INFECTIONS CAUSED BY MYCOBACTERIA OTHER THAN TUBERCLE (MOTT) BACILLI

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The prevalence and severity of primary mycobacteriosis prompted the studies of Robert Koch and others on its etiology that culminated in the discovery of the tubercle bacillus in 1882. Despite sporadic reports of isolation of many varieties of non-tuberculous mycobacteria from clinical specimens, only *Mycobacterium tuberculosis* and *M. bovis* were taken seriously as a cause of human disease(1), while the other isolates were given dismissive epithets such as "atypical", "pseudotubercular" and "tuberculoid" bacilli. Also, as their classification was in chaos, they were dubbed "anonymous" mycobacteria. Interest in their role as pathogens of man commenced in earnest in the 1950s with description of two distinct diseases, namely, swimming pool granuloma and Buruli ulcer caused by *M. marinum* and *M. ulcerans,* respectively and tuberculosis-like pulmonary disease(2).

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Nomenclature

The "atypical" mycobacteria isolated from clinical material have been called by many names--paratubercle, pseudotubercle, anonymous, atypical, nontuberculous, opportunistic, tuberculoid and mycobacteria other than tubercle (MOTT) bacilli. The disease associated with them has been termed--pseudotuberculosis, mycobacteriosis, nontuberculous mycobacterial infection and tuberculosis due to one of the specific mycobacteria by species name. None of the proposed names is without criticism, but the most appropriate and least offensive, in our opinion, is MOTT bacilli. Certainly "atypical", "anonymous" and "unclassified" are no longer acceptable. "Opportunistic" is not entirely appropriate. It is also not right to call the disease "tuberculosis" because of the pronounced differences between the two infections in pathogenesis, epidemiology, prognosis and response to treatment(3).

Runyon(4) in 1959 provided early leadership in the classification of these mycobacteria and on the basis of type of colony, rate of growth, pigment production and simple biochemical reactions divided them into four groups. Subsequent workers have suggested different biochemical tests in order to improve the identification of isolates but the basic format has remained unchanged. From the clinical point of view, however, these mycobacteria are divisible into three groups (*Table I*):

(*a*) The major pathogens,

(*b*) Opportunistic pathogens, and

(*c*) Saprophytic species.

At present, 54 species of mycobacteria are recognised. In the practice of medicine,

TABLE I--*Classification of Mycobacteria, Bartmann, 1988*(5)

Group	Runyon Group	Strictly or potentially pathogenic	Rarely or never pathogenic
Slow growing or "non culturable"		*M. tuberculosis* *M. bovis* *M. africanum* *M. ulcerans* *M. leprae*	
Photochromogens	I	*M. kansasii* *M. marinum* *M. simiae* *M. asiaticum*	
Scotochromogen	II	*M. scrofulaceum* *M. szulgai* *M. xenopi*	*M. gordonae* *M. flavescens*
Non photochromogens	III	*M.A.I.* *M. malmoense* *M. hemophilum*	*M. terrae* *M. triviale*
Rapid growing	IV	*M. fortuitum* *M. chelonei*	*M. vaccae* *M. smegmatis* *M. phlei*
Pathogenic in animals		*M. lepremurium* *M. microti* *M. paratuberculosis* *M. farcinogenes* *M. senegalense*	

it may be most useful to refer initially to MOTT bacilli by major grouping as proposed by Runyon and, subsequently, by species when laboratory identification is complete. Because of slow growth of some isolates precise identification can require up to several months to complete.

Epidemiology

While *M. tuberculosis* is almost always transmitted directly from one individual to another, disease due to MOTT bacilli, is rarely, if ever, transmitted in this manner. Almost, all cases follow acquisition of the bacilli from their natural inanimate environment. The number of cases of MOTT infection in a given area depends on the number of potentially pathogenic strains in the environment, the opportunities that they have for contact with the human population and the susceptibility of the potential hosts. The relative frequency of infection is determined by the distribution of the various species of MOTT bacilli in the environment(2).

