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Original article

Epidemiological Study of Mycosis in Bronchopulmonary Infections and Antifungal Susceptibility Testing in Diyala, Iraq

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Abstract
Fungal lung diseases, encompassing a wide range of pathogens and their associated clinical symptoms, are a major global health challenge. The nature and severity of the disease depend on both host immunity and the infecting fungal strain. This study aimed to isolate and Identification of fungi causing respiratory infection and allergic bronchopulmonary mycosis and study their response to locally available antifungals. Fifty-three samples (21 females and 32 males) were collected from patients with respiratory infections and allergic bronchopneumonia in Diyala\ Iraq. The samples were examined by culture media and microscopically to determine the fungal species. Antifungals were tested by the disc diffusion method. ABPM was detected in 69.8 % of the study group with highly significant differences (P≤0.01). Males were more affected than females, with a percentage of 78.1% with significant differences (P≤0.05). Respiratory Mycosis (RM) was more prevalent in the age group (40- 49). There were highly significant differences in fungal infections among age groups. *Candida* species were the most isolated species that caused RM, followed by *Aspergillus* spp. *Penicillium* spp. were detected in 18.9%, *Cryptococcus and Blastomyces* were detected in four (10.8%) isolates each, *Cladosporium, Mucor,* and *Alternaria* were detected in one (2.7%) isolate each. Some samples contain more than one species. 24.3% of fungal infections were detected in patients in Diyala Central Prison, Baqubah city, showing 18.9% of fungal infections. *Candida* and *Cryptococcus* were most affected by Clotrimazole and Fluconazole. Amphotericin and Clotrimazole have good effects on *Penicillium. Alternaria* was sensitive to Clotrimazole, Fluconazole, and Amphotericin B.
Key Words: Antifungals, Bronchopulmonary Mycosis, Respiratory Infections, Fungal Susceptibility, Diyala.

Introduction

Fungal lung diseases have been on the rise in recent decades, posing a unique challenge to clinicians worldwide. The clinical spectrum of illness is diverse, ranging from hypersensitivity reactions to colonization to invasive disease [1]. Respiratory fungal diseases occur in various forms, the most common of which is pneumonia. Fungal infections usually occur in immunocompetent individuals and are caused by inhalation of large amounts of fungal components (e.g., histoplasmosis). However, there is a limited number of pathogens that attack immunocompetent individuals and cause severe infections. The most common of these are *Aspergillus, Pneumocystis jiroveci, Scedosporium, Fusarium, Candida, Cryptococcus*, and members of the order Mucorales within the subphylum Mucorales [2]. Allergic bronchopulmonary mycoses (ABPM) are a complex group of lung diseases caused by overactivation of the immune system against a variety of fungi that colonize the airways of patients with chronic respiratory diseases (CRDs), the most common of which is asthma [3]. ABPM refers to allergic fungal diseases caused by fungi other than *Aspergillus* [4]. ABPM is less common than allergic bronchopulmonary aspergillosis (ABPA), and most cases have been described in case series [5].
Fungi that cause ABPM include *Candida albicans, Alternaria, Bipolaris, Penicillium,* and *Trichosporon* [6]. Although there are no globally accepted diagnostic criteria for ABPM, the diagnostic criteria for ABPA are often used, substituting various other fungi for *Aspergillus fumigatus* [7]. Patients with chronic obstructive pulmonary disease (COPD) are at direct risk of death from invasive aspergillosis [8]. Despite increasing awareness of the risk of fungal infections, adequate screening, diagnosis, and surveillance remain lacking, with the focus often on the pathogen. Antifungal resistance can result from inadequate drug dosing and a lack of effective diagnostics [9]. This study is the second of its kind conducted in Diyala, Iraq, to isolate and identify fungi that cause respiratory tract infections and allergic bronchopulmonary fungal disease, and to investigate their response to locally available antifungal drugs.

