Clinical significance and antimicrobial susceptibility of rapidly growing mycobacteria

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Atypical mycobacteria are ubiquitous in nature and widely distributed in water, soil and animals. Although a large number of species have been identified, only a few have clinical interest in humans. The most prevalent rapidly growing mycobacteria (RGM) in human infections are Mycobacterium fortuitum group, M. chelonae group and M. abscessus; other species are minority and only referred to opportunistic infections.

During the past three decades we have observed a notable increment of infections caused by RGM, both localized and disseminated, as well as nosocomial outbreaks of contaminated medical equipment. Pulmonary, skin and soft tissue are the most frequent locations. Other infections include keratitis, endophthalmitis, arthritis, osteomyelitis, endocarditis, meningitis, peritonitis, urinary tract infection, chronic otitis media after tympanostomy tube implantation and catheter-related bacteremia. They are mostly due to accidental inoculation from trauma, surgery, injection or aspiration. There is no evidence of interhuman transmission.

The microbiological diagnosis of RGM infections includes direct microscopic observation of the microorganism in clinical samples and culture in selective media: Löwenstein-Jensen solid medium and Middlebrook 7H9 broth. The identification to the species level is really important to direct the antimicrobial treatment. The taxonomic identification is performed by phenotypic, biochemical and chromatographic techniques, as well as molecular biology techniques: solid-phase hybridization, nucleic acid sequencing (16S rRNA gene) or polymorphism analysis of restriction fragments of the hsp65 gene (PRA or PCR-RFLP).

The treatment of infections caused by RGM differs from that of other mycobacteriosis like tuberculosis, owing to the variable in vitro susceptibility of this group. The RGM are resistant to conventional antituberculous drugs but can be susceptible to other broad spectrum antibiotics. The susceptibility to antibiotics varies among different species. The broth microdilution is the recommended method to determine it. Susceptibility tests offer guidance on clinical treatment.

In this chapter, we comment relevant aspects of human infections by rapidly growing mycobacteria, including biology, epidemiology, pathology, microbiological diagnosis, taxonomic identification, antimicrobial susceptibility and treatment.

Key words Mycobacterium, rapidly growing mycobacteria, atypical mycobacteria, Mycobacterium fortuitum, Mycobacterium chelonae, Mycobacterium abscessus, antimicrobial agents, antituberculous drugs.

1. Introduction

The genus Mycobacterium is included in the family Mycobacteriaceae and the order Actinomycetales, phenotypically most closely related to members of Nocardiaceae, Rhodococcus and Corynebacteriaceae. Mycobacteria appear as straight or slightly curved rods between 0.2-0.6 µm wide by 1.0-10 µm long, Gram positive, non-motile, non-spore forming, obligate aerobes. Their cell wall has a high lipid content, responsible for acid resistance in Ziehl-Neelsen stain. They are intracellular organisms resistant to the environmental conditions. Many Mycobacterium species readily adapt to growth on very simple substrates but some species can be very difficult to culture. Optimum growth temperatures widely vary according to the species and range from 25°C to over 50°C.

A natural division occurs between slow growth (>7 days) and rapid growth (<7 days) species. Mycobacteria that form clearly visible colonies to the naked eye within seven days on subculture are termed rapid growers, while those requiring longer periods are termed slow growers. Some mycobacteria produce deep yellow to orange colonies when grown in the presence of either the light or dark (chromotrophs), others produce non-pigmented colonies when grown in the dark and pigmented colonies only after photoactivation (photochromogens), and others are non-pigmented in the light and dark or have a pale yellow, buff or tan pigment that does not intensify after light exposure (non-chromogens) [1,2]. From a microbiological point of view mycobacteria are classified into six major groups proposed by Runyon, according to the growth rate and pigmentation to growth in Löwenstein Jensen solid medium: I. Slow growth photochromogens; II. Slow growth schotochromogens; III. Slow growth non-chromogens; IV. Rapid growth photochromogens; V. Rapid growth schotochromogens; VI. Rapid growth non-chromogens.

Atypical or environmental mycobacteria are known as all those who are not part of Mycobacterium tuberculosis and Mycobacterium leprae. They have an identical morphology but show some differences in their growth in culture, lipid constituents and their biochemical, antigenic and genetic profiles. Most are environmental mycobacteria that seldom
cause disease in humans. Nearly a hundred of rapidly-growing mycobacteria (RGM) have been identified. Although the general recognition of RGM can be made with confidence, further species identification becomes difficult, particularly by biochemical methods, as with many nontuberculous slow growers. Although many ones have been reclassified as species, particularly members of the Mycobacterium fortuitum complex. Mycobacterium fortuitum group includes M. fortuitum, M. peregrinum, M. mucogenicum, M. senegalense, M. mageritense and the several recently described species: M. septicum, M. alvei, M. houstonense, M. boenickei, M. conceptionense, M. porcinum, M. neworleansense y M. brisbanense. Mycobacterium chelonae group includes M. chelonae and M. abscessus. Mycobacterium mucogenicum group includes M. mucogenicum, M. aubagnense and M. phocicum. Mycobacterium smegmatis group includes M. smegmatis, M. goodii and M. wolinskyi [3,4].

