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Isolation and identification of bacteria and fungi from patients with otitis media in the city of Hawija and studying the sensitivity of antibiotics to them

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Abstract :

The study included isolation and identification of bacteria and fungi that cause otitis media, and they were diagnosed by routine laboratory methods. The diagnosis of bacteria and yeasts was confirmed using the Vitek 2 compact system.

Atotal of 216 samples of Ear, Nose Throat (ENT) collected from patients suffering from Otitis media in Hawija General Hospital for the period from September 2022 to March 2023.Their ages were 1-70 years .

The results of culture on the media, Blood agar, MacConkey agar, Sabouraud dextrose agar, showed that there was a bacteria and fungi infection with otitis media in 177samples, with a rate of 82%, and that the highest percentage of bacterial infection was in the age group 1-20 years, with a number of 85 sample with a rate of 50.61%. As for fungal infections, the highest infection rate was in the age group 21-40 years with 63 samples and a rate of 55.25%. The study showed that males are more with bacteria than females with a rate of 53.57%, and females are more infected than males in the case of a fungal, with a rate of 60.52%.

The isolation results showed that 63 samples with a rate of 35.6% of the samples positive for culture showed a single bacterial growth 6 samples with a rate of 3.4%showed a single fungal growth and 3 samples with a rate of 1.7% showed a growth of *Candida spp*. Only 27 samples with a rate of 15.3% showed mixed growth of bacteria and fungi, and 78 samples with a rate of 44% showed mixed growth of *bacteria and candida*.

The study showed that Gram-negative bacteria predominated 62% out of 114 samples, while the Gram-positive bacteria percentage was 32% out of 54 samples. As for fungi 81samples were belong to *Candida spp*.in rate of 37.5% .

Most of the infections of otitis media due to bacteria occurred in the young age group within the range 1-20 years, with a rate of 50.61%. As for fungi, most of them were in the age group 21-40 years, at a rate of 55.25%.

The sensitivity of the bacteria was tested for 10 types of commonly used antibiotics, as it was found that the bacteria showed high resistance to Amoxicillin and Amoxicillin/Clavulanic acid, while the antibiotics Ciprofloxacin and Levofloxacin showed high effectiveness against most bacterial isolates

The sensitivity of the fungi to 6 types of commonly used antibiotics was tested The fungal isolates showed high resistance to Amphotericin-B, while the antibiotics Nystatin and Ketoconazole showed high efficacy against most of the fungal isolates.

Keywords: Bacteria, Fungi, Middle ear. Hawija.

عزل وتشخيص البكتريا والفطريات من المرضى المصابين بالتهاب الاذن الوسطى في مدينة الحويجة ودراسة حساسية المضادات الحيوية عليها

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مستخلص

تضمنت الدراسة عزل وتشخيص البكتريا والفطريات المسببة لالتهاب الاذن الوسطى Otitis Media وشخصت بالطرق المختبرية الروتينية ، وتم تأكيد تشخيص البكتريا والخمائر باستخدام جهاز Vitek 2 compact system .

تم جمع 216 عينة من الأشخاص المصابين بالتهاب الأذن الوسطى والذين تتراوح أعمارهم بين 70-1 سنة من المراجعين لشعبة الانف والاذن والحنجرة Ear Nose Throat (ENT) في مستشفى الحويجة العام للفترة من ايلول 2022 الى أذار2023 .

اظهرت نتائج الزرع على الاوساط Blood agar، MacConkey agar، Sabouraud dextrose agar وجود اصابة جرثومية بالتهاب الاذن الوسطى بلغت 177عينة وبنسبة ٪82، وان اعلى نسبة اصابة بكتيرية كانت في الفئة العمرية 20-1 سنة بعدد 85 عينة وبنسبة ٪50.61، اما بالنسبة للإصابات الفطرية فقد كانت اعلى نسبة اصابة في الفئة العمرية 40-21 سنة بعدد 63 عينة وبنسبة ٪55.25، وبينت الدراسة بأن الذكور أكثر إصابة من الإناث بنسبة ٪53.57 ، والاناث اكثر اصابة من الذكور في حال المسبب فطري بنسبة ٪60.52.

