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Molecular Characterization of Virulence and Antibiogram profile of *Pseudomonas aeruginosa* Isolated from Chronic Suppurative Otitis Media Patients, Al-Nasiriyah City, Iraq.

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***Abstract*— This study was conducted to characterize the virulence of *P.aeruginosa* isolated from Chronic Suppurative Otitis Media (CSOM) in a particular area at the south of Iraq, Al-Nasiryah City. Conventional bacteriological methods were used to isolate and identify *P.aeruginosa*. Polymerase chain reaction (PCR) with a specific primer that targeting outer membrane Liporpotien L gene (*OprL*) was used to detect *P.aeruginosa*. The following virulence genes were screened; *ToxA*, *LasB* and *LasA*. Results indicated that the majority of CSOM was attributed to bacterial infection(83.4%) in comparison with only 2(5.6%) were fungal infections. *P.aeruginosa* was the most predominant species (36.7%) followed by *S.aurues*, Proteus Spp. and *E.coli*. Virulence profile revealed that all strains of *P.aeruginosa* were harboring at least one virulence gene. *ToxA* gene was found in most strains (90.1%) followed by *LasB* gene (81.8%) and *LasA* gene (63.6%). According to different combination of virulence genes, *P.aeruginosa* can be categorized into four genotypes with high prevalence (63.6%) of the most virulent genotype (*ToxA+ LasA+ Las B+)* followed by genotype (*ToxA+ LasA- Las B+*) (18.6%) while the genotypes (*ToxA+ LasA- LasB and ToxA+ LasA- LasB-*) were found in lowest percentage (9.1%). Additionally, all strains were able to form a biofilm with varying propensity however, the strong biofilm strains were found in a high percentage (72.7%) in genotype (*ToxA+ LasA+ Las B+*). Antibiogram analysis revealed that high resistance rate against otototic antibiotics including; Erythromycine, Ciprofloxacin(63%), Gentamycin, Ofloxacin and Ceftriaxone were(47.4%, 47.3% and 42.1% respectively). In conclusions, the *P.aeruginosa* causing CSOM in Al-Nasiriyah city is highly virulent and have a great ability to form biofilm, which could protect it from most commonly used antibiotics especially those used in Al-Nasiriyah, so it is highly recommended to seek for alternative treatment.**

Keywords: P.aeruginosa, Otitis Media, Biofilm, Antibiogram, Iraq

I. Introduction

Chronic Suppurative Otitis Media (CSOM) defined as a chronic middle ear and mastoid cavity inflammation, characterized by perforated tympanic membrane and persistence ear discharge (otorrhea) which lasting two weeks according to the World Health Organization(WHO) definition (World Health Organization, 2003). The disease mainly occurs during the first 6 years of childhood life, usually as s sequel of failure or incomplete Acute Otitis Media (AOM) treatment (Li, Hotez, Vrabec, & Donovan, 2015) however, occurring in the adults is not uncommon (Li *et al*., 2015; Orji FT, 2013; World Health Organization, 2003). The pathogenesis of CSOM has not yet understood however, the interaction between host factor (the abnormal function of Eustachian tube), environment, and microbial infection, thought to provide a fertile ground for the development of CSOM (Mittal *et al*., 2015b). Many bacterial species have implicated as the causative agents of CSOM including; Pseudomonase, *Staphylococccus aureus*, Proteues spp., Klebsiellia spp, and *E.coli* (Mittal *et al.*, 2015a; Molla, Tiruneh, Abebe, & Moges, 2019; World Health Organization, 2003). Studies that undertook the bacterial profile of CSOM from various parts of the world were almost agreed that most frequently encountered bacterium species in the CSOM were *P.aeruginosa* (Ettehad, G. H., Refahi, S., Nemmati, A., Pirzadeh, A. & Daryani, 2006;Ahmad, 2013). It appears that there is a global consensus that *P.aeruginosa* is the most likely bacterial species that cause CSOM.

