Review Article
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Infections due to non-tuberculous mycobacteria (NTM)

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The membership list of genus mycobacterium is ever expanding and it has grown to 95 in year 2003. While leprosy and tuberculosis are specific diseases caused by mycobacteria, other members are usually saprophytes but can be opportunistic and at times deadly pathogens. These other mycobacteria are referred to as atypical mycobacteria, non-tuberculous mycobacteria (NTM) or mycobacteria other than tubercle bacilli (MOTT). These organisms can produce localized disease in the lungs, lymph glands, skin, wounds or bone. Occasionally they may produce disseminated disease. Of the more than 90 known species of NTM, about one third have been associated with disease in humans. The species causing human disease are : *Mycobacterium avium, M. intracellulare, M. kansasii, M. paratuberculosis, M. scrofulaceum, M. simiae, M. habana, M. interjectum, M. xenopi, M. heckeshornense, M. szulgai, M. fortuitum, M. immunogenum, M. chelonae, M. marinum, M. genavense, M. haemophilum, M. celatum, M. conspicuum, M. malmoense, M. ulcerans, M. smegmatis, M. wolinskyi, M. goodii, M. thermoresistible, M. neoaurum, M. vaccae, M.palustre, M. elephantis, M. bohemicam* and *M. septicum*. Isolation of these mycobacteria from representative specimens and their rapid identification is very important as the treatment strategy for tuberculosis and other mycobacterioses is different. Several biochemical, chemical (lipid) and molecular techniques have been developed for rapid identification of these species. Along with suggestive clinical features, poor response to antitubercular treatment and repeated isolation of the organisms from the clinical specimens these techniques can help in establishing correct diagnosis. Further, many drugs like rifampicin, rifabutin, ethambutol, clofazimine, amikacin, new generation quinolones and macrolides effective against mycobacterial infections are available that can be used in appropriate combinations and dosage to treat these infections.

Key words Atypical mycobacteria - MOTT - non-tuberculous mycobacteria

Genus Mycobacterium has 95 well characterized species[1]. Over the centuries two well known mycbacterial species, namely, *Mycobacterium tuberculosis* and *M.leprae* have been the known causes of immense human suffering. Most of other mycobacteria are present in the environment as saprophytes. Their pathogenic potential has been recognized since the beginning of last century[2]. These organisms in the past have been called atypical mycobacteria, the term first coined by Pinner[3]. These have been increasingly recognised to cause pulmonary and non pulmonary infections[3-8], which is in part explained by the increase in the number of susceptible/immunocompromized individuals such as those suffering from acquired immuno deficiency syndrome (AIDS) and is also due to better recognition of their role through more sensitive and specific techniques[9,10]. The diseases caused by these mycobacteria have varied manifestations, are not usually transmitted from man to man and have been broadly grouped as mycobacterioses. In the immunocompromised individuals the infections due

to non-tuberculous mycobacteria (NTM) have been observed to be an important cause of morbidity and mortality in western countries[9]. Besides being known as atypical, these mycobacterial species have been given various names like anonymous, non-tuberculous, environmental, opportunistic and mycobacteria other than tubercle bacilli (MOTT). None of these terms have been universally accepted and the name NTM seems to be more acceptable. It has been endorsed by American Thoracic Society (ATS) in their statement[9,10].

Distribution in the environment

The distribution of NTM and the incidence of disease caused by them is perhaps not fully understood in most parts of the world. NTM are widely distributed in nature[11] and have been isolated from natural water, tap water, soil, water used in showers and surgical solutions. In United States most of isolates were *M. avium, M. kansasii* and *M. fortuitum*[12]. There have been some reports from Japan[13], UK[14] and India[15-21]. In most of Indian studies *M. tuberculosis* has been found as major cause of mycobacterial infections and the proportion of NTM has been considered low. Species like *M.fortuitum, M. avium, M. scrofulaceum etc*., have been isolated in different studies[17-21]. As the culture with strict criteria is still not routinely done in most parts of India and there is a tendency to ignore such isolates as cotaminants, it would be difficult to comment on the exact magnitude of the problem. Though *M.tuberculosis* has been observed to be the main secondary infection in the reported cases of AIDS in India[22] the future scenario is yet to unfold.

