Mycobacterial Skin Infections

Domenico Bonamonte
Gianni Angelini
Editors

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Editors
Domenico Bonamonte
University of Bari "Aldo Moro"
Department of Biomedical Science and Human Oncology
Bari
Italy

Gianni Angelini
University of Bari "Aldo Moro"
Dermatology
Bari
Italy

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To Davide
Elizabeth
Gianmarco

Preface

The overview of mycobacterial skin infections presented in this work is justified by the current worldwide increase in diseases induced by both old and new mycobacteria. Until the late 1980s, such diseases had been notably declining, but after that period, the trend reversed, and an exponential increase began to be observed. There are various reasons for this rise not only in developing countries but even in urban areas in the most developed nations.

Being the cause of tuberculosis and leprosy, the *Mycobacterium* genus has probably caused mankind more suffering than all the other bacterial genera combined. Moreover, in addition to the obligate pathogens, namely, the *Mycobacterium tuberculosis* complex and *M. leprae*, this genus is continually being enriched by the addition of many other species. Although usually encountered as environmental saprophytes, in some conditions that depend on the host immune status, they can induce extremely severe, sometimes fatal, clinical pictures.

It is well known that tuberculosis (TB) is one of the oldest diseases ever to affect mankind, but nowadays, it is emerging once more as an enormous and growing global health problem.

According to the World Health Organization (WHO) (Global Tuberculosis Report 2016, World Health Organization), in 2015 there were an estimated 10.4 million new (incident) TB cases worldwide, of which 5.9 million (56%) were among men, 3.5 million (34%) among women, and 1.0 million (10%) among children. HIV-infected people accounted for 11% of all new TB cases. Six countries accounted for 60% of the new cases: in descending order, India, Indonesia, China, Nigeria, Pakistan, and South Africa.

The rate of decline in TB incidence remained worldwide at only 1.5% from 2014 to 2015. This need to accelerate to a 4–5% annual decline by 2020 to reach the first milestones of the End TB Strategy. The WHO End TB Strategy (approved by the World Health Assembly in 2014), in fact, calls for a 90% reduction in TB deaths and an 80% reduction in the TB incidence rate by 2030, compared with 2015.

According to many authors, the high present TB incidence is attributable to increased levels of poverty in our overpopulated world, to the breakdown of health-care systems and to the impact of the HIV/AIDS pandemic. Further complicating the situation, there has been a worrying rise in multidrug-resistant TB (MDR-TB) (defined as resistance to isoniazid and rifampicin), and now we also face the recent emergence of extensively drug-resistant TB (defined as resistance to many other

drugs). In 2015, there were an estimated 580,000 new cases of MDR-TB. India, China, and the Russian Federation accounted for 45% of the combined total of 580,000 cases. The WHO estimated that in 2015 there has been 1.4 million TB deaths and an additional 0.4 million deaths resulting from TB infection among HIV-infected people. Although the number of TB deaths fell by 22% between 2000 and 2015, TB remained one of the top 10 causes of death worldwide in 2015.

Extrapulmonary TB manifests only in 8.4–13.7% of cases, while cutaneous TB comprises only a small proportion (<1–2%) of all cases of TB. All the same, in view of the current high prevalence of TB worldwide, these numbers become significant. Assuming that 1% of all cases of TB will have cutaneous involvement, dermatologists in countries such as India, where 1,847,000 new cases of TB were reported in 1999, must expect to see an annual incidence of about 18,000 cases of cutaneous TB. In any event, factors such as HIV infection and migration are ensuring that cutaneous TB will necessarily have to be included in differential diagnosis made by dermatologists all over the world.

Apart from *M. tuberculosis*, TB in man is induced also by *M. bovis*, which has a greater number of potential hosts than the tubercle bacillus. Various studies have shown that 0.3–1.5% of TB in humans is due to *M. bovis* in developed countries. Due to inappropriate methods for identifying the pathogen, in developing countries the incidence is unknown, but is most likely higher than in industrialized countries. In 1992, the European Union declared infection by *M. bovis* one of the four most important zoonoses (European Union. Council Directive 29/117/EEC, 1992. Off J Eur Comm 1993; L62:38), and in 1995, the WHO labeled the infection a global emergency (Guidelines for speciation within the *Mycobacterium tuberculosis* complex. Geneva: World Health Organization, 1995). Among the various modes of transmission of this organism to man (respiratory, gastrointestinal), we must include direct skin contact that occurs mainly in some occupational fields.

Meanwhile, the modes of transmission of *M. leprae* are still not fully understood. There is solid evidence of transmission among contacts, as well as of zoonotic leprosy in southern states in the USA. Current findings suggest that aerosols/droplets and shedding of bacteria in the environment, as well as skin-to-skin contact, are possible culprits. Globally, the number of leprosy cases is now below the elimination threshold of 1 case/10,000 people, as defined by the WHO. In various countries and subnational regions, however, the number of leprosy patients is still above this threshold. Moreover, despite the near-universal use and efficacy of multidrug therapy, the annual number of newly detected cases has remained fairly constant at around 200,000–300,000 cases in the last years. This demonstrates that the current control measures are insufficient to arrest the process of leprosy transmission.

