

Isolating and Identification Some Types of Bacteria that Cause Otitis Media and Detecting their Resistance Towards Antibiotics

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Abstract

One hundred ear swabs were collected from patients attending Hawija General Hospital, Azadi Teaching Hospital, and outpatient clinics in Kirkuk Governorate, for ages ranging between 2-45 years, During the period from October 27, 2022 to January 3,2023. The results showed that 91(91%)of samples have positive growth on MacConkey, mannitol and blood agar. While the remaining samples 9 (9%) had no growth. The samples that showed positive bacterial growth were identified as 139 isolates. The number of Gram positive isolates was 91(65.5%), while the number of negative isolates was 48 34.5%. The isolates were identified by routine biochemical tests, and the diagnosis was confirmed by Vitek2 compact system device. The percentage of isolated bacterial species were Staphylococcus aureus 34.53%, Staphylococcus epidermidis 22.30%, Proteus mirabilis 8.63%, Morganella morganii 6.47%, Enterobacter sakazakii 6.47%, Staphylococcus saprophyticus 4.32%, E.coli 3.60%, Klebsiella pneumonia 3.60% and each of Kocuria kristinae, Proteus vulgaris, Comamonas testosterone 2.88%, and Staphylococcus lentus 1.44%. The results of the sensitivity test to ten types of antibiotics, namely Amikacin, Ceftazidime, Nitrofurantion, Imipenem, Azithromycin, Tetracycline, Cefotaxime, Tobramycin, Ofloxacin, Oxacillin, showed that all isolates were 100% resistant to the antibiotics Cetazidime and Cefotaxime, while they showed varying resistance to the rest of the antibiotics. Antibiotics: All isolates showed 100% sensitivity to Antiimipenem.

1. Introduction:

Otitis media is an infection of the middle ear cavity Caused by bacteria, virus or fungi [1]. Bacterial infection is a term for bacteria that cause diseases when they enter the body or when they are not in their normal places. Like the skin disrupt, for example, causing multiple infections until it reaches the bloodstream, causing a state of "Septicemia" that leads to death. The most common species of bacteria that cause middle ear infections are (Staphylococcus spp., pseu-

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domonas aeruginosa, Proteus spp, Streptococcus spp, Klebsiella pneumonia, and Escherichia coli) [2]. The connection of the middle ear to the upper respiratory system led to its contamination with pathogens with this system such as bacteria, viruses, and others, expose to the external environment through the tympanic membrane also led to its exposure to many opportunistic pathogens that cause middle ear infection [3]. Middle ear infection occurs in children and adults, but its incidence in children is more than in adults [4]. Some studies indicated that more than 80% of children suffer from this type of inflammation in the United States [5]. Early detection of this disease is very important despite the absence of a reliable diagnostic test in the early stages of this disease. Inaccurate diagnosis leads to delayed treatment, misuse of treatment, or excessive intake, which increases the risk of complications

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and increases resistance to Antibiotics [2].

The sensitivity of all isolates was tested against ten types of antibiotics, including Amikacin, Ceftazidime, Nitrofurantion, Imipenem, Azithromycin, Tetracycline, Cefotaxime, Tobramycin, Ofloxacin, Oxacillin, The results of the susceptibility test showed that all of the studied isolates, gram positive and gram negative, were 100% resistant to the antibiotics Ceftazidim and Cefotaxime used in the study, while they showed variable resistance to the rest of the types of antibiotics. All isolates also showed absolute sensitivity to the antibiotic Imipenem by 100% for all isolates.

This study was aimed to: Isolation and identification of some of bacterial species that cause otitis media in Kirkuk governorate and determined its sensitivity to different types of antibiotics to recommend avoiding the use of antibiotics to which the isolates under study show multiple resistance.

2. Methods and Materials:

2.1 Sample Collection:

The study Included 100 samples collected from people attending the ear and nose consultation unit Section Throat Nose Ear (ENT) at Hawija General Hospital and Azadi Hospital Educational and outpatient clinics in Kirkuk Governorate for ages ranging from 2-45 years and for the period from October 27, 2022 to January 3, 2023, these samples were taken by rotating the sterile cotton swab inside the middle ear and placing in the middle ear and then placed in the carrier medium transport media until transported to the laboratory.

2.2 Isolation and Identification:

Swabs cultured on Blood Agar, MacConkey agar and Mannitol salt agar, and incubated aerobically at 37°C for 18-24 hours to observe the growth of bacteria, shape, size, and the changing in the colour of the medium, etc. A primary diagnosis based on phenotypic traits [6].

