Diagnostic Microbiology and Infectious Disease 94 (2019) 248–254

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Mycobacteriology

Current significance of the *Mycobacterium chelonae-abscessus* group

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ARTICLE INFO

Article history:
Received 19 April 2018
Received in revised form 30 January 2019
Accepted 31 January 2019
Available online 10 February 2019

Keywords:
Mycobacterium chelonae-abscessus group
Mycobacterium abscessus group
Infections produced by *Mycobacterium chelonae-abscessus* group

ABSTRACT

Organisms of the *Mycobacterium chelonae-abscessus* group can be significant pathogens in humans. They produce a number of diseases including acute, invasive and chronic infections, which may be difficult to diagnose correctly. Identification among members of this group is complicated by differentiating at least eleven (11) known species and subspecies and complexity of identification methodologies. Treatment of their infections may be problematic due to their correct species identification, antibiotic resistance, their differential susceptibility to the limited number of drugs available, and scarcity of susceptibility testing.

1. Introduction

The *Mycobacterium chelonae-abscessus* group (MC-AG) is composed of a group of organisms that continue to gain notoriety by producing disease in humans. The spectrum of individual species and subspecies producing increased types and numbers of infections is predominately due both to greater awareness and to more accurate methods of identification. MC-AG may produce severe infections which can be difficult to treat because of their high levels of antibiotic resistance. The *M. abscessus* group undoubtedly shows both a greater pathogenic potential and antibiotic resistance as compared to its counterpart, the *M. chelonae* group. Identification to the subspecies level and detection of the erythromycin ribosomal methylase *(erm)* (41) gene may be required for some *M. abscessus* group infections due to the differential antibiotic resistance found in these 3 different subspecies. This review provides an overview of the pathogenic burden of the MC-AG while focusing on the more virulent *M. abscessus* group. Infections caused by other members of the MC-AG are reviewed in Table 1.

2. Taxonomy

The taxonomy of the MC-AG has undergone a number of revisions over recent years with at least 11 species and subspecies recognized (Table 1). *M. chelonae* was first recognized as a species in 1923 (Bergey, 1923) and was described in more detail in 1972 (Stanford et al., 1972), while *M. abscessus* was established in 1953 (Moore and Frerichs, 1953) (Table 1). The taxonomic future of the *M. abscessus* group may be the most debated issue among acid-fast bacilli researchers during the next few years (Bryant et al., 2016; Leao et al., 2009, 2011; Sassi and Drancourt, 2014). It is unclear whether the members of the *M. abscessus* group will eventually be classified as a subspecies or as a full species.

3. Natural habitats of MC-AG

Members of the MC-AG are opportunistic pathogens that are normal inhabitants of the environment (Halstrom et al., 2015). Humans are believed to be infected through exposure to environmental sources (Falkinham III, 2002). It has been thought that humans are not a source of infection; however, a recent study suggests indirect transmission among cystic fibrosis patients may occur and that transmission through aerosols may be possible (Bryant et al., 2016).

Water is thought to be the most common source of infection. Rapidly growing mycobacteria, including members of the MC-AG, have been found in surface salt water and fresh water sources throughout the world (Appelgren et al., 2008; Covert et al., 1999; Gruft et al., 1981; van Ingen et al., 2009). They are also thought to be present in most municipal water sources (Chang et al., 2002; Primm et al., 2004). Their presence in public water sources is due in part to their high chlorine

Abbreviations: NTM, nontuberculous mycobacteria; MTB, *Mycobacterium tuberculosis*; *Mycobacterium chelonae* group, *Mycobacterium chelonae* subsp. *chelonae*, *Mycobacterium chelonae* subsp. *bovis* and *Mycobacterium chelonae* subsp. *gwanakae*; MC-AG, *Mycobacterium chelonae-abscessus* group; *M. abscessus* group, includes *Mycobacterium abscessus* subsp. *abscessus*, subsp. *massiliense*, and subsp. *bolletii* (for the purposes of this review, we have adopted the subspecies as the correct nomenclature). Note: Species-level identification of the *M. abscessus* group and the *M. chelonae* group is not consistent in the literature therefore, unless the organisms are clearly identified as a specific species, the organism name is denoted as a group.; MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight mass spectrometry.

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https://doi.org/10.1016/j.diagmicrobio.2019.01.021
0732-8893/

Table 1
Members of the MC-AG (Parte, 2018).