The experience we gained over several years of assiduous attendance of the Hansenian Colony in Gioia del Colle (Bari, Italy), one of the largest in Europe, validated by clinical-therapeutic studies of the disease, has been of inestimable aid in writing the relative chapter about leprosy.

To compound the issue, nontuberculous mycobacteria (NTM) are common in nature, and, except perhaps in extreme environments such as deserts and polar regions, humans are regularly exposed to them. Unlike the obligate pathogens,

NTM may occur as transient commensals on the skin and in various other organs (the pharynx, lower urethra, gastrointestinal tract), so their isolation does not necessarily indicate the presence of disease. The pathogenicity of NTM is directly correlated to the host immune system, and in fact since the 1930s, many species have been strongly associated with some human diseases. The development of new diagnostic tools has resulted in a huge increase in the number of infections associated with specific species, as well as the number of new species identified as causal agents. NTM can cause a vast spectrum of diseases that are often quite difficult to diagnose. A high suspicion index is important, and the diagnostic conclusions must be based on a number of microbiological and radiological investigations. It must also be borne in mind that the search for bacilli in various clinical specimens may not always be positive. Even histopathology is not definitive because granulomatous tubercle findings are rare, whereas nonspecific inflammatory aspects are common.

The aim of this work is, therefore, to offer dermatologists a tool to help them recognize the different clinical mycobacterial manifestations, make a prompt diagnosis, and institute effective treatment. Unfortunately, older dermatologists tend to have become less familiar with these very important diseases in recent decades, while younger dermatologists have perhaps never encountered them before. By contrast, the current globalization process, and all its accompanying implications as regards infectious diseases that may be more or less widespread in the different countries, “demands” as wide as possible a knowledge of these among clinicians.

Bari, Italy
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Gianni Angelini, MD
Domenico Bonamonte, MD, PhD

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Gianni Angelini
Domenico Bonamonte

Contents

Contributors

Gianni Angelini, MD Professor of Dermatology, Former Professor and Chairman, University of Bari "Aldo Moro", Bari, Italy

Domenico Bonamonte, MD, PhD Associate Professor of Dermatology, Department of Biomedical Science and Human Oncology, University of Bari "Aldo Moro", Bari, Italy

Angela Filoni, MD University of Bari "Aldo Moro", Bari, Italy

Paolo Romita, MD University of Bary "Aldo Moro", Bari, Italy

Pietro Verni, MD University of Bari "Aldo Moro", Bari, Italy

Michelangelo Vestita, MD University of Bari "Aldo Moro", Bari, Italy

Mycobacteria

1

Domenico Bonamonte, Paolo Romita,
Pietro Verni, and Gianni Angelini

As compared to other Schizomycetes, mycobacteria (from the Greek muxes:mycetes and bacterion: rod) present some atypical characteristics that cause them to resemble mycetes. Nevertheless, there is no doubt as to their status as bacteria: they belong to the order of the *Actinomycetaceae*, and the family of *Mycobacteriaceae.* This family includes only the *Mycobacterium* genus, that accounts for more than 170 species, many of which are of no clinical interest [1, 2].

Despite a few peculiar differences observed in some species, the morphology of mycobacteria tends to be homogeneous. They are long, slender bacilli ranging from 1 to 5 μm in length and 0.2–0.6 μm in diameter. Coccobacillary forms, although rarely found in pathological samples, are common in colony-forming preparations. Mycobacteria have no locomotor organules and are strictly aerobic or microaerophylic, as well as being Gram-positive and pleomorphic. Their pleomorphism is due to the fact that they normally grow in filaments, sometimes branched (hence the name of this genus, to indicate their resemblance to mycetes, that being moulds, have a filament-like branched morphology), that fragment into bacilli or coccobacilli.

Mycobacteria are not mobile nor sporogenic, and except for a few species, show fairly slow reproductive rhythms. In fact, while mean duplication time is about 20 min in *Enterobacteriaceae*, in the case of mycobacteria it exceeds 15 h. Except

D. Bonamonte, MD, PhD (✉)
Department of Biomedical Science and Human Oncology,
University of Bary “Aldo Moro”, Bari, Italy
e-mail: domenico.bonamonte@uniba.it

P. Romita, MD • P. Verni, MD
University of Bary “Aldo Moro”, Bari, Italy

G. Angelini, MD
Professor of Dermatology, Former Professor and Chairman, University of Bary “Aldo Moro”,
Bari, Italy

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for *M. leprae*, all the species can be cultured, even if most of them require very complex culture media. The colonies show morphological differences among the species but generally feature a rough surface.

1.1 Habitat and Diffusion

Mycobacteria are widespread in the environment; apart from in man, they are present in all types of water (including domestic), in the soil, air, plants, foods and in various hot- and cold-blooded animals. Mycobacteria have the same degree of resistance to heat and ultraviolet rays as other bacteria, but are more resistant to drying and chemical disinfectants, probably due to their high lipid content.