Moreover some have been described in the recent years by molecular biology methods [3,4].

Table 1 shows the rapidly growing mycobacteria species identified until recent years, classified according to the production of pigment.

<table>
<thead>
<tr>
<th>Photochromogens rapidly growing mycobacteria</th>
<th>Schotochromogens rapidly growing mycobacteria</th>
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<tbody>
<tr>
<td>M. marinum</td>
<td>Pik-red pigment</td>
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<td>M. novocastrense</td>
<td>M. engbaecki</td>
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<td>M. rhodochrous</td>
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<tr>
<td><strong>Non-chromogens rapidly growing mycobacteria</strong></td>
<td><strong>Yellow-orange pigment</strong></td>
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<td>M. acapulcense</td>
<td>M. achiense</td>
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<td>M. aurum</td>
<td>M. chlorophenolicum</td>
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<td>M. cosmeticum</td>
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<td>M. confluens</td>
<td>M. duvalii</td>
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<td>M. elephantis</td>
<td>M. flavescens*</td>
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<td>M. febrile</td>
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<td>M. flaveum</td>
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<td>M. gilvum</td>
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<td>M. monacense</td>
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<td>M. neoaurum</td>
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<td>M. obuense</td>
<td>M. poriferae</td>
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<td>M. porcinum</td>
<td>M. rhodesiae</td>
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<td>M. salmoniphilum</td>
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<td>M. senegalense</td>
<td>M. vanbaalenii</td>
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<td>M. senegalense</td>
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<td>M. vaccae</td>
<td><strong>Irregular pigment</strong></td>
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<td>M. savaje</td>
<td>M. austroafricanum</td>
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<td>M. confluens</td>
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<td>M. smegmatis</td>
<td>M. thermoresistibile</td>
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<td>M. thamnopheos</td>
<td>M. vaccae</td>
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<td>M. wolynski</td>
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Atypical mycobacteria are ubiquitous in nature and widely distributed in water, soil and animals. Water and soil are the main sources of infection in human infections. RGM can also be found in dust, rocks and bioaerosols. These organisms have been increasingly identified from environments with harsh conditions (low nutrients, low pH, and extreme temperatures) [7]. Biofilm formation is a successful survival strategy for these very hydrophobic organisms. In fact, their presence in early biofilms in water pipes may make them real biofilm “pioneers”. Rapidly growing mycobacteria are difficult to eradicate with common decontamination practices and are relatively resistant to standard disinfectants such as chlorine, organomercurials, and alkaline glutaraldehydes [8]. Dispersal from biofilms may be a mechanism of shedding from a device or water pipe to infect the patient. In piped water systems, multiple mycobacterial species have been described. In hot water systems, several thermophilic mycobacteria can survive and have been reported to cause outbreaks or pseudo-outbreaks [9]. In cold water systems, Mycobacterium fortuitum, M. chelonae, M. abscessus and Mycobacterium macrogeneicum have been found. Because both cold and hot water temperatures exist in nosocomial settings, it is not surprising to see an array of species responsible for infection.

2. Infections caused by rapidly growing mycobacteria

Rapidly-growing mycobacteria have emerged as significant human pathogens, causing various infections in healthy and immunocompromised hosts. The first cases of disease caused by atypical or environmental mycobacteria were described in the decade of the fifties. The set of these diseases is called mycobacteriosis. For many years these infections were occasional, but in the last 15 years they have become relatively common. The majority of infections are due to accidental inoculation from trauma, surgery, injection or aspiration, however there is no evidence of transmission from person to person. Noteworthy it is the fact that most infections occur in patients with any underlying disease or risk factor added. The development of modern methods of microbiological diagnosis has allowed the isolation and identification of a big number of new species, some of them difficult to grow and complex to characterize, which are related to nosocomial and serious infections in immunocompromised patients with malignancies [3,4,7,10]. The new liquid culture media using Middlebrook broth to detect the growth in automated reading systems, the high pressure liquid chromatography (HPLC), which determines the composition of mycolic acids, and the amplification technology to analyze genetic variability restriction profiles of the amplified hsp-65 gene by PCR-RFLP (Restriction Fragment Length Polymorphism) or the sequencing of 16S ribosomal RNA, have significantly contributed to the identification of the new species [1,2,7,11,12].

