومة اكثر للمضادات الحيوية من البكتريا الموجبة لصبغة كرام. بعد التنمية تم اجراء فحوصات كيميائية حيوية لمعرفة نوع البكتريا وتم تاكيد منها الاخطر امراضية بواسطة تفاعل البلمرة المتسلسل جينيا .

اظهرت النتائج تنوع في البكتريا المسببة لالتهاب الاذن مع تفوق لاصابات*Pseudomonas spp.* بنسبة (60%)، وبكتريا *Pseudomonas aeruginosa* بنسبة (17%) مقارنة باصابات *Klebsiella spp.* بنسبة *(4%)* واصابات *Proteus Vulgaris* بنسبة (13%) و*Morganella morganii* بنسبة (4%).

Introduction:

Otitis is inflammation infects the ear. It divides into two types; first, *Otits* media related to middle ear infection. Second, "external otitis", "Swimmer's ear"[1] and "Pseudomonas Otitis"[2] are inflam-mationS of the outer ear and ear canal. Although several subtypes of otitis media are distinguished, the term is commonly used synonymously with acute otitis. It is very common in childhood but may occur at any age of human [3].

Along with otitis media, external otitis is one of the two human conditions mostly called "otalgia". Inflammation of the ear canal is the major of this disorder. This kind of inflammation can be secondary to

eczema (dermatitis) only, with no microbial infections, or it can be caused by active bacterial or other infection such as fungal infection [4,5,9]. In either case, but more often with bacterial infection, the skin of the ear canal swells and may become painful. Otitis media is caused by a bacterium or may cause by virus in the middle ear. This case of infection commonly results from another illness-cold, flu or allergy-that induces recongestion and swelling of the eustachian tubes, nasal passages, and throat [5]. Here we address four different bacteria that we found it among twenty three patients infected with otitis. *Pseudomonas aeruginosa* is a Gram negative bacteria, aerobic, with unipolar motility[10]. An opportunistic human pathogen, also is an opportunistic pathogen of plants[6]. *P. aeruginosa* is the type species of the genus *Pseudomonas*.[3].*P. aeruginosa* secretes a many different pigments, including pyorubin (red-brown), pyocyanin (blue-green), and pyoverdine (yellow-green and fluorescent)[10]. Second, *Proteus vulgaris* is a rod-shaped, a gram-negative bacterium that inhabits the tracts of intestine of humans and animals. It can be found in soil, water and faeces. It considered an opportunistic pathogen of humans. It is known to cause infections of urinary tract and wound infections[6]. Third, *Klebsiella*, is a genus of Gram negative, non-motile, rod-shaped bacteria with capsulea containing a prominent polysaccharide-based. *Klebsiella* organisms can lead to a wide range of disease cases, notably urinary tract infections, pneumonia, septicemia, and soft tissue infections [6]. Fourth, *Morganella morganii* is a Gram negative bacteria. *M. morganii* has a commensal relationship within the intestinal tracts of mammals, humans, and reptiles as normal flora. Although *M. morganii* has a wide distribution, it is classified an uncommon cause of community-acquired infection and it is common often encountered in postoperative and other nosocomial infections such as urinary tract infections[5].

In general, *Pseudomonas spp.* is the one of the common organism associated with chronic otitis in human[3]. Objectively the ear is often ulcerated and filled with exudates with rod-shaped bacteria. As well as, it is the most common cause of infections of burn injuries and common in the outer ear (external otitis), Here we address three major bacteria that we found it among twenty three patients infected with Otitis[3,11-17].

The Azurin gene was selected as a screening agent in confirming crucially the *Pseudomonas aeruginosa*. Azurin is protein produced by only *P. aeruginosa* and other rare few bacteria[17].

Material and methods:

Sample collection:

The exudates of twenty three different patients suffering from Otits have been collected from July 2013 to end of September 2013. The samples took from the junction of the horizontal and vertical canal using a sterile mini-tip culturette and submitted for aerobic culture on MacConkey agar plates.

Media:

MacConkey agar and Kligler Iron agar that we used, were purchased from Sigma Company, both media were recommended for differentiation of Gram-negative bacilli from clinical specimens. Additional chemicals; Indole, Simmen Citrate and Urea test were purchased from Merck Millipore company.

The samples of exudates were grow on MacConkey agar for 24 hours in 37°C, Every single colony of different samples was tested biochemically and inoculated in biochemical media tubes including (Kligler Iron agar, Indole, Simmen Citrate and Urea Test).Tubes incubated aerobically at 35 ± 2°C and examined for growth after 18-24 hours.

