Chapter 6

Discussion
TB control programme of India reports 2,31,121 cases of extra pulmonary tuberculosis (EPTB) in the year 2010, constituting 16% of all the new TB cases (www.tbcindia.nic.in) and lymph node tuberculosis (LNTB) comprised of more than half of these cases.

Patients with enlarged lymph nodes – cervical, axillary and rarely inguinal are commonly met with in the clinical practice in India. Such patients are usually treated as tuberculous on the basis of clinical diagnosis. There is widespread prevalence of non specific tuberculin allergy associated with infections of unclassified mycobacteria. Hence, the present study attempted to look at the utility of FNA culture in diagnosing LNTB in a public health perspective.

The present study aims to isolates the mycobacteria and other non tuberculous mycobacteria (NTM) causing lymph node tuberculosis with major objectives (a) to assess the diagnostic role of cytology and bacteriology of fine needle aspirate of lymph node tuberculosis and (b) to know the drug susceptibility pattern of Mycobacterium tuberculosis causing lymph node tuberculosis.

Of these 200 specimens of clinically diagnosed lymph node tuberculosis, 178 (89%) were proven positive by one or either of the
laboratory tests. Tuberculous lymphadenitis diagnosis by H & E cytology was positive in 161 (80.5%) aspirates and by bacteriology (i.e. by ZN smear and culture) 107 or 53.5% were proven positive, of these 41 (20.5%) positive by ZN smear and 76 (38%) were proven positive by culture.

**6.1 Site of Lymph node infection**

Findings of the present study show that 200 patients with enlarged lymph nodes were examined of them 179 were from cervical region followed by 21-axillary lymph nodes. These observations suggest that the cervical lymph nodes were the most common site for tuberculous lymphadenitis and axillary lymph nodes are less commonly affected in tuberculosis. These findings are similar to those made by Miller (1934), Jones (1951), German and associates (1956), Lester and Jones (1956), Schless and Wier (1957), Miller and Cashman (1958) and Doctor (1964).

Miller (1934) in a series of 536 cases of tuberculous lymphadenitis observed 25 cases of axillary and 6 cases of inguinal tuberculosis lymphadenitis. Doctor (1964) from India reported very low incidence of tuberculous involvement of inguinal lymph nodes. In summary, it can be said that Axillary and inguinal lymph nodes are
involved in tuberculous process though quite infrequently in a large series of patients with lymphadenitis.

### 6.2. Age and Gender

Lymph node enlargement was observed more commonly in the age group of 21-30 and 31-40 years and the maximum number of patients of lymph node enlargement being seen in the age group of 21-30 (fig 5.1) both in males and females. The cytologic and bacteriologic confirmation of tuberculous lymphadenitis was also more frequent in this age group both in males and females indicating that tuberculous lymphadenitis is no more a disease of children.

Thompson (1940) studied 224 patients from Clare Hall Sanatorium during 1934 to 1936. He observed that the great majority of cases occurred between ages of 5 to 14 years and that females outnumbered the male patients. Siddiqui (1949) in a study of 140 patients of lymphadenitis during 1945-46, observed high prevalence in age groups 6 to 15 and 16 to 25 years and in females. Jones (1951) in a study of 51 Nigerian Africans of tuberculous lymphadenitis observed that 53 percent in children of the age of 0—14 years. In his study the prevalence was higher in males than in females. German, Black and Chapman (1956) in a study of 47 veterans suffering from tuberculous adenitis observed 50 percent cases in the age group of 20
to 29 and the rest in the higher age groups. Wilmot, James and Reilly (1957) in a study of 81 patients of tuberculous cervical adenitis observed that 54 percent of patients were under the age of 15 years. Schless and Weir (1957) in a study of 120 cases of TB lymphadenitis observed that about 75 percent of cases were in the age group of 21 to 30 years and that 85 percent were males. Tuberculous adenitis which was a disease of childhood during early years of the century, is now seen in young adults.

It has been observed in the present study that females have outnumbered males. Our findings are in conformity with other studies on LNTB reporting a female preponderance (Martin-Bates et al. 1993, Pamra & Mathur et al. 1974.) Similarly, cases proved to be tuberculous by bacteriology or histopathology were about one and half times more frequent in females than in males and this difference in males and females was seen in all age groups inspite of the fact that the proportion of enlarged lymph node was higher in male than in females in the age groups of 0 to 14 years and 45 years and above. These observations suggest that tuberculosis lymphadenitis is more a disease of females than of males. In literature there are conflicting reports regarding prevalence of this disease in male and females. Thompson (1940), Siddiqui (1949) observed higher prevalence in females while Jones (1951) and Schless & Wier (1957) made the
contrary observations. Murthy from India (Murthy KJR et al., 2001) observed in his study that female tuberculous patients were significantly higher.

6.3. Diagnosis and Demonstration of Mycobacteria in Lymph nodes

The diagnosis of lymph node tuberculosis is frequently based on the clinical symptoms, such as swelling of lymph nodes, occasionally with discharge of pus; in addition to general symptoms such as weight loss, fever, etc (Cadre et al. 1991). However, in the present study a small proportion of clinically suspected patients, was negative for tuberculosis by all the laboratory tests that were undertaken. This indicates that diagnosis based on clinical findings alone may lead to over or under diagnosis due to atypical clinical presentation simulating other inflammatory and neoplastic conditions (Gupta et al. 1993).

More than three quarters of the LNTB patients studied, were positively diagnosed by either one or more of the laboratory tests. Cytological examination of the fine needle aspirate seems to be the most sensitive test, followed by bacteriological tests (ZN staining and culture on LJ) (Table 5.9), for diagnosing LNTB. However, considering
the ability of the bacteriological tests to yield a definitive diagnosis, attempts should always be made to include these tests.

