Research Square

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Pattern of Isolated Fungi from Bronchoalveolar Lavage among Patients with Uncontrolled or Difficult-to-Treat Bronchial Asthma

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Research Article

Keywords: Asthma, bronchoalveolar lavage, Candida, Aspergillus, fungal colonization, severe asthma

Posted Date: August 10th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10547776/v1

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Additional Declarations: No competing interests reported.

Abstract

Background

Difficult-to-treat and uncontrolled asthma remain major clinical challenges, accounting for a disproportionate burden of exacerbations, impaired quality of life, and healthcare utilization. Increasing evidence suggests that airway fungal colonization and infection, particularly with *Candida* and *Aspergillus* species, may contribute to disease severity through persistent airway inflammation, immune dysregulation, and reduced responsiveness to therapy.

Aim

The aim of this work was to assess the prevalence and pattern of fungi isolated from bronchoalveolar lavage (BAL) in adults with uncontrolled or difficult-to-treat bronchial asthma and evaluate its relation to asthma control, lung function, radiological findings, and laboratory parameters.

Patients and methods

: This prospective cross-sectional observational study included 59 adult asthmatic patients recruited from Chest medicine department, Mansoura University Hospital and Damietta Chest Hospital in the period from May 2024 to May 2026. All patients had uncontrolled asthma or asthma with frequent exacerbations despite guideline-directed therapy. All patients underwent clinical evaluation, spirometry with salbutamol reversibility testing, chest CT, laboratory investigations and bronchoalveolar lavage (BAL). BAL samples were examined by direct microscopy for fungi and cultured on Sabouraud dextrose agar. Patients were classified as having no fungal infection, probable fungal infection, or fungal colonization according to semi-quantitative fungal growth and modified EORTC/MSG/SECURE criteria.

Results

Mean age of the studied cases was 32.69 ± 10.48 years; 64.4% of them were female. 26 patients (44.1%) fulfilled the criteria of probable fungal infection, 11 patients (18.6%) were classified as having fungal colonization, while 22 patients (37.3%) showed no evidence of fungal infection. BAL culture revealed no fungal growth in 22 patients (37.3%), *Candida* isolated in 19 patients (32.2%), and mixed *Candida* plus *Aspergillus* growth isolated from 18 patients (30.5%). Probable fungal infection was significantly associated with poor asthma control (P = 0.032), lower FEV1 (P = 0.045), more bronchiectasis on CT chest (P = 0.023).

Conclusion

Isolated fungi from BAL, particularly Candida and mixed Candida and Aspergillus growth, was common among adults with uncontrolled or difficult-to-treat asthma. Probable fungal infection was associated with poorasthma control, lower FEV1 and with bronchiectasis, suggesting a potential pathogenic role of airway fungi in severe asthma phenotypes.

Introduction

Bronchial asthma is a chronic heterogeneous inflammatory disorder of the airways characterized by variable respiratory symptoms and expiratory airflow limitation. Although most patients achieve adequate control with low to moderate doses of inhaled corticosteroids and bronchodilators, up to 5–10% develop difficult-to-treat or uncontrolled asthma with frequent exacerbations and impaired quality of life [1, 2].

Many factors contribute to poor asthma control, including incorrect inhaler technique, poor adherence, comorbidities, and persistent exposure to irritants and allergens [3, 4]. In recent years, several studies had linked fungal sensitization, colonization and infection to severe asthma phenotypes, fixed airflow limitation and bronchiectasis [5, 6, 7, 8]

Candida and Aspergillus species are the most commonly isolated fungi from respiratory samples in asthmatic patients, yet the clinical significance of lower-airway fungal growth remains debated. Distinguishing colonization from clinically relevant infection is challenging, particularly in immunocompetent hosts where systemic signs may be absent and radiological findings overlap with other conditions [9, 10].