PCR reaction:

Primers have been designed for Azurin gene (1287 bp) from Gen Bank: M30389.1.

Forward primer:

5- ATGCTACGTAAACTCGCTG-3,

Reverse primer:

5- TCACTTCAGGGTCAGGGTG-3,

The primers were purchased from Bioneer Company, South korea as well as 2X master mix for PCR product generation. The following conditions have been used:

- Initial denaturation 94°C for 4 minutes.
- Denaturation 94°C for 30 seconds.
- Annealing 55°C for 35 seconds.
- Extension 72°C for 35 seconds.
- Final extension at 72°C for 10minutes.

32 cycles for polymerization has been used. The generated PCR product (447bp) that shown in figure-4 was electrophoresed and analyzed on 1.5% agarose gel.

Results:

Using a selective media for Gram-negative bacteria, MacConkey agar used to grow Gram negative bacteria and differentiate them for lactose fermentation, (figure-1). This is a useful due to differentiation between the types of *Pseudomonas* species as well as other Gram- negative bacteria.

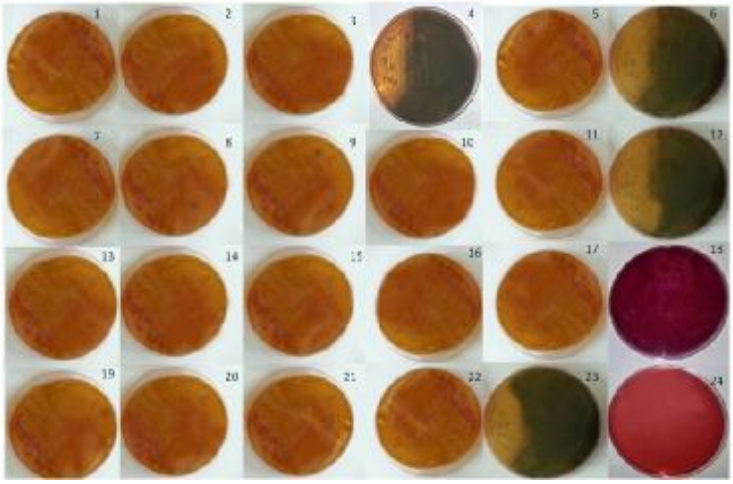
Twenty three samples screened on MacConkey agar and eighteen samples showed brown colonies color, four samples were showed as green colonies but only one showed the red colonies.

The brown colonies represented many different species with non lactose fermentation as diagnosed biochemically in figure-1and table-1, *Pseudomonas spp.*, *Proteus Vulgaris* and *Morganella morganii*, respectively, but only one sample showed its ability to ferment the lactose on MacConkey agar that was *Klebsiella spp.*

Other bacterial types were confirmed and identified biochemically. Three plates (numbers, 19-21) were diagnosed as *Proteus Vulgaris*, fourteen plates were diagnosed as *Pseudomonas spp*. All Four plates with green colonies color were diagnosed biochemically and confirmed genetically as *Pseudomonas aeruginosa*, figure (2 and 3).

The percentage level of bacteria types causes otitis was calculated, 60% of them showed infection with *Pseudomonas* spp. 17% showed infection with *Pseudomonas aeruginosa.* 4%, 13% and 4% showed infection with *Klebsiella sp. Proteus Vulgaris* and *Morganella morganii* respectively, (figure-2).

(A)



(B)

Number	Bacteria	Plate 's number	color of colonies
1	*Pseudomonas spp.*	14	Brown
2	*Pseudomonas aeruginosa.*	4	Green
3	*Klebsiella spp.*	1	Red
4	*Proteus vulgaris*	3	Brown
5	*Morganella morganii*	1	Brown

Figure- 1: Growth bacteria of different gram- negative bacteria on MacConkey agar:
(A): Plates:
[1] Fourteen plates (numbers, 1-3, 5, 7-11 and 13-17) were diagnosed as *Pseudomonas spp.*
[2] Four plates (numbers, 4, 6, 12 and 23) were diagnosed as *Pseudomonas aeruginosa*.
[3] Three plates (numbers, 19-21) were diagnosed as *Proteus Vulgaris* .
[4] One plate (number, 18) was diagnosed as *Klebsiella spp.*
[5] One plate (number, 22) was diagnosed as *Morganella morganii* .
[6] Plate number 24 was no growth (only MacConkey agar).
(B) Represent the statistics, types and shape of bacteria that were grown on MacConkey agar.