RNTCP recommends cytology and ZN smear of fine needle aspirate for diagnosing LNTB (Arora & Rajnish Gupta 2006). Cytological picture of other chronic granulomatous conditions may sometimes mimic LNTB, leading to over diagnosis, hence ZN smear may facilitate for more specific diagnosis. However, mycobacteria in the aspirates are present in too small a number to be picked up by ZN smear alone. Hence addition of culture may be more useful for enhancing both sensitivity as well as the specificity (Table 5.8).

In this study, the sensitivity of the diagnosis was further improved by adding culture to cytology and ZN smear (Table 5.8). Despite the fact that mycobacterial culture facility is not widely available in India, culture should always be attempted, wherever resources permit. We found that nearly five percent of LNTB cases were diagnosed only by mycobacterial culture; neither by cytology nor by ZN smear. These cases would have been missed if only cytology and smear were considered for diagnosis (Table 5.8). Moreover culture is considered as the gold standard test, where the presence and characters (species & anti-tuberculosis drug susceptibility) of mycobacteria can be well demonstrated.
Cytology positive aspirates showing negative bacteriological findings reported in the study, might perhaps be due to the presence of very few organisms that were missed by both smear and culture. In addition, as reported by Verenkar et al, there could have been a possibility for collecting an unrepresentative specimen of the lymph node material (Verenkar et al. 1996). Possibility of granulomatous findings from other causes can not be ruled out (Gupta et al 1993).

Though FNA cytology is presumed to be an established test for diagnosing LNTB (Norman et al 1997), six percent of the aspirates which were positive by culture could not show the cytological features suggestive of tuberculosis. This suggests the need to supplement mycobacterial culture to cytology while diagnosing LNTB. This may be especially useful in diagnosing LNTB in HIV positive patients as evidenced by the observation that, all the cytology negative aspirates from HIV positive patients yielded positive mycobacterial cultures. Atypical clinico-pathological presentation of tuberculosis in patients with advanced HIV infection, which has already been reported, confirms this finding (Srikantam et al. 2006).

Findings from the present study emphasize a definite need for a combination of tests for diagnosing LNTB. Though, it is not widely practiced, culture of FNA may be an additional and more specific tool.
It is therefore suggested that bacteriological examination including mycobacterial culture may be considered wherever possible for diagnosing LNTB in RNTCP set up.

Diagnosis of lymph node TB is one of the most interesting areas prompting for the research. With the increasing number of lymph node TB cases in TB control programme that need a specific diagnosis, attempts should always be made to investigate the utility of various available techniques. Our observations suggest that FNA cytology is the most simple and sensitive test for the diagnosis of LNTB, however, supplementing this with FNA culture would increase the sensitivity in addition to giving a highly specific diagnosis. FNA culture facility should be made available at least in a few referral centers to help in more accurate diagnosis in problem cases.

### 6.4. Mycobacterial Species

Historically *Mycobacterium tuberculosis* and *Mycobacterium bovis* have caused the preponderance of human disease. NTM earlier thought to be rare in clinical specimens, are being increasingly reported particularly in HIV-positive patients. The rise in the incidence of NTM disease has accelerated rapidly since the first reports of nontuberculous mycobacterial disease in AIDS patients in 1982 (Joseph O Falkinham, 1996). The actual frequency and type of
mycobacterial disease varies from region to region and between ethnic groups.

Respiratory infections due to NTM are often associated with various predisposing lung conditions (e.g., pneumoconiosis) or amongst patients who were exposed to dust. NTM disease was not exclusively pulmonary, *Mycobacterium scrofulaceum* was found to be the causative agent of cervical lymphadenitis in children (Wolinsky, 1979), *Mycobacterium marinum* infections were found principally in the skin and were associated with cuts or abrasions and exposure to aquaria or swimming pools, or occupation in the fishing industry (Zeligman, 1972).

Pang reporting from Western Australia noted 118 children under the age of 7, suffering from mycobacterial lymphadenitis, the disease was caused by *Mycobacterium tuberculosis* in five (4%), the *Mycobacterium avium* complex in 87 (74%) and *Mycobacterium scrofulaceum* in 23 (20%), whereas in the 54 adults aged 15 years and over, *Mycobacterium tuberculosis* infections was 48 (89%), *Mycobacterium avium* complex 1 (1.9%) and *Mycobacterium scrofulaceum* in 2 (4%) (Pang, 1992).

On reviewing the literature it is observed that there are three distinct phases as far as the type of the causative agent responsible
for the lymphadenitis is concerned. In the first phase, the bovine strain of Mycobacterium tuberculosis appeared to be responsible for majority of tuberculous lymphadenitis in Western countries, especially in children below the age of 15 years. Mitchell (1917) from Edinburgh, observed that 88 percent of cases of adenitis in children were due to bovine bacillus and he could demonstrate tubercle bacilli in 20 percent of the samples of local milk supply. Griffith (1937) found that tubercle bacilli isolated from patients with cervical adenitis were bovine type in about 50 percent of cases both in England and Scotland.

Boyd (1944) observed that bacilli were usually of bovine type in case of lymphadenitis in children. In New York, Park (1910) reported 34 percent of all cases and 54 per cent in children of bovine type.

Park and Krumwiede (1910) typed the infection in 54 cases of cervical adenitis. One third of these were of bovine type. The percentage of bovine infection of lymph nodes in New York city fell from 30 per cent to 16.6 per cent because of general adoption of pasteurisation of milk. There was a remarkable reduction in USA as a whole because of this measure and because of eradication of tuberculosis in cattle. Thus in USA as early as in 1910 cases of lymphadenitis due to human type of tubercle bacilli predominated
Discussion

except in children in the age group of less than 5 years. The reduction in incidence of bovine tuberculosis was observed at a later stage in England. Bovine infection is not responsible for “human” type of M. tuberculosis and Lester (1951), Rich (1951) and members of American Trudeau Society’s Committee on therapy (1954) as well as Wilmot and associates (1957) were of the opinion that there was little reason to think that tuberculosis of cervical lymph nodes was frequently caused by infection with tubercle bacilli of bovine type and that the human type of tubercle bacillus was responsible for almost all cases of lymph node tuberculosis. The third phase has mainly begun in USA where the disease caused by human tubercle bacilli is well under control and this phase may be termed as an era of “Unclassified Mycobacteria”. There are, many reports of cervical lymph adenitis caused by mycobacteria other than M.tuberculosis (Weed and associates 1956, Prissick and Masson, 1956 and Chapman and Guy, 1959).

