



ISOLATION OF BACTERIA, WITH SPECIAL REFERENCE TO (DETECTION) MYCOBACTERIUM TUBERCULOSIS, AND FUNGAL AGENTS CAUSING LOWER RESPIRATORY TRACT INFECTIONS IN HIV POSITIVE PATIENTS.

Microbiology

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ABSTRACT

INTRODUCTION: The respiratory tract is a common site for opportunistic infections in persons living with HIV (PLHIV). Bacterial, mycobacterial, and fungal pathogens commonly cause pneumonia. The present study was conducted to determine the causative agents of LRTI in PLHIV.

MATERIALS AND METHODS: 100 HIV patients diagnosed with lower respiratory infection were enrolled as subjects. Expecterated and induced sputum were processed, and bacterial and fungal pathogens were identified as per standard guidelines.

RESULTS: Bacterial agents were the most common pathogens (36.0%), followed by Mycobacterium tuberculosis (31.0%) and fungal pathogens (19.0%). Sputum microscopy was positive for AFB in 13% of the cases, while CBNAAT detected 31% of the cases.

CONCLUSION: Bacterial and mycobacterial agents form the leading cause of lower respiratory infections in PLHIV. CBNAAT helps in increased case detection in lesser time to diagnose pulmonary TB in PLHIV as compared to conventional sputum microscopy.

KEYWORDS

CBNAAT, HIV, LRTI, microscopy, Tuberculosis

INTRODUCTION:

Infection with HIV results in AIDS as the end-stage disease with impaired immune defence mechanisms. The respiratory tract is a common site for opportunistic infections. Lower respiratory tract infections are caused by bacterial, mycobacterial and fungal organisms. *Mycobacterium tuberculosis* is the pathogen causing Tuberculosis, and it remains a global health menace, especially in developing countries like India'. It is essential to diagnose LRTI in HIV infected patients, especially Tuberculosis, as early as possible to avoid case fatalities.

AIMS AND OBJECTIVES:

- To isolate the causative bacterial and fungal agents causing lower respiratory tract infections in HIV positive individuals
- For diagnosing pulmonary TB infection, determining the efficacy of:
 - direct microscopy before and after sputum concentration technique by modified Petroff's method
 - CBNAAT/GeneXpert

MATERIALS AND METHODS:

STUDY GROUP:

HIV seropositive patients clinically diagnosed to have lower respiratory tract infection attending the department of Pulmonology were enrolled in the study.

INCLUSION CRITERIA:

Patients who are 13 years and above and who are HIV seropositive presenting with a clinical picture of Lower respiratory tract infection.

EXCLUSION CRITERIA:

- Patients who have been administered antibiotics/antifungals before sample collection
- Patients who were a known case of pulmonary tuberculosis and are on anti-tubercular treatment.
- Patients who are relapse, default, and failure cases.

METHOD:

CASE HISTORY IN DETAIL:

Demographic details and clinical history of the patient was taken. Clinical history of the patient and investigation done in relation to their condition is kept confidential.

SAMPLE COLLECTION²:

Spot and early morning expecterated sputum samples were collected separately in sterile containers. For patients who are unable to expectorate sputum, induced sputum samples are collected using a Nebulizer and 3% hypertonic saline.

SAMPLE PROCESSING²:

Sputum specimens were examined macroscopically and were stained by Gram's stain. The quality of the sample was assessed by Bartlett's score. Samples with a final score of 1 or more were considered suitable for further processing.

BACTERIAL PROCESSING²:

All the samples were inoculated onto Mac Conkey Agar, 5% Sheep Blood Agar, Nutrient agar, and Chocolate agar and incubated at 37°C for 18-24 hours. Significant growth after incubation was further identified by conventional microbiological techniques as per standard protocol.

FUNGAL PROCESSING²:

10% KOH mounts and India ink mounts were prepared to look for fungi and encapsulated yeasts, respectively. The samples were then inoculated onto Sabouraud's dextrose agar with chloramphenicol and incubated at 28°C for 4-8 weeks. Growth on the media was identified by conventional microbiological techniques as per standard protocol.

