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Review

Mycobacterium avium: an overview

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ABSTRACT

Mycobacterium avium is an environmental microorganism found in soil and water sources worldwide. It is the most prevalent species of nontuberculous mycobacteria that causes infectious diseases, especially in immunocompromised individuals. This review discusses and highlights key topics about *M. avium*, such as epidemiology, pathogenicity, glycopeptidolipids, laboratory identification, genotyping, antimicrobial therapy and antimicrobial resistance. Additionally, the main comorbidities associated with *M. avium* infection are discussed.

1. Introduction

Nontuberculous mycobacteria (NTM) are a diverse and large group of species included in the *Mycobacterium* genus. The prevalence of infectious diseases caused by NTM has increased worldwide, and among the most frequent species involved are the *M. abscessus* complex, *M. kansasii* and the *M. avium* complex (MAC) [1–3]. These species are disseminated in the environment, and some are recognized as important human pathogens. Infection acquisition can occur through inhalation, ingestion and dermal contact from environmental sources or medical devices [4,5]. Recently, person-to-person transmission of *M. abscessus* has been reported among patients with cystic fibrosis (CF) [6].

The MAC accounts for approximately 80% of pulmonary diseases caused by NTM in different geographic regions, including countries such as Ireland, South Korea and the United States of America (USA) [7], and has recently been included in the group "*Tuberculosis-Simiae*" by the proposed new taxonomic classification of the genus *Mycobacterium.* Although the classification has not been approved, in this new proposal, MAC was included in the group of the major human pathogens together with *M. tuberculosis* [8–10].

The two main species belonging to the MAC are *M. avium* and *M. intracellulare.* However, new molecular evidence suggests the inclusion of new species such as *M. chimaera, M. colombiense*, *M. vulneris*, *M. arosiense*, *M. bouchedurhonense, M. yongonense, M. timonense, M. marseillense, M. paraintracellulare* and *M. lepraemurium* [5,11]. *M. avium*, the main species of the MAC, was isolated for the first time in 1890 from a chicken with cavitary disease similar to tuberculosis; nevertheless, cases in humans were only first identified in 1930 [12]. This species comprises a heterogeneous group of four subspecies: *M. avium* subsp. *avium*, *M. avium* subsp. *paratuberculosis*, *M. avium* subsp. *silvaticum* and *M. avium* subsp. *hominissuis* (Table 1) [13,14].

M. avium, a slow-growing mycobacteria, can be an opportunistic intracellular pathogen with the ability to persist within the macrophages and resist the immune mechanisms of the host. This mechanism may be influenced by glycopeptidolipids (GPLs), which are present in the cell wall [15]. The respiratory tract is the most common site of infection, and the typical pulmonary disease manifests in the nodular/bronchiectatic form or in the fibro-cavitary form when it develops as a secondary complication. In addition to lung disease, *M. avium* can invade the lymph nodes, bones, joints, skin and soft tissue and can spread systemically. The infection may result in severe disease or even death if untreated or improperly treated, especially among immunocompromised individuals. The symptoms of infection are nonspecific and include malaise, cough, fever, weakness, dyspnea and hemoptysis [16–18].

Because of the importance of *M. avium* in clinical management, this review aims to explore epidemiology, pathogenicity and GPLs, laboratory identification, genotyping, antimicrobial therapy and antimicrobial resistance related to this pathogen. Additionally, we also discuss the main comorbidities associated with *M. avium* infections.

2. Epidemiology

In general, diseases caused by NTM are not notified to the public health authorities in most countries, making it difficult to compare the incidence and prevalence of these infectious diseases in distinct

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Table 1
The four subspecies of *M. avium* and related diseases.

Species	Subspecies	Clinical Manifestation	Host
M. avium	*avium*	Tuberculosis-like disease	Birds
	paratuberculosis	Disease of Johne and possibly Crohn's Disease	Humans and ruminants
	silvaticum	Tuberculosis-like disease	Pigeons
	hominissuis	Pulmonary infection, cervical lymphadenitis, disseminated infection	Humans and pigs

Reference: Turenne et al., 2007 (modified).

geographical areas. Before the onset of AIDS (acquired immunodeficiency syndrome), pulmonary infections caused by *M. kansasii* were more common than those caused by MAC species, mainly in Europe. Currently however, MAC species, especially *M. avium,* are responsible for most pulmonary infections caused by NTM worldwide [7,19], as shown in Fig. 1.

A Japanese study found that the incidence of MAC infection increased from 5.2/100,000 in 2007 to 13.1/100,000 in 2014. Pulmonary diseases caused by *M. avium* were more prevalent in the north and east of Japan, while those caused by *M. intracellulare* were more prevalent in the south and west of Japan [20]. In Taiwan and China, the most frequent MAC species was *M. intracellulare* (25.0%) [21,22], while in Korea, *M. avium* was the most prevalent (88%) [23].

Similarly, in Europe, *M. avium* is also the most prevalent MAC species among pulmonary and extrapulmonary samples, as observed in studies conducted in Denmark (50.7%), Portugal (58.0%) and Italy (41.5%) [24–26]. In addition, a study conducted in England described an increase in the incidence of MAC infection (1.3/100,000 in 2007 to 2.2/100,000 in 2012); however, the MAC species were not differentiated [27].

In many low-income countries, there has also been an increase in the identification of MAC infections over the years. Unlike the 1992 panorama in Uganda, where a study did not find any cases of MAC infection [28], in 2016, MAC accounted for 49.4% of pulmonary infections due to NTM in Zimbabwe [19].

In the Americas, the prevalence of MAC pulmonary and extrapulmonary infections is not very different from that of other continents. *M. avium* was the most frequent isolate among MAC species in the USA (54%), and in Brazil, it was most frequent among NTM species (33.3%) [29,30].

Some possible explanations for the increase in the number of infection cases due to NTM, MAC and *M. avium* could be related to the improvement of laboratory diagnostic capability as well as greater awareness of these infections in clinical settings.

3. Glycopeptidolipids: structure, serotype and pathogenicity

Elucidation of the structure and function of the cell envelope of MAC species and subspecies/serotypes is essential to understand the pathogenesis of infections due to these microorganisms [31]. Mycobacteria are characterized by a hydrophobic lipid-rich cell wall and by the presence of three types of insoluble components composing their core: arabinogalactans, peptidoglycans and mycolic acids [32]. In particular, mycolic acids present in the cell wall of MAC species, which are responsible for a hydrophobic structure that accounts for their fast acid properties, are densely packed with a variety of GPLs, which are a class of glycolipids that are surface exposed. This class of glycolipids from MAC species differs from typical glycolipids of *M. tuberculosis* (i.e., sulfolipid) and is responsible for the characteristic morphology of MAC colonies and immunomodulatory properties [15,32,33].

Considered important cell surface antigens, GPLs are structurally composed of a common moiety of oligosaccharide linked at the D-*allo*Thr of the core (Fig. 2) [34,35]. Most NTM produce this GPLs core, which forms the nonpolar and serotype-nonspecific GPL (nsGPL). However, in MAC species, an oligosaccharide appendage linked to the nsGPL produces a polar and serotype-specific GPL (ssGPL), conferring immunogenicity [36–38]. The presence of ssGPL is responsible for the subdivision of MAC species in at least 31 serotypes and can influence the immune response of the host [31,36,39,40].

