NON – TUBERCULOUS MYCOBACTERIA–AN OVERVIEW

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(**Received original, December 1985; revised version for publication. January 1986)**

There have been sporadic reports of non-tuberculous mycobacteria (NTM) causing disease in the early part of this century. isolation of NTM from patients with pulmonary disease became more common after sputum culture for the diagnosis of pulmonary tuberculosis had become routine by the 1950s[1]. Since then important strides have been made in the taxonomy and identification of non-tuberculous mycobacteria, as well as clarifying their role as human pathogens and importance in human pulmonary disease. It is now well established that mycobacteria other than mammalian tubercle bacilli and *M.leprae.* are important human pathogens.[2,3]

Various names have been suggested for this group of organisms such as para-tubercle bacilli, pseudo-tubercle bacilli, unclassified mycobacteria, anonymous mycobacteria, atypical mycobacteria, opportunistic mycobacteria, tuberculoid mycobacteria and mycobacteria other than tubercle bacilli (MOTT). The name non - tuberculous mycobacteria (NTM), now used by the International Working Group on Mycobacterial Taxonomy (IWGMT) is gradually gaining acceptance among mycobacteriologists.

NTM as human pathogens

Most of the information available now on NTM infections are from Western Australia, Japan, United States, England and Wales. NTM which have been encountered in local lymphadenitis in children are *M.scrofulaceum* and *M.avium complex* followed by *M.kansasii* and *M.fortuitum complex.* Skin and soft tissue infections like swimming pool granuloma sporotrichoid, local abscess and Buruli (Bairnsdale) ulcer are known to be caused by *M.mainum, M.fortuitum complex* and *M.ulcerans. M.kansasaii* and *M.avium complex* followed by *M.fortuitum complex* and *M.marinum* are NTM which are involved in skeletal tissue infection whereas *M.avium complex* and *M.kansasii* followed by *M.fortuitum complex* and *M.scrofulaceum* have been reported in disseminated infection. NTM which have been isolated from adults with chronic pulmonary disease are *M.avium* complex and *M.kansasii* followed by *M.xenopi, M.szulgai, M.simiae, M.scrofulaceum* and *M.fortuitum* (Table 1).

TABLE-I

SUMMARY OF NTM DISEASES OF MAN'

Disease	Common species	Others
Chronic pulmonary diseases in adults	*M.avium* complex *M. kansasii*	*M. xenopi* *M. szulgai* *M. simiae* *M. scrofulaceum* *M. fortuitum*
Local lymphadenitis in children	*M. scrofulaceum* *M. avium complex*	*M. kansaii* *M. fortuitum complex*
Skin and soft tissue:		
Swimming pool granuloma	*M. marinum*	
Sprotrichoid	*M. marinum*	
Local abscess	*M. fortuitum complex.*	
Buruli (Bairnsdale) ulcer	*M. ulcerans*	
Skeletal (bone, joint, tendon)	*M. kansasii* *M. avium complex*	*M. fortuitum complex* *M. marinum*
Disseminated	*M. avium complex* *M. kansasii*	*M. fortuitum complex* *M. scrofulaceum*

* Adopted from Emanuel Wolinsky (1979)[1]

NTM associated with human pulmonary disease
Mycobacterium avium complex
(M. avium intracellulare)

M. avium and *M. intracellulare* strains resemble each other so closely that the distinction cannot be made by common laboratory examinations. Seroagglutination affords the most useful method of separation in the laboratory. Anz and associates (quoted by Wolirsky) studied the relationship between virulence and serotype among *M.avium* complex strains. They found that types 1, 2 and 3 usually were fully virulent, resembling the classic *M.avium* and the others showing variable degrees of virulence or no virulence were termed 'intracellulare group'. A co-operative study of the IWGMT found that strains could not be differentiated from each other by numerical analysis.

Mycobacterium kansasii

Strains are easily recognised by their photochromogenicity and the appearance of red crystals of ß-carotene on prolonged exposure to light. They must however be distinguished from other photochromogenie strains, namely, *M.marinum, M. simiae. M.szulgai* and *M.vaccae. M.kansasii* characteristically show large cross bars in acid fast smears of sputum. All strains appear to have a uniform antigenic structure and there is only one type by seroagglutination.

Mycobacterium xenopi

It is a scotochromogen producing a yellow pigment of variable intensity. The optimum temperature for growth is approximately 43°C. In smears the cells are long and slender, tapered at both ends and paliisaded. Beck and Stanford found[4] four species specific antigens and report that the strains were serologically homogenous.