أظهرت نتائج العزل ان 63 عينة بنسبة 35.6 ٪ من العينات الموجبة للزرع ظهر فيها نمواً بكتيرياً منفرداً و 6 عينات بنسبة 3.4 ٪ ظهر فيها نموا فطرياً منفرداً و3 عينات بنسبة 1.7 ٪ ظهر فيها نمواً لأنواع المبيضات Candida spp. فقط و 27 عينة بنسبة 15.3 ٪، ظهر فيها نمواً مختلطاً بين البكتريا والفطريات و 78 عينة بنسبة 44 ٪، ظهر فيها نمواً مختلطاً بين البكتريا والمبيضات .

بينت الدراسة سيادة البكتيريا السالبة لصبغة كرام اذ بلغت نسبتها ٪62 من 114 عينة بينما بلغت نسبة البكتريا الموجبة لصبغة كرام ٪32 من 54 عينة أما بالنسبة للفطريات فقد بينت الدراسة سيادة خمائر الـ Candida spp. بعدد 81 عينة وبنسبة ٪37.5 .

حيث حصلت اغلب إصابات التهاب الأذن الوسطى بسبب البكتريا في الفئة العمرية الصغيرة ضمن المدى 1- 20 سنة بنسبة ٪50.61. أما بسبب الفطريات فكانت اغلبها في الفئة العمرية 40-21 سنة بنسبة ٪55.25 .

اختبرت حساسية البكتريا لـ 10 انواع من المضادات الحيوية شائعة الاستعمال إذ وجد ان البكتريا اظهرت مقاومة عالية لمضادا Amoxicillin و Amoxicillin+Clavulanic acid في حين أظهر المضادان Ciprofloxacin و Livofloxacin فعالية عالية ضد معظم العزلات البكتيرية.

واختبرت حساسية الفطريات لـ 6 انواع من المضادات الحيوية شائعة الاستعمال إذ وجد ان الفطريات المعزولة اظهرت مقاومة عالية لمضاد Ampho-tericin-B في حين أظهر المضادان Nystatin و Ketoconazole فعالية عالية ضد معظم العزلات االفطرية .

الكلمات الدالة : البكتريا ، الفطريات ، الاذن الوسطى . الحويجة .

Introduction

Ear infection (otitis media) is a major public health issue in developing countries, with considerable infection rates and financial costs to patients, communities, and healthcare facilities. Even though it can affect adults, it is a commonly encountered ailment in children, resulting in numerous postoperative appointments in both modern and developing economies (Hailegiyorgis *et al*., 2018)

Otitis media of all kinds is a widespread disease that affects large numbers of people in different parts of the world, causing an important health problem, especially in early childhood, as it was found that 60-80% of children have frequent otitis media during the first years of life (Kalcioglu *et al*., 2006). It also affects the lining of the mucous membrane of the middle ear and its groove (Mufath *et al*., 2015). The World Health Organization (WHO) estimated in 2015 that more than 5% of the world's population, 328 million adults and 32 million children, suffer from hearing loss as a result of infection. (Elmanama *et al*., 2014). Infection occurs through the entry of the causative agent (bacteria, fungi or viruses) into the space of the ear directly or after the infection of the respiratory system through the nose or larynx through the Eustachian canal (Oguntibeju, 2003). There are many factors that play an important role in the occurrence of infection, such as age, gender, race, seasonal changes, physiological factors, as well as the economic and social level.

The disease exists in three phases, the first stage is Acute Otitis media it is an inflammation of the periosteal mucous membranes lining the middle ear cavity. It may be accompanied by purulent fluids as a result of attacking different types of microorganisms. It is a common disease in childhood and one of the important causes of hearing loss, which is a dangerous problem for infants and children, especially in the first two years of the child's life (Al-Obaidi, 2006). The second stage is Chronic otitis media is a chronic inflammation of the middle ear groove, characterized by intermittent or continuous fluid flow through the perforation of the tympanic membrane, which may lead to hearing loss (Hellier, 2018). And the third phase, Otitis media with effusion, is characterized by the presence of a flow of non-purulent or puru-

lent fluid in the middle ear without the appearance of acute signs (Rosenfeld *et al*., 2016).

Several types of microorganisms are involved in increasing the rate of disease. Among the most important bacterial isolates causing otitis media are *Psedomonasa spp, Proteus spp, Staphlococcus spp, Klebsiella spp.* As for fungal causes, they include *Aspergillus spp, Candida spp* (Punia *et al*., 2019).

