ISSN: 0067-2904

Pathogenicity of Fungi Isolated from the Respiratory System of Patients in Selected Hospitals in Baghdad

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Received: 13/2/2025 Accepted: 17/6/2025 Published: 30/6/2026

Abstract

Fungal infections of the respiratory system have increased in recent years with the increase in the number of fungal spores due to pollution, so there was a need for a study focusing on fungal species that infect the respiratory system, studying their pathogenicity and diagnosing them accurately to be able to give appropriate treatment and save patients. The study aimed to isolate and diagnose pathogenic fungi from patients with respiratory diseases and to indicate their pathogenicity by studying their virulence, toxicity, and impact on the immune system to know the extent of the danger of these fungi as well as their impact in causing serious opportunistic diseases for patients with respiratory injuries. The current study obtained 22 different fungal isolates belonging to 203 blood and sputum samples collected from people with respiratory diseases of different ages. The age of those affected of both sexes ranged between 17-73 years. Isolation was carried out from different hospitals in Baghdad, including Kadhimiya Teaching Hospital, Medical City, Euphrates Hospital, and Yarmouk Hospital, from 25/3/2024 to 29/5/2024, to detect fungal species in people suffering from respiratory diseases. The fungus was diagnosed based on external appearance, microscopic diagnosis and biochemical tests. The results of isolation from the blood showed the highest incidence of *Penicillium chrysogenum* (32.34%), which is the highest among isolated fungi, followed by *Penicillium verrucosum,* with a recurrence rate of 14.7%. In contrast, yeasts had the highest frequency of isolation from sputum smears which is 29.38% *Candida albicans*, 20.34% *Candida aruis*, 10.17% *Candida kruse*, 10.17% *Candida tropicals,* and 7.91% *Candida glabrata* followed by the genus *Aspergillus* spp., with the incidence of the species *Aspergillus terreus* was 18.08% and *Aspergillus ochraceus* was 1.13% the second highest incidence of sputum smears. All isolates conducted tests of the virulence factors to know the severity of the pathogenicity of isolated fungi, and the results showed that *Candida albicans* was the main yeast capable of germ tube formation. The study also showed the production of eight isolates of the enzyme proteases and phosphatase. The susceptibility to the formation of mycotoxins by *Aspergillus* fungi was examined under ultraviolet light, which led to the fluorescence of the toxins in blue and green. The ELISA test, which has high sensitivity and excellent specificity, was performed to detect interleukin, and no significant cross-reaction or interference was observed between IL4, IL5, IL17, and their analogues. The results of the study showed a high percentage of interleukin for the three types that were used in the study.

Keywords: pathogenic fungi, pathogenicity, interleukins, mycotoxins, respiratory system, virulence factors.

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القدرة المرضية للفطريات المعزولة من الجهاز التنفسي لمرضى في مستشفيات مختارة في بغداد

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الخلاصة

زادت الالتهابات الفطرية للجهاز التنفسي في السنوات الأخيرة مع زيادة عدد الجراثيم الفطرية بسبب التلوث، لذلك كانت هناك حاجة لدراسة تركز على الأنواع الفطرية التي تصيب الجهاز التنفسي ودراسة إمراضيتها وتشخيصها بدقة لنتمكن من إعطاء العلاج المناسب وإنقاذ المرضى. هدفت الدراسة إلى عزل وتشخيص الفطريات المسببة للأمراض من مرضى أمراض الجهاز التنفسي وبيان إمراضيتها من خلال دراسة ضراوتها وسميتها وتأثيرها على جهاز المناعة لمعرفة مدى خطورة هذه الفطريات وكذلك تأثيرها في التسبب في أمراض انتهازية خطيرة للمرضى الذين يعانون من إصابات الجهاز التنفسي. حصلت الدراسة الحالية على 22 عزلة فطرية مختلفة تعود ل203 عينه دم وقشع جمعت من اشخاص مصابين بامراض الجهاز التنفسي لمختلف الاعمار، تراوحت اعمار المصابين من كلا الجنسين بين 17–73 سنة. تم العزل من بعض مستشفيات مدينة بغداد منها مستشفى الكاظمية التعليمي، مدينة الطب، مستشفى الفرات و مستشفى اليرموك للفترة من 2024/3/25 الى 2024/5/29 لغرض الكشف عن الانواع الفطرية للاشخاص الذين يعانون من امراض في الجهاز التنفسي. تم تشخيص الفطريات بالاعتماد على المظهر الخارجي، التشخيص المجهري والاختبارات البايوكميائية. اظهرت نتائج العزل من الدم اعلى نسبة ظهور للفطر *Penicillium chrysogenum* 32.34٪ هي الأعلى بين الفطريات المعزولة، يليه فطر *Penicillium verrucosum* بنسبة تكرار 14.7٪. بينما حصلت الخمائر أعلى معدل تكرار للعزل من مسحات القشع وهي *Candida albicans* 29.38٪ ، *Candida aruis* 20.34٪، *Candida kruse* 10.17٪ ، *Candida tropicals* 10.17% و *Candida glabrata* 7.91٪، يليه جنس *Aspergillus spp*. ، حيث كان معدل ظهور الأنواع *Aspergillus terreus* 18.08٪ و *Aspergillus ochraceus* 1.13٪ ثاني أعلى نسبة ظهور من مسحات القشع. اجريت على جميع العزلات اختبارات عوامل الضراوة لمعرفة شدة امراضية الفطريات المعزولة حيث اظهرت النتائج ان للخمائر القابلية على تكوين انبوب الانبات حيث انتجت خمائر المبيضات الانبوب.كما ان للفطريات قابليه على تحلل الدم من النوع الفا وكاما . كذلك بينت الدراسة انتاج ثمان عزلات لانزيم البروتينيز والفوسفاتيز. تم فحص القابلية على تكوين الافلاتوكسين لانواع فطريات الرشاشيات تحت الاشعة فوق البنفسجية والتي ادت الى تألق السموم باللون الازرق والازرق والاخضر. تم اجراء اختبار الاليزا والذي يتميز بحساسية عالية وخصوصية ممتازة للكشف عن الإنترلوكين ولم يلاحظ أي تفاعل متقاطع أو تداخل كبير بين IL4 و IL5 و IL17 ونظائرها. أظهرت نتائج الدراسة ارتفاع نسبة الإنترلوكين للأنواع الثلاثة التي تم استخدامها في الدراسة.