During the past three decades there has been a significant increase in post-traumatic and post-operative infections due to these organisms, and in recent years RGM have been frequently associated with localized and disseminated infections, including outbreaks of infection due to contamination of medical equipment. However, to consider the RGM as pathogenic, it is necessary to analyze the clinical and laboratory data, the source of isolation and the crop characteristics, to demonstrate the presence of the isolated species in other patient samples or repeated crops of the same sample, and to observe the patient's evolution after specific treatment.

Rapidly growing mycobacterial infections have been increasingly reported within a medical or paramedical scenario associated to:

- **Catheters**: indwelling venous access catheters, vascular shunts, epidural catheters or Tenckhoff catheters, in relation to immunosuppression, long duration of the catheter placement and prior antimicrobial therapy. They are cause of bacteraemia, catheter tunnel infection, meningitis or peritonitis [2,10,13-15].
- **Dialysis procedure**: both intravascular and peritoneal mechanisms of renal replacement therapy, in relation to contamination of the aqueous solutions used to sterilize the reusable dialysis filters, the catheter insertion site, the tunneling tract and/or the peritoneum. It leads to bacteraemia or peritonitis [16,17].
- **Injections**: contaminated solutions of local anesthetic agents, steroids dispensed in multiuse vials, adrenal cortex injections in individuals following naturopathic or weight loss programs, reused needles or rinsed in tap water, etc. resulting in abscesses formation.
- **Surgical supplies**: contaminated surgical instruments, implants, prosthetic valves, tympanostomy tubes, suture material or solutions [2,7,18].
- **Surgery**: laser vision-correction surgery, facial procedures, abdominoplasty, liposuction, breast reduction or augmentation, mammoplasty and nipple piercing, as causes of post-surgical infections. Contributing factors may include the use of alternative medicine providers and the performance of these practices in freestanding surgical centers not routinely monitored by infection-control committees or equivalent oversight bodies [19-21].

Pulmonary disease may be associated with structural lung disease and impaired clearance of the organisms, as it is seen in patients with cystic fibrosis, bronchiectasis, and chronic vomiting. Clinically the infection can range from an asymptomatic, indolent disease with minimal clinical symptoms to severe bronchiectasis and cavitary lung disease [22]. Hypersensitivity pneumonitis is mostly seen in people working with metal-working fluids that are contaminated with mycobacteria, although it may also occur after contact with indoor hot tubs, spas and swimming [23-25].

Disseminated infections are characterized by the presence of non-contiguous multiple nodular lesions, usually in the extremities but rarely affecting organs, and sometimes accompanied by fever. The finding of a disseminated disease.
should alert the clinician to an immunocompromising condition, such as malignancy, transplantation, HIV infection, cell-mediated immunity defects, lymphoma, leukemia, corticosteroid therapy, chronic renal failure, collagen vascular disease or defects in cytokine pathways [26].

Other infections caused by RGM include keratitis, endophthalmitis, arthritis, osteomyelitis, endocarditis, meningitis, lymphadenitis, peritonitis, urinary tract infection, hepatitis, chronic otitis media after tympanostomy tube implantation, mastoiditis, pacemaker leads infection, tenosynovitis, pleural infection and furunculosis after whirlpool footbaths [10].

3. Rapidly growing mycobacteria of clinical interest

As a result of the widespread use of 16S ribosomal RNA gene sequencing, about fifty RGM species have been described but only a few have clinical significance. From a clinical point of view RGM are mainly opportunistic pathogens. The species most commonly recovered from patients belong to the Mycobacterium fortuitum complex, Mycobacterium chelonae, Mycobacterium abscessus, Mycobacterium mucogenicum, and Mycobacterium smegmatis, reported almost everywhere worldwide. Other RGM species may occasionally cause disease in humans.

Table 2 shows rapidly growing mycobacteria according to the production of pigment and its importance in human infections.

