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Isolation and Identification of Opportunistic Yeast and Mold Causing Otitis Media in AL- Najaf Province, Iraq

Ehab Y. Jabber[1*], Salim Shamkhi Jaafar[2], Athraa Harjan Mohsen[3]

[1,2]DNA Research Center, University of Babylon, Hilla, Iraq
[3]Dept. Biology, Faculty of science, Kufa University, Najaf, Iraq

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***Corresponding author:** Ehab Y. Jabber
DNA Research Center, University of Babylon, Hilla, Iraq

Abstract | **Original Research Article**

Otitis media is an inflammation or infection of the middle ear. It may be caused by a cold, sore throat, or respiratory infection. This research aims to isolate and identify the types of opportunistic fungi and yeasts that cause this infection. *Aspergillus* and *Candida spp* are the most frequently isolated fungi in patients with otomycosis. Fifty samples were collected from patients with otitis media who visited the ENT consulting clinic at Al-Sadder Medical City. In AL-Najaf Governorate during the period from September 2024 to February 2025 for the purpose of isolating opportunistic fungi resulting from otitis media. Their ages ranged between 7-40 years. The results showed that using different methods including direct diagnosis and incubation in the laboratory, the ratio of females to males was as follows: - Females [25] 15 60% and males [25] 10 40%. The results showed that the incidence of otitis media in females was higher than males in the group of patients aged between [21-30] years. The results of this study revealed that the incidence rate in females was higher than males in patients with hypertension, diabetes and cancer, reaching 12 [48%], 9 [36%] and 4 [16%] respectively. It also revealed that the infection rate for males and females, especially in the city, was higher than in the countryside, as the percentage of males reached 18 [72%] in the city and 7 [28%] in the countryside, and females 15 [60%] and 10 [40%] male. It was found that 7 species of opportunistic fungi and yeast belonging to 4 fungal genera were isolated and diagnosed by grown on two different culture media because it the most widely used in various research and cultivation of fungi used for different purposes. with the genus *Aspergillus flavus* being the most prominent, followed by the genus *Aspergillus terreus*.

Keywords: Otitis media, Opportunistic fungi, Middle ear infection, *Aspergillus spp.*, *Candida spp*., Mold, External ear.

1. INTRODUCTION

Fungi, like other microorganisms, play an important role in nature. There are more than 50,000 species of fungi that live in nature in different ways and play a fundamental and important role in breaking down and analyzing organic materials in different environments. From a medical perspective, there are about 80 species of molds, filamentous fungi, and yeasts that have the ability to cause various diseases to humans and animals alike [1]. Otomycosis, also referred to as ear infectious disease, affects the external ear canal and is more common in warm, humid, and dusty environments [2]. It causes inflammation. *Aspergillus* and *Candida* are common fungal species that are used as causative agents or microorganisms. *Candida albicans* causes white, fluffy otorrhoea, while *Aspergillus niger* and *Aspergillus fumigates* give off a black pepper-like appearance [3].

Otomycosis can be caused by a swimming habit [not drying the ear after swimming], persistent and recurrent infections in the ear, using ear drops without a doctor's advice, using steroid-containing medications and oil, excessive ear wax buildup, not cleaning the ear [4] Opportunistic fungal infections have become more significant in medicine in recent years due to weakened immune systems. But these kinds of fungi are extremely widespread across all demographic groups, and they primarily infect immunocompetent hosts [5]. It is important to take extra precautions to avoid hearing loss. Treatment ought to be started as soon as possible, particularly for inpatients with weakened immune systems [6].