In particular, *P.aeruginosa* was blamed for the progressive destruction of middle ear tissues through its toxins and enzymes(World Health Organization, 2003). *P.aeruginosa* is a gram-negative bacterium that has high adaptability and intrinsic resistance to antibiotics, enables it to colonize a variety of settings(Gellatly & Hancock, 2013). It has been proposed, that during otitis media, the ear environment became suitable for the colonization of *P.aeruginosa*. Once colonization of ear, it is

difficult to eradicate, taking the advantage of its toxins and enzymes that damage the ear tissue. *P.aeruginosa* able to avoid host immune response through minimizing blood flow and forming a shell that protects themselves(Mittal *et al*., 2015a). *P.aeruginosa* has a number of exotoxins and enzymes that secreted passively or through secretion system, most importantly; exotoxin A (toxA), protease (lasA) and elastaese (lasB), all these virulence factors secreted via secretion system type II(Najafi, Kafil, Shokrian, & Azimi, 2015a).

Recent studies have linked the pathogenesis of CSOM with biofilm production, biofilm firmly attached to damaged tissue and resist antibiotics(J.C. Post, P. Stoodley, L. Hall-Stoodley, 2004; Wang, J. C., Hamood, A. N., Saadeh, C., Cunningham, M. J., Yim & Cordero, 2014). *P.aeruginosa* considered a model of biofilm-producing bacteria as it has been found in biofilm form in many infections including CSOM(Dohar *et al*., 2005). The therapeutic (antimicrobial drugs) choices for CSOM are becoming limited through the continued emergence of multidrug resistance strains of *P.aeruginosa*. Hence it is important to highlight the virulence and antimicrobial resistance of local strain of the *P.aeruginosa*. Thus, the overall aims of this study were to identify the virulence factors possessed by this bacterium, and looking for its antibiogram profile to provide the local physicians with best antimicrobial treatment recommendations.

II. Materials and Methods

A. Study Design, Area and Population

This hospital-based study was carried out in the Ear, Nose and Throat (E.N.T) clinic of Al-Habobbi hospital at Al-Nasiriyah city which is geographically located at the south of Iraq with estimated population 400249. The ENT clinic health services covering the entire Al-Nasiriyah city. Attending this clinic was most often for Otitis Media (O.M) reason. The study population was the out-patients with ear discharge for at least two weeks and had perforated tympanic membrane as diagnosed with an otoscope by otologist of the E.N.T Clinic. Exclusion criteria were; patients with intact tympanic membrane, patients with tympanostomy tube, and patients who had antibiotics therapy for last three days before attending the Clinic.

B. Sample Collection

Samples collection was conducted in the period extended from October to December 2019 on daily surveillance for CSOM patients, during this period 36 ear swabs were aseptically collected by the clinician at ENT clinic, following the standard technique as follows: the auditory canal was firstly cleaned with 70% ethanol then sterile swab was inserted carefully without touching the auditory canal until reached the tympanic membrane. The swabs were directly transported to Microbiology lab at Technical Institute-Southern Technical University at Al-Nasiriya City within less than one hour, for bacteriological cultivation, identification.

C. Bacteriological Cultivation and Identification

Swabs were streaked on Blood Agar (BA) medium and incubated aerobically for 24 hours at 37 Cº. The blood agar plates that showed no bacterial growth for 48 hours were discarded as negative growth. On blood agar plate, the colonies that showed positive Oxidase test were picked carefully and subcultured on nutrient agar medium and incubated at 37Cº for 24 hours. Identification was accomplished based on pyocyanin production on nutrient agar medium plates, gram staining, growth at 42Cº and API20NE (Biomereux, France). The identified isolates were sub-cultured in Brain Heart Infusion Broth (BHIB) for further works.

D. Molecular Identification and Screening for Virulence Factors

- **Genomic DNA Extraction**

The brain heart infusion broth cultures were subjected to DNA extraction using DNA extraction Kit (Jenaid DNA extraction kit, Thailand). The amount and purity of extracted DNA were estimated using NanoDrop.