<table>
<thead>
<tr>
<th>Group</th>
<th>Opportunistic pathogens</th>
<th>Casual pathogens</th>
<th>Rarely pathogens</th>
<th>Usually nonpathogens</th>
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<tbody>
<tr>
<td>Photochromogens</td>
<td><em>M. marinum</em></td>
<td><em>M. novocastrense</em></td>
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<td>Schotochromogens</td>
<td><em>M. aurum</em></td>
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<td><em>M. thermoresistibilis</em></td>
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<td><em>M. vaccae</em></td>
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<td>Schotochromogens</td>
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<td>Non-chromogens</td>
<td><em>M. abscessus</em></td>
<td><em>M. bolletii</em></td>
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<td><em>M. chelone</em></td>
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<td><em>M. fortuitum</em></td>
<td><em>M. brusie</em></td>
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<td><em>M. mucogenicum</em></td>
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<td><em>M. confectionen</em></td>
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<td><em>M. goodi</em></td>
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<td><em>M. houstonense</em></td>
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<td><em>M. immunoogenesis</em></td>
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<td><em>M. peregrinnum</em></td>
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<td><em>M. porcinum</em></td>
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<td><em>M. pulveis</em></td>
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* It also may be slow growth.
The RGM species which are considered opportunistic pathogens are the following ones:

- **Mycobacterium marinum**, which typically grows optimally at 30°C, often within the range of time considered to define the mycobacterial species as rapid growth, but when the culture is incubated at 37°C it appears as a slow-growing species. This makes the species can be included both among the rapid and the slow-growing mycobacteria. *Mycobacterium marinum* causes swimming pool or aquarium granuloma, in patients with epidemiological history of contact with contaminated water in swimming pools or aquariums, where the mycobacterium enters the skin through continuity’s solutions. Once in the body, it causes an indolent granulomatous lesion that may end fistulized. Much more rarely, *M. marinum* associates to other medical conditions, such as bone and joint infections, tenosynovitis, arthritis and osteomyelitis [27-31]. Disseminated infection is exceptional [32].

- **Mycobacterium abscessus** is an ubiquitous organism that can be isolated from different aquatic habitats and soil and may contaminate water supplies, reagents and washing solutions for hospitals. This is due to its ability to survive in the absence of nutrients and within a wide range of temperatures. It often causes pulmonary infection, chronic lung disease, endocarditis, chronic otitis media, disease after laser in situ keratomileusis surgery, surgical wound, post-injection, catheter and hemodialysis-related infections, as well as disseminated infections in immunosuppressed patients [25,33-41].

- **Mycobacterium chelonae** is one of the most pathogenic RGM which shows greater resistance to antibiotics. The most common clinical picture is the skin disease, sometimes disseminated, generally in patients under immunosuppressive therapy for solid organ transplant, rheumatoid arthritis or other autoimmune processes. It can also produce traumatic localized infection (cellulitis, abscesses and osteomyelitis), surgical wound infection, post-infection disease and that related to intravascular catheters [26,34,42-46].

- **Mycobacterium fortuitum** has been found in sternal wound infections, post-injection abscesses, surgical and traumatic wound-related disease, traumatic osteomyelitis, cellulitis, mastitis, peritonitis and, among others, intravenous catheter-related infection. Otherwise pulmonary and disseminated infections are rare [26,34-36,47-53].

- **Mycobacterium mucogenicum** owes its name to the mucoid appearance of its colonies. Although it has been frequently recovered in drinking water and spitting, being a simple contaminant, the mycobacterium’s pathogenic capacity has been demonstrated in several nosocomial outbreaks in patients under dialysis, intravenous catheter-related infections, central nervous system diseases, respiratory infections, skin and soft tissue infections, bacteremia and disseminated infections [19,36,54-57].

The implication as pathogens of other RGM is reduced to sporadic isolated cases:

**Chromogenic species**

- **Mycobacterium aurum** has been described as the causative agent of catheter-related bacteremia in some immunocompromised patients, and bilateral pneumonia in a patient receiving infliximab therapy [58-60].

- **Mycobacterium cosmeticum** has been recovered from a footbath drain and a granulomatous subdermal lesion in a patient undergoing mesotherapy [61].

- **Mycobacterium flavescens**, classified as both rapid and slow-growing because of its intermediate growth rate, has been held responsible for pulmonary infection, keratitis, osteomyelitis, gluteal abscess and disseminated post-injection infection [62-64].

- **Mycobacterium neoaurum** has been reported in catheter-related bacteremia, endocarditis and meningocerephalitis [65-70].

- **Mycobacterium phlei** has been associated to peritonitis as a complication of chronic peritoneal dialysis, septic arthritis, infection of the foot and cardiac device-related infections [71-74].

- **Mycobacterium smegmatis** has been described as the causative agent of pulmonary disease, catheter-related bacteremia, skin and soft tissue infection, endocarditis, arthritis, osteomyelitis, lymphadenitis and disseminated infection [75-81].

- **Mycobacterium thermorresistibile** is a species that is characterized by its ability to grow at 52°C and has been described as a cause of lung infection and skin and soft tissue infection after surgery [82-85].

- **Mycobacterium vaccae** has been reported to cause skin and lung infection [86,87].

