Iraqi Journal of Biotechnology, 2025, Vol. 24, Special Issue 2, 192-199

Molecular and Microscopic Diagnosis of Pathogenic Fungi from Patients with Respiratory Disease in Baghdad

[1]Teba Saad Akram, [2]Teeba Hashim Mohammad

[1]Department of Biology, Collage of Science for Women, University of Baghdad, Baghdad, Iraq
[2]Department of Biology, Collage of Science for Women, University of Baghdad, Baghdad, Iraq

Received: February 20, 2025 / Accepted: May 13, 2025 / Published: November 16, 2025

Abstract: This study aimed to isolate and identify fungal species from patients exhibiting symptoms of asthma, bronchial allergy, and chronic respiratory diseases. Samples were collected from patients attending several hospitals in Baghdad-Baghdad Teaching Hospital (Medical City), Al-Kadhimiya Teaching Hospital, Al-Furat Teaching Hospital, and Al-Yarmouk Hospital-between February and May 2024. A total of 203 samples were obtained from patients diagnosed with respiratory conditions by specialized physicians. Twenty-two fungal isolates were identified, including 15 from blood samples and 7 from sputum samples. The patients included 16 males and 13 females, aged between 24 and 70 years, Fungal identification was initially conducted using light microscopy and biochemical tests based on standard taxonomic keys. Further confirmation was achieved through molecular diagnosis using PCR. Seven fungal isolates were selected for molecular analysis, revealing four isolates of *Penicillium chrysogenum*, one *Aspergillus ochraceus*, one *Penicillium consobrinum*, and one *Alternaria alternata*. PCR amplification targeting the 18S rRNA gene produced specific bands at 476 bp, 612 bp, and 720 bp, confirming the identification. DNA sequencing of the PCR products showed 100% similarity to reference strains in the NCBI BLAST database, confirming the accuracy of the fungal identification.

Keywords: pathogenic fungi, respiratory infections, fungal taxonomic keys, PCR technology.

Corresponding author: (Email: teba.saad1602d@csw.uobaghdad.edu.iq)

Introduction

Fungal infections of the respiratory system rank third after bacterial and viral infections in terms of prevalence and clinical impact (1). The incidence of allergic and chronic respiratory diseases has been increasing, particularly in industrialized countries, despite advances in medical science (2). Globally, respiratory diseases account for nearly one-third of infection-associated deaths (3). However, fungal respiratory infections are often underreported and overlooked in clinical diagnostics (4).

Fungi, whether endemic or opportunistic, can cause significant pulmonary complications. Opportunistic fungal infections are especially prevalent among immunocompromised individuals, including those with chronic conditions such as asthma, tuberculosis, bronchitis, and various malignancies (5). Several host-related factors such as age, gender, immune status, diabetes, obesity, and use of immunosuppressive drugs contribute to fungal susceptibility (6). Fungal spores are ubiquitous in the environment and can enter the lungs through inhalation. During high-spore seasons, airborne concentrations may exceed 50,000 spores/m^3, increasing the risk of both

community-acquired and nosocomial infections (7). Once inhaled, spores can disseminate into alveolar spaces and bloodstream, triggering inflammatory responses. Like other pathogens, fungal infections follow a cascade of pathogenic steps: adherence, invasion, proliferation, and immune evasion (8).

Traditional phenotypic diagnosis, though valuable, is time-consuming and highly dependent on the skill of the microbiologist and environmental growth conditions (9). In contrast, molecular techniques, particularly PCR and DNA sequencing, offer rapid, specific, and sensitive alternatives for fungal identification (10). DNA barcoding using conserved genetic markers such as the internal transcribed spacer (ITS) region has emerged as a robust method for distinguishing fungal species, even among closely related taxa(11). PCR enables the amplification of target DNA fragments in a matter of hours, reducing diagnosis time significantly (12). Therefore, this study aimed to isolate and identify fungal species from patients with respiratory diseases using both conventional and molecular diagnostic techniques.

Materials and Methods

Samples Collection

A total of 203 clinical samples were collected from patients diagnosed with respiratory diseases by specialist physicians in Baghdad hospitals between February and May 2024. The samples included blood and sputum specimens. Blood samples (5 mL) were collected under aseptic conditions using EDTA tubes and stored in cold sterile boxes until cultured. Sputum samples were collected in sterile containers after fasting for 4–6 hours, sputum.