- **Amplification of Identification and Virulence Genes**

For identification of *P.aeruginosa* species, *Oprl L* gene which encoded for outer membrane Lipoprotein was used. For screening of virulence factors the following genes; *ToxA* (coding for Exotoxin A), *LasB* (coding for elastase) and *LasA* (coding for protease) were screened using Polymerase Chain Reaction (PCR) with specific primers whose sequences, annealing temperature and product size presented in table(1). The PCR reactions were done in 0.2 ml tube containing; 5 µl template DNA, 0.5 µl (10 Pico mole) of each forward and reverse primer, 5 µl of Premix (AccuaPure, Bioneer Korea) and the remaining of volume was completed with deionized distal water. The thermo-cycler machine (Bioneer Korea) was set to fulfill the thermo-cycle conditions presented in the table(1). Visualization of the product was conducted by transferring 10 µl of the products to previously prepared wells in agarose gel(2%), stained with Ethidium bromide. Electrophoresis was conducted under 105 V and 400 mA for 55 Minutes. The product sizes were estimated in comparison with molecular markers (1Kpb and 10Kbp Ladder) and Image Analyzer version 0.4 software for precise determination of product size. Gel documentation device (Atta, Japan) was used for photographing and analysis.

E. Antibiogram Analysis

All *P.aerugionsa* isolates were tested against 8 commonly used antibiotics to treat CSOM including those prescribed at ENT clinic of Al-Habobbi hospital. Kirby-Bauer, disk diffusion method was conducted using Muller-Hinton Agar medium. The following antibiotics are used;Piperacillintazobactam,Amikacin(10µg),Gentamycin(10µg),Ceftriaxone,Erythromycin(15µg),Ciprofloxcin(5 µg), Ofloxacin(30 µg), co-trimoxazole (1.25/23.75 µg). The results were recorded as sensitive and resistance according to criteria of CLSI2018(27th ed. CLSI supplement M100. Wayne, 2017).

F. Biofilm assay

Microtiter plate protocol was employed to quantitatively determine the *in vitro* biofilm production as described by Igbinosa *et al* .,(2015). An overnight *P.aeruginosa* brain heart infusion(BHI) broth culture was prepared, the cell density was adjusted to 0.2 of optical density (OD=600) using a spectrophotometer. 20µl of bacterial broth was transferred to plate wells containing 180 µl BHI and 20µl of D.W was added to negative control wells. The plate was incubated at 37C for 24 hours. Using a multichannel pipette, the wells were aspirated and washed three times with phosphate buffered slain (PBS,PH=7.4). Crystalviolate (1%) was added (200 µl) to each well including control negative and lets stand for 30 minutes. By the end of incubation, the stain was removed by multichannel pipette and the wells were washed three times with PBS. Plate was allowed to air dry at room temperature, then 150 µl absolute ethanol was added to each well for removing extra stain. The plate inverted on filter paper to remove the ethanol. Cell adherence was measured by microliter plate reader (Biotech, USA) at wave length 570 mm. biofilm propensity was recorded as in comparison with control negative value as follow; non biofilm (OD of isolate $\geqslant$ OD of control negative), weak biofilm (OD control negative < OD of

isolate < 0.1), moderate biofilm (OD isolate > 0.12), strong biofilm (OD isolate >1).

Table (1): The primers sequences and thermo-cycle conditions for amplification of targeted identification and virulence genes of

Type of microbial infection	No.	Species	Total	P-Value
Mono-microbial infections	14 (46.7%)	*P.aeruginiosa*	8(57.1%)	P>0.05
		S.aureus	6(42.8%)	
Poly-microbial infections	16 (53.4%)	With *P.aeruginosa*	3 (18.7%)	P<0.05
		Without *P.aeruginosa*	13(81.3%)	
Total			30	

Genes	Sequences	Cycles (No.)	Annealing Temp.(C°)	Product size bp	Reference
OprL	F:ATGGAAATGCTGAAATTCTCT GCT R:CTTCTTCAGCTCGACGCGACG	30	55	504	(Vos et al., 1997)
ToxA	F:GACAACGCCCTCAGCATCACC AGC R:CGCTGGCCCATTCGCTCCAGC GCT	35	67	396	(Najafi, Kafil, Shokrian, & Azimi, 2015b)
LasB	F:ACAGGTAGAACGCACGGTTG R: GATCGACGTGTCCAAACTGC	30	50	1220	(Najafi et al., 2015b)
LasA	F: GCAGCACCAAAGATCCC R: GAAATGCAGGTGCGGTG	30	57	1075	(Najafi et al., 2015b)

G. Statistical Analysis

All data were computerized and analyzed using Microsoft excel version 2010. Percentages were used for bacterial isolation, biofilm and antibiotics resistance. Statistical significant were calculated at 0.05 level using Chi-Square online program.

