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The Frequency and Sensitivity Pattern of *Pseudomonas aeruginosa* among Otitis Media patients in Nasiriyah City

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ABSTRACT

Otitis Media considered as one of the most common types of ear infections, as well as the most blamed etiology is bacterial. *Pseudomonas aeruginosa* is the most diagnosed pathogens causing these infections. *P. aeruginosa* has virulence factors that lead to damage to the mucosa of the middle ear. A total of two hundred and ten (210) samples collected from Otitis media patients whom consulting the Ear, Nose, and Throat (ENT) section in AL-Habbobi Teaching Hospital in Al-Nasiriyah City, Southern-Iraq. During the period from August 2018 to January 2019. *P. aeruginosa* accounted for 65 samples (30.95%). Almost of isolates were sensitive to Carbapenem. The present study concludes females constitute a high percentage of infection with Otitis media (P-value= 0.01) and younger ages are more exposed to the risk of Otitis media (P-value = 0.36). The present study aimed to detect the relationship between the prevalence of Otitis media and the parameters associated with patients like gender, age, housing, and the study period. Also studying the pattern of antibiotics Sensitivity for the targeted bacteria and detection of some virulence factors.

Keywords: Otitis media, *Pseudomonas aeruginosa,* Sensitivity test, Nasiriyah.

INTRODUCTION

Otitis Media

Otitis Media (OM) is a term referring to an infection of the middle ear. It is mostly caused by an accumulation of the fluid behind the eardrum, that result of obstruction the Eustachian tube. It is considered one of the most common infections in children because the Eustachian tube in children is shorter and more horizontal than adults, which it is formed of more flaccid cartilage, that it makes opening easily [1]. Clinically, Otitis Media (OM) include a broader range of disease, Acute Otitis Media (AOM), Chronic Suppurative Otitis Media (CSOM), and Otitis Media with Effusion (OME) [2]. The pathogenesis and the etiology of Otitis media are multiple including infections, allergy, genetic, racial, environmental, and social factors [3]. *P. aeruginosa* has the ability to colonize several sites because it contains effective adhesion mechanisms, Multidrug resistance, Biofilm formation and ability to survive in low nutritional requirements [4]. The incidence of the disease differs between the children and the adults. Children tend to develop AOM because the anatomical structure and the immune system are immature. While the ear infections in the adult are usually chronic infection [5]. Otitis media if untreated or left with incomplete treatment may be lead to complications, due to for many reasons like antimicrobial-resistant bacteria, or unknown causes. Other factors may be lead to complications such as immunological or anatomical factors, congenital malformations and the soft bony structures of kids may be more exposed to infection [6].

P. aeruginosa is infrequently found in nasopharynx by comparison with other types of bacteria. It is very rarely isolated from a healthy person ear canal [7, 8]. *Pseudomonas aeruginosa* can grow in strict conditions like the absence of special nutrition and can proliferate at the room temperature, and it has highly resistant to the antibiotic, all these causes making it difficult to treat [9]. A previous study in Baghdad

province isolated *P. aeruginosa* from Otitis media with a percentage of (30%) [10]. Globally, *P. aeruginosa* was isolated from these infections in Nigeria with a percentage of (28.8%) [11].

Virulence factors have a significant pathological role in adhering, colonization, the survival of microbe and lastly in the invasion of tissues [12]. Hemolysin is an enzyme produced by Gram-negative bacteria and Gram-positive such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Vibrio Vulnificus*, *Salmonella enterica*, *Proteus vulgaris* and *Proteus mirabilis* [13].

Pyocyanin is a blue-green pigment considered one of the most notable phenotypic characteristics of *P. aeruginosa*. Pyocyanin created in big quantities, particularly when bacteria are cultivated in low-iron media [14]. This pigmentation result from the excretion of phenazine compound pyocyanin (PCN), that acts as an important virulence factor in this bacteria, phenazine compound pyocyanin is readily isolated from the ear discharges and the sputum from *P. aeruginosa* infections [15].

The present study aimed to:

1- Detection of the relationship between Otitis media prevalence and the parameters associated with patients like gender, age, housing, and the study period.
2- Studying the pattern of antibiotic Sensitivity of *P. aeruginosa.*
3- Detected of some virulence factors of *P. aeruginosa* such as Hemolysin, and Pyocyanin.