Recent molecular techniques and analyses have revealed that the *Aspergillus niger*, *A. niger* being the most common causes of otomycosis. Despite their rarity, *A. welwitschiae*, *A. awamori*, and *A. foetidus* have also been reported to be the species responsible for this infection [7]. The precise molecular identification of these black fungi is essential for the development of

rapid diagnostic tests for *Aspergillus* otomycosis and the selection of the best course of treatment for the different species in this complex. Although species of the *A. flavus* complex are also frequently isolated, *A. terreus*, *A. fumigatus, A. versicolor, and A. luchuensis* are less frequently the cause of infections than the previously mentioned molds [8]. A frequent cause of otomycosis, aside from *Candida albicans*, has also been identified as *C. parapsilosis*, especially in Europe where it is highly prevalent. Other Candida species, such as *Candida lustaniae*, *Candida guilliermondii*, *Candida famata*, *Candida tropicalis*, *Candida krusei*, and *Candida glabrata*, have also been isolated and identified as the etiological agents of the external auditory canal infection, despite their relatively low incidence [9]. Also found to colonize external auditory canal skin and possibly be the source of the infection is *C. auris*, a highly pathogenic fungal pathogen that is still in its infancy. This has the potential to spread and is highly concerning because of its multi-drug resistance [10].

The intricate structure of the ear aids in both hearing and maintaining balance. various types of ear problems in patients of various ages. Otitis externa, an inflammatory condition that can be acute or chronic, affects 4 out of every 100 people. Acute cases account for 3-5% of cases, while chronic cases affect 3-5% of the population. Some of the studies [11,12] reported otomycosis in Iraq.

A study found that whereas children under 4 had acute ear infections, patients in the 15–44 age range experienced chronic infections [13].

In particular, many species of mold and yeast have been found in Africa [14], Central America [15], South America [16], Asia [17-19] and Central America [20]] Seasonality has not been observed in surveys carried out in Europe [8].

The outer surface of the ear has a higher potential for fungal colonization because it is a humid area with a pH that is favorable for microbes [21]. The precise microbiological test and clinical observation morganism responsible for the infection must be identified. Before, it was believed that otomycosis was exclusively caused by fungi, but additional research revealed that bacteria can also cause this infection [22].

Candida albicans is observed to have a stronger correlation with otomycosis than any other species. Compared to *A. niger*, *A. fumigatus* is more common to cause invasive otomycosis [23]. These saprophytic microbes can become pathogenic if the balance between fungal and bacterial growth is disturbed, especially if non-specific and specific body defense mechanisms are compromised [24].

Otomycosis is caused by two different kinds of factors: environmental and host-derived. It should come as no surprise that tropical and subtropical areas have the highest prevalence of otomycosis since these areas have the warm, humid climate that is the main external risk factor for the disease [25,26].

Otomycosis may also develop as a result of an earlier external auditory canal bacterial infection that was managed with topical antibiotics. In patients with untreated dermatomycosis, the illness may also arise from autoinfection of the canal [27,2]].

Fungal ear infection is an outer and middle ear infection where one or both ears can become inflamed, with dry, itchy skin, and possibly a smelly discharge. It can become chronic but often is easily treated with antifungal Occasionally, otitis media is usually caused by *Aspergillus spp*, Candida spp or other pathogens such as herpes virus [29].

The primary means of acquisition of *Aspergillus* is the inhalation of airborne conidia, which are released from environmental sources such as soil, decomposing organic material, and contaminated food. A study from Lass-Flörl and colleagues demonstrated *A. terreus*-contaminated potted plants in the hospital to be the most probable source of invasive infection in patients with hematological disease. Hospital-acquired [nosocomial] infections with *A. terreus* have been mainly associated with immunocompromised hosts and environmental disturbances, such as construction sites and/or renovation work in units for high-risk patients [30-32] Such pathogenic fungal species have the ability to cause severe harm to humans due to the secondary metabolites they produce during their growth, which are characterized by toxic properties. At the same time, some of them can be used as antibiotics such as Cyclosporine, Penicillin, and other well-known and commonly used organic compounds [33]. Although this small percentage of pathogenic species is of less importance compared to bacteria and viruses, they, especially the opportunistic fungi, have become of increasing importance [34].