1.2 Cellular Structure

Under the electronic microscope, mycobacteria are seen to have a very thick cell wall, inwards from which extend the first sprouts of the transverse septa, that will lead to cell division (mesosomes). The components of the cell wall include peptidoglycan and diaminopimelate, as well as polysaccharides (glucan, mannitol, arabinogalactan, arabino-mannitol). There is a wealth of cytoplasmic inclusions, especially lipids, glycogen and polymetaphosphates.

Most of the properties of mycobacteria are directly or indirectly owed to the structure of the cell wall. The lipid content, that is already very high overall in mycobacteria cells (25%, that is 10–15 times higher than that of other bacteria) reaches extremely high values at the level of the cell wall (more than 60% of the dry weight). Another important characteristic of mycobacteria, that is unusual among Schizomycetes, is the presence of some large compounds with associated sugars and glycoproteins, that therefore give rise to the formation of glycolipids and glycopeptidolipids called mycosides.

The basic constituents of mycosides are mycolic acids, that are often esterified in lipid molecules. These are beta-hydroxylated fatty acids, characterized by a long saturated chain (ramified in the alpha position) of carbon atoms (from 83 to 93). These mycolic acids are not present in other bacteria species. At the level of the external surface of the cell wall, mycolic acids have the function of phagic receptors. The proportion of different types of mycosides remains constant within each single species, and is therefore also a valid aid for taxonomic purposes.

Another very important class of lipids, that is also present in corynebacteria, is that of waxes, fatty acid esters (including mycolic acids) and alcohols. One mycoside that has undergone close study is dimycolil-trehalose, known as the cord factor because when tubercular mycobacteria lack this substance, they lose the ability to develop as tortuous bands on particular culture media. This characteristic is likely due to the virulence of these bacteria. In fact, the greater the cord factor content the more virulent the species. Moreover, this factor is lethal in mouse, inhibiting the migration of polymorphonuclear cells [3]. However, the virulence seems to be also

correlated to a sulfonate glycolipid that is present in high quantities in those species featuring this property.

Mycobacteria waxes are subdivided into four classes (A, B, C, D): the most important is wax D, that has an immunological adjuvant action, boosting the production of antibodies against other antigens that may be present (in particular of proteinaceous type).

1.3 Antigenic Structure

Various antigens have been identified in mycobacteria, many of which are common to several species. Some antigens crossreact with the Nocardia and Corynebacterium genuses, whose cell wall has some affinities with that of mycobacteria.

As stated above, the most abundant substances in the chemical composition of mycobacteria are lipids, that determine many of their peculiar properties. These are not important from the antigenic standpoint because they are non immunogenic, but it seems that they may act as haptens.

Polysaccharide antigens consist largely of arabino-galactan; from the immunological standpoint they are haptens, that can stimulate the production of antibodies only if inoculated together with the entire bacterial cell.

Proteic antigens are complex and quite difficult to purify. They are weakly immunogenic, although their activity is reinforced by class D waxes, that act as adjuvants. These antigens are responsible for delayed hypersensitivity reactions, as induced by the tuberculin test.

1.4 Sensitivity to Antibiotics

Mycobacteria behave rather differently toward antibiotics as compared to other microorganisms. For example, *M. tuberculosis* is resistant to all the antibiotics normally used to treat Gram-positive and Gram-negative infections, but it is sensitive to a small number of drugs that are therefore denominated antitubercular; sensitivity to these drugs is generally fairly constant, at least as regards those strains isolated from untreated subjects. By contrast, resistance may arise during treatment, related to various factors. It should also be borne in mind that in vitro susceptibility results are not always correlated with the same drug results in vivo.

Most of the clinical pictures induced by mycobacteria are treated with one or two active drugs. The association of drugs is necessary to reduce the likelihood of onset of resistant mutants. It is estimated, for example, that in a tubercle bacilli culture the frequency of isoniazide resistant mutants is 1 in 10^5, of streptomycin mutants 1 in 10^6, while the frequency of mutants resistant to both drugs is lower, being 1 in 10^{11} [2].

It is not necessary to test the drug sensitivity of most of the strains isolated if the patient has never previously taken antitubercular drugs. In any case, there is no need to wait for the results of susceptibility tests before starting treatment, that can be begun with drugs known to be more active against the strain isolated. Instead, it is

important to determine the sensitivity of the isolated strain when the patient has already undergone antitubercular treatment (owing to the common onset of resistance), or shows worsening during the treatment, or else when the culture does not become negative within 4–6 months from the start of treatment or if a nontubercular species is isolated.

1.5 Nontubercular Bacteria

The existence of mycobacteria other than *M. tuberculosis* complex and *M. leprae* has been known since the time of the discovery of the tubercle bacillus in the late 1800s. They are ubiquitous in the environment and have been variously denominated "atypical", "anonymous", "opportunistic", or "non classified". These terminologies have since been abandoned and these mycobacteria are now denominated "nontubercular mycobacteria" (NTM). This denomination can be considered to correspond to the acronym MOTT (Mycobacteria Other Than Tubercle bacilli) used by Anglo-Saxon writers. Some authors prefer to use the denomination "environmental mycobacteria" in view of their ubiquity in the environment [4, 5].