Methods

Study Group

Fifty-three sputum samples (21 females and 32 males) were collected from patients with respiratory infections and allergic bronchopneumonia from Baqubah teaching hospital and some private clinics in Diyala\ Iraq, in the duration from December 2025 to early February, with different age groups from 20 years to 75 years.

Ethical approval

The study was approved by the ethical committee for research at Diyala University. The samples were collected and disposed of as per the rules and regulations of the Ministry of Health. In addition, the patients

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were conversant that their samples and their data information, including age, gender, and place of residence, would be used for research studies.

Sample size calculation

This study used the following equation for calculating the adequate sample size,

$$n = \frac{z^2\, p(1-p)}{d^2}$$

Where n is the sample size, z at a 95% confidence level is 1.96, P is the expected prevalence (50%), and d is the precision (corresponding to the effect size).

This means that to achieve a 95% confidence level that the true value is within ±5% of the sample value, 47 or more samples are required; to achieve a 95% confidence level that the true value is within ±10% of the test value, 35 or more samples are required.

Sample Isolation and Identification

All sputum samples were examined directly with KOH 10% to determine fungal hyphae or any fungal structures to confirm fungal respiratory infections, then cultured on Sabouraud Dextrose Agar (SDA) with Ampicillin (500mg /liter) and Streptomycin (1g\ g/liter) at 35- 37 ºC with daily examination till growth appeared. Fungal isolates were examined microscopically by lactophenol cotton blue and Indian Ink for *Cryptococcus. We* used the Slide culture technique for molds microscopic examination.

Antifungals Susceptibility Test

The isolates were activated by using a modified new process which give good results; the activation saline is prepared by adding (9gm) of NaCl and (2 gm) dextrose to 100 distill water then sterilize the solution by autoclave, let the solution cool then add 5ml in clean test tube and inoculate it with fungal colony, incubate at 35 c for 3 hours. Then the activated isolates were cultured on Mueller-Hinton plates with 3% glucose the antifungal discs (Nystatin Nc 50 mg, Chlotrimazole cc 10 mg, Amphotericin AP 100 mg and Fluconazole Flc 10 mg) were placed on the inoculated plate, the plates were incubated within (30mins) for 18-24h at 35ºC in an inverted position. After incubation.

Statistical Analysis

The Statistical Package for Social Sciences (SPSS) (2019) program was used to determine the effects of different groups or factors on the study parameters. The chi-square test was used to compare the significance percentages (at probabilities of 0.05 and 0.01 in this study).

Results

Distribution of ABPM among the study group

This study shows that 37 (69.8 %) of 53 sputum samples were positive for ABPM and 16(30.2%) were negative, with highly significant differences (P≤0.01) (Figure 1).

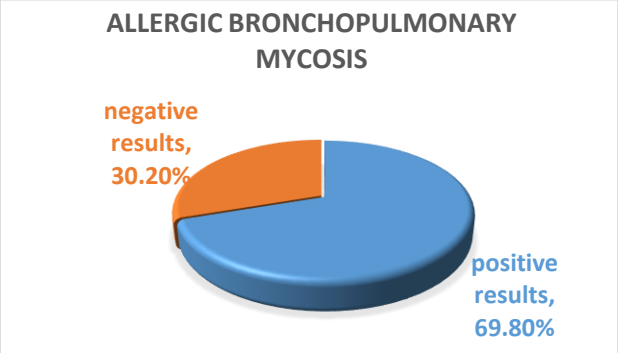

Figure 1. Distribution of ABPM among the study group

Prevalence of respiratory mycosis (RM) among genders

The data of this research collected randomly, included both sexes were 21(39.6%) out of 53 females and 32 (60.4%) were males, 12 (57.1%) out of 21females were give positive results for respiratory mycosis (RM) while 25 (78.1%) out of 32 males were positive (table 1). Statistical analysis shows that there were significant differences (P≤0.05) in respiratory mycosis among males and females.

