Acta Scientific Microbiology (ISSN: 2581-3226)

Volume 8 Issue 4 April 2025

Research Article

Isolation of Bacterial Agents Causing LRTI in HIV Positive Patients; Radiological Corelation and CD4 Status from a Tertiary Care Centre

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Received: February 03, 2025

Published: March 05, 2025

Abstract

Introduction: Bacterial pneumonia is reported to be more frequent in HIV positive patients as compared to seronegative and the rate of bacterial pneumonia increased with decreasing CD4 lymphocyte counts. The aim of the present study is to ascertain the spectrum of bacterial pathogens causing lower respiratory tract infections in HIV positives in western Rajasthan, microbiological and radiological correlation and to find out variation in CD4+T Lymphocytes, CD8 +T Lymphocytes and CD4/CD8 ratio in HIV positive and HIV negative patients having LRTI.

Material and Methods: A case control prospective study conducted. With a preset protocol a history of clinical signs, and symptoms, radiological findings, routine lab. Investigations was collected. CD4+ T and CD8 + T Lymphocyte counts of the samples under study was estimated by Flow Cytometry using BD FACS Calibur.

Results: Polymicrobial isolation was found to be more in study group. Among the bacterial isolates in the study group, S aureus was isolated in 42.8%, K. Pneumoniae 11.9%, E. coli 4.7% and P. aeruginosa in 9.52%. M tuberculosis was isolated in 8% in the study group. Radiological findings like focal consolidation and military pattern was observed in the study group with M. Tuberculosis isolation. In the study group, mean CD4 counts 214 ± 120 cells/ul, mean CD8 counts 940 ± 168 cells/ul and CD4/CD8 ratio: 0.23 ± 0.11, was found.

Conclusion: HIV positive Patients with LRTI should undergo diagnostic evaluation for specific pathogens that would alter the standard treatment. Also, low CD4 counts predisposes the HIV positive patients to bacterial LRTIs.

Keywords: LRTI; HIV; Polymicrobial

Introduction

AIDS (Acquired Immunodeficiency Syndrome) caused by Human Immunodeficiency virus (HIV) is the most important public health problem in 21st century. In India, an estimated 21.17 lakh people are living with HIV (PLHIV) in 2015. Rajasthan contributes 5% (1.43 lakh) of the total PLHIV in India [1].

Lower respiratory tract infections (LRTI) in AIDS include bronchitis, bronchiolitis and pneumonia. Among the HIV-associated pulmonary complications, opportunistic pneumonias are major causes of morbidity and mortality. The spectrum of HIV-associated opportunistic pneumonias is broad and includes bacterial, mycobacterial, fungal, viral, and parasitic pneumonias [2].

Bronchitis is usually preceded by an upper respiratory tract infection or forms a part of clinical syndrome in diseases such as influenza, rubella, pertussis, scarlet fever and typhoid fever. Chronic bronchitis with a persistent cough and sputum production appears to be caused by a combination of environmental factors such as smoking, and bacterial infection with pathogens such as *H. influ-*

enzae and *S. Pneumonia*. Bronchiolitis is a disease of infants and is caused primarily by respiratory syncytial virus (RSV), other viruses causing bronchiolitis includes parainfluenza virus, adenovirus, influenza virus and occasionally *M. pneumoniae* [3].

Mycobacterial infection, including both tuberculosis (TB) and non-tuberculous mycobacterial infection mainly Mycobacterium avium complex (MAC), is an important cause of morbidity and mortality in immunocompromised patients, particularly in AIDS patients. According to a report from the World Health Organization in 2009, among the 9.27 million cases of TB, approximately 14.8% occurred in HIV-positive patients with about half of million deaths from HIV-infected TB patients. Fatality rate among HIV-infected TB cases remains 13–14% against less than 4% in HIV negative TB cases [4]. Tuberculosis ranks as the most common respiratory infection in HIV patients in developing countries. About 55 -89% of AIDS cases in India were found to be suffering from extensive pulmonary tuberculosis [5]. The implication of HIV infection is that it activates dormant tuberculosis to rapid disease progression of tuberculosis and death [6].

The annual incidence of bacterial pneumonia in HIV-seropositive patients ranges from 5.5 to 29 per 100, compared with 0.7 to 10 per 100 in HIV-seronegative patients [7]. One of the main cause of morbidity and mortality in HIV infected patients is due to bacterial pneumonia. Community acquired pneumonia in adults is most commonly due to bacterial infection.

