

## Research Article

# Bacterial and fungal agents causing lower respiratory tract infections in patients with human immunodeficiency virus infection

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## ABSTRACT

**Background:** HIV the causative agent of acquired immunodeficiency syndrome (AIDS) was considered a global problem after its discovery in 1981. Lower respiratory infections are 25 fold more common in HIV cases, than in the general community, occurring in up to 90 cases per 1000 person-years. The present study was conducted to determine the regional profile of respiratory pathogens including opportunistic implicated in lower respiratory tract infections in HIV cases.

**Methods:** 200 HIV patients diagnosed with lower respiratory infection were enrolled as subjects. Expecterated, induced sputum and Broncho alveolar lavage were processed and bacterial and fungal pathogens were identified as per standard guidelines.

**Results:** *M. tuberculosis* was the common pathogen (44.08%), followed by bacterial (21.71%) which include predominant Gram negative bacteria. *Candida sp* was the common fungal pathogen (16.45%) followed by *Aspergillus sp* (12.83%), *Cryptococcus sp* (1.97%). Most important observation of the study was isolation of nine cases of *Pneumocystis jiroveci* pneumonia.

**Conclusions:** Bacterial and fungal agents form the main cause of lower respiratory infections in HIV individuals. Most of them are polymicrobial and an increasing trend of pneumocystis pneumonia is observed.

**Keywords:** HIV, Broncho alveolar lavage, *Pneumocystis jiroveci*, *Aspergillus sp*

## INTRODUCTION

HIV the causative agent of acquired immunodeficiency syndrome (AIDS) was considered a global problem after its discovery in 1981.<sup>1</sup> A 27% increase, is noted in a number of HIV cases worldwide from 1999 to 2009. Though its entry into India is late in 1984, it has reached an epidemic proportion in some of the states. There are 21.17 lakhs cases of HIV in India, with an adult (15-49 years) prevalence of 0.26% (0.22%–0.32%).<sup>2</sup> *Pneumocystis carinii* pneumonia was the cause of death in first cases of AIDS, signifying the importance of

respiratory infections in HIV-infected cases.<sup>3</sup> Up to 70% of HIV cases develop respiratory infections of infectious aetiology during the evolution of the disease. Lower respiratory infections are 25 fold more common in HIV cases, than in the general community, occurring in up to 90 cases per 1000 person-years.<sup>4</sup> Pulmonary infections remain a leading cause of morbidity and mortality and one of the most frequent cause of hospital admissions in HIV-infected cases worldwide with 20-25 episodes per 100 hospital admissions per year.<sup>5,6</sup> There are major differences in the spectrum of pathogens of respiratory infections in India and the west. However, the relative

importance of infections and the pathogens differ from region to region. In Indian scenario, with the introduction of HAART, a significant reduction of respiratory infections has been mentioned in few studies, although some studies don't agree with these findings.

The present study was conducted to determine the regional profile of respiratory pathogens including opportunistic implicated in lower respiratory tract infections in HIV cases. This knowledge regarding the pathogens will be useful in recognition and timely intervention for proper management of HIV cases.

## METHODS

### Study design and group

The study was conducted at Narayana general and super specialty hospital. Between January 2015 and December 2015, Two hundred consecutive patients who were diagnosed with lower respiratory tract infection by the clinician and tested positive for HIV attending the Department of Pulmonology and Department of General medicine, as inpatients and out patients were enrolled in the study. The study was approved by the institutional ethical committee and research committee of the hospital. All the demographic data of the enrolled subjects who consented for the study were collected in a predesigned protocol form which included age, mode of transmission, presenting complaints, stage of HIV, marital status etc. Patients of paediatric age, less than 20 years, confirmed cases of pulmonary and extra-pulmonary tuberculosis, on antifungal and antibiotics [except Trimethoprim + sulphomethoxazole as part of HAART (Highly active antiretroviral therapy)] are excluded from the study. Staging of the patient was at the discretion of the physician, following WHO guidelines.