Prevalence of respiratory mycosis among age groups

The age groups were divided into six age groups (10 ±1 years within one period). Figure 2 shows that the highest percentage of RM was 43% within the age group (40- 49), followed by the age groups (30 -39) and (60 -69), the twenties and fifties age groups gave the same percentage, 8%. The lowest number of infections was in older ages ≥ 70, which was 2.7%, with highly significant differences (P≤0.01).

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Table 1. The distribution of RM according to gender

Gender	Positive	Negative	Total	P-value
Female	12 (57.1%)	9 (42.9%)	21 (39.6%)	0.512 NS
Male	25 (78.1%)	7 (21.9%)	32 (60.4%)	0.0015 **
Total	37 (69.8%)	16 (30.2%)	53	0.0039 **
P-value	0.0326 *	0.617 NS	0.131 NS	---

** (P≤0.05) - Significant, ** (P≤0.01) - Highly significant.*

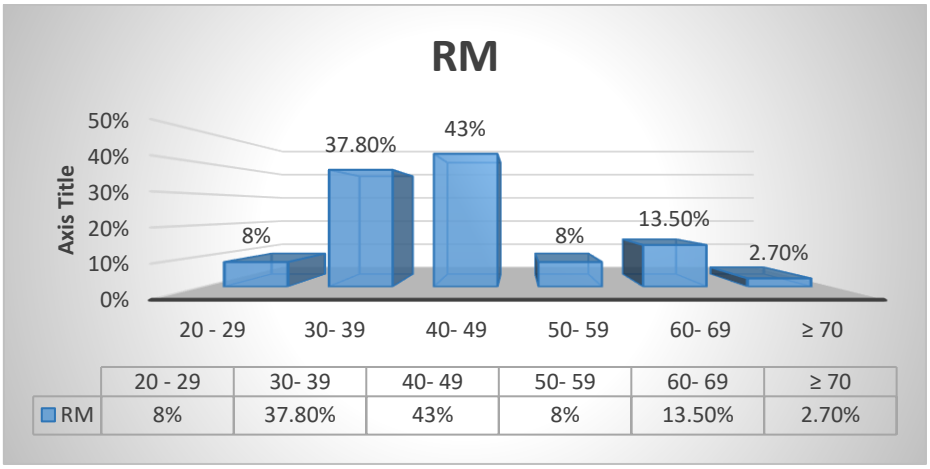

Figure 2. Detecting respiratory fungal infections due to age periods

Detection of the pathogenic fungal species by Sabouraud Dextrose Agar and microscopic examination

Laboratory examination on SDA culture media and microscopic examination (Figure 3) shows that different fungal species cause respiratory infections. *Candida* species were the most isolated species that cause RM, which were 12 (32.4%) isolates out of 37 positive fungal growths, followed by 8 (21.6%) isolates of *Aspergillus* spp. *Penicillium* spp. were detected in 18.9%. *Cryptococcus and Blastomyces* were detected in four (10.8%) isolates each; *Cladosporium*, *Mucor,* and *Alternaria* were detected in one (2.7%) isolate each. Some samples contain more than one species, such as *Blastomyces* and *A. niger or Blastomyces* with *Penicillium.* Others contain *Penicillium* and *A. niger* (Table 2).

Table 2: Fungal spp. identified in this study

Fungal species	Number of fungal species among 37 positive samples (%)
Penicillium	7 (18.9%)
Candida	12 (32.4%)
Aspergillus	8 (21.6%)
Cryptococcus	4 (10.8 %)
Blastomyces	4 (10.8%)
Mucor	1 (2.7)
Alternaria	1 (2.7%)
Cladosporiom	1 (2.7%)
Total**	41

*** The total of fungal species is more than the positive RM samples because some cases contain more than one species.*

Distribution of respiratory mycosis according to place of residence

The results of this study show the distribution of respiratory mycoses among Diyala cities (Table 3)