Davis and Comstock (1961) reported that in a community of south eastern United States, 70 per cent of cases of granulomatous cervical lymphadenitis in children were not caused by M. tuberculosis but by atypical mycobacteria. Sinclair and Mittal (1972) from All India Institute of Medical Sciences, New Delhi, in a study of 31 strains of atypical mycobacteria isolated from various sources, observed two cases of cervical adenitis caused by such strains.
In developing countries the situation is different and very little is known regarding lymphadenitis due to NTM from India. *Mycobacterium tuberculosis* has always been found as the major cause of mycobacterial infection, and the proportion of NTM has varied from less than 1 to 8 percent (Pathak *et al*., 1973; Hardas and Jayaram, 1984; Paramasivan *et al*., 1986, Nataraj et al 2002, Jesudasan et al 2005, Prasnta RM & Ashok Kumar J 2009.). Culture with strict criteria is still not routinely practiced. Therefore, it would be difficult to comment on the exact magnitude of the problem of infection due to NTM.

In this prospective study, data on 200 lymph node aspirates from tuberculosis suspects shows that the commonest age group affected was 21-30 years followed by 31–40 years (Table 5.1). This was also noted by Subrahmanyam (Subrahmanyam, 1993). In the USA and the UK, the highest incidence of tuberculous lymphadenitis in patients occurs between 25 to 50 years of age (Alvarez and McCabe, 1984; Monie *et al*., 1982). The ratio of males to females in this study was 1:1.3, which is similar to that found by Subrahmanyam (1:1.3), Dandapat *et al* (1:1.2), and Arora *et al* (1:1.3) (Subrahmanyam, 1993; Dandapat *et al*., 1990; Arora and Rajnish Gupta, 2006). In the present study 28 patients with LNTB were co-infected with HIV and majority of them are in the age group of 21-30.
As per the findings from other studies from India, *Mycobacterium tuberculosis* still appears to be the most common causative agent of tuberculous lymphadenitis. Two NTMs (one each of *Mycobacterium kansasii* and *Mycobacterium fortuitum*) and 74 *Mycobacterium tuberculosis* were isolated.

Nataraj *et al*., from Mumbai reported NTM isolation rate of 3.8% (Nataraj *et al*., 2002) and MV Jesudason *et al*, from Vellore reported NTM isolation rate of 3.9% (Jesudason and Gladstone, 2005). The prevalence of NTM observed in the present study is two out of 76 (2.6%). The two NTMs were isolated from HIV sero-negative patients. Isolation of these NTM strains shows that these strains are not only opportunistic infections but re-emerging as potential primary pathogens. This calls for an increasing surveillance for NTM in this part of the country.

NTM are widely distributed in the environment, and it is believed that infections by NTM are on the rise in many developed and developing countries. Immunosuppression is considered to be a predisposing factor. However in this study, the tubercular lymphadenitis due to NTM is seen in a very small number of cases. These NTM (*M.kansasii* and *M.fortuitum*) are isolated from non-immunosuppressed cases while *Mycobacterium tuberculosis* was
isolated from all immunosuppressed cases. Further large scale studies are needed to clearly delineate the role of NTM in non-pulmonary infections amongst immunocompromised and immuno-competent patients.

Occurrence of lymphadenitis due to infection by NTM has been reported from other parts of the world, where tuberculosis has declined and the relative frequency of NTM has risen especially in immunocompromised individuals.
6.4.1. *M. kansasii*

*Mycobacterium kansasii* is an acid-fast bacillus (AFB) that is readily recognized by its photochromogenicity, which produces a yellow pigment when exposed to light. In 1953, Buhler and Pollack first described the bacterium. Under light microscopy, its appearance is relatively long, thick, and cross-barred.

The most common presentation of *M kansasii* is a chronic pulmonary infection that resembles pulmonary tuberculosis. It may also infect other organs. Incidence of infection has increased because of the HIV/AIDS epidemic. It is the second most common nontuberculous, opportunistic mycobacterial infection associated with AIDS, surpassed only by *Mycobacterium avium* complex (MAC).

6.4.1.1. Epidemiology of *M. kansasii* Infection

The AIDS epidemic has had a striking effect on the frequency of disease caused by individual nontuberculous mycobacterial species, especially the *M. avium* complex, but not necessarily *M. kansasii*. In certain parts of the world before the appearance of the AIDS epidemic, *M. kansasii* infections were more common than *M. avium* complex infections. In southeastern England over the period from 1977 to 1984, the number of cases of mycobacterial disease caused by *M.*
K. kansasii was larger than the number caused by M. avium complex but still smaller than the number of M. xenopi cases (Yates et al., 1986). In Wales, there were 143 pulmonary M. kansasii infections compared with 41 M. avium complex pulmonary infections over the period from 1952 to 1978 (Jenkin 1981).

In fact, over the period from 1953 through 1970, there was an increase in the incidence of M. kansasii infections in Wales (Paull 1973). In Texas, over the period from 1967 to 1976, there was an increase in the number of cases of M. kansasii infection and there were approximately twice the number of M. kansasii cases as M. avium complex cases (Ahn et al., 1979). The number of M. kansasii isolates submitted to the Centers for Disease Control and Prevention is quite variable (Good 1980, Good 1982), and certain areas, including Louisiana, have large numbers (Witzig et al., 1995). In New Orleans, the number of cases of M. kansasii infection among human immunodeficiency virus (HIV)-infected individuals doubled from July 1984–January 1988 to July 1988–June 1991 (Witzig et al., 1995).