Today, more than 170 species have been identified (www.bacterio.net/mycobacterium.html), thanks to the advent of molecular techniques and 16s rRNA gene sequencing for defining new species [6–9]. There is no evidence of human-to-human or animal-to-human transmission of infections due to NTM. The source of infection in man is always the environment, even if the precise source is not always identified [10].

1.6 Classification

In 1959 Runyon proposed a classification of mycobacteria in four groups, based on the speed of growth and the pigmentation of the colonies (Table 1.1) [11]. It was later seen that there were several strains within each group, and also that the different strains could elicit different reaction patterns from those considered as typical. Certain species may therefore belong to more than one group; for example, *M. szulgai*, that features slow growth, is scotochromogenic if incubated at 37°C and photochromogenic when incubated at 22–25°C. Many species may be lightly pigmented and so misinterpreted, causing classification errors.

Table 1.1 Classification of nontubercular mycobacteria according to Runyon

Group I: photochromogens	*M. asiaticum, M. kansasii, M. marinum, M. simiae*
Group II: scotochromogens	*M. flavescens, M. gordonae, M. scrofulaceum, M. szulgai, M. xenopi*
Group III: non chromogens	*M. avium, M. intracellulare, M. haemophilum, M. malmoense, M. terrae, M. nonchromogenicum, M. triviale, M. gastri*
Group IV: rapidly growing mycobacteria	*M. chelonae, M. fortuitum, M. phlei, M. smegmatis, M. vaccae*

Moreover, *M. tuberculosis, M. africanum, M. bovis, M. leprae* and *M. ulcerans* are not included in the Runyon classification. It is now therefore largely of historical interest and has been replaced by more precise methods for determining the different species [12, 13].

In the Runyon classification the first group includes slow growth photochromogenic species, i.e. those that are able to produce pigment only after exposure to light. The second group is that of slow growth scotochromogenic (or true chromogenic) species that can produce pigment even in the dark (but the *M. xenopi* species is assigned by some authors to the third group). The third group includes slow growth nonchromogenic species, and the fourth, rapidly growing species.

1.7 Complexes

Depending on their bio-physiological appearance, the different mycobacteria species are generally grouped in "complexes" named after the most representative species. However, some species, that show no resemblance to any of these groups, or whose characteristics are poorly delineated, are not included in any of the complexes.

Table 1.2 reports only some of the complexes, in particular those species that most commonly cause disease in man.

Table 1.2 The most common complexes and most representative species isolated in man

1- Mycobacterium tuberculosis complex
a. M. tuberculosis
b. M. bovis
c. M. africanum
2- Mycobacterium avium complex
a. M. avium
b. M. intracellulare
c. M. xenopi
3- Mycobacterium scrofulaceum complex
a. M. scrofulaceum
b. M. simiae
4- Mycobacterium gordonae complex
a. M. gordonae
b. M. szulgai
5- Mycobacterium kansasii complex
a. M. kansasii
b. M. gastri
6- Mycobacterium terrae complex
a. M. terrae
b. M. nonchromogenicum
c. M. triviale
7- Mycobacterium fortuitum complex
a. M. fortuitum
b. M. chelonae
8- Mycobacterium parafortuitum complex
a. M. parafortuitum
b. M. vaccae

1.8 Microscopic Examination

This examination has a major diagnostic importance owing to the peculiar property of mycobacteria, namely that they are acid-fast. This property seems to be linked to the lipid component of the cell wall: the mycolic acids form a complex with fuchsin, impeding its release despite alcohol and acid treatment.

The most commonly employed staining method is Ziehl–Neelsen, that involves the use of fuchsin and heat. Preparations are observed in immersion (1000×) and the bacilli stain red against a blue background.

Fluorescent microscopy is also based on acid-fastness: in this case after the fluorochrome has stained the mycobacteria they do not release it despite alcohol and acid treatment [14]. The reagent is fluorochrome (0.1 g auramine-O in 10 ml of 95% ethanol; 3 ml of liquid phenol in 87 ml of H_2O). Preparations must be observed under a blue light fluorescence microscope (mean excitation wave length 460 nm). There are some advantages of this method as compared to Ziehl–Neelsen staining, namely faster reading thanks to the greater width of the microscope observation field and the lesser importance of observer chromatic discrimination.

A slide treated with auramine can be counterstained with Ziehl–Neelsen if it may help to interpret any fluorescent bodies with an untypical morphology.

Kinyoun staining, that also employs fuchsin, is similar to Ziehl–Neelsen staining but has the advantage of not requiring the use of heat. Preparations are observed using a 100× oil immersion objective.

The preparation findings are generally reported as semiquantitative assessments, indicating the presence of bacilli as + (sporadic bacilli), ++ (bacilli in several microscope fields), and +++ (numerous bacilli in all the fields observed). This assessment provides the clinician with useful indications as regards the effects of therapy, and can also help to identify the onset of resistance (observation of a high bacterial load after microscope monitoring had demonstrated the reduction or absence of bacilli).

