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Comparison of Phenotypic Methods for Detection of Aspergillus species among Clinically Suspected Pulmonary Tuberculosis Cases

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Abstract

Background: Pulmonary *Aspergillosis* has shown a significant rise in recent years, so its early diagnosis and laboratory detection is essential. Phenotypic methods such as, microscopy and culture remain widespread and essential tools for diagnosis of *Aspergillus* infection. **Objective:** The objective of the study was to compare phenotypic methods, such as microscopy and culture for detection of *Aspergillus species* from sputum samples of clinically suspected pulmonary tuberculosis cases. **Methodology:** This cross-sectional study was conducted from January 2021 to December 2021 in the Department of Microbiology of Sir Salimullah Medical College, Dhaka. A total of 176 sputum samples were collected from clinically suspected pulmonary tuberculosis patients. *Mycobacterium tuberculosis* was detected by GeneXpert from the sputum sample. *Aspergillus* was identified by KOH mount microscopy and culture on Sabouraud's chloramphenicol agar media with gentamicin. Species identification of *Aspergillus* was done by colony characteristics and lactophenol cotton blue staining using direct microscopy. **Results:** In this study, out of 176 sputum samples, *Mycobacterium tuberculosis* was detected by Gene Xpert in 28(15.91%) sputum samples. 24(13.64%) Aspergillus species were detected by culture and 20(11.36%) were detected by microscopy. Aspergillus fumigatus 12(50%) was the commonest isolated species, followed by A*spergillus niger* 7(29.17%). **Conclusion:** Morphological identification systems, such as traditional culture methods and direct microscopic examination, have been always played a dominant role in *Aspergillus* identification.

Keywords: Pulmonary aspergillosis; *Mycobacterium tuberculosis*; phenotypic methods

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Introduction

In developing nations, *Aspergillus* infections are becoming more common in immunocompetent hosts. These infections typically occur in the presence of other pulmonary and systemic abnormalities such fibrotic lung disease, suppurative infections, pre-existing cavities, bullas, or cyst[1]. It can also cause acute pneumonia in immunocompetent hosts which is invariably fatal[2].

Pulmonary tuberculosis remains the most important cause of subacute and chronic respiratory morbidity which most often leaves behind a scarred pulmonary parenchyma. It provides sufficient oxygen and necrotizing tissue to facilitate the growth of many microorganisms, including *Aspergillus species*[3]. More than 95.0% of pulmonary tuberculosis cases have been documented in developing countries where there is limited diagnostic and therapeutic equipment[4]. Respiratory tract *aspergillosis* has symptoms and radiological features similar to tuberculosis, which can cause it to be mistakenly identified and treated as tuberculosis. Fungal infections are more likely to occur as a result of long-term tuberculosis therapy, such as

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the long-term use of antibiotics, which depress the immune system and increases the opportunity of infections. Fungal infection can occur in the early stage of tuberculosis, but doctors usually prescribe only anti-TB drugs that make it difficult for patients to get well due to coexistence of fungal infections[5].

Human immunodeficiency virus infection, uncontrolled diabetes mellitus, cancer, recent corticosteroid therapy, immunosuppressive drugs, chemotherapy, and solid organ transplantation or thoracic surgery are well recognized risk factors for aspergillosis[6]. The co-infection of *Mycobacterium tuberculosis* with pulmonary aspergillosis may influence the course of treatment and may show more complications. The combined *Aspergillus* coinfection rate among patients with pulmonary tuberculosis is 15.4% in Asia and Africa[7]. There is an estimated prevalence of 3 million cases of chronic *pulmonary aspergillosis* worldwide of which 1.2 million are thought to represent complications of pulmonary tuberculosis[8].

Aspergillus species are the most frequent cause of invasive mold infections. Although over 180 species are found within the genus, three species such as *Aspergillus flavus*, *Aspergillus fumigatus*, and *Aspergillus terreus*, account for most cases of invasive aspergillosis (IA), with *Aspergillus nidulans*, *Aspergillus niger*, and *Aspergillus ustus* being rare causes of IA[9]. The ability to distinguish between the various clinically relevant *Aspergillus species* may have diagnostic value, as certain species are associated with higher mortality and increased virulence and vary in their resistance to antifungal therapy.