Mycobacterium szulgai

The strains show strong catalase activity, positive nitrate reduction test, slow Tween hydrolysis, positive aryl sulfatase test and a distinctive lipid compound. It was noted by Wolinsky and reported by Schaefer and co-workers that many of the strains tested were scotqchromogenic at 37°C, but photochromogenie at 25°C. All strains have the same species specific antigen and are homogenous by seroagglutination.

Mycobacterium simiae

The species shows positive niacin test, weak photochromogenicity and strong catalase activity Strains belonging to serotype 1 are homogenous in their biochemical reactions whereas serotype 2 strains are heterogenous. Antigenic cross reactivity between strains of *M.simiae* and *M.avium complex* has been noted.

Mycobacterium scrofulaceum

The colonies are smooth and globoid and pigmentation varies from light yellow to deep orange which usually darkens on prolonged exposure to light. Because of variable pigmentation of both species and the similarity of biochemical reactions, surface antigens and drug resistance, it has been suggested that, *M.scrofulaceum* could be classified as a member of the *M.avium complex.* A study of the lipids led to the conclusion that organisms classified as *M.scrofulaceum* should be regarded as pigmented types of *M.intracellulare.*

Riesinkov and Dawson (as quoted by Wolinsky)[1] coined the name, MAIS complex, for *M.avium-intracellulare-scrofulaceum.* All but a few strains of *M.scrofulaceum* may be classified by seroagglutinarion into one of three types.

Mycobacterium fortuitum complex
(M. fortuitum chelonei)

These strains show rapid growth at temperatures ranging from 25°C to 40°C. The colonies may be smooth or rough, or mixtures of both varieties, and they are usually not pigmented. The aryl sulfatase test is strongly positive. The nitrate reduction test is positive in strains of *M.fortuitum* and negative in cultures of *M.chelonei.* Strains of *M.chelonei* were homogenous, while two types of *M.fortuitum* could be recognised by seroagglutination.

Source of NTM

Numerous studies have shown that the NTM may be found everywhere in the environment: water, soil and dust, milk and animals. Most common are the rapidly growing strains, including *M.fortuitum,* and the tap water bacillus, now known as *M.gordonae. M.kansasii, M.xenopi* and strains of *M.avium-intracellulare-scrofulaceum* complex have been isolated from water. The mycobacteria recovered from raw milk included rapidly growing strains, *M.gordonae,* and strains that resembled *M.avium intracellulare* and *M.scrofulaceum.* Strains of MAIS complex have also been isolated from soil and dust. Strains of *M.avium* and *M.scrofulaceum* have been shown to remain viable in the soil for a period of two to four years. In another study, 16 species were tested. Those

that were considered to have multiplied in the soil included *M.fortuitum* and *M.chelonei. M.szulgai* probably had multiplied while *M.simiae, M.xenopi* and two serotypes of *M.intracellulare* survived without apparent multiplication. Strains of the MAIS complex have also been recovered from pigs and cattle.

Transmission

Since the organisms are ubiquitously present in nature, the environment must be considered as the source of infection. Evidence for person to person transmission is wanting and there is no information so far pointing to a vector. Direct transmission from animal to human also does not seem to occur. The distinct geographic patterns of *M.kansasii* and of *M.avium intracellulare* mycobacterioses indicate environmental origins. There is some evidence for a mode of dispersal through water. They may also enter the body through the respiratory tract or from the stomach and bowel where they are present.

Pathogenicity

Experiments have shown that in general, healthy animals are quite resistant to infection by NTM and special procedures have to be adopted to produce lesions of any magnitude in the experimental animals. Because NTM are widespread and may colonise a person for a short or long period of time, a fundamental question arises as to how and when such organisms produce disease in humans. The possibilities are (1) their infectivity dose may be very large and small numbers may not be able to produce disease. Evidence for this is lacking; (2) long standing colonisation may eventually result in invasion. Evidence for this is very scanty; and (3) some alteration in the host defence mechanism, either locally or throughout the host immune system, may take place either on a temporary or permanent basis. There is some evidence for this last possibility.

Some predisposing conditions to invasion by NTM have also been investigated. Most of the reports emphasise that NTM pulmonary disease usually occurs in middle-aged men who have some chronic lung disease. Pre-existing pulmonary lesions are noted in most of the cases of *M.fortuitum chelonei. M.scrofulaceum, M. avium intracellulare* and *M.kansasii* disease. It must be emphasised, however, that cases do occur in women, in younger men, and in middle aged men without apparent lung disease or deficiency of cellular immunity.