3. Diagnosis:

3.1 Microscopic Examination:

Microscopic examination of bacteria was performed by preparing a thin smear of the colony using a sterile loop, placing it on the glass slide containing a drop of sterile distilled water, then leave it for five minutes at room temperature to dry, and then heat fixed by passing the glass slide over the flame rapidly several times, then staining with Gram stain, drying it, and examining it with an optical microscope under power (100 X) using an oil lens to observe the shape of bacterial cells, their aggregation, and their interaction with the dye [7].

3.2 Biochemical Tests:

Biochemical tests for gram positive and gram negative don according to methods described by [8],[9], [10], [11].

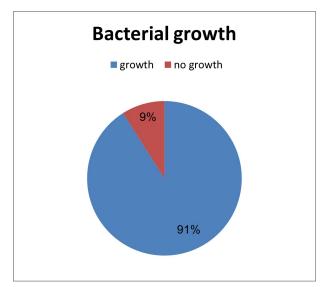


Figure 1. Number and percentages of samples isolated from patients with otitis media and their percentage.

3.3 Antibiotics Susceptibility:

The sensitivity of bacterial isolates to antibiotics was tested using Kirby-Baure method, The sensitivity of all isolates was tested towards ten types of antibiotics: Amikacin $10\mu g$, Ceftazidime $30 \mu g$, Nitrofurantion $100\mu g$, Imipenem $10\mu g$, Azithromycin $15\mu g$, Tetracycline $10\mu g$, Cefotaxime $10\mu g$, Tobramycin $10\mu g$, Ofloxacin $5\mu g$, Oxacillin $5\mu g$, according to [12].

4. Results and Discussion:

4.1 Isolation:

The results showed that 91(91%) of samples have bacterial growth on Blood agar, Mannitol salt agar, and MacConkey agar. These results were close to that reported by previous studies and for each of the researchers,[13], [14] which was 95.5-93% respectively, While the remaining samples 9(9%) had no growth, as shown in Figure 1, the reason for the lack of growth may be attributed to the appearance of bacterial growth until the pathogen is not bacterial. It may be viral, fungal, or types of anaerobic bacteria or Mycoplasmas that can be isolated by usual culture methods, or the reason may be due to doses of antibiotics taken by patients, which have been proven information about it in the form for injured people [15].

4.2 Identification:

Bacterial isolates were initially identified based on their phenotypic characteristics on different culture media, Gram staining was also used to observe the response of the cells to the dye, their shapes, sizes, and method of aggregation. The Gram-negative bacterial species appeared as red to pinkcolored, thin-shaped bacilli under a light microscope and the initial diagnosis is based on the shape, color, size and texture of the colonies, as well as the smell resulting from their growth, Staphylococcus species appeared as spherical shapes cluster, and in purple color, on mannitol salt agar medium. It differentiates from *Staph aureus* bacteria that appeared its colonies are large and golden in color due to their ability to ferment the mannitol sugar and the color of the medium changed from pink to yellow. Due to the presence of neutral red evidence, which distinguishes it from Coagulase negative Staplylococci, pink in color, small in shape, not fermenting mannitol, and from other species that are not fermenting mannitol sugar, such as Staph. epidermidis and Staph. Saprophyticus [16].

The results of current study agreed with what was reported by [17], who explained that these bacteria have the ability to ferment mannitol sugar on mannitol salt medium. As for the type *K. kristinae*, it appeared under the microscope in the form of small and medium-sized cells, and some of these isolates were arranged in a spherical shape. These colonies appeared as white, with a colonies are white, whitish or yellowish color, and some are small, and some are large, smooth, round and raised on the nutrient agar medium.

4.3 Biochemical Tests:

Biochemical tests were performed to ascertain the genus of bacteria and based on what was stated by [8], [9], [10] and [11] as shown in Table 1 and Table 2. The diagnosis was confirmed using the VITEK2 compact system device.

4.3.1 Biochemical Tests for Gram- Positive Bacteria:

Staph aureus colonies appeared yellow in color because they fermented mannitol sugar, while other types remained that did not ferment it, and it were characterized as being positive for the coagulase test, while other types were negative.

Staph lentus was characterized as being positive for the oxidase test, while the other species were negative for this test. All positive species were characterized as positive for the catalase test.