Predominance of M. kansasii infections among nontuberculous mycobacterial infections in immunocompetent individuals is not the case worldwide. In Japan, the majority of nontuberculous mycobacterial lung infections were caused by the M. avium complex
over the period from 1971 to 1979 (Tsukamura et al., 1981) and 1971 to 1984 (Tsukamura et al., 1988). The number of pulmonary infections caused by *M. kansasii* also rose during those periods (Tsukamura et al., 1988). *M. kansasii* infections are rare in Australia (Dawson et al., 1974). In British Columbia, the number of *M. avium* complex infections far exceeds the number due to *M. kansasii* (Isaac-Renton et al., 1985). Among isolates submitted to the Centers for Disease Control and Prevention for identification in 1979, 18.4% were *M. avium* complex and 3.3% were *M. kansasii* (Good 1980). In a southern California hospital between 1971 and 1981, 12 patients with *M. kansasii* pulmonary disease and 15 with either *M. avium* or *M. intracellulare* were identified (Gorse et al., 1983). *M. avium* complex cases predominated among cases in Virginia over the period from 1970 to 1979 (Kim et al., 1981). In Virginia, the prevalence of *M. kansasii* infections was almost four times higher in the first half of the period 1970 to 1979 than in the second half (Kim et al., 1981), suggesting a change in the epidemiology of these two mycobacterial species.

By contrast, over the period from 1984 to 1992, there has been a 10-fold increase in the number of *M. avium* complex infections compared with *M. kansasii* infections in England (Yates et al., 1993). The recent shift to a predominance of *M. avium* over *M. kansasii*
infections is not always the case. In Zurich, Switzerland, there was no change in the prevalence of *M. kansasii* infections over the period from 1983 to 1988 (Debrunner et al., 1992). In New Orleans, the increase in *M. kansasii*-HIV coinfecion has paralleled the increase in AIDS cases (Witzig et al., 1995).

As has been the cases with disease caused by other nontuberculous mycobacteria, *M. kansasii* disease in immunocompetent patients is primarily pulmonary (Choudhri et al. 1995, Debrunner et al. 1992, Grose et al. 1983). In only 1 of 42 immunocompetent New Orleans patients with *M. kansasii* disease was the infection disseminated (Witzig et al. 1995). In addition, there are often predisposing lung conditions. Among 154 patients with pulmonary *M. kansasii* disease in Great Britain, 33 had pneumoconiosis and another 31 were coal miners or steelworkers or worked in dusty conditions (Jenkins, 1981). In a study of 12 patients with *M. kansasii* pulmonary infections in southern California, 7 had preexisting pulmonary disease and 3 reported exposures to dusts (Gorse et al. 1983). *M. kansasii* has been isolated from sputum samples collected from a patient with cystic fibrosis (Heurlin & Petrini, 1993). In addition to pulmonary disease, cervical lymphadenitis caused by *M. kansasii* has been found in children (Colville, 1993, Gorse et al. 1983).
Discussion

*M. kansasii* pulmonary disease in immunodeficient patients (e.g., those with AIDS) can be disseminated or exclusively pulmonary (Witzig et al. 1995). In a study of 49 HIV-infected individuals with *M. kansasii* coinfection, 32 (65%) had pulmonary infections only and 17 (35%) had disseminated infections (Witzig et al. 1995). Thirteen of the *M. kansasii*-infected HIV-positive patients were also infected with *M. avium* complex and six patients had disseminated infections with both *M. kansasii* and *M. avium* complex (Witzig et al. 1995). In another study of 19 HIV-infected patients with a median CD4+ count of 49 cells per mm (74%) had pulmonary disease only and the remainder had disseminated infections (Levine & Chaisson. 1991). Disseminated *M. kansasii* disease has been found in other groups of AIDS patients (Portaels et al.1998, Sherer et al.1986). Cutaneous *M. kansasii* infections have been found in both immunosuppressed and immunocompetent patients (Breathnach et al.1995).

6.4.1.2. Characteristics of *M. kansasii*

*M. kansasii* is a slowly growing photochromogen that grows over a range from 32 to 42°C; there is no growth at 45°C (Jenkins et al.1982). Isolates produce both catalase and nitrate reductase and hydrolyze Tween 80 (Jenkins et al.1982). These characteristics form the basis for the identification of *M. kansasii*. *M. kansasii* isolates with strong catalase activity are more virulent (Steadham. 1980). Like other
nontuberculous mycobacterial species, individual isolates demonstrate colonial variation (Jenkins et al. 1982). The genetic basis for that variation is unknown.

There appears to be some genetic diversity among \textit{M. kansasii} isolates recovered throughout the world (Ross et al. 1992). Almost all isolates of \textit{M. kansasii} from Australia, Japan, and South Africa reacted with either of two \textit{M. kansasii}-specific DNA probes (Huang et al. 1991); isolates from Belgium and Switzerland less frequently reacted with either probe (Ross et al. 1992). Sequences of 16S rRNA were different for the probe-negative isolates, but all were similar to the 16S rRNA sequence of \textit{M. kansasii} (Ross et al. 1992). A probe prepared from a highly repeated DNA element from \textit{M. tuberculosis} (Ross et al. 1992) demonstrated that there were five different RFLP patterns among isolates reacting with an \textit{M. kansasii} -specific probe (Ross et al. 1992). In addition to an RFLP type seen in European and Australian isolates, there was a unique South African RFLP type (Ross et al. 1992, Ross et al. 1992). Distinct RFLP types were also seen among the isolates that did not react with the \textit{M. kansasii} specific probe (Ross et al (a). 1992, Ross et al (b). 1992).
6.4.1.3. Sources of M. kansasii

There have been a number of reports documenting the presence of M. kansasii in water samples. It has seldom been recovered from soil. M. kansasii isolates of the same phage type as those isolated from patients in the coal-mining provinces of Limburg and Rotterdam have been recovered from drinking water distribution systems in the Netherlands (Engel et al. 1980, Engel et al. 1981). Other investigators have reported the isolation of M. kansasii from tap water and shower heads (Bailey et al. 1970, Kaustova et al. 1971, Maniar & Vanbuckethout. 1976, McSwiggan & Collins. 1974). M. kansasii-like mycobacteria were recovered from water and soil samples collected in Cardiff, Wales (Colville. 1993). M. kansasii was shown to be capable of surviving in water for up to 12 months (Joynson. 1979) but incapable of long-term survival in soil (Joynson. 1979, Suwankrughasn & Leat. 1977). Those reports prompted the hypothesis that infection by M. kansasii occurs via an aerosol route (Collins & Yates. 1984).