Bronchoalveolar lavage with flexible bronchoscopy permits targeted sampling of the lower respiratory tract and is widely used to diagnose respiratory fungal infections or colonization [11, 12]. However, local data regarding the pattern of fungi isolated from BAL in adults with difficult-to-treat asthma and their relationship to clinical, functional and radiological features are limited.

The aim of this study was to assess the pattern and prevalence of fungi isolated from BAL in adult patients with uncontrolled or difficult-to-treat bronchial asthma in our locality and to evaluate the association between fungal burden and asthma control, pulmonary function, radiological abnormalities and basic laboratory findings.

Patients and Methods

This prospective cross-sectional observational study was conducted at the Department of Chest Medicine, Mansoura University Hospital, and Damietta Chest Hospital between May 2024 and May 2026 after approval by the Institutional Research Board of the Faculty of Medicine, Mansoura University (code no. MS.24.03.2743).

A total of 59 adult patients (≥ 18 years) with clinically and spirometrically confirmed bronchial asthma, diagnosed according to the Global Initiative for Asthma (GINA) were enrolled in the study.

Inclusion Criteria

- Patients aged ≥ 18 years with clinically and spirometrically confirmed bronchial asthma according to GINA recommendations.
- Uncontrolled asthma despite appropriate therapy or with frequent asthma exacerbations.
- Radiological findings suggestive of fungal infection requiring further evaluation.

Exclusion Criteria

- Patients refused or unfit for bronchoscopy.
- Presence of other major pulmonary diseases that could confound the diagnosis as chronic obstructive pulmonary disease, interstitial lung disease (ILD), lung cancer, pneumonia, cystic fibrosis and bronchiectasis as the primary diagnosis.
- Unstable cardiovascular disease.
- Pregnancy.
- Abnormal coagulation profile or bleeding disorders.
- Systemic antifungal therapy within the preceding 3 months.
- Severe acute asthma exacerbation requiring emergency management.

All patients underwent detailed history taking, physical examination, and assessment of asthma control using the Asthma Control Questionnaire based on GINA 2024 criteria. Spirometry with bronchodilator reversibility testing was performed following ATS/ERS standards; a positive reversibility was defined as an increase in FEV1 of more than 12% and more than 200 mL after inhalation of 200–400 µg salbutamol, with greater confidence if the increase exceeded 15% and 400 mL [13, 14]. Chest CT scans were obtained to evaluate radiological abnormalities. Routine laboratory investigations included complete blood count and coagulation profile, and all patients underwent 12-lead ECG before bronchoscopy.

FOB was carried out according to British Thoracic Society (BTS) guidelines. BAL was performed by instilling 120 mL in 20 mL aliquots of sterile normal saline at room temperature into the affected segment or the middle lobe or the lingula if there were no radiological abnormalities. BAL then gently aspirated and immediately transported to the mycology laboratory under aseptic conditions. After centrifugation, BAL sediments were examined by direct microscopy using lactophenol cotton blue/KOH preparations and cultured on Sabouraud dextrose agar, with or without antibacterial agents, at 25°C and 35–37°C for up to four weeks.

Yeast and yeast-like colonies were identified phenotypically based on colony morphology and microscopic features, whereas filamentous fungi were identified by macroscopic characteristics and

microscopic morphology. Semi-quantitative assessment of fungal growth was performed using colony-forming units per milliliter (CFU/mL), classifying growth as mild (≤ 10^2 CFU/mL), moderate (> 10^2–10^4 CFU/mL) or heavy (> 10^4–10^6 CFU/mL).

Modified EORTC/MSG/SECURE criteria, incorporating BAL semi-quantitative culture, host factors and radiological findings, were used to categorize patients into no fungal infection, probable fungal infection or colonization groups.

Statistical analysis

Data analysis was performed by SPSS software, version 26 (SPSS Inc., PASW statistics for windows version 26. Chicago: SPSS Inc.). Qualitative data were described using number and percent. Quantitative data were described using mean± Standard deviation for normally distributed data after testing normality using Kolmogrov-Smirnov test. Significance of the obtained results was judged at the (0.05) level.