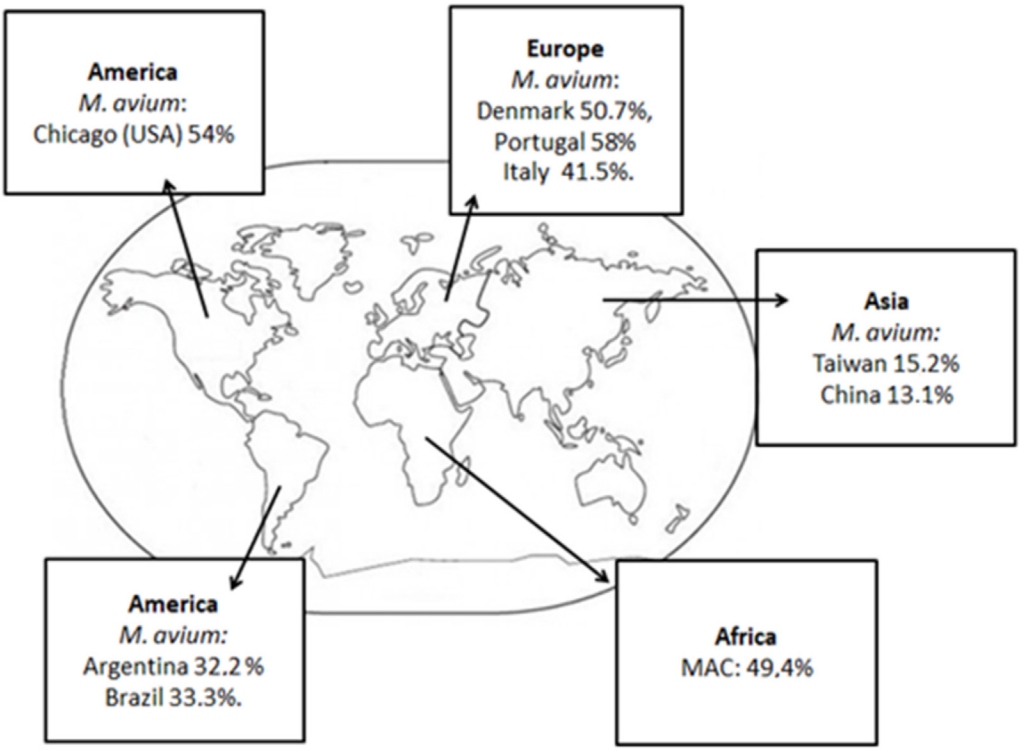

Fig. 1. Geographic representation of the prevalence of *M. avium* in NTM infections [19,21,22,24–26,29,30,117].

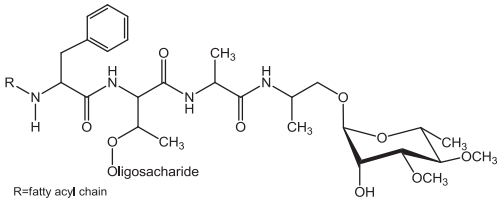

Fig. 2. Chemical structure of glycopeptidolipid (GPL).

Apparently, some serotypes may be specific to individual species of MAC. For example, serotypes 1 to 6, 8 to 11 and 21 belong to *M. avium*, and most of the strains belonging to serotypes 7, 12 to 20, 23 and 25 were identified as *M. intracellulare* [41]. More recently, some of these serotypes (1, 4, 8 and 9) were associated with AIDS patients infected with *M. avium* [40]. Furthermore, serotype 4 of *M. avium* was associated with a worse prognosis in lung disease [42], while serotypes 1 and 8 were associated with a higher ability to infect and survive *in vitro* within human macrophages [37]. After the establishment of the initial infection, *M. avium* is phagocytosed by macrophages, as is the case for most pathogenic mycobacteria. However, the presence of GPLs in *M. avium* may promote a change in macrophage functions, preventing phagosome-lysosome fusion and consequently limiting exposure to hydrolytic enzymes [34,43]. Therefore, these GPLs serve as a protective barrier against the host cell-mediated immune response in the phagosome [37,43,44].

This ability of GPLs to support the survival of mycobacteria in macrophages occurs through the interaction of these GPLs with mannose receptors that are present on the surface of macrophages. The importance of this receptor/oligosaccharide interaction has been shown by experiments in which the elimination of the variable oligosaccharide resulted in consequent abrogation of phagosome-lysosome inhibition [45,46].

Therefore, GPLs are key molecules for the survival of *M. avium* in macrophages and the regulation of the host innate immunity response, and are thus considered a major *M. avium* pathogenicity factor. In this sense, Bhatnagar and Schorey [43], considering the knowledge that the GPLs accumulates within the cell and has a long half-life within a phagosome, determined whether GPLs can enter the endocytic pathway and follow trafficking within infected cells. Their results showed that exosomes released from macrophages infected with *M. avium* contained GPLs that is able to interact with uninfected macrophages, leading to the retention of GPLs in these uninfected cells and inducing an amplification of the proinflammatory response. In the infected host, the production of pro- and anti-inflammatory cytokines can be induced through the interaction of GPLs with Toll-like receptors (TLRs), initiating an inflammatory response [39,47,48]. This is a very important phenomenon because the effects of infection are therefore amplified without the necessary presence of the microorganism.

Moreover, another important role of *M. avium* GPLs is its relationship with biofilm formation. The synthesis of GPLs is important for adhesion and initial establishment of biofilms [49]. It is known that mycobacteria can translocate on the surface of solid growth medium by a sliding motility mechanism and can also form biofilms [50,51]; in these two processes, the surface of the mycobacterial cell is in direct contact with an abiotic surface, and the cells slide or stick to it, respectively. It has been shown that inactivation of the genes involved in GPLs biosynthesis in *M. avium* results in a significant impairment of the ability of the bacteria to form a biofilm on a polyvinyl chloride (PVC) surface [52]. Thus, it is suggested that there exists a close relationship between sliding mobility, biofilm formation and the amount of GPLs [50,53]. Additionally, it has been shown that *M. avium* biofilm formation may play a role in the survival of virulent strains both in the environment and the host. In this sense, biofilms may play an important role in the maintenance of pulmonary mucosal infection, increasing the difficulty of treatment [53].

4. Laboratory identification

Although *M. avium* infections are not considered a public health problem, this microorganism represents an important challenge in clinical practice because it is difficult to diagnose and treat. In fact, the clinical manifestations of infectious diseases caused by *M. avium* and the radiographic findings are very similar to diseases caused by other species of mycobacteria, including *M. tuberculosis* [34].

4.1. Serology and lipid analysis

In the 1960s, Schaefer et al. observed that opportunistic mycobacteria could be classified by agglutination reactions, which are based on differences in the oligosaccharide compositions of GPLs; at that time, it was the preferred method of identifying MAC members [54]. However, interlaboratory reproducibility was poor, and problems with autoagglutination, failure to react with any serum or agglutination with two or more antisera were common. Although these tests were useful in the past, their limitation in the accurate identification of MAC species caused them to be discontinued in laboratory practice [55].

Later, it was shown that thin-layer chromatography (TLC) could be used to characterize the different lipid profiles of MAC species and to complement the use of serum agglutination for classifying *M. avium*. This provided a link between serotype specificity and lipid surface composition [56]. Several chromatography techniques were tested by mycolic acid analysis, which were adequate with 95.7% specificity and 100% sensitivity for MAC species [57]. However, the patterns generated by chromatography for *M. avium* and *M. intracellulare* were very similar. In addition, with this method, *M. avium* subsp. *paratuberculosis* could not be differentiated from the other subspecies of *M. avium* [58,59].

Years later, an enzyme immunoassay (EIA) was developed to diagnose disease caused by the MAC using the specific GPLs core as the antigen. This technique confirmed that the GPLs core antibody was in the sera of immunocompetent patients with MAC disease [60,61]. Thus, quantification of the GPLs core antibody was evaluated as a clinical tool for serodiagnosis, and it was observed that the levels of antibodies (IgG, IgA and IgM) were elevated in the serum of patients with lung disease due to MAC species but not in patients with lung disease caused by *M. tuberculosis, M. kansasii* or in healthy subjects. Among the immunoglobulin classes, the best sensitivity (84%) and specificity (100%) were obtained with IgA [62,63]. Recently, it has also been suggested that high levels of anti-GPLs IgA may reflect disease activity and help to predict the response to treatment in patients with MAC disease. This is very relevant because it is still a challenge to differentiate between infection and colonization when the laboratory identifies NTM in clinical samples [64].

4.2. Microscopy and culture

In many countries, especially in areas with a high incidence of tuberculosis and poor resources, the diagnosis of MAC infection is mainly based on the detection of acid-fast bacilli (AFB) by smear microscopy. This characterizes the first bacteriological evidence of the presence of mycobacteria in a clinical sample. Although this method is widely used due to its low cost and rapid results, it has low sensitivity and does not identify the mycobacterial species [65].

The gold standard method for detection and characterization of mycobacteria is culture in solid or liquid medium. Although semi-automated liquid culture can detect growth around ten days, the time necessary for performing identification and susceptibility testing is still considerable, lasting days or weeks [66,67]. Although the identification of mycobacterial isolates can be based on growth rates, colony pigmentation and biochemical tests such as niacin production and nitrate reduction in addition to serology, these tests may result in errors in the identification of the *Mycobacterium* species. For example, MAC species

present a wide range of colony variability, from smooth to rough and from not pigmented to cream-colored to bright yellow, resembling many other mycobacterial species. In this sense, molecular methods have been demonstrated to be superior to the phenotypic and serological tests for *M. avium* identification [14,67].