The reports of successful isolation of M. kansasii stand in contrast to other studies in which M. kansasii was not found in the environment. For example, no M. kansasii isolates were recovered from a variety of water samples collected in and around Cleveland, Ohio (Goslee & Wolinsky. 1976), and from soils collected in the environs of Houston, Texas (Jones & Jenkins. 1965), or Cleveland,
Ohio (Wolinsky & Rynearson. 1968). In addition, *M. kansasii* was not recovered from water samples collected in New Hampshire, Boston, Massachusetts, Finland, Kenya, and Zaire (von Reyn et al. 1993) and soil samples collected in Uganda (Eaton et al., 1995). Because *M. kansasii* disease is found in urban as opposed to rural areas (Wolinsky 1979), failure to recover *M. kansasii* from rural New England, Finland, and Africa is expected. A long-term study of *M. kansasii* in tap water demonstrated that isolation was intermittent, which suggests why some investigators have failed to recover *M. kansasii* from that source (Collins et al. 1984). There have been a few reports of isolation of *M. kansasii* from other types of samples. *M. kansasii* has been isolated from the tissue of 1 of 193 feral pigs in Australia (Corner et al. 1981).

### 6.4.1.4. Risk Factors for *M. kansasii* Infection

Risk factors for *M. kansasii* infection include preexisting pulmonary disease, cancer, and alcoholism (Johanson & Nicholson. 1969, Witzig et al. 1995). A report of recovery of *M. kansasii* from a patient with cystic fibrosis (Heurlin & Petrini 1993) suggests that this inherited disease may also be a risk factor for *M. kansasii* infection. Preexisting pulmonary diseases include pneumoconiosis (Jenkins. 1981), chronic obstructive pulmonary disease (Gorse et al. 1983), and impaired ventilatory function (Ahn et
al. 1976). In a 1988 review of nontuberculous pulmonary infections in patients admitted to a Toronto hospital, 8 (9%) of 89 patients had infections that were caused by *M. kansasii*, and of those 8 patients, 5 had underlying lung disease, 3 had chronic liver disease, 2 smoked, and 2 reported alcohol abuse (Contreras et al. 1988). No common risk factors could be identified among the remaining patients. Persons in occupations in which dust is generated, such as coal mining, steelmaking, and glass manufacturing, are also at risk for *M. kansasii* infection (Bailey et al. 1974, Gorse et al. 1983, Owens et al. 1988). In British Columbia, *M. kansasii* isolates came from a portion of the province where smelting was a major industry (Isaac-Renton et al. 1985). Based on the finding that the rate of increase of *M. kansasii* disease in New Orleans has paralleled the rise in AIDS in Louisiana (Witzig et al. 1995), geography appears to be a risk factor for *M. kansasii* disease.

In a demographic study of pulmonary disease caused by *M. kansasii* in Texas, it was reported that cases were significantly more likely to come from urban than rural areas and that patients were unlikely to have Spanish surnames (Ahn et al. 1979). In Japan, most *M. kansasii* lung infections were found in Tokyo (Tsukamura et al. 1988, Tsukamura et al. 1981). Prior nocardiosis was associated with
M. kansasii infection in cardiac allograft recipients (Simpson et al. 1982).

It is logical that immunodeficiency is a risk factor for M. kansasii infection. Immunodeficiency and the attendant low CD4 cell count appear to predispose patients to dissemination of infection (Breathnach et al. 1995, Levine & Chaisson 1991, Witzig et al. 1995). In a retrospective study of 49 M. kansasii-infected human immunodeficiency virus (HIV)-positive patients in New Orleans, 35 (71%) were cigarette smokers and only 1 had preexisting pulmonary disease (Witzig et al. 1995). Risk factors for cutaneous M. kansasii infection include systemic illness, immunosuppression, skin pathology, or exposure to M. kansasii-contaminated water (Breathnach et al. 1995). However, there has not been a significant rise in the number of M. kansasii infections since the start of the AIDS epidemic in southeastern England (Yates et al. 1993), underscoring the possible role of geography in M. kansasii epidemiology.

6.4.1.5. Chemotherapy of M. kansasii Infections

In most studies of treatment of M. kansasii infections, rifampin has been included along with other antimycobacterial drugs (Gorse et al. 1983, Heurlin & Petrini 1993). Combinations that have been used include rifampin, streptomycin, isoniazid, and ethambutol (Ahn et

Some isolates of *M. kansasii* are resistant to low concentrations of streptomycin or isoniazid, and most are resistant to high concentrations of pyrazinamide (Wallace RJ Jr et al. 1990) and *p*-aminosalicylic acid (Ahn et al. 1981). Among *M. kansasii* isolates recovered from HIV-infected patients in New Orleans, 40 of 43 were susceptible to 1 mg of rifampin per ml, 29 of 39 were susceptible to 2 mg of streptomycin per ml, 12 of 37 were susceptible to 0.2 mg of isoniazid per ml, and 26 of 38 were susceptible to 0.5 mg of ethambutol per ml (Witzig et al. 1995). The New Orleans patients were treated with a triple-drug regimen of isoniazid, rifampin, and either ethambutol or pyrazinamide (Witzig et al. 1995). Drug combinations including rifampin were more effective in clearing infection within 4 months than were combinations lacking rifampin (Ahn et al. 1981, Pezzia et al. 1981).
6.4.2. *M. fortuitum*

*Mycobacterium fortuitum* is a nontuberculous mycobacterium (NTM), a grouping that encompasses all mycobacteria outside of the *Mycobacterium tuberculosis* complex. *M fortuitum* is classified in the Runyon group IV, rapidly growing mycobacteria. It has been found in natural and processed water sources, as well as in sewage. Distribution is probably worldwide.