The current study aimed to isolate and diagnose bacteria and fungi that cause otitis media and studying the sensitivity of bacterial and fungal isolates to antibiotics and the study of the effect of some factors such as age, gender and educational level on the spread of the disease.

Materials and Methods

Samples collection

The study included the collection of (216) swabs from patients attending the consulting clinic/ear, nose and throat (ENT) department at Hawija General Hospital/Kirkuk Governorate for the period from September 2022 to March 2023. With the help of specialized doctors residing in this division, and after diagnosing the symptoms by them, samples were collected according to what was stated in (Jik *et al*., 2015) using cotton swabs with Transport media .The swabs were transferred to the microbiology laboratory to isolate and diagnose the disease-causing germs according to the approved standard methods.

Culture of sample

The samples taken from the patients were cultured in Sabouraud dextrose agar, MacConkey agar, Blood agar media. The dishes were incubated aerobically at a temperature of 37C^{o} for a period of 18-24 hours to observe the growth of bacteria and 5 days to observe the growth of fungi. A sub culturing is done from one of the isolated colonies on other selective media to obtain pure bacterial and fungal cultures with Single colonies of the same gender of a species (Ghasemi *et al*., 2020).

Diagnosis of bacterial isolates

The phenotypic characteristics of each of the colonies and bacterial cells growing on different culture media were studied, including the study of bacterial colonies in terms of color, shape, consistency, edges, and growth or lack of growth on differential media and selective media. As for the pheno-

typic characteristics of cells, they included Bacterial cell shape, the regularity of bacterial cells with each other and the nature of their interaction with Gram stain.

Microscopic examination

The microscopic examination of the bacteria was carried out by preparing a thin smear from the colony using a sterile loop and placed on a glass slide containing a drop of sterile distilled water and brushed well and then left on a hot plate to dry and then stained with gram stain, dried and examined by light microscopy under force (100X) using an oil lens to observe the shape of bacterial cells, their aggregation, and their interaction with the dye (Levinson, 2016).

Diagnosis of bacteria using biochemical tests

These tests included Oxidase test, Catalase test, Indole test, Urease hydrolysis test, Motility test, Methyl red test, Voges Proskauer ,H2S, Mannitol fermentation test, Simmon citrate test.

Diagnosis of fungal isolates

Molds of the genus *Aspergillus niger* and *Candida spp*. were diagnosed based on examining the external appearance of the colonies, which included the shape, diameter, surface, height, and color of the colony on the obverse side and the reverse side, its colonial texture, and growth rate after it was cultured on (SDA) media (De Hooge *et al*., 2000).

Microscopic examination

A part of the colony to be examined was taken by means of the vector (Inoculation loop) and mixed with a drop of lactophenol blue cotton dye, then the sample was spread on a glass slide and covered with a cover slide, after that it was examined by light microscopy under power 10X and 40X to note the pseudohyphae and spores. A second smear was taken on another sterile glass slide, stained with gram stain, then placed on a flame, and examined to note budding (Ellis *et al*., 2007).

Diagnosis of fungi using biochemical tests

These tests included the Growth test on chrome agar *candida* (CAC) , Urease Test , Germ tube forming test , Growth test at 45°C , Chlamydospores forming test.

Diagnostics with the VITEK2 compact system

The Vitek 2 compact system device, equipped by Bio Merieux, was used to diagnose bacteria and fungi with a high degree of accuracy, as this device in-

cludes (64) biochemical tests that are used in the diagnosis of bacteria (Pincus, 2006).

Testing the sensitivity of bacterial isolates to antibiotics

The isolated bacteria were tested for sensitivity to (10) types of antibiotics using the disc diffusion methods.The standard Kirby and Bauer method was used to test the antibiotic susceptibility of bacteria (Bensons,2002) .