Predisposing factors

It is a common observation that environmental mycobacteria cause disease in individuals who offer some opportunity due to altered local or systemic immunity[3,4,9,10,23-26]. Chronic obstructive pulmonary diseases, emphysema, pneumoconiosis, bronchiectasis, cystic fibrosis, thoracic scoliosis, aspiration due to oesophageal disease, previous gastrectomy and chronic alcoholism are some of conditions which have been linked to disease due to NTM. While the reasons may be less clear in conditions like adenitis in children, such factors may be quite obvious in other conditions like bronchiectasis, surgical procedures, injections, break in skin surface due to wounds and generalized immune deficiency states like AIDS, use of immunosuppressive agents as used in transplant patients *etc*[9,10]. The mechanisms of pathogenesis of NTM are not very clear and have not been adequately investigated. The lipid rich outer envelope of the organisms may be important as first defence but specific moeities on the surface may be the important factors. Very low CD4 counts in AIDS patients and defective cytokine response(s) have been linked to severe infections due to *M.avium* from the common sources like potable water[26].

Clinical manifestations

Of the 95 known species of mycobacteria, nearly one third have been observed to be associated with disease in humans. The species of NTM associated with human disease are : *M.avium, M.intracellulare*[25-30], *M.kansasii*[31-33], *M.paratuberculosis*[34-36], *M.scrofulaceum*[37], *M.simiae*[7,8], *M.habana*[7], *M.interjectum*[38], *M.xenopi*[39-41], *M.heckeshornense*[42], *M.szulgai*[43], *M.fortuitum*[18,44,45], *M.immunogenum*[46], *M.chelonae* [20, 47], *M.marinum*[48-50], *M.genavense*[51,52], *M. bohemicum*[53], *M.haemophilum*[54-56], *M.celatum*[57], *M.conspicuum*[58], *M.malmoense*[59,60], *M.ulcerans*[61], *M.smegmatis*[62], *M.wolinskyi* and *M.goodii*[63], *M.thermoresistible*[64], *M.neoaurum*[65], *M.vaccae*[66], *M.palustre*[67], *M.elephantis*[68], and *M.septicum*[69] and *M.nonchromogenicum*[70].

Non-tuberculous mycobacteria have been reported to cause localized or disseminated disease depending on local predisposition and/or degree of immune deficit[3,4-9]. In non-HIV patients, different NTM may cause localized pulmonary disease, adenitis, soft tissue infections, infections of joints/bones, bursae, skin ulcers and generalized disease in individuals like leukaemia, transplant patients *etc*[3,4,9]. In AIDS patients the manifestations may range from localized to disseminated disease[9,71]. Clinical features will include local organ specific signs and symptoms to persistent high grade fever, night sweats, anaemia and weight loss in addition to nonspecific symptoms of malaise, anorexia, diarrhoea, myalgia and occasional painful adenopathy.

(a) Pulmonary infections due to NTM: *M.avium-intracellulare* complex (MAC) strains have been a major cause of pulmonary and other infections in the pre-AIDS era also[9,10]. MAC infections were commonly seen in chronic bronchitis, bronchiectasis and in chronic obstructive airway disease in the pre-AIDS era in geriatric patients. Since long, *M. kansasii* has been considered an important cause of pulmonary disease[4,9]. *M.scrofulaceum* has been shown to be the cause of localized pulmonary infections[9,10]. *M. xenopi*, an unusual bacterium with optimal growth temperature of 45^0 C has been encountered as a pathogen in patients with other underlying lung diseases[39-41]. One instance of an outbreak of pulmonary disease due to this organism from hot water supply of a hospital has been reported[9]. *M.simiae* [3, 4, 9], *M.habana*[8], *M.szulgai*[19,43], *M.fortuitum*[18], *M.vaccae*[66] , *M.malmoense*[59,60] are other pathogens reported to be associated with pulmonary infections. *M.heckeshornense* is a new slow growing species of mycobacteria which has been shown to be associated with cavitary disease in immunocompetent individuals[42].

