



Original Article

Bacterial Isolates of Lower Respiratory Tract infection among subjects with and without HIV from South India

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ABSTRACT

SUMMARY LRTI is defined as respiratory infection occurring below the cricoid's cartilage. As HIV positive patients are susceptible to many lower respiratory infections (LRTI). This study aimed at to find out the prevalence and pattern of various bacterial isolates of LRTI among subjects with and without HIV. Expecterated sputum was collected and subjected for microscopic examination. The sputum specimens were inoculated into Blood, MacConkey's and Nutrient agar. They were evaluated clinically, radiologically and microbiologically. Tuberculosis infection was diagnosed by sputum and its smear growth on Lowenstein-Jensen culture media, among 143 patients, 16 (24.6%) of the 65 HIV positive and 36 (46%) of the 78 HIV negative. Single bacterial infection with *Streptococcus pyogenes* and *Staphylococcus aureus* were more in HIV+TB+ patients (i.e. 9% and 27.2% respectively) than HIV+TB-. The prevalence of *Klebsiella* sp. infection was higher (18.9%) among HIV+TB- patients, but HIV-TB- patients failed to show the presence of any such organisms. The occurrence of *S.pyogenes* and *S.aureus* (16% and 25%) were more among HIV-TB+ patients than HIV-TB- patients (2.2% and 4.5%). Findings suggest that gram-positive bacterial infections were common in TB patients irrespective of HIV status. HIV positives patients were more prone for gram negative bacterial infections.

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Introduction

The HIV/AIDS pandemic has had a major impact on the epidemiological dynamics of tuberculosis (TB) and other respiratory infections. TB has become the most common life-threatening opportunistic infection (OI) in patients with HIV/AIDS. HIV and TB are intricately linked to malnutrition, unemployment, alcoholism, drug abuse, poverty and homelessness (1). Among the various opportunistic infections, respiratory infections account for up to 70% of AIDS cases defining illness (2). Their relative importance differs in different parts of the world. Sinusitis has also been reported to occur frequently in HIV-infected people who contribute to lower respiratory tract infections (LRTI) (3). The co-infection of respiratory pathogens is quite common (4) and the etiology of LRTIs is diverse and complicated (4).

The incidence and severity of bacterial LRTI increase with the degree of immunosuppression especially after the CD4 count falls below 200 cells/mm³. The present study was undertaken to find out the prevalence and pattern of various bacterial isolates of LRTI among subjects with and without HIV, and to compare them with published reports.

MATERIALS AND METHODS

A cross sectional study was carried among out patients attending of Thoracic medicine division of Government Rajaji Hospital, Madurai after Institutional Ethical clearance, over a consecutive period of seven months in 2014. Written informed consent was also obtained from each participant. Thus 143 patients with LRTI who satisfied inclusion and exclusion criteria and willing to participate in the study were alone considered. They were evaluated clinically, radiologically and microbiologically. Patients presenting with persisting cough for more than three weeks suggestive of LRTI who have not taken any modern medicine either from hospital or pharmacy or elsewhere were alone included for the study.

Patients with malignancy, diabetes mellitus, chronic respiratory disorders with acute exacerbations or *pneumocystis carinii* infection and other end organ disorders were excluded clinically and by laboratory means. Pregnant women and patients on immunosuppressive or any other antimicrobials or suffering from other serious illnesses were excluded.

An early morning expecterated sputum after brushing teeth and warm water gargling was collected separately in a sterile containers from each patient, included in the study. Pulmonary tuberculosis was considered based on positive microcopy for acid fast bacilli (AFB) at least on two sputum and/or a growth in Lowenstein-Jensen (L.J) medium (5).

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The sputum specimens were also inoculated into Blood agar (10% sheep blood), MacConkey's agar and Nutrient agar. Any significant bacterial growth was further processed as per standard procedures to identify the pathogens. Also the sputum sample was incubated at 37°C for 24 hours to isolate bacterial pathogens. HIV infection was diagnosed by performing two ELISA tests with two different kits according to the WHO recommendation for developing countries (Inno test, Belgium and Lab system, Finland). If both tests were positive, the patient was designated as HIV positive. All the data were analyzed using chi-square test.