A single pure colony was transferred by sterilized metal carrier from a 24-hour-old culture into tubes containing 5 ml of nutrient broth media and incubated at 37°C for 24 hours, then the turbidity of the growth was compared with the turbidity of the standard fixed-turbidity solution McFarland's solution which is equivalent to (1.5 x 10^8) cells / ml, and in the event that the turbidity is not equal, the physiological solution is added to these tubes until the turbidity is equal to MacFarland's solution. Then the plates were left to dry at room temperature for a period of (10-15) minutes for evaporation to occur. After that, the antibiotic tablets were placed in an appropriate shape and dimensions on the surface of the media using sterile forceps, at the rate of 5 tablets per plate, then the plates were incubated at a temperature of 37°C for 24 hours, and the diameter of inhibition was measured from the other side of the plate using a transparent ruler, and then compared to the numbers indicated in Standard tables defined by the National Committee for Clinical Laboratory Standards (NCCLS 2022) for the determination of Resistance and Sensitive Bacteria

***Candida spp.* sensitivity testing for antifungal**

The isolated *Candida spp.* were tested for sensitivity to 6 types of antibiotics using the disc diffusion methods (Espinel-Ingroff and Canton, 2007), as a single pure colony was transferred by means of a sterilized mineral carrier from a 24 hour into tubes containing 5 ml of normal saline physiological solution and mixed well, then the turbidity of the growth was compared with the turbidity of the standard turbidity solution (McFarland's solution) which is equivalent to (1.5 x10^8) cells / ml, and in case the turbidity is not equal, the physiological solution is added to These tubes until the turbidity is equal to MacFarland's solution, and using sterile cotton swabs, the yeasts whose sensitivity is to be tested are spread on Sabouraud dextrose agar, then the

dishes were left to dry at room temperature for (15) minutes for impregnation to occur, then the antibiotic tablets were placed in an appropriate shape and dimensions on the surface of the media using sterile forceps at the rate of 5 tablets per dish, then the dishes were incubated At a temperature of 37 °C for 24 hours, the diameter of inhibition was measured from the other side of the plate using a transparent ruler, and then compared with the numbers fixed in the specified standard tables.

***Aspergillus niger* sensitivity testing for antifungal**

Six types of antifungals were used to test the sensitivity of the *Aspergillus niger* fungus to them. Medium plates of Sabouraud dextrose agar containing chloromphenicol were prepared. Then 1 cm of fungal tissue was taken and added to 3 ml of normal saline. Then the sample was mixed well until the suspension was formed, then the fungal suspension was placed. On the dishes Sabouraud dextrose agar media and leave the dishes for 10 minutes until it absorbs the moisture. The antibiotic tablets were then placed in an appropriate shape and dimensions on the surface of the media using sterile forceps, 6 tablets per dish. The dishes were then incubated at a temperature of 28°C for 5-10 days. The diameter of inhibition was measured on the other side of the dish using a transparent ruler and then compared to the numbers listed in the tables. The specified standard (Al-Bajilan, 2016).

Results and Discussion

Isolation

The samples that gave bacterial growth were 177 samples, at a rate of 82% , while 39 samples, at a rate of 18% , did not give any bacterial , fungi growth despite the presence of inflammation in the patients. It is believed that the reason may be due to the patient's use of therapeutic antibiotics for a period before taking the sample as shown in Table No. (1)

Table (1) Results of primary isolation of ear swabs and their percentage

Culture result	No.	Ratio(%)
positive growth	177	82%
negative growth	39	18%
Total	216	100%

The culture-positive samples were distributed among 63 samples with a rate of 35.6% in which a single bacterial growth appeared, 6 samples with a rate of 3.4% in which a single fungal growth appeared, and 3 samples with a rate of 1.7% in which a yeast growth appeared. *Candida* only, and 27 samples with a rate of 15.3% showed mixed growth between bacteria and fungi, and 78 samples with a rate of 44% showed mixed growth between bacteria and *candida* as shown in Table (2).

Table (2) Number and percentage of types of bacterial isolates isolated during the study

Totals of bacterial isolates	No.	Ratio(%)
Single bacterial isolates	63	35.6%
Single fungal isolates	6	3.4%
Single Candida isolates	3	1.7%
Isolates of common bacteria and fungi	27	15.3%
Isolates of bacteria and *Candida spp*.	78	44%
Total	177	100%

Bacterial genera isolated from otitis media

The results of the study showed, as shown in Table (3), that the bacterium *Psedomonas aeruginosa* was the main cause of otitis media in the city of Hawija, which represented 66 isolates, a rate of 39.28% of the total of 168 isolates, followed by *Staphylococcus aureus* as a pathogen in the second degree in causing middle ear infections by 48 isolates, a rate of 28.57%, then *Proteus mirabilis*, ranked third after *Psedomonas aeruginosa* and *Staphylococcus. aureus* as a major cause of otitis media, as it was isolated from 24 pathological cases with different clinical manifestations. It was 14.29% *Escherichia coli* ranked fourth in causing otitis media, as it was coliform bacteria that caused 15 pathological cases with a rate of 8.9%, and *Klebsiella pneumoniae* came after *E.coli* in the fifth place with a rate of 5.35%, and *Staphylococcus epidermidis*, as it was the pathogen in six cases, with a rate of 3.6%.