Blood Samples

Blood samples were collected by drawing blood from the vein area. This method was done after the specialist doctor diagnosed people suffering from respiratory diseases such as tuberculosis, asthma, bronchitis, COPD, sinusitis, and pneumonia. The blood drawing area was sterilized with a sterile cotton ball with 70% ethyl alcohol after wearing gloves and drawing 5 ml of blood with a needle and placing the blood in a test tube, which is an ETDA tube. After that, the tube was placed in a cold, sterile box until planting on SDA medium for a maximum period of one day, each patient's information was recorded on his own blood tube.

Sputum Samples

Sputum samples are taken from the patient after ensuring that he has not eaten for a period ranging between 4-6 hours. The patient is given a sterile, tightly closed tube in which he places a small sample of sputum. The patient’s information is recorded on it and it is placed in a sterile box and cultured directly on SDA medium.

Sample Culture

Blood samples taken from patients were cultured by placing an SDA medium in a sterile dish, then placing it over the patient's blood sample and allowing it to solidify, then transferring it to the incubator. As for sputum samples, the sample was taken from the tube in which the sample was collected using a sterile loop and the sample was placed on a dish containing a medium that was prepared and stored in the refrigerator, the dishes were kept in the incubator at room temperature (25-28) °C for a period of (5-7) days with continuous monitoring (11).

Direct Microscopic Examination

To examine blood and sputum samples for fungal presence, a small portion of the fungal growth from cultured samples was directly observed. The dishes were examined after 7 days of culturing on Sabouraud Dextrose Agar (SDA) to identify developing fungal colonies. Morphological

identification of the isolates was conducted using taxonomic classification keys (12, 13, 14), based on the characteristics of colony shape, color, texture, and conidial head structure. Additionally, the shape, color, and size of conidial heads were observed.

For accurate diagnosis, a direct microscopic examination was performed using the glass slide culture technique. A drop of distilled water was placed in the center of the slide, followed by transferring a small section of the fungal growth using a sterile loop. A cover slip was placed gently on top, applying minimal pressure. The fungal species were identified and classified based on their microscopic characteristics. The frequency and percentage of each fungal species were calculated using two formulae (15).

$$\%appearanc = \frac{No.of\ samples\ species\ appeared}{Total\ number\ of\ samples} \times 100$$

$$\%frequency = \frac{No.of\ single\ species\ isolates}{Total\ number\ of\ all\ isolates} \times 100$$

Molecular diagnosis

PCR, the primary DNA amplification technique, uses genus-specific primers to pinpoint distinct genes, providing a dependable and rapid means of identifying clinical samples with enhanced precision and sensitivity. PCR methods exhibit versatility in identifying an extensive array of organisms through specific primers that amplify minute gene segments (9).

Detection ITS1 L and ITS4 gene using PCR

The extracted DNA of fungi Isolated by polymerase chain reaction using forward TCCTCCGCTTATTGATATGC-3`with a yield of 102 bp fragment. While the forward primer for the reverse primer was 5' CCAAATAGTGACGAGTTAGG-3', with a yieldof164 bp fragment. All primers were designed according. Primer selection

The primer was used for detection of ITS gene as Table (1).

Table (1): Primer sequences of ITS1 and ITS4 gene.

Primer Name	Sequences	Annealing Temp. (°C)	Product Size (bp)	Reference
ITS1	5`-TCCGTAGGTGAACCTGCGG-3`	55	~500-700	16,17
ITS4	5`-TCCTCCGCTTATTGATATGC-3`			

These primers were supplied by Microgen Company in a lyophilized form. Lyophilized primers were dissolved in a nuclease free water to give a final concentration of 100pmol/µl as a stock solution. A working solution of these primers was prepared by adding 10µl of primer stock solution (stored at freezer -20 C) to 90µl of nuclease free water to obtain a working primer solution of 10pmol/µ.

Table (2): Preparing of the Primer.

