



Mycobiota of Ear Canals Associated with Recovered Individuals from Covid-19 and Vaccination Based on Molecular Techniques



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Abstract

Background: The human body harbors diverse mycobiome and is often neglected as a potential cause of the disease and may network with various sites of the human body. **Objective:** The purpose of the present study was to investigate the fungal flora of ears of the recovered individuals from COVID-19 and its vaccination. **Methodology:** The study involved 80 adults who attended Imam Hussain Hospital in Karbala, Iraq, between November 2022 and January 2023. COVID-19 antibody titers were measured in all participants. In addition, ear swab samples were collected and cultured for fungal isolation, followed by phenotypic and molecular identification, as well as sequence analysis of the isolated fungi. **Results:** The COVID-19 Antibody Titer test was performed after collecting blood from the elbow veins of participants. Depending on the results of antibody titer, individuals were divided into four groups: recovered individuals who were infected and vaccinated, infected and not vaccinated, as well as not infected and vaccinated, and not infected and not vaccinated as controls. Specimens from the external ear canals of split participants were collected for fungal isolation. After applying molecular and morphological techniques for fungal identification, the results revealed that various mycobiota were distributed differently in the four study groups, with merely sharing four species were 28 fungal species and 60 strains were isolated from 160 ear swabs obtained from the external ear canals of 80 recovery individuals as well as controls. **Conclusion:** This study first documents the post-COVID-19 ear mycobiota, adding 80 new sequences globally. Future mycobiota profiling in Iraq is vital to understand disease links, aiding management of upcoming pandemics and vaccine impacts. [*Bangladesh Journal of Infectious Diseases, June 2026;13(1):171-180*]

Keywords: Ear canal mycobiota; recovered COVID-19 individuals; vaccinated individuals; molecular techniques

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Introduction

Varied microorganisms colonize healthy human bodies, known as microbiota, associated beneficially as well as destructively with numerous disease etiologies. Mycobiomes represent 1.0% of microorganisms found in/on the human body but play a prime role in maintaining homeostasis

through the production of antimicrobial peptides and protection against external harmful microorganisms¹. The mycobiome may network with various sites of the human body. The external auditory canals in human ears are a microenvironment for diverse microorganisms. However, limited studies have investigated and

detected the mycobiota of the external ear canals in healthy humans. Around the world, some studies have recognized *Candida albicans*, *C. parapsilosis*, *Malassezia furfur*, and *Aspergillus flavus*, as well as species of *Alternaria*, *Cladosporium*, and *Penicillium* as the fungal flora of healthy individuals². Yet, exploration of the mycobiota of external auditory canals of humans has not been studied in Iraq. These microorganisms may alter from being commensals to pathogens based on the immune system conditions. Molecular markers of fungi like DNA, RNA, and peptidoglycans may be translocated into the bloodstream, leading to the triggering of a systemic immune response and remotely causing disease from the original site of fungal infection³.

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has rapidly spread across the globe, accounting for almost 300 million cases of coronavirus disease 2019 (COVID-19) so far. The SARS-CoV-2 infection cause a reduction in T cells, weakening the immune system and increasing the patient's risk of developing fungal infections⁴. Fungal infections associated with COVID-19 can enhance the pathogenicity and virulence of COVID-19 infection and were estimated to be 40% of COVID-19 cases, leading to the death of the patients⁵. The external ear canals may be one of the ways for COVID-19 to reach and cause middle ear infections. The positive nucleic acid test of SARS-CoV-2 in the middle ear effusion has been demonstrated⁶. In less than a year, scientists have developed many safe and effective vaccines. In Iraq, the Pfizer vaccine was the most preferred⁷. The few investigated studies have shown changes in gut and oral microbiomes with some alterations and proliferations of pathogenic organisms after vaccinations⁸. However, vaccination effects on the mycobiome have not yet been analyzed. The current study is the first report in Iraq and around the world based on our knowledge. The goals of this study were to explore the mycobiota of the external auditory canals in Iraqi healthy and recovered COVID-19 individuals. Also, the study focused on detecting any alterations in the fungal assemblages before and after taking the COVID-19 vaccine using both morphological and molecular techniques.

Methodology

Study Settings and Population: The study involved 80 healthy adult volunteers with various COVID-19 infection and vaccination backgrounds. The involved volunteers had attended the hospital of Imam Hussain in Karbala, Iraq, from November

2022 to January 2023. After agreeing, participants underwent a comprehensive interview to assemble sociodemographic data, medical history, and other relevant aspects.

COVID-19 Antibody Titer test: The COVID-19 Antibody Titer test using the mini-VIDAS (BioMérieux, France) was conducted as described in detail in Hameed et al., 2025⁹ to detect COVID the recovered and non-COVID-infected individuals. Following the acquisition of antibody titer findings, the study groups were divided into four subsets, with 20 individuals for each. These groups were COVID-19 infected with vaccination (IV); and with no vaccination (INV) as well not infected with vaccination (NIV) and with no vaccination (NINV). Ethical considerations were conducted and approved by the local ethics committee, the Al-Qasim Green University, and Karbala Health Directorate (based on the research committee letter 8.10.2022 dated 30.10.2022).