The reason for the dominance of *Psedomonas aeruginosa* is that it is more of the secondary invaders entering the ear through contaminated med-

ical tools and through the occurrence of scratches, as it is one of the opportunistic pathogens, and it is one of the most important bacterial types acquired from hospital infections. It has the ability to survive in disinfectants and some types of sterilizers (Moradadi *et al*., 2017), cases of eliminating infections caused by this type of bacteria are of high difficulty due to its wide resistance to many types of antibiotics, *Psedomonas aeruginosa* bacteria possess many virulence factors, as they secrete a large number of metabolites that play a major role in their pathogenicity, including: Lipase, Fibrinolysin, Leukocidin, Haemolysin, Protease, Elastase, Exotoxin, Lecithinase, and Collagenase. The results of the study agree with previous studies, including the study of (Ferede *et al*., 2001), and the study of (Osazuwa *et al*., 2011). Which confirms that *Psedomonas aeruginosa* is the most common cause of otitis media infection.

Staphylococcus aureus is the only species of the genus Staphylococcus of clinical importance to humans. Most of its strains produce coagulase enzyme, cause acute otitis externa or the so-called swimmers ear infection. Using earphones can facilitate the spread of these bacteria, and skin allergies contribute to the exacerbation of the infection (Frothingham, 2019). Two studies agree with the results of previous studies on the middle ear, which showed that *Staphylococcus aureus* comes as a pathogen mainly in causing infections in the middle ear, including a study (Al-Lahibi, 2021) .

The results of the current study were consistent with the findings of (Juyal *et al.*, 2014) who isolated *Staphylococcus epidermidis*, these bacteria are non-pathogenic and exist in a symbiotic relationship on the skin , joints and human ear canals and is present in large numbers everywhere, which leads to repeated contamination of collected samples. From or through the skin and become opportunistic and cause infection when the resistance of the middle ear decreases as a result of the entry of other microbes or as a result of the use of antibiotics (Greenwood *et al*., 2012).

Table (3) Percentages of bacteria isolated from patients with otitis media

Bactria	Total isolates	Ratio(%)
Psedomonas aeruginosa	66	39.28%
Staphylococcus aureus	48	28.57%
Proteus mirabilis	24	14.29%
Escherichia coli	15	8.9%
Klebsiella pneumoniae.	9	5.35%
Staphylococcus epidermidis	6	3.6%
Total	168	100%

Fungi isolated from otitis media

In the current study, there were (114) fungal isolates from patients with otitis media from a total of 216 samples, as these isolates constituted (52.77%), and the isolated fungi belonged to the genus *Candida spp*. and *Aspregillus niger*. Where the yeasts of *Candida spp*. were the most prevalent in the isolated samples, as the number of isolated samples belonging to *Candida spp*. was 81 samples with a rate of 37.5%, and the type *C. parapsilosis* prevailed over the rest of the species, as shown in Table (4), while the fungus *Aspergillus niger* reached the number The samples were 33 samples, with a rate of 15.27%.

The aforementioned results converged with what was found by (Pontes *et al*., 2009), as it was found that the genus *Candida spp*. main cause of infection,

Table (4) Percentages of fungi isolated from patients with otitis media

fungi	Total isolates	Ratio(%)
Aspergillus niger	33	28.95
Candida parapsilosis	39	34.2
Candida krusie	15	13.16
Candida glabrata	12	10.53
Candida albicans	9	7.9
Candida tropicalis	6	5.26
Total	114	100%

The relationship of some factors with the incidence of otitis media

1- Distribution of cases of bacterial otitis media according to age

The results in table 5 showed that the highest infection rate was among the first age group (1-20) years with a rate of (50.61%), followed by the age group (21-40) years with a rate of (29.17%), then the age group (41-60) years with a rate of (14.87%, while the age groups over 60 years old had a rate of (5.35%).