However, we wish to underline once more here that a negative result of a bacterioscopic examination is not a certain indication of negative results; in fact, the limit of detection of the current microscope observation method is calculated to be about 5×10^4 bacilli per ml of sample material [15]. Similarly, a positive result is not equivalent to an etiological diagnosis of the species and hence a specific disease, since examination at the microscope only reveals the presence of acid-fast bacilli, that may or may not be pathogenic.

1.9 Culture Media

All mycobacteria are obligate parasites, saprophytic or intermediate forms, that differ as regards nutrient requirements and hence culture methods. Some species grow on fairly simple culture media while others need media enriched by particular substances (potato flour, egg, albumin, glycerine, casein hydroxylate, oleic acid, various salts, vitamins), and others can only be cultured in living cells. In general they show a fairly slow growth, ranging from about 3 days to 8 weeks or more, depending on the cell division time, that ranges from 2 to more than 20 h.

Mycobacteria culture media are generally fairly complex because they are particularly demanding microorganisms. Liquid media are normally unsuited to the isolation of mycobacteria from biological samples. Exceptionally, they may be used for seeding sterile matter (CSF, blood, cavitary fluids), while they are largely used to enrich pure culture media and to prepare bacterial suspensions for sensitivity or typing tests [15]. The most common liquid media are the Dubos, Middlebrook 7H9 and Middlebrook 7H12 broths (the latter being used only for radiometric culture methods). Solid media, in practice the only ones employed for primary isolation, have an egg or synthetic base. The former contain, together with egg, natural substances (potato flour, glycerol, milk, mineral salts); the addition of malachite green has the function of inhibiting the development of any associated flora that have survived the decontamination process. Among the various natural solid media those most commonly employed are Jehnsen modified Löwenstein (Löwenstein-Jehnsen, certainly the one in most widespread use), Petragnani medium, the American Thoracic Society medium, and the International Union Tuberculosis Medium. Some species of mycobacteria require specific substances to foster growth: for example, *M. bovis* needs pyruvate, instead of glycerol, as carbon source [15]. The basic ingredients of synthetic media are agar and oleic acid; Middlebrook 7H10 and 7H11 are among the best known. Added with the single antimicrobial agents, these media are used to perform sensitivity testing.

The pathological material used for seeding to isolate mycobacteria is often contaminated by other microbial flora and so needs to be adequately decontaminated to eliminate concomitant flora. These have a faster growth and so could impede mycobacterial development by invading the entire medium. When reading and reporting the results, a positive response to a culture medium is always preceded by ascertainment that the colony-forming bacilli are acid-fast. In general, the results of microscopy and culture coincide but positive results to one and negative to the other may occur. Unless gross errors have been made, positive results to microscopy and negative to culture may be due to the presence of non vital bacilli (this is not infrequent in patients undergoing treatment) or the use of a harmful decontaminant, and the reverse result, to a minor mycobacterial load in the sample.

1.10 Biological Testing

Biological testing in guineapigs, by subcutaneous inoculation of test matter in the inguinal region, is no longer performed because the animals have a reduced receptivity to some mycobacterial strains. In fact, they are refractory not only to NTM, but also to isoniazide- and rifampicin-resistant strains of *M. tuberculosis.*

1.11 Identification

In itself, a positive culture provides some information about the species, according to the type of material from which the strain was isolated, the temperature and the growth speed of the primary culture, the morphology and pigmentation of the

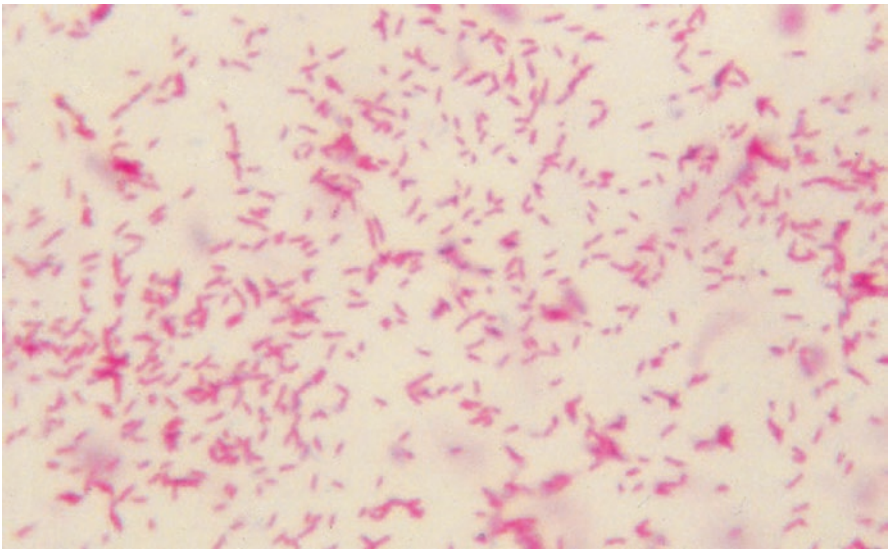

Fig. 1.1 *Mycobacterium fortuitum*

colonies obtained (that may range from yellow to a deep orange, due to the production of carotene crystals).