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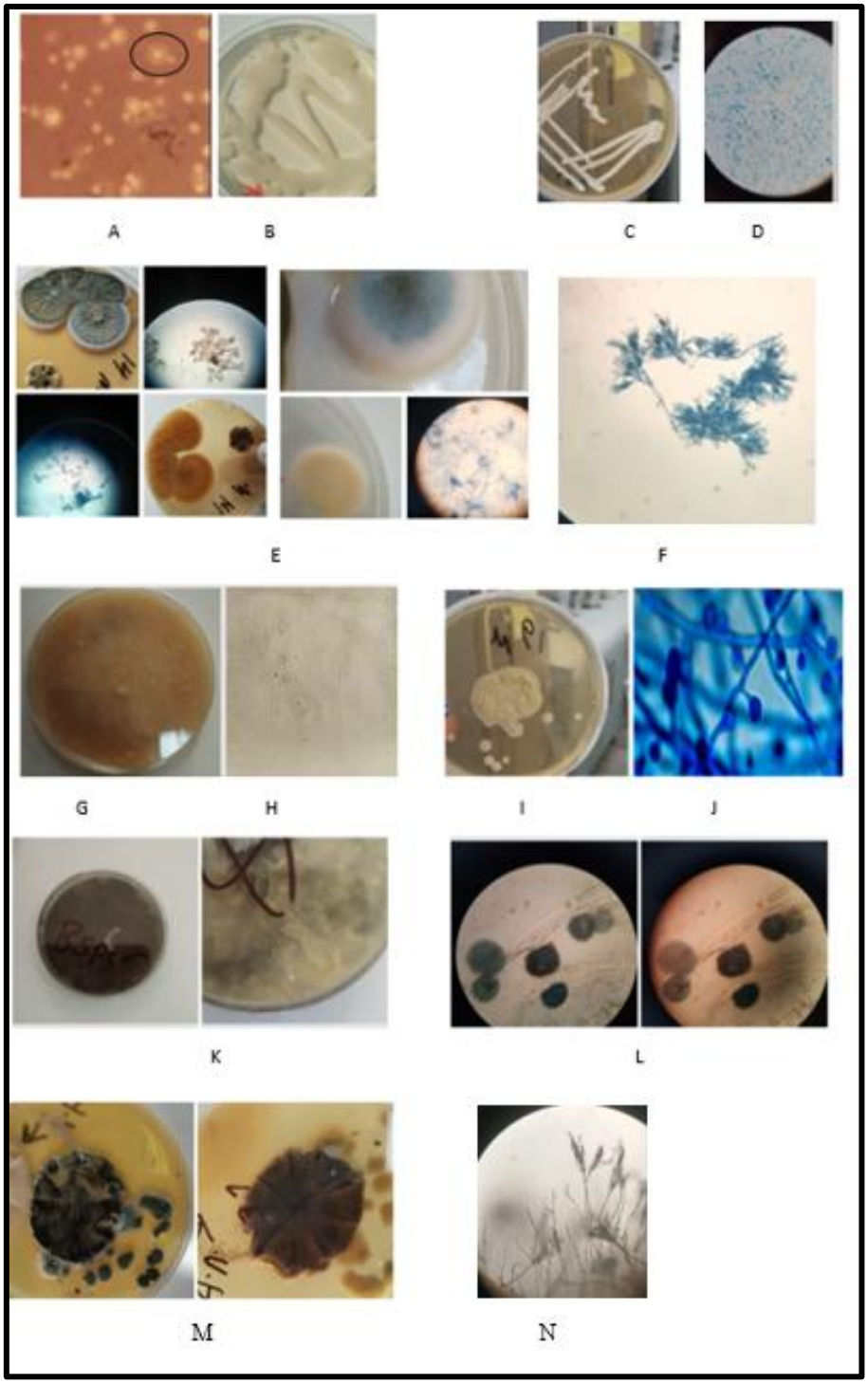

