Original Article

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Fungal Isolates in Sputum Samples of Multidrug-resistant Tuberculosis Suspects

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Abstract

Background: Fungal pulmonary infection has been emerging recently due to widely used broad-spectrum antibiotics and steroids. Due to immune deficiency or suppression in tuberculosis (TB), patients are easily vulnerable to opportunistic fungal infections. The aim of the present study was to isolate fungi from sputum samples of multidrug-resistant TB (MDR-TB) suspects which may help in the correct diagnosis of these patients.

Materials and Methods: In the present study, 100 sputum samples of MDR-TB suspects received in culture and drug susceptibility testing lab under RNTCP at Andhra Medical College, Visakhapatnam, were included. Gram's-stain and Ziehl–Neelsen (ZN) stain were done for all the samples. Before processing the samples for TB culture and polymerase chain reaction, they were inoculated on two sets of sabouraud's dextrose agar with gentamicin and incubated one set at 25°C in biological oxygen demand incubator and another set at 37°C in the incubator. The fungi were isolated and identified as per the standard procedures in the lab. No growths observed after 4 weeks of incubation were taken as sterile.

Results: Out of 100 samples, 62% were positive for opportunistic fungi, and out of these, 34 (54.8%) were yeasts, whereas 28 (45.2%) were filamentous fungi. *Candida* non albicans were the predominant yeasts 16, followed by *Candida albicans* 14 and *Cryptococcus* species 4. Among filamentous fungi, *Aspergillus niger* was the predominent isolate 10, followed by *Aspergillus fumigatus* 8, *Aspergillus flavus* 4, *Penicillium* species 4, and *Rhizopus* species 2. Out of 100 samples, 54 were positive for acid-fast bacilli by ZN stain.

Conclusion: As there is a high prevalence of fungal infections in TB patients, routine screening for fungal infection is recommended for proper diagnosis and early treatment.

Key words: *Aspergillus* species, *Candida* non albicans, Multidrug-resistant tuberculosis suspects, Opportunistic fungal infections, Sabouraud's dextrose agar, Screening

INTRODUCTION

Tuberculosis (TB) is principally a disease of poverty, with 95% of cases and 98% of deaths occurring in developing countries.[1] Globally, about 3% of all newly diagnosed patients have multidrug-resistant TB (MDR-TB).[2] India accounts for one-fifth of the global incidence of TB and is on top of the list of 22 high TB burden countries.[3]

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Fungal pulmonary infection has been emerging recently due to widely used broad-spectrum antibiotics and steroids.[4] It can be acquired primarily or secondarily in TB, immunodeficiency patients, other chronic disease such as diabetes mellitus or malignancy, HIV and may worsen the primary disease.[5-9] Due to immune deficiency or suppression in TB, patients are easily vulnerable to opportunistic fungal infections.[10] The clinical features of mycotic infections and coexisting bacterial pneumonia alter the manifestations of TB.[11] There is increasing awareness among clinicians and microbiologists pertaining to importance of infections caused by opportunistic fungi.[12-14] The aim of the present study was to isolate fungi from sputum samples of MDR-TB suspects by conventional culture which may help in the correct diagnosis of these patients.

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MATERIALS AND METHODS

In the present study, 100 sputum samples of MDR-TB suspects received in culture and drug susceptibility testing lab under RNTCP at Andhra Medical College, Visakhapatnam, were included. Gram'-stain and Ziehl–Neelsen (ZN) stain were done for all the samples. Before processing the samples for TB culture and polymerase chain reaction, all the samples were inoculated on two sets of sabouraud's dextrose agar with gentamicin and incubated one set at 25°C in biological oxygen demand incubator and another set at 37°C in the incubator. The yeasts were isolated within 24-48 hours and identified as per the standard procedures in the laboratory such as germ tube test, corn meal agar, hicrome agar, urease test, and capsular stain. The inoculated specimens were incubated up to 4 weeks, and growths were observed regularly and identified by lactophenol cotton blue mounts and slide culture techniques. No growths observed after 4 weeks of incubation were taken as sterile. Direct KOH mountings were not done as the samples were from MDR-TB suspects.

RESULTS

Out of 100 samples, 62% were positive for opportunistic fungi, and out of these, 34 (54.8%) were yeasts, whereas 28 (45.2%) were filamentous fungi (Table 1). Out of 34 yeasts isolated, *Candida* non albicans were predominant 16 (47%) followed by *Candida albicans* 14 (41.2%) and *Cryptococcus* species 4 (11.8%). Among *C. nonalbicans*, *Candida tropicalis* 8, *Candida krusei* 4, and *Candida glabrata* 4 were isolated (Table 2). Out of the 28 filamentus fungi, the predominant isolates were *Aspergillus niger* 10 (35.6%) followed by *A. fumigatus* 8 (28.6%), *A. flavus* 4 (14.3%), *Penicillium* species 4 (14.3%), and *Rhizopus* species 2 (7.2%) (Table 3). Out of 100 samples, 54 were positive for acid-fast bacilli by ZN stain.

Table 1: Distribution of culture positivity (*n*=100)

Culture positive	(*n*=62) (%)	Culture sterile (*n*=38)
Yeasts	34 (54.8)	
Filamentous fungi	28 (45.2)	
Total	62	38

Table 2: Distribution of yeasts (*n*=34)

Isolate	*n* (%)
C. albicans	14 (41.2)
C. tropicalis	8 (23.4)
C. krusei	4 (11.8)
C. glabrata	4 (11.8)
Cryptococcus spp.	4 (11.8)
Total	34

C. albicans: Candida albicans, C. tropicalis: Candida tropicalis, C. krusei: Candida krusei, C. glabrata: Candida glabrata

Table 3: Distribution of filamentous fungi (*n*=28)

Isolate	Number (%)
A. niger	10 (35.6)
A. fumigatus	8 (28.6)
A. flavus	4 (14.3)
Penicillium spp.	4 (14.3)
Rhizopus spp.	2 (7.2)
Total	28

A. niger: Aspergillus niger, A. flavus: Aspergillus flavus, A. fumigatus: Aspergillus fumigatus

DISCUSSION

Fungal infections remain a leading cause of infectious morbidity and mortality in heavily immunosuppressed patients.[15-20] Many clinicians miss fungal pulmonary infection because it does not show specific clinical manifestations and usually hindered by other diseases and cause high rates of morbidity and mortality.[21] Early initiation of effective antifungal therapy and reversal of underlying host defects remain the cornerstones of treatment for pulmonary fungal infections.[22] Therefore, there is an acute need for proper diagnosis of the opportunistic fungal pathogens, especially in TB patients.[23] In the present study, fungi were isolated in 62% of samples which correlates with Jain *et al.*,[24] who reported 53%, Yadu *et al.*,[25] 49%, and Bansod and Rai[11] 46%. Yeasts were isolated in 54.8% and filamentous fungi in 45.2% of samples in the present study which correlates with Yadu *et al.*, who reported 62.3% and 37.7%. *C. albicans* was isolated in 41.2% among yeasts which correlates with Yadu *et al.,* (34.7%). *C. non albicans* and *Cryptococcus* species were isolated in the present study which correlates with Bansod and Rai, Yadu *et al.* The predominent filamentus fungi isolated in the present study were *Aspergillus* species which correlates with Osman *et al.,* Yadu *et al.,* and Ekenna *et al.*[26]

CONCLUSION

As there is a high prevalence of fungal infection in TB patients, routine screening for fungal infection is recommended for proper diagnosis and early treatment.

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