NTM disease of the lung, like tuberculosis, seems to select the upper lobes, the main route of infection being hematogenous seeding of upper lobes from a primary site or lymph nodes, However, the evidence is scanty regarding inhaled and aspirated mycobacterial particles settling in the upper lobes. These mycobacteria apparently prefer the higher oxygen tension in the upper lobe alveoli, There exists some experimental evidence for the possibility that a failure or overloading of the clearing mechanisms may be basic to the development of pulmonary mycobacteriosis in humans. The other suggested alternative cause is the less efficient clearing mechanisms of the upper lobe.

Diagnosis of NTM pulmonary disease

In tuberculosis, recovery of *M.tuberculosis* from the patient is generally equivalent to diagnosis of disease as no true carrier stage exists. NTM, on the other hand, are environmental saprophytes and, in terms of their total numbers, only a very minute proportion is ever involved in disease process. Hence, criteria for diagnosis of pulmonary disease due to NTM are rather more strict than for tuberculosis. The American Thoracic Society has given the following criteria for diagnosis of disease due to NTM:

1. "Evidence such as infiltrate, visible on chest skiagram of disease, the cause of which has not been determined by careful clinical and laboratory studies; and

2. Either (a) isolation of the same strain of mycobacteria repeatedly, usually in the absence of other pathogens, or (b) isolation of the same strain of mycobacterium from a closed lesion from which the specimen has been collected and handled under sterile conditions, for example, an abscess or biopsy tissue. Occassional isolation of these organisms from sputum, throat washings and gastric aspirates in the absence of related disease may occur and are not considered significant diagnostically".

Pulmonary disease due to NTM in other countries

In the USA, a 1979 surveys of 49 public health laboratories showed that *M.tuberculosis* is the most commonly isolated mycobacterial species of clinical significance followed by *M.avium complex* and *M. fortuitum complex. M.kansasii* and *M.scrofulaceum* accounted for three percent and two percent of the isolates respectively. Among saprophytic species *M.gordonae* was isolated most often. In Japan, although the incidence of lung disease due to *M.tuberculosis* is steadily decreasing, the

incidence of lung disease due to NTM has remained almost at the same level over the years. The non-tuberculous mycobacteria that cause disease most frequently belong to the *M.avium-intracellulare complex.* In a recent report by Tsukamura, et al[6] in 537 cases of disease due to non-tuberculous mycobacteria, 89.6% was due to *M.avium-intracellulare* followed by *M.kansasii* (8%) and *M.fortuitum (1.3%).* He estimated that the incidence of lung disaese due to non-tuberculous mycobacteria in Japan to be 0.9 to 1.9 per 10 population per year in 1971-1979. More or less similar distribution of pathogenic strains were isolated in Western Australia and in many centres in Europe including England.

TABLE–2

PULMONARY DISEASE DUE TO NTM SUMMARY OF REPORTS FROM WORKERS IN INDIA*

References	Region	No. of cultures tested	NTM Isolates	Major type
Mahapatra (1961)	Lucknow	125	5	Non-chromogens
Thomas *et al (1961)*	Madras	287	–	–
Kaur Chitkara (1964)	Delhi	50	10	Non-chromogens
Bhathena *et al* (1964)	Delhi	28	1	Scotochromogens
Kulkarni Moller (1971)	Madanapalli	–	4	Battey type
Lal *et al* (1972)	Delhi	280	32	Non-chromogens
Saran (1973)	Patna	100	2	Photochromogens
Mukhopadyaya (1979)	Madanapalli	43929	5	*M. interacellulare*

* Adopted from Ramakrishnan (1961)[9]

Pulmonary disease due to NTM in India

From India, there are only very few documented case reports available so far. Between the years 1961-1979, eight such reports have been published by Indian workers. Three groups reported non-chromogens as the major type, while each of the rest reported non-chromogens, *M. intracellulare.* Battey type photochromogens and scotochromogens as the major type respectively (Table 2).