The overall rate of bacterial pneumonia in HIV infected persons is approximately six times greater than in general population. Bacterial pneumonia in HIV infected individuals is usually community acquired. Regardless of age or coexisting, *Streptococcus pneumonia* (causing 80% of all community acquired bacterial pneumonia) is the most prevalent lower respiratory infection in AIDS. Hemophilus influenza, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* account for most of the remainder of cases. Atypical agents such as Legionella pneumophilia and Mycoplasma pneumonia, are rarely diagnosed in HIV infected patients with community acquired pneumonia. AIDS patients with advanced immunosuppression are also vulnerable to lots unusual infections including *Nocardia asteroids*, *Rhodococcus e*qui, *Bartonella henslae* and *Bartonella quintana* [8].

HIV infected individuals with bacterial pneumonia usually have the same signs and symptoms as the general population. Typically such patients present with a relatively rapid onset of clinical symptoms, such as productive cough, fever, shaking chills, pleuritic chest pain and dysnea. Symptoms are usually present for less than one week before a patient seeks medical attention [2].

There are four important differences between bacterial pneumonia occurring in HIV infected patients and in HIV seronegative patients, high frequency of bacteremia, unusual radiographic abnormalities, high rate of pleural effusion and bacterial pneumonia due to opportunistic bacteria particularly *N. asteroides* and *R. equi* [9].

Etiologic diagnosis of pneumonia on clinical grounds alone is almost impossible [8]. The challenge of HIV infection is that the clinical and radiographic presentations of HIV-associated opportunistic pneumonias overlap and also that persons with HIV infection may present with more than one concurrent pneumonia [2].

The aim of the present study is to ascertain the spectrum of bacterial pathogens causing lower respiratory tract infections in HIV positives in western Rajasthan, microbiological and radiological correlation and to find out variation in CD4+TLymphocytes, CD8 +T Lymphocytes and CD4/CD8 ratio in HIV positive and HIV negative patients having LRTI.

Material and Methods

This is a case control prospective study conducted at the department of microbiology of Dr SN Medical college and associated group of hospitals, Jodhpur (Rajasthan) over a period of sixteen months.

All patients with clinical and radiological findings of LRTI attending the Integrated Testing and counselling centre (ICTC) were included in the study. The study included two groups -study group and control group. The study group included 100 HIV positive patients with LRTI and the control group included 50 HIV negative patients with LRTI. With a preset protocol a history of clinical signs, and symptoms, radiological findings, routine lab. Investigations was collected.

The HIV status was confirmed by three ELISA/rapid (E/R) tests based on different antigen or principal as per NACO guidelines [10]. Informed consent of patients enrolled under study was taken and confidentiality of their HIV status maintained. Ethical clearance for the study was taken from college research review committee.

Collection of sample

An early morning expectorated sputum sample from patients enrolled in study and control group was collected. Sputum was evaluated macroscopically and microscopically by Bartlett's Grading system. A final score of 0 or less indicates either lack of inflammatory response or presence of significant salivary contamination thus invalidating the specimen. Gram' staining, KOH, Ziehl Neelsen (Z-N) and modified Z-N using 1% sulphuric acid was done. Modified Petroff' method was used for decontamination of sample for AFB culture.

Sputum culture

Sputum samples were inoculated on sheep blood agar (5%), MacConkey's agar, chocolate agar and Sabouraud s dextrose agar. CA plate were incubated under microaerophilic (5-10% C02) conditions for 24-48 hrs while blood agar and MacConkey agar were incubated aerobically at 37 ◦ c for 24 hrs. Two SDA tubes were inoculated at 25 ◦ c and 37 ◦ c for each sample Also, sputum samples processed by modified Petroff's method were inoculated on Lowenstein –Jensen (LJ) media for culturing Mycobacteria and Nocardia [11]. LJ was incubated at 37 ◦ c for 8 weeks. The identification of significant isolates was carried out by standard microbiological techniques [12].

CD4+T and CD8+T Lymphocyte counts estimation

CD4+ T and CD8 + T Lymphocyte counts of the samples under study was estimated by Flow Cytometry using BD FACS Calibur (Becton Dickinson Immunocytometry system, San Jose, CA, USA) using manufacturer's instructions [13].

Statistical analysis

Data was analysed using the Statistical Package for Social Sciences software (SPSS version 16). P <0.05 were considered to be statistically significant.

Results

Male: Female ratio was found to be 2.7:1 in the study group and 1.7:1 in the control group. Most common age group affected was 31-47 yrs followed by 21-30 and minimum in >50 yrs. Most of the HIV positive patients with LRTI presented with generalized weakness-53% followed by pyrexia-36%, weight loss 34%. HIV1 predominance was observed as all samples in study group found to be HIV-1 reactive. Table 1 shows the distribution of pathogenic isolates from both groups.

Polymicrobial isolation was found to be more in study group (5%) than in the control group (2%).

Table 1: Showing prevalence of pathogenic isolates from both the groups.