Materials and Methods

Specimens

A total of 210 of middle ear swab samples were collected from Otitis media patients attending to the ENT Department in AL-Habbobi Teaching Hospital in Thi-Qar province, which includes both sexes of different ages. 99 males and 111females. The age groups of the patients ranged from less than ten years to more than fifty years. During the period from August (2018) to January (2019). These samples were collected by the ENT physicians by using a sterile swab. The information for each

patient was recorded in a special form, including name, age, gender, housing.

Isolation and identification

P. aeruginosa isolates culturing on Blood agar, MacConkey agar, and Cetrimide agar. Also diagnosed with different biochemical tests, including Oxidase test, Catalase test, gram staining, and phenotypic characteristics, the diagnosis was confirmed with VITEK-2 compact System (BioMerieux, France) at probability level between 98%-99%. *P. aeruginosa* in blood agar appears as a large, gray to dark colonies and some isolates were beta hemolysis. While in MacConkey agar *P. aeruginosa* was non-lactose fermenter with pale colonies, All *P. aeruginosa* isolates were grown on cetrimide agar as a selective medium for this bacteria to distinguish it from other species in this genus [16].

Detection of some Virulence Factors

Hemolysin production

A blood agar plate (5%) was inoculated with bacteria and incubated at (37 °C) for (24 h) for detection of beta hemolysis. Hemolysin production was detected by the presence of a clear zone around the colonies (complete lysis for RBCs) [17].

Pyocyanin production

Young colonies at (18-24 h) were inoculated on Muller-Hinton agar and incubated at (37°C) for (24 h). This medium is used for detection the

ability of *Pseudomonas aeruginosa* to produce pyocyanin pigment, which considered as an important diagnostic for this bacteria [18].

Antibiotic sensitivity test

In this study used 12 antibiotics from different classes (Bioanalyse, Turkey and Mast Group, England) Amikacin (10 µg), Aztreonam (30 µg), Cefepime (30 µg), Ceftazidime (30 µg), Ciprofloxacin (10 µg), Gentamicin (10 µg), Imipenem (10 µg), Levofloxacin (5 µg), Meropenem (10 µg), Ofloxacin (5 µg), Piperacillin (100 µg), Tobramycin (10 µg). Antibiotic sensitivity test was performed using Kirby Bauer disc diffusion method [19]. A bacterial suspension was prepared by inoculated 2-3 fresh colonies at (24 h) into test tube contains (3 ml) of normal saline. The suspension turbidity was compared with McFarland turbidity (0.5) that equal to 1.5×10^{8} CFU/ml. The bacterial suspension for each isolate was streaked in three different directions on Muller-Hinton agar plates by using a sterile cotton swab after pressed the swab several times on the inside wall of the tube to remove the excess inoculum. The plates were left to dry for (10 minutes) at room temperature. The antibiotic discs were applied to the medium surface by using a sterile forceps and the plates were incubated at (37°C) for (24 h). The diameter of the inhibition zone for each antibiotic disc was measured by using a plastic ruler (mm), and the results were interpreted based on CLSI, 2018 [20].

Statistical Analysis

The results in the present study were evaluated statistically via Chi-square by using Statistical Package for Social Sciences (SPSS) program version 23 at a probability of ($P \leq 0.05$) as a significant level between the parameters of the present study like gender, age, the study period, residency and some of virulence factors.

Results

After samples collection and performing all the diagnostic tests, the positive culture of *P. aeruginosa* was 65 samples (30.95%). The present study results for total samples revealed females more infected with Otitis media with111 cases (52.86%) and 99 cases (47.14%) for males. with regard to the relationship between *P. aeruginosa* rate infections with gender. Females recorded with 40 cases (61.54%), compared to males with 25 cases (38.46%), there was a significant difference (P-value= 0.01) in gender distribution (Figure 1).