RESULTS

Among the 143 patient, there were 103 males and 40 females. Their age ranged from 29 to 52, with a median and mean age of 34 and 36 years respectively. HIV was positive in 65 of them. Mycobacterium was grown in L.J. medium in 75% of sputum smear positive cases. X-ray revealed one or other form of infiltrative lesion in all those cases positive for AFB. Pulmonary tuberculosis (PT) was diagnosed by sputum studies among 36 (46.1%) of the 78 HIV negative and 16 (24.6%) of the 65 HIV positive, and the difference was significant ($P < 0.03$). The odds ratio and risk ratio (with confidence interval) for acquiring PT among HIV positive group were 0.38 (0.17 - 0.83) and 0.53 (0.33 - 0.87) respectively. (Chi-square $\chi^2 = 7.11$).

The patients were classified into four groups based on presence or absence of HIV and TB infection viz., Group 1 with HIV infection alone, Group 2 with both HIV and TB positive, Group 3 with TB alone, Group 4 with neither HIV nor TB. The distribution of cases in each group was 49, 16, 36 and 42 respectively. The nature of the bacterial isolates in each group is shown in Table 1.

The prevalence of isolation of *S.aureus* and *S.pyogenes* was significant ($P < 0.05$) in those patients with TB positive alone (group 3) compared to other Group (Group 1, 2 and 4). Gram negative organisms were isolated significantly more in HIV positive group (Group 1) than negative. The polymicrobial isolates were significantly more ($P < 0.05$) in HIV infected than HIV negative group Table 1. However inter group significance could not be assessed with reference to polymicrobial infection, as isolates were small in number. During the study period *S.pneumoniae*, and *H.influenzae* were not isolated in any of the group.

DISCUSSION

Identification of invading microbial organisms is essential to institute appropriate therapy and also alerts the treating physician on the anticipated complications. Interestingly, wide variations were observed in the pattern and prevalence of microbes in different group, which might be related to baseline health, nutritional status, habits like smoking, etc., as described by (6). The results of present study are discussed in relation to other studies on the prevalence of bacterial isolates from patients with LRTI including tuberculosis infection in the ensuing paragraphs.

Tuberculosis (TB) and HIV epidemic are intertwined. In India TB prevalence is 0.4% (3.5 million cases) (7). Hence the efforts are taken to diagnose, treat and control the same by Revised National Control Tuberculosis Programme (RNTCP). PT was noted in 16 of the 65 HIV positive and 36 of the 78 HIV negative and the difference was statistically significant ($P < 0.03$). In the present study PT was significantly more among HIV negative than HIV positive (46.2% Vs 24.6) and the difference was statistically significant. The prevalence of tuberculosis among HIV infected individuals varied from 25 to 65% (1) and the present observations falls on the lower side. bacilli are widely considered the major pathogens responsible for LRTIs

The probable reasons for increased occurrence of PT among non HIV group were attributable to the selection criteria, socio-cultural changes, environmental factors, habits, poor nutritional status, contact with other PT cases, endemicity, altered metabolic status, host immune status and genetic make up or a combination of them (8) & (9).

The probable explanation that could be offered for less prevalence of PT among HIV positive cases in the study area are free facilities for earlier diagnosis of HIV infection, availability and utilization of Integrated Counseling and Testing Centre (ICTC) in almost all government health care settings in this part of the country, awareness of predisposition of HIV patients for PT among health care providers, intensive follow up programme and nutritional support provided by non governmental organizations (NGO's) to HIV infected individuals and availability of Highly Active Anti Retroviral Therapy (HAART) within 50 to 100 Kilometers.

Pattern and prevalence of pathogenic microbes in HIV positive TB negative patients (Group 1).

The pattern and prevalence of pathogenic microbes in HIV positive TB negative patients were *Klebsiella pneumoniae* (22.4%), *P.aeruginosa* (20.4%), *S. pyogenes* (12.2%) and *E. coli* (4%). *Klebsiella* infection predominated in this study. Similar observations were made earlier by (10). The isolation of this organism varied from 3.5% to 10% among HIV positive patients of other centers (2) and (11). Increased susceptibility to *Klebsiella pneumoniae* infection in HIV might be due to their susceptibility or acquiring infection from community or malnourishment (12).