MYCOBACTERIAL PROCESSING^{2,3,4}:

Ziehl Neelsen (ZN) stain was performed on the sputum specimens to look for acid-fast bacilli and graded according to RNTCP guidelines. A portion of the specimen was concentrated by Modified Petroff's method and again stained by Ziehl Neelsen method and examined for acid-fast bacilli. All the specimens were subjected to CBNAAT/GeneXpert for detection of *Mycobacterium tuberculosis* and Rifampicin resistance.

RESULTS:

Out of 100 samples, pathogenic organisms were detected in 86 samples. The majority of the pathogens were detected in the age group 51-60 years. A major number of the samples were positive for the pathogenic organism in males. Tables I and II show the distribution of pathogens detected and the different bacterial pathogens isolated, respectively.

Table 1: Spectrum Of The Pathogens Causing Lrti

Type of pathogen	Total number of positives (n=86)	Percentage
Bacterial	36	41.9%
Fungal	19	22.1%
Mycobacterial	31	36.0%

Table 2: Distribution Of Bacterial Pathogens Isolated

Organism isolated	Number of isolates (n=36)	Percentage
<i>Staphylococcus aureus</i>	11	30.6%
<i>Klebsiella pneumoniae</i>	11	30.6%
<i>Pseudomonas aeruginosa</i>	6	16.7%
<i>Citrobacter species</i>	3	8.3%

<i>Acinetobacter species</i>	3	8.3%
<i>Escherichia coli</i>	1	2.7%
<i>Proteus mirabilis</i>	1	2.7%

On fungal culture, 19 fungal pathogens were isolated, which were *Candida albicans*. No filamentous fungi were isolated.

On direct microscopy, six samples were positive for AFB. The RNTCP grading of the six positive samples is shown in table III.

Table 3: Distribution Of Results According To Rntcp Grading Of AFB Smears

Grade	Number of positive samples
0	94
Scanty	2
1	2
2	1
3	1

After concentration by Modified Petroff's method, totally 13 samples were positive for AFB on microscopy. Seven samples that were missed on direct ZN staining were detected. Figure 1 and 2 show ZN stained smears of sputum before and after concentration techniques, respectively.

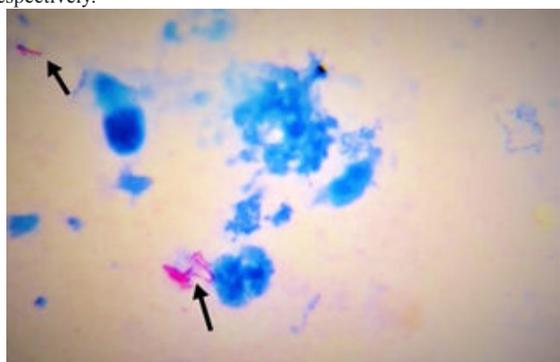


Fig. 1: Zn Stained Sputum Before Being Treated By Modified Petroff's Method)

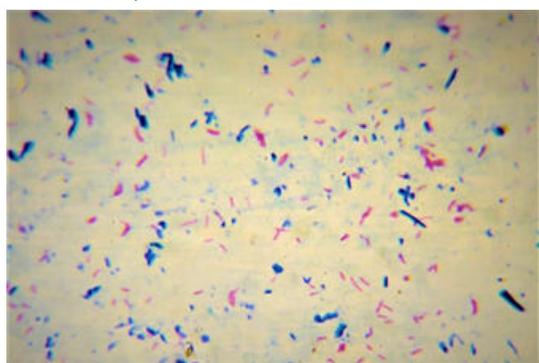


Fig. 2: Zn Stain Of Sputum After Treating With Modified Petroff's Method

A total of 31 samples were tested positive by CBNAAT. These 31 samples are inclusive of the samples that were smear positive for AFB (13). CBNAAT had detected *M.tb* in 18 samples that were missed on microscopy. This comparison is shown in table IV.

Table 4: Comparison Of AFB Staining With CBNAAT

	AFB Staining	Number of positive samples detected by CBNAAT	Total
Number of positives	13	18	31

CBNAAT had detected *Mycobacterium tuberculosis* in 25 samples that were graded 0 (RNTCP guidelines) by direct microscopy. These 25 samples constitute the seven samples that were positive for microscopy after modified Petroff's method and 18 samples that were negative for AFB on microscopy. None of the samples were positive for ZN staining and negative for CBNAAT, thereby ruling out the

possibility of non-tuberculous mycobacterial (NTM) infection. All the 31 samples were negative for Rifampicin resistance.