The diagnosis of *pulmonary aspergillosis* often poses a difficult diagnostic challenge due to the signs and symptoms in most cases of *aspergillosis* being non-specific and radiological findings are of little diagnostic help. Conventional diagnosis of *Aspergillus* infection relies on the identification of pathogens utilizing morphological characteristics specific to genus and species. Despite recent advances in the molecular diagnosis of *Aspergillus*, direct microscopy remains an essential tool for both detection of the organisms in the sample, and identification of the growth in culture to the species level[10]. Confirmation of microscopic findings by culture is always desirable and, in most cases it is essential for definitive identification of the pathogen. Culture needs repeated isolation of the same *Aspergillus species* as part of the differentiation between colonization and infection[11]. Antigen assays such as the galactomannan and glucan detection system are frequently used, yet these tests are costly and vary in sensitivity and specificity depending on the patient population involved[12].

Due to the significance of *Aspergillus species* and their association with pulmonary tuberculosis, as well as lack of comprehensive research about detection of *Aspergillus* in Bangladesh, this study was conducted to compare phenotypic methods, such as microscopy and culture for detection of *Aspergillus species* from sputum samples from patients with suspected pulmonary tuberculosis.

Methodology

Study Settings and Population: This cross-sectional descriptive study was conducted over a period of 12 months from January 2021 to December 2021 in the Department of Microbiology at Sir Salimullah Medical College, Dhaka, Bangladesh. A questionnaire and a check list were the tools of data collection. The study protocol was approved by protocol approval committee of the Ethical Review Committee of Sir Salimullah Medical College and Mitford Hospital, Dhaka, Bangladesh. Individuals who presented with clinical symptoms of pulmonary tuberculosis were included in the study. Apparently healthy individuals without any clinical symptoms of pulmonary tuberculosis or who with HIV seropositive results, history of diabetes mellitus, patient on immunosuppressive drug and patient who had current treatment with any antifungal drugs were excluded.

Specimen collection: Early morning sputum was collected following standard procedure from the patients attending the department of Microbiology of Sir Salimullah Medical College for examination of sputum samples for Gene Xpert[14].

Laboratory Procedures

Identification of Mycobacterium tuberculosis by Gene Xpert: Only 1st sputum sample was subjected to Gene Xpert. Gene Xpert MTB/RIF assay is a nucleic acid amplification (NAA) test which identify DNA of Mycobacterium Tuberculosis complex (MTBC) and resistance to rifampicin (i.e.mutation of the rpo B gene) in less than 2 hours. This system integrates and automates sample processing, nucleic acid amplification and detection of target sequences. Interpretations were MTB detected with rifampicin sensitive, MTB detected without rifampicin resistant and MTB not detected.

Detection of Aspergillus species: ATCC strains of Aspergillus could not be collected, therefore Aspergillus fumigatus obtained from BSMMU was

taken and considered as control strain.

Direct Microscopic Examination: Consecutive three early morning sputum samples were taken for microscopic examination. KOH mount procedure was done following standard procedure[15]. Hyaline, septate hyphae, 3 to 5 µm in diameter with characteristic dichotomous branching and an irregular outline, was recorded as positive in direct microscopy.

Culture of the Specimen: Consecutive three early morning sputum samples were taken for culture. Prior to inoculation, the medium was warmed to room temperature and the agar surface dried. All collected specimens were primarily cultured in Sabouraud's chloramphenicol agar with gentamicin in screw capped test tube and also in petridish. The culture tubes were incubated at 37^{o}C, evaluated daily for fungal growth. Test tubes that have no growth at the 4th week was taken as negative and disposed appropriately. The cap of the test tube was partially unscrewed to ensure oxygen availability[16]. Identification of *Aspergillus species* by colony morphology. Fungi growth on cultures were identified by morphologic characteristics using both macroscopic and microscopic features such as colony growth rate, color of the colony on surface and reverse, elevation, texture and colony size were captured[16].