Study of NTM pulmonary disease from the Tuberculosis Research Centre (TRC), Madras

Pulmonary disease due to NTM spanning more than two decades upto 1979 has been reported from TRC, Madras[9]. Out of a total of 5,435 patients admitted to 12 successive chemotherapy studies 25 (0.45%) repeatedly excreted organisms that were not typical human tubercle bacilli; 14 excreted NTM from the start indicating primary infection with absence of bacteriological evidence of isolation of *M.tuberculosis* and 11 had lesions colonised with NTM following a period of quiescent pulmonary tuberculosis. Among patients with probable primary disease due to NTM, 10 out of the 14 yielded photochromogens and four were non-chromogens, and in patients with probable colonisation of quiescent lesions, three yielded Photochromogens, one scotochromogen and seven non-chromogens (Table 3).

TABLE 3

PULMONARY DISEASE DUE TO NTM

(TRC RETROSPECTIVE STUDY)

	Early excretors		Late excretors		
Age	Males	Females	Males	Females	Total
> 30	1	1	2	3	7
31-39	6	1	0	2	9
< 40	5	0	2	2	9
Photochromogens	10		3		13
Scotochromogens	–		1		1
Non-chromogens	4		7		11
Total	14		11		25

Failure to respond to treatment with standard drugs occurred in nearly 50% of patients as evidenced by absence of radiographic improvement and persistent sputum positivity. There were 10 deaths among the 25 patients. There is some evidence to show that rifampicin containing regimens could be beneficial from the therapeutic point of view in the treatment of some of the cases with disease due to NTM (Table 4).

TABLE 4

NTM PULMONARY DISEASE-RESPONSE TO TREATMENT

(TRC RETROSPECTIVE STUDY)

Response	Early excretors	Late excretors	Both groups
Good	4	3	7
Poor	8	3	11
Not assessable	2	5	7
Total	14	11	25

A later study conducted in 1981 by Paramasivan et *al*[7] has shown that non-tuberculous mycobacteria (NTM) were obtained from 8.6% of 16,907 sputum specimens in a trial in the Chingleput district of Tamil Nadu to test the efficacy of BCG vaccination in the prevention of tuberculosis However, only 0.6% of 672 autoclaved specimens cultured as part of a quality control procedure yielded positive cultures of NTM. This finding substantiates that the NTM were truly derived from the sputum of the BCG trial subjects. The mycobacterial species could be identified in 966 (96.6%) of the first 1000 isolates of NTM; 54.6% were potential pathogens and 73.0% were slow growing. The species isolated most frequently were *M.avium intracellulare (22.6%* of all NTM). *M.terrae* (12.5%) and *M.scrofu/aceum (10.5%).*

Treatment of NTM pulmariary disease

Treatment depends on the site and extent of disease due to NTM and the nature of infecting organisms. *M. kansasii* is generally susceptible to most of the anti-tuberculosis drugs and sputum conversion occurs in 4-6 months in 96% of patients, comparable to that of patients with *M. tuberculosis.* However, the picture is different with infection caused by the *M. avium intracellulare* group. A high proportion of organisms are resistant *in vitro* to isoniazid, PAS, rifampicin, ethambutol and streptomycin. Often, however, the resistance is not total. Susceptibility to ethambutol and cycloserine is reported frequently. Lester, a pioneer in this field suggests 5-drug combination with capreomycin. Other drugs that may occasionally have a place in combination are kanamycin, erythromycin and pyrazinamide. Since medical treatment is so unsatisfactory, surgical intervention may have to be considered in patients who fail to respond to chemotherapy, within a period of six months.

In 30% of patients with acquired immune deficiency syndrome (AIDS) *M. avium intrecellulare* has heen isolated and followed at National Institute of Health (NIH), USA, and in these patients therapy consisting of conventional anti-tuberculous drugs has been almost invariably ineffective. Over 90% of the clinical isolates have been resistant to isoniazid, rifampicin and streptomycin. While ansamycin and clofazimine each show excellent *in vitro* activity, a two-drug regimen using ansamycin 150 mg and clofazimine 100 mg per day, respectively, has not been clinically effective. Some success has been reported for multiple drug regimens that include third generation cephalosporine or amikacins.

Conclusion

Assuming that most of the NTM originate from sputum specimens, are they chance contaminants from the environment, do they colonise the respiratory tract and are they responsible for clinical disease? If NTM, isolated from 4.5% of the sputum specimens of patients examined in Madras City and Bangalore was often responsible for clinical disease, this would have been noted in the reports of the chemotherapy studies. However, as reported by Ramakrishnan[9] only 21 (0.4%) of a total of 4943 patients in 12 chemotherapy studies between 1958 and 1978 yielded NTM on repeated occasions and of these only 12 yielded NTM without *M. tuberculosis* on admission to the study suggesting that disease due to NTM is rare. By contrast, NTM isolated in technically advanced countries, where the prevalence of tuberculosis is low, are more often the cause of disease. NTM isolates from Indian patients, in general appear to be either occuring as contaminants or organisms that colonise the respiratory tract over a period without pathological significance. The distinction between these two possibilities can only be made by further studies on the isolation in pure culture of a particular species of NTM from serial sputum samples from the same patient.