III. Results

Isolation Rate of *Pseudomonas aeruginosa*

In general, the bacterial growth was observed in 30(83.4%) out of 36 ear swabs, 4 (11.1%) revealed no growth, while the remaining 2(5.6%) revealed yeast (*Candida albicans*). Thirty ear swabs samples showed growth of four bacterial species, *P.aeruginosa*, *S.aureus*, proteus spp. and *E.coli*, table (2). *P.aeruginosa* was predominant species 11/30(36.7%) when compared with an isolation rate of other bacterial species which collectively constitute 19/30(63.3%). *P.aerugniosa* was isolated in pure culture in 8/11(72.7%), while 3/11(27.3%) were in mixed growth with other bacterial species. On the other hand, Proteus spp. and *E. coli* were found only in mixed growth.

Table(2): *P.aeruginosa* isolation rate from patient with Chronic Suppurative Otitis Media

No. of ear swabs	Bacterial Growth				No bacterial growth	
	P.aeruginosa		Other bacterial species		Fungal Growth	No growth at all
	Pure	Mixed	Species	No.		
36	8(72.7)	3(27.3)	*S.aureus*	8	2(5.6)	4(11.1)
			Proteus spp	6		
			E.coli	5		
Sub-Total	11(36.7%)		19(63.3)			
P-value	p>0.05					
total	30 (83.4)				6(1.7%)	

In regard with the type of microbial infection table(3), this study found that 14/30(46.7%) were mono-microbial infection, majority of these infection 8(57.1%) were accounted to *P.aeruginosa*, while only 6(42.9%) were caused by *S.aureus* with no statistical differences(P>0.05).

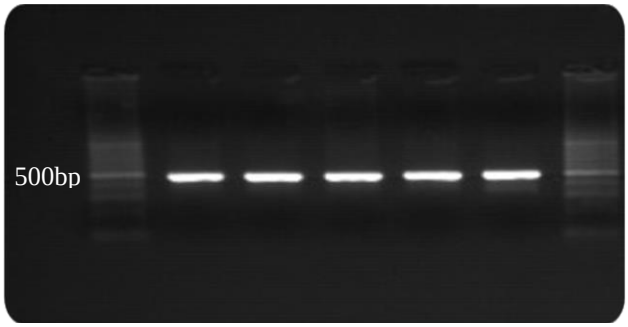
Table(3):Poly-microbial and Mono-Microbial infections of Chronic Suppurative Otitis Media

The ear swabs that yield polymicrobial growth 16(53.4%), *P.aeruginosa* was found in 3(18.7%) of these samples. Statistically significant differences(p<0.05) was found between poly-microbial infections with *P.aeruginosa* and those for other bacterial species.

Molecular study

Identification of *P.aeruginosa*

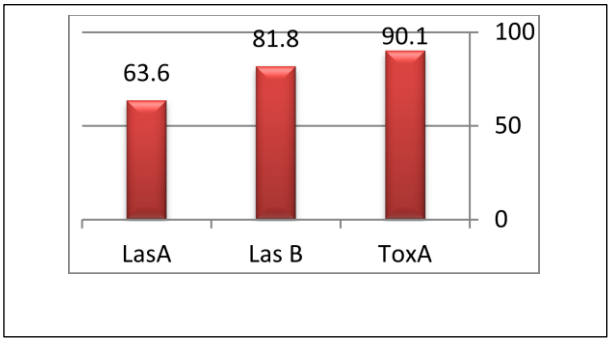
The results of amplification of identification gene (*oprL*) revealed, that all (100%) phenotypically and biochemically identified *P.aeruginosa* were positive for *oprL* gene which identify the bacterium to species level. Figure (1).