Chi-Square test and Monte Carlo tests were used to compare qualitative data between groups as appropriate.

One Way ANOVA test was used to compare more than 2 independent groups with Post Hoc Tukey test to detect pair-wise comparison.

The Spearman's rank-order correlation is used to determine the strength and direction of a linear relationship between two non-normally distributed continuous variables and / or ordinal variables.

Results

Table (1):Age, sex and smoking characteristics of studied cases:

	n=59	**%**
Age (years) (mean±SD)	32.69±10.48(18-59)	
Sex		
Males	21	35.6
Females	38	64.4
Smoking		
Non-smoker	40	67.8
Ex-smoker	5	8.5
Passive smoker	14	23.7

Table (1) presents the studied cases had a mean age of 32.69±10.48 years, with females representing 64.4% of participants and non-smokers forming the largest smoking-status category at 67.8%, indicating that the sample was predominantly female and largely non-smoking.

Table (2): Relation between sociodemographic, clinical characteristics and fungal infection pattern among studied cases

	No fungal infection	**Probable fungal infection**	**Colonization =11**	**Test of significance**
	n=22	**n=26**		
Age (years) (mean±SD)	32.86±9.60	29.54±9.66	39.82±11.29	F=4.13 P=0.02*
Sex				
Males	8(36.4)	12(46.2)	1(9.1)	χ^2 =4.64
Females	14(63.6)	14(53.8)	10(90.9)	P=0.098
Smoking				
Non-smoker	18(81.8)	16(61.5)	6(54.5)	Mc=4.38
Ex-smoker	2(9.1)	2(7.7)	1(9.1)	P=0.357
Passive smoker	2(9.1)	8(30.8)	4(36.4)	
Oral corticosteroids				
No use	12(54.5)	14(53.8)	8(72.7)	χ^2 =1.28
Use	10(45.5)	12(46.2)	3(27.3)	P=0.531
Asthma control				
Uncontrolled	4(18.2)	10(38.5)	0	χ^2 =6.91
Partially controlled	18(81.8)	16(61.5)	11(100)	P=0.032*

Table 2 present age and asthma control which showed statistically significant associations with fungal infection pattern among the studied cases (P = 0.020 and P = 0.032, respectively). Patients with fungal colonization had a significantly higher mean age compared with the other groups. Uncontrolled asthma was more frequent among patients with probable fungal infection. In contrast, sex, smoking status, and oral corticosteroid use were not significantly associated with fungal infection pattern (P > 0.05).

Table (3): Relation between laboratory findings, pulmonary function, and fungal infection patterns in studied cases:

(mean±SD)	No fungal infection n=22	Probable fungal infection n=26	Colonization n=11	Test of significance
Neutrophils%	64.18±9.11	58.77±7.15	60.64±11.19	F=2.31 P=0.109
Eosinophils %	1(0-8)	1(0-8.5)	0.3(0-5.0)	KW=2.58 P=0.275
WBCS (n/cmm)	8.89±1.50	9.10±2.67	7.99±2.67	F=0.911 P=0.408
FEV1	66.91±16.52	62.77±15.46	76.45±8.28	F=3.26 P=0.045*
FEV1/FVC	70.88±12.16	65.34±13.34	76.15±9.44	F=2.31 P=0.109

F: One Way ANOVA test ,KW: Kruskal -wallis test , *statistically significant , data expressed as mean ±SD except eosinophils , which are expressed as median (range).

Table (3)present the Laboratory findings, including neutrophil percentage, eosinophil percentage, total white blood cell count which showed no statistically significant differences among the studied groups (P > 0.05). In contrast, among pulmonary function parameters, FEV1 showed a statistically significant association with fungal infection pattern (P = 0.045), with the colonization group demonstrating the highest mean value, whereas FEV1/FVC ratio did not differ significantly among the studied groups (P > 0.05).