Isolation of MOTT Bacilli Around the World

In USA(6), *M. avium-intracellulare* (*MAI*) was isolated in 18% of samples followed by *M. fortuitum* (6%), *M. Kansasii* (3%) and *M. scrofulaceum* (2%), while the commonest mycobacterium isolated was *M. tuberculosis* (68%). Of the 5182 isolates from specimens in Buenos Aires in 1977-78; 99% were *M. tuberculosis* and only 62 cultures from 10 patients were MOTT bacilli. *M. kansasii* was isolated from 4 patients, *MAI* from 3, *M. fortuitum* from 2 and *M. marinum* from 1. In 1982-84 of the 13,544 mycobacterial cultures obtained from 7,672 patients, only 437 (3.22%) were MOTT bacilli(7), while in Sweden out of the 196 cultures of MOTT bacilli isolated by the various laboratories during 1974 and 1975, 7% were *MAI,* 7% were *M. xenopi* and 16% rapid growers *M. fortuitum* or *M. chelonei.*

In India, Shriniwas and Bhatia(8) studied the isolation of MOTT bacilli from sputum samples between 1964-70 and reported that there was a gradual increase in the isolation of these bacteria. The rate of isolation of MOTT bacilli amongst the total mycobacterial isolates increased from 2.68 to 13.64% during this period. Most (54.7%) of the isolates were identified as scotochromogens. Shriniwas(9) characterised 527 acid fast isolates obtained from 11,928 clinical samples submitted to our laboratory between 1969-71 and reported that 473 (81.7%) were identified as *M. tuberculosis* while the remaining 106 (18.3%) were MOTT bacilli. There were 22 isolates of *M. kansasii* 49 were identified as scotochromogens, 27 as *MAI* and 8 as rapid growers. Das *et al.*(10) obtained 168 mycobacterial isolates from various clinical samples. On species identification, 154 (99.7%) were identified as *M. tuberculosis* and only 14 (8.3%) were MOTT bacilli belonging to seven different species. They also reviewed the Indian literature regarding isolation of "atypical" mycobacteria. The isolation of MOTT bacilli from clinical samples in these studies ranged from 0.7% in Madras to 9% in Rajasthan. Nain *et al.* (11) and Chakrabarti *et al.*(12) have also reported similar findings. The drawback with most of these studies is that they are based on laboratory isolation of MOTT bacilli. Shanker *et al.*(13) tried to remedy this by following a strict criteria for identification of pulmonary infection with MOTT bacilli and reported that out of the 3943 patients undergoing treatment at a tuberculosis clinic only two patients could be considered as suffering from MOTT bacilli disease while in 45 patients MOTT bacilli appeared to colonise the lungs without producing any disease. They concluded that MOTT bacilli did not appear to cause much concern in India.

Kotian *et al.*(14) brought forth data to indicate that patients susceptible to mycobacteriosis in a high tuberculosis incidence area will develop tuberculosis rather than one of the other mycobacteriosis. However, mycobacteriosis following successfully treated tuberculosis is more likely to be due to MOTT bacilli even in high incidence areas of tuberculosis.

MOTT bacilli occur in soil and water, and the human population is constantly exposed to them through ingestion, inhaling aerosols and inoculation of traumatised skin (*Table II*). MOTT bacilli could be isolated from about 25% of treated and natural water specimens in USA(15). MOTT bacilli were isolated from 76% of soil, 67% of sewage, 43% of well water and 7% of house dust specimens in Korea. Nine MOTT bacilli species were also isolated from samples of raw milk.