Pathogen isolated	Study group (n = 100)	Control group (n = 50)
Mycobacteria	8 (8%)	2 (4%)
Other Bacteria	29 (29%)	9 (18%)
Fungal Agents	5 (5%)	1 (2%)
Polymicrobial	5 (5%)	1 (2%)
Total	42 (42%)	13 (26%)

Out of 59 clinically diagnosed patients of TB who were on Anti tubercular treatment (ATT) or taken ATT, 2 (3.38%) were culture positive in study group and from clinically diagnosed TB patients not on ATT positivity was 54.54% in study group and 40% in control group. Table 2 shows distribution of bacterial pathogens from both the groups. Table 3 shows microbiological and radiological correlation in the study group. In the control group M. Tuberculosis was found to associated with diffuse consolidation, cavitary lesions radiologically while other bacteria, associated with focal consolidation.

Table 2: Shows distribution of bacterial pathogens from both the groups.

Isolates	Study group (n = 100)	Control group (n = 50)
Staphylococcus aureus	18 (62.06%)	4 (44.4%)
Klebsiella pneumoniae	5 (17.24%)	2 (22.22%)
Escherichia coli	2 (6.6%)	1 (11.11%)
Pseudomonas aeruginosa	4 (13.79%)	3 (33.33%)
Total	29 (29%)	9 (18%)

No significant difference was found in male and female study groups with their respective control group for haemoglobin, TLC, Lymphocyte counts and lymphocyte percentage. Table 4 shows mean CD4 and CD8 counts and their ratios in both groups.

Table 3: Shows microbiological, clinical and radiological correlation in the study group.

Clinical Diagnosis	Radiology	Isolates
Lobar pneumonia	Focal Consolidation Focal Consolidation/cavitary lesion	*K. pneumonia, S. aureus, E. coli P. aeruginosa*
Pulmonary Tuberculosis	Focal Consolidation/Bilateral Infiltration/Intracavitary lesions/Miliary/ mottling	*M. tuberculosis*

Table 4: Showing mean ± SD (Standard deviation) CD4 and CD8 counts and their ratios in both groups.

	CD4 (cells/ul)	CD8 (cells /ul)	CD4/CD8 ratio
Study Group (n = 100)	214 ± 120	940 ± 168	0.23 ± 0.11
Control group (n = 29)	860 ± 160	644 ± 138	1.71 ± 0.47

Table 5: Shows male/female distribution of CD4, CD8 and CD4/CD8 ratio in both groups.

	Study group	Control group	p-value
CD4 (cells/ul)	221 ± 117	860 ± 155	0.0001
Males Females	249 ± 77	782 ± 197	<0.0001
CD8 (cells/ul)	977 ± 292	643 ± 219	0.0001
Males Females	1038 ± 462	592 ± 86	0.0002
CD4/CD8 ratio	0.24 ± 0.12	1.71 ± 0.48	<0.0001
Males Females	0.29 ± 0.09	1.48 ± 0.51	<0.0001

Discussion

In the present study, male: female ratio was found to be to be 2.7: 1. This is in accordance with Kothari K., *et al*. who reported M:F ratio as 5:1 in their study. This may be due to ambulatory nature and high risk behaviour of male population [14].

HIV 1 predominance is observed in the present study. This is consistent with the findings by Kamal HA., *et al*. who also reported HIV 1 as the predominant virus prevalent in Mumbai [15].

In the present study most of the HIV positive patients were in the age group 31-40 yrs followed by age group 21-30 yrs. Our findings are consistent with other studies elsewhere in India (2015, 2008) [16].

In the present study, most of the patients presented with generalised weakness (53%) followed by fever (36%), weight loss (34%), mucocutaneous manifestations (28%) and arthralgia (22%). Kothari Kand Goyal S also reported fever 96% and weight loss 66% as commonest initial presentations. Bollinger RC., *et al*. in their study conducted at pune observed fever 48% and joint pain 28% as commonest presentations [17].

Polymicrobial etiology was found to be 5% in HIV reactive patients which is a significant finding indicating severity of infection in this group. This may be due to immunosuppression associated with HIV infection. Bacterial pneumonia is reported to be more frequent in HIV positive patients as compared to seronegatives and the rate of bacterial pneumonia increased with decreasing CD4 lymphocyte counts [18]. HIV sero-positive patients were found to be infected with one or more microbial pathogens [28].

In the present study, M tuberculosis was isolated in 8% in the study group. No atypical mycobacteria were isolated. Other bacteria isolated formed 29% and fungal agents 5%. In the control group, *M. tuberculosis* isolation was 2%, Other bacteria 9% and fungal agents 1%. Shah RR in his study reported 21% mycobacterial isolation, other bacteria 18.41%, fungal agents 6.87% in HIV positives while 12% other bacteria in HIV positives.