Specimen Processing and Morphological Identification

Identification: The current descriptive study was performed to investigate the fungal flora of the recovered ear individuals from COVID-19 and its vaccination. A total of 160 swabs were cautiously collected from the right and left ears of 80 adult participants under aseptic conditions after being clinically examined and diagnosed. Then, for each individual, the swabs were separately placed into a sterile tube, immediately transferred to the laboratory, and individually cultured on the surface of two Sabouraud's dextrose agar (SDA) plates. The plates were incubated at 37°C and observed daily for the first week with data. After incubation, the fungal growth was purified by transferring into new SDA plates for phenotyping and identification. The Species distribution across the four investigated groups was detected. The frequency percentages of isolated species in each group were estimated as well as in each gender. Percentages of colony forming

Molecular Identification and Bioinformatics

Analysis: The fungal forms of purified cultures were transferred to Eppendorf tubes for DNA extraction. The genomic DNA of 80 isolates was extracted using Fungi genomic DNA preparation kits (Favorgen) following the manufacturer's instructions. Later, the concentration of the extracted DNA was estimated using the Nanodrop and was frozen at 4°C until used. The PCR amplification was completed using the primer pair ITS1 and ITS4, targeting the conserved regions of the ribosomal DNA region (47). The PCR reaction mixtures, cycle parameters, and analyzed products

were performed as described in Hameed et al⁹. The PCR products of 80 isolates from the four investigated groups were purified and sequenced using the Sanger sequencing method by Macrogen Company (Seoul, Korea). The DNA sequences were manually edited using Geneious software 9.0 (3) and collectively identified using the Basic Local Alignment Search Tool (BLAST) based on the specific criteria.

Statistical Analysis: Statistical analysis was performed by Windows based software named as Statistical Package for Social Science (SPSS), versions 22.0 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Continuous data were expressed as mean, standard deviation, minimum and maximum. Categorical data were summarized in terms of frequency counts and percentages. Chi-square test was used for comparison of categorical variables and Student t test was applied for continuous variables. Every effort was made to obtain missing data. A two-sided P value of less than 0.05 was considered to indicate statistical significance.

Ethical Consideration: Ethical considerations were conducted and approved by the local ethics committee, the Al-Qasim Green University, and Karbala Health Directorate (based on the research committee letter 8.10.2022 dated 30.10.2022).

Results

Antibody titer test: The current study of 80 individuals divided into four groups based on COVID-19 and vaccination backgrounds successfully divided the study individuals into the four groups based on the IgG and IgM results.

Species distribution across four investigated groups: The presented results showed there were 28 fungal species and 60 strains isolated from 160 ear swabs obtained from the external canal ears of 80 recovery individuals as well as controls. After being identified morphologically and verified genetically, these fungal species represented 11 genera, 8 families and 9 orders, most of which were related to the phylum Ascomycota.

The sequences of molecular identification species were submitted to GenBank under accession numbers OR673615-OR673676, and OR673715. Many of these were the first submitted Iraqi sequences in the world database. *Penicillium chrysogenum*, *P. polonicum*, *P. crustosum*, *Aspergillus neoniger*, *A. aflatoxiformans*, *Curvularia buchloes*, and *Cladosporium endophyticum* are the first records from humans or the environment in Iraq.

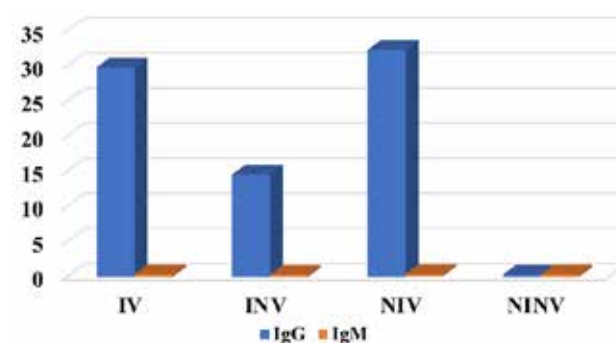


Figure I: The Immunoglobulin results of the COVID-19 Antibody Titer test for the healthy individuals with various COVID-19 and vaccination histories; IV: infected, vaccinated; INV: infected, not vaccinated; NIV: not-infected, vaccinated; NINV: not-infected, not-vaccinated.

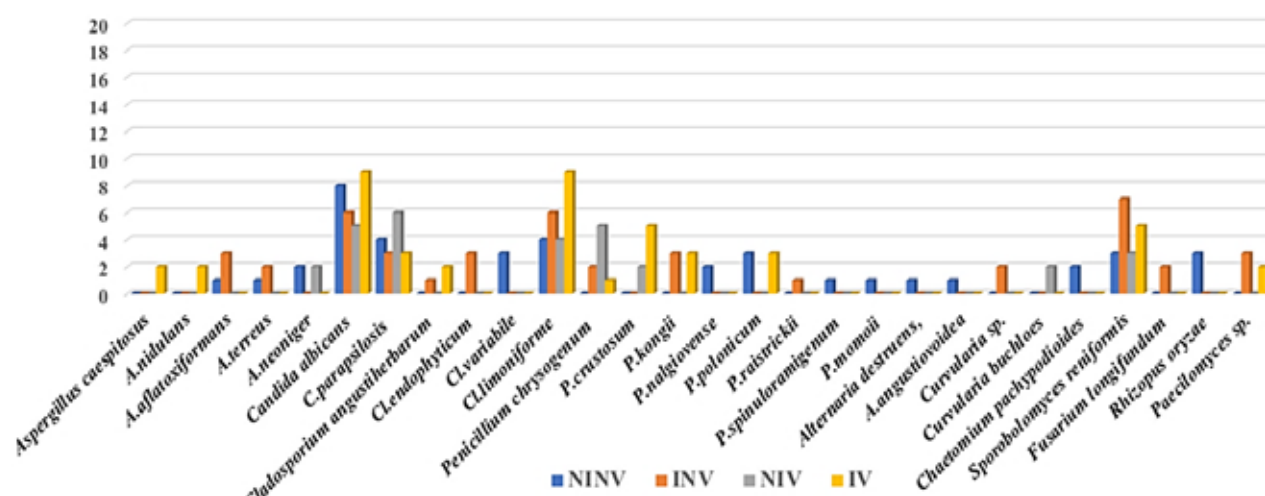


Figure II: Species-distribution groups of the four investigated groups: infected and vaccinated (IV), infected and not vaccinated (INV) individuals as well as not infected and vaccinated (NIV), and not infected and not vaccinated individuals as controls (NINV).