Perhaps the reason for the emergence of the highest incidence of infection in the first age group is due to the incompleteness of their immune system and the nature and characteristics of the Eustachian tube, and the use of contaminated tools and inserting them into the ear and poor hygiene play a major role in causing infection (Naqvi *et al*., 2019). The lowest incidence is in the fourth age group, and this may be due to the lack of patients in this category for consulting clinics due to their advanced age. These results are consistent with (Morris and Leach, 2009).

Table (5) Percentage of bacterial otitis media by age group

Age group (years)	Males		Females		Total	Ratio(%)
	No.	%	No.	%		
(1-20)	48	28.57	37	22.04	85	50.61
(21-40)	30	17.86	19	11.31	49	29.17
(41-60)	9	5.35	16	9.52	25	14.87
More than (60)	3	1.78	6	3.57	9	5.35
Total	90	53.56	78	46.44	168	100

2 - Distribution of fungal otitis media cases according to age

The results in table 6 showed that the highest infection rate was among the first age group (21-40) years at a rate of (55.25%), followed by the age group (41-60) years at a rate of (21.94%), then the age group (1-20) years at a rate of (17.55%). As for the age groups over 60 years, their percentage was (5.26%).

In general, many researchers, including (Gharaghani *et al*., 2015), found an increase in the rate of fungal ear infection with increasing the age of the patient, and (Gonsalves *et al*., 2008) mentioned that older ages are more susceptible to infection with

opportunistic fungi when compared to younger groups, and this is due to Lack of movement and activity, poor blood circulation, immunity and metabolic processes, and a decrease in infection in the category (>20) years, given that *candida* is an opportunistic infection that depends mainly on the immune status.

Table (6): Percentage of fungal otitis media by age group

Age group (years)	Males		Females		Total	Ratio(%)
	No.	%	No.	%		
(1-20)	10	8.78	10	8.78	20	17.55
(21-40)	27	23.65	36	31.59	63	55.25
(41-60)	8	7.02	17	14.92	25	21.94
Morethan (60)	0	0	6	5.26	6	5.26
Total	45	39.45	69	60.55	114	100%

2 - Distribution of otitis media cases by gender

The results of the current study showed that infection due to bacteria is more common in males than in females. As shown in Table (7), males represent 90 patients with a rate of (53.57%), while females represent 78 patients with a rate of (46.43%). This is consistent with many studies, including the study of (Al-Ani, 2020). The ratios of males to females were as follows (45.09% - 54.91%). And work, as well as hormonal changes, and males are more likely to visit hospitals and private clinics in the current circumstances.

As for the infection of the middle ear due to fungi, the results of the study showed that the infection of females is higher than that of males As shown in Table (8), distributed among (69) injuries in females, at a rate of (60.52%), and (45) injuries in males, at a rate of (38.48%) of the total number of injuries. The reason for the high rate of injury in females is due to the nature of female work at home during the day, which It makes them more susceptible to infection with fungi due to the spread of fungi and their spores throughout the corners of the house (Shehab, 2005). The results agreed with what was indicated by (Tasic-Otasevic *et al*., 2020) and (Mofatteh *et al*., 2018).

Table (7) Percentages of bacterial isolates that cause bacterial otitis media, by gender

Bacteria	Female		Male		Total	
	No.	Ratio(%)	No.	Ratio(%)	No.	Ratio(%)
P. aeruginosa	33	19.64	33	19.64	66	39.28
S. aureus	21	12.5	27	16.07	48	28.57
Proteus mirabilis	12	7.15	12	7.15	24	14.3
Escherichia coli	9	5.36	6	3.57	15	8.93
Klebsiella pneumoniae.	6	3.57	3	1.78	9	5.35
S. epidermidis	0	0	6	3.57	6	3.57
Total	81	48.22	87	51.78	168	100

Table (8) Percentages of fungal isolates that cause fungal otitis media, by gender

Fungi	Female		Male		Total	
	No.	Ratio(%)	No.	Ratio(%)	No.	Ratio(%)
Aspergillus niger	21	18.42	12	10.52	33	28.94
C. albicans	3	2.63	6	5.27	9	7.9
C. parapsilosis	24	21.05	15	13.16	39	34.21
C. tropicalis	3	2.63	3	2.63	6	5.26
C. glabrata	12	10.52	0	0	12	10.52
C. krusie	6	5.27	9	7.9	15	13.17
Total	69	60.52	45	39.48	114	100

Distribution of otitis media cases according to educational level

The results of the current study showed that the prevalence of otitis media in the uneducated group of patients was high, 62.7%, with 111 infections, while the prevalence of the disease among the educated was lower than it was among the uneducated, at 37.3%, with 66 infections, as shown in Figure (1). This result coincided with the results reached by some researchers such as (Parry and Ronald, 2002) when they noticed the predominance of infection among the uneducated group and its lack among the educated, and it may be due to the low cultural and educational awareness.