1. Introduction

Pathological fungi invade different parts of the body, such as the lungs, genital, skin, and other organs, where they travel from their habitats in soil, water, and air and become pathogenic. Although healthy people are not affected by fungal infections, the effect is temporary because the human body can resist invading fungal blackboards and overcome them due to its immunity against disease [1]. Recently, fungal diseases have become more prevalent and appear to patients with frightening clinical symptoms that lead to lethality. The difficulty of dealing with these fungal diseases lies in the fact that they grow and multiply slowly. The infection is initially hidden, in addition to the fact that the diagnosis is not an easy task as it depends mainly on the phenotypic and microscopic characteristics of the

fungus. Many people believe that fungi only cause diseases of athlete's foot, ringworm, and blanching of the tongue, unaware that it may be a cause of death due to opportunistic diseases that cause systemic diseases in humans [2]. Respiratory injuries are one of the most important infections a person suffers from, targeting the upper and lower respiratory tract [3, 4]. Fungal infections of the lower respiratory tract are considered the most dangerous, as they include (lungs, bronchi, and larynx). The patient suffers from these injuries without knowing the main cause of the injury, as the fungus has a susceptibility to fungal fibrosis within the tissues [5]. Fungi that infect the lungs enter through inhalation, where large numbers of fungal spores are carried on dust grains that are not visible to the naked eye and carried by the wind for hundreds of kilometers. If they are inhaled too much, the body cannot get rid of them because of their large molecular weight in addition to the virulence factors that make them hidden in relation to bodily defenses [6]. Candidiasis causes about 20 diseases, and about 90% of systemic infections are caused by *Candida albicans*, and there are other types of disease, such as *Candida glabrata*, *Candida krusei*, *Candida tropicalis* and *Candida parapsilosis* [7].
Everyone breathes in airborne fungal conidia due to their great quantity in the atmosphere, which is about 1–100 conidia per 3 meters. This could be the cause of the high frequency of nosocomial and community-acquired infections in both immunocompetent and immunocompromised individuals [8, 9]. Fungal infection involves some fundamental processes, just like any other microbial disease, including adhesion and penetration of the host tissue, then growth, colonization, and spread throughout the tissues, and avoidance of the host immune system and tissue damage [10]. Over the past few decades, serious fungal illnesses have become more common, and the human body can be invaded by opportunistic pathogens from the genera *Candida* and *Aspergillus*, which can cause deep-seated mycoses of nearly all internal organs or infections of the mucosa and skin, particularly in people with impaired immune systems [11]. The issue of severe nosocomial fungal illnesses has gotten worse during the past few decades. Only a small number of fungal species are harmful to people [9, 10].

The human respiratory system has been exposed to inhaling the spores of these fungi, which may lead to the development of over-sensitization or asthma in a certain percentage of people [3, 4]. These fungi may settle and grow in the tissues of the nose and lungs, causing, slowly, objective infections and fibers, and then the destruction of the blood hairs of the nose and lungs occurs among immunocompromised people or those treated with steroids or carcinogen inhibitor drugs, in a healthy person does not easily develop diseases of these fungi [12]. It is not easy to control the spread of fungus spores in the environment of homes, hospitals, and food factories, but the rate of contamination can be reduced by reducing the accumulation of air dust by keeping places and foods rich in proteins and sugars clean, and not exposing them to dust during their preparation [13, 14]. They must also be kept in dry places or in refrigerators and in hospitals. Periodic sterilization of patients' rooms and corridors must be taken into account to reduce the chances of reproduction and spread of fungi.