Figure(1): Product of oprL gene after electrophoresis in agarose gel(2%) under 105 V for 55 minutes. M, molecular marker(1Kbp), lanes, 1,2,3,4,5, the product size (504bp).

Molecular Screening of Virulence genes

Virulence genes were detected in all Paeruginosa stains with varying rates (figure 2). The most frequently detected virulence gene was ToxA 10/11(90.1%), followed by LasB 9/11 (81.8%) and LasA 7/11(63.6%). The expected products of ToxA, LasB and LasA genes were presented in figures (3,4,5, respectively).

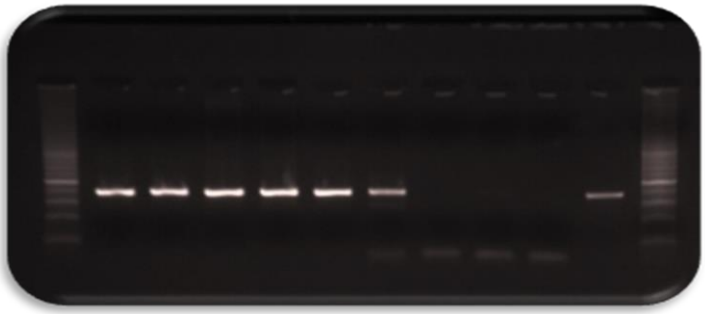


Figure(2): Detection rates of the three virulence genes in *P.aeruginosa* isolated from patients of Chronic Suppurative Otitis Media (CSOM).

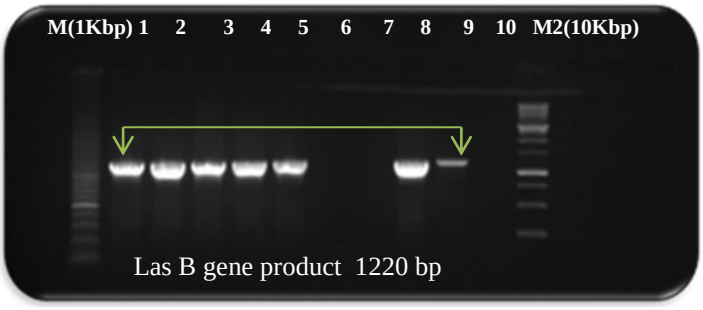
Based on the combination of three virulence genes, the eleven P.aeruginosa isolates were categorized into four genotypes, table (4). Most strains of P.aeruginosa 7/11(63.6%) in the studied population were belong to genotype (ToxA+ LasA+ Las B+) that harbour the three virulence gens, and 2/11(18.2%) were belong to pathotype (ToxA+ LasA- Las B+) that harbour tow virulence genes (exotoxin A and protease). The genotype that has a combination of exotoxin A and elastase A (ToxA+ LasA+LasB-) and the genotype that harbour only exotoxin A (ToxA+ LasA- LasB-) was found in low percentage (9.1%).

Table(4): genotypes of *P.aeruginosa* isolated from CSOM based on different combination of virulence

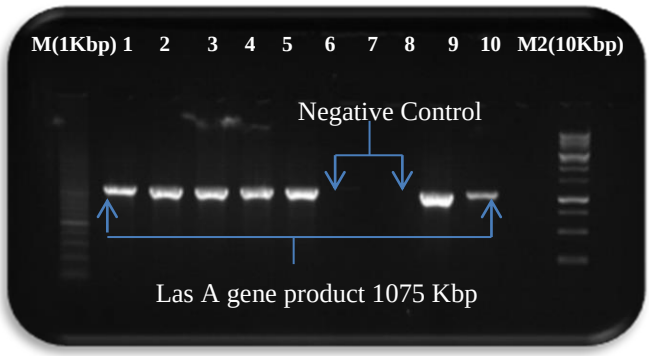
genotypes	No. of isolates	%
$ToxA^+$ $LasA^-$ $LasB^-$	1	9.1%
$ToxA^+$ $LasA^+$ $lasB^-$	1	9.1%
$ToxA^+$ $LasA^-$ $Las\ B^+$	2	18.2%
$ToxA^+$ $LasA^+$ $Las\ B^+$	7	63.6%
Total	11	



Figure(3): Product of *ToxA* gene after electrophoresis in agarose gel(2%) under 105 V for 55 minutes which give product size396bp. M, molecular marker (1Kbp), lanes, 1,2,3,4,5,6 and 10 positive amplification. Lans 7,8 and 9 negative control.