TABLE II-- *Reservoirs of Mycobacteria and their Relationship to Human Disease*

Mycobacterium	Human pathogen	Reservoir
M. tuberculosis	Yes	Humans
M. bovis	„	Humans, cattle
M. kansasii	„	Water, cattle
M. marinum	„	Fish, water
M. simiae	„	Primates
M. scrofulaceum	„	Soil, water
M.A.I.	„	Soil, water
M. gordonae	No	Water
M. gastri	„	Soil, water
M. smegmatis	„	Urogenital flora

The mycobacteria are very hydrophobic on account of their thick, waxy cell walls yet, paradoxically, their natural habitats are waterly ones–mud, marshes, ponds, rivers and estuaries. They prefer a slightly acidic environment. In view of their water-repellent coats, they are often found on air-water interfaces; indeed they have been termed the "ducks of the microbial world". This situation enables them to derive oxygen from the atmosphere and nutrients from the water–such nutrients probably arising from decomposing vegetation(16).

It is now clear, contrary to earlier views, that the genus *Mycobacterium* is essentially one of environmental saprophytes and that pathogenicity is not their usual behaviour. Thus, the major pathogens *M. tuberculosis* and *M. leprae* are in fact "atypical" mycobacteria although, paradoxically, this term is usually applied to the typical saprophytic species(17). Despite their widespread distribution in nature, many of the potentially pathogenic MOTT bacilli appear to be relatively more common in certain geographic locations.

Clinical Diseases

Disease due to MOTT bacilli is indistinguishable clinically, radiologically and histopathologically from tuberculosis. There are four main types of diseases that MOTT bacilli are associated with (*Table III*):

(*i*) Pulmonary
(*ii*) Localised lymph node infection
(*iii*) Localised soft tissue infection
(*iv*) Disseminated disease

As in the case of tuberculosis, the lung is the organ most frequently involved in MOTT infection. In many cases there is a predisposing factor such as residual cavities from past tuberculosis, dust associated disease, chronic bronchitis, cystic fibrosis, cancer of lung, HIV infection and other immunosuppressive conditions and autoimmune diseases. Occasionally though, infections occur in the apparently healthy subjects. The forms of nonpulmonary disease are similar to those caused by *M. tuberculosis*. Thus single or multiple lesions may occur in the cervical lymph nodes, traumatised skin, bone, urinary tract, CNS and less frequently, virtually any other system or organ(2,18,19).

Laboratory Diagnosis

MOTT bacilli abound in the environment and colonize water pipes and taps(20), they even occur in taps of distilled water supplies and in deionizer resins(21). Great care must therefore be taken in the collection of specimens and in their bacteriological examination. The diagnosis of MOTT infection is made bacteriologically; only culture procedures can unequivocally determine the species responsible. Once a culture is obtained, a decision on its clinical relevance must be made. Isolation of a

TABLE III--*Clinical Syndromes Associated with MOTT Bacilli*

Syndrome	Relatively common cause	Less frequent cause
Cervical or other lymphadenopathy (especially in children)	*M. scrofulaceum*	*M.A.I.* *M. kansasii* *M. fortuitum* *M. chelonei*
Chronic bronocho-pulmonary disease (adults)	*M. kansasii* *M.A.I.*	*M. szulgai* *M. simiae* *M. xenopi* *M. scrofulaceum* *M. chelonei*
Disseminated disease	*M.A.I*	*M. kansasii* *M. fortuitum* *M. chelonei* *M. scrofulaceum*

MOTT bacillus from a biopsy, together with histological evidence of granulomatous disease, usually poses no interpretational problem. Most specimens, however, are sputum and although an isolate may indicate disease it may also indicate transient contamination of the mouth or pharynx or a benign colonisation of damaged portions of the respiratory tract. Thus, unless invasive techniques are used, diagnosis usually rests on repeated isolation over several weeks, a high clinical suspicion of active disease and the thorough exclusion of other causes of the signs and symptoms. Even greater care must be taken in bacteriological examination of the urine as the urethra and external genitalia are frequently contaminated with acid fast bacilli(2).