Primer Name	Vol. of Nuclease Free Water (µl)	Concentration (pmol/µl)
ITS1	300	100
ITS4	300	100

The primers which used in PCR for detection fungi Were designed in this study by using NCBI-Gen bank and primer2 plus design online, fungal incubated 37°C and cultured in Sabouraud dextrose broth (SDB) for

24 and 48 h before proceeding to the DNA extraction by Kits used to extract the DNA equipped by the company Promega U.S. the Amplification reaction mixture PCR master mix was prepared by using (AccuPower PCR Premix Kit) and this master mix was done according to company instructions. Finally, the PCR products were analyzed by agarose gel electrophorese (16)

DNA Extraction

DNA concentration and purity were measured using a Quintus fluorimeter. All the isolates had DNA concentrations ranging between 45–97 ng/μL, and the DNA purity was 1.7–2.0.

Reaction Setup and Thermal Cycling Protocol

The components of the PCR mixed reaction mentioned above were put in a typical PCR tube together with PCR Premix, a lyophilized substance that contained every other component needed for the PCR reaction. After that, the tube spent three minutes in an Exispin vortex centrifuge. Proceed to the Mygene PCR Thermal Cycler after that, table (3).

Table (3): PCR Reaction Components.

No. of Reaction	3	**Rxn**	Annealing temperature of primers	55
Reaction Volume /run	25	ng/μL	No. of PCR Cycles	30

PCR Program

A standard PCR thermal cycler system was used to accomplish the PCR thermos cycler conditions for the gene, as indicated in the accompanying table (4). In this study, DNA sample of fungal isolates has been selected to detect the ITS1 and ITS4 diagnostic gene. The PCR products have been confirmed by the analysis of the bands on gel electrophoresis (1.5% agarose at 75 Volt for one hour) and by comparing their molecular weight DNA ladder.

Table (4): Condition of PCR reaction.

Steps	Temperature	Time	Cycle
Initial Denaturation	95	05:00	1
Denaturation	95	00:30	30
Annealing	55	00:30	
Extension	72	00:30	
Final extension	72	07:00	1
Hold	10	10:00	

Standard Sequencing

PCR product was sent for Sanger sequencing using ABI3730XL, automated DNA sequences, by Macrogen Corporation – Korea. The results were received by email then analyzed using generous software (17).

Results and Discussions

Fungi Isolated from the blood

Table (5) refers to fungi isolated from the blood throughout the study period, where the table reveals that the percentage of the appearance of a fungus *Penicillium chrysogenum* 32.34% is the highest among the isolated fungi, followed by a fungus *Penicillium verrucosum* with a repetition rate 14.7% (table 5). These findings are consistent with (18) where *Penicillium spp*. had the highest rate of appearance when isolated from blood, indicating the pathogenicity of this fungus, as *Penicillium spp*. may analyse blood and cause systemic illnesses (19).

Table (5): Types of Fungi isolated from the blood specimen.

No.	Isolation Type	Occurrence	Frequency	Percentage of Frequency
1	*Penicillium verrucosum*	4	15	14.7%
2	*Penicillium marneffei*	1	6	5.88%
3	*Penicillium chrysogenum*	4	33	32.34%
4	*Yeast*	1	1	0.98%
5	*Alternaria alternata*	2	4	3.92%
6	*Paecilomyces spp.*	1	3	2.94%
7	*CladosporiumCladosporioites*	2	6	5.88%
8	*Blastomyces Dermatitidis*	1	2	1.96%
9	*Trichderma Spp.*	1	1	0.98%
10	*Stemphylium*	1	5	4.9%
11	*Hortaea Werneckii*	1	3	2.94%
12	*Aspergillus Terreus*	2	7	6.86%
13	*Mucor Irregularis*	2	2	1.96%
14	*Curvularia* spp.	1	2	1.96%
15	*Aspergillus Ochraceus*	1	8	7.84%
	Total	25	98	

Table (6) also indicates fungus isolated from individuals with respiratory illnesses who visited Baghdad hospitals. The data suggest that yeasts had the highest frequency rate*: Candida albicans* 29.38%, *Candida aruis* 20.34%, *Candida kruse* 10.17%, *Candida tropicals* 10.17%, and *Candida glabrata* 7.91%. These findings are similar with (20). The genus Aspergillus spp. came in second, with species *Aspergillus terreus* appearing at 18.08% and *Aspergillus ochraceus* at 1.13%. This result is consistent with (21), as the study demonstrates the emergence of many types of fungi in people with respiratory diseases, as these fungi are opportunistic fungi that exploit viral infection and weaken the immune system, allowing them to penetrate into weak places in the body, such as the respiratory system. If the body resists.