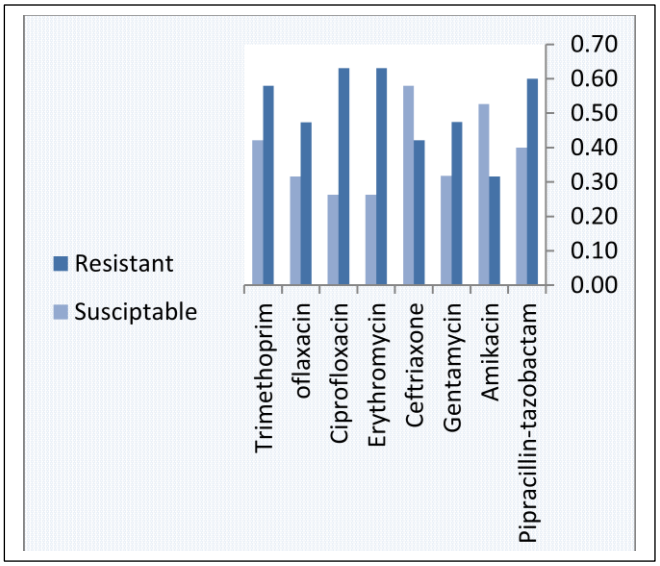
Figure(4):Product of lasB gene after electrophoresis in agarose gel (2%) under 105 V for 55 minutes which give product size 1220 bp. M1(1Kbp),M2 (10Kbp) molecular markers. Lanes, 1,2,3,4,5, 8,9 positive amplification. Lanes 6,7,10 negative control



Figure(5): product of lasA gene after electrophoresis in agarose gel(2%) under 105 V for 55 minutes which give product size 1075bp. M,molecular marker , lanes, 1,2,3,4,5,8,9 positive amplification. Lanes 6,7,10 negative control.

Antibiotics resistance profile

The results of the antibiogram analysis are illustrated figure (6). The highest resistance was recorded against Erythromycin, Ciprofloxacin(63.1%) and Trimethoprim (57.9%). Also, this study found the resistance to Gentamycin, Ofloxacin and Ceftriaxone were(47.4%, 47.3% and 42.1% respectively). Most isolates (60.%) were susceptible to Piperacillin-tazobactam.



Figure(6): Antibiogram of *P.aeruginosa* isolated from CSOM patients

Biofilm production

This study found that all *P.aeruginosa* strains (100%) were biofilm producer with varying strength. Most strains 8/11(72.7%) were strong producer, and 3/11(27.3%) were moderate biofilm producer. Table,(5). According to genotypes, the production of biofilm correlate with occurrence of virulence genes. According to the results of this study (table,5), the strong biofilm production was found at high percentage (75%)in the genotype that harbor three

virulence genes(*ToxA+ LasA+ Las B+*). Only one strain (12.5%) of each genotype (*ToxA+ LasA- Las B+*) and genotype (*ToxA+ LasA+lasB-*) was strong biofilm producer. While, the genotype *ToxA+ LasA- LasB-* that harbored only one virulence gene (*ToxA* gene) was moderate biofilm producer.