Table-1: Assay of biochemical reactions was used in bacterial diagnosis.

Bacteria	OXID	IND	CIT	MOTI	URE	GAS	LACT	MAN	H2S	SUC	GLU
Pseudomonas sp.	+	-	+	+	-	-	-	-	-	-	-
Proteus Vulgaris	-	+	-	+	+	**D**	-	-	+	-	-
Klebsiellasp.	-	-	+	-	+	+	+	+	-	+	-
Morgonella morganii	-	+	-	**D**	+	**D**	-	-	+	-	-

OXID: Oxidase; IND: Indole; CIT: Simmen Citrate; MONT: Semi-Sold-Manitol; URE: Urea Test; GAS: C0² (Kligler Iron test); LAC: Lactose (Kligler iron test); SUC: sucrose (Kligler iron test); (D) Sign means different (+/-).

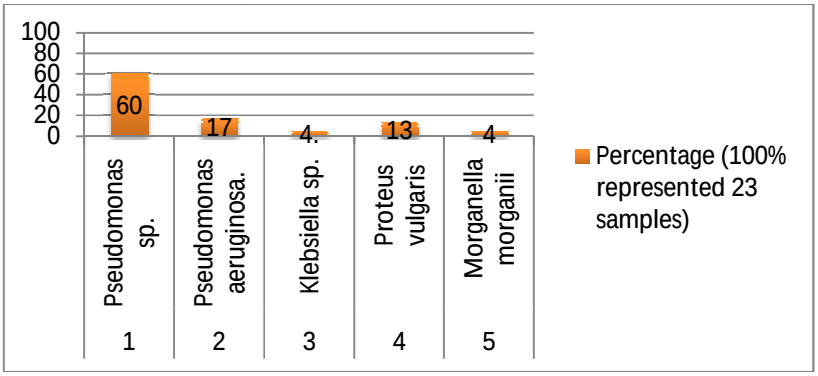


Figure-2: The percentage levels of bacterial growth on MacConkey agar for 23 samples of patients were infected with otitis.

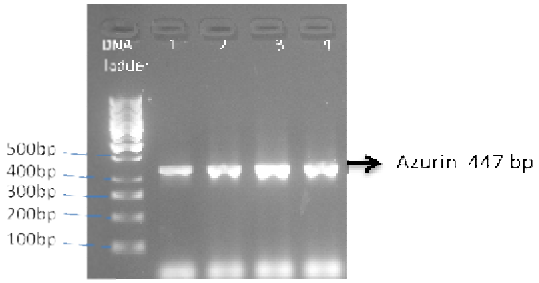


Figure-3: Electrophoresis of Azurin product 447bp loaded in 1.5 % of Agarose gel for 40 minutes (100volt) for *Pseudomonas aeruginosa.*

Lane 1: PCR product of plate number 4 .
Lane 2: PCR product of plate number 6 .
Lane 3: PCR product of plate number 12.
Lane 4: PCR product of plate number 23 .