Skin Test

A variety of skin test reagents have been prepared from various species of MOTT. These include purified proteins derivatives like:

(*i*) PPD-A from *M. avium*
(*ii*) PPD-B from *M. intracellulare*
(*iii*) PPD-F from *M. fortuitum*
(*iv*) PPD-G from *M. scrofulaceum*
(*v*) PPD-Y from *M. kansasii*

Cross reactivity among these reagents due to shared antigens have limited their use primarily to epidemiologic studies. However, Vandiviere *et al.*(22) have demonstrated a correlation of skin test profiles of PPD-S (*M. tuberculosis*) and five MOTT bacilli with the etiology of pulmonary disease determined subsequently by culture. They also found this method to be useful in selecting therapy for culture negative patients. The use of multiple simultaneous antigen skin test would, therefore, seem to hold promise as a method of rapid diagnosis in the future(23).

American Thoracic Society(24) recommended that to make a definite diagnosis of MOTT infection, the criteria are: (*i*) Evidence, such as infiltrate visible on a chest roentgenogram of disease, the cause of which has not been determined by care-

ful clinical and labortory studies, and (*ii*) Either isolation of multiple colonies of the same MOTT bacillus repeatedly, usually in the absence of other pathogens, or isolation of MOTT bacillus from a closed lesion from which the specimen has been collected and handled under sterile conditions.

Salient Features of Some Important MOTT Bacilli

M. Kansasii

This bacillus produces a yellow pigment when exposed to light and therefore is classified as a photochromogenic acid fast bacillus. The bacillus is relatively long and thick, and often appears cross barred. Infection with *M. kansasii* may confer immunity to subsequent challenge with other MOTT bacilli and cross reaction with skin test reagents prepared from other species are quite common. It characteristically produces a chronic lung infection that closely resembles pulmonary tuberculosis, though the symptoms tend to be milder and may be totally overshadowed by symptoms of underlying chronic obstructive pulmonary disease. The infection is frequently progressive in nature. It can infect extrapulmonary tissues directly, often as a result of inoculation or after hematogenous dissemination. Cervical and other local lymphadenitis have been reported occasionally(23). Its strains are among the most predictably sensitive of all the MOTT bacilli. There is a good *in-vivo* correlation to *in-vitro* susceptibility reports. The bacilli are sensitive to rifampicin (RMP), ethambutol (EMB), ethionamide (ETH) and cycloserine (CS) but resistant to para amino salicylic acid (PAS) and pyrazinamide (PZA). *M. kansasii* must be classified as mainly resistant against isoniazid (INH) and streptomycin (SM), however, many strains show only a partial resistance to both the drugs.

M. kansasii infections should be treated with triple drug combinations including RMP, one of the aminoglycosides and either EMB or ETH. Sputum conversion can be obtained in these infections with practically the same speed as in *M. tuberculosis* infections (5-6 weeks). It has been recommended that treatment should continue for 18 to 24 months(5).

Scotochromogens

This group comprises of slowly growing mycobacteria that produce pigments when growing in the dark. *M. scrofulaceum* is an important pathogenic member of this group. It is longer, thicker and more coarsely beaded than *M. tuberculosis*. But the vast majority of scotochromogenic mycobacteria are nonpathogenic or very weakly virulent. They are ubiquitous and frequently contaminate specimens.

M. scrofulaceum

It is a relatively frequent cause of lymphadenitis and an occasional cause of disease in other tissues. Lymphadenitis due to this organism occurs most commonly in children of 1 to 3 years of age. Secondary cases in household are rare. Characteristically, the nodes enlarge slowly over a period of weeks. There are very few local systemic symptoms. Slight pain may be elicited upon manipulation. Untreated, the infection will point to the surface, rupture, form a draining sinus and eventually calcify. Normal chest *X*-ray findings and unilateral location may be some indicators to suggest infection due to MOTT bacilli rather than *M. tuberculosis*(23).

Excision of involved nodes and overlying sinus tract, if present, is almost always curative. Some advocate the use of chemotherapy, but this is seldom necessary with prompt diagnosis and surgery. However, strains of this group are resistant to INH, PAS and PZA. More strains are resistant than sensitive to SM, EMB, thiosemicarbazone (TSC) and viomycin (VM). About 50% are sensitive to RMP. The strains are sensitive to ETH, CS, and kanamycin (KM)(5).