4.3.2 Biochemical Tests for Gram-Negative Bacteria:

The results of the current study showed that all Gramnegative bacteria gave a negative result for oxidase test, except for *Comamonas testosterone* was positive for oxidase test, and showed that both *E. coli*, *P. mirabilis* was a positive for catalase test, while the species *Klebsiella pneumoniae*, *Proteus vulgaris*, *Comamonas testosterone*, *E. sakazakii*, and *Morganella morganii* were all positive for catalase test. And the results showed that *E. coli*, *Morganella morganii* and *Proteus vulgaris* was a positive for indole test, while the species the *Klebsiella pneumoniae*, *Proteus mirabilis*, *Comamonas testosterone* and *E. sakazakii* were all negative for Indole test, as shown in Figure 2.

This study showed that *E. coli*, *P. mirabilis*, *P. vulgaris* and *Morganella morganim* gave a positive result, while the species the *Klebsiella pneumoniae*, *Comamonas testosterone*

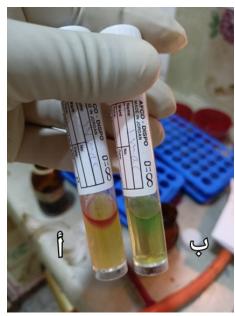


Figure 2. A: *E. coli* bacteria that are positive for the indole test and form a pink ring.

B: *P. mirabilis* bacteria that are negative for the indole test and have a yellow or yellow green ring.

and *E. sakazakii* were all negative for methyl red test, as seen in Figure 3.

The results also showed that *P. mirabilis, Klebsiella pneumoniae and E. sakazakii* was a positive for Voges–Proskauer test, while *E. coli, Morganella morganimm Comamonas* testosterone and *P. vulgaris* gave a negative result. And all the species *P. mirabilis, P. vulgaris, Klebsiella pneumoniae* and *E. sakazakii* appeared positive for Citrate utilization test, while *E. coli, C. testosterone* and *Morganella morganimm* was negative for citrate test. The results of the current study showed that all Gram-negative bacteria have ability to move, except *Klebsiella pneumoniae* and *Morganella morganimm* bacteria was negative for motility test, As shown in Table 2.

4.4 Identification by Vitek-2 Compact System:

The diagnosis of five bacterial isolates was confirmed using the Vitek-2 compact system, two samples for Staph. aureus bacteria and two samples for *Staph. lentus* bacteria and one sample of the *K. kristinae* bacteria. The purpose of this test is to diagnose the bacteria and to confirm the phenotypic and biochemical tests, as shown on the diagnosis form for the Vitec device for each sample.

4.5 Percentage Distribution of Bacterial Species Causing Otitis Media:

Several studies studies confirm that the most common bacteria isolated from festering middle ear infections are: *E. coli, Staph. aureus, Proteus spp., Ps. aeruginosa, Klebsiella spp.*, The types of isolated bacteria may vary depending on

Table 1. Shows the results of bacterial test of Gram-positive bacteria which indicate to the species of the identified bacteria.

Tests	TSI			Oxidase	Catalase	Coagulase	Mannitol fermentation	Novobiocin antibiotic
Bacterial species		Gas	H2S					
Staph. aureus	K/ A	+	-	-	+	+	+	S
Staph. lentus	K/ A	-	-	+	+	-	-	S
Staph. epidermidis	K/ A	-	-	-	+	-	-	S
Staph. saprophyticus	A A	-	-	-	+	-	-	R
Kocuria kristinae	A A	-	-	-	+	-		

Table 2. SShows the results of bacterial test of Gram- negative bacteria which indicate to the species of the identified bacteria.

Tests	Gram stain	Oxidase	Catalase	Indol test	Methyl red test	Motility	TSI / H2S	Citrate utilization test	Voges- Proskauer test
Bacterial species									
Escherichia coli	-	-	+	+	+	+	A A-	-	-
Proteus mirabilis	-	-	+	-	+	+	A A+	+	+
Morganella morganii	-	-	+	+	+	-	K A-	-	-
Enterobacter sakazakii	-	-	+	-	-	+	A A-	+	+
Klebsiella pneumonia	-	-	+	-	-	-	A A-	+	+
Proteus vulgaris	-	-	+	+	+	+	K A+	+	-
Comamonas testosterone	-	+	+	-	-	+	K K-	-	-

the geographical region, season, age, and number of samples isolated.