A series of tests is then employed to make a definitive identification of the species and subspecies. For *M. tuberculosis* tests of niacin, nitrates and catalase heat inactivation are generally sufficient. In the case of NTM, as well as different culture tests (media incubated at different growth temperatures, one of which will be wrapped in aluminum foil to protect it from light; media containing specific nutrient substances), biochemical tests and some other complementary tests are necessary (e.g. sensitivity to ciprofloxacin to discriminate *M. fortuitum*, sensitive, from *M. chelonae*, resistant), and must all be performed in a single session (Fig. 1.1).

1.12 Rapid Methods

Compared to conventional tests, so-called rapid tests allow faster positive results to be obtained. The radiometric method is based on the discrimination of radiolabeled CO_2 released by the metabolic activity of the microorganism on a substrate containing C^{14} [16]. A beta-counter removes culture medium (7H12 broth) daily from the flask, and from a gas sample, measures the radioactivity, transforming the resulting value into a growth index. Use of this method speeds positivization time by 30% [15].

The molecular probes technique is based on the following principle: as well as nucleotide sequences that may be more or less homologous to those of other species (the homology varies according to the philogenetic distance), living organisms possess some entirely species-specific sequences in their genome. These specific genome fractions can be used as probes once they have been identified, cloned and made available

in single helix form. The genomic material of a species to be identified is added with a probe of known specificity: if there are complementary nucleic acid filaments in the reaction mix (this condition will occur only if the unknown microorganism belongs to the same species as the probe) they will unite to form a double helix. The probe target can be denatured nuclear DNA or ribosomal RNA; in the latter case the sensitivity of the method is enormously increased because there are about 10,000 ribosomes per cell. This technology is widely employed in the mycobacteriological field thanks to the availability of specific probes for clinically important mycobacteria.

Chromatography is based on analysis of the lipids making up the bacterial wall: the different mycobacterial species can be identified on the basis of the differences in lipid content. The various chromatography techniques separate and identify the single lipid components of the cell wall of the species under study and the species can then be identified on the basis of analysis of the lipid pattern [15].

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Cutaneous Tuberculosis

2

Domenico Bonamonte, Angela Filoni, Pietro Verni, and Gianni Angelini

2.1 History

Tuberculosis (TB) is one of the most ancient diseases of humankind; it spread around the globe together with human migration as people moved to populate the earth [1–3]. The disease manifestation has always been characterized by great epidemics followed by periods of quiescence: in total, *Mycobacterium tuberculosis* may have killed more people on earth than any other microbial pathogen [1].

The modern molecular biology techniques and genome sequencing of several strains of *M. tuberculosis,* together with the low mutation rate of the germ, have made it possible to estimate the time of origin of TB. An early progenitor of *M. tuberculosis* was present in East Africa about 3 million years ago, and presumably infected early hominids [4]. However, the various members of the *M. tuberculosis* complex (*M. tuberculosis*, *M. africanum*, *M. canettii*, *M. microti, M. caprae, M. pinnipedii, M. bovis*) likely date back to a common African ancestor about 35,000–15,000 years ago [4–6]. The current strains of *M. tuberculosis* seem to have originated from a common ancestor about 20,000–15,000 years ago [7]. The current circulating strains fall into six major lineages, all present in East Africa, and with a variable global circulation [8]; they diversified between 250 and 1000 years ago [9].

D. Bonamonte, MD, PhD (✉)
Department of Biomedical Science and Human Oncology, University of Bary "Aldo Moro", Bari, Italy
e-mail: domenico.bonamonte@uniba.it

A. Filoni, MD • P. Verni, MD
University of Bary "Aldo Moro", Bari, Italy

G. Angelini, MD
Professor of Dermatology, Former Professor and Chairman, University of Bary "Aldo Moro", Bari, Italy

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Skeletal abnormalities due to TB have been observed in ancient Egyptian mummies dating back 5000 years [10, 11], and their cause has been ascertained thanks to studies of DNA amplified from tissues of the mummies [12, 13]. In India, texts dating back 3300 years speak of TB, as do 2300 year old texts from China [14]. TB is also mentioned in the Biblical books Deuteronomy and Leviticus [15]. In classical Greece, TB was well known, and named phthisis (consumption, wasting disease) [16]. Hippocrates described the clinical picture and claimed that the disease has a predilection for young adults and that "consumption" is the main symptom. Also the Greek physician Galen wrote about TB in the second century AD; he recommended treatment with fresh air, milk, and sea voyages [1]. Bony tuberculosis has been demonstrated in Peruvian mummies, and it is certain that the disease was present in America prior to the arrival of European explorers [17–20]. In various sites scattered all over Europe, TB epidemics occurred during the millennium that followed the fall of Rome in the fifth century [21].