Table (6): Types of Fungi Isolated from Sputum specimen.

No.	Isolation Type	Occurrence	Frequency	Percentage of Frequency
1	*Penicillium Chrysogenum*	1	8	9.04%
2	*Alternaria alternate*	2	2	2.26%
3	*Aspergillus Terrus*	4	16	18.08%
4	*Mucor Irregularis*	1	1	1.13%
5	*Aspergillus Niger*	6	11	12.43%
6	*Curvularia*	2	4	4.52%
7	*Aspergillus Ochraceus*	1	1	1.13%
8	*Candida tropicals*	1	9	10.17%
9	*Candida albicans*	5	26	29.38%
10	*Candida glabrata*	1	7	7.91%
12	*Candida. kruse*	1	9	10.17%
13	*Candida aruis*	1	18	20.34%
14	*Coccidioides immitis*	1	1	1.13%
	Total	27	113	

Extraction of Genome DNA

To verify genomic DNA isolation, PCR products were separated and electrophoresed on 1.5% agarose gels (w/v) in 1x Tris-borate buffer at pH 8.3. Under UV light, genomic DNAs

appeared as a smear with varying molecular weights. The amplified DNA bands were seen using ethidium bromide staining and photographed under ultraviolet light fig. (1). DNA from isolated strains was effectively retrieved. Electrophoresis analysis validated the PCR amplification results on all isolates' retrieved DNA, the primer effectively amplified ribosomal DNA in all investigated strains, yielding around 600 bp products in fungus, Figure 1 shows that PCR technique may identify species-specific DNA polymorphisms in fungi using agarose gel electrophoresis (22).

PCR showed good specificity while screening blood samples, the use of PCR to detect fungus DNA has been criticized for its potential invalidity due to the prevalence of molds in the environment and contamination the risk of contamination (23,24).

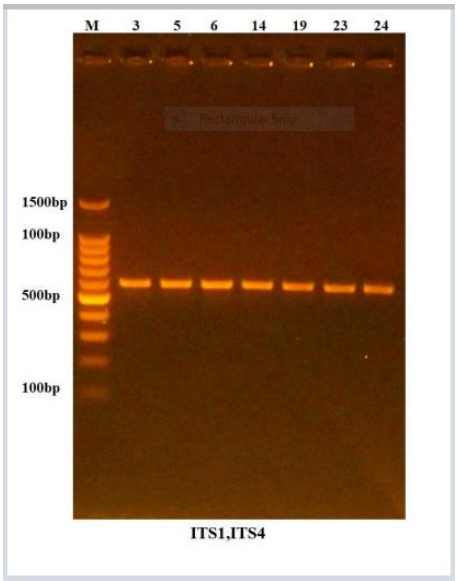

Figure (1): Gel electrophoresis of ITS gene of fungal isolates using (1.5%agarose gel electrophoresis, 75 Volt for 1 hour). M: 100bp ladder marker. Lane 1-7 resemble PCR products with 600 Pb.

The Result of BLAST DNA sequencing:

The genomic DNA was analyzed for the primers of each of the diagnostic gene and the coding gene Forward and reverse, The (ITS1, ITS4) genes sequencing with molecular weights (600pb) respectively, service to Hitachi Company (Japan) the results available on the global website of the gene bank (National Center for Biotechnology Information) (NCBI) showed 100% close identity of the isolates with respect to NCBI blast reference isolates) as Table (7).

Table (7): Identity of The Fungi Isolate from Hitachi Company (Japan) To NCBI BLAST.