Percentages of species found in the fourth investigated groups

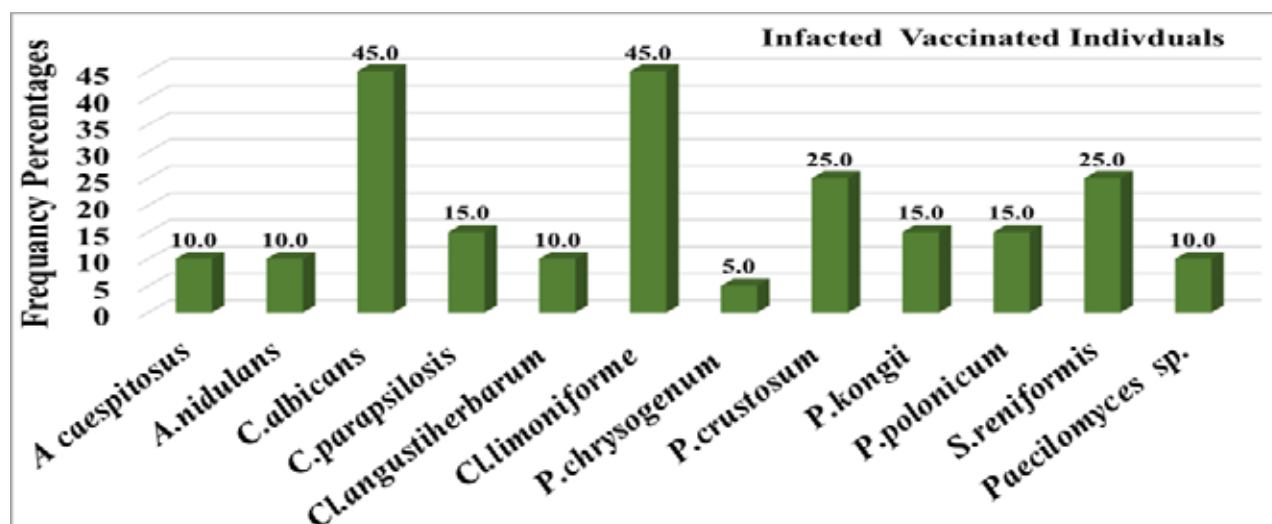


Figure III: Percentages of Species Located in Outer Ear Canals of the Infected and Vaccinated Set

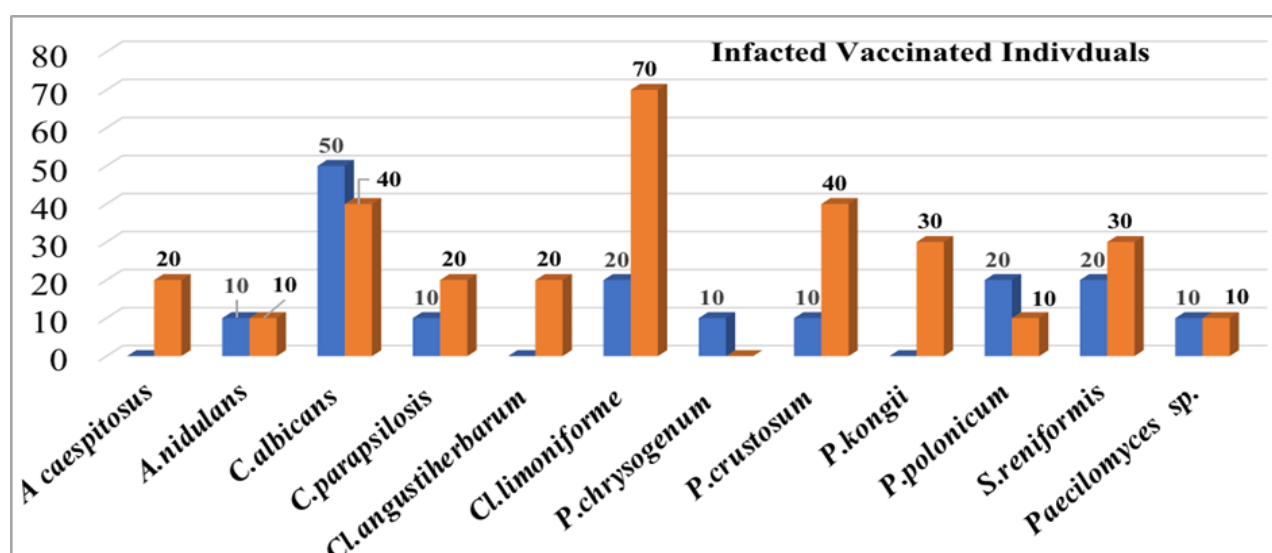


Figure IV: Frequency Percentages of Isolated Species from the Outer Ear Canals of Infected, Vaccinated Individuals. Orange and blue colors presented the species in females and males, respectively

