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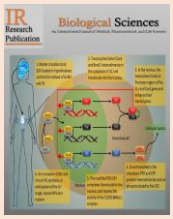

Epidemiological distribution of Fungal Infections Isolates from Bronchoalveolar Lavage (BAL) in Patients suffering from Chronic Respiratory Diseases in Babylon Province

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ABSTRACT

Fungal infections of the lower respiratory tract (LRT) showed a significant clinical challenge specifically in patients with compromised pulmonary function. Early and accurate identification of these pathogens is crucial for effective management of this disease. To determine the prevalence, species distribution, antifungal susceptibility patterns, and independent risk factors associated with fungal isolation from (BAL samples) in Babylon Province, Iraq. A hospital-based cross-sectional study was conducted at Imam Al-Sadiq Teaching Hospital for the periods (October 1,2025 – December 31, 2025), involving (96 patients) with clinical and radiological suspicion of pulmonary mycosis. Furthermore, fungal identification was performed using standard mycological techniques (KOH, SDA, and API 20C AUX). Antifungal susceptibility testing followed (CLSI M27) and (M38) guidelines. Fungal growth was detected in 41.7% (40/96) of BAL samples. Candida were the most common isolate (45%), followed by Aspergillus (22.5%) and Mucorales (20%). Non-albicans Candida accounted for 55% of Candida isolates. Fluconazole resistance was observed in 28% of Candida isolates. Diabetes mellitus and prolonged corticosteroid exposure were significantly associated with fungal isolation ($p<0.05$). The study showed a significant presence of Candida and Mucor infection in the lower respiratory tract (LRT) of patients in Babylon province. Finally, suggest that demographic factors such as (age and gender) may influence the distribution patterns of specific fungal pathogens infection. Therefore, focusing the need for rapid diagnostic approaches and the study represented an emerging shift toward resistant (non-albicans species) and emphasizes the necessity of susceptibility-guided antifungal therapy in future.

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Introduction

Fungal infections of the pulmonary system develop when a pathogenic fungus infects the lungs and respiratory system of the human body, normally via spores of the fungal body found in the environment (1, 11). Additionally, the pulmonary system is the primary target for the infections. The infections can also spread to other parts of the body system, especially for patients with certain risk factors (2, 4). Consequently, over the recent past, the rate of systemic fungal infections has risen remarkably, especially for immunocompromised patients (3). This can be attributed to various clinical factors, including the increased rate of the use of antibiotics, immunosuppressive agents, medical procedures, as well as the increased rate of comorbid conditions such as (AIDS), cancer, and immune senescence. (4,6,11).

Pulmonary fungal infections have Shifting from rare opportunistic infections to major clinical challenges in both immunocompromised and chronically ill populations. Global epidemiological shifts indicate increasing prevalence of non-albicans Candida and filamentous fungi, accompanied by rising antifungal resistance. This evolution is strongly associated with prolonged antibiotic exposure, intensive care admission, corticosteroid therapy, diabetes mellitus, and invasive pulmonary procedures.

Additionally, clinical data which collected in several regions indicate that candidiasis is a leading cause of fungal-related mortality, accounting for (50 - 71%) of deaths in hospital settings (7, 15). Subsequently, Candida albicans remains the most prevalent species in these cases, there is a notable emergence of non-albicans species, especially in Intensive Care Units (ICUs) (5, 8). Therefore, epidemiological studies in Europe have reported higher mortality rates for non-albicans infections (47%) compared to *C. albicans* (32%), often due to the multi-drug resistant nature of these strains (7,13). Furthermore, rare molds such as Mucor and Fusarium are increasingly identified in patients with hematological malignancies, further complicating therapeutic outcomes (4,9). Consequently, the other significant respiratory threats include Aspergillus, Cryptococcus, and Pneumocystis jirovecii, fungal infection include in other study (10, 16, 17).

Although in Iraq environmental dust exposure, high diabetes prevalence, and expanding ICU services may further shape the regional fungal landscape. However, robust analytical studies incorporating antifungal susceptibility testing and regression-based risk modeling remain limited. Whereas, this study integrates microbiological characterization with inferential statistical modeling to provide clinically applicable regional data.

Medical advances are still under development, and the fungal pathogens are still a challenge for modern medicine. Moreover, studies have shown the prevalence of the fungal infections among the patients suffering from pulmonary diseases in Iraq. (18, 19). Following this, a previous study was conducted to determine the prevalence of opportunistic fungal pathogens, which cause infections for the patients suffering from COPD, in the Babylon province of Iraq. (21, 22). Accordingly, the main aim of the current study was to determine the prevalence of fungal pathogens in the BAL fluids of the patients suffering from respiratory distress in the Imam Al-Sadiq Teaching Hospital, Al-Hilla, Babylon province, Iraq.