In the present study *M. tuberculosis* was isolated in 8% patients. The low isolation of *M. tuberculosis* might be due to the factor that 27% of the patients were o ATT and 32% had taken ATT in the past. Only 11% were not taking any ATT. Zar HJ., *et al*. reported *M. tuberculosis* as important cause of acute pneumonia in areas with high tuberculosis prevalence [19]. *M. tuberculosis* was isolated in 83.3% of HIV positive patients in a study by K Shreevidya., *et al*. [20].

Among the bacterial isolates in the study group, *S aureus* was isolated in 42.8%, *K. Pneumoniae* 11.9%, *E. coli* 4.7% and *P. aeruginosa* in 9.52%. VV Shailja., *et al*. in their study of 130 patients with LRTI, of which 100 were HIV reactive reported 30 mycobacterial isolates, 9 fungal isolates and 31 bacterial isolates of which *K. Pneumoniae* 32.26%, *S. pneumoniae* 25.81%, *S. aureus* 12.90% and *p. aeruginosa* in 9.68% [21]. Schneider RF., *et al*. reported *S. pneumoniae* as the most common, *H. influenzae, S. aureus, E.coli* and other gram negative organisms responsible for the remainder of bacterial pneumonia cases [22]. Other studies by Robert E Hirschtick., *et al*. Yarlagadda P reported varied percentage of different bacteria [18,23]. Ojha CR., *et al*. reported *K. pneumoniae* was the predominant bacterial pathogens, followed by *M. Tuberculosis*, *E. coli* and *S. pneumonia*, *S. aureus*, P. *aeruginosa*, *M. catarrhalis* and *H. Influenza* [28].

Lubega G., *et al*. reported *Moraxella species* (27.4%), *Streptococcus pneumoniae* (25.4%), *Haemophilus influenza* (22.4%), *Mycobacterium species* (4.5%), *Pseudomonas species* (4.0%), *Staphylococcus aureus* (4.0%), *Escherichia coli* (1.0%), *Klebsiella species* (1.0%) and other bacteria (10.4%) [29]. This may be due to prevalence of different types of bacteria in different populations.

The typical radiographic appearance of bacterial pneumonia CXR (chest X-ray) in AIDS is the same as that in an immune competent host with lobar or segmental consolidation but progressing rapidly, with frequent multilobar or bilateral disease [24]. The most common radiographic pattern in bacterial pneumonia is focal consolidation which typically presents in either a segmental or lobar distribution [30]. Parapneumonic effusions and empyema, especially if due to *S. pneumoniae* or *S. aureus* are common in HIV infection. Most persons with *S. pneumoniae* or *Haemophilus* pneumonia present with unilateral, focal, segmental or lobar consolidation, occasionally accompanied by pleural effusion. However, the frequency of these radiographic findings depends in part on the underlying bacterial pathogen. In the present study two *P. aeruginosa* isolates in the study group were found to be associated with cavitary lesion on chest X-ray while no cavitary lesion was found in the control group. Also, it is reported that pneumonia due to *P. aeruginosa* or *S. aureus* is often associated with cavitation.

Radiological findings like focal consolidation and military pattern was observed in the study group with M. Tuberculosis isolation. Similar findings have been reported by Badie BM., *et al*. [25] HIV infection leads to increased frequency of atypical radiographic features in patients with PTB with a tendency towards the pattern of primary disease [26]. These features include an increased frequency of lymphadenopathy and pleural effusions, less typical disease and less cavitations.

The present study reports mean CD4 counts 214 ± 120 cells/ul, mean CD8 counts 940 ± 168 cells/ul and CD4/CD8 ratio as 0.23 ± 0.11 in the study group. Krishna Ray., *et al*. reported mean CD4 140 ± 105 cells/ul, CD8 counts 1010 ± 579 cells/ul and CD4/CD8 ratio as 0.15 ± 0.1 in HIV positives. The receptor for HIV virus is CD4 antigen, hence the virus primarily affects CD4 T cells (helper/inducer lymphocyte). HIV infection reduces the absolute CD4 lymphocyte count and inversion of CD4/CD8 T cell ratio occurs later because of rise in the number of CD8 T cells. Bharati., *et al*. reported 53 % patients with LRTI between 201-500 cells/ul [27].

Conclusion

It is recommend that patients with suspected bacterial pneumonia should undergo diagnostic evaluation for specific pathogens that would alter the standard treatment (e.g., *P. aeruginosa* or *S. aureus*) whenever the presence of these pathogens is suspected on the basis of epidemiological, clinical, and radiographic clues. Early diagnosis would prevent unnecessary prescription of antibiotics, detect drug resistant strains early. And help in antibiotic stewardship.

Low CD4 counts predisposes the HIV positive patients to bacterial LRTIs. As the rate of bacterial pneumonia increases with declining immune status in HIV infection, one would expect that the overall rate of bacterial pneumonia would decline with enhanced immune function associated with highly active antiretroviral.

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