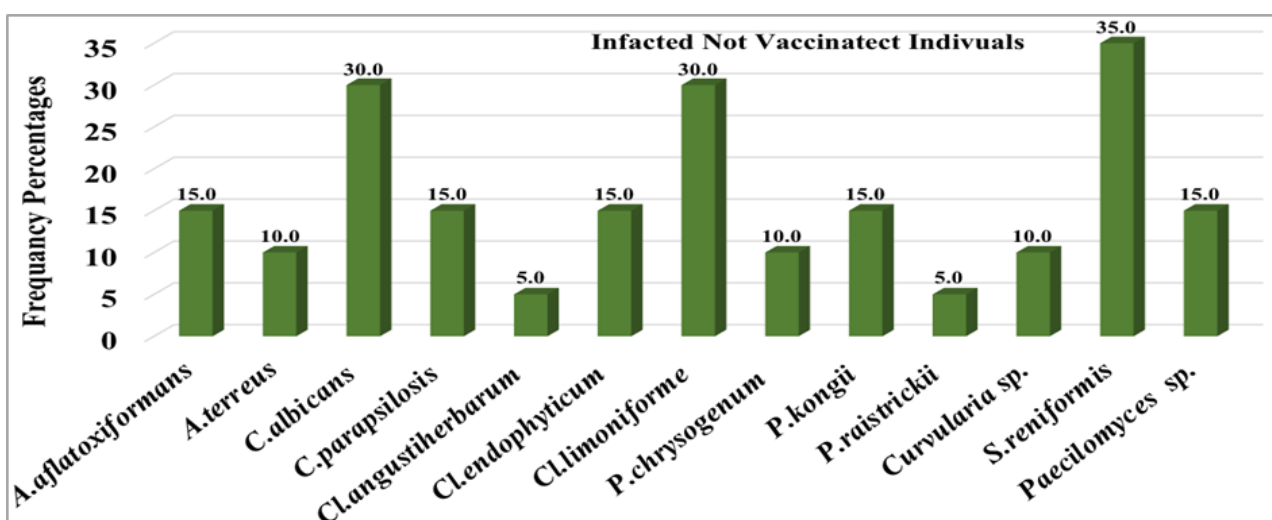


Figure V: Percentages of Species Located in the Outer Ear Canals of Infected and Non-Vaccinated Individuals

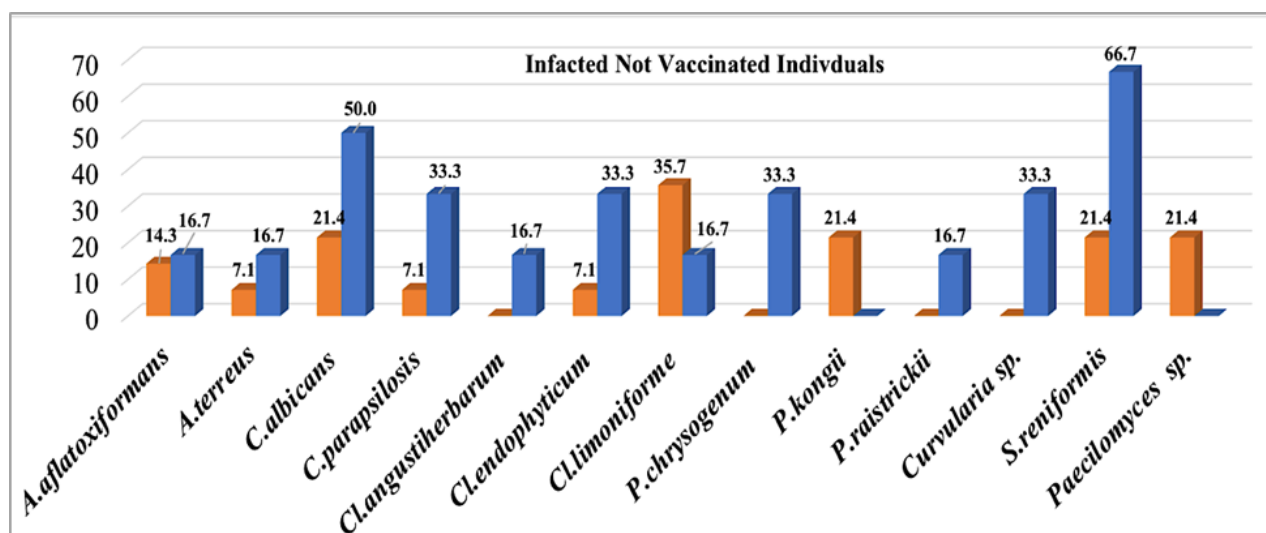


Figure VI: Frequency Percentages of Species Isolated from the Outer Ear Canals of Infected Non-Vaccinated Individuals. The orange and the blue colors represent the fungal species in females and males, respectively

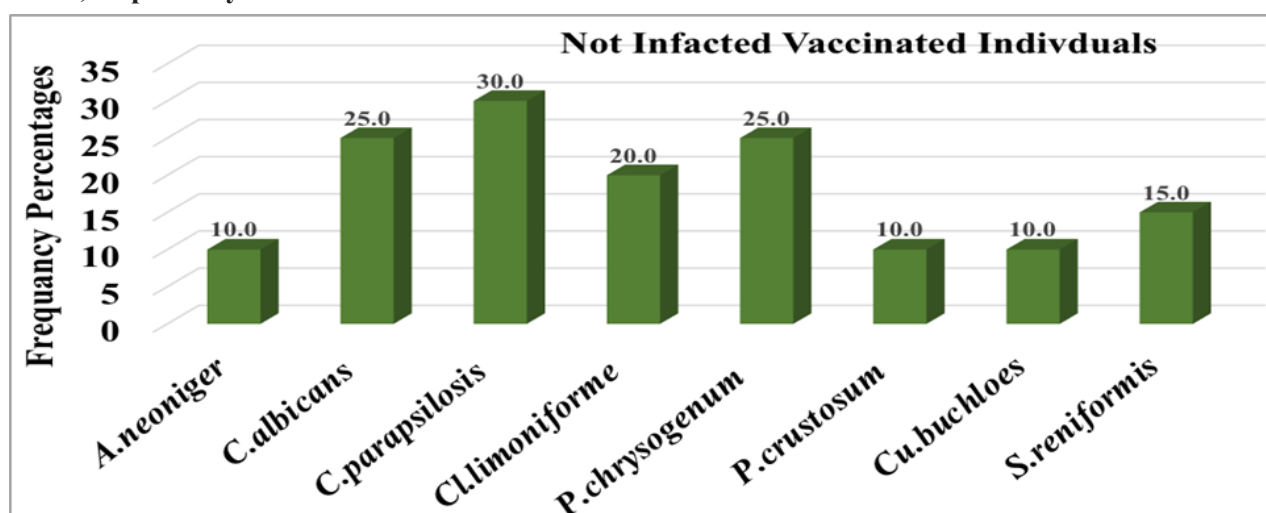


Figure VII: Percentages of fungal species isolated from the outer ear canals of not infected and vaccinated individuals.