Lactophenol Cotton Blue Staining: A clean glass slide was taken, and a drop of LPCB stain was put on it. A small fragment of a colony (cottony, woolly, or powdery) picked from the midpoint of the culture was placed on it and covered with a clean coverslip. Using 10x and 40x magnifications of the microscope, characteristics including conidial heads, color, size, length, vesicles form, arrangement of phialides, and the type of hyphae were recorded[17].

Statistical Analysis: Informed written consent was taken from each patient. Before collecting specimen, each patient was interviewed and relevant information was recorded systematically in a pre-designed standard data sheet. In analysis of results, the data obtained were entered in Microsoft Excel sheet. Chi-square was used to determine the association between two categorical variables. Values of $p \leq 0.05$ at 90.0% interval were considered statistically significant.

Ethical Clearance: All procedures of the present study were carried out in accordance with the principles for human investigations (i.e., Helsinki Declaration 2013) and also with the ethical guidelines of the Institutional research ethics. Formal ethics approval was granted by the local ethics committee. Participants in the study were informed about the procedure and purpose of the study and confidentiality of information provided. All participants consented willingly to be a part of the study during the data collection periods. All data were collected anonymously and were analyzed using the coding system.

Results

The present study was conducted on a total 176 suspected patients of *pulmonary tuberculosis* and three morning sputum were collected from them. Table 1 showed identification of *Mycobacterium tuberculosis* by Gene Xpert. Out of 176 sputum samples, 28(15.91%) were positive by Gene Xpert.

Table 1: Identification of *Mycobacterium tuberculosis* by GeneXpert (n=176)

Gene Xpert	Frequency	Percent
MTB Detected	28	15.9
MTB not detected	148	84.1
Total	**176**	**100.0**

Detection of Aspergillus by KOH mount microscopy and culture showed that out of 176 samples, 20(11.36%) Aspergillus were identified by KOH mount microscopy and, 24(13.64%) were isolated by culture. No significant difference was found among two detection method (Table 2).

Table 2: Detection of Aspergillus species by different phenotypic methods (n=176)

Methods	Positive	Negative	P value
Culture	24(13.64)	152(86.36)	>0 .05
Microscopy	20(11.36)	156(88.64)	

Note: Chi-square test was done to analyze the data. p <0.05=significant.

Comparison of culture with microscopy in detection of Aspergillus species from sputum showed that out of 24 culture positive Aspergillus, 20 were positive by KOH microscopy. In culture negative sputum samples, no Aspergillus was detected in microscopy.152 sputum was found negative by both diagnostic tests. Culture yielded more detection of Aspergillus species (Table 3).

Table 3: Comparison of Culture and Microscopy for Detection of Aspergillus from Sputum (n=176)

Microscopy	Culture		Total
	Positive	Negative	
Positive	20	0	20(11.7%)
Negative	4	152	156 (88.6%)
Total	**24**	**152**	**176(100.0%)**

Note: Culture is regarded as gold standard[18]

Table 4: Distribution of *Aspergillus species* among Total Isolated *Aspergillus* (n=24).

***Aspergillus* species**	**Frequency**	**Percent**
A. fumigatus	12	50.0
A. niger	7	29.2
A. flavus	4	16.7
A. terreus	1	4.2
Total	**24**	**100,0**

Table 4 showed distribution of *Aspergillus species* among total isolated *Aspergillus*. Out of 24 *Aspergillus species*, *Aspergillus fumigatus* was the commonest isolated species while *Aspergillus terreus* 1(4.2%) was the least common species.

Discussion

The burden of pulmonary *Aspergillus* infection is increasing and often misdiagnosed as pulmonary tuberculosis in developing countries where the prevalence of pulmonary tuberculosis is high[19]. As these patients present with non-specific clinical and radiological findings, an early microbiological diagnosis is essential to prevent its dissemination.