Table (4): Relation between radiological findings and fungal infection pattern among studied cases:

Radiology findings	No fungal infection n=22	Probable fungal infection n=26	Colonization n=11	Test of significance
No radiological abnormalities	12 (54,5)	6(23,1)	4(36.4)	χ^2=5.05 P=0.08
Air trapping	14(63.6)	12(46.2)	6(54.5)	χ^2=1.47 P=0.480
Bronchiectasis	5(22.7)	16(61.5)	4(36.4)	MC=7.55 P=0.023*
Mycetoma	2(9.1)	2(7.7)	0	MC=1.02 P=0.600
Others	1	8	2	

MC: Monte Carlo test , χ^2=Chi-Square test , *statistically significant , data expressed as number (%) .

Table (4) demonstrates that bronchiectasis as statistically significant association with fungal infection pattern (P=0.023), being most frequent in the probable fungal infection group 61.5%, whereas other radiological findings were not significantly associated.

Table (5): Relation between radiological findings and type of isolated fungi among studied case

Radiology findings	Isolated fungi			Test of significance
	No growth n=22	Candida plus aspergillus n= 18	Candida n=19	
No radiological abnormalities	12(54.5)	4(22.2)	6(31.6)	χ^2=4.81 P=0.09
Air trapping	14(63.6)	10(55.6)	8(42.1)	χ^2=1.92 P=0.382
Bronchiectasis	5(22.7)	12(66.7)	8(42.1)	MC=7.82 P=0.023*
Mycetoma	2(9.1)	2(11.1)	0	MC=2.10 P=0.350
Others	1	4	7	

MC: Monte Carlo test , χ^2=Chi-Square test , *statistically significant , data expressed as number (%)

Table (6): Relation between laboratory findings, pulmonary function and type of isolated fungi among studied cases

(Mean ±SD)	Isolated fungi			Test of significance
	No growth n=22	Candida plus aspergillus n= 18	Candida n=19	
Neutrophils%	64.18±9.11	56.56±8.71	61.95±7.44	F=4.13 P=0.02*
Eosinophils %	1(0-8)	0.55(0-8.5)	0.8(0-8	Kw=0.575 P=0.750
WBCS (n/cmm)	8.89±1.50	8.44±1.81	9.08±3.33	F=0.373 P=0.690
FEV1	66.91±16.52	66.39±12.84	67.26±17.16	F=0.014 P=0.986
FEV1/FVC	70.88±12.16	68.38±12.66	68.72±13.98	F=0.227 P=0.798

F**:** One-way ANOVA test; KW: Kruskal–Wallis test, Statistically significant at P ≤ 0.05, Data are expressed as mean ± SD except eosinophil count, which is expressed as median (range).

Table (6) show no statistically significant differences as regarding laboratory findings, including eosinophil percentage, total white blood cell count among the studied groups (P > 0.05). However, neutrophil percentage showed a statistically significant association with BAL results (P = 0.020).

Regarding pulmonary function tests, FEV1 showed a statistically significant difference among the studied groups (P = 0.045), whereas FEV1/FVC ratio showed no statistically significant difference (P > 0.05).

Discussion

This prospective cross-sectional study enrolled 59 adults with uncontrolled or difficult-to-treat bronchial asthma.

The studied population consisted predominantly of young adults with a mean age (32.69 ± 10.48 years), and females represented nearly two-thirds of the study (64.4%). Neither sex nor smoking status was significantly associated with fungal infection pattern. Similar observations have been reported in previous studies demonstrating that demographic variables have limited influence on fungal isolation compared with disease-related factors such as airway inflammation, corticosteroid exposure, impaired mucociliary clearance, and structural airway abnormalities [15].