Figure 3: A- microscopic examination with Indian Ink 100X shows the gelatinous capsule around the yeast cell., B- Cryptococcus spp. grow as glistening mucoid colonies on SDA, C- D- Candida spp. SDA, lactophenole cotton blue 100X, E-F- Penicillium spp. on SDA and lactophenole cotton blue 40 X, G- Mucor spp. on SDA, H- microscopic examination by slide culture technique 40X, I- Blastomyces on SDA, J- lactophenole cotton blue 100x, K-L- Aspergillus niger on SDA, and slide culture examination 40X, M- Cladosporium on SDA, N- microscopic examination by slide culture technique 40X

Table 3. Distribution of respiratory mycosis according to place of residence (some of Diyala cities)

Place of residence	Number of infection (%)
Baqubah	7 (18.9%)
Khan Bani Saad	3 (8.1%)
Kanaan	2 (5.4%)
Buhriz	1 (2.7%)
Al-tahreer	3 (8.1%)
Hayu almuealimin	1 (2.7%)
Shaftah	2 (5.4%)

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Muqdadiyah	2 (5.4%)
Alrazi	1 (2.7%)
Khanaqin	4 (10.8%)
Jalulaa	2 (5.4%)
Diyala Central Prison	9 (24.3%)
Total	37

Antifungal susceptibility test

Four antifungals were tested in this study according to availability in pharmacies which are Nystatin Nc 50 mg, Chlotrimazole cc 10 mg, Amphotericin AP 100 mg and Fluconazole Flc 10 mg, by using disc diffusion method on Mueller Hinton agar for yeast and fungi, at the present study we attempt to use antifungal disc diffusion method on molds instead of the usual method (poisoning media with antifungals) because the difficulties that faced us to fined powder antifungals. These results show that yeasts (*Candida* and *Cryptococcus*) were most affected by Clotrimazole and Fluconazole. They showed less inhibition zone for Nystatin and Amphotericin B. Some of *the Cryptococcus* isolates give a large inhibition zone for Fluconazole (4.5 mm) while others are resistant. It appeared that Clotrimazole is the best antifungal affected against yeast spp. the same results for molds spp. in this study, it appeared that *Penicillium* is the most fungus affected by these antifungals and its shows high sensitivity against Clotrimazole. Amphotericin and Clotrimazole have good effects with an inhibition zone (1.7 mm on average). *Alternaria* was sensitive to Clotrimazole and Fluconazole, and Amphotericin B (Figure 4).

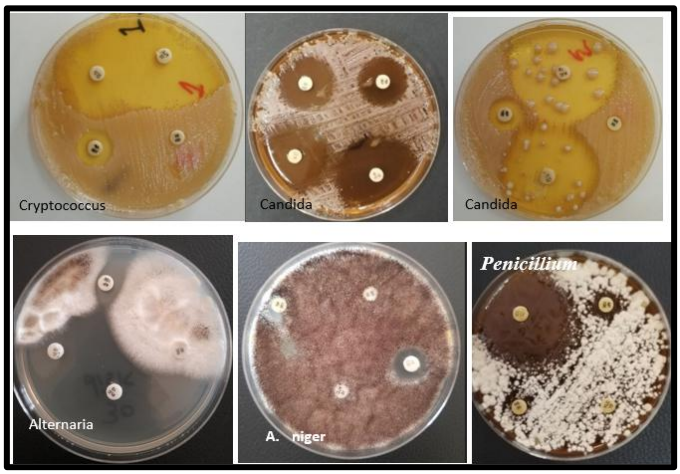

Figure 4: Inhibition zones of antifungals

Discussion

Fungal lung disease remains epidemiologically heterogeneous and is influenced by geography, environment, and host comorbidities [1]. This study shows that males were more prevalent with ABPM than females, with significant differences. These results agree with Mahor *et al.* (2024) [10]. These results indicate that age affects the predisposition to infection with various fungal diseases as a result of the different physiological factors and the different nature of the body, Different activity, and frequent exposure to pathogens.