Table(5): biofilm production of four genotypes of *P.aeruginosa* strains isolated from patients of CSOM.

genotypes	Biofilm Production Strength		
	Weak (%)	Moderate (%)	Strong (%)
$ToxA^+$ $LasA^-$ $LasB^-$	-	1	0(0%)
$ToxA^+$ $LasA^+$ $lasB^-$	0	0	1(12.5%)
$ToxA^+$ $LasA^-$ $Las\ B^+$	0	1	1(12.5%)
$ToxA^+$ $LasA^+$ $Las\ B^+$	0	1	6 (75%)
Total	0	3(27.3%)	8(72.7%)

IV. Discussion

The present study focused on the molecular characterization of *P.aeruginosa* as the most common cause CSOM, along with an investigation of its ability to resist most commonly used antibiotics. This study found that the bacterial infection rate (83.4%) was higher than the fungal recovery rate(5.2%). This finding was in agreement with other studies (Madana, Yolmo, Kalaiarasi, Gopalakrishnan, & Sujatha, 2011; Rajat P, Deepak J, 2013; Seid, Deribe, Ali, & Kibru, 2013),as it has been accepted that bacterial infections are the most common cause of SCOM(Mittal *et al*., 2015b; Verhoeff, Van Der Veen, Rovers, Sanders, & Schilder, 2006). The low infection rate of fungal species in this study could be contributed to the climate of Iraq particularly, south of country (Thi-Qar province, were the study conducted) which is not humid this explanation depend on the fact that, only regions with high humidity recorded high fungal recovery in patients with CSOM (Verhoeff *et al*., 2006). The finding of this study regarding the *P.aeruginosa* predominance in CSOM was in agreement with local studies; Alsaimary, *et al* (19.4%)27Al-Hilli,201528, Aldhaher and Hassan,2018(Aldhaher & Hassan, 2018; Arwa Hammodi Karim, 2005). Similarly, the finding of this study regarding the predominance of *P.aeruginosa* was also in agreement with other global studies(Chirwa *et al.*, 2015; Madana *et al.*, 2011; Rath, Das, & Padhy, 2017). The predominance of *P.aeruginosa* in CSOM can be accounted to their minimal nutritional requirement and potential ability to resist antibiotics(Pollock .M, 1996). On the other hand, in contrast to Sied A, *et al* .,2013; Seid et al., 2013; and Molla R *et al* .,2019, who reported that Portues spp. was the most prevalent species among CSOM patients. This variation could be attributed to the small sample size of this study population, anaerobic bacterial species which not included in this study and geographical variation. Finding of this study regarding the type of microbial infections (Poly-microbial & Mono-Microbial) was inconsistent with Al-marzoqi A.H, *et al*.,2013; Al-marzoqi, Hussein, Mohammad, Taee, & Yheea, 2013, who found that poly-microbial infection was higher than monomicrobial infection. In this regard results of the current study was in harmony with the well-known fact that microbial infection of the ear usually polymicrobial (Bakaletz, 2010; Mittal *et al*., 2015b). For precise identification of Pseudomonas spp to species level, lipoprotein L (OprL) gene was selected with specific primer designated by Vos *et al*., 1997, this primer showed great specificity to detect Pseudomonas to species level and it is applicable for routine detection of *P. aeruginosa* in clinical samples, has been used in several studies; Najafi *et al*., 2015b; Neamah & Department, 2017).

In this study all *P.aeruginosa* (100%) showed positive for at least one virulence genes. The detection rate of *ToxA* gene 90.1% was in consistence with the result obtained by Auda *et al* .,(2015) ;Auda, Kadmy, Naseer, Ali, & Muslim, 2015; and Neamah & Department, (2017). Most *P.aeruginosa* (81.9%) in this study harbour gene (*LasB*) which coded for elastase enzyme, this result was in agreement with Al-Shwaikh,(2019); Al-Shwaikh & Al-Arnawtee, 2019 who found that the detection rate of this gene 82% in otitis media samples. Production of biofilm was found in all *P.aeruginosa* strains, this finding was in agreement with Dohar J.E.,(2005); Dohar *et al*., (2005) who concluded in his study that the CSOM is a biofilm disease. Similarly, finding of this study was in agreement with Abdelshafy I.A (2015) . on the other hand, our finding was higher than those recorded by Pinar *et al* ., (2008) and Lee *et al* .,(2009). The higher biofilm percentage in this study could be explained by the type of bacterial species as this study focused on *P.aeruginosa* which is known to have a high propensity of biofilm production. It is estimated that about 10% of *P.aeruginosa* virulence genes are controlled by quorum sensing (QS) system. The cell survival, biofilm production and virulence factors are depending on these quorum sensing, particularly, lasl QS regulates the biofilm production and secretion system II (both LasA and LasB produce through this system)16. Thus this could explain the high biofilm propensity of strains that harbour the three virulence factors (*ToxA+ LasA+ Las B+).*