4.6 Testing the Sensitivity of Gram-negative and Positive Bacteria to Antibiotics:

A test was conducted for the sensitivity of 139 Grampositive and Gram-negative bacteria isolates to 10 different types of antibiotics, by measuring the diameter of the inhibition zone and comparing it with standard diameters. These antibiotics are: AK, CAZ, F, IMP, AZM, TE, CTX, TOB, OFX, OX, as shown in Table 4.

The results of the current study showed that Staph. aureus bacteria was sensitive to the antibiotics: Imipenem 100%, Ofloxacin 54.17%, Tobramycin 54.17%, and resistant to the antibiotics Cefotaxime 100%, Ceftazidime 100%,

Amikacin 77.08%, Oxacillin 77.08%, Azithromycin 68.7%, Tetracycline 60.42%, Nitrofurantion 60.42% [18]. As indicated in Table 4 and Figure 4, these results were close to the study conducted by [19] in which there results appeared that *Staph. aureus* was 82.9% resistant to ceftazidime and 89.5% sensitive to imipenem, as in Figure 4.

The results of the current study showed that *E. coli* bacteria were 100% sensitive to Imipenem, and resistant to Amikacin 100%, Nitrofurantion 100%, Cefotaxime 100%, Oxacillin 100%, Tetracycline 100%, Ceftazidime 100%, Tobramycin 100%, Azithromycin 80%, Ofloxacin 60%. These results are completely consistent with a study conducted in Bangladesh by [20], who showed that *E. coli* bacteria are 100% sensitive to the antibiotic Imipenem. As for the antibiotic Ceftazidime, the current results differed from the results



Figure 3. A: *P. mirabilis* was positive for methyl red test by appearance of red color.

B: *K. pneumonia* was negative for the methyl red test it didn't changing the color of the MR_*VPmedium*.

of the study of [21], as the resistance rate of these bacteria to it was 60%. The current results converged with the results of the study conducted by [22], where the rate of resistance of *E. coli* bacteria to Tobramycin was 89.6%. The results of this study differed from the study conducted by [23], in which the resistance of *E. coli* bacteria to Tobramycin was recorded at a rate of 75%, as in Figure 5.

And showed results for Staph. epidermidis bacteria was 96.77% sensitive to Imipenem, and resistant to Cefotaxime 100%, Ceftazidime 100%, Tobramycin 93.5%, Amikacin 93.5%, Tetracycline 93.5%, Nitrofurantion 71%, Oxacillin 71%, Ofloxacin 67.7%, Azithromycin 58%, these results matched with the results of the study of [24]] regarding the antibiotic Imipenem in that it was 100% sensitive to it, and it differed with the same study regarding the antibiotic Ceftazidime in that it was 60% sensitive to it. These results were consistent with a study conducted by [22], which showed that the resistance rate of Staph. epidermidis isolates for Tobramycin antibiotic is 100%, and Staph. saprophyticus was sensitive to Imipenem 96.77%, and resistant to Cefotaxime 100%, Ceftazidime 100%, Tobramycin 93.5%, Amikacin 93.5%, Tetracycline 93.5%, Nitrofurantion 71%, Oxacillin 71%, Ofloxacin 67.7%, Azithromycin 58%, this results were close for the antibiotic Azithromycin, with the results of [25], they recorded a resistance rate of 50%, while the results for the antibiotics Oxacillin, Tobramycin, and Ofloxacin differed with the results of the same researchers, who stated that they were resistant to the first by 25% and sensitive to the second and third by 100%, this may be the reason for resistance to antibiotics by Gram-positive bacteria such as Staph. aureus and Staph. epidermidis is produced by B-lactamase enzymes, or the cause may be due to the occurrence of a mutation [26], or it may be Sometimes the reason is due to the type of antibi-

Table 3. Represents the percentages of the types of bacteria isolated under study from otitis media.

Bacterial species	Isolate numbers	percentage
Staph. aureus	48	34.53%
Staph. epidermidis	31	22.30%
Proteus mirabilis	12	8.63%
Morganella morganii	9	6.47%
Enterobacter sakazakii	9	6.47%
Staph. saprophyticus	6	4.32%
E.coli	5	3.60%
Klebsiella pneumonia	5	3.60%
Kocuria kristinae	4	2.88%
Proteus vulgaris	4	2.88%
Comamonas testosterone	4	2.88%
Staph. lentus	2	1.44%
Total	139	100%

otic used or the use of small doses with low concentrations for the purpose of treating bacterial infections, as it prevents the growth of bacteria sensitive to these concentrations, but leaves a small number of them to grow and become more resistant [27].