**Non-chromogenic species**

- **Mycobacterium bolletii** has been recovered from infections after laparoscopic and cosmetic surgery, respiratory and disseminated infections [88-91].

- **Mycobacterium bonickei** have been isolated in osteomyelitis, surgical and traumatic wound infections [6].

- **Mycobacterium brisbanense** has been held responsible for surgical and traumatic wound infections, osteomyelitis and catheter-related bacteremia [6,36].

- **Mycobacterium brumae** has been associated to catheter-related bacteremia [36,92].
• **Mycobacterium conceptionense** has been involved in infections after face rejuvenation with fat grafting and breast implant surgery, subcutaneous abscess and post-traumatic osteitis [93-96].
• **Mycobacterium goodii** causes cellulitis, bursitis, osteomyelitis, post-traumatic wound infection, surgical infection and chronic lung disease [5,97-103].
• **Mycobacterium houstonense** has been reported in surgical and traumatic wound infections and osteomyelitis [6].
• **Mycobacterium immunogenum** has been associated to catheter-related infection, skin infection, disseminated infection, keratitis, respiratory infection and arthritis [23,100-103].
• **Mycobacterium mageritense** is responsible for surgical and catheter infections and severe sinusitis [104].
• **Mycobacterium massiliense** has been found in infections after laparoscopic and cosmetic surgery, cutaneous infection in a "hot spa" and pneumonia [88,105-107].
• **Mycobacterium neworleansense** has been related to surgical and traumatic wound infections and osteomyelitis [6].
• **Mycobacterium peregrinum** has been reported in infections of lung and sternal wounds, cutaneous disease, infections related to surgical site and catheter-related infections [22,108-112].
• **Mycobacterium porcinum** has been involved in peritonitis in a patient under continuous ambulatory peritoneal dialysis, osteomyelitis and catheter-associated bacteremia [6,36,113,114].
• **Mycobacterium senegalense** has been described in catheter-associated bacteremia [36,115].
• **Mycobacterium septicum** has been isolated in infections after laparoscopic and cosmetic surgery, cutaneous infection in a "hot spa" and pneumonia [88,105-107].
• **Mycobacterium wolinskyi** has been associated to cellulitis, osteomyelitis, and surgical wound infection following facial plastic surgery [5].

Other species has been isolated from human samples:

**Chromogenic species**

• **Mycobacterium novocastrense** from a biopsy of a cutaneous granulomatous lesion, and expectorations [118,119].
• **Mycobacterium austroafricanum** from a patient with arthritis [120].
• **Mycobacterium confluentis** from sputum [121].
• **Mycobacterium frederiksbergense** from infection after mesotherapy and soft tissue infection [46,122].
• **Mycobacterium hassiacum** from urine [123].
• **Mycobacterium hodleri** from an opportunistic infection in the course of a rheumatoid arthritis [120].
• **Mycobacterium holsaticum** from sputum, urine, and gastric fluid [124].
• **Mycobacterium laeticola** from catheter-related bacteremia [125].
• **Mycobacterium mamaceae** from infection of the hand and pulmonary tumor [126,127].
• **Mycobacterium rhodesiae** from peritonitis in continuous ambulatory peritoneal dialysis [128].
• **Mycobacterium rhodochrous** from cutaneous lesion and pericarditis [129,130].
• **Mycobacterium tokiense** from caseous necrotic granuloma in the pituitary stalk [131].

**Non-chromogenic species**

• **Mycobacterium alvei** from sputum [132].
• **Mycobacterium aubagnense** from respiratory infection and sepsis [57].
• **Mycobacterium barrusiae** from a patient with chronic pneumonia [133].
• **Mycobacterium canariesense** from the blood of a patient with febrile syndrome [134].
• **Mycobacterium elephantis** from the sputum and granulomatous tissue of an axillary lymph node [135].
• **Mycobacterium hackensackense** from a patient with sepsis [136].
• **Mycobacterium phocaicum** from catheter-associated bacteremia [57].