5. Molecular methods

Molecular methods can be used for identification either from the DNA extracted from isolates or directly from the clinical sample.

5.1. Identification

Among the molecular methods, the amplification of DNA by polymerase chain reaction (PCR) has been the technique of first choice. *M. avium* has different mobile genomic elements called insertion sequences (IS) that can be used as targets for PCR. Thus, there are four IS described: IS*1311*, IS*900*, IS*901* and IS*1245*, which are present in high copy numbers in the genome of *M. avium* strains isolated from humans. They can also be used to differentiate subspecies because IS*900* is specific for *M. avium* subsp. *paratuberculosis* and IS*1245* is not present in *M. avium* subsp. *paratuberculosis* [68,69]. Another option available in reference laboratories, which may be adaptable in clinical practice for the detection of *M. avium*, is PCR restriction fragment analysis (PRA) of a 441-bp portion of the 65-kDa heat shock protein gene (*hsp65*) sequence. Additionally, there is (partial) gene sequencing, such as for *hsp65* (heat shock protein), *rpoB* (encodes the β subunit of RNA polymerase*)* and the 16S rRNA-23S rRNA internal transcribed spacer (ITS), which offers high discriminatory power and can identify up to the subspecies level. The sequencing of these genes allows discrimination at the species level but is only feasible for laboratories with access to sequencing facilities. As with all gene sequencing methods, the integrity and continuous updating of the gene sequence databases remains major limitation [70–72].

To facilitate the identification process, commercially available tests based on reverse hybridization of DNA have been developed. The main advantage is that, from specific oligonucleotide probes fixed in membrane strips, it is possible to detect *M. avium* and a wide variety of mycobacterial species. Some examples of these tests are AccuProbe (Gen-Probe, San Diego, Calif.), INNO-LiPA mycobacteria v2 (Innogenetics, Ghent, Belgium), GenoType® *Mycobacterium* CM (Hain LifeScience, Reutlingen, Germany), BluePoint MycoID (Bio Concept Corporation, Taiwan), and Speed-oligo *Mycobacterium* assay (Vircell SL, Santa Fe, Granada, Spain) [73–76].

The most recent breakthrough in the field of molecular biology is whole-genome sequencing (WGS). This technique can potentially identify genetic determinants that characterize species diversity and strain specificity. WGS enables the study of multiple genetic regions that may be associated with antibiotic resistance, virulence, and/or host relationships to NTM. Although WGS is not widely available in clinical or reference laboratories, it is undoubtedly emerging as an important tool for the genetic characterization of NTM, specifically *M. avium* [72].

In summary, the lack of tools with an affordable cost for a rapid and accurate diagnosis remains critical. Thus, a low-cost method for identification at the species level is still necessary because infections with different species of mycobacteria require different management approaches [4].

6. Genotyping

In addition to the identification of the species involved in the infectious process, molecular biology tools have allowed for genotyping and differentiation of the multiple isolates of the same species. These genotyping methods have played an important role in the differentiation of microbial isolates, and among other possibilities, they can establish epidemiological links and identify outbreaks [77,78].

The main techniques are PCR based on commercial repetitive sequence (rep-PCR), amplified fragment length polymorphism (ALFP), multispacer sequence typing (MST) and pulsed-field gel electrophoresis (PFGE) [79–82]. Restriction fragment length polymorphism (RFLP) using IS*1245* as a probe is the standard method for the genotyping of *M. avium* strains. This insertion element was not identified in *M. intracellulare* or any of 18 other species of mycobacteria tested [68,83].

However, the utility of these methods is limited because they vary in terms of quantity and purity of DNA required for analysis and portability or the ease with which results can be compared through different analysis among laboratories. In the case of RFLP using IS*1245*, it has a reduced resolution when *M. avium* strains have few or no IS*1245* sequences. These methods are applicable only to cultivable strains (large quantities of high-quality DNA required) and require complex band pattern analysis [68,83,84].

In contrast, a molecular typing method called mycobacterial interspersed repetitive unit-variable number tandem repeats (MIRU-VNTR) has been used for genotyping different mycobacterial species, including *M. avium* isolates. The MIRU-VNTR, eight loci have been mores used; however, seven, 16 and 20 loci tests are also available [84–86]. The main advantages are that this technique is easy to perform, produces rapid results, and displays reproducibility and high discriminatory power [78,84].

7. Antimicrobial therapy

Isolation of *M. avium* from patients with NTM disease symptoms does not mean that the microorganism is the cause of the disease. Indeed, many mycobacteria can only colonize patients without contributing to the progression of the disease. The clinician, therefore, must always know the context in which the isolate was obtained to accurately assess the clinical significance of its isolation. Thus, the ATS (American Thoracic Society) issued diagnostic criteria to assist in diagnosis of NTM disease cases and distinguish them from simple colonization. Clinical, radiographic and microbiological evidence are required. Once the decision to start treatment of *M. avium* infection has been made, treatment regimens should be formulated according to established guidelines and/or drug susceptibility testing (DST) [2,4,87].

The recommended standard therapy for infections caused by *M. avium* consists of combination therapy with one macrolide such as azithromycin or clarithromycin in combination with ethambutol and rifamycin. The dose and duration of therapy are described in Table 2. Aminoglycosides such as streptomycin and amikacin are also used as second-line antimicrobials. Patients should be treated until culture-negative on therapy for at least one year [4,88].

The second-line antimicrobials for *M. avium* are usually reserved for cases that showed failure, intolerance or adverse effects for one or more of the first-line agents. In view of emerging resistance to macrolides, a more aggressive therapy, usually including the use of an injectable aminoglycoside and possibly lung resection should be considered. The treatment success rate reported for *M. avium* infections is only 50–55% [88,89].

The therapy represents a great challenge due to its duration, toxicity and high cost. In addition, it is prone to fail due to the intrinsic resistance of *M. avium* and its predisposition to develop acquired resistance during treatment [4,88].

Recently, other antimicrobials have been proposed as an alternative for treating *M. avium* infections, from repurposed antimicrobials such as clofazimine [90] to new ones such as bedaquiline. Recently, a study with several NTM showed the high activity of bedaquiline against *M. avium* [91,92]. Another option available is liposomal amikacin for inhalation (LAI) for which it was observed that, when added to a multiple drug regimen, it produced improvements in culture conversion and was possibly associated with improvements in functional capacity, resulting in greater efficacy and lower systemic toxicity [93].

Table 2
Recommended treatment in accordance with the American Thoracic Society.

Local of infection	Treatment	Notes
Lung	Clarithromycin (1000 mg) or Azithromycin (500 mg) + Rifampicin (600 mg) + Ethambutol (25 mg/kg)	Three times a week, addition of Amikacin or Streptomycin at the beginning of treatment (3 months) in patients with severe pulmonary disease
Extrapulmonary (skin, tissues, tendons and joints)	Surgical excision (or surgical debridement) and chemotherapy	Whether the three-drug regimen in this setting would be adequate, it is not known. The optimal duration of treatment is also unknown, but drug treatment usually lasts from 6 to 12 months.
Lymphadenitis	Surgical excision of the lymph nodes involved (cure rate of 90%)	It may be carried out a scheme based on macrolides in patients who present an extensive lymphadenitis or a negative response to surgical treatment.
Disseminated infection	Clarithromycin (1000 mg/day) or Azithromycin (500 mg/day) + Ethambutol (15 mg/kg/day)	Patients with HIV infection: addition of rifabutin, especially in patients with advanced immunosuppression and high loads of mycobacteria in blood or in the absence of anti-retroviral therapy. Patients without HIV infection: streptomycin and amikacin

Reference: Griffith 2007 (modified).

8. Antimicrobial resistance

The intrinsic resistance of mycobacteria to antimicrobials can be mainly attributed to the structure of a cell wall, which is surrounded by a capsule of noncovalently attached polysaccharides, proteins and a small amount of lipids, which include GPLs and phenolic glycolipids. The cell wall gives mycobacteria remarkable impermeability to external substances, a critical determinant of virulence for these organisms. However, clinically significant levels of resistance generally require participation of additional resistance mechanisms [33,50].