The hyperactivity of the atmosphere, high temperature, and lack of ventilation in hospitals, factories, and houses help speed up the growth and spread of fungi [15, 16].
In hospitals, a vast range of opportunistic and pathogenic fungi are thought to flourish. Some fungi are common causes of infections, whereas others are uncommon. [1]. Some *Aspergillus* molds, including *Aspergillus flavus*, *Aspergillus parasiticus,* and the uncommon *Aspergillus nomius*, produce aflatoxins (AFs), a type of mycotoxin, as their primary harmful secondary metabolites. Aflatoxin types B1, B2, G1, B2a, and G2a can be distinguished from one another. B1 > G1 > B2 > G2 is its toxicity order [9].

The study aimed to isolate and diagnose pathogenic fungi from patients with respiratory diseases and to indicate their pathogenicity by studying their virulence, toxicity, and impact on the immune system to know the extent of the danger of these fungi as well as their impact in causing serious opportunistic diseases for patients with respiratory injuries.

2. Materials and Methods

Ethics approval

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

2-1 Collection of samples

2.1.1 Blood

The collection was carried out from different hospitals in Baghdad, including Kadhimiya Teaching Hospital, Medical City, Euphrates Hospital, and Al-Yarmouk Hospital from 25/3/2024 to 29/5/2024, to detect fungal species in people suffering from respiratory diseases.168 blood samples were collected from people with respiratory diseases**;** the blood was drawn using a sterile needle and placed in a special tube for storing blood, which is an ETDA tube. The sample was quickly cultured for a period not exceeding one day and cultured on Sabouraud dextrose agar medium by using sterile dishes in which SDA medium was placed and the 5 ml blood sample was placed in it. After that, the dishes were kept in the incubator at a temperature of 25 and examined under a microscope after 5-7 days [17].

2.1.2 Sputum

A total of 35 sputum samples were collected from patients with respiratory diseases using a sterile cup. Then, the cotton swabs were placed on a sterile dish containing SDA medium. The dishes were kept in a sterile incubator at 25°C for 2-5 days and examined under a microscope.

2-2 Cultivation

2.2.1 Sabouraud dextrose agar media

It was prepared according to the company instructions after sterilization, using an autoclave for 15 minutes, and then the antibiotic chloramphenicol was applied to prevent bacterial growth. This medium was used to culture blood samples as well as sputum.

2.2.2 Chromogenic agar media (for Candida spp.)

Forty-two grams of medium in 1000 ml of distilled water. Heat gently to boil with constant stirring to dissolve the medium completely without using an autoclave. Pour the prepared medium into the dishes at a temperature of 40-50 ° C, and then the candida was inoculated for the purpose of diagnosing it after 48 hours [16].

2-3 Microscopic examination

After the fungus purification process and the obtaining of pure cultures, the fungus was examined by a light microscope, and the fungus was diagnosed based on the shape of the filaments, budding, and the shape of the spores. The test results of each colony identified were recorded by recording the name of each fungus after being diagnosed by taxonomic keys [18, 19, 20].

2-4 Examinations for the virulence factors of fungi that invade the blood

Germ tube test

A test tube was filled with 0.5 ml of human blood serum, and after a day, yeast colonies were introduced. For two to three hours, the mixture was then incubated at 37°C. A drop of the suspension was put on a sterile glass slide after incubation, covered with a cover slip, and seen under a light microscope to check for the presence of germ tubes, which are tiny, thread-like structures [21].

Phospholipase activity

Phospholipase activity was assessed using egg yolk agar, which is made up of Sabouraud Dextrose Agar supplemented with 10% sterile egg yolk, 0.005 M $CaCl_2$, and 1 M NaCl. After inoculating the agar surface with a 10 µL aliquot of fungal suspension, the mixture was incubated for 48 hours at 37 °C. A precipitation zone forming around the colony was a sign of active phospholipase [22]

Proteinase activity

This test was performed on a medium prepared by dissolving 11.7 g of yeast carbon, 0.1 g of yeast extract, 2 g of bovine serum albumin in 200 ml of distilled water [23].

Hemolytic activity

An agar plate containing 7% sheep blood was used to measure haemolytic activity [13]. A translucent or semi-transparent area surrounding the inoculation site was regarded as a sign of hemolytic activity. Iron that encourages microbial development is released when hemolysis lyses red blood cells (RBCs) by creating pores or holes in their membranes [24].