4. Microbiological diagnosis of infections by rapidly growing mycobacteria

The microbiological diagnosis of rapidly growing mycobacteria infections includes direct microscopic observation of the microorganism in the samples, culture in selective media and identification of the isolated species by phenotypic, biochemical, molecular and chromatographic techniques [137]. The finding of acid-fast bacilli (AFB) in stained smears by the Ziehl-Neelsen or auramine techniques examined under a microscope is the first evidence of the presence of mycobacteria in a clinical specimen. Accompanied by clinical data it can help to establish the presumptive diagnosis of mycobacteriosis, but we must bear in mind that all mycobacteria share the acid-resistance and microscopic morphological characteristics what does not differenciate between species. Crop and identification are necessary requisites for the diagnosis.
Estas micobacterias se aislaron tras cultivo en medio sólido de Lowenstein-Jensen y en medio líquido Middlebrook 7H9 procesado en el sistema automatizado Bactec MGIT 960 (Becton-Dickinson, Reino Unido). La identificación de las cepas se realizó mediante técnicas fenotípicas (temperatura de crecimiento, velocidad de crecimiento en medio sólido y formación de pigmento), técnicas bioquímicas (reducción de nitratos, producción de arilsulfatasa y ureasa, hidrólisis del tween 80, crecimiento en presencia de ClNa al 5% y en agar de Mac Conkey sin cristal violeta y utilización de manitol, inositol y sorbitol), y el método molecular INNO-LiPA Mycobacteria v2 (Innogenetics, Bélgica)Samples can be grown in solid and liquid media. The traditional method for cultivating mycobacteria includes inoculation of egg-based medium such as Lowenstein-Jensen, but also media without egg, such as Middlebrook 7H10 and 7H11 are used. At present, it is recommended a primary culture of all samples in liquid media (Middlebrook 7H9 medium supplemented with enrichment substrates and inhibitors for bacteria and fungi) and incubation and reading in automated systems such as Bectec MGIT 960, MB/BacT Alert 3D or ESP Culture System II.

There are species of RGM with growth special features. The isolation of M. haemophilum requires culture media containing hemin (medium with 1% ferric citrate ammonium), and grows best at incubation temperature of 30-32°C. We must also account for species that need lower or higher growth temperatures, as M. marinum (30°C) and M. thermorresistibile (52°C).

The taxonomic identification is performed by phenotypic techniques (growth temperature, growth rate on solid medium and pigment formation), biochemical techniques (reduction of nitrate, production of arilsulfatase and urease, tween 80 hydrolysis, growth in the presence of NaCl 5% and MacConkey agar without crystal violet and the use of mannitol, inositol and sorbitol), chromatographic techniques, and molecular biology techniques: solid-phase hybridization (INNO-LiPA Mycobacteria, GenoType Mykobacterien), sequencing of the 16S ribosomal RNA gene, polymorphism analysis of restriction fragments of the hsp65 gene (PRA or PCR-RFLP). Molecular biology techniques recognize either lipopolysaccharides, specific proteins or certain sequences of DNA, allowing an increased sensitivity compared to that of conventional tests used in the microbiological diagnosis. They enable the identification of microorganisms difficult to culture, dangerous to manipulate or impossible to identify by conventional methods and the detection of microorganisms in dormant state, as microbial genetic information does not depend on the viability of microorganisms. These methods permit the direct analysis of genes in the DNA or, alternatively, gene transcription in the form of RNA, which is also useful for the direct detection of microorganisms in clinical samples, substituting microscopy, little specific and sensitive, and overcoming the slowness or failure of the crop. They are fast and sensitive methods which allow preliminary recognition of new mycobacterial taxa and have a high biological safety, because the stability of DNA provides a safe handling and storage, apart from maintaining a proper cost-benefit ratio. A major disadvantage they present, apart from potential contamination and direct use limitations on clinical samples, is that there is little marketing of many of these techniques and some, such as sequencing, require high initial investment. But several of them may be perfectly set in laboratories without requiring a large expenditure with little maintenance and good performance [138-147].

### 5. Antimicrobial susceptibility of rapidly growing mycobacteria

The management of rapidly growing mycobacteria infections comprises medical treatment with various antimicrobial agents based on susceptibility patterns, sometimes besides surgical treatment as in the case of lymphadenitis and skin and soft tissue infections. The treatment of infections caused by RGM differs from that of other mycobacterioses like tuberculosis, owing to the variable in vitro susceptibility of this group. RGM are resistant to conventional antituberculous drugs but can be susceptible to other broad spectrum antibiotics. Moreover, different species show a great variability in their response to commonly used antimicrobials in clinical practice. This situation motivates to correctly identify each clinical isolate and study its susceptibility pattern. Series studies with large numbers of strains can guide the empiric therapy, given the global knowledge of the susceptibility of various species of mycobacteria in specific geographic areas. However, it is recommended an individual study of each isolate, because the differences in the results of susceptibility may have a considerable significance for treatment.

Although there is no unanimous agreement on the indications to carry out susceptibility studies in mycobacteria, some recommended scenarios are:

- Clinically significant isolates in patients previously treated with macrolides
- Bacteremia in patients who are on prophylaxis with macrolides
- Isolates in patients who relapse during treatment with macrolides
- Initial isolates in patients with firmly diagnosed disseminated or respiratory disease

In addition, susceptibility studies should be repeated in patients with chronic pulmonary or disseminated disease at three and six months respectively, whether they show no improvement or a clinical deterioration and cultures remain positive.