In addition to the cell wall, the intrinsic antimicrobial resistance can be due to the production of enzymes that degrade or inactivate antimicrobials and by the extrusion of antimicrobials through transporter proteins, also called efflux pumps [94].

Another mechanism of resistance observed in *M. avium* is the development of biofilms in the environment, such as in water distribution systems, in medical devices and possibly in human airways [95]. Cell surface hydrophobicity allows *M. avium* cells to adhere to a wide variety of surface materials, thus reducing the interaction with charged ions in suspension. The biofilm allows *M. avium* survival of traditional disinfection procedures and resistance to chlorine and acidic pH. Studies have shown that in patients with pulmonary infections, such as those occurring frequently in bronchiectasis and CF, the inefficiency of antimicrobials used in the infection treatment is related to the microorganism's ability to form biofilms in human airways [53,96]. The reduction in antimicrobial susceptibility observed in biofilms may be caused by a lack of physical penetration of the antimicrobial in the biofilm, by the limitation of nutrients that leads to slow growth, or by the existence of a stationary phase for many of the cells in a biofilm [97].

Acquired resistance in *M. avium* could be mediated by mutations in genes involved in the mechanism of action of the antimicrobial. Mutations in the *rrl* gene, which encodes 23S rRNA, confer resistance to macrolides and were mainly described at positions 2058 and 2059 (*Escherichia coli* numbering). Furthermore, mutations in the *rrs* gene, which encodes 16S rRNA, are responsible for aminoglycoside resistance, and these mutations occur mainly at position 1408 (*E. coli* numbering) [98–100].

Regarding rifamycins (e.g., rifampicin and rifabutin), resistance is conferred by mutations in the *rpoB* gene. Mutations in codons 426–452 and 544 (*E. coli* numbering), associated with rifamycin resistance in *M. tuberculosis*, have also been demonstrated in *M. avium* [98,101]. Acquisition of ethambutol resistance appears to be a multistep process involving an increase in the expression level of the *emb* operon and a selection of mutations. Resistance to ethambutol is attributed to a mutation in the *embB* gene, which encodes a target enzyme involved in arabinogalactan biosynthesis [98].

Efflux, in addition to being involved in intrinsic resistance, was also described as associated with acquired resistance in *M. avium*. Two efflux pumps of *M. avium*, MAV_1406 and MAV_3306, orthologs of the pumps Rv1258c and Rv1473 coded in *M. tuberculosis,* were induced by exposure to azithromycin and were related to the appearance of resistant phenotypes. Due to the important role of the efflux mechanism in *M. avium*, efflux inhibition has been widely studied, aiming at restoring antimicrobial activity [102,103].

As many traditional and well-tolerated antimicrobials are not effective against *M. avium*, antimicrobial susceptibility testing is recommended. The antimicrobials tested are those that demonstrated activity on a given species and must be evaluated when acquired resistance is suspected, i.e., in the case of recurrent infection or a previous treatment. Although the gold standard method for DST in *M. avium*, as well as NTM, is broth microdilution, other methods with a molecular approach to detect drug resistance have been employed, such as the sequencing and line probe hybridization assay [104,105].

9. *Mycobacterium avium* and comorbidities

9.1. Human immunodeficiency virus (HIV)

HIV infection predisposes patients to the development of opportunistic infections by mycobacteria. Since the beginning of the AIDS epidemic, the incidence of infections by *M. avium*, especially disseminated infections, has increased dramatically. In Brazil, a recent study found a 55% rate of coinfection with *M. avium* among NTM isolates from HIV patients [106]. The *M. avium* infection may remain latent until the immune system of the individuals has been seriously compromised. In this sense, it is important to consider the stage of immunosuppression, which is defined through the count of T CD4 cells in HIV-positive patients [107,108].

According to a prospective cohort study conducted in Africa, the T CD4 cell count in HIV patients can predict the patient's odds of developing opportunistic infectious diseases. In 721 HIV-positive patients evaluated, the incidence of NTM infection was 9.7-fold higher in patients with CD4 cell counts below 100 cells/mm^3 compared to patients with CD4 cell counts above 100 cells/mm^3. Moreover, the development of disseminated disease due to *M. avium* in patients infected by HIV usually occurs when they have a CD4 cell count less than 50 cells/mm^3 [109–111], and mycobacterial infections accelerate HIV disease progression. Although it is still not clear whether mycobacterial factors or processes are directly or indirectly involved in increasing HIV titers during coinfection, it is suggested that mycobacterial stimulation of macrophages may promote the spread of HIV. This is an extremely

important observation because these infections can remain asymptomatic while silently promoting viral proliferation in HIV patients [112].

9.2. Pulmonary disease

The prevalence of pulmonary diseases caused by NTM is increasing throughout the world. In a study conducted in Taiwan, pulmonary NTM infection was the most common type of infection in 93.6% of cancer patients, with lung cancer being the most common type [113]. Bronchiectasis, lung cancer and chronic obstructive pulmonary disease (COPD), associated with immunosuppression, increase the risk of colonization and infection by *M. avium* [7].

Studies have reported that *M. avium* is the most common isolate of NTM in patients with bronchiectasis and lung cancer [113,114]. In addition, the presence of underlying pulmonary diseases and/or immunosuppression may be a possible factor for the development of chronic lung disease by *M. avium* [4,87].

M. avium also has a high prevalence in CF, a genetic disorder that generates defects in the CF transmembrane conductance regulator gene product, resulting in abnormally thickened airway secretions, chronic bacterial infection of the airways and early death [115,116].

One of the main problems associated with *M. avium* chronic lung infection is the limited response to therapy. Patients infected with *M. avium* in the lung tend to respond to therapy initially, but recurrence of infection shortly after the end of treatment is common [4]. The clinical course of pulmonary infection by *M. avium* is suggestive of the presence of a biofilm, which would explain the chronic nature of the infection and the poor response to treatment. Interestingly, Yamazaki et al. [49] showed that *M. avium* can form biofilms in the bronchiolar and bronchial mucosa, protecting itself from the immune response, and subsequently cross the mucosal barrier of the bronchiolar or bronchial airways.

In addition, serotyping of MAC isolates was shown to be important for assessing both the chemotherapy outcome and the prognosis of lung disease due to MAC species. Serotype 4 was associated with a poor prognosis of lung disease due to MAC species because most patients with pulmonary disease caused by this serotype had worsening chest radiographic findings and did not respond to multiple drug chemotherapy [42,64].

10. Final considerations

M. avium has been one of the most frequent species of NTM isolated worldwide. Some issues related to this mycobacteria such as the success of treatment and the evolution of resistance remain poorly elucidated. Additionally, clinical and laboratory diagnosis of *M. avium* continues to be a challenge in clinical practice.

Another important dilemma is the difficulty in recognition of patients with an increased risk for infection with *M. avium*. Although some predisposing conditions are very clear (e.g., HIV), many others remain weakly understood. Although preexisting lung diseases, including COPD and CF, are clear risk factors, it remains impossible to predict which patient will develop a disease caused by *M. avium*. Finally, we emphasize the need for investigations regarding the survival of *M. avium* in the environment, its sources and routes of infection, and the development of effective elimination methods. In addition, accessible, highly sensitive and specific methods for screening potential *M. avium* infection cases and an effective medical treatment aimed at controlling *M. avium* infections should be considered public health priorities.