Local Isolate of Fungi Isolate NCBI-BLAST of Fungi					Identity %
No.	**Isolation Type**	**Size (bp)**	**Country**	**Access number**	**Identity %**
1	*Penicillum chrysogenum*	571	Japan	PP101523.1	99.81%
2	*Penicillum consobrinum*	571	Japan	MG490873.1	100.00%
3	*Aspergillus Ochraceus*	571	Japan	OM952173.1	98.81%
4	*Alternaria alternata*	571	Japan	PP967930.1	100.00%

The introduction of DNA amplification using PCR has enabled the creation of rapid and extremely sensitive detection, in vitro, conventional PCR allows for the amplification of DNA or RNA via

repeating cycles. This technology has been viable for microbiology organism diagnosis since the early 1990s due to the determination of many particular DNA fragments (25) ,The PCR methodology is superior to other parasitological procedures such as microscopy or culturing, especially for samples with low parasite burdens ,PCR can also be used to quantify parasites and monitor illness progression, as well as predict and control therapy outcomes (26). Fungal species identification is critical for making informed therapeutic decisions about the importance of a specific isolate as well as the dosage and duration of antifungal medication. Culture and microbiological determination of the species of fungi from clinical material normally require up to several days, while DNA extraction and amplification of the PCR results and diagnosis of the fungal species can be achieved within 12 hours(27).

Ethical approval

In Iraq, this study was approved by the Ethical Committee, Department of Biology. College of Science for Women, University of Bagdad and the Iraqi Ministry of Health, Baghdad, Iraq under the reference number 22/359 in January 14, 2024.

Conclusion

Identification of fungi is important for diagnosis and epidemiological studies that are currently widespread, and for taxonomic and population genetic investigations for drug selection for disease treatment. Our study has demonstrated the usefulness of PCR for the detection of fungal DNA. This study provides accurate molecular detection using universal primer pairs, which can be used individually or in combination with each other to detect the presence of fungi in suspected patients with respiratory problems, so it is preferable to use them in central laboratories instead of classical low-resolution tests, these results indicated the high prevalence of fungi among hospital patients, especially those with weak immunity. Therefore, this method is considered the fastest and most accurate in detecting the presence of fungi.

References

1. Brown, G. D.; Ballou, E. R.; Bates, S.; Bignell, E. M.; Borman, A. M.; Brand, A. C., *et al.* (2024). The pathobiology of human fungal infections. *Nature reviews microbiology*, *22*, 687-704.
2. Storfie, E. R. and Saville, B. J. (2021). Fungal Pathogen Emergence: Investigations with an Ustilago maydis × Sporisorium reilianum Hybrid. Journal of Fungi, 7, 672.
3. AL-Shamary, M. G. (2020). Fungal and Immunological Study for Some Patients, with Aspergillosis Mold That Caused Respirtory System Diseases in Baqubah city. Diyala, Iraq: A Thesis Submitted to the Council of College of Education for Pure Science/Diyala University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biology.
4. Mohammed, S. R. and AL-Jibouri, M. H. (2015). Isolation and identification of fungi from two hospitals in Baghdad city and effect of disinfectants on some fungi. Iraqi Journal of Science, 56(1C), 673*682.
5. AL-Ammari, A. M.; Hussain, A. F. and Ismael, T. K. (2019, 11). Prevalence of Fungal Pathogens among Respiratory Tract Infections. Fungal Pathogens in RTIs, 22(8).
6. Alghazali, N. A. (2022). Isolation and identification of the fungi Rhizoctonia solani and Fusarium oxysporum that cause diseases of the roots and bases of the stems of periwinkle plants (Cartharanthus roseus) in some nurseries and their biological and chemical control. Karbala, Iraq: A Thesis Submitted to The Council of the College of Agriculture / University of Karbala as Partial Fulfillment of the Requirement for Degree of Master of Sciences in Agriculture – Plant Protection.
7. Owaid, R. S. (2019). Molecular study of most prevalent fungi isolated from human and horses. Baghdad, Iraq: A Thesis Submitted to the Council of the College of Veterinary Medicine at the University of Baghdad in Partial fulfillment of the Requirements for the Degree of Master