Resistance to the antibiotics Oxacillin and Ofloxacin arises as a result of a change in the affinity of the antigen with the target, either as a result of mutations or the cause may be the efflux pump or the acquisition of genes that confer resistance [28].

While the results differed with the study of the researcher [29], he stated that the rate of resistance to the antibiotic Cefotaxime was 76% - 26.7%, respectively. The results showed that P. mirabilis bacteria were sensitive to the antibiotics Imipenem 100% and Ofloxacin 58.33%, and resistant to the antibiotics: Cefotaxime 100%, Ceftazidime 100%, Tobramycin 100%, Oxacillin 100%, Tetracycline 100%, Azithromycin 91.67%, Amikacin 91.67%, Nitrofurantion 91.67%. Our results agreed with the study of researchers [30] as they showed that the sensitivity of P. mirabilis bacteria to the antibiotic Imipenem was 97.2%, and the results differed with the study Researchers [31], [32] reported that the resistance rate of isolates of these bacteria to Imipenem was 16.2% -15%, respectively. The reason for the resistance of some bacterial genera and species to many antibiotics, especially the species of the Enterobacter family of Gram-negative bacteria, K. pneumonia, P. mirabilis, and E. coli, is due to several reasons, including the production of enzymes that destroy the beta-lactam ring present in the composition of antibiotics belonging to the group Beta-lactams [33], or their possession of a capsule, or their possession of efflux pumps, or their formation of a biofilm substance that surrounds colonies of bacterial cells, providing them with a type of protection that greatly

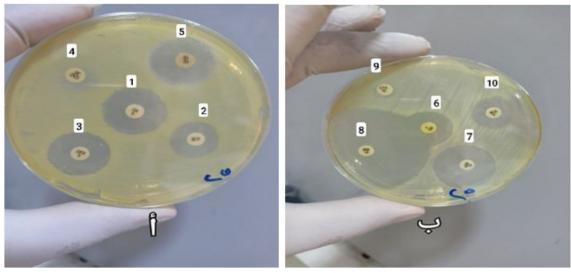


Figure 4. Susceptibility of *S. aureus* to antibiotics OX(1) AK(2) OFX(3) CAZ(4) TE(5) F(6) AZM(7) IPM(8) CTX(9) TOB(10)

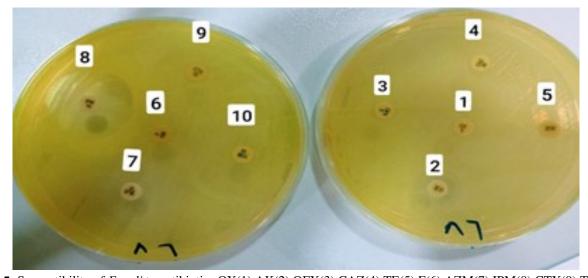


Figure 5. Susceptibility of *E. coli* to antibiotics OX(1) AK(2) OFX(3) CAZ(4) TE(5) F(6) AZM(7) IPM(8) CTX(9) TOB(10)

Bacterial species	Total No.	P	λK	F		OFX		IPM		OX		TE		AZM		CAZ		CTX		ТОВ	
No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No	%		
Staph. aureus	48	37	77,1	29	60,4	22	45,5	0	0	37	77,1	29	60,4	33	68,7	48	100	48	100	22	45,5
Staph. epidermidis	31	29	93,5	22	71	21	67,7	1	3,23	22	71	29	93,5	18	58	31	100	31	100	29	93,5
P. mirabilis	12	11	91,6	11	91.6	5	41.6	0	0	12	100	12	100	11	91.6	12	100	12	100	12	100
M. morganii	9	9	100	9	100	7	77.8	0	0	9	100	9	100	8	88.8	9	100	9	100	9	100
E. sakazakii	9	9	100	9	100	8	88.8	0	0	9	100	9	100	7	77.7	9	100	9	100	9	100
Staph. saprophyticus	6	3	50	4	66.7	3	50	0	0	4	66.7	4	66.7	5	83.4	6	100	6	100	4	66.7
E. coli	5	5	100	5	100	3	60	0	0	5	100	5	100	4	80	5	100	5	100	5	100
K. pneumonia	5	5	100	5	100	3	50	0	0	5	100	5	100	5	100	5	100	5	100	5	100
K. kristinae	4	4	100	4	100	4	100	0	0	4	100	4	100	4	100	4	100	4	100	4	100
P. vulgaris	4	4	100	4	100	3	75	0	0	4	100	4	100	3	75	4	100	4	100	4	100
C. testosteroni	4	4	100	4	100	3	75	0	0	4	100	4	100	3	75	4	100	4	100	4	100
Staph. lentus	2	0	0	2	100	0	0	0	0	2	100	0	0	0	0	2	100	2	100	0	0