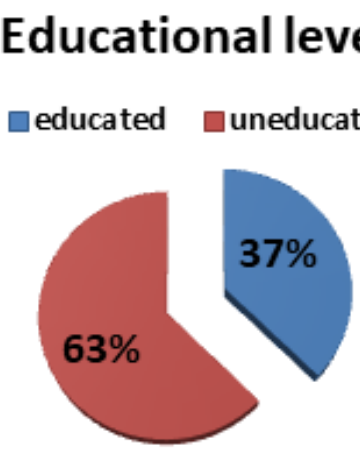

Figure (1) The relationship of otitis media with the educational level

Resistance of bacterial isolates to antibiotics

A sensitivity test was conducted using 10 antibiotics used to treat otitis media for 168 bacterial pathogen isolated from ear swabs of patients with otitis media, as shown in the table (9). The results of the current study showed that the isolates of *Psedomonas aeruginosa* a very high resistance by 100% against all the antibiotics used in the study except for Ciprofolxacin and Levofloxacin, as these isolates were sensitive to these two antibiotics by 100%. As for *Staphylococcus aureus*, they were sensitive to antibiotics Rifampicin, Ciprofoxacin, Trimethoprim and Levofloxacin at 100% while 100% resistant to antibiotics (Amoxicillin/ Clavulanic acid, Azithromycin, Ceftriaxone, Ceftazidime, Amoxicillin). *Proteus mirabilis* was 100% resistant to all types of antibiotics used, except for Ciprofolxacin and Levofloxacin, where it was 100% sensitive to these two antibiotics. *E. coli* showed 100% resistant to Rifampicin, amoxicillin/ clavulanic acid and amoxicillin, while these isolates were sensitive to azithromycin, ceftriaxone, ceftazidime, ciprofloxacin, trimethoprim and levofloxacin. *Klebsiella pneumoniae* are the most sensitive isolates to the antibiotics used in the current study, as they were sensitive to Azithromycin, Ceftriaxone, Ciprofloxacin, Trimethoprim, Doxycycline, and Levofloxacin by 100%, but they were resistant to Ri-

fampicin, Amoxicillin/ Clavulanic acid, and Amoxicillin. with a rate of 100% . Finally, *Staphylococcus epidermidis*, as the sensitivity of this bacterium to antibiotics Rifampicin, Ciprofloxacin, Trimethoprim Doxycycline and Levofloxacin reached 100%, while it was resistant to antibiotics Amoxicillin/ Clavulanic acid, Ceftriaxone, Ceftazidime and Amoxicillin by 100%. The results of the current study are illustrated, that all bacterial isolates had high resistance to Amoxicillin/ Clavulanic acid and Amoxicillin, The reason for this is due to several mechanisms, the most important of which is the ability of the bacteria to produce the beta-lactamase enzyme (Burgos sunchez *et al*., 2000).

The results in the current study showed the high resistance shown by the isolates under study towards the antibiotics used in a very large way, except in some exceptional cases. The reason for this may be due to the development of these bacterial races of resistance mechanisms that enabled them to curb the power of these antibiotics, such as the fact that the outer walls of these bacterial races contain special proteins such as the PER-1 protein (efflux pump) have the ability to release various antibiotics outside the cell. It may also be known that the reason for these genera to possess resistance is that they contain the resistance plasmid R - Plasmid, which plays an essential role in conferring resistance to many antibiotics. life (Rashid *et al.,* 2007).

We note from the foregoing The rise in bacterial resistance to a large number of antibiotics is due to the incorrect and indiscriminate use of medications by infected people without resorting to specialist doctors (Ventola , 2015).

Through the results that appeared, it can be said that the best antibiotics for treating otitis media are Ciprofloxacin and Levofloxacin in the first place, and this result is consistent with the study (Sadiq, 2015) and the study (Owaied, 2020). It is one of the most effective antibiotics for treating otitis media.