Zuo *et al.* (2020) show that there are no differences in age and sex ratio between study groups [11]. An Iranian study showed that there were no significant differences in fungal infections among age groups [12]. According to Mahor *et al.* (2024) the most common age group affected was 30 to 50 years old [10]**,** Which are so close with current results, in addition to that in a previous study in Diyala\ Iraq it appeared that the age group (30- 41) have the most prevalence with respiratory mycosis [13], nine of samples were belong to prisoners in Diyala jail with ages thirties to forties and all were show RFI. Different fungal species caused respiratory infections. This result agrees with other previous research that *Candida* species predominate in single isolates, followed by *Cryptococcus neoformans, Penicillium* spp., *Aspergillus fumigatus,* and *Aspergillus niger,* which is similar to the result shown by El-Badrawy *et al.* [14]. Another study in Iraq, Diyala in the same line of this study *Aspergillus* spp. was the most prevalent species fallowed by *Penicillium* spp. the same results for *Alternaria* spp. with a percentage of 2.38 % compared with 2.7% for the current results [13].

Although *Penicillium* spp. Rarely cause human infections, this study showed that a high proportion of *Penicillium* spp. were isolated from respiratory tract infections. These samples were mainly isolated from prisoners at Diyala Central Prison with persistent respiratory symptoms and allergies (Table 3). Although human infections with *Penicillium* spp. are rare, they appear to be highly virulent and resistant to antifungal drugs. *Candida* species are part of the normal human flora and are the most common opportunistic fungal pathogens. Candidal pneumonia remains difficult to diagnose and most often occurs in immunocompromised individuals. It has rarely been reported in immunocompetent individuals [15].

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Candida colonization of the human respiratory tract increases the risk of bacterial infection by interacting with pathogens [16]. *Candida* species have also been shown to cause allergic bronchopulmonary mycosis, so sensitization to colonized *Candida* species can lead to respiratory symptoms [5]. 62.93% of Aspergillus species were isolated from sputum, with *Aspergillus fumigatus* being the most common [17]. This result is significantly higher than the current results, possibly because the previous study included a larger number of cases than the current study.

The most common diseases associated with the filamentous fungus *Aspergillus* include allergic bronchopulmonary aspergillosis, sensitization, aspergilloma, and chronic and invasive pulmonary aspergillosis [1]. Immunocompromised individuals may acquire aspergillosis through inhalation of airborne fungal conidia, leading to pulmonary aspergillosis and disseminated infection [18]. *Cryptococcus* is a species of pathogen that is recognized as a pathogen that infects humans and animals and is known to cause severe lung disease [19]. Although Cryptococcal and *C. gattii* infections share clinical features, species-specific differences must be noted, including indicators of lung disease such as lung masses [20]. If *Cryptococcus* or *C. gattii* is detected in a sputum sample, it should be reported to the clinician for further investigation for cryptococcosis by chest X-ray and lower respiratory tract sampling [21]. Cryptococcal infection occurs through inhalation of dried yeast cells or basidiospores into the lungs. Human-to-human transmission has not been confirmed [22]. These results indicate that one case was infected with *Alternaria* in a 45-year-old woman, and another case was reported as *Cladosporium*, both at 2.7%.

Globally, Alternaria was first described as an ABPM in a case report by Chowdhary *et al.* (2012) and subsequently described by Jo et al. in East Asia in 2024 [6,7]. Locally, this study was referenced to a second study reporting respiratory tract infection with *Alternaria*, which is consistent with the study by Adeeb *et al.* (2021) [13]. *Cladosporium*, a radiotrophic dark-colored fungus commonly found in decaying leaves and branches of citrus trees, can cause opportunistic infections, including subcutaneous and deep infections in humans and animals [23]. Wang *et al.* reported a case of *Cladosporium* infection in a 68-year-old farmer who had a positive bronchoalveolar lavage [24]. In another case, *Cladosporium* was reported to cause complicated pneumonia. The patient was a 51-year-old male, a heavy smoker, and was severely dependent on alcohol. It was concluded that this patient was at high risk for pathogen aspiration, which was emphasized by his extensive smoking history and severe alcohol use disorder. [25].