DISCUSSION:

This study is undertaken to determine the various bacterial, mycobacterial, and fungal agents causing LRTI in HIV infected cases.

The etiological agent was identified in 86% of the cases, and no pathogen was detected in 14% of the cases. This is comparable to studies conducted by Kandati et al. and Palewar S et al., in which the causative agent of LRTI was isolated in 89.5% and 71.43% of the cases respectively^{5,6}. In the present study, the unidentified pathogens may be attributable to viral etiology of LRTI.

The mean age in this study population is 49.82. According to national data, the most commonly affected age group is 21-40 years⁷. As this age group is more occupationally active, they are more exposed to pathogens, which may be contributory to the higher incidence of LRTI in this age group.

In the present study, amongst the causative agents of LRTI, the percentage of bacterial pathogens was 41.9%, fungal pathogens 22.1%, and *Mycobacterium tuberculosis* 36.0% as shown in table 1. Comparison of the spectrum of pathogens causing LRTI in this study with other studies is shown in figure 3.

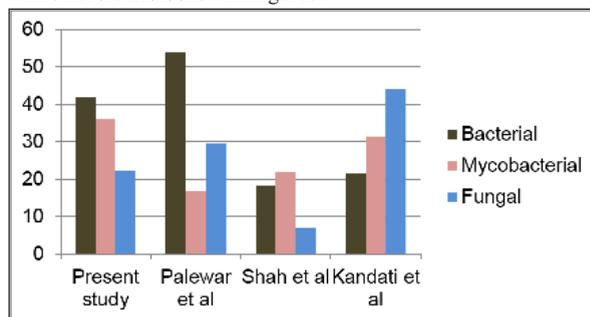


Fig. 3: Spectrum Of The Pathogens Causing Lrti In Other Studies^{5,6,8}.

Table II shows that *Klebsiella pneumoniae* (30.6%) and *Staphylococcus aureus* (30.6%) were the most common bacterial isolates causing LRTI followed by other Gram-negative bacteria. Table V shows the bacterial organisms isolated in the present study compared to other studies.

Table 5: Comparison of bacterial isolates causing LRTI in various studies^{5,6,8,9}.

	Present study	Yadav et al	Palewar et al	Kandati et al	Shah et al
<i>Klebsiella pneumoniae</i>	30.6%	27.0%	15.21%	-	-
<i>Staphylococcus aureus</i>	30.6%	20.8%	13.44%	-	-
<i>Pseudomonas aeruginosa</i>	16.7%	4.2%	-	15.15%	-
<i>Acinetobacter species</i>	8.3%	-	-	15.1%	6.21%

Although criteria for diagnosis of pulmonary Candidiasis is controversial, in this study *Candida* in sputum was regarded as a pathogen based on the presence of numerous polymorphonuclear leucocytes along with plenty of pseudohyphae in direct Gram's stain and isolation of *Candida* from repeat sample¹⁰.

In this study, *Candida albicans* was the only fungal pathogen isolated (22.1%). Similarly, it was the most common fungal pathogen isolated in studies conducted by Palewar et al. (5.21%) and Manjunath et al. (45.0%)^{6,12}. It has been observed that although *Candida albicans* is the most common fungal pathogen of the respiratory tract, non-albicans *Candida* infections in HIV infected patients are on the rise in India from the past few years¹¹.

No filamentous fungi were isolated in this study. The findings of this study are against the findings of Kandati et al. and Chandwani et al. who reported the isolation of filamentous fungi in 12.83% and 27.7% cases respectively^{3,13}. However, Shah et al. reported a low prevalence

as they isolated filamentous fungi in 3 out of 961 cases (0.31%)⁸. Advanced immunosuppression, i.e. CD4+ T-cell count <100 cells/ μ L, leads to failure in eradication of the fungus¹⁴. Chandwani et al., concluded that Candidal infections of the respiratory tract occurred in patients with CD4+ count <350 cells/ μ L and filamentous fungal infections occurred in patients with CD4+ count <100 cells/ μ L¹³. Prompt initiation of antiretroviral therapy as soon as the patient is tested seropositive for HIV, monitoring of CD4+ T-cell counts (both under ART centre) and geographic/climatic variation may explain the absence of filamentous fungal infections in this study group.

