Chapter -1

Bacterial Isolates of Lower Respiratory Tract infection among subjects

with and without HIV
Bacterial diseases are responsible for a significant proportion of the morbidity and mortality seen in HIV population. Bacterial infections were the leading cause of death in HIV-infected patients. The course of certain bacterial infections does not differ from that in the immunocompetent host, whereas other bacterial infections are notable for an increased incidence, a more fulminant course, invasive disease, and unusual rates of relapse. Hence this study was undertaken.

**Objective**

In this chapter following things are focused. 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. Also to find out the prevalence of leprosy among TB and HIV/AIDS patients.

**Ethical clearance and informed consent**

The study was approved by the Institutional Ethical Committee (IEC), Government Rajaji Hospital and Madurai Medical College, Madurai, Tamilnadu.

Written informed consent was also obtained from the patients or guardian and controls of the entire study subjects.

**Conflict of interest**

Conflict of interest: nil.

**HIV test**

HIV infection was diagnosed by performing 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. HIV test was carried out after counseling and written informed consent of the patients.

A prospective observational study was carried out among patients attending of Thoracic medicine division of Government Rajaji Hospital, Madurai after Institutional Ethical clearance, over a consecutive period of three months in 2007. Written informed consent was also obtained from each participant. LRTI is defined (Okesola and Ige, 2008) as respiratory infection occurring below the cricoid cartilage. Thus, 143 patients with Lower Respiratory Tract Infections (LRTI) who satisfied inclusion and exclusion criteria and willing to participate in the study were alone considered. They were evaluated clinically, radiologically and microbiologically, dermatologically, 1500 patients were recruited. All the data were analyzed using chi-square test.

**Inclusion criteria**

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.

**Exclusion criteria**

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

Specimen collection and transport

The successful isolation and identification of the organism, as in all microbiological procedures depends on the quality of the specimen obtained and appropriate processing of the samples. Therefore, efforts were made to collect 5ml specimens in a sterile, leak proof, disposable and appropriately labeled containers. Patients were asked to cough deeply and to bring forth the purulent sputum from within lungs. Once the sputum was collected, the lid of container was tightly closed without any leakage. All the samples collected in the hospital were transported to the laboratory within one to two hours of collection. The sputum was examined for purulence, blood tinged and viscosity.

An early morning expectorated sputum after brushing teeth and warm water gargling was collected separately in a sterile containers from each patient, was included in the study. Every patient with 3 sputum samples examined for AFB (Acid Fast Bacillus) as per RNTCP guidelines (Selvakumar et al., 2005). 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 (LJ) medium, (Ramachandran et al., 2003). Also patients with Leprosy were considered based skin smear positive microcopy by Ziehl-Neelsen (Z.N.) stain. The samples were transported in transport media (MacConkey, Nutrient and Blood agar).

Ziehl-Neelsen (ZN) staining techniques

Freshly filtered carbol fuchsin was poured on the slide covering the entire smear. Heat was applied underneath until steam rises from the stain. Care was taken not to dry the stain. The hot carbol fuchsin was allowed to act for at least 5 minutes.
After that the stained slides was washed with running tap water, taking care to control the flow of water so as to prevent washing away the smear.

The slide was poured completely with acid alcohol for 1-2 minutes. It was gently washed with running tap water and then 1% methylene blue was added as a counter stain for 30 seconds. It was finally washed as before with water and kept the slides on the hot plate to dry. All slides were examined under oil immersion objective lens of the microscope (100 x) (Selvakumar et al., 2002).

**Sputum culture for Acid Fast Bacilli using modified Petroff’s method**

The 40 gram sodium hydroxide was dissolved in 1000ml distilled water, and distributed in 200 ml of solution in conical flasks covered with gauze cotton wool as stopper. To each 10 ml of sputum were added with the two volume of 4 % sodium hydroxide. Care was taken to avoid contact between the specimen bottle rim and the sodium hydroxide flask. And then the samples were carefully capped, ensuring the same time that the bottle tops are not broken. Then the bottles were shaken by hand for one minute, again placed in an orbital shaker - and left to shake gently for 20 minutes. Then the flasks were centrifuged for 15 minutes at 4000 (rpm). The supernatant was poured into the disinfectant bath and the rim of each bottle was wiped with sterile filter paper (Petroff, 1915).