### Specimen collection

Early morning expectorated sputum was collected in sterile, plastic disposable containers after advising strict precautions. Induced sputum specimen [By aerosol Nebulisation of 3% hypertonic saline] is collected from patients who were unable to expectorate or produce good quality sputum, following all universal precautions under supervision by a pulmonologist. Broncho alveolar lavage fluid by bronchoscopy was collected by trained pulmonologist following universal precautions, from patients who were negative for *Mycobacterium tuberculosis* by sputum smear microscopy. All the specimens were further processed for staining, wet mount, culture under bio-safety cabinet- III.

### Microbiological workup

#### Microscopy

All the specimens collected were given a unique Lab ID. Smears from the purulent portion of good quality

expectorated, deposit of centrifuged induced sputum were made and performed gram stain, Zeil- Nelson staining, Modified acid fast staining by using 1% H<sub>2</sub>SO<sub>4</sub> for *Nocardia*, Toluidine-Blue stain for detection of Cysts of *Pneumocystis jiroveci* and finally KOH wet mount for fungal elements. BAL fluid was cytocentrifuged and the sediment performed ZN staining and observed under microscope.

### Culture

Material from the sputum and BAL fluid were inoculated on 5% sheep blood agar, heated blood agar, McConkey agar and incubated at 37<sup>0</sup>c and also with 5 -10% CO<sub>2</sub> for bacterial isolation. Hi-CHROME Candida agar for speciation of Candida and [Himedia Laboratories, Mumbai] sabourads Dextrose agar [SDA] with antibiotics in duplicate is incubated at 37<sup>0</sup>c and 25<sup>0</sup>c for 2-4 weeks and observed for fungal growth at regular intervals. Identification of isolates was done by a battery of standard microbiological tests.<sup>7,8</sup>

Antibiotic sensitivity testing for bacterial isolates was done by Kirby -Bauer disc diffusion test using CLSI guidelines.<sup>9</sup>

### Identification of fungal isolates

*Candida* sp on Hi-CHROME agar was identified by the colour of the colony as per manufacturers manual. *C. albicans* was identified further by germ tube test and Chlamydospore formation on Corn meal agar. *Cryptococcus* was identified on SDA by mucoid colony and demonstration of capsule by Indian ink preparation. *Aspergillus* was identified by colony morphology on SDA, lactophenol cotton blue mount and Riddle's slide culture technique for speciation.<sup>10</sup> Cysts of *Pneumocystis jiroveci* were identified by microscopy alone using Toluidine Blue staining. (Blue colored honeycombed appearance)

Identification of *Mycobacterium tuberculosis* was done by Zn staining as per RNTCP guidelines.<sup>11</sup> Cultivation of *Mycobacterium* was not done for further differentiation from atypical *mycobacteria* due to lack of facilities.

HIV status of the patient was confirmed at integrated counselling testing centre, Dept of Microbiology. ERS tests [ELISA, Rapid, and Simple tests] were done to Confirm and assess the HIV antibody status of the individual as per the recommendations of National AIDS control organization [NACO].<sup>12</sup>

### Statistical analysis

All the data of the subjects, including age, mode of transmission, staging of HIV, clinical profile, presenting complaints were collected and entered in a predesigned protocol form. Data was entered by trained paramedical personnel in Statistical package for social sciences

(SPSS) version 15 and analyzed. The mean, median were calculated. P value <0.005 was considered significant.

## RESULTS

During the study period of one year, two hundred diagnosed cases of HIV in various stages, with confirmed LRTI were enrolled in the study. The mean age of the patients was 55.21±15.28 years, with 44% in the age group of 41-60 years which is the main resource and productive age group of the country followed by 61-80 years (34%). Females outnumbered males in our study with 55.5%.