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References

[1] Egelund EF, Fennelly KP, Peloquin AC. Medications and monitoring in NTM infections. Clin Chest Med 2015;36(1):55–66. https://doi.org/10.1016/j.ccm.2014.11.001.
[2] Stout JE, Koh WJ, Yew WW. Update on pulmonary disease due to nontuberculous mycobacteria. Int J Infect Dis 2016;45(1):123–34. https://doi.org/10.1016/j.ijid.2016.03.006.
[3] Kim N, Jongyoun Y, Chulhun LC. Recovery rates of non-tuberculous mycobacteria from clinical specimens are increasing in Korean tertiary-care hospitals. J Kor Med Sci 2017;32(1):1263–7. https://doi.org/10.3346/jkms.2017.32.8.1263.
[4] Griffith DE, Aksamit T, Brown-Elliott BA, Catanzaro A, Daley C, Gordin F, et al. An official ATS/IDSA statement: diagnosis, treatment, and prevention of non-tuberculous mycobacterial diseases. Am J Respir Crit Care Med 2007;175(1):367–416. https://doi.org/10.1164/rccm.200604-571ST.
[5] Tortoli E. Microbiological features and clinical relevance of new species of the genus *Mycobacterium*. Clin Microbiol Rev 2014;27(4):727–52. https://doi.org/10.1128/CMR.00035-14.
[6] Bryant JM, Grogono DM, Rodriguez-Rincon D, Everall I, Brown KP, Moreno P, et al. Emergence and spread of a human-transmissible multidrug-resistant non-tuberculous mycobacterium. Science 2016;354(6313):751–7. https://doi.org/10.1126/science.aaf8156.
[7] Prevots DR, Marras TK. Epidemiology of human pulmonary infection with non-tuberculous mycobacteria: a review. Clin Chest Med 2015;36(1):13–34. https://doi.org/10.1016/j.ccm.2014.10.002.
[8] Oren A, Garrity G. List of new names and new combinations previously effectively, but not validly, published. Int J Syst Evol Microbiol 2018;68(7):1411–7. https://doi.org/10.1099/ijsem.0.001149.
[9] Lory S. Rosenberg E, DeLong E, Lory S, Stackebrandt E, Thompson F, editors. "The family mycobacteriaceae," in the prokaryotes actinobacteria New York, NY: Springer; 2014. p. 571–5. https://doi.org/10.1007/978-3-642-30138-4.
[10] Gupta RS, Lo B, Son J. Phylogenomics and comparative genomic studies robustly support division of the genus M*ycobacterium* into an emended genus *Mycobacterium* and four novel genera. Front Microbiol 2018;9:67. https://doi.org/10.3389/fmicb.2018.00067.
[11] Van Ingen J, Turenne CY, Tortoli E, Wallace JR, Brown-Elliott B. A definition of the *Mycobacterium avium* complex for taxonomical and clinical purposes, a review. Int J Syst Evol Microbiol 2018;68:3666–77.
[12] Field SK, Fisher D, Cowie RL. *Mycobacterium avium* complex pulmonary disease in patients without HIV infection. Chest 2004;126:566e81. https://doi.org/10.1378/chest.126.2.566.
[13] Rindi L, Garzelli C. Genetic diversity and phylogeny of *Mycobacterium avium*. Infect Genet Evol 2013;21:375–83. https://doi.org/10.1016/j.meegid.2013.12.007.
[14] Turenne YC, Wallace RJ, Behr MA. *Mycobacterium avium* in the postgenomic era. Clin Microbiol Rev 2007;20(2):205–29. https://doi.org/10.1128/CMR.00036-06.
[15] Schorey SJ, Sweet L. The mycobacterial glycopeptidolipids: structure, function, and their role in pathogenesis. Glycobiology 2008;18(11):832–41. https://doi.org/10.1093/glycob/cwn076.
[16] Rose JS, Bermudez LE. *Mycobacterium avium* biofilm attenuates mononuclear phagocyte function by triggering hyperstimulation and apoptosis during early infection. Infect Immun 2014;82(1):405–12. https://doi.org/10.1128/IAI.00820-13.
[17] Johson MM, Odell JA. Nontuberculous mycobacterial pulmonary infections. J Thorac Dis 2014;6(3):210–20. https://doi.org/10.3978/j.issn.2072-1439.2013.12.24.
[18] Falkinham JO. Environmental sources of nontuberculous mycobacteria. Clin Chest Med 2015;36(1):35–41. https://doi.org/10.1016/j.ccm.2014.10.003.
[19] Chin'ombe N, Muzividzi B, Munemo E, Nziramasanga P. Molecular identification of nontuberculous mycobacteria in humans in Zimbabwe using 16S ribosequencing. Open Microbiol J 2016;10:113–23. https://doi.org/10.2174/1874285801610010113.
[20] Namkoong H, Kurashima A, Morimoto K, Hoshino Y, Hasegawa N, Ato M, et al. Epidemiology of pulmonary nontuberculous mycobacterial disease. Jpn Emerg Infect Dis 2016;22(6):1116–7. https://doi.org/10.3201/eid2206.151086.
[21] Chien JY, Yu CJ, Hsueh PR. Identification of nontuberculous mycobacteria in MGIT by matrix-assisted laser desorption/ionization mass spectrometry. Future Microbiol 2016;11(8):1025–33. https://doi.org/10.1128/JCM.00819-13.
[22] Wang HX, Yue J, Han M, Yang JH, Gao RL, Jing LJ, Yang SS, Zhao YL. Nontuberculous mycobacteria: susceptibility pattern and prevalence rate in Shanghai from 2005 to 2008. Chin Med J (Engl) 2010;123(2):184–7. https://doi.org/10.5588/ijtld.14.0140.
[23] Jang MA, Koh WJ, Huh HJ, Kim SY, Jeon K, Ki CS, et al. Distribution of non-tuberculous mycobacteria by multigene sequence-based typing and clinical significance of isolated strains. J Clin Microbiol 2014;52(4):1207–12. https://doi.org/10.1128/JCM.03053-13.
[24] Hermansen S, Ravn P, Svensson E, Lillebaek T. Nontuberculous mycobacteria in Denmark, incidence and clinical importance during the last quarter century. Sci Rep 2017;7:6696. https://doi.org/10.1038/s41598-017-06931-4.
[25] Dabó H, Santos V, Marinho A, Ramos A, Carvalho T, Ribeiro M, Amorim A. Micobactérias não tuberculosas em espécimes respiratórios: significado clínico em um hospital terciário no norte de Portugal. J Bras Pneumol 2015;41(3):292–4. https://doi.org/10.1590/S1806-37132015000000005.
[26] Rindi L, Garzelli C. Increase in non-tuberculous mycobacteria isolated from