Otitis media is a major health problem and occurs with a high incidence and prevalence in both developed and developing countries [35] It can be either suppurative or non-suppurative. The inflammation often begins when infections that cause sore throat, cold, or other respiratory or breathing problems spread to middle ear. [36] Otitis media could be of viral or bacterial origin. It could present an acute or chronic course [37].

Aspergillus spp., together with *Candida spp.*, are the most common causative agents of fungal otitis [38,39]. It is still controversial whether fungi are the true pathogens or rather a result of the compromised immune system after the bacterial infection [40]. In fact, most of the otomycosis cases are related to a prolonged topical antibiotic and steroid use. Other causative factors of fungal otitis include humid climate, immunodeficiency

syndromes, diabetes and history of prior otologic procedures [39,40].

Otomycosis may be either subacute or acute and is characterized most commonly by ear itching [pruritus], discomfort, otalgia, malodorous discharge, otorrhea, scaling, sometimes hearing loss, and/or a feeling as if something is in the ear canal [41]. The most common causative agents of otomycosis are molds of the genus *Aspergillus* and yeasts of the genus *Candida*, particularly *Aspergillus* and *Candida*, *C. albicans* [42].

Most fungal Otitis externa are superficial but they may result in severe complications, such as malignant Otitis externa, which is typically associated with diabetes, AIDS, and other immunocompromising conditions. Malignant Otitis externa is an invasive infection of tissues around the external auditory canal and may lead to further complications, such as cranial nerve palsies [43-45]. *Aspergillus species* are among the most common fungi in malignant Otitis externa [44]. Fungal Otitis externa may develop into otitis media and mastoiditis via tympanic membrane perforation or tympanostomy [46].

Otomycosis is often diagnosed clinically based on the history and examination of EAC, revealing accumulation of whitish or blackish debris, discharge, or erythema. However, the clinical presentations of otomycosis can be indistinguishable from the bacterial otitis externa, and paraclinical methods should be used to confirm the diagnosis [47].

Opportunistic fungal infections have grown in significance in human medicine recently, possibly as a result of the rise in patients with immune system impairments [48].

2. MATERIAL AND METHODS

2.1. Ethical Statement and Participant Enrollment

The study received ethical approval from the Ethics Committee of the University of Babylon [Approval No. Z 221201, Date.2025.03.12] and was conducted in accordance with the principles of the Helsinki Declaration. All procedures adhered to the approved guidelines, and written informed consent was obtained from all participants prior to sampling.

In the study group, the following details were assessed: disease symptoms, previous topical/systemic treatment, history of ear surgery, general history including additional diseases [diabetes, hypertension, immunodeficiency and cancer], and epidemiological data such as age and sex. The total number of samples was 50 samples were collected from cases of otitis media that were clinically diagnosed by an ENT specialist. The samples were taken from patients of different ages ranging from [7-40 years] for both sexes.

The samples were collected in the [Ear, Nose and Throat] Consultation Department / Al-Sadr Teaching Hospital in Najaf Governorate for the period from September 2024 to February 2025.samples were collected according to what was stated in [49] 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. and the following tests were performed: -

2.2 Microscopic Examination

A glass slide was prepared for direct examination as follows:

A- 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] mediaA small amount of 10% potassium hydroxide [KOH] was placed on a clean glass slide. Then, an ear swab was taken and wiped over the glass slide [50].

B- The glass slide was gently heated by passing it through a flame two or three times or placing it on a hot plate for 30-60 seconds. Avoid heating to boiling point, as this leads to crystallization of the KOH, and tested under the light microscope. then 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 [50].

C- The glass slide was examined under a microscope, indicating whether the test was positive or negative based on the presence or absence of fungal hyphae, spores, or yeast cells.