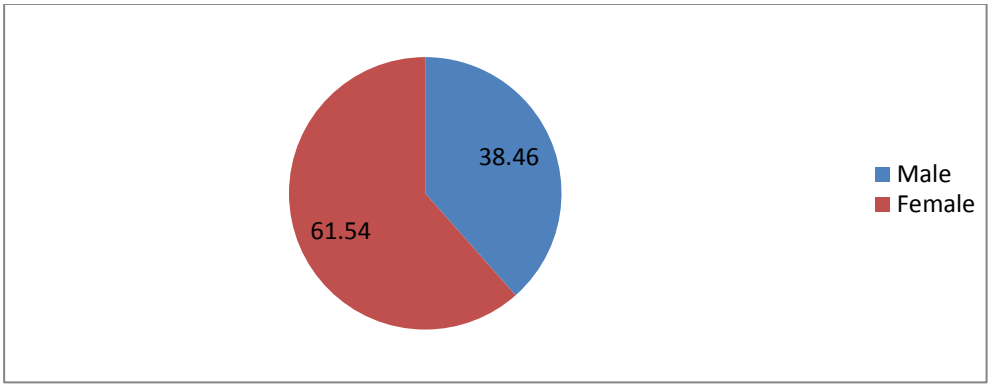

Figure (1): Patients distribution according to the gender.

With regard to the age, the present study showed the predominance the third group of age (21-30) years for total samples with percentage (21.43%). The highest incidence of Otitis media caused by *P. aeruginosa* also was in the age group of (21-30) years with 15 patients (23.08%). While the lowest occurrence was noticed with patients in the groups of (41-50) and more than 50 years with a percentage of (12.31%) for both. Statistically, have not a significant difference among these groups (P-value = 0.36) (Table 1).

Table 1: Patients distribution according to age groups

No.	Age	No. of Total Isolates	%	No. of *P. aeruginosa* Isolates	%
1	<10	37	17.62	10	15.38
2	11-20	39	18.57	12	18.46
3	21-30	45	21.43	15	23.08
4	31-40	39	18.57	12	18.46
5	41-50	21	10	8	12.31
6	>50	29	13.81	8	12.31
	Total	210	100	65	100

The relationship between the study period parameter and Otitis media for total samples revealed the highest percentage of Otitis media was in November (24.76%). Also, this study showed the highest percentage for *P. aeruginosa* targeted infections with 19 patients (29.23%) in November. While the lowest rate of infection was in January (3.08%), there was a significant difference (P-value = 0.00) between *P. aeruginosa* infections rates and the months of years (Table 2).

Table 2: Patients Distribution according to the study period

Month	No. of Total Isolates	%	No. of *P. aeruginosa* Isolates	%
August	28	13.33	9	13.85
September	31	14.76	10	15.38
October	45	21.43	14	21.54
November	52	24.76	19	29.23
December	40	19.05	11	16.92
January	14	6.67	2	3.08
Total	210	100	65	100

According to the residency, the total samples revealed the highest percentage of Otitis media was in urban 130 patients (61.90%) and in rural 80 patients (38.10%), with respect to *P. aeruginosa* infections were 36 patients (55.38%) in urban and lower in rural with 29 patients (44.62%), there have not a significant difference according to the otitis media occurrence with *P. aeruginosa* and the residency (P-value = 0.31) (Figure 2).

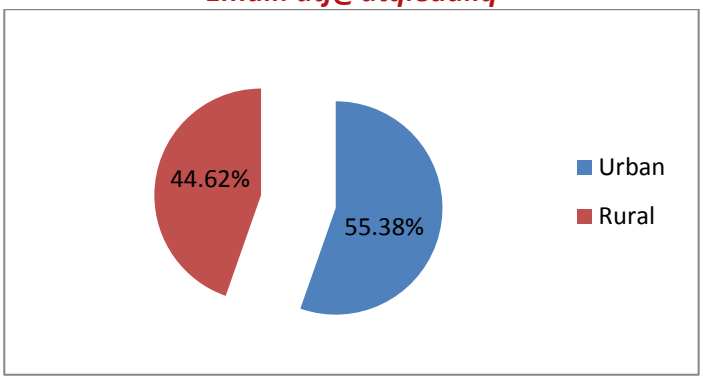

Figure 2: Distribution of the patients according to residency

Virulence factors

The present study results showed the ability of *P. aeruginosa* isolates to hemolysin production in 49/65 (75.38%) of the isolates, there was a significant difference in the production of this enzyme (P-value= 0.00). Pyocyanin production was detected on Muller Hinton agar, 32/65 (49.23%) isolates were Pyocyanin Production, there was a non-significant difference in the production of pyocyanin (P-value= 0.84) (Figure 3).