Table 4. Represents the percentages of the types of bacteria isolated under study from otitis media.

hinders and prevents antibiotics from reaching the bacterial cell [34], and that the excessive use of antibiotics has led to an increasing rate of the development of resistance to them and its spread throughout the world, and that when bacteria coexisting in the human and animal bodies are exposed to antibiotics, they will also develop resistance to these antibiotics, and worse than that, bacteria that are resistant to antibiotics It can spread to other people and the environment[35], [36].

5. Conclusions:

It is concluded from the study as follows:

- 1- The infection of the middle ear is more common in male than female. It is estimated that the number of infected males in our study is 51 isolation in the rate of 51%, while, in females is 49 isolation in the rate of 49%.
- 2- Kinds of bacteria gram-positive to stained fumarate of greater isolation in the inflammation of the middle ear in account of 91 isolation in the rate of 65.5%, while, another Kinds of gram-negative to stained is about 48 isolations at rate 34.5%.
- 3- It is estimated that isolations of bacteria staph. aureus 48 isolation in the rate 34.53%, it was the greater rate in the involved positive Kinds, while, the greater rate of bacteria Kind gram-negative to Stained isolation, it was bacteria proteus mirabilis in the number of isolation is 12 in the rate 8.63%.
- 4- All isolations in the field of study show the absolute Sensitivity to anti-biotic (imipenem), while, some isolations from staph. aureus and proteus mirabilis show various sensitivity to anti-biotics ofloxacin and tobramycin. Thus, all isolations are distinguished by absolute resistance to anti-biotic ceftazidime and cefotaxime.

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Data Availability Statement: All of the data supporting the

findings of the presented study are available from corresponding author on request.

Declarations:

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: The manuscript has not been published or submitted to another journal, nor is it under review.

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عزل وتشخيص بعض انواع البكتيريا المسببة لالتهاب الاذن الوسطى والكشف عن مقاومتها تجاه المضادات الحيوية

سهيل نجم عبدالله الجبوري 1* ، شيماء ناجي دحام 2 قسم علوم الحياة ، كلية العلوم ، جامعة تكريت ، تكريت ، العراق 2 قسم الكيمياء ، كلية العلوم ، جامعة تكريت ، تكريت ، العراق 2

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الخلاصة

تضمنت الدراسة جمع 100 عينة من المرضى المراجعين الى مستشفى الحويجة العام ومستشفى آزادي التعليمي والعيادات الخارجية وتضمنت الدراسة جمع 100 عينة من المرضى المراجعين الى مستشفى الحويجة العام ومستشفى آزادي التلاثة بيناير 2023 ، اظهرت نتائج العزل أن الاولي الثلاثة بينيا 90 من العينات أعطت نمواً ايجابياً على اوساط العزل الاولي الثلاثة المحتوباً موجباً شخصت الى 139 عزلة العينات التي اعطت نمواً بكتيرياً. العينات التي اعطت نمواً بكتيرياً موجباً شخصت الى 139 عزلة بنسبة 34.5% ، شخصت الى 139 العزلات الموجبة لصبغة كرام هي 91 عزلة بنسبة \$6.5% اما السالبة فكان عددها 48 عزلة بنسبة 34.5% ، شخصت العزلات باستعمال الاختبارات البايوكيميائية الروتينية وتم تأكيد التشخيص بواسطة جهاز \$150 بنسبة \$140 بنسبة \$22.30% ، نشخصت كليولات باستعمال الاختبارات البايوكيميائية الروتينية وتم تأكيد التشخيص بواسطة بهاز \$140 بنسبة \$14

الكلمات الدالة : عدوى الأذن الوسطى؛ البكتيريا المسببة للأمراض؛ النضادّات الحيوية.

التمويل: لايوجد.

بيان توفر البيانات: جميع البيانات الداعمة لنتائج الدراسة المقدمة يمكن طلبها من المؤلف المسؤول. اقرارات:

تضارب المصالح: يقر المؤلفون أنه ليس لديهم تضارب في المصالح.

الموافقة الأخلاقية: لم يتم نشر المخطوطة أو تقديمها لمجلة أخرى، كما أنها ليست قيد المراجعة.