The number of Mycobacterium tuberculosis cases detected by GeneXpert in this investigation was 28(15.9%), which is approximately identical to the number of cases detected by GeneXpert in an Indian study by Verma et al[20] from 125 clinically suspected TB cases. 67(67.0%) MTB cases were discovered in a study by Jahan et al[21] from Bangladesh, which was undertaken in the same institution as the current study. A declining rate of pulmonary tuberculosis was emphasized by two separate investigations from the same institution. Study conducted by Helb et al[22] showed that 62 (58%) *Mycobacterium tuberculosis* was detected out of 107 clinical sputum samples. Haider et al[23] from Malaysia showed that out of 125 sputum samples Gene Xpert could detect tuberculosis from only 8(6.4%) samples. These variations might be due to geographical variation and difference in case selection. Also, environment of Malayasia is different from Bangladesh. Possibly Gene Xpert MTB/RIF assay made dramatic improvements for the rapid detection of *Mycobacterium tuberculosis*, which offers promising advancements in TB control initiatives in Bangladesh.

In the present study, out of 176 suspected TB patient, 20(11.36%) was detected as *Aspergillus species* by direct microscopic examination. Study conducted by Sharma et al[24] from India found 20(13%) samples positive by KOH mount microscopy. Examination of sputum by Mwaura et al[25] revealed that 37.8% samples were positive by direct microscopic examination. Present research work excluded patients from study who had diabetes mellitus, human immunodeficiency virus infection or who were taking immunosuppressive agent, which might have reflected in lower detection rate of *Aspergillus species*. These observations had demonstrated the diagnostic importance of direct microscopy of sputum samples.

Current study revealed that 24 (13.64%) *Aspergillus* was detected by culture in Sabouraud 's chloramphenicol agar with gentamicin which was comparable with the study by Kurhade et al[26] where they observed 16.0% of respiratory specimens were culture positive for *Aspergillus*. Similar observations were found by the study of Sharma et al[24] who found 17(11.0%) culture positive *Aspergillus species* from sputum samples. Prabha et al[27] from India found 13(15.0%) culture positive cases from sputum sample.

In the present study, *Aspergillus fumigatus* 12(50.0%) had been reported as the most common species isolated from sputum sample and was responsible for most cases of *aspergillosis* followed by Aspergillus niger 7(29.2%), Aspergillus flavus in 4(16.7%) and Aspergillus terreus in 1(4.2%). The study of Sani et al[12] and Nasir et al[28] showed that the most common *Aspergillus species* was *Aspergillus fumigatus* followed by *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus terreus* which was parallal with the current study. In the study conducted by Bitew et al[19] from Ethiopia found *Aspergillus niger* as the commonest isolated species. Iqbal et al[29] found *Aspergillus flavus* (47.2%) as most common *Aspergillus species*. The wide variation in the species distribution of *Aspergillus* might be due to the differences in climatic conditions, (temperature and humidity) which affected the growth of *Aspergillus*, season of study period, environmental conditions (winds, dust particles in air) of the sample area and predisposing conditions of the study population. Most studies reported that *Aspergillus fumigatus* was the major cause of *pulmonary aspergillosis* in contrast to other *Aspergillus species*. This could have been due to the ability of *Aspergillus fumigatus* to grow abundantly everywhere, produce tiny conidia that easily penetrate deep into the alveolar region and grows at 37^{o}C there, producing pulmonary infection[13].

Conclusion

The current study highlights that routine screening of sputum samples for the presence of *Aspergillus* by a

conventional microscopy along with culture should be done. There was a growing realization during the conduct of the study that *Aspergillus species* may also play a role in the deterioration of pulmonary diseases and exacerbations in individuals lacking classical risk factors. Fungal pathogens should be investigated in all presumptive *pulmonary tuberculosis* cases to avoid misdiagnosis, unnecessary use of antibacterial chemotherapy, delayed antifungal intervention and unwarranted morbidity and mortality.