In order to optimize susceptibility testing and facilitate the interpretation of susceptibility results, the former National Committee for Clinical Laboratory Standards (NCCLS), now known as Clinical and Laboratory Standards Institute (CLSI), recently published guidelines and recommendations for testing nontuberculous mycobacteria (CLSI, M24-A, 2003) [148,149]. It contains revised guidelines for the testing of M. tuberculosis complex and newly proposed
guidelines for the testing of some nontuberculous mycobacteria, including some rapidly growing mycobacteria (Mycobacterium fortuitum group, Mycobacterium chelonae, and Mycobacterium abscessus), Mycobacterium avium complex, Mycobacterium kansasii, and Mycobacterium marinum, as well as Nocardia species and other aerobic Actinomycetes.

CLSI in vitro susceptibility testing and guidelines for microbroth dilution consists of a panel of antimicrobial agents, including macrolides, aminoglycosides, fluoroquinolones, cefoxitin, imipenem, linezolid, tigecycline, doxycycline, minocycline and trimethoprim-sulfamethoxazole. The CLSI M2-A broth microdilution technique is performed in broth Mueller-Hinton broth, with a final concentration of microbial inoculum of 1x10⁴ to 5x10⁸ cfu/ml, incubation at 30°C and reading after 72 hours. Quality controls are those recommended by the CLSI M2-A document with the addition of other inhouse controls. Mycobacterium peregrinum ATCC 700686 and Enterococcus faecalis ATCC 29212, are to be tested on the RGM plate. The interpretation criteria to class a strains as being susceptible or resistant are included in the mentioned document, except for tigecycline which must be interpreted following the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [150]. Other useful methods are Sensititre broth microdilution, E-test, Kirby Bauer disk diffusion, flow cytometry and radiometric ones [151-154]. The use of methods such as agar diffusion with disks is not recommended, because they are not well standardized and the results do not correlate well with those of the reference method.

Susceptibility profiles of clinically significant RGM species may be useful in differentiating the M. fortuitum group from the M. chelonae group. Members of the M. fortuitum group are usually susceptible to ciprofloxacin and ofloxacin, while M. chelonae and M. abscessus are resistant to these agents. The same applies to cefoxitin and tobramycin, M. chelonae, characterizes by high MICs of cefoxitin (>64 mg/L) and susceptibility to tobramycin (MIC ≤ 4 mg/L), whereas M. abscessus shows lower MICs of cefoxitin (≤64 mg/L) and resistance to tobramycin (MIC of >8 mg/L).

6. Treatment of diseases caused by rapidly growing mycobacteria

Identification to the species level is important, because there are predictable antimicrobial susceptibility patterns. Los resultados de nuestro estudio coinciden con los de la mayoría de los trabajos publicados y sugieren que, entre los antimi crobianos disponibles, la amikacina es el más efectivo para el tratamiento de las infecciones producidas por las MCR, y que M. fortuitum es más sensible al conjunto de antimicrobianos que el resto de las especies. Several authors have assessed the susceptibility of some species in different geographic areas, using microdilution in broth and agar methods, but there are few publications in this respect [7,155-158]. Statements of the técnica de E-test evaluada por nosotros parece ser útil para determinar la sensibilidad de MCR, sencilla de realizar y asequible a cualquier laboratorio. Infectious Diseases Society of America and the American Thoracic Society [159] regarding nontuberculous mycobacteria suggest that susceptibility data should be reported and used as a clinical guidance for treatment.

Among the available antimicrobials, aminoglycosides are important parenteral antibiotics used in the treatment of RGM infections, being amikacin the most active and the most effective drug [36]. Clarithromycin is the second most active drug. Among macrolides, 70% of RGM are susceptible to azithromycin. Clarithromycin has good activity against M. fortuitum but not against M. chelonae [50,160]. Mycobacterium abscessus typically shows susceptibility to clarithromycin and azithromycin. Recently, there has been found intrinsic resistance to macrolide antibiotics among several RGM species, such as M. magdalenense, M. boenicei, M. goodii, M. houstonense, M. neworleansense, M. porcinum, and Mycobacterium wolinskyi [36,104,161]. Rapidly growing mycobacteria can develop macrolide resistance by specific mutations in the peptidyltransferase region of the 23S ribosome gene [162,163]. Because of this property, we do not recommend the use of monotherapy for infections due to RGM, especially when there is a large organism burden or when macrolide’s MICs are in the 4-8-µg/mL range. Ciprofloxacin and imipenem are also active against most RGM strains except for M. chelonae and M. abscessus [33,36,164,165]. The 8-methoxy fluoroquinolones (gatifloxacin, moxifloxacin) may be effective in some rapidly growing mycobacteria infections [165,166].