(b) Lymph glands: MAC isolates have been reported to be the cause of lymphadenitis[9,10]. *M. scrofulaceum* whose distribution in nature closely resembles that of *M.avium*, has also been found to be a common cause of cervical lymphadenitis in western countries[9] and has been reported from India as well[21]. *M.bohemicum*[53], *M.szulgai*[43] and *M. interjectum*, a new species resembling *M.scrofulaceum* have been isolated from cases of lymphadenitis[38].

(c) Bone, joints and bursae: MAC isolates, *M.szulgai*[43], *M.fortuitum*[3,4,9], *M.non-chromogenicum* and *M.kansasii* have been reported to be the cause of bone and joint infections[9].

(d) Cutaneous infections: *M.szulgai*[43], *M.marinum*[3,4,48-50], *M.ulcerans*[61] and *M.vaccae*[66] have been reported to be a cause of skin infections. *M.marinum* species has been recognized as a causative organism of swimming pool granuloma or fish tank granuloma. It causes papular lesions in the extremities and may be confused with sporotrichosis [3,4,48-50]. *M.ulcerans* is established cause of buruli ulcer[61]. *M.vaccae*[66] has also been reported to be a cause of skin infections.

(e)Wound infection and sepsis: *M. fortuitum* causes pyogenic lesions in the soft tissue, joints, bursae and injection abscesses[3,4,9,10]. While *M.chelonae abscessus* is a well known cause of wound infections[47], a new related species *M.immunogenum* has been recently been recognized as a cause of sepsis[46]. *M. marinum* also causes infections of bones/joints/tendon sheaths specially in AIDS patients[50]. *M.smegmatis*[62], *M. wolinskyi* and *M. goodii* [63] , *M. thermoresistible*[64] and *M. palustre*[67] have been reported to cause wound infections and also bacteraemia. Members of *M.terrae* complex (*M. terrae, M. nonchromogenicum* and *M. triviale*) may be associated with mycobacterial disease. Occasionally *M.nonchrmogenicum* and *M.chelonae* have been identified as causes of acupuncture induced infections[70]. *M.septicum* a new rapidly growing species has been reported to be associated with catheter related bacteremia[69].

(f) Crohn's disease: Advances in molecular techniques have by and large established the aetiology of regional ileitis (Crohn's disease) due to *M. paratuberculosis* , a species closely related to *M. avium*. Members of this species have been reported to be the causative agents of enteritis (Johne's disease) in cattle, goats and sheep and can be characterized rapidly with the help of molecular techniques[34,35]. By using gene probes and *in situ* hybridization strategy *M.paratuberculosis* has been linked to the aetiology of Crohn's disease in man[35,36] with reasonable certainty.

(g) Disseminated disease in immunocompromized individuals: Important NTM causing disseminated disease in immunocompromized cases including:

(*i*) *MAC*: Unlike tuberculosis, the strains of MAC are pathogens of very low virulence and despite being commonly found in the environment rarely cause disease[11,26]. In western countries, infections due to members of MAC have been frequently reported in AIDS patients [27-30]. Certain specific serotypes of *M.avium*[9,24], plasmid containing *M.avium*[29], and in some European and African countries certain restricted fragment length polymorphism (RFLP) types of *M. avium* have been found to be more commonly isolated from AIDS patients[24,30]. As compared to *M. intracellulare, M. avium* appears to

have greater predilection for causing disease in AIDS cases[24]. Further, these may cause mixed infections along with other NTM such as *M.kansasii*[27] and *M.simiae*[28] *etc*. MAC usually produce clinical disease only when the CD4 count is very low (<50 cells/ml) towards the end of natural history of disease, seen in 4 to 5 per cent of HIV infected patients. MAC strains isolated from AIDS cases in Africa have been shown to be different from those of western strain[24]. In AIDS patients, the portal of entry of MAC is thought to be mainly through the gut[26]. Persistent high grade fever, night sweats, anaemia and weight loss in addition to nonspecific symptoms of malaise, anorexia, diarrhoea, myalgia and occasional painful adenopathy are common signs and symptoms associated with MAC disease in AIDS cases.