It was the Frenchman René Théophile Hyacinthe Laënnec, the inventor of the stethoscope, who first elucidated the pathogenesis of TB [22]; in 1819, in his book "D'Auscultation Mediate" he described the physical signs of the pulmonary disease and introduced the terminology that is still in use today [23]. By the Laënnec era, TB was diffuse in Europe and deadly epidemics struck down thousands every year. This was why John Bunyan gave it the famous definition by which it is still known in Anglo-Saxon countries today, "the Captain of all these men of death" [2]. In 1865, the French military surgeon Jean-Antoine Villemin (1827–1892) demonstrated the infectious nature of TB by inoculating a rabbit with a small quantity of purulent matter extracted from a tuberculous cavity in a man who died of TB: 3 months later, extensive TB was revealed at autopsy [1].

On March 24, 1882, Hermann Heinrich Robert Koch made his presentation, "Die Aetiologic der Tuberculose", to the Berlin Physiological Society, where he illustrated the tubercle bacillus that he had identified. He was awarded the Noble Prize in Medicine in 1905 for this discovery [24]. In 1907, Clemens von Pirquet, a pediatrician in Vienna, used a vaccination lancet to introduce a small amount of tuberculin intracutaneously [25, 26]. In 1908 Charles Mantoux introduced the use of a cannulated needle and syringe to inject tuberculin intracutaneously, and during the 1930s Florence Seibert developed a purified protein derivative (PPD); this is the form that is still currently used [1].

A more direct aid to public health arrived with Albert Calmette and his associate Camille Guérin, who set up a TB vaccine with attenuated *M. bovis*. The BCG (Bacille Calmette-Guérin) vaccine was experimented in 1921: the first recipient was a child born of a mother who died of pulmonary tuberculosis; the child survived and did not develop TB. In the following 7 years, more than 100,000 children were immunized, including Calmette's children, and from France the use of the vaccine spread all over Europe [27–29].

The history of TB changed radically after the introduction of chemotherapy. The first drugs to be used, both bacteriostatic, were para-amino salicylic acid (PAS), discovered by Jorgen Lehmann in 1943, and thiosemicarbazone, discovered by Gerhard Domagk [1]. In 1944, streptomycin, the first both antibiotic and

bactericidal agent effective against *M. tuberculosis* was isolated [16, 30]. Isoniazide, the first oral mycobactericidal drug, followed in 1952 and rifampicins in 1957, triggering a new era in the treatment of the infection, that finally led to the closure of the sanatoriums.

2.2 Epidemiology

TB is ranked among the leading 10 causes of death worldwide, and is one of the worst kinds of epidemics that humankind has ever had to face; it is a reflection of our incapacity to alleviate poverty [31]. More than 20 years after the World Health Organization (WHO) declared TB a global emergency [32], the disease is still a major cause of human suffering and death, and is a pandemic of devastating proportions [31]. The progress made in combating the disease and the renewed efforts made recently are aimed at eradicating TB as a public health problem by 2050, the target adopted by the international community [33]. However, new challenges such as the persistent adverse social conditions, high rates of migration of infected people from areas of relatively high prevalence to low endemic areas, the co-existent human immunodeficiency virus (HIV) epidemic, and the appearance of extensively drug-resistant TB have all contributed to worsen the pandemic and offset the efforts made in the last years.

In 2008, the WHO estimated that there were 9.4 million incident cases (139 cases per 100,000 population) worldwide, 3.6 million of which were women. There were also 11 million prevalent cases of TB (164 cases per 100,000 population) [34]. South-East Asia and African regions accounted for 3.2 million and 2.8 million new cases, respectively, while 80% of the 9.4 million new cases were recorded in India (1.9 million), China (1.3 million), and 20 other countries. In 2008, there were an estimated 1.3 million deaths (20/100,000 population) among HIV-negative incident cases, 0.5 million of which were women. An additional 0.5 million deaths occurred among HIV-positive subjects with incident TB. Thus, in 2008 the total number of deaths worldwide attributable to TB amounted to 1.8 million [31].

2.2.1 Impact of the HIV Epidemic

In recent years, the epidemiology of TB has been adversely affected by the HIV pandemic: in fact, HIV infection is now the most important predisposing factor to the development of active TB. Moreover, TB and HIV infection pose the two greatest global public health threats owing to their high morbidity and mortality rates [35, 36]. This is particularly evident in Sub-Saharan Africa, where more than 20 million of the globally estimated 33 million patients with HIV type 1 infection live [37].

TB is an opportunistic infection in subjects infected by HIV. While the risk of developing post-primary TB later in life is about 10% in a non-immunocompromised patient infected by *M. tuberculosis* who has overcome the primary infection, the risk is multiplied 20–37 times in an HIV-positive patient [38, 39]. Patients with both HIV

and TB infection have a 10% risk per year of developing TB [40]. The association between the two infections is due to a synergic interaction of HIV and *M. tuberculosis*: HIV induces immunosuppression and so is an important risk factor for the progression of infection by the tubercle bacillus; meanwhile, the tubercle bacillus accelerates the progression of HIV infection.