M. avium-intracellulare

Besides superficial lymphadenitis and rare disseminated disease which occurs mainly in immunocompromised patients these organisms can cause chronic, indolent, often cavitary disease, especially in persons with a reduced local or systemic resistance. If untreated, the disease usually deteriorates. Infections with *MAI* are difficult to treat as most of the isolates are resistant to all antitubercular drugs than *M. kansasii*. The isolates are however, sensitive to ansamycin, a spiro-piperidyl derivative of rifampicin S(5).

M. ulcerans (M. buruli)

Deeply undermined skin ulcers with extensive necrosis produced by mycobacteria were first observed in the 1940s in Australia and in tropical Africa. The causative agent has optimum growth at about 33°C. In its natural course the disease runs for several years and ends in healing with defects that are often severe. The isolates are uniformly sensitive to RMP. Most strains are sensitive to SM, VM, KM and CS, and resistant to INH, PAS, TSC, ETH, EMB and PZA. They are also sensitive to clofazimine and to co-trimoxazole.

Up to now chemotherapy alone has been unsuccessful. At present excision on the nodules in the preulcerative stage or extensive surgery and skin grafting in the ulcerative stage are the methods of choice(5).

M. fortuitum Group

They resemble diphtheroids on Gram staining. On primary isolation, they may require 2 to 30 days for growth. Thereafter, they usually grow in 1-3 days. They flourish on most routine laboratory media as well as those designed for isolation of mycobacteria. They may be recovered readily from soil, dust and water. They have been isolated from tap water, chlorinated water supply and moist areas of the hospitals. Human infections are acquired by inoculation after accidental trauma, surgery or injection(23). The typical disease produced by the complex is soft tissue infection after a penetrating trauma, but other infections, including pulmonary and systemic ones, have been observed(25).

The isolates are regularly resistant to INH, SM, PAS, EMB RMP, CS, TSC and PZA. Many strains are sensitive to ETH, while a minority to KM and VM. Ansamycin is ineffective as also clofazimine but some strains have been shown to be sensitive to the combination of clavulanic acid and amoxicillin, tetracyclines and the newer quinolones. But treatment with the antitubercular regimes are ineffective and the effects of even combinations of 4 to 6 drugs have not been impressive. Surgical intervention is necessary in most cases of soft tissue infections. Especially difficult to treat are disseminated infections and the response to therapy may vary with immune status of the patient. Treatment with amikacin (15 mg/kg/day) plus cefoxitin

(200 mg/kg/day up to 12 g/day) with oral probenecid seems to be the most promising regimen at present(5).

Banks and Jenkins(26) performed *in-vitro* drug sensitivity tests–both alone and in paired combinations–on a few strains of MOTT bacilli and reported that while most of the strains were resistant to INH, SM, EMB and RMP when tested alone, growth of the same strains was inhibited when a combination of drugs was used. They postulated that similar effects *in-vivo* might be responsible for the difference observed between the *in-vitro* sensitivity and clinical responses of the patients.

MOTT Bacilli and AIDS

Organisms that belong to the *MAI* complex are the most common cause of systemic bacterial infections in patients with AIDS. The typical setting of *MAI* disease in the patient with AIDS is as a so called "late" opportunistic infection. Usually, the patient has had one or more episodes of *Pneumocystis carinii* pneumonia and often other opportunistic infections. Although the patient may respond to treatment, *MAI* presents in a patient whose clinical status is deteriorating. *MAI* can be cultured or stained from body fluids, stool, bone marrow or aspirates from upper gastrointestinal tract endoscopic procedures(27).

Tuberculosis was not mentioned as a manifestation of AIDS in early description of the disease from USA and Europe; the association with tuberculosis was first recognised in Haitians and intravenous drug abusers with HIV infection. In developing countries, tuberculosis is now recognised as one of the commonest opportunistic infections in patients seropositive for HIV(28). Tuberculosis is an "early" opportunistic infection in AIDS and is sometimes detected a few months before the patient is diagnosed as suffering from AIDS. It appears that the immunosuppression required for reactivation of dormant *M. tuberculosis* in patients is low. While persons not previously exposed to *M. tuberculosis* would pick up environmental mycobacteria only when the immunodepression is severe. *MAI* in AIDS is generally a subterminal infection, the patient surviving for only a few months after the diagnosis is established.