(*ii*) Infections due to *M.kansasii* have become more important in AIDS cases and are common in individuals with severe immunodeficiency state[27,31-33]. While *M.kansasii* isolates are generally more resistant to antimicrobial agents than *M. tuberculosis*, therapeutic responses to multiple drug therapy have been usually found to be good[6,33].

(*iii*) *M. scrofulaceum (M. marianum*) may cause adult pulmonary disease and disseminated infections in patients with AIDS[9,37].

(*iv*) *M. xenopi* causes disease with clinical manifestations similar to those of MAC in advanced AIDS patients[40]. One instance of an outbreak of pulmonary disease due to this organism from hot water supply in a hospital has been reported[9].

(*v*) *M. simiae* has been recognized as an agent of human pulmonary disease in AIDS as well as non-AIDS cases[3,4,9,28].

(*vi*) *M. fortuitum-M. chelonei complex*: These rapidly growing organisms have been frequently isolated from soil in different countries including India, and are well known cause of soft tissue infections like injection abscesses and wound infections[4-8,9,18-20,44-47]. These organisms can also cause generalized disease in immunocompromized hosts and present as subcutaneous nodules; similar picture may be shared by other NTM like *M. kansasii*[9,44].

(*vii*) *M. genavense*: These organisms initially isolated from AIDS patients with advanced disease, have been reported from many countries[51-52]. These organisms need enriched medium with mycobactin J and often grow after prolonged incubation periods. Weight loss, fever, abdominal pain and diarrhoea are the common presenting symptoms in infected individuals.

(viii) M. haemophilum is a fastidious slow growing organism and requires enriched chocolate agar, haemin or ferric ammonium citrate for its growth. It grows well at a comparatively lower temperature of 30°C. It has been recognized as a cause of life threatening infections in immunocompromised individuals like AIDS cases and bone marrow transplant recipients[54-56].

(*ix*) Several other species of NTM namely *M. celatum*[57], *M. conspicuum*[58], and *M. malmoense*[59,60] have been isolated from AIDS patients.

Diagnostic procedures

Due to ubiquitous presence of NTMs in the environment, establishing the causative relationship depends upon appropriate sampling and strict laboratory practices as contamination needs to be ruled out. These issues have been extensively debated and some broad guidelines are available[9,10].

(a) Diagnosis of pulmonary disease: The clinical presentation of the pulmonary disease due to NTM may be like tuberculosis. Infection due to NTM should be suspected specially in cases in whom initial antitubercular treatment (ATT) has not produced the desired response. This should be corroborated by repeated isolation of the same NTM from sputum or bronchoalveolar lavage. Infection with NTM may be asymptomatic or subacute or chronic illness resembling pulmonary tuberculosis. While radiological appearances are usually like in tuberculosis with cavities and infiltrates, thin walled cavities with lesser parenchymal infiltrates have been described as suggestive features [9,10]. The changes may be uni- or bilateral and more than one lobes may be involved. High resolution computed tomography (CT) scanning may show clusters of

small nodules associated with areas of bronchiectasis in the lower and middle zones. Pleural thickening and effusion is uncommon. Bronchoscopy is very useful to obtain lavage samples for culture and to obtain biopsy samples. Tissue biopsy showing granulomatous inflammation, which may or may not contain acid fast bacilli (AFB) and a positive culture, even if the sample was smear negative is considered as most appropriate strategy to establish the diagnosis.

(b) Other clinical forms: Infections with NTM should be considered in the differential diagnosis of any chronic infection, pyrexia of unknown origin and localized clinical disease (abscess, ulcers, nodules, infiltrates *etc*.) not responding to antibiotics. Attempt then should be made to repeatedly demonstrate and isolate the NTM from such lesions using most stringent criteria and precautions.

(c) Strategy to diagnose NTM infections

As many of the NTM are not amenable to routine antituberculosis therapy, it is important to correctly identify the causative mycobacteria and if required determine their sensitivity profile. Main strategic points are:

(*i*) *Specimens*: As far as possible the specimen should be directly from lesion/organ concerned[9,10]. For such purposes biopsies and procedures like bronchoalveolar lavage have advantages. As NTM are more sensitive to agents like sodium hydroxide, the decontamination has to be gentler than *M.tuberculosis*. In case of the disseminated infections such as in AIDS patients with CD4 count less than 50 cells/mm isolation of mycobacteria from peripheral blood or bone marrow has been reported to be quite useful[72-75].