The bottles were filled with 20 ml of sterile distilled water, shaken by hand to mix the deposit and then recentrifuged for 15 minutes at 4000 rpm. The supernatant was poured into the disinfectant bath again and the rim of each bottle was wiped with sterile filter paper. Finally, the sediment of each sample was inoculated with a 5 mm diameter loop into previously numbered Lowenstein-Jensen (Hi-Media Ltd) slopes. Then the media were inoculated and incubated at 37°C. The cultures were left for 8 weeks for growth of AFB (Petroff, 1915).
Sputum culture for pyogenic bacteria

The sputum specimens was inoculated with a 5 mm diameter loop into previously numbered into Blood agar (5% sheep blood), MacConkey’s agar and Nutrient agar media. Any significant bacterial growth was further processed as per standard procedures to identify the pathogens. The sputum sample was incubated at 37°C to isolate bacterial pathogens (Pitchenik, 1986).

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. Two sputum were positive for AFB in 43 of the 60 and three in the rest of the sample. Mycobacterium was grown in L.J. medium in 75% of sputum smear (Fig-3), positive cases, X-ray revealed one or other form of infiltrative lesion in all those cases positive for AFB, (Fig-4). 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 $\chi^2 = 7.11$).
Figure-3. *Mycobacterium Tuberculosis* by Ziehl Neelsen staining.

Figure-4. *Mycobacterium Tuberculosis* by Lowenstein–Jensen medium (LJ).

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 (among 143
cases). The nature of the bacterial isolates in each group is shown in Figure 5.
The organisms showing irridiscent colonies with mawkish odour on nutrient agar, beta
hemolysis with serrated edges on Blood agar were subjected as Pseudomonas species.
They were further confirmed by biochemical tests like oxidase, indole, triple sugar iron
agar, citrate, urease, catalase, oxidation fermentation test. The gram negative
organism’s which were showing indole negative, non-fermenting with glucose, lactose
and sucrose, positive reaction for citrate, oxidase, urease and catalase by changing the
colour (did not ferment carbohydrates).

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 (1) than negative. The polymicrobial isolates were significantly more (P<0.05) in
HIV infected than HIV negative group shown in Figure 5.

However intergroup significance could not be assessed with reference to
colour (did not ferment carbohydrates). During the study period
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.
Figure 5. Pattern and Prevalence of bacterial pathogens in patients with lower respiratory tract infections

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 Wafaie et al., (2003). 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.
Prevalence of Pulmonary Tuberculosis

Tuberculosis (TB) and HIV epidemic are intertwined. In India TB prevalence is about 0.4% (3.5 million cases) (Technical guidelines TB control / Central TB Division, 1999) and hence efforts are taken to diagnose, treat and control the same by Revised National Control Tuberculosis Programme (RNTCP). PT was observed in 16 of the 65 HIV positive and 36 of the 78 HIV negative. 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% (Sharma et al., 2005) and the present observations fall on the lower side.

The probable reasons for increased occurrence of PT among non HIV group in different serious 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 (Coimbra et al., 2007 & Basta et al., 2006).

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 Counselling 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 (www.emg-hips.com/publications/Hima).
Pattern and prevalence of pathogenic microbes in HIV positive and 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. pyogens* (12.2%) and *E. coli* (4%). *Klebsiella* infection was predominated in this study. Similar observations were made earlier by Somporn et al., (2005). The isolation of this organism varied from 3.5% to 10% among HIV positive patients of other centres (Shailaja et al., & Sanjeev et al., 2004). Increased susceptibility to *Klebsiella pneumoniae* infection in HIV might be due to their susceptibility or acquiring infection from community or malnourishment (Schleicher et al., 2003).