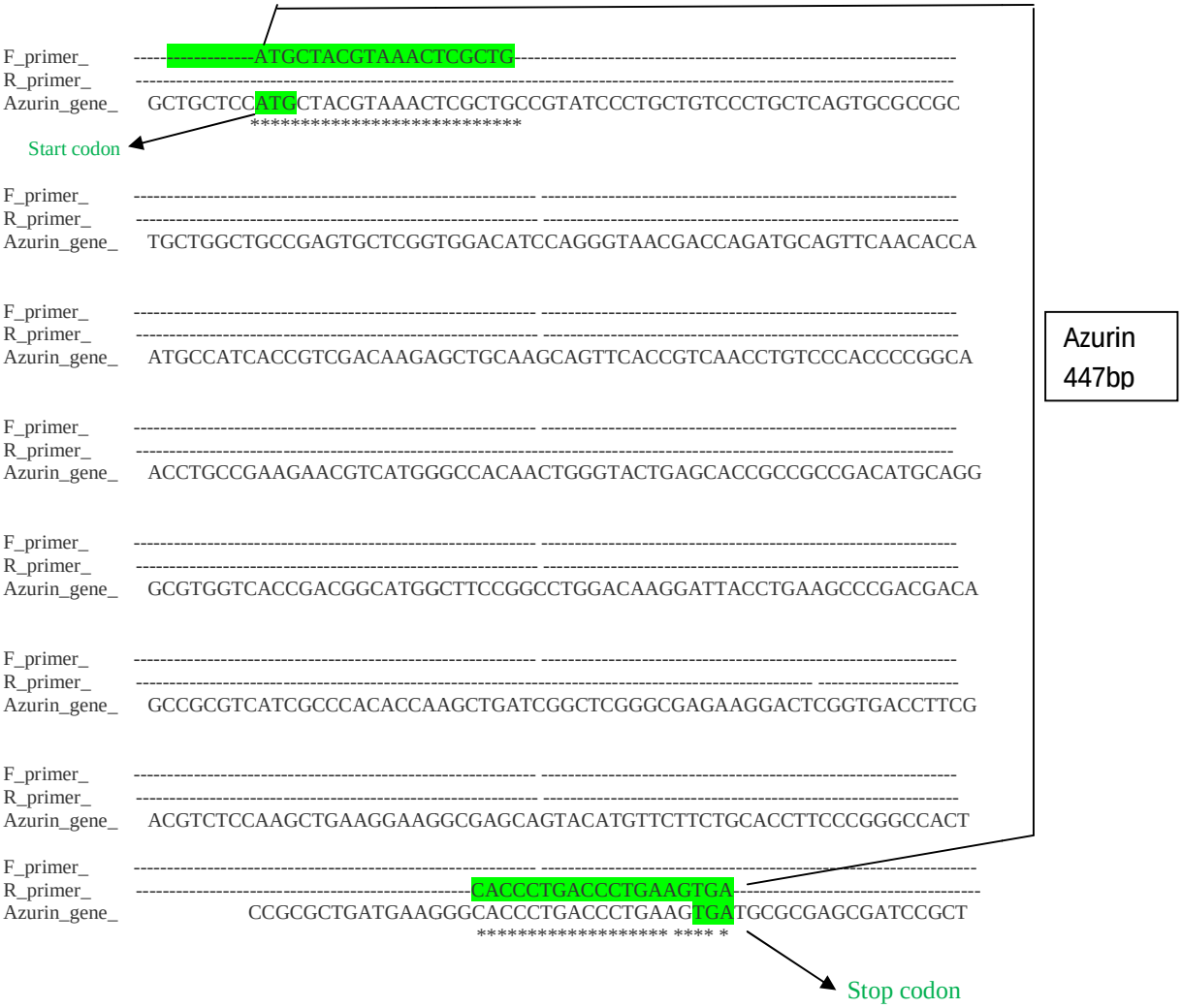


Figure-4: Alignments of primers with Azurin gene showing an amplification size of Azurin 447bp by PCR.

Discussion:

MacConkey agar was used selectively due to it can differentiate the characters; bile salts (inhibit most Gram positive bacteria), crystal violet dye (which also can inhibit certain Gram positive bacteria), neutral red dye (which stains bacteria fermenting lactose), lactose and peptone. *Pseudomonas spp.* played a majority role in causing Otitis as showed in figure-3 and was represented 60%.

Simply, the most risky bacteria and difficult in treatment compared with other found bacteria here were *Pseudomonas aeruginosa*, were represented 17%.

In otitis, the other types of bacteria displayed fewer than the *Pseudomonas* strains. The differentiation between *Pseudomonas* species and *Pseudomonas aeruginosa* colors return to availability of some other characters, such as the production of pyocyanin, pyoverdine,and pyorubin pigments[3].

The high pathogenisity of *Pseudomonas aeruginosa* in otitis excited us to genetically confirm it using a bacterial protein called arsenate reductase (Azurin gene, figure-3). Azurin is available in *Pseudomonas aeruginosa* and not available in the other bacteria excepting of Bordetella and alcaligenes genera.

Bacterial strains that found in external otitis probably exhibit the types of strains present in the natural habitat, as opposed to the strains that have adapted to the environment of other human infections[11-15]. So the Increased knowledge of the characteristics of the strains found in otitis is important in understanding the pathogenesis of the disease. Some researchers comfirmed the majority of *Pseudomonas aeruginosa* in causing Otitis[1-8, 11-17]. This research demonstrates the high dominancy of *Pseudomonas spp* compared with other bacteria.

Although the availability of *Pseudomonas aeruginosa in otits indicated* difficulties in leaving the infection without a suitable treatment,but that is paying attention to connected this disease with *Pseudomonas spp.*

The results showed the majority of *Pseudomonas sp. including Pseudomonas aeruginosa* (77%) (figure-2), but only 17% was highly pathogenic as diagnosed with genetic comfirmation *as Pseudomonas aeruginosa* compared to 4%, 13% and 4% that showed infection with *Klebsiella sp., Proteus Vulgaris* and *Morganella morganii, respectively*, figure (1and 2).

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