Mucormycosis is a rare, sometimes serious fungal infection that affects the sinuses, lungs, and occasionally the brain. This infection is caused by mucorales members including *Absidia, Rhizopus, Rhizomucor, Mucor,* and *Cunninghamella, Absidia, Rhizopus,* and *Rhizomucor* are the most common types [26]. He *et al.* (2021) reported a case of isolated pulmonary mucormycosis caused by *Absidia* in an adult male with no known immunodeficiency [27]. This agrees with current results. At the last decades, there has been a global increase in fungal infections; previous studies estimated that several fungal infections can be fatal [28]. Iraqi climate is very hot and dry and there were a repeated dust storms during summer in addition to dryness environment there were lack of vegetation cover, around cities, the small size and the huge numbers of spores; these factors encourage fungal airborne spores and conidia to disseminated and inhaled which result in respiratory allergies and may cause infections, Diyala province was rich in citrus trees and there were lots of village with domestic animals these environments increase the exposure to different fungal species specially *Penicillium* and *Aspergillus* spp. Uncontrolled diabetes, random antibiotic usage for long time i.e., broad-spectrum antibiotics and corticosteroids.

In addition to the antifungal classes, these reasons increase the resistance of pathogenic fungi, in addition to the lack of a fungal vaccine. Smoking is another factor that increases the susceptibility to fungal respiratory infections. The relation between fungal infection and asthma is not well studied. A study of chronic bronchitis in smokers seeking care found 64% to have asthma [29]. 24.3% of fungal infections were detected in patients in Diyala Central Prison. The high percentage of fungi isolated from patients in prison in the current study may related to the environmental conditions of prisons in terms of high humidity, insufficient exposure to sunlight, old buildings, in addition to reduced personal care and constant exposure to dust and infection. Antifungal drugs play a crucial role in managing invasive mycosis, which can be fatal if untreated.

There were lots of antifungal drugs in Iraqi pharmacies that used to control mycosis, such as topical miconazole and nystatin for oral candidiasis, followed by fluconazole and amphotericin B [30]. This study demonstrates that the antifungal drugs tested affect *Penicillium* sporulation. No sporulation was observed during the 7-day incubation period with antifungal tablets, whereas spore formation occurred after 4–5 days without antifungal use. Optimal treatment of severe disease requires prolonged induction therapy (amphotericin B and flucytosine) and consolidation therapy (fluconazole) under close clinical monitoring. Antimicrobial susceptibility testing is important for epidemiological purposes and in regions where the minimum inhibitory concentration of azoles, particularly fluconazole, is relatively high [21]. There are some limitations to this study in terms of the small size of the study sample and the lack of accurate diagnosis in hospitals regarding fungal respiratory infections compared to bacterial infections, in addition to the lack of the possibility of examining all patients under study with X-rays to observe fungal growth inside the lung.

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Conclusion

This study concluded that *Candida* spp. were the most causative for respiratory fungal infection, and allergic bronchopulmonary mycosis can be caused by multiple fungal agents, such as *Penicillium, Chladosporium, Alternaria,* in addition to *Aspergillus*. Clotrimazole shows good results against fungi isolated from ABPM and respiratory mycosis; these fungi can affect the respiratory tract mostly in humid, hot, and dusty environments, which are considered optimal conditions for spores' formation and spread.

Acknowledgment

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Conflict of Interest

The author declares that there are no conflicts of interest regarding the publication of this manuscript.

Ethics Approval and Consent to Participate

The study was approved by the ethical committee for research and higher studies of the Iraqi Ministry of Health. Informed consent was obtained from each participant.

Availability of Data and Materials

Data produced through this study are available from the corresponding author upon reasonable request.

Funding Statement

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Authors' Contributions

Mohammed N A is responsible for the design of this study, analyzing the results, and writing the manuscript, in addition to reading and approving the final version to be published.

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