The common presenting complaints were fever (71.2%), weight loss (69.9%) and loss of appetite (70%). Findings of our study are on par with findings of Kaur et al., 1992, Kumar & Goyal, 2001 and Anupriya Wadhwa et al.<sup>13-15</sup>

Heterosexual mode of transmission was the commonest (73.5%) mostly from males to females, followed by blood transfusion (11%), drug abuse (2.5%) and in 13% of cases, cause could not be ascertained. 67 (33.5%) were in WHO Stage-I of HIV followed by, 62 (31%) and 42 (21%) in WHO Stage-II & WHO Stage-III, only 29 (14.5%) in WHO Stage-IV. Expecterated and Induced sputum was collected from 125 and 75 cases, whereas BAL was collected from 87 (Table 1).

**Table 1: Demographic data of HIV cases.**

Demographic variables	No. of cases (percentage)
<b>Gender</b>	
Male	89 (44.5%)
Female	111 (55.5%)
<b>Age group in years</b>	
21-40	35 (17.5%)
41-60	88 (44%)
61-80	68 (34%)
>80	9 (4.5%)
Mean age	55.21±15.28
<b>Stage of HIV</b>	
I	67 (33.5%)
II	62 (31%)
III	42 (21%)
IV	29 (14.5%)
<b>Mode of transmission</b>	
Heterosexual	147 (73.5%)
Blood transfusion	22 (11%)
Drug abuse	5 (2.5%)
Not known	26 (13%)
<b>Specimens</b>	
Expecterated sputum	125
Induced sputum	75
BAL	87

In the present study, out of total 287 specimens processed for isolation of pathogens, 266 produced polymicrobial growth and 21 did not demonstrate any significant pathogens which may be due to viral or bacterial or fungal which could not be cultivated. Table 2 shows the distribution of various pathogens from the specimens. *M. tuberculosis* was the commonest pathogen 134/304 (44.08%) followed in order by other bacterial (66/304, 21.71%), *Candida* sp (50/304, 16.45%), *Aspergillus* sp (39/304, 12.83%), *Cryptococcus* sp (6/304, 1.97%) and *Pneumocystis jiroveci* (9/304, 2.96%). *Klebsiella pneumoniae* (14), *Acinetobacter baumannii* (10), *Pseudomonas aeruginosa* (10), *Methicillin-resistant staphylococcus aureus* (8), *Coagulase negative staphylococcus aureus* (7), *Escherichia coli* (7), *Streptococcus pneumoniae* (7) and *Nocardia* (3) were the bacterial isolates from the specimens.

Table 3 shows the isolation of bacterial isolates from various stages of HIV cases included in the study.

**Table 2: Distribution of various isolates.**

Type of isolate	No. of cases	Percentage
<i>Mycobacterium tuberculosis</i>	134	44.08
Bacterial isolates	66	21.71
<i>Candida</i> sp	50	16.45
<i>Aspergillus</i> sp	39	12.83
<i>Cryptococcus</i> sp	6	1.97
<i>Pneumocystis jiroveci</i>	9	2.96
No Isolate	21	10.5

*Candida albicans* was isolated from 37 cases and *C.tropicalis* from 17 cases. Six cases were confirmed as Cryptococcal pneumonia. *Aspergillus niger* was isolated from 32 and *Aspergillus flavus* from 7 cases. Cystic forms of *Pneumocystis jiroveci* were identified in 9 cases from BAL. Table 4 demonstrate the isolation of fungal pathogens from various stages of HIV.

Out of total 125 expecterated sputum specimens, 79 (63.2%) were MTB positive, 34 (45.3%) were MTB positive in induced sputum. Out of 87 BAL specimens, 21 (24.1%) were MTB positive clearly indicating an additional gain of 7.3% by screening of BAL fluid for *Mycobacterium tuberculosis* in sputum smear microscopy negative cases. The results were evaluated statistically which demonstrated very high significance [p<0.0001]. Out of 134 total MTB positive cases, 31 (23.1%) were in stage-I, followed by 33 (24.6%), 41 (30.6%) and 29 (21.6%) in Stage-II, II and IV.