(*ii*) *Histopathological examination*: Histopathology has been described to be useful specially for demonstration of granuloma for specimens from such as aspirates/ biopsies from bone marrow, liver or lymph nodes[10]. Histopathology was reported to be rapid and useful approach to diagnose infections due to MAC[74]. It may be advisable to include some *in situ* methods (antigen detection/gene probes) to confirm the histological diagnosis and aetiology directly with perhaps good speed and specificity.

(*iii*) *Cultivation*: Most of NTM can be grown on ordinary media for mycobacteria like Lowenstein-Jensen, Middlebrook and Dubos Broth/Agar[9,76]. While organisms like *M. hemophilum* may have special requirements like hemin which may be obtained from blood containing media -chocolate agar or supplement of ferric ammonium citrate, *M.genavense* and *M.paratuberculosis* will need media enrichment with mycobactin J. Radiometric systems such as BACTEC medium or an agar based isolator system have been reported to be highly sensitive for cultivation of MAC[3,4,9,12,73-77]. Different incubation temperatures such as 30° C for *M.ulcerans* and *M.marinum*, 37° C for most pathogens, 45° C for *M.xenopi etc.*, will have to be selected depending upon the suspected organisms.

(*iv*) *Identification of isolates by phenotypic characteristics*: Growth rates, colony pigmentation and biochemical tests such as niacin production, nitrate reduction, tween-80 hydrolysis, arylsulphatase, urease, tellurite reduction, thiophen-2-carboxylic acid hydrazide (TCH) sensitivity, catalase (qualitative and quantitative), growth on MaConkey agar, sodium chloride tolerance *etc*., are adequate to identify majority of clinically relevant mycobacteria[78,79]. This strategy is, however, time consuming and not conclusive for many isolates with variable characters. Analysis of the lipids of mycobacteria by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) has been recommended as an alternate approach. When used along with easy software programmes for rapid analysis, isolates from liquid/ solid medium can be rapidly identified[79,80].

Serotyping methods using serotype specific sera have been described for the members of mycobacterium avium intracellulare scrofulacecm (MAIS) complex[73,81]. Some serotypes[9,73] of *M. avium* have been shown to be preferentially associated with disease in AIDS patients. Isoenzyme and protein electropherogram based schemes for rapid identification and characterization of strains of *M. tuberculosis* and NTM have been developed which

may be used in small laboratory settings[82,83]. Based on measurement of immunological divergences in the structure of certain enzyme molecules like catalase[84] and superoxide dismutase[85], techniques have been described for identification of mycobacteria. All these techniques need some specialized infrastructure and expertise but are not difficult to set up.

(d) Identification and characterization of NTM by molecular methods

Based on new knowledge about the gene sequences of mycobacteria many gene probes for the identification of isolates as well as amplification of specific gene fragments from the lesions and mycobacterial culture isolates have been developed:

(*i*) *Gene probes*: For the identification of important NTM several gene probes have been developed and some are also commercially available[77,86-88]. With the help of these probes, growth from solid slants/ liquid cultures (*e.g.*, BACTEC) can be rapidly and reliably identified.

(*ii*) *Gene amplification methods*: Polymerase chain reaction (PCR) techniques for detection and rapid identification of various clinically relevant mycobacteria have been developed which include methods for concentration and detection of *M. avium, M. intracellulare* from the clinical specimens[89-91], *M. paratuberculosis* from clinical specimens and milk[34,92]. PCR assays using genus/ group specific amplification followed by restriction analysis have been described for the analysis of gene regions like 65 kD[93,94] and rRNA gene region which have been found to be useful for identification of different mycobacteria[95-99]. Besides the PCR-RFLP approach, PCR amplification followed by hybridization has been reported to be useful for *M. avium, M. chelonae, M. scrofulaceum, M. ulcerans* and other *mycobacteria*[100-103]. Most important and specific approach mainly applicable at reference level involves the amplification of 16S rRNA followed by sequencing and several species have been identified for the first time using this approach[104,105]. These PCR methods can be used for direct detection of mycobacterial pathogens[89-92] as well as for rapid identification of clinical isolates of NTM[93-104]. Keeping in view the diversity of these organisms present in different geographical locations, it is important to evaluate the usefulness of these techniques in different settings.