Surgical wound infections associated with cardiac bypass surgery (Wallace et al.1989) or augmentation mammaplasty (Clegg et al. 1983, (a)Wallace et al.1989) are often clustered and occur more
frequently in Texas and the southern coastal region of the United States. *M. fortuitum*, *M. chelonae*, and *M. abscessus* do not have the same likelihood of causing infection in different locations in the body. For example, *M. fortuitum* is responsible for the majority of infections following cardiac bypass surgery (Wallace et al. 1989) or augmentation mammoplasty ((a)Wallace et al. 1989). In contrast, skin and soft tissue infections are due to *M. chelonae* and *M. abscessus* (Ingram et al. 1993) and pulmonary infections are most often caused by *M. abscessus* (Griffith et al. 1993). These patterns may reflect the environmental source and route of infection, especially in cases of nosocomial infection. For example, in two outbreaks of sternal wound infection following cardiac bypass surgery, isolates from possible environmental sources were similar or identical to isolates from patients (Wallace et al. 1989).

The genetic and phenotypic heterogeneity of rapidly growing mycobacterial isolates recovered from infections following cardiac bypass surgery or mammoplasty suggests that the isolates were unrelated and probably came from local sources rather than from contaminated surgical materials or devices (Wallace et al. 1989, (a) Wallace et al. 1989). Other cases of infection caused by these species include prosthetic valve endocarditis caused by *M. chelonae* (Repath et al. 1976) and post-thoracotomy sternal osteomyelitis due to
*M.fortuitum* (Robicsek et al. 1978), sternotomy wound infections involving *M. fortuitum* (Yew et al. 1993), and augmentation mammaplasty (Clegg et al. 1983).

There have also been a number of reports describing corneal infections caused by rapidly growing mycobacteria (Dalovisio et al. 1981, Lazar et al. 1974, Levenson & Harrison. 1966, Willis & Laibson. 1971, Wunsh et al. 1969, Zimmerman et al. 1969). Those reports identified the causative organism as *M. fortuitum*. However, the changes in the taxonomy of the group and the fact that most rapidly growing mycobacteria recovered from corneal infections today are *M. chelonae* (Wallace. 1994) suggest that the earlier isolates were possibly *M. chelonae*, not *M. fortuitum*. There have been reports of the isolation of rapidly growing mycobacteria (Boxerbaum. 1980, Smith et al. 1984), *M. fortuitum* (Aitken et al. 1993, Kilby et al. 1992), and *M. chelonae* (Kilby et al. 1992) from sputum of patients with cystic fibrosis.

The clinical significance of the rapidly growing mycobacteria recovered from these patients was not investigated. However, death of a patient with cystic fibrosis was attributed to *M. fortuitum* infection in one case (Efthimiou et al. 1984). A large multicenter investigation of the prevalence and significance of nontuberculous mycobacterial infections in cystic fibrosis patients has been initiated. Cases of
disseminated *M. fortuitum* and *M. chelonae* infections in the United States over the period from 1978 to 1991 have been reported and reviewed (Ingram et al.1993). Of the 54 patients reviewed, 40 had skin involvement and 75% of the total had a history of fever (Ingram et al.1993). Eighty percent of all the patients had some underlying disease or condition and fell into three groups (Ingram et al.1993). Patients in the first group did not have cell-mediated immune deficiency but did have an underlying condition or disease. These included kidney transplantation, chronic renal failure, and collagen vascular disease (Ingram et al.1993). Infections were limited, for the most part, to the skin, and the probability of clearing the infections and symptoms was high (Ingram et al.1993). Patients in the second group had cell-mediated immune deficiency including lymphoma and leukemia. For these individuals, infection involved many organs and the prognosis for survival was very low, with only a 10% survival rate (Ingram et al.1993). The third group of patients had underlying diseases of great variety (e.g., carcinoma and anemia) but no impaired cell-mediated immunity. Of the 12 patients in this group, 7 died, principally those with some type of carcinoma (Ingram et al.1993). Survival of patients was associated with a lower degree of involvement of organs and better general health than for those who died. Thus, patients whose infection was limited to the skin and were without carcinoma or cell-mediated immune deficiency survived (Ingram et
Disseminated infection in 10 of 11 kidney transplant patients and all 9 patients with chronic renal failure was caused by *M. chelonae* (Cooper et al. 1989, Ingram et al. 1993). There was no other evidence of a bias in infections in any other groups of patients with disseminated disease (Ingram et al. 1993).

### 6.4.2.1 Characteristics of *M. fortuitum*, *M. chelonae*, and *M. abscessus*

Since 1986, there have been significant changes in the names and taxonomy of the rapidly growing mycobacteria as a result of studies of DNA-DNA hybridization (Domenech et al. 1994, Kusunoki & Ezaki, 1992, Le´vy-Fre´bault et al. 1986, Pitulle et al. 1992) and 16S rRNA RFLP comparisons (Domenech et al. 1994, Pitulle et al. 1992). In some cases, biovariant and subspecies designations have been dropped because a group met the criteria for species designation (Le´vy-Fre´bault et al. 1986, Rogall et al. 1990, Wallace. 1994).