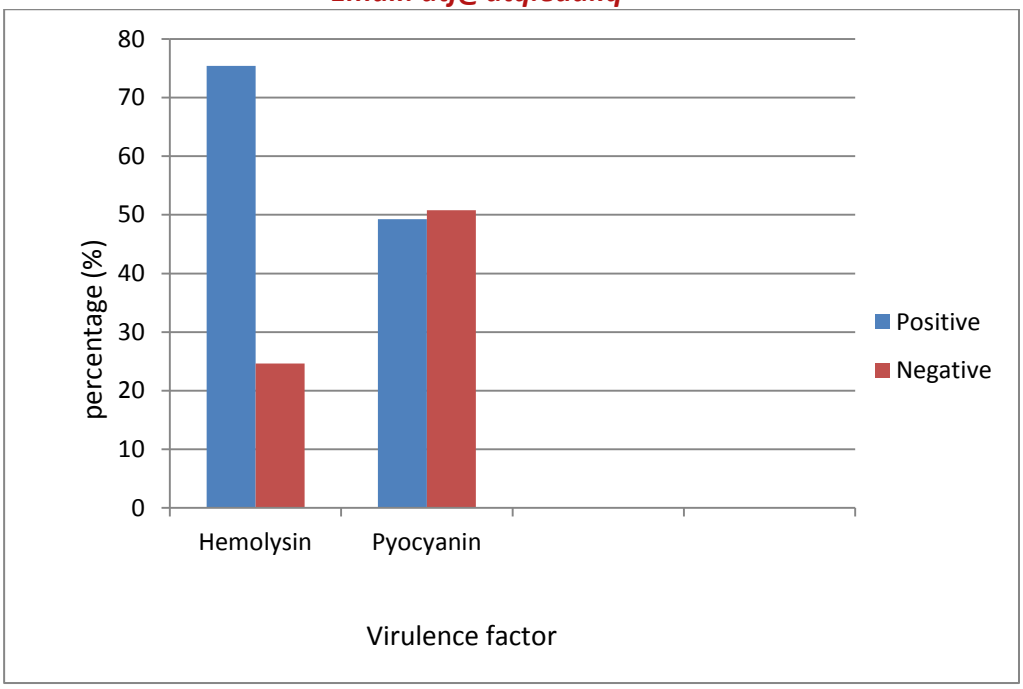

Figure (3): Percentages of virulence factors of *P. aeruginosa* isolates

Antibiotic sensitivity test of *Pseudomonas aeruginosa*

The antibiotic sensitivity results showed a high sensitivity of *P. aeruginosa* isolates to Carbapenems, 63/65 (96.92%) were sensitive to Imipenem. Followed by Meropenem with 61/65 (93.84%). *P. aeruginosa* showed high resistance to Cephalosporins 64/65 (98.46%) and 62/65 (95.38%) isolates were resistant to Ceftazidime and Cefepime, respectively (Figure 4).