Currently, the modality of treatment of CSOM depend on the aural toilet in combination with topical and systemic antibiotics. Some antimicrobial solutions are used such as acetic acid, Aluminum acetate and Iodine in resolving otoreah, unfortunately these solutions were not included in this study. The current study, found high resistance rate against commonly used ototic antiobiotics including; Ciprofloxacin, Ofloxacin which are considered first line treatment of CSOM. This finding was in agreement with Rath.S, *et al* .,(2017).

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التوصيف الجزيئي لعوامل ضراوة و مقاومة المضادات الحياتية لبكتريا الزوائف الزنجارية المعزولة من الاذن الوسطى التقيحي المزمن في مدينة الناصرية – العراق

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الخلاصة

اجريت هذ الدراسة لتوصيف فوعة بكتريا الزوائف الزنجارية المعزولة من اصابات التهاب الاذن الوسطى القيحي المزمن في منطقة محددة من جنوب العراق ، مدينة الناصرية. استخدمت الطرق البكتريولوجية التقليدية لعزل و تشخيص الزوائف الزنجارية ومن خلال تقنية تفاعل البلمرة المتسلسل و باستخدام بادئات متخصصة تستهدف الجين المشفر للبروتين الدهني الخارجي (Oprl) لتشخيص بكتريا الزوائف الزنجارية ، وتم تقصي ضراوة البكتريا من خلال تشخيص جينات الضراوة و التي شملت جين toxA, جين و LasB, جين . LasA بينت النتائج الى ان غالبية حالات التهاب الاذن الوسطى التقيحي المزمن تعود للإصابات بكتيرية (83,4%) بالمقارنة مع (5,6%) اصابات فطرية. ان بكتريا الزوائف الزنجارية كانت هي السائدة بنسبة 36,7% ، تلتها بكتريا المكورات العنقودية الذهبية و بكتريا جنس البروتيوس و بكتريا الاشيريشيا القولونية . كشف فحص الضراوة ، ان كل عتر الزوائف الزنجارية كانت حاوية على جين ضراوة واحد على الاقل حيث وجد جين toxAفي اغلب العتر بنسبة 90,1% و يليه جين LasB بنسبة 81,8% و جين LasA بنسبة 63,6%. و بالاعتماد على الاختلافات في تشخيص جينات الضراوة صنفت بكتريا الزوائف الزنجارية في هذه الدراسة الى اربعة انماط وراثية و سجل النمط الوراثي الاكثر ضراوة (ToxA+ LasA+ Las B+) اعلى نسبة 63,6% و يلية النمط الوراثي ((ToxA+ LasA- Las B+ بنسبة 18,6% اما النمطيين الوراثيين (ToxA+ LasA- LasB and ToxA+ LasA- LasB-) فقد سجلا اقل نسبة (9,1%) ، اضافة الى ذلك كانت كل العتر منتجة للبايوفلم لكن بقوة متفاوتة ومع ذلك اغلب العتر ذات الانتاج القوي كانت ضمن النمط الوراثي (ToxA+ LasA+ Las B+). اوضح اختبار المقاومة للمضادات الحياتية وجود مقاومة عالية للمضادات المستخدمة الالتهابات الاذن حيث كانت المقاومة للازثرومايسين و السبروفلوكساسين (63%) وكانت المقاومة للجنتامايسين و الاوفلوكساسين و السفترياكسون بنسبة47,4 و 47,3% و 42.1 % على التوالي. نستنتج من هذه الدراسة ان بكتريا الزوائف الزنجارية المسببة لالتهاب الاذن الوسطى التقيحي المزمن هي ذلت ضراوة عالية و لها قدرة على انتاج البايوفلم الذي يوفر الحماية لها من المضادات الحياتية الاكثر استعمالا في مدينة الناصرية لذا اصبح من الضروري جدا البحث عن بدائل علاجية.