Materials and Methods

Study design and setting

A hospital-based cross-sectional analytical study was conducted to evaluate the prevalence and distribution of fungal pathogens in the lower respiratory tract. The study was carried out at (Imam Al-Sadiq Teaching Hospital) in Hilla city, Babylon Province, Iraq, over a three-month period from (1, October 2025 to 31, December 2025).

Patient recruitment and ethics

A total of (96 hospitalized patients) presenting with clinical and radiological suspicion of pulmonary mycosis were enrolled in these study.

Inclusion criteria

Patients with chronic respiratory symptoms (e.g., COPD), radiological abnormalities, and at least one established risk factor, including diabetes mellitus, prolonged corticosteroid therapy as (Mometasone nasal spray) for allergy, or ICU admission to the hospital, in addition, must be ensure that the isolated fungal species represented active clinical infection rather than transient colonization, the study inclusion criteria were strictly limited to patients exhibiting both persistent clinical symptoms of respiratory distress and clear radiological abnormalities. In these study, the use of fiberoptic bronchoscopy for Bronchoalveolar Lavage (BAL) for sample collection was particularly chosen to avoid contamination from upper respiratory tracts, thereby increasing the diagnostic specificity of the isolates obtained.

Ethical consideration

The study was approved by the ethical committee of the College of Medicine, University of Babylon, and all participants provided informed consent prior to the study.

Sample collection bronchoalveolar lavage (BAL)

Fluid sample was used as a primary clinical sample to ensure accurate detection of deep-seated pulmonary fungal pathogens, and a specialized pulmonary physician collected (96) BAL samples using standard fiberoptic bronchoscopy under aseptic conditions to avoid contamination from the upper respiratory tract, and then samples were transported to the microbiology lab for processing analysis.

In the present study, isolation of fungi from the BALF samples was interpreted along with clinical and radiological features. The study only included those patients who presented with persisting respiratory symptoms and radiological abnormalities. This was done to ensure that the isolated fungal pathogens were not indicative of colonization but actual infection.

Laboratory identification and procedures

The diagnostic process used fundamental manual microbiological techniques to ensure accurate fungal pathogen identification in the lab, which include:

Direct microscopic examination

In the first steps, bronchoalveolar lavage (BAL) samples were analyzed using direct smears applied with 10% KOH and Lactophenol Cotton Blue staining to detection fungal elements such as hyphae, budding yeast cells, or cysts.

Culture and isolation

In laboratory, samples were inoculated onto specific media such as Sabouraud Dextrose Agar (SDA) containing chloramphenicol and CHROMagar Candida to isolate specific fungal genera. In the final procedure Plates were incubated at (30°C and 37°C) for up to 7 days.

In contrast, Pneumocystis jirovecii is not culturable with standard fungal media, identification was based on the presence of the organism under the microscope with characteristic cystic structures in the BALF smears after staining with Lactophenol Cotton Blue dye. Clinical correlation with radiological evidence of Pneumocystis pneumonia

Species confirmation

To ensure Candida were identified using (germ tube tests) and the (API 20C AUX system). Molds were identified based on macroscopic and microscopic morphological characteristics examination to distinguish between various genera and species, such as Candida albicans, Candida krusei, and filamentous fungi in all sample.

Antifungal susceptibility testing (AST)

In these study, antifungal susceptibility testing was applied according to the Clinical and Laboratory Standards Institute (CLSI) M27 guidelines for yeasts and M38 for molds. The broth micro dilution method was used to test the susceptibility of isolates to Fluconazole, Voriconazole, and Amphotericin B.

Statistical analysis

Statistical processing was performed using SPSS version (26). The analysis which included:

Descriptive statistics

Frequency, percentages, mean, standard deviation (SD), and coefficient of variation (CV) were calculated to describe the distribution and variability of infections.

Inferential Statistics

Categorical variables were compared using (Chi-square) or (Fisher's exact tests) and the t-test was applied to compare mean ages across genders in these study.

Risk Modeling

Multivariate logistic regression was used to identify independent risk factors, with Odds Ratios (OR) and 95% confidence intervals (CI) calculated and the statistical significance was set at a p-value (< 0.05).

Moreover, the corticosteroid exposure recorded in this study was mainly among patients exposed to topical or inhaled corticosteroids containing mometasone for chronic allergic or respiratory diseases. Even though systemic corticosteroids are commonly linked with IFIs, prolonged

corticosteroid therapy has also been known to reduce local resistance against fungal colonization in the respiratory tract.