Table 5 explains the comparison of isolation and gain of *M. tuberculosis* from sputum and BAL specimens. Bronchoscopy and screening of BAL fluid in HIV-positive individuals provides higher yield in identifying missed cases of MTB by sputum smear microscopy alone.

**Table 3: Distribution of bacterial isolates.**

Bacterial isolates	Number	Stages of HIV			
		I	II	III	IV
<i>Klebsiella pneumoniae</i>	14	4	3	4	3
<i>Acinetobacter baumannii</i>	10	3	3	3	1
<i>Methicillin resistant staphylococcus aureus</i>	8	1	1	4	2
<i>Coagulase Negative Staphylococcus aureus</i>	7	2	5	0	0
<i>Pseudomonas aeruginosa</i>	10	2	3	3	2
<i>Streptococcus pneumoniae</i>	7	1	3	1	2
<i>Nocardia asteroides</i>	3		3		
<i>Escherichia coli</i>	7	2	2	2	1
No growth of Bacteria	134				
<b>Total</b>	<b>66</b>	<b>15</b>	<b>23</b>	<b>17</b>	<b>11</b>

**Table 4: Distribution of fungal isolates.**

Fungal isolate	Number	Stages of HIV			
		I	II	III	IV
<i>Candida albicans</i>	37	8	7	10	12
<i>Candida tropicalis</i>	13	1	2	2	8
<b>Total</b>	<b>50</b>	<b>9</b>	<b>9</b>	<b>12</b>	<b>20</b>
<i>Cryptococcus sp</i>	6	2	0	4	0
<i>Aspergillus niger</i>	32	8	5	11	8
<i>Aspergillus flavus</i>	7	0	2	2	3
<b>Total</b>	<b>39</b>	<b>8</b>	<b>7</b>	<b>13</b>	<b>11</b>
<i>Pneumocystis jiroveci</i>	9	0	0	2	7

**Table 5: Isolation of *Mycobacterium tuberculosis* from specimens.**

Specimen	MTB+VE n (%)	MTB-VE n (%)	Total	p value
ES	79 (63.2%)	46 (36.8 %)	125 (43.55%)	< 0.0001 (VHS)
IS	34 (45.3%)	41 (54.7%)	75 (26.13%)	
BAL	21 (24.1%)	66 (75.9%)	87 (30.31%)	
<b>Total</b>	134 (46.69%)	153 (53.31%)	287 (100.0%)	

\*Chi-square test, VHS – very highly significant.

## DISCUSSION

In the present study, we found the mean age group was 55.21±15.28 years, with female preponderance (55.5%) which is in contrast with many of the studies. The most common age group in the study was 41-60 years which coincides with the findings of few studies in India elsewhere, but studies on age group are variable from place to place and region, national data suggests 21 -40 years as the most common age group and females accounted for 39%.<sup>16</sup> Heterosexual route was the most common mode of transmission in our study (73.5%) which coincides with the national data and studies all over, 22 cases through blood transfusion where majority were in geriatric age group and in 26 cases mode was not known.

Out of 200 HIV reactive cases, etiological agent for LRTI was identified in 179 cases, *M tuberculosis* was the

predominant pathogen isolated (44.08%) in the study which is on par with the findings of Shailaja et al, Mohanthy KC et al, but is in contrast with the findings of Shreevidya K et al and Sharma et al with high 83.3% and 71.1% isolation rate.<sup>17-20</sup> Atypical mycobacteria are more common in western countries than in India; however studies on prevalence of Atypical mycobacteria in HIV are few.