Dimorphism form of pathological fungi

Fungi isolated from the blood were tested for their ability to transform from yeast to filamentous form by taking one ml of blood, pouring the SDA medium into a sterile dish, and incubating it for seven days at a temperature of 37 ° C and 25 ° C to monitor the shift between mold and yeast forms. The appearance of the yeast form of the fungus that invades the patient's blood will be noticed [25].

2-5 Aflatoxin screening tests

The method of Thin Layer Chromatography (TLC) is a popular, easy, and economical way to find and analyze aflatoxins in fungi [26], TLC is used to detect aflatoxins from fungi by cultivating *Aspergillus flavus* and other aflatoxin-producing fungi on a clean medium (like SDA) for seven to ten days. After that, an organic solvent, like chloroform or an 80:20 methanol: water combination, is used to extract the toxin. The extract is then agitated and filtered [27]. A silica gel TLC plate is filled with the extract, and it is developed using an appropriate solvent, such as chloroform: acetone (9:1). Once the plate has dried, aflatoxins are detected as blue or green fluorescent spots when it is inspected under a UV lamp (365 coulombs) [28].

Measurement of human Interleukins (IL-4, IL-5, and IL- 17) by fungal infections

For serum collection, the samples were allowed to clot for 1 hour at room temperature or overnight at 2-8°C before centrifugation for 20 min at 1000×g at 2-8°C. Collect the supernatant to carry out the assay. The sandwich ELISA technique was used to measure the levels of the interleukins IL-4, IL-5, and IL-17. Commercial ELISA kits were used: An R and D Systems (USA) kit for IL-17 and Bio-Rad (USA) kits for IL-4 and IL-5. Despite using kits from several vendors, the assay process was the same for all of them. In a nutshell, each

interleukin's unique capture antibody was adhered to microplates beforehand. Upon blocking nonspecific binding sites, antigen-antibody interaction was allowed by adding patient serum samples and standards and incubating at 37°C. Application of the Streptavidin-HRP conjugate and biotinylated detection antibodies followed. Sulfuric acid halted the enzymatic reaction, which was initiated with a TMB substrate. Optical density was measured at 450 nm using a microplate reader, and cytokine concentrations were computed using the standard curves of each kit [29].

3. Results & Discussion

3-1 Isolation results for fungi isolated from the blood

Table 1 refers to fungi isolated from the blood during the study period, where the table shows that the percentage of the appearance of a fungus *Penicillium chrysogenum* (32.34%) was the highest among the isolated fungi, followed by the fungus *Penicillium verrucosum* with a repetition rate of 14.7%. These results are consistent with Azoulay *et al.,* where the *Penicillium spp*. had the highest rate of appearance when isolated from the blood, which suggests the pathogenicity of the fungus, as the *Penicillium* spp. also has the ability to hydrolyze blood and cause systemic diseases [6].

Table 1: Types of fungi isolated from the blood according to occurrence and frequency.

No.	Isolation Type	Occurrence	Frequency	Percentage of Frequency
1	*Penicillium chrysogenum*	4	33	32.34%
2	*Penicillium verrucosum*	4	15	14.7%
3	*Aspergillus ochraceus*	1	8	7.84%
4	*Aspergillus terreus*	2	7	6.86%
5	*Cladosporium cladosporioites*	2	6	5.88%
6	*Penicillium marneffei*	1	6	5.88%
7	*Stemphylium*	1	5	4.9%
8	*Alternaria alternata*	2	4	3.92%
9	*Paecilomyces spp.*	1	3	2.94%
10	*Hortaea werneckii*	1	3	2.94%
11	*Mucor irregularis*	2	2	1.96%
12	*Curvularia spp.*	1	2	1.96%
13	*Blastomyces dermatitidis*	1	2	1.96%
14	*Yeast*	1	1	0.98%
15	*Trichderma spp.*	1	1	0.98%
	Total	25	98	

Table 2 also indicates fungi isolated from sputum samples of patients suffering from respiratory infections during their visits to hospitals in Baghdad. The results show that yeasts had the highest frequency rate, such as *C.albicans* (29.38%), *Candida aruis* (20.34%), *Candida kruse* (10.17%), *Candida tropicals* (10.17%), and *Candida glabrata* (7.91%), followed by the genus *Aspergillus* spp., where the appearance rate of species *Aspergillus terreus* was18.08% and *Aspergillus ochraceus* was1.13% were the second highest. This result is consistent with Alhadidi, as the study proves the emergence of many types of fungi in people with respiratory diseases. These fungi are opportunistic fungi that exploit viral infection and weaken the immune system, which allows these fungi to penetrate weak places in the body, such as the respiratory system. If the body's resistance is weak to many diseases,

such as high blood pressure, diabetes, cancer, organ transplantation, or hospitalization, it leads to the invasion of fungi into the blood and the rest of the body's organs.

Table 2: Types of fungi isolated from sputum according to occurrence and frequency.