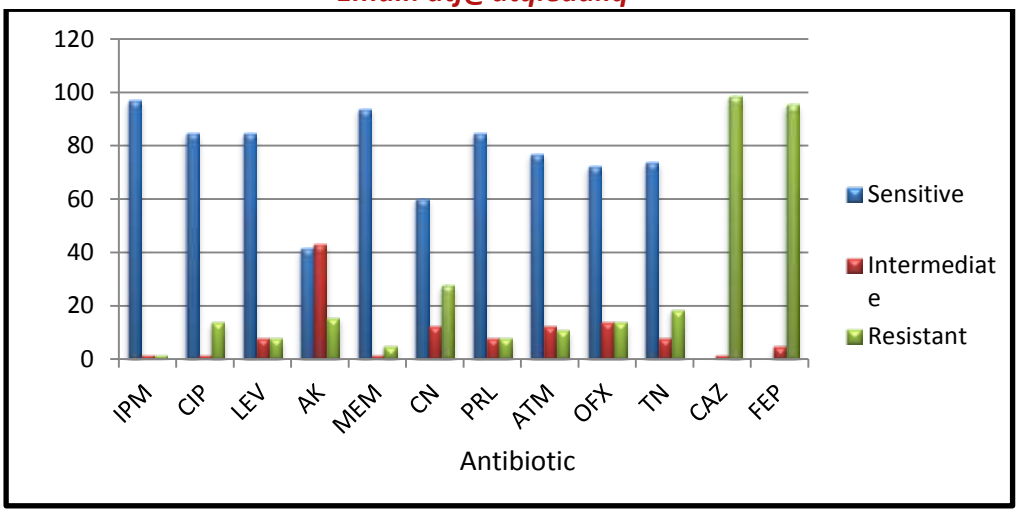

Figure (4): Antibiotic sensitivity of *P. aeruginosa*

Discussion

The results of the present study showed *P. aeruginosa* was the most common cause of Otitis media with the highest ratio of isolation. *P. aeruginosa* considered a ubiquitous microbe which exists in wide ranges of environments, developing mechanisms to antibiotic resistance persistently, ability to compete with other organisms, and widely distributed in the swimming water [21]. The present study results were inconsistent with other studies in Diyala and Baghdad provinces with a percentage of (30.6%, and 33.33%), respectively [22, 23]. Also agree with another previous study in Palestine, that showed the percentage of *P. aeruginosa* was (32.5%) [24]. On the other hand, the present study results conflicts with many former studies in Thi-Qar, and Kufa Cities, where recorded the percentage of *P. aeruginosa* as following (53.14%, 18%), respectively [25, 26]. The variations in the percentages of bacterial type

depend on the geographical regions, the environmental factors, and the health state of people [27].

The present study results revealed the percentage of Otitis media was higher in females compared with males. Higher rates of infections in females may be due to many reasons such as females constitute a higher percentage of the population surveyed in Iraqi society, based on the latest statistical account in Iraq was found that the females comprise 55% of the Iraqi population [28]. Other reasons may include direct contact with the polluted environment, and polluted water, as well as exposed to trauma that affect the ear due to rapid change in the pressure between the middle ear and the surrounding air [29, 30]. The present study results agree with a previous study in Baghdad province where mention the rates of infection in females (25%) compared with males (19%) [31].

The present study results showed the highest rates of Otitis media were located in the third group in the age (21-30) year. The high percentage of Otitis media in this age group, due to this group of the population are more exposed to several factors like environmental factors, practicing swimming in polluted water, smoking and different lifestyles [27]. *P. aeruginosa* is considered from the Contaminants of the water of the river, and using of these water in swimming is an important way of infection with this bacteria especially otitis media [32]. The present data discrepancy with a former study performed in India, where revealed the highest percentage of Otitis media was in the age (11-20) with 31 cases, while in the age (21-30) with 14 cases [33]. Also, a previous study in Korea showed the percentage of infection with *P. aeruginosa* in (21-30) age group was (4.1%) [34].

The current study data showed that the occurrence of Otitis media was the highest in November. In the present study the highest rates of infection in the Autumn season, especially in November due to the nasal allergy that is considered as a risk factor for occurrence otitis media. The role of inflammatory mediators like cytokines and colony stimulatory factors that secreted by mast cells in response to an allergen resulting in mucosal damage and increased secretions. 30-45% of patients with allergic rhinitis also suffering from otitis media. The allergic rhinitis patients have increased secretions in eustachian tubes and middle ear, that may be resulting in the development of otitis media [35]. This result seems to be approximated with a study conducted in Korea was reported the highest percentage of otitis media in Autumn season, the percentage of chronic suppurative Otitis media with *P. aeruginosa* in November was (24.5%) [36].

In the current study, the percentage of patients with Otitis media that caused by *Pseudomonas aeruginosa* was higher in urban than a rural area. Most patients attending the hospital from urban areas and the polluted environment compared with the rural area that considered predisposing factors for Otitis media, an earlier study performed in Al- Hawija City recorded a higher percentage of Otitis media in urban area (71.6%) compared with the rural area [37].

The present study showed a bit high beta hemolysis activity of the targeted bacteria. Hemolysin causes disrupting of tissue and releasing the host nutrients like iron this improves the ability of bacteria to grow and multiply within the host cell [38]. A predate study performed in Babyl

province reported (30%) of *P. aeruginosa* isolates have the ability to hemolysin enzyme production [39].

The present study showed that about half of *P. aeruginosa* isolates were positive to Pyocyanin Production. *P. aeruginosa* has a single polar flagellum is important to mediate chemotactic signaling when occurring changes in the environment as a response process for these changes, flagellum-deficient mutant strains are suffering from an inability to regulate Quorum sensing-signalling molecules that are basic for pyocyanin production [40, 41]. A previous study isolated *P. aeruginosa* from different clinical isolates included otitis media cases showed (78.66%) of isolates have the ability of Pyocyanin Production [42].