Exposure to MOTT Bacilli and Effectiveness of BCG

Stanford *et al.*(29) postulated that there were atleast two types of cell mediated immune (CMI) responses to mycobacteria (*i*) Koch phenomenon, an important part of which is the destruction of cells which contain mycobacteria or which have mycobacterial products on their surface, and (*ii*) Listeria type response which depends upon activated macrophages which are bactericidal and may provide a much better protective mechanism from infection.

They also suggested that bacilli differed in their capacity to induce the two patterns of responses. Some species can induce only the Listeria type of response while others can induce either pattern depending on their frequency in the environment and the particular mixture of species that are present. Among the MOTT bacilli that can induce only the Listeria type responses to newer tuberculins are– *M. nonchromogenicum, M. vaccae* and *M. leprae.* Species capable of inducing either type *of response* include *M. kansasii, M. scrofulaceum* and *M. tuberculosis.* The first type of response developed on meeting mycobacteria is likely to be the Listeria

type provided there is no over exposure to, or infection with, one the species that can induce the Koch type of response then the protective immunity will be enhanced by acquaintance with MOTT bacilli. But, if an individual has already developed the Koch type of response, or has suffered from *M. tuberculosis* infection, BCG vaccination will atleast temporarily reinforce this type of response and perhaps reduce the level of protective immunity already present. Rook *et al.*(30) working on this hypothesis then described a mouse model to validate the presence of two types of responses. Further evidence was provided by Brown *et al.*(31) but Smith *et al.*(32) using guinea pig model failed to demonstrate this effect.

Multiple skin testing surveys of school children (5 to 15 years) living in Agra showed very high levels of sensitisation to numerous MOTT bacilli and there was little difference in tuberculin positivity between those with or without BCG scars(33). While a similar survey in Ahmednagar showed that the rate of sensitisation was much lower and that the BCG vaccination scars were associated with considerable enhancement in sensitisation to tuberculin and other reagents(34). A similar survey in three cities of Vietnam indicated low levels of sensitisation in Hanoi and Ho Chi Minh city than in Nha Trang and indicated that a high effectiveness of BCG vaccination against tuberculosis correlated with a low prevalence of MOTT bacilli sensitisation(35). In the Indian situation, it has been proposed that in places like Ahmednagar, BCG could provide a considerable level of protection and a suitable age for BCG vaccination could be when the child first goes to school, while in situations like Agra, BCG may have to be given within the first two years of life for it to have any beneficial role. Kotila *et al.*(36) reported that the practice of giving BCG vaccination during the neonatal period prevented MOTT lymphadenitis in Sweden. Kathipari *et al.*(37) found that the neonates could be stimulated to produce CMI response to BCG and supported the practice of giving BCG at birth. Houston *et al.*(38) found that BCG protected 57% of children against infection, but the protection was lesser in children vaccinated before the age of six months.

MOTT Bacilli Vaccination against Leprosy

It was noted in Brazil more than 50 years ago that BCG vaccination induced a form of delayed type of hypersensitivity (Mitsuda positivity) to antigens of the leprosy bacillus. Since this kind of hypersensitivity is associated with the milder and self healing "tuberculoid" form of leprosy, interest arose in the possibility that BCG vaccine might protect against leprosy as well as against tuberculosis. Large trials have been undertaken in Venezuela and Malawi to evaluate the efficacy of combined live BCG and killed *M. leprae* vaccines for protection against leprosy. Field trials are also underway to explore the efficacy of killed MOTT bacilli for protection against leprosy(39). In India the two species tried have been the ICRC bacilli by Deo and coworkers and *Mycobacterium-W* by Talwar and colleagues

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