(*iii*) *DNA fingerprinting techniques*: DNA fingerprinting techniques are of interest for identifying the subtypes/strains which would be more commonly associated with disease and also to investigate hospital acquired infections. Several DNA fingerprinting techniques have been tried to investigate the diversity in NTM[106]. Techniques like pulsed field gel electrophoresis[107], random amplified polymorphic DNA (RAPD) - arbitrary PCR[108], rRNA gene polymorphism[109-111], typing using different insertion/repeat elements[53,112,113], plasmid typing[114] and single gene polymorphism[115] have been successfully used for molecular typing of NTM. Insertion sequence based RFLP methods have been described to be useful for characterization of *M. hemophilum*[55], *M. avium*[112] as well as *M. kansasii*[113]. Using these methods certain RFLP types of *M. avium* have been shown to be closely linked with disease in Europe and Africa[24,30]. In India, such information about molecular types of NTM is very scanty.

(e) Determination of sensitivity profiles

The drug susceptibility profile of NTM is usually quite different from *M.tuberculosis.* Firstly, these organisms are usually sensitive at high concentrations of antitubercular drugs[9,10], thus higher cut off values for deciding sensitivity/resistance are recommended. Secondly, rapid growing mycobacteria are usually resistant to rifampicin and isoniazid (INH) whereas these are sensitive to drugs like new generation macrolides, cephalosporins and sulphones. The media usually recommended for the sensitivity screening of *M. tuberculosis* are used for NTM also. Other media like chocolate agar/supplemented with ferric ammonium salts/mycobactins *etc*., will be needed for the sensitivity screening of fastidious species. Newer techniques like BACTEC and E test have been also found to be quite useful for sensitivity determination of rapid as well as slow growing NTM[116-118]. A new technique using recombinant strain

of *M.avium* expressing beta- galactosidase to evaluate the activities of antimycobacterial agents inside macrophages has been recently described[119].

Management of NTM infections

Prophylaxis, medical and surgical treatment are three important aspects of management of infections due to NTM[9,10].

(a) Prophylaxis: Chemoprophylaxis with antimycobacterial drugs such as rifabutin has been recommended as primary prophylaxis by US Food and Drug Administration to prevent and delay the onset of bacteraemia in AIDS patients[120]. There is a need to undertake studies in India to gain experience as profile of NTM causing infection in AIDS cases may be quite different from west and these infections may not be very important in our country.

(b) Surgical treatment: Surgical intervention has been recommended for the management of cervical lymphadenitis[121] and also as a last resort in the management of localized infections due to drug resistant organisms[122,123]. Additional antimycobacterial treatment is also considered to be beneficial by some investigators whereas others disagree[9]. Surgical debridement is required in cases with accumulation of pus, dead tissue and is indicated in infections due to *M.fortuitum-chelonae*[9] and in some cases of *M.marinum*[49] as an adjunct to antimycobacterial treatment.

(c) Medical treatment: Medical treatment for NTM infections should be based on background information about sensitivity profiles[9,10] which is very limited for NTM in India[10]. In United States, trends about the type of NTM isolates and their sensitivity profile have been studied over a long period of time and some broad principles of management have already been suggested by American Thoracic Society (ATS)[9]. An initial trial of 2 wk with ATT and repeated isolation of NTM is suggested as starting point. After that drug combinations and their dosage for treatment of various NTM infections differ significantly from tuberculosis. In general, dosage higher than recommended for the treatment of tuberculosis is recommended and requires intense monitoring of side effects of these drugs and combinations.