Table No. (9) Resistance of bacteria isolated from otitis media to antibiotics

Type of bacteria and number of isolates / Antibiotic	P. aeruginosa	*Staph. aureus*	*Proteus mirabilis*	*E. coli*	*K. pneumoniae*	*Staph. epidermidis*
Rifampicin	100%	0	100%	100%	100%	0
Amoxicillin+ Clavulanic acid	100%	100%	100%	100%	100%	100%
Azithromycin	100%	100%	100%	0	0	66.66%
Ceftriaxone	100%	100%	100%	0	0	100%
Ceftazidime	100%	100%	100%	73.33%	86.66%	100%
Ciprofloxacin	0	0	0	0	0	0
Amoxicillin	100%	100%	100%	100%	100%	0
Trimethoprim	100%	0	100%	0	0	100%
Doxycycline	100%	100%	100%	20%	0	0
Levofloxacin	0	93.75%	0	0	0	0

Resistance of fungal isolates to antibiotics

A sensitivity test was conducted using 6 antibiotics used to treat otitis media 114 fungal isolates isolated from ear swabs of patients with otitis media, as shown in Table (10). The results of the study showed that *Aspergillus niger* isolates showed the highest resistance to Amphotericin-B by 27.2%, while they were 100% sensitive to the two antibiotics Fluconazole and Nystatin. *Candida parapsilosis* showed the highest resistance against Amphotericin-B by 28.2%, while it was sensitive by (100%) against Ketoconazole. While *Candida krusie* yeast was the most antibiotic resistant species of *Candida* isolates It was 100% resistant to Amphotericin-B, Fluconazole, and Itraconazole, while it was 93% sensitive to the antibiotic Nystatin. *Candida glabrata* showed the highest resistance against Amphotericin-B by 66.7%, while it was 100% sensitive to the two antibiotics Fluconazole and Ketoconazole. *Candida albicans* was 66.7% resistant to Ketoconazole, while it was 100% sensitive to Fluconazole and Clotrimazole. Finally, the yeast *Candi-*

da tropicalis showed the highest resistance against the two antibiotics, Amphotericin - B and Nystatin, by 33.3%, while it was sensitive by 100% against the two antibiotics, Fluconazole and Ketoconazole.

The discrepancy of the results of the current study with the results of other studies in terms of resistance and sensitivity is due to the excessive use of antifungal and antibiotics and the environmental location of the isolates and personal practices (Aslam, 2016). The difference in diameters of inhibition from one species to another for *Candida* yeast species, and even within isolates of the same species, is a normal matter, and even the variation between isolates in terms of resistance and sensitivity. Because it depends on the environment (Mohammed, 2012).

Through the results that appeared, it can be said that the best antibiotics for the treatment of fungal otitis media are the antibiotics Nystatin and Ketoconazole. It is one of the most effective antibiotics for treating fungal otitis media.

Table No. (10) Resistance of fungi isolated from otitis media to antibiotics

Type of bacteria and number of isolates / Antibiotic	*Aspergillus niger*	*Candida parapsilosis*	*Candida krusie*	*Candida glabrata*	*Candida albicans*	*Candida tropicalis*
Amphotericin-B	27.3	28.2	100	66.7	22.2	33.3
Clotrimazole	15.2	12.8	20	25	0	16.7
Fluconazole	0	10.26	100	0	0	0
Ketoconazole	9.1	0	13.3	0	66.7	0
Itraconazole	24.2	7.7	100	16.7	22.2	16.7
Nystatin	0	5.13	6.7	8.33	11.1	33.3

Conclusions

The study showed that the most common bacterial species in otitis media is *Pseudomonas aeruginosa*, ranked first .As for fungi, the study showed the dominance of *Candida spp*.

The results of the study showed that males are more infected than females, if the cause is bacterial, and females are more infected than males in the case of

a fungal cause.

The majority of otitis media infections due to bacteria are in the age group (1-20) years, as for fungi, most of them were in the age group (21-40) years.

The results of sensitivity tests showed that the optimal antibiotics for the treatment of bacterial otitis media are Ciprofloxacin and Levofloxacin, while the two antibiotics, Nystatin and Ketoconazole, were common antibiotics used to treat fungal otitis media.

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