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

3-2 Virulence factors of fungi that invade the blood

3-2-1 Germ tube formation

The results of the germ tube test for yeast isolates showed that Candida species clearly formed germ tubes in all tested samples. Specifically, *Candida glabrata* produced germ tubes in all isolates; however, these structures appeared weak or distorted. The test was conducted by incubating the isolates in 0.5 ml human serum at 37 °C for three hours. No other yeast species formed germ tubes under the same conditions (Figure 1). These findings are consistent with those reported by Ali *et al.*, who demonstrated that the use of serum as an inducer enhances germ tube formation around yeast cells. In addition to playing a critical role in facilitating yeast entry into the bloodstream through epithelial cell layers, the germ tube is also believed to be involved in nutrient acquisition by the yeast [8].

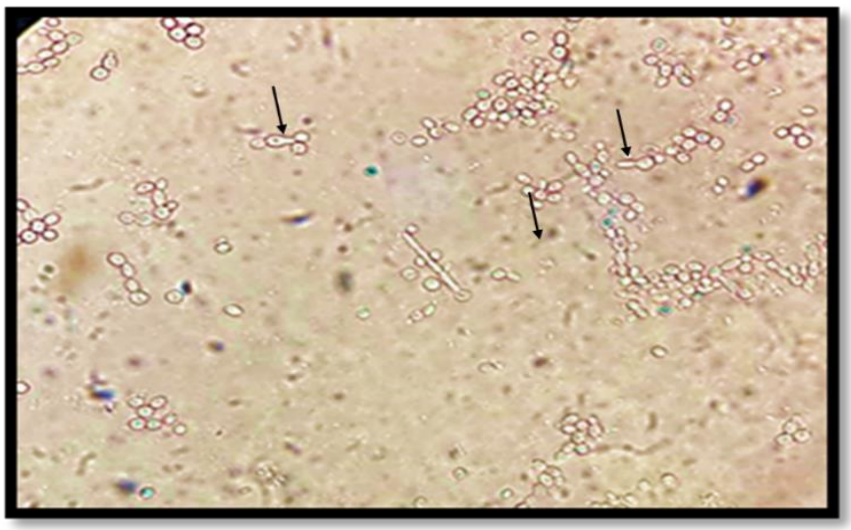

Figure 1: Germ tube of *Candida albicans* on a light compound microscope (40x).

3-2-2 Production of phosphatase and protease

The results of the study tests for the secretion of the enzyme phosphatase by pathogenic fungi showed that only eight species were able to produce the enzyme. *Penicillium chrysogenum* and *Trichderma spp.,*as shown in Table 3, were able to produce the phosphatase enzyme strongly by size inhibition of 3.7 and 3.8 cm, respectively. *Alternaria alternata* and *Candida spp.* had weak phosphatase production by inhibition 1.5 and 1.6 cm, respectively (Figure 2).

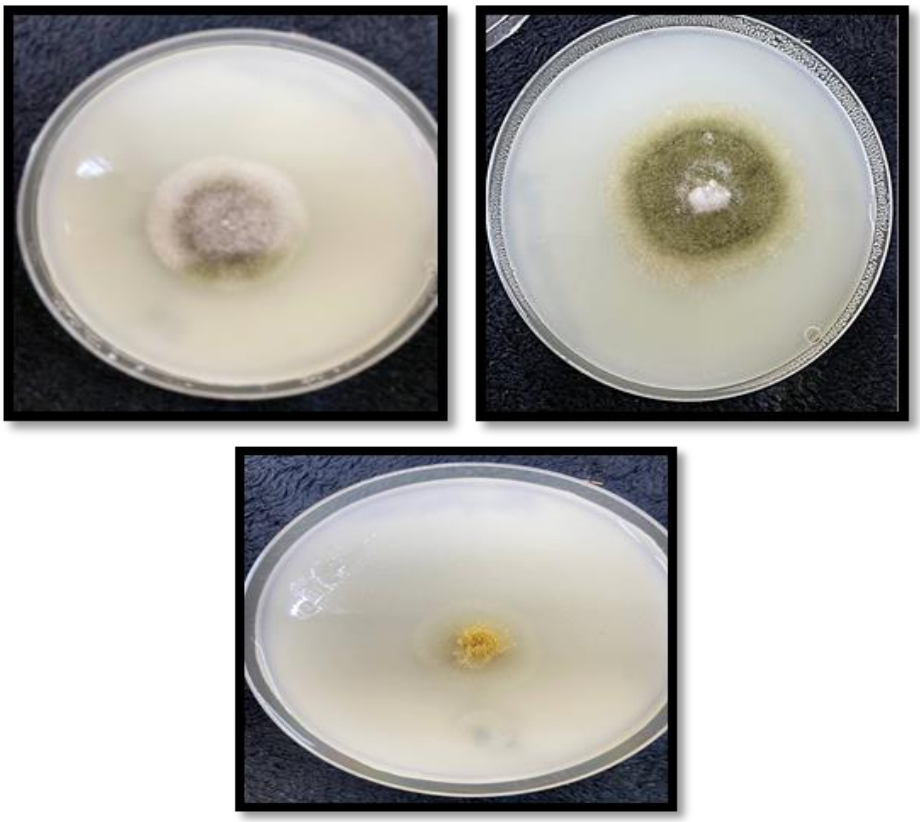

Figure 2: Inhibition zone around some isolates of fungi as a result of phosphatase enzyme production

Table 3: Types of fungi that can produce phosphatase enzyme with their size of inhibition.