(i) Treatment of M. avium complex: A variety of compounds and their combinations have been reported to be effective against MAC strains[124-140]. The compounds showing activity against MAC include rifampicin, rifabutin, clofazimine, ciprofloxacin, amikacin, ethambutol, azithromycin, clarithromycin, telithromycin, and INH. While the experience about many of the above compounds is still experimental[124,125,134-136], others have been tested in patients as well. Important regimens tried for the treatment of MAC infections include combinations of rifampin, isoniazid, ethambutol streptomycin[9,126]; ansamycins, clofazimine, ethambutol and isoniazid[127]; and amikacin, ethambutol, rifampin and ciprofloxacin[128]; rifabutin, clofazimine, INH, ethambutol[129]; rifampin, ethambutol, clofazimine and ciprofloxacin[130]. Clarithromycin and azithromycin have been considered two promising drugs for treatment of MAC infections[131-134]. Telithromycin has been observed to be active against *M.avium* in mice despite lacking significant activity *in vitro* and within macrophages[135]. Liposomized encapsulated clofazimine has been reported to be more effective than clofazimine alone in the experimental studies in mice[136]. A combination of clofazimine, rifampin, ethionamide, streptomycin and ethamutol has been observed to be effective against *M.intracellulare* infections[139]. Combination of amikacin and rifampicin which has been reported to be active against *M.avium* in mice, holds promise in management of infections in humans as well[141]. Except for ATS regimen[9] the duration of other regimens is not certain and more trials need to be carried out.

(*ii*) *Treatment of M. kansasii infections*: A regimen comprising of isoniazid, rifampin and ethambutol for a duration of 18-24 months has been recommended by ATS for the treatment of *M.kansasii* infections[9]. Sulphonamides and short course regimens for the treatment of rifampin resistant *M.kansasii* have been also been suggested[142,143].

(iii) Treatment of other NTM infections: Regimens used for MAC infections have been generally reported

to be good for the management of infections due to *M.malmoense, M.simiae, M.szulgai* and *M.xenopi* as well[9]. Rifampicin and ethambutol has been recommended for the management of *M. xenopi, M. kansasii, M. fortuitum*. For infections due to *M.marinum* and *M.szulgai* a combination of trimethoprim- sulfamethoxazole and doxycycline has been suggested[4,49]. There is extensive information which suggests that commonly used antitubercular drugs like rifampin and INH have no role to play in the treatment of infections due to *M. fortuitum chelonei* complex. Clarithromycin[144] and combination of amikacin and doxycycline have been observed to be effective against *M.fortuitum-chelonei* infections[145]. For the treatment of severe infections caused by these organisms cefoxitin, sulpha drugs and amikacin have been recommended[9]. For the treatment of *M. haemphilum* infections, a regimen comprising of rifampin, clofazimine and clarithromycin has been described to be effective[146]. For the treatment of infections due to other NTM, no specific recommendations are available. Antimicrobial drug screening results should guide the management of such infections.

Recent developments relevant in improving the therapy of NTM infections

There are several new developments which may have long-term effect on improving the therapy of NTM infections . Moxifloxacin, telithromycin and quinupristin and dalfopristin have been reported to be more effective against *M.marinum* than other macrolides and antibiotics[147]. It has also been reported that mycothiol deficient mutants of *M.smegmatis* are inhibited better by antibiotics[148]. Further C-8 halogen and methoxy moeties have been shown to enhance fluoroquinolone activity even in gyrase resistant mutants[149]. A new protein involved in intrinsic resistance to quinolone has been identified[150]. Further studies need to be done to reach at some meaningful conclusions.

It may be unrealistic to imagine common mechanisms of susceptibility/resistance in these diverse organisms. Several studies have shown that the mechanisms of drug susceptibility in NTM are quite distinct from *M.tuberculosis* . While mutations in gene targets like *rpo*B have been found be responsible for resistance to rifampicin in most isolates of *M.tuberculosis,* these are much less important in case of NTM. Other mutations seen in drug-resistant isolates of *M.tuberculosis* are not frequently found in drug resistant NTM[151-155]. In general, mechanisms like alterations in the permeability at cell wall[140] and efflux pumps appear to be far more important than mutations in the targets[151-153]. It would be appropriate to conclude that these mechanisms are by and large not well understood in NTM. More in depth studies for gaining better understanding of these mechanisms are required which may help in developing better diagnostics and therapeutics for the treatment of NTM infections.

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