2.3 Samples culture

The direct plate method was used to isolated. Cotton swabs were taken after direct microscopic examination and cultured on sterile glass plates containing Sabouraud's medium, supplemented with the antibiotic chloramphenicol [250 mg / L] to inhibit bacterial growth using the streaking method. Samples were incubated at 37°C for 24 hours and then at 25°C for 7–14 days until growth began. *Aspergillus* species were identified and characterized by microscopic characteristics and colony morphology, with other yeast species. To characterize the isolated molds and yeasts, we relied on the external appearance of the colony and morphological features such as the enlarged shape, color, and droplet of the colony, in addition to the diversity of microscopic features such as the massive shape, color of the conidia, and whether they were spiny or not. A portion of the colony was transferred and placed on a glass slide containing a drop of lactophenol stain. The isolated fungi were identified using microscope [51].

2.4 Preparation of culture medium:

All culture media were prepared according to the instruction of manufactures fixed on the container. They were sterilized by autoclave at 15 psi/inch² in 121 C° for 15 min. Sabouraud's dextrose agar media was boiled for 10 min without over heating 15ml of media were poured in disposable petirdish and stored at 4 C° untile used. while Sabouraud's dextrose agar media used freshly. The study was carried out by using two cultures media which prepared as following:

2.4.1 Sabouraud's dextrose agar [SDA] with chloramphenicol

This medium agar was prepared according to the manufacturer's instructions by suspending 65 gm of SDA powder in 1000 ml of distilled water, final pH was adjusted to 6.8 and then were added 250 mg / L from Chloramphenicol antibiotic prior to autoclaved This medium was used for cultivating pathogenic and commensal fungi and yeasts, Chloramphenicol is a broad-spectrum antibiotic inhibitory to a wide range of Gram-negative and Gram-positive bacteria [52].

2.4.2 Potato dextrose agar [PDA] with chloramphenicol

Potato dextrose agar was prepared according to the manufacturer's instructions by suspending 39 gm of PDA powder in 1000 ml of distilled water final pH was adjusted to 6.8 and then were added 250 mg / L from Chloramphenicol antibiotic prior to autoclaved, Chloramphenicol is a broad-spectrum antibiotic inhibitory to a wide range of Gram-negative and Gram-positive bacteria [52].

3. RESULTS & DISCUSSION

3.1 Patient

In the study group there were 25 men and 25 women at a median age of 7-40 years, all samples were collected from [ENT] Consulting. From in AL Sadder Medical City.In AL Najaf Governorate. The specimens were included: hypertension, diabetes and cancer patients which was 12 [48%], 9 [36%] and 4 [16%] female and 10 [40%],8[32%] and7[28%] in male respectively, as in table [1].

Table 1: A table representing the relationship between ear fungal infections and immunodeficiency patients

Sex	Immunocompromised state			No. positive
	Hypertension	Diabetes	Cancer	
Male	10 [40%]	8[32%]	7[28%]	25 100%
Female	12[48%]	9[36%]	4[16%]	25 100%

Otomycosis is a relatively common problem. In most cases it is localized in the external auditory canal and presents as an accumulation of typical hyphae or thick, fibrinous debris [53,54] They usually occur in immunocompromised patients and, if untreated, can lead to serious complications [55].

Among the results obtained, the genus *Aspergillus* spp. was dominant, accounting for 48.31% of the total number of isolates, a percentage higher than that recorded by [56, which amounted to 2.9%. The reason for the dominance of his genus is likely due to its high ability to withstand harsh environmental conditions, and its high capacity to produce enzymes and secondary metabolites, which enables it to exploit diverse food sources [57].

It was also found that the species *A. flavus* is the most prevalent among the species of the genus *Aspergillus*, representing 19.92% of the total collection of *Aspergillus* isolates. This is consistent with [58] This molds is more than other fungi.

The results showed that the genus *Candida spp*. came in second place in terms of total frequency. as its frequency reached 14% of the total number of isolates, which is higher than the rate recorded by [56] in Basra, which was 1.4%.

Have indicated [59,60] that humidity, hot weather, and the health status of the patient are factors that contribute to the infection of various areas of the body by *Candida spp*. As in table [2] and figure [1]. As the type of *Aspergillus spp.* appear in figure [2,3].