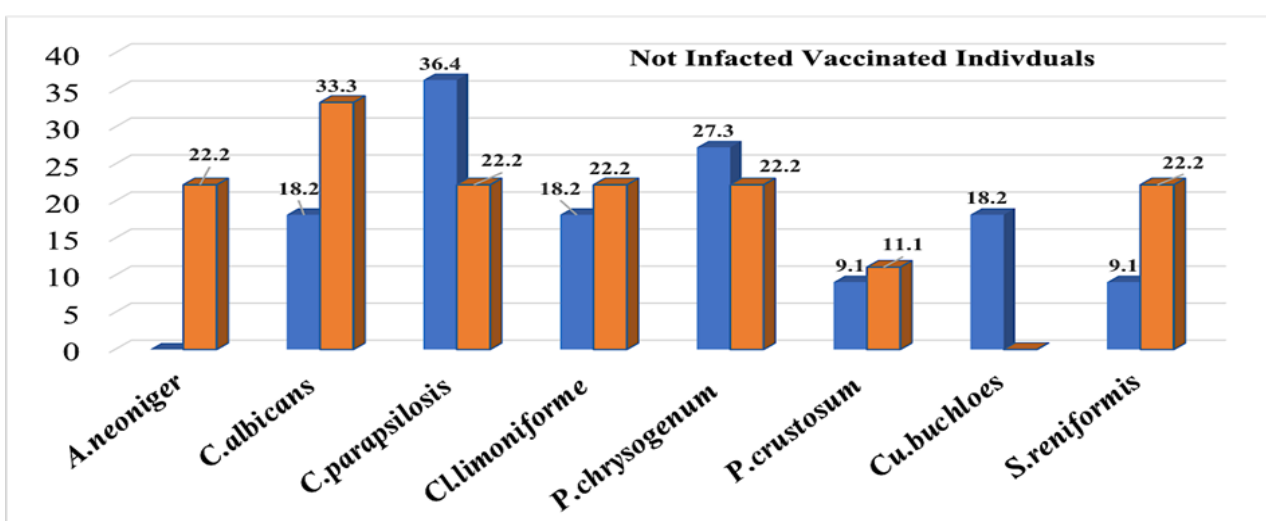


Figure VIII: The frequency percentages of species isolated from the outer ear canals of not infected vaccinated individuals. The orange and the blue colors presented the fungal species in females and males respectively.

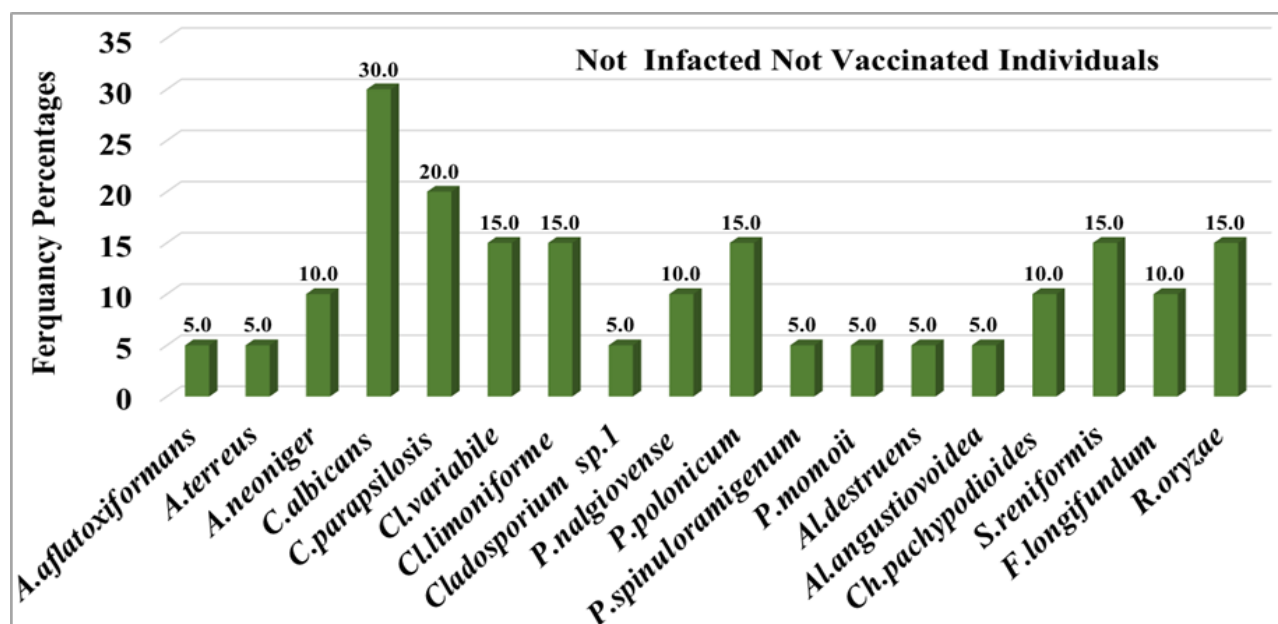


Figure IX: The frequency percentages of identified fungal species isolated from the outer ear canals of not infected and not vaccinated individuals.