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Conflict of Interest
The authors have no conflicts of interest to disclose.

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Authors' contributions
Rahat T, Afroz S, Sani UIJ conceived and designed the study, analyzed the data, interpreted the results, and wrote up the draft manuscript. Rahat T contributed to the analysis of the data, interpretation of the results and critically reviewing the manuscript. Haider SB, Nazrul AK involved in the manuscript review and editing. Afroz S, Sani UIJ, Haider SB as collector of Data and Data Analyst. All authors read and approved the final manuscript.

Data Availability
Any inquiries regarding supporting data availability of this study should be directed to the corresponding author and are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate
Ethical approval for the study was obtained from the Institutional Review Board. As this was a prospective study the written informed consent was obtained from all study participants. All methods were performed in accordance with the relevant guidelines and regulations.

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References

1. Jin E, Wang LM, Li QY, Feng X, Ma SL. Chronic necrotizing pulmonary aspergillosis in an immunocompetent patient: report of a rare case. Infection. 2014;42(3): 565-568.
2. Sridhar V, Rajagopalan N, Shivaprasad C, Patil M, Varghese J et al. Acute community acquired Aspergillus pneumonia in a presumed immunocompetent host. BMJ Case Rep. 2012;0920114866
3. Mathavi S, Shankar R, Sasikala G, Kavitha A. A study on mycotic infections among sputum positive pulmonary tuberculosis patients in Salem district. Indian J Sci Res. 2015;4(7):299-302
4. Amiri M, Siami R, Khaledi A. Tuberculosis Status and Coinfection of Pulmonary Fungal Infections in Patients Referred to Reference Laboratory of Health Centers Ghaemshahr City during 2007-2017. Ethiop J Health Sci. 2018; 28(6):683–690.
5. Bansod S, Rai M. Emerging of Mycotic Infection in Patients Infected with Mycobacterium tuberculosis. World J Med Sci. 2008;3(2):74-80
6. Singh RK. Chronic Pulmonary Aspergillosis in a Patient with AIDS. Cureus. 2021;12(4):14588.
7. Hosseini M, Shakerimoghaddam A, Ghazalibina M, Khaledi A. Aspergillus coinfection among patients with pulmonary tuberculosis in Asia and Africa countries; A Systematic Review and Meta-Analysis of Cross-Sectional Studies. Microbial Pathogenesis 2020;141, 104018.
8. Zarif A, Thomas A, Vayro A. Chronic Pulmonary Aspergillosis: A Brief Review. Yale J Biol Med. 2021;94(4):673–679.
9. Henry T, Iwen PC, Hinrichs SH. Identification of Aspergillus species using internal transcribed spacer regions 1 and 2. J Clin Microbiol 2000;38(4):1510-5
10. Zarrinfar H, Saber S, Kordbacheh P, Makimura K, Fata A, Geramishoar M, Mirhendi H. Mycological Microscopic and Culture Examination of 400 Bronchoalveolar Lavage (BAL) Samples. Iran J Public Health. 2012; 41(7):70-6.
11. Barberan J, Alcazar B, Malmierca E, Garcia F, Dorca J, Del C, et al. Investigator Group. Repeated Aspergillus isolation in respiratory samples from non-immunocompromised patients not selected based on clinical diagnoses: colonization or infection? BMC Infect Dis, 2012; 12:295
12.Ullmann A, Aguado J, Arikan-Akdagli S, Denning D, Groll A, Lagrou K, et al. Diagnosis and management of Aspergillus diseases: executive summary of the 2017 ESCMID-ECMM-ERS guideline. CMI. 2018;24:1-38.
13. Sani FM, Uba A, Tahir F, Abdullahi IN, Adekola HA, Mustapha J et al. Spectrum of pulmonary fungal pathogens, associated risk factors, and anti-fungal susceptibility pattern among persons with presumptive tuberculosis at Gombe, Nigeria. Int J Mycobacteriol. 2020;9:144-9
14. https://www.who.int/tb/laboratory/mycobacteriology-laboratory-manual.2014
15. Cheesbrough M. District Laboratory Practice in Tropical Countries, New York, USA: 2nd ed, Cambridge University Press. 2006; Part-2: 39-42.
16. Tonui J, Mureithi M, Jaoko W, Bii C. Spectrum of Filamentous Fungi from Sputum of Tuberculosis Relapse and Retreatment Patients in Tuberculosis Reference Facilities. OJMM. 2020;10:190-203
17. Muni S, Rajpal K, Kumar R, Kumari R, Sinha R, Kumar S et al. Identification of Fungal Isolates in Patients With Pulmonary Tuberculosis Treated at a Tertiary Care Hospital. Cureus. 2023;15(4):e37664
18.Singh R, Singh G, Urhekar AD . Incidence of Aspergillus