Table 2: Number and Percentage of fungi specie from clinical cases

Fungi species	Isolates No [%]
Aspergillus flavus	19.92
A. terreus	16.95
A. niger	12.95
A. Candidus	11.44
Penicillium	10.52
Candida albicans	14.22
Candida spp.	14
Total	100%

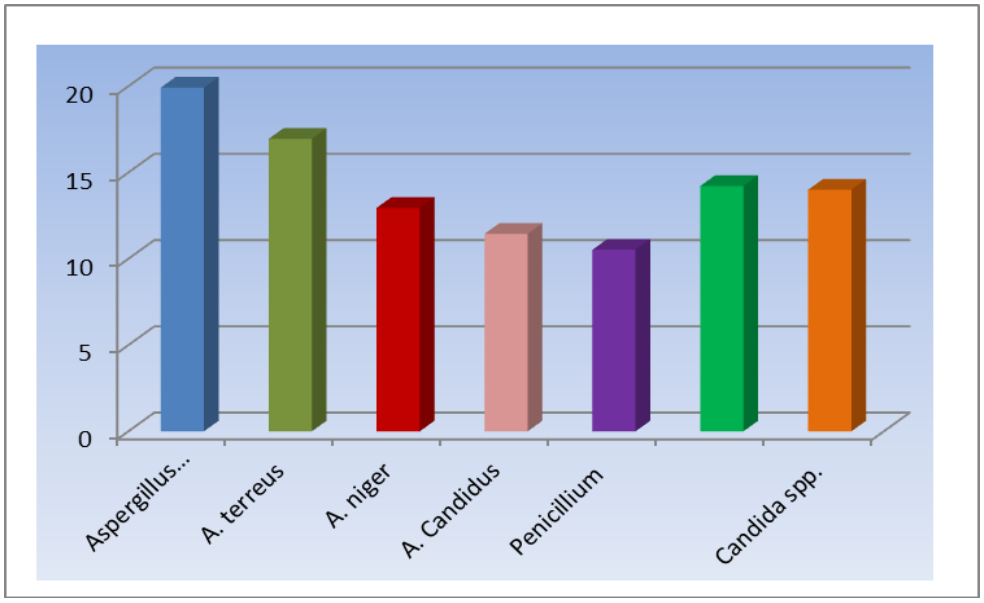

Fig. 1: The total percentages of the fungi isolates

In this study also was studied the relationship between infection rates and residential location, and it was found that people living in cities are more susceptible to infection than those living in rural areas especially in male, in this study we found that Otomycosis occur more commonly in female [especially housewife] than male and our finding confirmed the results other researchers have reported [61,62]. The results showed that the age group between 21-30 years had the highest infections for all etiologic agents, whether the causative was yeast or molds [63,64]. As in figure [4,5],due to environmental concerns, such as ongoing construction and improper waste disposal, which not only provide a suitable environment for the fungus to grow in the form of rotting plants and vegetables, but also provide a means for its spread in the form of dust particles, as swimming has been observed to be a predisposing factor for otomycosis as seen in a study, with higher prevalence recorded in the summer season This is consistent with [65,66]. But in the rural, female are more susceptible to ear infections due to using home remedies to get relief and use Mustard oil has also been reported as a common risk factor for ear infections used to relieve itching in the ear, this is agreement with [67,68]. As in figure [6]. Fungal Otitis Externa [Otomycosis] Associated with *Aspergillus Flavus* agree with [69].