HIV-TB co-infection is a catastrophic comradeship as weakened immunity caused by HIV predisposes an individual to Tuberculosis, and active Tuberculosis accelerates the progress of HIV disease¹⁵. There has been a paradigm shift in the diagnostic approaches towards TB, from the ancient sputum smear microscopy to the current era of molecular tests.

In this study, smear positivity improved from 19.35% when directly stained, to 41.94% when stained after treating with modified Petroff's method. This correlates with the findings of Tripathi et al., who showed that the positivity of ZN stain increased from 54.67% to 57.33% before and after concentration with modified Petroff's method, respectively³. Matte et al concluded in their study that specimen processing methods like digestion or liquefaction followed by centrifugation/prolonged gravity sedimentation/filtration increases sensitivity by 13 to 33 percent over direct microscopy¹⁶.

The sensitivity of sputum microscopy is further compromised in HIV positive patients, ranging from 43% to 51%. To overcome such shortcomings, WHO has endorsed the use of a cartridge-based nucleic acid amplification test, GeneXpert-Rif assay¹⁷.

All the samples in this study were subjected to CBNAAT. A total of 31 samples were positive for *Mycobacterium tuberculosis*. CBNAAT had detected 18 samples that were smear-negative.

Among 31 subjects with CBNAAT positivity, 41.9% were smear-positive and 58.06% were smear-negative for AFB (false negative). Among 69 cases negative for CBNAAT, 100% were negative for sputum microscopy. Thus, this observation shows that sputum microscopy had 58.06% false-negative rate compared to CBNAAT. The consequences of this can be delayed or misdiagnosed cases, contributing to delayed treatment with increased morbidity and mortality rates and continued spread of TB to contacts. Figure 4 shows comparison of performance of microscopy with CBNAAT in other studies.

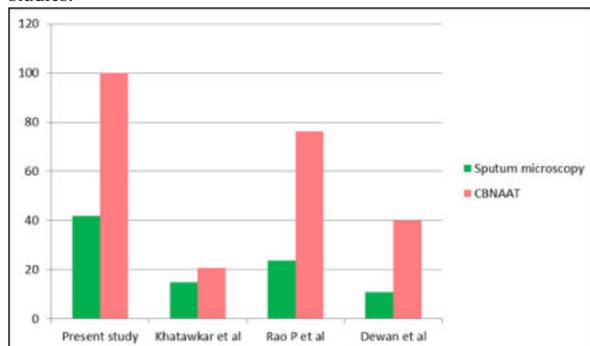


Fig. 4: Comparison Of Microscopy With CBNAAT In Various Studies^{18,19,20}.

None (0%) of the samples were resistant to Rifampicin. This is against the findings of Dewan et al. and Khatawkar et al. where Rifampicin resistance was detected in 25% and 16.1% of cases in their studies^{18,19}. Retrospective analysis showed that rifampicin resistance was seldom detected in patients being tested in this institute.

CONCLUSION

The causative agent of bacterial and fungal pneumonia should be identified to improve the prognosis of PLHIV affected with LRTI. In resource limited settings where sputum microscopy is the only means of diagnosing TB, decontamination, and concentration of sputum samples by modified Petroff's method can be routinely implemented to improve tuberculosis case finding. WHO recommends CBNAAT for

diagnosis of tuberculosis in PLHIV as it has greater efficacy than sputum microscopy, also helping in early diagnosis in less than 2 hours.

However, as ground realities go, it will take a long time for CBNAAT to replace conventional microscopy as a diagnostic test. Hypothetically, for an ideal test to diagnose tuberculosis, it should be available for use in peripheral centres where there are limited resources. The technology should be available to populations that need it most, but can afford the least. Currently, as such a diagnostic test does not exist, as of now; the closest to this is sputum smear microscopy. Till such a point of care diagnostic test is available; perhaps, sputum smear microscopy will stay around in our laboratories.

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