In patients with HIV infection, a defective *M. tuberculosis*-mediated alveolar macrophage apoptosis (a critical mechanism for the elimination of the tubercle bacillus) has recently been identified [41]. This defect reduces the killing of *M. tuberculosis* and increases the susceptibility to active TB, even among HIV-infected patients with relatively well preserved CD4+ T cells [42].

TB is observed in all stages of HIV infection. If more than 350 CD4+ T cells/mm^3 are present, the clinical and histopathologic features of TB are similar to those in subjects without HIV infection, featuring granuloma, with or without central caseation [43]. As the immunosuppression condition progresses, however, granulomas are unformed or absent but there are abundant tubercle bacilli and abscess formation in soft tissues, and disseminated TB is more frequent [44]. Despite antiretroviral therapy, that should reduce the risk of TB in most populations, the risk remains on average 5–10-fold higher than in the HIV-unaffected population, even if the therapy is shown to be active [45]. This may be partly explained by an incomplete restoration of the tuberculosis-specific immune response [46, 47].

TB induces the progression of HIV immunosuppression by means of several mechanisms [48]: the increase of HIV RNA (due to HIV replication in monocytes and macrophages through tumor necrosis factor-alpha and chemoattractant protein-1) [49–51], and transcriptional activation of latent HIV in alveolar macrophages [48].

Because the clinical severity of TB varies according to the host immune response to the infection, there is inevitably a wide range of TB symptoms, depending on the level of HIV-induced immunosuppression. In cases with modest immunosuppression, TB is similar to the condition in HIV-unaffected subjects. But as the immunosuppression progresses, the TB picture becomes "atypical", showing unusual radiological manifestations, non-reactive skin tests, and disseminated and extrapulmonary forms (Table 2.1) [52, 53].

Table 2.1 Clinical features of tuberculosis in patients with HIV infection (modified, by [35])

A.	In early HIV infection:
	Pulmonary disease with upper lobes involvement and cavitation
	Positive PPD in >50% of cases
	Good response to treatment
B.	In advanced HIV infection:
	Involvement of any organ
	Common extrapulmonary involvement (bones, joints, lymphatics, meningi, pleura, liver, kidneys, spleen, skin) and miliary dissemination, with manifestations similar to those in patients without HIV infection
	Unusual radiographic manifestations
	PPD positive in <40% of cases
	Good response to therapy but possible high early mortality

In view of the high frequency of coinfection, all patients infected by HIV must undergo screening for a latent TB infection or active TB, and conversely, subjects with TB must be tested for HIV infection [54]. The diagnostic tools employed to identify the two infections are well known but great care must be taken in interpreting the results due to the risk of false-negative results and the lack of specific symptoms [35]. To treat HIV/AIDS infection, the interested reader should consult the standard texts; therapy for TB, also in cases with coexisting HIV infection, will be considered in a later section. For preventive purposes, the *M. bovis* BCG vaccination is not recommended in patients with HIV [55, 56], because a study conducted in South Africa demonstrated that about 992 per 100,000 BCG-vaccinated HIV-infected children then developed disseminated BCG disease [57], compared to the estimated 5 per million in the general population [58]. It has also been demonstrated that in children with HIV infection the CD4+ and CD8+ T cell response to BCG was severely impaired, and hence little or no benefit was gained from the vaccination [59].

In any case, it should be borne in mind that TB is both preventable and treatable even in subjects with HIV infection.

2.2.2 Impact of Drug and Multidrug Resistance

Effective control of TB is threatened by the emergence of *M. tuberculosis* strains that are resistant to one or more of the standard anti-tuberculosis drugs [31, 60]. Such resistance is attributable to bacterial mutation in patients treated with inadequate or inappropriate drug regimens or poorly formulated combination drugs, and in patients receiving suboptimal therapy. This type of resistance is termed secondary or acquired resistance, whereas in a person infected by an already resistant strain, the resistance is denominated initial or primary resistance. Multidrug-resistant TB (MDR-TB) is defined as TB resistant to at least two of the most potent first-line drugs, namely isoniazid and rifampicin, while extensively drug-resistant TB (XDR-TB) is defined as MDR-TB plus additional resistance to second-line drugs, such as any of the fluoroquinolones and any of the three injectables (amikacin, kamamycin, and capreomycin) [31].

The factors contributing to acquired resistance are reported in Table 2.2 [60]. It should be noted that in cases of MDR-TB, patients must be treated with more costly, less effective and more toxic drugs for extended periods: as a consequence, many patients in developing countries are not treated and so spread the infection to other people. The WHO has carried out surveys of the prevalence of drug resistance in the world [61]. The prevalence of MDR among new cases of TB ranged from 0 to 28% across different settings over 15 years of surveillance [62]. A high prevalence of MDR-TB has been identified in some areas of the world (including Mexico, India, Peru, Mozambique, Sierra Leone, South Africa, Botswana, Guinea), and in particular in countries of the former Soviet Union and some provinces of China [31]. In addition to MDR-TB, combined data from 50 countries and territories suggest that 5.4% of all