No.	Fungi Type	Phosphatase production	Size of inhibition
1	*Penicillium chrysogenum*	Strong	(+++) 3.7 cm
2	*Cladosporium cladosporioites*	Weak	(+) 1.8 cm
3	*Trichderma spp.*	Strong	(+++) 3.8 cm
4	*Alternaria alternata*	Weak	(+) 1.5 cm
5	*Stemphylium spp.*	Weak	(+) 1.7 cm
6	*Aspergillus ochraceus*	Moderate	(++) 2.0 cm
7	*Candida spp.*	Weak	(+) 1.6 cm
8	*Blastomyces dermatitidis*	Moderate	(++) 2.1 cm

The results of the production of the protease enzyme showed that only eight samples produced this enzyme (Table 4). *Penicillium chrysogenum* produced the enzyme strongly where the inhibition size was 2.5 cm, while most of the remaining fungi isolates showed

weak enzyme production within the range of 1-2 cm, except for *Trichderma spp*. was moderate enzyme production and the inhibition size was 2 cm (Figure 3).

Table 4: Types of fungi that can produce protease enzyme with their size of inhibition.

No.	Fungi Type	protease production	Size of inhibition
1	*Blastomyces dermatitidis*	Weak	(+) 1.5 cm
2	*Alternaria, alternata*	Weak	(+) 1.2 cm
3	*Aspergillus ochraceus*	Weak	(+) 1.9 cm
4	*Alternaria, alternata*	Weak	(+) 1.1 cm
5	yeast	Weak	(+) 1.4 cm
6	*Penicillium chrysogenum*	Strong	(+++) 2.5 cm
7	*Cladosporium cladosporioites*	Weak	(+) 1.3 cm
8	*Trichderma spp.*	Moderate	(++) 2.0 cm

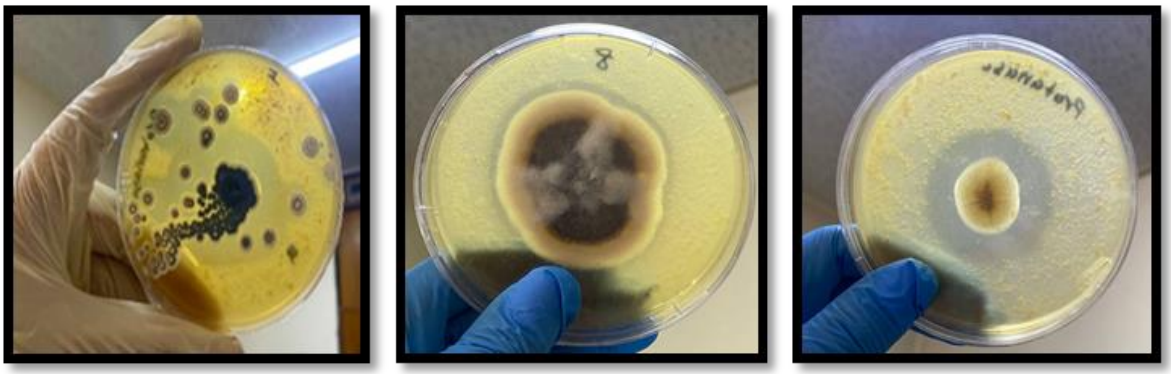

Figure 3: Inhibition zone around some isolates of fungi as a result of protease enzyme production.

It corresponds to Alhadidi, where the fungi that invade the blood are characterized by having virulent factors that enable them to destroy the immune system and penetrate into the respiratory system and other organs through the bloodstream. Phosphatase and proteinase destroy cells, proteins, and immunoglobulins from the extracellular background. They also disrupt phagocytosis in neutrophils and trigger inflammatory responses.

3-2-3 Examination of the dimorphic form of pathological fungi

The results showed the phenomenon of change in shape or dimorphism of pathological fungi, as some fungi appear on a media containing blood at a temperature of 37 ° C in the form of spherical or oval cells in the form yeasts and when converted to a media with a temperature of 25 ° C, they appear in a filamentous form, which indicates the presence of mycelia. This phenomenon is one of the most important phenomena that characterize pathological fungi, as the spherical or oval shape allows them to penetrate into the body's systems through the bloodstream and escape from the body's defenses; corresponds to Gnat *et al.*, where the fungus needs to change its shape to the shape of yeast, which is characterized by an oval or spherical shape to be able to invade many tissues of the body and this pattern is one of the most important patterns of virulence in pathological fungi [31].