As per the data of NACO, Bacterial infections form 7% of total infections; common etiological agents include *Staphylococcus aureus*, *Hemophilus influenzae* and *Streptococcus pneumoniae*. Among the total 66 bacterial isolates in the study other than *M. tuberculosis*, *Klebsiella pneumoniae* was predominant (21.2%) followed by *Acinetobacter baumannii* and *Pseudomonas aeruginosa* (15.2%) which coincides with the findings of Shailaja VV et al, Srividya et al. HIV patients are more susceptible or at increased risk of developing respiratory

infections predominantly by Capsulated organisms.<sup>21</sup> *Acinetobacter baumannii* was mostly isolated from hospitalized cases, it was hospital acquired. *Pseudomonas aeruginosa* is mostly community acquired and was notable for its drug resistance and patient expired due to pneumonia. Dropulic in his study explains that *P. aeruginosa* accounted for repeated infections in HIV cases and most of them were pneumonia.<sup>22</sup> Tcharman in his study in African population noted that *S. pneumoniae* was the most common pathogen (81%) from respiratory infections.<sup>23</sup> However, the decrease in bacterial infections can be explained by the reason that most of the cases of HIV were on cotrimoxazole prophylaxis along with HAART. Gram-negative bacteria isolates of the study demonstrated multi-drug resistance. *Pseudomonas aeruginosa* was sensitive to Imipenem and Piperacillin+tazobactam. *Acinetobacter sp* and other members of Enterobacteriaceae were sensitive only to imipenem in the study which coincides with the studies of Shailaja et al and Srividhya et al.

*Candida* constitutes the most common fungal pathogen in HIV positive cases. In our study 50, *Candida* was isolated of which 37 were *albicans* and 13 were *tropicalis*. *C. albicans* still remains as the major pathogen which is on par with the findings of Monika maheswari et al, Mourya et al and many other studies in India.<sup>24,25</sup> Some of the studies from abroad state that there is a shift in distribution of *Candida* species from *albicans* to Non *albicans* group in HIV individuals.<sup>26</sup>

Six cases of cryptococcal pneumonia were identified in the study, out of which 2 cases from stage-I and 4 from stage-IV. All the four cases of stage-IV were hospitalized and two expired. However data regarding the aetiology and prevalence of *Cryptococcus* in India are few and it is variable from place to place and region. Infections of lower respiratory tract with *Cryptococcus* in HIV positive cases indicate a lowered CD<sub>4</sub> counts which were not performed in the study due to lack of facilities which is a limitation of our study. Findings of our study correlate with the findings of Anupriya Wadhwa et al.<sup>15</sup>

39 cases of invasive pulmonary aspergillosis were confirmed clinically and radiologically. *Aspergillus niger* from 32 and *flavus* from 7 cases was isolated. The majority of the cases of invasive aspergillosis were from Clinical stage-III and IV. Majority of studies have reported invasive aspergillosis when CD<sub>4</sub> counts are less than 200/ $\mu$ l. The most important finding of our study is 9 cases of PCP were diagnosed, with 7 in stage –IV and 2 in stage-III. 5 of the cases in stage –IV expired. As compared to our study, Mishra et al reported a high prevalence of 25.34% of PCP from HIV cases, findings of Khan PA et al reported 1.87% which coincides with our study.<sup>27,28</sup> Earlier reports from India have reported its prevalence to be 17 to 55%.<sup>29,30</sup>

## CONCLUSION

Development of lower respiratory tract infections is a serious problem and mostly in HIV-positive patients. Most of the infections are Polymicrobial with *M. tuberculosis* as the common causative agent and other agents include various bacterial and fungal. *Pneumocystis jiroveci* as the cause of pneumonia in HIV positive cases is increasingly being reported which signifies the necessity of promoting awareness among the individuals and health care professionals. Early diagnosis and effective treatment is an important measure in the prevention of respiratory infections among HIV positive cases. Cheaper diagnostic methods and well-developed guidelines are to be developed for management of co-infections of *M. tuberculosis* and other fungal or bacterial agents. However limitations of our study included, the culture of *M. tuberculosis* could not be done, to differentiate from atypical mycobacteria. CD<sub>4</sub> Counts of HIV-positive individuals could not be estimated to assess the immune status of the individual for correlation with respiratory pathogens.

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