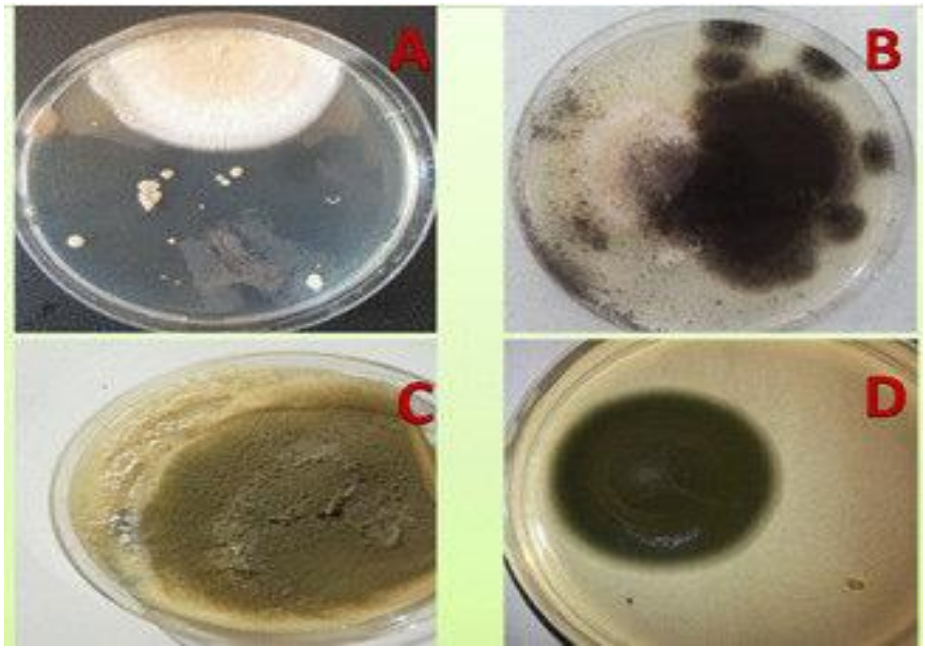

Fig. 2: *Aspergillus spp* grown on SDA at 37°C after 7 days of incubation. A: A. *terrus,* B: *A. niger,* C: *A. flavus,* D: *A. fumigatus* E:

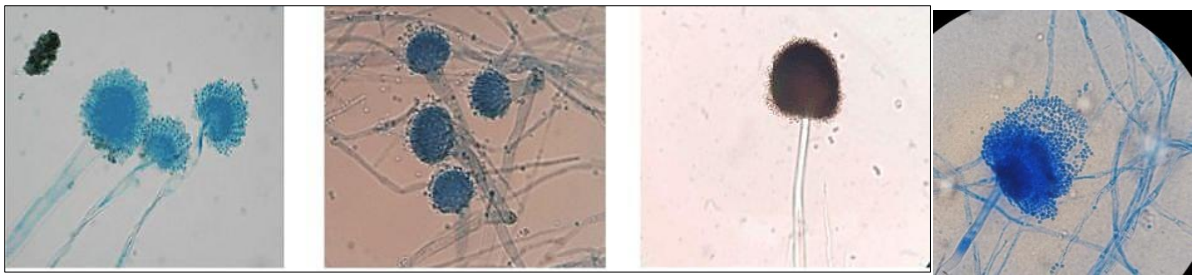

Fig 3: *Aspergillus spp.* Appear under microscop 40 Xdyed by lactophenolcotton blue. A: A. *flavus,* B: *A. fumigatus,* C: *A. niger,* D*: A. terrus*