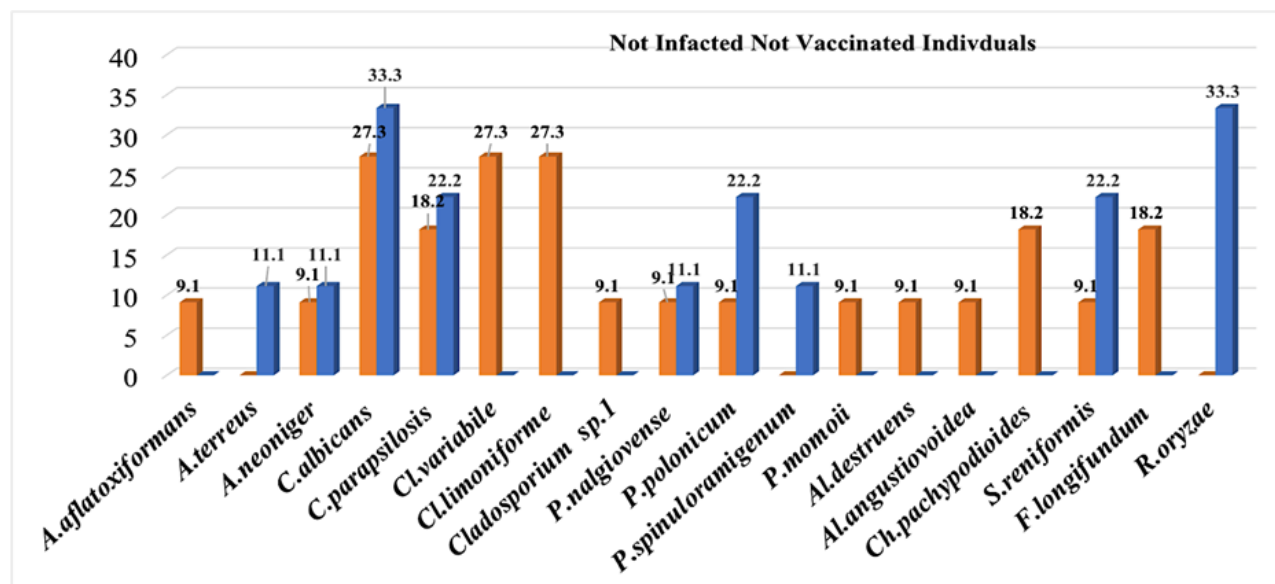


Figure X: The frequency percentages of species isolated from the outer ear canals of Not infected and not vaccinated individuals. The orange and the blue colors presented the fungal species in females and males respectively.

Percentages of colony-forming units for the fungal species among studied groups: The percentages of colony forming unites for the fungal species isolated from the outer ear canals were distinct between the four sets related to their mycobiota (Figure XI). The order of Eurotiales, consisting of *Aspergillus* and *Penicillium*, was the highest in all investigated groups compared to other orders. The infected vaccinated group had more species richness compared to the not infected vaccinated set (Figure XIA). In this group, a total of 57% of the colony forming unites were produced by species belonging

to Eurotiales, with a high proportion of species (Figure IIA). The remaining of colony forming unites was formed by species from Capnoidiales, Saccharomycetales and Sporidiobolales. The infected, not vaccinated sets presented six orders contributing to the colony forming unites (Figure XIB). The order Eurotiales, followed by Capnoidiales and Saccharomycetales accounted for most of the reproductive structures. These related to Eurotiales included six species of *Penicillium* and *Aspergillus*, while other orders had only seven species linked to *Cladosporium*, *Candida*,

Sporobolomyces, and *Curvularia*. There were five orders in not infected vaccinated individuals (Figure XIC).

The Eurotiales was also the most abundant species that occupied 39% of total spore forming unites represented as two species of *Penicillium* with 25% frequency and only *Aspergillus neoniger* with 14% frequency. About 61% of colony forming unites produced by four orders: Saccharomycetales, Capnoidiales, Pleosporales and Sporidiobolales. The figure 11 (D) shows that the *Penicillium* genus has four species with 25% frequencies while *Aspergillus* species were detected as three species with 14 % frequency. On the other hand, the three orders (Saccharomycetales, Capnoidiales and Pleosporales) consisting of one genus. Each has two species. The

other four orders; Hypocerales, Mucorales, Sporidiobolales and Sordariales observed only one genus and only one species.

Discussion

There were no significant differences between IgG values of IV and NIV sets compared to the INV, NINV groups. However, the IgG measurements of INV, NINV sets were statistically varied and lowest compared to IgG levels in the IV, NIV individuals. Also, there were no significant differences between females/males and infection /vaccination statuses with a P-value of 0.431 across the four sets (results not presented). These results agreed with another investigation: vaccination mimicked an infection, and several factors influence responding to vaccines and