Results

Demographic and clinical characteristics

In these study enrolled (96 symptomatic patients) with suspected pulmonary fungal infections. The study population consisted of about 54 males (56.3%) and 42 females (43.7%). Moreover, regarding clinical comorbidities, 38 patients (39.6%) were diabetic, 31 (32.3%) had a history of prolonged corticosteroid use (Mometasone Nasal spray), and 27 (28.1%) required ICU admission during the study period. Therefore, detailed demographic and clinical characteristics are presented in Table 1.

Prevalence and distribution of fungal isolates

Fungal growth was successfully detected in 40 out of 96 BAL samples (41.7%). Candida were the most predominant isolates, accounting for 18 cases (45%), with non-albicans (n=10, 25%) outnumbering Candida albicans (n=8, 20%). Other significant pathogens included Aspergillus (22.5%), Mucorales (20%), and Pneumocystis jirovecii (12.5%). The complete taxonomic distribution is presented in (Table 2) positive sample (n= 40).

Antifungal susceptibility testing (AST)

In vitro susceptibility testing of Candida positive isolates (n=18) revealed that 28% (n=5) were resistant to Fluconazole, particularly among non-albicans strains. However, high sensitivity rates were maintained for Voriconazole (89%) and Amphotericin B (100%). The Minimum Inhibitory Concentration (MIC) ranges and susceptibility profiles are presented in Table 3.

Risk factor analysis univariate and multivariate modeling

To determine the independent predictors of fungal infection, risk factors were analyzed using univariate and multivariate logistic regression. In the univariate analysis, Diabetes Mellitus (OR=2.9) and Corticosteroid use (OR=3.4) were significantly associated with fungal isolation. After adjusting for confounders in the multivariate model, Corticosteroid medication use remained the strongest independent risk factor (Adjusted OR) = 3.1, 95% (CI: 1.3–7.2, p=0.008), followed by Diabetes Mellitus (Adjusted OR = 2.5, 95% CI: 1.1–5.9, p=0.02).

Corticosteroids were mostly reported in the form of prolonged exposure to intranasal mometasone spray, which was used for allergic conditions. While systemic corticosteroids are more commonly linked with invasive fungal infections, prolonged exposure to local corticosteroids may play a role in immunosuppression of the mucosal immune system, leading to susceptibility for fungal colonization in the respiratory tract.

ICU admission did not achieve statistical significance in the final model which presented in (Table 4 and Table 5).

Discussion

The present study demonstrated a relatively high fungal recovery rate 41.7% (40/96) in bronchoalveolar lavage (BAL) samples from patients with chronic respiratory diseases in Al-Hilla, Iraq. While fungal recovery from BAL does not inherently confirm invasive infection, this substantial burden suggests that chronic respiratory distress may often mask underlying fungal colonization or subclinical infection. The findings of the study support the growing evidence of opportunistic fungal pathogens being identified in the patient with chronic respiratory disease and the hospitalized patient (4,5).

Candida species were found to be responsible for 45% (18/40) of all the fungal isolates. Among the candida isolates, non-albican candida species were found to be higher by 55% (10/18) compared to the candida albicans isolates (8/18). This is a reflection of the epidemiological findings of the gradual shift from the dominance of candida albicans to the non-albicans species. The increased prevalence of non-albicans species is of clinical importance since the non-albicans species are found to have reduced sensitivity to the commonly used azole antifungal agents. (12, 13, 8). Typically, our study is agreeing with findings in Babylon Province, where Candida was identified as the leading opportunistic isolate in patient suffering from COPD (21). Additionally, the detection of Mucorales isolates (20%) in our results was higher than reported in northern Iran patients (19). This discrepancy likely stems from the arid climate and increased dust exposure characteristic of central Iraq, which facilitates the dispersion of environmental fungal spores (24). Furthermore, our study disagrees with patterns observed in Cameroon (14). In terms of gender distribution,

Table 1. Baseline demographic and clinical characteristics of the study population (n=96).

Variable	Category	n (%)
Gender	Male	54 (56.3%)
	Female	42 (43.7%)
Diabetes Mellitus	Yes	38 (39.6%)
	No	58 (60.4%)
Corticosteroid Use	Yes	31 (32.3%)
	No	65 (67.7%)
ICU Admission	Yes	27 (28.1%)
	No	69 (71.9%)

Table 2. Distribution of Fungal Pathogens Isolated from BAL Samples n=40.

Fungal Species	Number	Percentage (%)
Candida albicans	8	20%
Non-albicans Candida	10	25%
Aspergillus	9	22.5%
Mucorales	8	20%
Pneumocystis jirovecii	5	12.5%
Total	40	100%

Table 3. Antifungal Susceptibility Testing of Candida Isolates positive sample n=18.