humans in Tuscany, Italy, from 2004 to 2014. BMC Infect Dis 2016;16:44. https://doi.org/10.1186/s12879-016-1380-y.
[27] Shah NM, Jennifer AD, Anderson LF, Lalor MK, Kim J, Thomas HL, et al. Pulmonary *Mycobacterium avium-intracellulare* is the main driver of the rise in nontuberculous mycobacteria incidence in England, Wales and Northern Ireland, 2007–2012. BMC Infect Dis 2016;16(1):195. https://doi.org/10.1186/s12879-016-1521-3.
[28] Morrisey AB, Aisu TO, Falkinham JO, Eriki PP, Ellner JJ, Daniel TM. Absence of *Mycobacterium avium* complex disease in patients with AIDS in Uganda. J Acquir Immune Defic Syndr 1992;5(5):477–8.
[29] Boyle D, Zembower Teresa R, Reddy Susheel, Qi Chao. Comparison of clinical features, virulence, and relapse among *Mycobacterium avium* complex species. Am J Respir Crit Care Med 2015;191(11):1310–7. https://doi.org/10.1164/rccm.201501-0067OC.
[30] Pedro HSP, Coelho AG, Mansur IM, Chiou AC, Pereira MI, Belotti NC, et al. Epidemiological and laboratorial profile of patients with isolation of non-tuberculous mycobacteria. Int J Mycobacteriol 2017;6(3):239–45. https://doi.org/10.4103/ijmy.ijmy_87_17.
[31] Fujiwara N, Nakata N, Naka T, Yano I, Doe M, Chatterjee D, et al. Structural analysis and biosynthesis gene cluster of an antigenic glycopeptidolipid from *Mycobacterium intracellulare*. J Bact 2008;190(10):3613–21. https://doi.org/10.1128/JB.01850-07.
[32] Brennan PJ. Structure, function, and biogenesis of the cell wall of *Mycobacterium tuberculosis*. Tuberculosis 2003;83:91–7. https://doi.org/10.1016/S1472-9792(02)00089-6.
[33] Brennan PJ, Nikaido H. The envelope of mycobacteria. Annu Rev Biochem 1995;64:29–63. https://doi.org/10.1146/annurev.bi.64.070195.000333.
[34] Kobayashi K. Serodiagnosis of *Mycobacterium avium* complex disease in humans: translational research from basic mycobacteriology to clinical medicine. Jpn J Infect Dis 2014;67(1):329–32. https://doi.org/10.7883/yoken.67.329.
[35] Hsu FF, Pacheco S, Turk J, Purdy G. Structural determination of glycopeptidolipids of *Mycobacterium smegmatis* by high resolution multiple-stage linear ion-trap mass spectrometry with electrospray ionization. J Mass Spectrom 2012;47(10):1269–81. https://doi.org/10.1002/jms.3070.
[36] Matsunaga I, Komori T, Ochi A, Mori N, Sugita M. Identification of antibody responses to the serotype-nonspecific molecular species of glycopeptidolipids in *Mycobacterium avium* infection. Biochem Biophys Res Commun 2008;377(1):165–9. https://doi.org/10.1016/j.bbrc.2008.09.09.
[37] Guirado E, Arcos J, Knaup R, Reeder R, Betz B, Cotton C, et al. Characterization of Clinical and Environmental *Mycobacterium avium* spp. Isolates and Their Interaction with Human Macrophages. PLoS One 2012;7(9):e45411https://doi.org/10.1371/journal.pone.0045411.
[38] Mukherjee R, Chatterji D. Glycopeptidolipids: immuno-modulators in greasy mycobacterial cell envelope. IUBMB Life 2012;64(3):215–25. https://doi.org/10.1002/iub.602.
[39] Rocco JM, Irani VR. *Mycobacterium avium* and modulation of the host macrophage immune mechanisms. Int J Tubercul Lung Dis 2011;15(4):447–52. https://doi.org/10.5588/ijtld.09.0695.
[40] Chatterjee D, Khoo KH. The surface glycopeptidolipids of mycobacteria: structures and biological properties. Cell Mol Life Sci 2001;58(14):2018–42. doi:1420-682X/01/142018-25.
[41] Saito H, Tomioka H, Sato K, Tasaka H, Dawson DJ. Identification of various serovar strains of *Mycobacterium avium* complex by using DNA probes specific for *Mycobacterium avium* and *Mycobacterium intracellulare*. J Clin Microbiol 1990;28(8):1694–7. doi: 0095-1137/90/081694-04$02.00/0.
[42] Maekura R, Okuda Y, Hirotani A, Kitada S, Hiraga T, Yoshimura K, et al. Clinical and prognostic importance of serotyping *Mycobacterium avium-Mycobacterium intracellulare* complex isolates in human immunodeficiency virus-negative patients. J Clin Microbiol 2005;43(7):3150–8. https://doi.org/10.1128/JCM.43.7.3150–3158.
[43] Bhatnagar S, Schorey SJ. Exosomes released from infected macrophages contain *Mycobacterium avium* glycopeptidolipids and are proinflammatory. J Biol Chem 2007;282(35):25779–89. https://doi.org/10.1074/jbc.M702277200.
[44] Barrow WW. Processing of mycobacterial lipids and effects on host responsiveness. Front Biosci 1997:d387–400.
[45] Shimada K, Takimoto H, Yano I, Kumazawa Y. Involvement of mannose receptor in glycopeptidolipid-mediated inhibition of phagosome-lysosome fusion. Microbiol Immunol 2006;50(3):243–51. https://doi.org/10.1111/j.1348-0421.2006.tb03782.x.
[46] Sweet L, Singh PP, Azad KA, Rajaram MVS, Schlesinger LS, Schorey JS. Mannose receptor-dependent delay in phagosome maturation by *Mycobacterium avium* glycopeptidolipids. Infect Immun 2010;78(1):518–26. https://doi.org/10.1128/IAI.00257-09.
[47] Kitada S, Nishiuchi Y, Hiraga T, Naka N, Hashimoto H, Yoshimura K, et al. Serological test and chest computed tomography findings in patients with *Mycobacterium avium* complex lung disease. Eur Respir J 2007;29(6):1217–23. https://doi.org/10.1183/09031936.00061806.
[48] Horgen L, Barrow ELW, Barrow WW, Rastogi N. Exposure of human peripheral blood mononuclear cells to total lipids and serovarspecific glycopeptidolipids from *Mycobacterium avium* serovars 4 and 8 results in inhibition of TH1-type responses. Microb Pathog 2000;29(1):9–16. https://doi.org/10.1006/mpat.2000.0358.
[49] Yamazaki Y, Danelishvili L, Wu M, Hidaka E, Katsuyama T, Stang B, et al. The ability to form biofilm influences *Mycobacterium avium* invasion and translocation of bronchial epithelial cells. Cell Microbiol 2006;8(5):806–14. https://doi.org/10.1111/j.1462-5822.2005.00667.x.
[50] Martinez A, Torello S, Kolter R. Sliding motility in mycobacteria. J Bacteriol 1999;181(23):7331–8. PMID: 10572138.
[51] Recht J, Martinez A, Tornello S, Kolter R. Genetic analysis of sliding motility in *Mycobacterium smegmatis*. J Bacteriol 2000;182(15):4348–51. https://doi.org/10.1128/JB.182.15.4348-4351.2000.