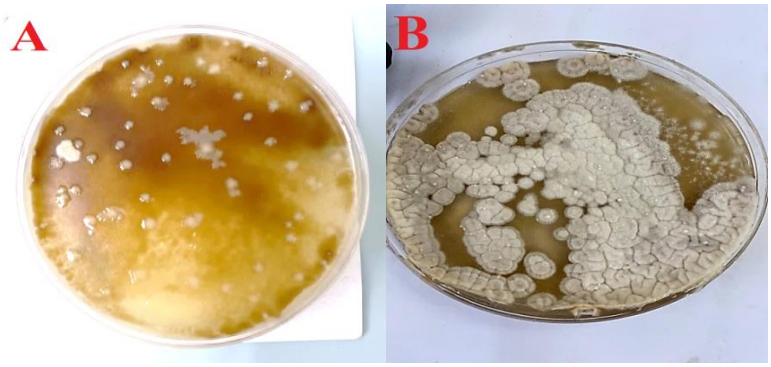

Figure 4: dimorphic form of pathological fungi (*Coccidioides immits*). (A) SDA contains blood at a temperature of 37°C, and (B) SDA is without blood at a temperature of 25 °C.

3-2-4 Hemolysis results

The results indicated that some of the isolates were α-hemolysis, while others were γ-hemolysis.

Table 5: α-hemolysis and gamma (γ) hemolysis of some of the isolates.

No.	Isolation Type	Hemolysis Type
1	*Penicillium verrucosum*	∂
2	*Penicillium marneffei*	γ
3	*Penicillium chrysogenum*	∂
4	*Yeast*	∂
5	*Alternaria alternata*	γ
6	*Paecilomyces spp.*	γ
7	*Cladosporium cladosporioites*	γ
8	*Blastomyces dermatitidis*	∂
9	*Trichderma Spp.*	∂
10	*Stemphylium*	γ
11	*Hortaea werneckii*	γ
12	*Aspergillus terreus*	∂
13	*Mucor irregularis*	∂
14	*Curvularia* spp.	γ
15	*Aspergillus ochraceus*	∂
16	*Aspergillus niger*	∂
17	*Coccidioides immitis*	∂

Fungi can form the phenomenon of dimorphic form that enables them to penetrate through the bloodstream and infect the organs of the body, which is one of the most important factors of virulence possessed by pathological fungi. As well as the ability to hydrolyze the blood of the ∂ and γ type and its inability to analyze blood completely, and this is consistent with *Alhadidi.*

3-3 Statement of toxicity of fungi that invade the blood

The existence of aflatoxins was indicated by their distinctive fluorescence, which was blue at 425 nm and blue-green at 450 nm when exposed to UV light. Aflatoxin-producing *Aspergillus flavus*, *Aspergillus terreus*, and *Aspergillus niger* were isolated from blood samples. These results are consistent with those of Nofiyanti *et al.,* who confirm the effectiveness of UV screening in identifying aflatoxins because of their hazardous and carcinogenic properties [32].

3.4 Affected human Interleukins (IL-4, IL-5, and IL- 17) by fungal infections

ELISA typically uses concentrations between 7.8 and 500 pixels/ml, which are as follows: 500 pg/ml, 250 pixels/ml, 125 pg/ml, 62.5 pg/ml, 31.2 pg/ml, 15.6 pg/ml, and 700 pg/ml. According to ELISA analysis, the lowest doses of IL4, IL5, and IL17 found are less than 3.5 pg/ml. Minimum detection (LLD), the test's sensitivity, was defined as the lowest concentration of the material that could be differentiated from zero. Using the average optical density value of twenty-zero standards and adding two standard deviations, the concentration of interleukin was calculated [31]. This test has high sensitivity and excellent interleukin detection specificity, and no cross-reaction or significant interference was observed between IL4, IL5, IL17, or their analogues [33]. The results of the study showed a high rate of interleukin for the three types that were used in the study, which shows the effect of immunity and defenses of the body with fungal infection causing damage to the respiratory system. In addition to that, the results of the ELISA tests showed higher rates or percentages for women than men, as shown in Table 6. Normal values for the three interleukins are IL-4: less than 5 pg/ml, IL-5: less than 10 pg/ml, and IL-17: less than 15-20 pg/ml

Table 6: Some immunological tests for some interleukins show high rates as a result of fungal infection.