Species identification of rapidly growing mycobacteria is important, because the members differ in their susceptibilities to different antibiotics (Wallace. 1994). Measurement of the extent of DNA-DNA hybridization between rapidly growing mycobacteria demonstrated that *M. fortuitum*, *M. chelonae*, and *M. abscessus* are distinct species (Kusunoki i& Ezaki. 1992, Le´vy-Fre´bault et al. 1986).
Phylogenetic analysis of 16S rRNA sequences demonstrated that *M.fortuitum*, *M. abscessus*, and *M. smegmatis* belonged to a unique tight, distinct cluster (Rogall et al. 1990). Biovariants of *M.fortuitum* have been distinguished on the basis of sequence differences in 16S rRNA genes (Kirschner et al. 1992). *M. fortuitum*, *M.chelonae*, and *M. abscessus* can be distinguished from one another and from other rapidly growing mycobacteria on the basis of a number of cultural and biochemical tests (Kusunok i& Ezaki. 1992, Le’vy-Fre’bault et al.1986).

For several of the tests (i.e., growth on MacConkey agar medium and growth in 5% NaCl), the response is influenced by the temperature of incubation (Kusunok i& Ezaki. 1992). *M. fortuitum* isolates grow at 43°C, grow on MacConkey agar medium at 37°C, grow in 5% NaCl at 37°C, have nitrate reductase activity, and are unable to utilize citrate as the sole carbon source (Kusunok i& Ezaki. 1992, Le´vy-Fre´bault et al.1986). *M. chelonae* isolates do not grow at 43°C, do not grow on MacConkey agar medium at 37°C, do not grow in 5% NaCl at 37°C, lack nitrate reductase activity, and utilize citrate as the sole carbon source (Kusunok i& Ezaki. 1992, Le´vy-Fre´bault et al.1986). *M. abscessus* isolates do not grow at 43°C, grow on MacConkey agar medium at 37°C, grow in 5% NaCl at 37°C, lack nitrate reductase activity, and are unable to utilize citrate as the sole
carbon source (Kusunoki & Ezaki. 1992, Le’vy-Fre’bault et al.1986). These and other tests can be used for identification of other rapidly growing mycobacteria isolated from clinical and environmental sources (Kusunoki & Ezaki. 1992, Le’vy-Fre’bault et al.1986).

Rapidly growing mycobacteria have been shown to be responsible for degradation of a number of novel compounds. *Mycobacterium vaccae* has been reported to degrade alkanes (Coleman & Perry. 1984), acetone, benzene, dioxane, styrene (Burback & Perry. 1993, Burback et al. 1994), and trichloroethylene (Burback & Perry. 1993, Burback et al. 1994, Wackett et al. 1980) and to degrade and transform sterols (Vorbrodt et al. 1991). In addition, on the basis of the characteristics of growth, it is likely that those unidentified nontuberculous mycobacterial isolates reported to degrade a variety of other novel compounds are rapidly growing mycobacteria as well.

### 6.4.2.2 Source of *M. fortuitum*, *M. chelonae*, and *M. abscessus*

*M. fortuitum*, *M. chelonae*, and *M. abscessus* are widely distributed in the environment in relatively large numbers. Caution should be exercised in interpreting many reports describing the isolation of individual rapidly growing species from environmental samples, because of the changes in names and taxonomy. They have
been isolated from freshwater rivers and lakes, seawater, and wastewater from hospitals (Viallier. 1973), from animal drinking troughs (Beerwerth. 1973), and from soils (Jones & Jenkins. 1965, Paull. 1973). *M.*fortuitum* was found in water samples collected from ponds, canals, and swamps in Germany (Beerwerth. 1973) and in water samples collected in Zaire (Portaels 1973).

Although an early systematic search for mycobacteria in drinking water showed that rapidly growing mycobacteria were seldom recovered (Goslee & Wolinsky. 1976), a 1984 review documented reports of recovery of *M.*fortuitum* and *M.*chelonae* (and probably *M.*abscessus* as well) from drinking-water samples (Collins et al. 1984). A recent study has documented their presence and measured the numbers of *M.*fortuitum and *M.*chelonae* organisms in drinking water as well (Fischeder et al. 1991). *M.*fortuitum, *M.*chelonae, and *M.*abscessus can grow in distilled water (70). Although neither *M.*chelonae nor *M.*abscessus can grow at 42°C, they were recovered in large numbers from a hot water drinking water distribution system (Fischeder et al. 1991). Because nontuberculous mycobacteria are relatively resistant to chlorine (Carson et al. 1978, Haas et al. 1983), it is likely that *M.*fortuitum, *M.*chelonae, and *M.*abscessus in water distribution systems can survive and even grow on the nutrients in the water or in biofilms (Schulze-Röbbecke & Fischeder. 1989).
growth may be stimulated by the presence of novel compounds such as polyhalogenated phenols (Nohynek et al. 1993) and pentachlorophenol (Uotila et al. 1992) in polluted natural waters or water distribution systems.

There is evidence that the environment is the source of rapidly growing mycobacteria that infect patients. Isolates of *M. fortuitum* and *M. abscessus* recovered from a water bath in an operating room had the same patterns of susceptibility to antimicrobial agents, multilocus enzyme electrophoresis patterns, and plasmids as did *M. fortuitum* and *M. abscessus* isolates from patients following cardiac bypass surgery (Wallace et al. 1989). The availability of those techniques, as well as methods for comparison of rRNA RFLP patterns (Yew et al. 1993), will permit the identification of sources and routes of infection by rapidly growing mycobacteria. Soil is another habitat for *M. fortuitum*. *M. fortuitum* has been recovered from a variety of soils in the northern (Wolinsky & Rynearson. 1968) and southern (Jones & Jenkins. 1965) United States, Wales (Paull. 1973), and Zaire (Portaels 1973).