[52] Yamazaki Y, Danelishvili L, Wu M, MacNab M, Bermudez LE. *Mycobacterium avium* genes associated with the ability to form biofilm. Appl Environ Microbiol 2006;72(1):819–25. https://doi.org/10.1128/AEM.72.1.819–825.2006.
[53] Carter G, Wu M, Drummond DC, Bermudez LE. Characterization of biofilm formation by clinical isolates of *Mycobacterium avium*. J Med Microbiol 2003;52(pt9):747–52. https://doi.org/10.1099/jmm.0.05224-0.
[54] Schaefer WB. Serologic identification and classification of the atypical mycobacteria by their agglutination. Am Rev Respir Dis 1965;92(Suppl):85–93. https://doi.org/10.1164/arrd.1965.92.6P2.85.
[55] Tsang AI, Denner JC, Brennan PJ, McClatchy JK. Clinical and epidemiological importance of typing of *Mycobacterium avium* complex isolates. J Clin Microbiol 1992;30(2):479–84. doi: 0095-1137/92/020479.
[56] Jenkins PA, Marks J, Schaefer WB. Thin-layer chromatography of mycobacterial lipids as an aid to classification: the scotochromogenic mycobacteria, in- cluding Mycobacterium scrofulaceum, M. xenopi, M. aquae, M. gordonae, M. flavescens. Tubercle 1972;53(2):118–27. PMID: 5040575.
[57] Glickman SE, Kilburn JO, Butler WR, Ramos LS. Rapid identification of mycolic acid patterns of mycobacteria by high-performance liquid chromatography using pattern recognition software and a *Mycobacterium* library. J Clin Microbiol 1994;32(3):740–5. PMID: 8195387.
[58] Dei R, Tortoli E, Bartoloni A, Simonetti MT, Lillini E. HPLC does not differentiate *Mycobacterium paratuberculosis* from *Mycobacterium avium*. Vet Microbiol 1999;65(3):209–13. https://doi.org/10.1016/S0378-1135(98)00292-2.
[59] Butler WR, Thibert LM, Kilburn JO. Identification of *Mycobacterium avium* complex strains and some similar species by high performance liquid chromatography. J Clin Microbiol 1992;30(10):2698–704. PMID: 1400970.
[60] Kitada S, Maekura R, Toyoshima N, Fujiwara N, Yano I, Ogura T. Serodiagnosis of pulmonary disease due to *Mycobacterium avium* complex with an enzyme immunoassay that uses a mixture of glycopeptidolipid antigens. Clin Infect Dis 2002;35(11):1328–35https://doi.org/10.1086/344277.
[61] Kobashi Y, Mouri K, Obase Y, Kato S, Oka M. Serological assay by use of glycopeptidolipid core antigen for *Mycobacterium avium* complex. Scand J Infect Dis 2013;45(4):241–9. https://doi.org/10.3109/00365548.2012.714904.
[62] Kitada S, Maekura R, Toyoshima N, Naka T, Fujiwara N, Yano I, et al. Use of glycopeptidolipid core antigen for serodiagnosis of *Mycobacterium avium* complex pulmonary disease in immunocompetent patients. Clin Diagn Lab Immunol 2005;12(1):44–51. https://doi.org/10.1128/CDLI.12.1.44–51.2005.
[63] Kitada S, Mishiuchi Y, Hiraga T, Naka N, Hashimoto H, Yoshimura K, et al. Serological test and chest computed tomography findings in patients with *Mycobacterium avium* complex lung disease. Eur Respir J 2007;29(6):1217–23. https://doi.org/10.1183/09031936.00061806.
[64] Jhun BW, Kim SY, Park HY, Jeon K, Shin SJ, Koh WJ. Changes in serum IgA antibody levels against the glycopeptidolipid core antigen during antibiotic treatment of *Mycobacterium avium* complex lung disease. Jpn J Infect Dis 2017;70(5):582–5. https://doi.org/10.7883/yoken.JJID.2016.523.
[65] Cudahy P, Shenoi S. Diagnostics for pulmonary tuberculosis. Postgrad Med J 2016;92(1086):187–93. https://doi.org/10.1136/postgradmedj-2015-133278.
[66] World Health Organization (WHO). Laboratory services in TB control, part I: organization and management, vol. 1. 2015. Geneva.
[67] Van Ingen J. Microbiological diagnosis of nontuberculous mycobacterial pulmonary disease. Clin Chest Med 2015;36(1):43–54https://doi.org/10.1016/j.ccm.2014.11.005.
[68] Guerrero C, Bernasconi D, Burki T, Bodmer, Telenti A. A novel insertion element from *Mycobacterium avium*, IS*1245*, is a specific target for analysis of strain relatedness. J Clin Microbiol 1995;33(2):304–7. PMCID: PMC227937.
[69] Moravkova M, Hlozek P, Beran V, Pavlik I, Preziuso S, Cuteri V, Bartos M. Strategy for the detection and differentiation of *Mycobacterium avium* species in isolates and heavily infected tissues. Res Vet Sci 2008;85:257–64.
[70] Hoza AS, Mfinanga SG, Rodloff AC, Moser I, König B. Increased isolation of non-tuberculous mycobacteria among TB suspects in northeastern Tanzania: public health and diagnostic implications for control programmes. BMC Res Notes 2016;17(9):109. https://doi.org/10.1186/s13104-016-1928-3.
[71] Zwaan R, Van Ingen J, Van Soolingen D. Utility of rpoB gene sequencing for identification of nontuberculous mycobacteria in The Netherlands. J Clin Microbiol 2014;52(7):2544–51. https://doi.org/10.1128/JCM.00233-14.
[72] Brown-Elliott BA, Philley JV. Rapidly growing mycobacteria. Microbiol Spectr 2017;5(1). https://doi.org/10.1128/microbiolspec.TNMI7-0027-2016. 0027-2016.
[73] Viljanen MK, Olkkonen L, Katila ML. Conventional identification characteristics, mycolate and fatty acid composition, and clinical significance of MAIX AccuProbe-positive isolates of *Mycobacterium avium* complex. J Clin Microbiol 1993;31(5):1376–8. Doi: 0095-1137/93/051376-03$02.00/0.
[74] Somoskovi A, Salfinger M. Nontuberculous mycobacteria in respiratory infections: advances in diagnosis and identification. Clin Lab Med 2014;34:271–95https://doi.org/10.1016/j.cll.2014.03.001.
[75] Chien JY, Chang TC, Chiu WY, Yu CJ, Hsueh PR. Performance assessment of the BluePoint MycoID Plus kit for identification of *Mycobacterium tuberculosis*, including rifampin- and isoniazid-resistant isolates, and nontuberculous mycobacteria. PLoS One 2015;10(5):e0125016https://doi.org/10.1371/journal.pone.0125016.
[76] Ramis IB, Cnockaert M, Von Groll A, Mathys V, Simon A, Tortoli E, Palomino JC, Silva PEA, Vandamme P, Andre E, Martin A. Evaluation of the Speed-Oligo