Descriptive Statistics						
	IL4		**IL5**		**IL17**	
	Female	Male	Female	Male	Female	Male
Mean	470.003	225.311	424.673	239.021	485.201	250.201
Std. Error of Mean	48.010	24.495	24.214	16.700	29.931	24.531
Std. Deviation	173.101	88.318	87.305	60.214	107.920	88.449
Minimum	226.395	66.242	226.981	128.596	365.663	101.108
Maximum	928.712	345.381	514.624	310.522	701.906	419.952

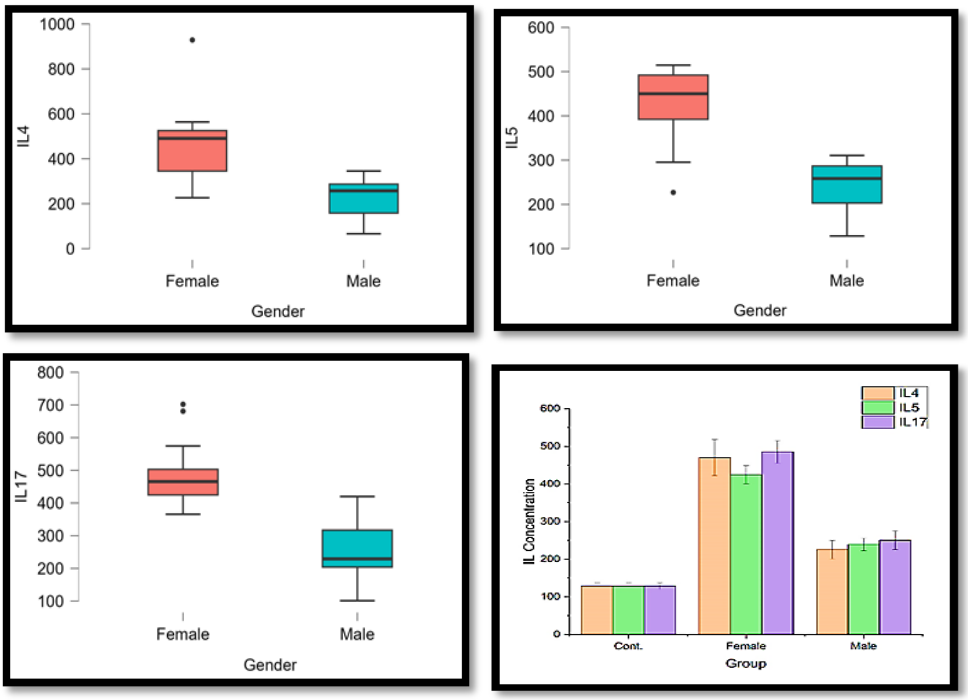

Figure 6: Some immunological tests for IL4, IL5, and IL17 show high rates as a result of fungal infection.

The results presented in Table 6 show a significant increase in the levels of interleukins IL-4, IL-5, and IL-17 in patients with fungal infections compared to normal values observed in healthy individuals. The maximum level of IL-4 reached 928.712 pg/mL in females and 345.381 pg/mL in males, while normal values do not exceed 0.88 pg/mL. This marked elevation indicates a strong immune response mediated by T helper type 2 (Th2) cells, which are responsible for producing cytokines such as IL-4 and IL-5 that promote antibody-mediated immunity against extracellular pathogens including fungi. Similarly, IL-5 showed maximum levels of 514.624 pg/mL in females and 310.522 pg/mL in males, significantly exceeding normal levels. IL-17 levels also rose to 701.906 pg/mL in females and 419.952 pg/mL in males, compared to a normal value of approximately 37.6 ± 35.3 pg/mL. These substantial increases reflect a pronounced inflammatory response and reveal that females exhibit a higher immune response than males across all examined immune parameters, suggesting potential sex-related differences in immune system activation during fungal infections. These findings are consistent with previous observations by Pathakumari *et al.,* who emphasized the critical role of cytokine-mediated immunity in defense against invasive fungal infections.

4. Conclusions

The results of the isolation of fungi from patients with respiratory diseases at different ages showed the emergence of a large number of fungal species, including *Penicillium spp*. and *Candida* yeasts. It was also noted after conducting many tests of virulence factors on these fungi that produce various types of enzymes that affect cells and may lead to their destruction, such as the enzyme phosphatase and the enzyme protease. Also, after the toxicity test on some types of Aspergilloses, which appeared during the study, the ability of these species to produce dangerous aflatoxin affects the cell directly. Finally, the results of immunological tests confirmed the extent to which some interleukins are affected by fungal infections, as shown in the tables mentioned in the study. It was concluded from this study that fungi that invade the respiratory system affect cells and can lead to their destruction as a result of the virulence of the pathogen.

Acknowledgement

We would like to thank the Department of Biology Sciences, College of Science for Women, University of Baghdad, and all members of the National Center for Blood Diseases and Tuberculosis Institute for their assistance in collecting and storing blood and sputum samples. We also thank all doctors at the Respiratory Diseases Consultation Clinic at Baghdad Teaching Hospital in Medical City for their help and advice.

Conflict of interest

The authors have no conflict of interest

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