Similarly, *P. aeruginosa* isolation from HIV positive patients have been classically reported as late events in the course of the disease and is usually life threatening and nosocomially acquired (Domingo et al., 1998). Prevalence of *P. aeruginosa* infections in HIV positive patients varied in different series published from different countries. e.g., 2.5% in Paris, France (Meynard et al., 1999), 2.6% in Florida, USA (Afassa and Green., 2000), 8% in San Francisco, USA (Doyle et al., 1995) and 8% in Chennai, South India (Shankar et al., 2005). In the present study (Figure 5) *Pseudomonas* infection was more (20.4%) than published reports (Afessa et al., 2000). This may be related to selection of cases or community acquired infection.

Polymicrobial infections of *Klebsiella pneumoniae / S. pyogens* occurred in 12.3% (8/65) and *S. pyogens / E.coli* 4.6 % (3/65) of patients with isolated HIV infection (Group1). This may 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 (Shailaja et al., 2004). In a study conducted among HIV positive African adults, *Streptococcus*
was dominant opportunistic infection and the prevalence was 81% (Tchmaran et al., 1997).

**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* in 37%, *Klebsiella pneumoniae* 18.7% and *S. pyogens* 18.7%. The occurrence of 10.8% *Klebsiella pneumoniae* observed by Somporn et al., (2005) was nearer to the present report, where as isolates of *Klebsiella pneumoniae* (58%) in a series for Malawi. The variations may be related to biological or environmental reasons. The occurrence of *S. aureus* and *S. pyogenes* was comparatively lower in this series than Shankar et al., (2005) who observed more *S. aureus* (34%) and *S. pyogens* (31%) among HIV and TB positive in Chennai. On the other hand Shailaja et al., (2004) 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 (Group3) in the present study were *Staphylococcus aureus* (25%), *Streptococcus pyogens* (16.7%) *P. aeruginosa* (13.9%) and *Klebsiella sp* (8.3%). The isolates observed by Arora et al., (1999) among HIV negative TB positive cases at New Delhi with reference to *P. aeruginosa* (12%) and *Klebsiella sp.* (10.3%), which were almost similar to the present study. Isolation of *Staphylococcus aureus* was only 1.7% in Delhi. Rana et al., (2005) 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/ Strepococcus pyogens* also observed 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 isolates were in small number.

**Prevalence of Leprosy among TB and HIV/AIDS patients**

Among the 1500 patients, there were 1033 males and 467 females. Their age ranged from 30 to 58, with a median and mean age of 34 and 36 years respectively. Both (HIV and TB) infection was positive in 200 of them. Of these 200 cases one leprosy was positive and two patients (1/1300) were positive in the rest used by Z.N. techniques.

The lower prevalence of Leprosy 0.002% (3/1500) was seen in HIV and TB patients. This might be due to their similarities could be responsible for immune modulation and could probably be the reason for differential susceptibility / association to these two important mycobacterial infections (Ustianowski *et al.*, 2006). Also serious implications for programs to control leprosy, through awareness, new case detection, early diagnosis and management. The published epidemiological data are limited in quality but show neither an increased HIV prevalence among leprosy cases nor an alteration in clinical spectrum of leprosy among coinfected patients. Some data suggested that immune-mediated reactions that complicate leprosy occur at a higher frequency in coinfected patients. Leprosy has now been reported presenting as immune reconstitution disease among patients commencing highly active antiretroviral treatment (http://doctorshangout.com/forum/topics/less-incidence-of-leprosy-in).
Conclusion

Our findings revealed that HIV positive patients are susceptible to develop LRTI due to polymicrobial agents, gram negative organisms and mycobacterium tuberculosis. Hence, LRTI infection identified in HIV positive patients with or without TB, required aggressive therapy. Also, HIV infection was positively associated with bacterial infections in addition to pulmonary tuberculosis.