Antibiogram results for the studied isolates showed high sensitivity to Imipenem and Meropenem (Carbapenems). The penems (Meropenem and Imipenem) antibiotic structure differs a little of the penicillins structure. These antibiotics possess a wider spectrum of activity against gram-positive and gram-negative bacteria. Due to they are more reluctant to beta-lactamase hydrolysis [43]. The present study agrees with a previous study in Najaf province found the percentage of Imipenem sensitivity was (100%) [44].

The current study result reported high sensitivity of Ciprofloxacin, Levofloxacin, and Ofloxacin (Fluoroquinolones). Fluoroquinolones group have a broad spectrum of activity for microbes and active against *P. aeruginosa*. This group targets the bacterial topoisomerase II, and DNA gyrase and consequent inhibition the DNA replication. The resistance to fluoroquinolones is developed as a reflection of a mutation that resulting

from the selective pressure of the random usage of these antibiotics [45]. The present study results approximated with an earlier study in Basrah City showed the percentage of Ciprofloxacin sensitivity (75%) [46]. A quondam study was performed in Diala province found all isolates were sensitive to Ofloxacin [22].

The sensitivity results of Amikacin, Gentamicin, and Tobramycin (Aminoglycosides) in the present study showed a low percentage by comparison with other studies. The current study results disagree with a previous study in Baqubah City that showed the percentages of Gentamicin and Amikacin sensitivity were (81%, and 85%), respectively [47]. A former study carried out in Baghdad province revealed Tobramycin active against (67.7%) of isolates [48]. In the present study, Amikacin sensitivity percentage disagrees with most previous studies,that revealed high sensitivity to Amikacin. Whereas with regard to our study revealed (43.07%) were intermediate isolates, this means most isolates tend to become more resistant.

The present study showed the majority of isolates were sensitive to Piperacillin (which belongs to Penicillins) and Aztreonam (Monobactams). Aztreonam is a monobactam antibiotic, it has great activity against gram negative, and also active against β-lactamase producing bacteria [49]. This result came differently from a predate study in Najaf province that found from total 40 isolates only (19 and 14) isolates were sensitive to Piperacillin and Aztreonam, respectively [44].

The current study results showed high resistance to Cephalosporine generations. The development of resistance to Cephalosporin generations

is considered a warning sign to stop the random antibiotic using. Because these groups are considered a potent anti-Psuedomals [50, 51]. A previous study performed in Baghdad province found the sensitivity percentages of Ceftazidime and Cefipime (81.8%, and 0.0%), respectively [48].

Conclusion

Pseudomonas aeruginosa seems to be the predominant pathogen in Otitis media patients. Patient's gender may consider as a risk factor in Otitis media infections. Younger ages were the most infected with Otitis media by *P. aeruginosa*. The majority of isolates were resistant to Cephalosporins.

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تواتر ونمط الحساسية للزائفة الزنجارية بين مرضى التهاب الاذن الوسطى في مدينة الناصرية

المستخلص

يعد التهاب الأذن الوسطى أحد أكثر أنواع التهابات الأذن شيوعا. بالاضافة الى ان المسببات الاكثر هي بكتيرية. الزائفة الزنجارية هي أكثر الممرضات المشخصة التي تسبب هذه العدوى. تمتلك الزائفة الزنجارية عوامل ضراوة تودي الى تلف الغشاء المخاطي المبطن للاذن الوسطى. تم جمع مئتان وعشرة عينة من المرضى الذين راجعوا قسم الانف والاذن والحنجرة في مستشفى الحبوبي التعليمي في مدينة الناصرية جنوب العراق. خلال الفترة من شهر أب 2018 الى شهر كانون الثاني 2019. شكلت الزائفة الزنجارية 65 عينة بنسبة (30.95%) . وكانت معظم العزلات حساسة لصنف الكاربابينيم. وخلصت الدراسة الحالية الى ان الاناث يشكلن نسبة عالية من الاصابة بالتهاب الاذن الوسطى (P-value= 0.01). وان الشباب هم الاكثر تعرضا لخطر الاصابة (P-value = 0.36). تهدف الدراسة الحالية الى تحديد العلاقة بين نسبة التهاب الاذن الوسطى وبعض العوامل المرتبطة بالمرضى مثل الجنس والعمر ومنطقة السكن وفترة الدراسة وكذلك تحديد بعض عوامل الضراوة للبكتريا المستهدفة.

الكلمات المفتاحية: التهاب الاذن الوسطى, الزائفة الزنجارية, أختبار الحساسية ,الناصرية.