The choice of treatment varies according to three main factors: the clinical presentation, the implicated mycobacterial species and the immune status of the patient. In vitro resistance to most first-line TB drugs justified, until recently, the need for drug treatment associations [167]. There are no clinical trials comparing different treatment regimens, but in order to select an appropriate treatment, due to the variability in susceptibility among species, it seems necessary to perform an in vitro testing in all significant samples, including the vast majority of traditional antibiotics. Skin infection usually resolves spontaneously or sometimes after surgical debridement. For major infections it is recommended the use of intravenous amikacin (10-15 mg/kg in 2 doses) and cefoxitin (1-2 g iv every 6-8 h for continuous infusion) for a minimum of two weeks. Imipenem is a reasonable alternative to cefoxitin if the mycobacterium is resistant to this drug. In severe infections treatment must prolong a minimum of 4 months or 6 months in the case of bone infections. For lung infections 6-12 months of treatment could be enough.

Table 3 lists the recommended antimicrobial agents for the treatment of RGM infections and the treatment regimens normally used [7].
# Table 3. Antimicrobial agents used to treat infections due to rapidly growing mycobacteria (Modified from reference [21]).

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th>Treatment regimen</th>
<th>Possible activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefoxitin</td>
<td>1-2 g iv every 6-8 h (for continuous infusion) or 200 mg/kg maximum 12 g/day</td>
<td><em>Mycobacterium abscessus</em></td>
</tr>
<tr>
<td>Imipenem</td>
<td>0.5 g iv every 6 h or 1 mg iv every 12 h</td>
<td><em>Mycobacterium abscessus</em></td>
</tr>
<tr>
<td>Amikacin</td>
<td>10-15 mg/kg iv 3 times per week to achieve levels of 20-40 mg/L</td>
<td><em>Mycobacterium abscessus</em></td>
</tr>
<tr>
<td>Tobramycin</td>
<td>2.5 mg/kg iv every 12 h</td>
<td><em>Mycobacterium chelonae</em></td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>500 mg orally twice daily or 15-30 mg/kg maximum 1g/day</td>
<td><em>Mycobacterium abscessus</em></td>
</tr>
<tr>
<td>Azithromycin</td>
<td>250-500 mg orally daily or 3 times per week</td>
<td><em>Mycobacterium abscessus</em></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>500-750 mg orally twice daily</td>
<td><em>Mycobacterium abscessus</em></td>
</tr>
<tr>
<td>Doxycycline/Minocycline</td>
<td>100 mg orally twice daily</td>
<td><em>Mycobacterium abscessus</em></td>
</tr>
<tr>
<td>Trimethroprim-sulfamethoxazole</td>
<td>1 double-strength tablet twice daily</td>
<td><em>Mycobacterium smegmatis</em></td>
</tr>
<tr>
<td>Linezolid</td>
<td>600 mg iv or orally daily</td>
<td><em>Mycobacterium chelonae</em></td>
</tr>
<tr>
<td>Tigecycline</td>
<td>50 mg iv twice daily</td>
<td><em>Mycobacterium abscessus</em></td>
</tr>
</tbody>
</table>

The treatment of infections by *M. marinum* might involved the surgical excision of the lesion in some cases or the use of different antibiotic regimens with classical tuberculostatics as rifampicin or ethambutol, or other antibiotics such as minocycline, tetracycline, cotrimoxazole, or more recently, levofloxacin or clarithromycin. Spontaneous recovery has been rarely described [168].

En publicaciones recientes se postula la utilización de nuevos antimicrobianos para el tratamiento de las infecciones por micobacterias atípicas, entre los que se citan las nuevas quinolonas, linezolid, tigeciclina, telitromicina o isepamicina. Recent publications postulates the use of new drugs for the treatment of atypical mycobacterial infections, including the new quinolones, linezolid, tigecycline, telithromycin or isepamicin. The in vitro activity of these drugs appears to be good but clinical experience is still limited [154,156,166,169-171]. Según nuestros resultados, tigeciclina presenta una excelente actividad para todas las especies de MCR; linezolid es efectivo frente a *M. chelonae*, pero algo menos frente a *M. fortuitum* y *M. abscessus*, en el caso de quinupristina/dalfopristina, se puede decir que es infeciaz frente a la mayoría de las especies de MCR [15,18,22-24].

**References**