Infections in Patients in a Tertiary Care Hospital in Navi Mumbai. Fungal Genom Biol,2015;5:127
19. Bitew A, Bati S. Profiling of potential pulmonary fungal pathogens and the prevalence of the association between pulmonary tuberculosis and potential fungal pathogens in presumptive tuberculosis patients referred to Saint Peter's Specialized Tuberculosis Referral Hospital, Addis Ababa, Ethiopia. SAGE Open Med. 2021;9:1-9
20. Verma D, Sharma R, Chand AE, Saxena A. The application of gene xpert for the diagnosis of mycobacterium tuberculosis and MDR TB in Kota region. Int J Med Res Rev 2016;4(10):1863-1870.
21. Jahan H, Jhora S, Habib Z, Yusuf M, Ahmed I, Farzana A and Parveen R. Diagnostic Evaluation of GeneXpert MTB/RIF Assay for the Detection of Rifampicin Resistant Mycobacterium tuberculosis among Pulmonary Tuberculosis Patients in Bangladesh. J Tuberc Res. 2016;4:55-60.
22. Helb D, Jones M, Story E, Boehme C, Wallace E, Ho K et al. Rapid Detection of Mycobacterium tuberculosis and Rifampicin Resistance by Use of On-Demand, Near-Patient Technology. J Clin Microbiol. 2010; 51 :132-144.
23. Haider Al, Darraji HA, Razak HA, Ng KP, Altice FL, Kamarulzaman A. The diagnostic performance of a single GeneXpert MTB/RIF assay in an intensified tuberculosis case finding survey among HIV-infected prisoners in Malaysia. PLoS One. 2013; 8(9) :73717.
24. Sharma A, Bisht D, Das S, Rai G, Dutt S, Arora VK. Molecular Detection of Aspergillus in Sputum of Patients with Lower Respiratory Tract Infections. Int J appl basic med. 2020; 10(2): 86–90.
25. Mwaura EN, Matiru V, Bii C. Mycological findings of sputum samples from pulmonary tuberculosis patients attending TB clinic in Nairobi, Kenya. Virol Mycol. 2013; 2(3):1-6.
26. Kurhade AM, Deshmukh JM, Fule RP, Chande C and Akulwar S. Mycological and serological study of pulmonary aspergillosis in central India. IJMM. 2002; 20(3):141–144.
27. Prabha R, G, Dhivya & S, Pramodhini & Easow, J M. A bacterial and fungal profile of sputum samples in a tertiary care hospital in Puducherry. IJMR.2021; 8(1): 53-57.
28. Nasir IA, Shuwa HA, Emeribe AU, Adekola HA, Dangana A. Phenotypic profile of pulmonary aspergillosis and associated cellular immunity among people living with human immunodeficiency virus in Maiduguri, Nigeria. Tzu Chi Med J. 2019; 31(3):149-15328.
29. Iqbal N, Irfan M, Mushtaq A, Jabeen K. Underlying Conditions and Clinical Spectrum of Chronic Pulmonary Aspergillosis (CPA): An Experience from a Tertiary Care Hospital in Karachi, Pakistan. J Fungi. 2020; 6(2):41