TABLE 5

ISOLATION OF NTM FROM SAMPLES OBTAINED IN DIFFERENT STUDIES DURING 1981

Study Area	Total number of specimens processed	Specimens with growth of NTM on one or both slopes	
		No.	%
Trivellore	16907	1457	8.6
Tambaram	3576	270	7.6
Madras City	24121	1095	4.5
Bangalore City	12909	587	4.5
Hona Kona	12830	334	2.6

TABLE-6

SPECIES LEVEL IDENTIFICATION OF 1000 NTM STRAINS FROM THE BCG TRIAL AREA

Growth rate	Potential pathogens Species	Isolates No.	%	Non-pathogens Species	Isolates No.	%
slow	*M. avium/intracellulare*	226	42.9	*M. terrae* complex	125	28.5
	M. scrofulaceum	105	19.9	*M. flavescens*	67	15.3
	M. asiaticum	15	2.8	*M. gordonae*	66	15.0
	M. marinum	10	1.9	*M. triviale*	33	7.5
	M. malmoense	9	1.7	*M. gastri*	18	4.1
	M. kanasasii	7	1.3	*M. nonchromogenicum*	0	0.0
	M. szulgae		1.3			
	M. heemophilum	6	1.1			
	M. xenopi	5	0.9			
	M. ulcerans	5	0.9			
	M. simiae	1	0.2			
	Total	396	75.1	**Total**	309	70.4
rapid	*M. fotuitum*	76	14.4	*M. vaccae*	54	12.3
	M. chelonei	55	10.4	*M. phlei*	34	7.7
				M. smegmatis	19	4.3
				M. toakienses $	11	.5
				M. aurum	5	1.1
				M. thermoresistable	2	0.5
				M. aichiense $	2	0.5
				M. parafortuitum	1	0.2
				M. neoaurum	1	0.2
				M. fortuitum/ (thermophium)	1	0.2
				Other species†	0	0.0
	Total	131	24.9	**Total**	130	29.6
	Total pathogens	527	100.0	Total non-pathogens	439	100.0
		Not identified	34			

$ classified according to Tsukamura, but their taxonomic position is unsettled.

† *M. chitae. M. gadium. M. gilvum. M duvalii*

REFERENCES

1. Emanuel Wolinsky (1979). Non-tuberculous Mycobacteria and associated diseases. Ame. Rev. Respir. Dis. *119:* 107-159.
2. Jenkins, P.A. (1979). Pulmonary infections with opportunistic mycobacteria– "Mycobacterial infections". Selected abstracts from a scientific meeting of the British Society for the Study of Infection Ed. by Steen, J.S.M. pp 17-23.
3. John S. Chapman (1982). The atypical mycobacteria. Ame. Rev. Respir. Dis. *125:* 119-124.
4. Beck and Stanford (1968) Mycolacheicum Xeopei : A sturdy of sixteen strains. Tubercle, *49,* 226-234.
5. Good R.C. and Snider, D.E. (1982): isolation of non-Tuberculous mycobacteria in the United States. 1980. J. Infect. Dis. *146,* 829-833
6. Tsukamura. M.. Shimotde. H., Kita. N et al (1981) : Epidemiologic studies of lung disease due to mycobacteria other than M. Tuberculosis in Japan. Rev. Infect Dis. *3,* 997-1007.
7. Paramasivan, C.N.. Govindan, D.. Prabhakar, R.. Somasundaram P.R., Subbammal, S. and Tripathy, S.P. (1985) Species level identification of Non-Tuberculous Mycobacteria from South Indian BCG Trial area during 1981. Tubercle. *66:* 9-15.
8. Henry Masur (1985). New concepts in the therapy of infections in the Acquired Immunodeficiency Syndrome. P 803. In: Fauci, A.S. Moderator. The Acquired Immunodeficiency Syndrome: an update. Ann. Intern. Med. *102:* 800-813.
9. Ramakrishnan, C.V. (1981). Pulmonary disease due to atypical mycobacteria: A retrospective study from South India. Rev. Infect. Dis. *3:* 1090-1092.

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