Similarly, *P.aeruginosa* isolation from HIV positive patients has been classically reported as late events in the course of the disease and is usually life threatening and nosocomially acquired (13). Prevalence of *P.aeruginosa* infections in HIV positive patients varied in different series published from different countries. e.g., 2.5% in Paris, France (14), 2.6% in Florida, USA (15), 8% in San Francisco, USA (16), and 8% in Chennai, South India (17). In the present study *Pseudomonas* infection was more (20.4%) than published reports (15). This may be related to selection of cases or community acquired infection.

Polymicrobial infections of *Klebsiella pneumoniae* / *S. pyogenes* occurred in 12.3% (8/65) and *S.pyogenes* / *E.coli* 4.6 % (3/65) of patients with isolated HIV infection (Group1). This may be due to declining immune status in these individuals. More over bacterial infection contributed to 7% of opportunistic infections in HIV positive patients of which *S.pneumoniae*, *H.influenzae* and *S.aureus* were frequently isolated (2). In a study conducted among HIV positive African adults, *Streptococcus sp* was dominant opportunistic infection and the prevalence was 81 % (18).

Pattern and prevalence of pathogenic microbes in HIV positive and TB positive patients (Group-2).

The monomicrobial isolates found in HIV and TB positive patients (group 2) were *S.aureus* (37%), *Klebsiella pneumoniae* (18.7%) and *S. pyogenes* (18.7%). Somporn *et al* observed the occurrence of *Klebsiella pneumoniae* in 10.8% of their series which was nearer to the present report and in contrast to observations made from Malawi (58%). The variations may be related to biological or environmental reasons. The occurrence of *S.aureus* and *S. pyogenes* were comparatively lower in this series than (17), who observed (34%) *S.aureus* and (31%) *S.pyogenes* among HIV and TB positive where as 2 observed *S.aureus* in 12.9% of their HIV and TB positive patients at Hyderabad.

Pattern and prevalence of pathogenic microbes in HIV negative TB positive patients (Group 3).

Bacterial isolates in HIV negative TB positive patients (Group 3) in the present study were *Staphylococcus aureus* (25%), *Streptococcus pyogenes* (16.7%), *Paeruginosa* (13.9%) and *Klebsiella* sp (8.3%). Arora *et al* observed (19) *Paeruginosa* (12%) and *Klebsiella* sp (10.3%) among HIV negative TB positive cases at New Delhi which were almost nearer to the present study. Isolation of *Staphylococcus aureus* was only 1.7% in Delhi. Rana *et al* (20) observed the prevalence of *Pseudomonas* (45.5%) and *Klebsiella* (36.4%) more among HIV negative TB positive patients in Aligarh (U.P.). Polymicrobial infection with *Klebsiella pneumoniae*/*Streptococcus pyogenes* also observed only 11.1% in this study.

Pattern and prevalence of pathogenic microbes in HIV negative TB negative patients (Group 4)

The lower prevalence of *Streptococcus pyogenes* (2.2%) and *S.aureus* (4.5%) was seen in HIV negative TB negative patients. This might be due to their innate ability to withstand against infection, when compared to other groups. Statistical significance could not be assessed, as the isolations were in small number.

Strength and limitations: The strength of the study was uniform criteria adopted for selection of cases and standard methods followed for sputum collection and processing. The limitations were non evaluation of immunological parameters of these patients.

In conclusion, bacterial infections were noticed more among HIV infected individuals but pulmonary tuberculosis was less in contrary to the expectations. The occurrence of mixed pulmonary infections due to *Klebsiella* and *S.aureus* in HIV positive patients coming from the community, indicates the prevalence of such organisms in the community and the susceptibility of these patients to the same. The wide variations for the prevalence and pattern observed in the present study are in contrast to published literature.

Key message: HIV positive patients are susceptible to develop LRTI due to polymicrobial agents, gram-negative organisms and mycobacterium tuberculosis. Hence, LRTI infection, if identified in HIV positive patients, they require specific diagnosis and aggressive therapy if the duration of infection is prolonged.

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Conflicts of interest statement

The authors have no conflicts of interest concerning the work reported in this paper.

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