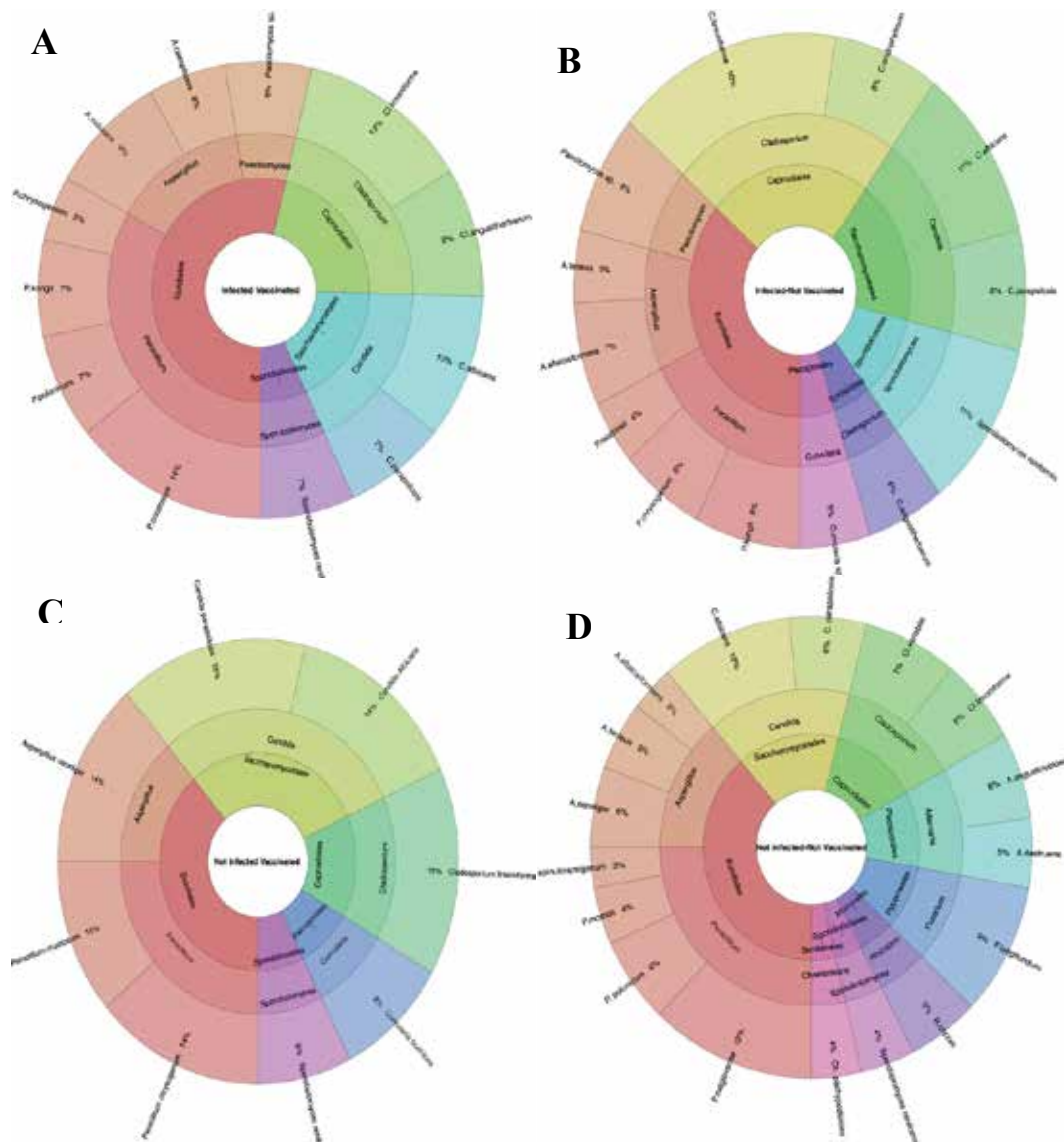


Figure XI: The percentages of colony forming unites for the fungal species isolated from the outer ear canal of individuals infected vaccinated individuals (A) and infected not vaccinated individuals (B). Not infected vaccinated individuals (C) and Not infected not vaccinated (D).

infections¹⁰.

Diverse mycobiota were distributed differently in the four study groups. *Candida albicans*, *Candida parapsilosis*, *Cladosporium limoniforme*, and *Sporobolomyces reniformis* were shared in all investigated groups.

The presented results of the recovered patients who were infected and vaccinated displayed 12 fungal species involved in the structure of their outer ear canals. *Penicillium* was found to be the most diverse genus, with 4 species, followed by *Candida*, *Cladosporium*, and *Aspergillus*. In that fungal structure, *Candida albicans* and *Cladosporium limoniforme* were the highest frequency 45.0% of individuals, followed by *Penicillium crustosum* and *Sporobolomyces reniformis* with a prevalence 25.0% of sampled peoples¹¹. The percentages of species frequency were mostly distributed differently based on gender. *Cladosporium limoniforme* was the highest frequency in 70.0% of females compared to 20% of males, while *C. albicans* had the highest prevalence in 50.0% of males compared to 40.0% of females. Among these species, *P. crustosum*, *Sporobolomyces reniformis*, and *Candida parapsilosis* had the highest frequencies of 40, 30, and 20.0% in females, respectively¹². The results for the recovered patients' group with COVID-19 and without coronavirus vaccination (INV) revealed 13 isolated species. Unlike the IV set, there were 3 species of *Penicillium* and 2 species of *Cladosporium*. *Sporobolomyces reniformis* was the most prevalent 35.0% of individuals, followed by *Candida albicans* and *Cladosporium limoniforme* with a frequency 25.0% each. The interactions between gender and species revealed that most species were isolated from both genders, with the highest prevalence of the common species observed in the male ear canals.

Candida species exist as a normal commensal flora of the human body¹³. *Candida albicans* and *Candida parapsilosis* have been isolated from the normal human external ear¹⁴. However, they are considered opportunistic pathogens. *Candida albicans*, for example, is isolated in 50.0% to 70.0% of the cases of immunocompromised hosts and hospitalized patients¹⁵. *Cladosporium limoniforme* is first recorded in Iraq. The species has been isolated from plants and hypersaline water from a few countries around the world¹⁶. Few species of *Cladosporium* have been reported as etiologic agents in humans; most of its species are isolated from plants and known to be one of the most airborne fungi causing respiratory allergic diseases¹⁷. *Sporobolomyces reniformis* is also the first record in Iraq related to basidiomycetous yeasts. The species was recently recorded in 2020 from plant

surfaces¹⁸. Generally, fungi are ubiquitous with a wide range of enzymatic ability and diverse reproductive strategies that give an opportunity to colonize new habitats. Recent studies on isolation of fungal species from a variety of human specimens suggested there has significantly increased the diversity of known genus species and, potentially, the range of species of clinical interest¹⁹.