Science in Veterinary Medicine\ Zoonotic Diseases.
8. Mashari, H. R. (2024). Isolation and Identification of Fungi from Human, Sheep carcasses and Equipments in Baghdad Slaughterhouses. Baghdad, Iraq: A Thesis Submitted to the Council of the College of Veterinary Medicine/ University of Baghdad, as a Partial Fulfillment of the Requirements for the Degree of Master.
9. Latteef, N. S. (2024). Molecular Detection of Some Salmonella spp. in Chicken from Local Markets in Baghdad. Iraqi Journal of Biotechnology, 23(3), 165-173.
10. Bayram, D. M. and Ali, H. Z. (2017). Molecular Detection of Suspected Leishmania Isolates Using Polymerase Chain Reaction. Journal of Science, 58(4B), 2076-2082.
11. Salah, N. S.; Risan, M. H. and Muhsen, T. A. (2020). Isolation and characterization of Candida glabrata from clinical specimens in some hospitals in Baghdad. Arabian Journal of Scientific Research, 2(10), 1-9.
12. Bulman, S. and Braselton, J. P. (2014). Rhizaria Phytomyxea in The Mycota Systematics and Evolution, VII part A: (2nd ed.). Berlin, Heidelberg: Springer-Verlag, 99-112.
13. Larone, D. H. and Washington, D. C. (2007). Medically important fungi-a guide to identification. American Society for Microbiology.
14. Hawksworth DL, D. L. and Lücking, R. (2017). Fungal diversity revisited: 2.2 to 3.8 million species. Microbiol Spectrum, 5(4), 1-17.
15. Abed, F. D.; Saadedin, S. M. and Shareef, H. K. (2021). Molecular Deduction of Aspergillus flavus Isolated from Wheat Grains in Karbala City. Iraqi Journal of Biotechnology, 20(1), 46-53.
16. Sadeq, A. M. and Ismail, Z. Z. (2024, 3). Microalgae Growth in a Biocathode-Photosynthesis Microbial Desalination Cell: Molecular Characterization, Modeling Study, and Performance Evaluation. Iraqi Journal of Chemical and Petroleum Engineering, 25(1), 1-12.
17. Donnelly, J. P.; Chen, S. C.; Kauffman, C. A.; Steinbach, W. J.; Baddley, J. W.; Verweij, P. E., *et al*. (2020, 9). Revision and Update of the Consensus Definitions of Invasive Fungal Disease from the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium. Definitions of Invasive Fungal Disease, 71, 1367-1376.
18. Farah, Al- Qursh, H. and Ali, I. A. (2023). Molecular Detection of Candida spp. Isolated from Female Patients Infected with COVID-19 in Baghdad City. Iraqi Journal of Biotechnology, 22(1), 238-244.
19. Hasan, A. M. and Abdul Majeed, S. M. (2017). Isolation and identification of opportunistic fungi from patients with different types of leukemia in Baghdad province. Iraqi Journal of Biotechnology, 16(3), 216-222.
20. Chong, W. H. and Neu, K. P. (2021). Incidence, diagnosis and outcomes of COVID-19- associated pulmonary aspergillosis (CAPA): a systematic review. Journal of Hospital Infection, 113, 115-129.
21. Risan, M. H. (2016). Molecular Identification of Yeast Candida glabrata from Candidemia Patients in Iraq. Iraqi Journal of Science, 57(2A), 808-813.
22. Loffler, J.; Hebart, H.; Schumacher, U.; Reitze, H. and Einsele, H. (1997, 12). Comparison of Different Methods for Extraction of DNA of Fungal Pathogens from Cultures and Blood. JOURNAL OF CLINICAL MICROBIOLOGY, 35(12), 3311–3312.
23. MacKnight, K. T. (2004, 1). The Polymerase Chain Reaction (PCR): The Second Generation of DNA Analysis Methods Takes the Stand. Santa Clara High Tech. L.J.; 20(1), 95-164.
24. Lackner, N.; Posch, W. and Flörl, C. L. (2021). Microbiological and Molecular Diagnosis of Mucormycosis: From Old to New. Microorganisms, 9(1518), 2-16.
25. Hariharan, G. and Prasannath, K. (2021, 1). Recent Advances in Molecular Diagnostics of Fungal Plant Pathogens: A Mini Review. Front. Cell. Infect. Microbiol.; 10, 600234. Larone, D. H. and Washington, D. C. (2007). Medically important fungi-a guide to identification. American Society for Microbiology.
26. Gharban, H. A. (2024). First genotyping confirmation of Pichia kudriavzevii in subclinically mastitic cows in Iraq. Revista de Ciências Agroveterinárias, 23(3).
27. Kennedy, A. H.; Schoch, C. L.; Marrero, G.; Brover, V. and Robbertse, B. (2022, 6). Publicly Available and Validated DNA Reference Sequences Are Critical to Fungal Identification and Global Plant Protection Efforts: A Use-Case in Colletotrichum. Plant Dis.; 106(6), 1573–1596.