Age was significantly associated with fungal infection pattern P = 0.02, with patients in the colonization group being significantly older than those without fungal growth or those with probable fungal infection. Increasing age has been recognized as a risk factor for fungal persistence within the respiratory tract because of age-related decline in mucociliary clearance, cumulative airway remodeling, repeated exposure to antimicrobial therapy, and impaired innate immune defenses. Similar associations between increasing age and fungal isolation have been reported in studies evaluating chronic respiratory diseases [16]

Fungal growth was detected in 62.7% of BAL specimens obtained from adults with uncontrolled or difficult-to-treat asthma, highlighting the substantial burden of airway fungi in this population. Candida species were the predominant isolates, either alone or in combination with Aspergillus species. Similar findings have been reported in studies evaluating fungal isolation from the lower respiratory tract of patients with chronic airway diseases [17].

A significant relationship was observed between fungal involvement and asthma control. Patients with probable fungal infection were more likely to have uncontrolled asthma, supporting previous evidence that fungal-associated airway disease contributes to persistent airway inflammation and poor clinical outcomes [18].

Pulmonary function analysis demonstrated a significant association between fungal infection pattern and FEV1, with lower values observed among patients with probable fungal infection. This finding is consistent with previous studies showing that fungal sensitization and fungal airway disease are associated with impaired lung function and more severe asthma phenotypes [19].

As regard relation between radiological findings and type of isolated fungi among studied case, Bronchiectasis was significantly associated with fungal involvement and was more frequent among patients with probable fungal infection, P = 0.023.

This observation agrees with previous reports describing bronchiectasis as a common radiological feature of fungal airway diseases, particularly Aspergillus-related airway disorders [20].

The predominance of Candida should be interpreted cautiously because respiratory isolation does not necessarily indicate invasive disease. Nevertheless, the observed association between fungal burden, poor asthma control, and reduced lung function suggests that Candida may have clinical relevance beyond simple colonization in some patients [21].

Conclusion

Fungal isolation from bronchoalveolar lavage (BAL) was common among patients with uncontrolled bronchial asthma, with approximately two-thirds of the studied patients demonstrating either probable fungal infection or fungal colonization. *Candida* species were the most frequently isolated fungi, either alone or in combination with Aspergillus species.

Patients with probable fungal infection exhibited poorer asthma control, lower pulmonary function, and a higher prevalence of bronchiectatic changes compared with those without fungal isolation, suggesting a potential association between airway fungal burden and asthma severity. These findings highlight the possible role of fungal organisms in persistent airway inflammation and disease progression in selected patients with difficult-to-control asthma.

Bronchoscopy with BAL may provide valuable microbiological information in asthmatic patients with recurrent exacerbations, persistent respiratory symptoms, or radiological abnormalities when routine evaluation fails to identify an underlying cause.

Limitations:

The present study was limited by its single-center design, relatively small sample size, and reliance on conventional fungal culture without routine molecular or immunological fungal testing. Despite these limitations, the findings demonstrate a high prevalence of airway fungal colonization and probable fungal infection among patients with uncontrolled or difficult-to-treat asthma and support the potential value of BAL mycological assessment in selected cases. Future multicenter studies incorporating molecular and immunological diagnostics are needed.

Declarations

Ethics approval and consent to participate: approved by the Institutional Research Board of Faculty of Medicine, Mansoura University (code no:MS.24.03.2743) .

All participants gave their written informed consents prior to participation in the study.

During the research, all personal information was kept confidential, ensuring complete privacy for participants.

The study was registered at ClinicalTrials.gov number :**(NCT06376617).**

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Acknowledgements:

Not applicable.

Financial support and sponsorship:

Not applicable

Author contribution: S.S.A was involved in the design of the study, recruitment of patients, clinical assessment, interpretation of results, methods, and preparation of the manuscript.

M.K.E.B; conceptualization, methodology, supervision, writing, review and editing.

A.Y.M and T.A.E.S; were involved in the analysis of data, supervision, and review of the manuscript.

M.A.E.: validation, microbiological data interpretation, and writing, review and editing.

All authors read and approved the final manuscript.

Availability of data and materials: the data used in this study are not publicly available due to patient confidentiality but may be obtained from the corresponding author upon reasonable request and with appropriate institutional approval.

Conflicts of interest: the authors declare no competing interests.

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