Antifungal Agent	Sensitive n (%)	Resistant n (%)	MIC Range (µg/mL)
Fluconazole	13 (72%)	5 (28%)	0.25–64
Voriconazole	16 (89%)	2 (11%)	0.03–8
Amphotericin B	18 (100%)	0 (0%)	0.125–2

Table 4. Univariate analysis for Fungal Isolation (n=96).

Risk Factor	**Odds Ratio (OR)**	**95% CI**	**p-value**
Diabetes Mellitus	2.9	1.3–6.5	0.01
Corticosteroid Use	3.4	1.5–7.8	0.004
ICU Admission	2.1	0.9–4.8	0.07

Table 5. Multivariate logistic regression for fungal isolate.

Variable	Adjusted OR	95% CI	p-value
Diabetes Mellitus	2.5	1.1–5.9	0.02
Corticosteroid Use	3.1	1.3–7.2	0.008

reinforcing the need for the necessity for region-specific surveillance to allow for environmental/demographic variation. In contrast, Predictors of Fungal Isolation - a multivariate analysis using logistic regression identified two primary independent predictors for the recovery of fungi such as Prolonged Corticosteroid Therapy (Adjusted OR = 3.1, p = 0.008) This was found to be the most powerful predictor, as observed in the studies by Sai Saran and Azim (3). Additionally, Corticosteroids inhibit the activity of macrophages and neutrophils, thereby creating a conducive environment for the transition of fungi from colonization to tissue invasion. On the contrary, Diabetes Mellitus (Adjusted OR = 2.5, p = 0.02) in the context of "immunometabolic" disorders due to hyperglycemia, including the impairment of neutrophil chemotaxis, is conducive for the growth of fungi, especially Mucorales. This is in conformity with multicenter studies from Egypt and Jordan and others (25).

Moreover, although "patient with (ICU admission)" was significant in the univariate analysis, it did not have statistical independence in the adjusted model. This shows that the ICU admission status is a surrogate for comorbid states rather than being directly related to the recovery of the fungus (2).

Lastly, antifungal resistance and clinical implications covered a percentage of (28%) resistance rate to fluconazole among Candida isolates, mainly caused by non-albicans Candida spp. such as C. krusei, which is a major clinical problem as shown in this study. These findings are consistent with the recent trend reported by (12, 13). Finally, antifungal susceptibility testing revealed a notable resistance rate to fluconazole among Candida isolates, particularly among non-albicans species. The presence of azole resistance represents an important clinical concern because fluconazole is frequently used as empirical therapy in many healthcare settings. In contrast, high susceptibility rates were observed for voriconazole and amphotericin B, indicating that these agents may remain effective treatment options for fungal infections in this region.

Study limitations

Despite the clinical implications of these findings, certain limitations should be taken into consideration. For one, this study was carried out in a single hospital setting with a relatively small sample size, which may limit its scope or application to other areas in Iraq. Second, this study used conventional microbiological methods to identify fungal pathogens. Although these methods are still widely used today, integration with molecular techniques such as PCR as a molecular diagnostic test or MALDI-TOF could improve the accuracy of fungal pathogen identification and detection. Lastly, this study was only carried out over a span of three months and may not take into consideration any possible seasonal variation in environmental exposure to fungal pathogens. Future research should be carried out over a longer span to obtain a more detailed understanding of the epidemiology of fungal infections in Iraq.

Conclusion

This research will give a definitive characterization of the fungal infections that exist within the lower respiratory tract of patients within the Al-Hilla area in Iraq. The results will give a conclusive ending to the fact that fungal colonization and infections within the respiratory tract of patients with chronic respiratory distress syndromes are highly prevalent, with the main causative agent being Candida and Mucor.

The high incidence rate of the isolation of non-albicans Candida spp. especially Candida krusei from elderly male patients indicates a developing epidemiological shift that requires a transition from empirical to sensitivity-based therapy. In addition, the high incidence rate of filamentous fungi such as Mucor and Rhizopus in the Babylon province in Iraq indicates a strong environmental influence on pulmonary infections that requires a high level of caution. This research will also give a conclusive ending to the fact that the utilization of BAL fluid as a diagnostic tool for the identification of pulmonary mycoses infections is critical in the field of medicine.

Ethical Approval

The study protocol was approved by the Ethical Committee of the College of Medicine, University of Babylon, On the other hand, all participants provided written informed consent was obtained from all participants.

Journalism Ethics Considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Contribution of Authors

M. K. Alkreami Conceptualization, Methodology, Formal Analysis, Laboratory Investigation, and Writing Original Draft Preparation. U. H. K. AL-Janabi Supervision, Data Validation, Review, and Editing of the Final Manuscript.

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Conflict of Interest

The authors declare no conflicts of interest.

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Data Availability

All data generated or analyzed during this study are included in this published article.

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