The current mycobiota results detected that the number of species was reduced in IV and INV groups, 13 and 12 species, respectively, compared to the NINV group, control, including 18 species. However, the NIV group had 8 species, the lowest diversity. Alterations in the fungal composition of the recovery individuals with COVID-19-infected and vaccinated status were detected, unlike controls, NIV and NINV individuals. When comparing between INV and NINV groups, the results showed a decrease and alteration in the species structure in agreement with observations of the oral fungi in COVID-19 recovered patients²⁰. Nevertheless, an increase in fungal diversity and shifts in the patients with COVID-19 characterized the intestinal mycobiomes from the time of hospitalization until discharge, affecting disease progression²¹. Compared to other groups, the highest prevalence of *C. albicans* in the recovered IV group may be related to the prior COVID-19 infections, in agreement with the oral mycobiome report²². Unlike the previous report, the *Cladosporium* species was higher in both the recovered IV and INV groups. Certain populations, such as older adults, immunocompromised people, and those on immunomodulation drugs, may have reduced levels of protection after both vaccination and infection, leading to changes in the occurrence percentages of fungal flora and/or promoting secondary fungal infections²³. As verified from the four assemblies in the current study, *Cl. limoniforme* may be a common species in indoor and outdoor environments in Iraq. This species has been isolated previously in a few countries since being first recorded in 2015 from plant materials and hypersaline water. Alterations in fungal assemblages of IV and INV groups may relate to the potential influence of vaccination on general human health and the imbalance between fungal invasiveness and the immune reaction²⁴. The prevalence of some species such as *Penicillium chrysogenum* isolated from the IV, INV, and NIV sets may decline the numbers and frequency of observed species due to their antibacterial and antifungal activities²⁵. Similarly, *Penicillium crustosum* was recovered from IV and NIV subjects whilst *Penicillium polonicum* was isolated from IV and NINV individuals. *Penicillium*

crustosum was collected from indoor dust of Mosul buildings and poultry feed in Iraq *Penicillium polonicum* was isolated from soil and plant as endophytes²⁶.

In this study, unique species were also documented for specific individual groups. For example, *Aspergillus caespitosus* and *Aspergillus nidulans* were also isolated merely in the IV set whilst *Aspergillus aflatoxiformans* and *Aspergillus terreus* were recovered from INV and NINV groups. Similarly, *Aspergillus neoniger* was detected in both NIV and NINV individuals. Isolation of species known to be human pathogens may provide an indicator that the mycobiota of groups were related to previous COVID-19 infection and candidate to development of COVID-19-related diseases. For example, *Aspergillus nidulans* is an opportunistic fungal pathogen in patients with immunodeficiency²⁷. It is important to note that *Aspergillus* species are commonly associated with ear infections. In fact, it has been reported that 90% of fungal infections of the human ear are associated with *Aspergillus*²⁸. *Aspergillus terreus* is air born fungus and the most common *Aspergillus* species can cause *aspergillosis* in the respiratory system and is isolated from human and sheep respiratory infections in Al-Qadisiyah Province²⁹.

The conditions of the immune system and its response to infections or even vaccinations have a crucial influence on detecting and be colonized by fungal propagules and are structured by mycobiota compositions. Across the four studied groups in Iraq, the highest percentage frequency of colony forming unites was species related to Eurotiales, a large order of Ascomycetes and the most abundant group³⁰. although its species were distributed differently. Most of the isolated species are *Aspergillus* and *Penicillium*, widely dispensed as the indoor air, human pathogens and mycotoxin producers, as well as antibiotic producers or pharmaceuticals³¹.

Although the species frequency was altered, both Capnodiales, Saccharomycetales were the second most abundant species, contributing to structuring fungal compositions by colony forming unites. The introduced species in the current results belong to genera with species richness, found in almost every cubic meter of air and produce airborne conidia in tremendous numbers. With the proliferation of a single mycelial endpoint into multiple spore-forming cells and spores in dry chains, for example, they play an essential role in that allocation³². Most of them produce single- or multicelled survival spores, are heat- and UV-resistant, and are able to germinate under low biological requirements with highly

enzymatic activity³³. The conditions of the immune system and its response to infections or even vaccinations have a crucial influence on detecting and be colonized by fungal propagules and are structured by mycobiota compositions³⁴.

Conclusion

In conclusion, this study is the first in Iraq and successfully divided the study individuals into four groups based on the IgG and IgM results. Later, the mycobiota of the human external ear canals and its association with COVID-19 and its vaccines was profiled and provided valuable insights into the potential impacts of COVID-19 infection and vaccination on fungal colonization after recovery. Many of the identified species were recorded for the first time and uploaded molecularly to the NCBI database from Iraq. The comprehensive analysis of mycobiota composition and its association with diseases of the human body in Iraq is urgently needed in case of future pandemics.

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None

Conflict of interest:

The Author has declared no conflict of interest in this work.

Financial Disclosure

No funding is received for this work.

Authors' contributions

The whole work was done by Sulaf Adil Chyad, under the supervision of Dr. Rajaa Abdulrazzaq Al Anbagi.

Data Availability

Any questions regarding the availability of the study's supporting data should be addressed to the corresponding author, who can provide it upon justifiable request.

Ethics Approval and Consent to Participate

All experimental procedures for animals were conducted in accordance with approved ethical controls and after obtaining approval at Al-Qasim Green University, College of Biotechnology, under approval number: UOK.VET.BI.2026.177.

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