Although those reports did not assign any of the rapidly growing isolates recovered from soils to the species *M. chelonae* or *M. abscessus*, it is clear from the characteristics of the strains that many could have been *M. chelonae* or *M. abscessus*. *M. fortuitum* and *M.*
cheloneae (and possibly M. abscessus) are able to colonize and survive in soils (Suwankrughasn & Leat. 1977). Therefore, it is unlikely that M. cheloneae and M. abscessus are absent in soils. As was suggested for waters, it is likely that the growth of pathogenic rapidly growing mycobacteria would be stimulated by the presence of novel compounds such as polyhalogenated phenols (Nohynek et al. 1993) and pentachlorophenol (Uotila et al. 1992) in polluted soils. In addition to soil and water, M. fortuitum has been isolated from raw milk (Dunn & Hodgson. 1982) and from tissue samples of feral buffaloes (Hein & Tomasovic. 1981). M.cheloneae (and possibly M. abscessus too) has been found frequently in sphagnum vegetation in Sweden, Norway, and Germany (Kazda et al. 1979) and was responsible for infections in numbats in the Perth Zoo (Gaynor et al. 1990). Evidence of prosthetic valve endocarditis caused by M. cheloneae (Repath et al.1976) suggests that the replacement valves can be contaminated with M.cheloneae.

6.4.2.3 Risk Factors for M. fortuitum, M. cheloneae, and M. abscessus Infection

Because of the widespread presence of M. fortuitum, M.cheloneae, and M. abscessus in the environment and drinking water systems, everyone is exposed to these rapidly growing mycobacteria. In addition, exposures to contaminated solutions (e.g., gentian violet)
(Safraneck et al. 1987), medical equipment (e.g., bronchoscopes) (Wallace. 1994), and materials used in surgery (e.g., prosthetic heart valves) (Repath et al. 1976) are risk factors for infections by rapidly growing mycobacteria. Risk factors for pulmonary infections caused by *M. fortuitum* or *M. abscessus* included previous or current mycobacterial disease, cystic fibrosis, bronchiectasis, and chronic vomiting (Griffith et al. 1993). Risk factors for localized skin or soft tissue infections were trauma or surgery (Wallace et al. 1992). Risk factors for one of the three groups of patients with disseminated *M. chelonae* or *M. abscessus* infections included kidney transplantation, collagen vascular disease, corticosteroid therapy, or chronic renal failure (Ingram et al. 1993). Corticosteroid therapy was also associated with *M. chelonae* infection (Ingram et al. 1993). Cell-mediated immunity defects, lymphoma, and leukemia were risk factors for a second group (Ingram et al. 1993). Corticosteroid therapy and chronic renal failure were also risk factors for catheter-associated infections caused by rapidly growing mycobacteria (Wallace. 1994).

### 6.4.2.4 Chemotherapy of *M. fortuitum, M. chelonae, and M. abscessus* Infections

Like the slowly growing nontuberculous mycobacteria, *M. fortuitum, M. chelonae, and M. abscessus* are resistant to many of the antimycobacterial drugs (Seidel & Horhold. 1992, Wallace. 1994).
The presence of tetracycline resistance genetic determinants in members of the *M. fortuitum* group (Pang et al. 1991) clearly precludes the use of tetracyclines. Susceptibility testing is useful in guiding the choice of antibiotic therapy (Swenson et al. 1985, Wallace. 1994). *M. fortuitum* strains are susceptible to amikacin, ciprofloxacin, sulfonamides, cefoxitin, and imipenem (Wallace. 1994, Wallace et al. 1990). *M. chelonae* strains are susceptible to amikacin, tobramycin, and imipenem and resistant to cefoxitin (Wallace. 1994, Wallace et al. 1990). Strains of *M. abscessus* are susceptible to amikacin and cefoxitin (Wallace. 1994, Wallace et al. 1990). All three species are susceptible to clarithromycin (Brown et al. 1992) and azithromycin (Wallace. 1994), and a multidrug regimen including clarithromycin has been used to successfully treat *M. abscessus* infections (Mushatt & Witzig. 1995).
6.5. Drug resistance:

Development of drug resistance is common to all pathogenic bacteria and *M. tuberculosis* is not an exception. Nearly seventy two (71.6%) percent (53/74) of the isolates were susceptible to all the drugs, and resistance was observed in 28.3% isolates. Any resistance to isoniazid (H) was 27%, Rifampicin (R) 5.4%, Streptomycin (S) 6.7%, and none was resistance to Ethambutol (E). Resistance to single drug was observed in 13/74 (17.5%) isolates. Mono resistance to H was 12 (16.2%); R was 1(1.3%). None of the isolates was mono resistant to either S or E. Combined resistance to H and R (MDR) was observed in five (6.5%) isolates. Proportion of drug resistance for each drug was higher in isolates from previously treated lymph node cases than that from new cases. Out of four MDR from LNTB group, three were from patients who were previously treated for either pulmonary or LNTB. One of these patients was an MDR pulmonary TB, who later relapsed with LNTB. In India, the proportion of multi drug resistance (MDR) in new pulmonary cases is approximately 3%, and the proportion among previously treated cases was 30%. MDR of 0.7% was reported in newly treated pulmonary TB patients in Hyderabad area. Similar study from this region has reported 15% of MDR (Chitra et al, 2003). An earlier study on LNTB from Mumbai, reported, 1% MDR in new cases and 16% in previously treated cases (Mahadev et al. 2005 & Anuradha et
al. 2006). Our study reports 2.3% MDR in new LNTB cases and 30% in patients who had previous anti TB treatment.

Drug resistance in LNTB may not have serious public health implications, but for each affected person, the swollen lymph node would be a medical problem that needs an immediate intervention. Owing to the serious nature of MDR TB, and its reported implications in treatment failure and relapses in pulmonary TB patients (IUATLD 1996), attempts should therefore be made to study prevalence of drug resistant LNTB in the programme conditions. Our findings suggest that, multi drug resistance is present in LNTB cases in this part of the country. This justifies the need for mycobacterial culture of fine needle aspirates and drug susceptibility tests, wherever the resources permit (Anuradha et al. 2006). Collection of data on drug sensitivity pattern and knowledge about the MDR from large number of isolates would be helpful in formulating the treatment policies for LNTB patients.

We conclude from the present study that lymph node infections due to NTM seem to occur in HIV negative patients also. Further studies on role of NTM in human diseases should be examined in more detail and on a larger scale. Information is urgently needed for proper diagnostic procedures and for adequate treatment of NTM induced disease.