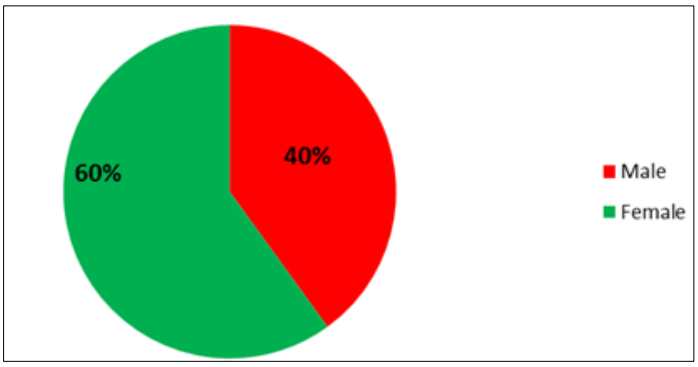

Fig. 4: Sex distribution of study subjects

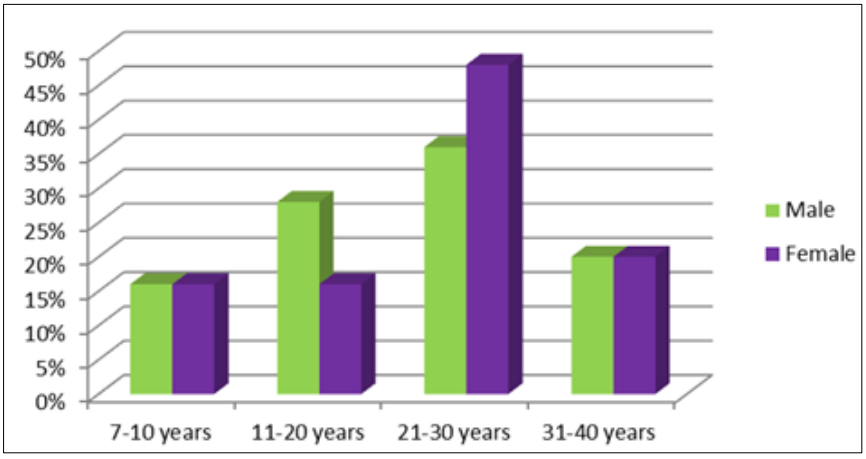

Fig. 5: No. of positive percentage infection according to the age of patients

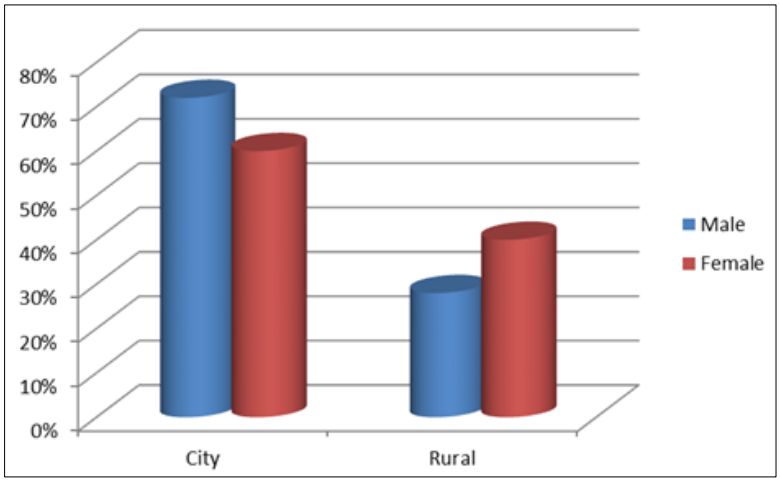

Fig. 6: Sexes distribution of infections by place of residence

4. CONCLUSION

The results showed that the incidence of otitis media in females was higher than males in the group of patients aged between [21-30] years. The results of this study revealed that the incidence rate in females was higher than males in patients with hypertension, diabetes and cancer, reaching 12 [48%], 9 [36%] and 4 [16%] respectively. It also revealed that the infection rate for males and females, especially in the city, was higher than in the countryside, as the percentage of males reached 18 [72%] in the city and 7 [28%] in the countryside, and females 15 [60%] and 10 [40%] male.

5. DECLARATIONS

Ethics approval and consent to participate

The study received ethical approval from the ethics committee of the University of Babylon [Approval No. Z 221201, Date.2025.03.12] and was conducted in accordance with the principles of the Helsinki Declaration.

Authors' contributions: All authors contributed to the article, read and agreed approved the submitted version.

Conflict of Interests: The authors declare that they have no competing interests.

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Availability of data and material

All data and materials are available in the paper and supplemental files

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