Mycobacteria assay for the identification of nontuberculous mycobacteria. J Med Microbiol 2015;64:283–7. https://doi.org/10.1099/jmm.0.000025.
[77] Radomski N, Thibault CV, Karoui C, Cruz K, Cochard T, Gutierrez C. Determination of genotypic diversity of *Mycobacterium avium* subspecies from human and animal origins by mycobacterial interspersed repetitive-unit–variable-number tandem repeat and IS1311 restriction fragment length polymorphism typing methods. J Clin Microbiol 2010;48(4):1026–34. https://doi.org/10.1128/JCM.01869-09.
[78] Thibault VC, Grayon M, Boschiroli ML, Hubbans C, Overduin P, Stevenson K, Gutierrez MC, Supply P, Biet F. New variable number tandem-repeat markers for typing *Mycobacterium avium subsp. paratuberculosis* and *M. avium* strains: comparison with IS*900* and IS*1245* restriction fragment length polymorphism typing. J Clin Microbiol 2007;45:2404–10. https://doi.org/10.1128/JCM.00476-07.
[79] Horan KL, Freeman R, Weigel K, Semret M, Pfaller S, Covert TC, van Soolingen D, Leao SC, Behr MA, Cangelosi GA. Isolation of the genome sequence strain *Mycobacterium avium* 104 from multiple patients over a 17-year period. J Clin Microbiol 2006;44(3):783–9. https://doi.org/10.1128/JCM.44.3.783–789.2006.
[80] Pfaller SL, Aronson TW, Holtzman AE, Covert TC. Amplified fragment length polymorphism analysis of *Mycobacterium avium* complex isolates recovered from southern California. J Med Microbiol 2007;56:1152–60. https://doi.org/10.1099/jmm.0.47075-0.
[81] Cayrou C, Turenne C, Behr MA, Drancourt M. Genotyping of *Mycobacterium avium* complex organisms using multispacer sequence typing. Microbiology 2010;156(p3):687–94. https://doi.org/10.1099/mic.0.033522-0.
[82] Biet F, Sevilla IA, Cochard T, Lefrançois LH, Garrido JM, Heron I, Juste RA, et al. Inter- and Intra-subtype genotypic differences that differentiate M*ycobacterium avium subspecies paratuberculosis* strains. BMC Microbiol 2012;12:264. https://doi.org/10.1186/1471-2180-12-264.
[83] Van soolingen D, Bauer J, Ritacco V, Leão SC, Pavlik I, Vincent V, et al. IS*1245* restriction fragment length polymorphism typing of *Mycobacterium avium* isolates: proposal for standardization. J Clin Microbiol 1998;36(10):3051–4. Doi: 0095-1137/98/$04.0010.
[84] Inagaki T, Kei N, Tetsuya Y, Kazuya I, Makoto M, Taku N, et al. Comparison of a variable-number tandem-repeat (VNTR) method for typing *Mycobacterium avium* with mycobacterial interspersed repetitive-unit–VNTR and IS1245 restriction fragment length polymorphism typing. J Clin Microbiol 2009;47(7):2156–64. https://doi.org/10.1128/JCM.02373-08.
[85] Gioffré A, Muñoz CM, Pinedo AFM, Vaca R, Morsella C, Fiorentino MA, Paolicchi F, et al. Molecular typing of Argentinian *Mycobacterium avium subsp. paratuberculosis* isolates by multiple-locus variable number-tandem repeat analysis. Braz J Microbiol 2015;46(2):557–64.
[86] Leão C, Canto A, Machado D, Sanches SI, Couto I, Viveiros M, Inácio J, Botelho A. Relatedness of M*ycobacterium avium* subspecies h*ominissuis* clinical isolates of human and porcine origins assessed by MLVA. Vet Microbiol 2014;173(1):92–100.
[87] Nunes-Costa D, Alarico S, Dalcolmo MP, Correia-Neves M, Empadinhas N. The looming tide of nontuberculous mycobacterial infections in Portugal and Brazil. Tuberculosis (Edinb) 2016;96(1):107–19https://doi.org/10.1016/j.tube.2015.09.006.
[88] Griffith DE, Brown-Elliott BA, Langsjoen B, Zhang Y, Pan X, Girard W, et al. Clinical and molecular analysis of macrolide resistance in *Mycobacterium avium* complex lung disease. Am J Respir Crit Care Med 2006;174(8):928–34. https://doi.org/10.1164/rccm.200603-450OC.
[89] Huang CC, Wu MF, Chen CH, Huang CW. In vitro activity of aminoglycosides, clofazimine, D-cycloserine and dapsone against 83 *Mycobacterium avium* complex clinical isolates. J Microbiol Immunol Infect 2017;S1684–1182(17):30081–6https://doi.org/10.1016/j.jmii.2017.05.001.
[90] Jarand J, Davis JP, Cowie RL, Field SK, Fisher DA. Long-term follow-up of *Mycobacterium avium* complex lung disease in patients treated with regimens including clofazimine and/or rifampin. Chest Infect 2016;149(5):1285–93https://doi.org/10.1378/chest.15-0543.
[91] Philley V, Wallace RJJ, Benwill JL, Taskar V, Brown-Elliott BA, Thakkar F, Aksamit TR, Griffith DE. Preliminary results of bedaquiline as salvage therapy for patients with nontuberculous mycobacterial lung disease. Chest Infect 2015;148(2):499–506. https://doi.org/10.1378/chest.14-2764.
[92] Pang Y, Zheng H, Tan Y, Song Y, Zhao Y. In vitro activity of bedaquiline against nontuberculous mycobacteria in China. Antimicrob Agents Chemother 2017;61:e02627https://doi.org/10.1128/AAC.02627-16.
[93] Olivier KN, Griffith DE, Gina E, McGinnis JP, Micioni L, Liu K, et al. Randomized trial of liposomal amikacin for inhalation in nontuberculous mycobacterial lung disease. Am J Respir Crit Care Med 2017;195(6):814–23. https://doi.org/10.1164/rccm.201604-0700OC on October 17, 2016.
[94] Nash KA. Multidrug resistance in mycobacteria. Curr Clin Micro Rep 2016;3(1):53–61. https://doi.org/10.1007/s40588-016-0032-8.
[95] Carter J, Young LS, Bermudez LE. A subinhibitory concentration of clarithromycin inhibits *Mycobacterium avium* biofilm formation. Antimicrob Agents Chemother 2004;48(1):4907–10. https://doi.org/10.1128/AAC.48.12.4907–4910.2004.
[96] Falkinham JO. *Mycobacterium avium* complex: adherence as a way of life. AIMS Microbiol 2018;4(3):428–38. https://doi.org/10.3934/microbiol.2018.3.428.
[97] Brown M, Allison DG, Gilber P. Resistance of bacterial biofilms to antibiotics: a growth rate related effect? J Antimicrob Chemother 1988;22(6):777–83. PMID: 3072331.
[98] Brown-Elliott BA, Nash KA, Wallace Jr. RJ. Antimicrobial susceptibility testing, drug resistance mechanisms, and therapy of infections with nontuberculous mycobacteria. Clin Microbiol Rev 2012;25(3):545e82. https://doi.org/10.1128/CMR.05030-11.
[99] Renvoisé A, Brossier F, Galati E, Veziris N, Sougakoff W, Aubry A, et al. Assessing primary and secondary resistance to clarithromycin and amikacin in infections due to *Mycobacterium avium* complex. Antimicrob Agents Chemother 2015;59:7153–5. https://doi.org/10.1128/AAC.01027-15.
[100] Pfister P, Jenni S, Poehlsgaard J, Thomas A, Douthwaite S, Ban N, Böttger EC. The structural basis of macrolide–ribosome binding assessed using mutagenesis of 23S rRNA positions 2058 and 2059. Biochem Mol Biol J 2004;342(5):1569–81. https://doi.org/10.1016/j.jmb.2004.07.095.
[101] Obata S, Zwolska Z, Toyota E, Kudo K, Nakamura A, Sawai T, et al. Association of *rpoB* mutations with rifampicin resistance in *Mycobacterium avium*. Int J Antimicrob Agents 2006;27(1):32–9. https://doi.org/10.1016/j.ijantimicag.2005.09.015.
[102] Rodrigues L, Sampaio D, Couto I, Machado D, Kern WV, Amaral L, Viveiros M. The role of efflux pumps in macrolide resistance in *Mycobacterium avium* complex. Int J Antimicrob Agents 2009;34(6):529–33. https://doi.org/10.1016/j.ijantimicag.2009.07.010.
[103] Schmalstieg AM, Srivatava S, Belkaya S, Deshpande D, Meek C, Leff R, Van Oers NS, Gumbo T. The antibiotic resistance arrow of time: efflux pump induction is a general first step in the evolution of mycobacterial drug resistance. Antimicrob Agents Chemother 2012;56(9):4806–15. https://doi.org/10.1128/AAC.05546-11.
[104] Mougari F, Jade L, Nicolas V, Christine B, Beatrice B, Wladimir S, et al. Evaluation of the new GenoType NTM-DR kit for the molecular detection of antimicrobial resistance in non-tuberculous mycobacteria. J Antimicrob Chemother 2017;72(6):1669–77. https://doi.org/10.1093/jac/dkx021.
[105] Clinical and Laboratory Standards Institute (CLSI). Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes—second edition: approved StandardM24-A2. Wayne, PA, USA: CLSI; 2011.
[106] Riello FN, Rebecca TS, Araújo SB, Moreira TA, Goulart RL, Goulart IMB. Diagnosis of mycobacterial infections based on acid-fast bacilli test and bacterial growth time and implications on treatment and disease outcome. BMC Infect Dis 2016;16:142. https://doi.org/10.1186/s12879-016-1474-6.
[107] Collins LF, Clement ME, Stout JE. Incidence, long-term outcomes, and healthcare utilization of patients with human immunodeficiency virus/acquired immune deficiency syndrome and disseminated *Mycobacterium avium* complex from 1992–2015. Open Forum Infect Dis 2017;4(3):1–7. https://doi.org/10.1093/ofid/ofx120.
[108] Agizew T, Basotli J, Alexander H, Boyd R, Letsibogo G, Auld A, et al. Higher-than-expected prevalence of nontuberculous mycobacteria in HIV setting in Botswana: implications for diagnostic algorithms using Xpert MTB/RIF assay. PLoS One 2017;12(12):e0189981https://doi.org/10.1371/journal.pone.0189981.
[109] Aliyu G, El-Kamary SS, Abimiku A, Brown C, Tracy K, Hungerford L, et al. Prevalence of Non-Tuberculous Mycobacterial Infections among Tuberculosis Suspects in Nigeria. PLoS One 2013;8(5):e63170https://doi.org/10.1371/journal.pone.0063170.
[110] Bonard D, Messou E, Seyler C, Vincent V, Gabillard D, et al. High incidence of atypical mycobacteriosis in African HIV-infected adults with low CD4 cell counts: a 6-year cohort study in Cote d'Ivoire. AIDS 2004;18:1961–4. PMID:15353985.
[111] Procop GW. HIV and mycobacteria. Semin Diagn Pathol 2017;34(4):332–9. https://doi.org/10.1053/j.semdp.2017.04.006.
[112] Ganji R, Dhali S, Rizvi A, Sankati S, Vemula MH, Mahajan G. Proteomics approach to understand reduced clearance of mycobacteria and high viral titers during HIV-mycobacteria co-infection. Cell Microbiol 2016;18(3):355–68. https://doi.org/10.1111/cmi.12516.
[113] Lai CC, Tan CK, Cheng A, Chung KP, Chen YC, Liao HC, et al. Nontuberculous mycobacterial infections in cancer patients in a medical center in Taiwan, 2005-2008. Diagn Microbiol Infect Dis 2012;72(2):161–5. https://doi.org/10.1016/j.diagmicrobio.2011.10.006.
[114] Mirsaeidi M, Hadid W, Ericsoussi B, Rodgers D, Sadikot TR. Non-tuberculous mycobacterial disease is common in patients with non-cystic fibrosis bronchiectasis. Int J Infect Dis 2013;17(11):e1000–4. https://doi.org/10.1016/j.ijid.2013.03.018.
[115] Wu UI, Holland SM. Host susceptibility to non-tuberculous mycobacterial infections. Lancet Infect Dis 2015;15(8):968–80. https://doi.org/10.1016/S1473-3099(15)00089-4.
[116] Qvist T, Gilljam M, Jönsson B, Taylor-Robinson D, Fangel JS, Wang M, et al. Epidemiology of nontuberculous mycobacteria among patients with cystic fibrosis in Scandinavia. J Cyst Fibros 2015;14(1):46–52https://doi.org/10.1016/j.jcf.2014.08.002.
[117] Imperiale B, Zumarraga M, Gioffré A, Di Giulio B, Cataldi A, Morcillo N. Disease caused by non-tuberculous mycobacteria: diagnostic procedures and treatment evaluation in the North of Buenos Aires Province. Rev Argent Microbiol 2012;44:3–9.