Organism	Type strain with references*	Macrolide resistance mechanisms	Pathogenic potential and infections produced	Identification methods	Antibiotic susceptibility
Mycobacterium abscessus subsp. *abscessus*	ATCC 19977T =CIP 104536T = DSM 944196T = JCM 13569T (Kusunoki and Ezaki, 1992; Moore and Frerichs, 1953)	*Erm* (41) gene: macrolide resistance may be induced or produced when either subspecies contain this gene; alternate macrolide resistance gene for both subspecies is the gene *rrl*.	Opportunist: most virulent of the MC-AG chronic respiratory disease (Jarand et al., 2011; Roux et al., 2009), skin and soft tissue (Galil et al., 1999; Liu et al., 2013; Uslan et al., 2006) skeletal and disseminated infections (possibly catheter-related) (Brown-Elliott & Wallace, 2005a; Apiwattankul et al., 2015; Hibi et al., 2017; Mooren et al., 2017) * *Most published studies on infections do not distinguish between the three subspecies	*M. chelonae* complex vs. *M. abscessus* complex: HPLC, MALDI-TOF successful for identification; for the subspeciation of *M. abscessus* complex MALDI-TOF is variable (Body et al., 2018; Fangous et al., 2014; Girard et al., 2016; Kodana et al., 2016; Mather et al., 2014; Mediavilla-Gradolph et al., 2015; Suzuki et al., 2015). Definitive identification of the *M. abscessus* subsp. organisms: sequencing gene targets including (*rpoB*), *hsp65*, *sodA*, and/or *ITS* region. 16S rRNA is not successful (Macheras et al., 2009; Ringuet et al., 1999). Other target genes are needed to definitely identify other MC-AG organisms (see below)	Amikacin: 90–100% Cefoxitin: 7–75% Clarithromycin: 79–100% Clofazimine: >90% Tigecycline: 100% (van Ingen et al., 2012)
Mycobacterium abscessus subsp. *bolletii*	CIP 108n541T = CCUG 50184T (Adekambi et al., 2006)				
Mycobacterium abscessus subsp. *massiliense** *Some authorities recognize *M. massiliense* as a full species	CIP 108297T = CCUG 48898T (Adekambi et al., 2004)	Nonfunctional, truncated *erm* (41); gene may also contain the functional macrolide resistance gene, A2058G/C			
Mycobacterium chelonae subsp. *chelonae*	ATCC 35752T = CCUG 47445T = CIP 104535T = DSM 43804T= JCM 6388T (Bergey, 1923; Stanford et al., 1972)	No detectable *erm* gene; may harbor the alternate resistance gene, *rrl* A2058G/C	Opportunist: 2nd most virulent member of the MAC group Skin and soft tissue (Kennedy et al., 2012) Eye infections: 2nd leading type of infection (Moorthy et al., 2012) Chronic respiratory disease, (uncommon as compared to *M. abscessus*) (Griffith et al., 1993): found in cystic fibrosis disease, Skeletal (Wallace et al., 1992) Disseminated infection is associated with organ transplants, diabetes mellitus, malignancy, long-term corticosteroid administrational, immunosuppressant therapy, and tumor necrosis factor-alpha (TNF-α) inhibitors (Griffith et al., 2007). Catheter-related infections (Song et al., 2012)	See above	Amikacin: 78–100% Cefoxitin: 5–67% Clarithromycin: 49–89% Clofazimine: 90% Tigecycline: 23–27% (van Ingen et al., 2012)
Mycobacterium chelonae subsp. *bovis*	ATCC 35752T(=CCUG 47445T = CIP 104535T = DSM 43804T = JCM 6388T = NCTC 946T) and QIA-37T (=KCTC 39630T = JCM 30986T) (Kim et al., 2017)	No documentation of inducible clarithromycin resistance (Kim et al., 2017)	Likely cattle pathogen	Distinctive MALDI-TOF profile as compared to *M. chelonae* subspecies chelonae) (Kim et al., 2017); sequencing of 16S rRNA, *hsp65*, and *rpoB* indicates that *M. chelonae* subsp. *bovis* is closely related to *M. chelonae* subsp. *chelonae* (Kim et al., 2017).	Similar clarithromycin susceptibility pattern as *M. chelonae subsp. chelonae*
Mycobacterium chelonae subsp. *gwanakae*	MOTT36WT = KCTC 29127T = JCM 32454T (Kim et al., 2018)	No documentation of inducible clarithromycin resistance (Kim et al., 2018)	Unknown; potential opportunist; isolates recovered from sputum samples (Kim et al., 2018)	Have different MALDI-TOF profiles from those of *M. chelonae* subsp. *chelonae* and *M. chelonae* subsp. *bovis*	More resistant to various antibiotics than *M. chelonae* subsp. *chelonae* except for

(continued on next page)

Table 1 *(continued)*

Organism	Type strain with references*	Macrolide resistance mechanisms	Pathogenic potential and infections produced	Identification methods	Antibiotic susceptibility
				Sequence ID of the *rpoB* and *hsp65* genes distinguishes from other members of the *M. chelonae* group (Kim et al., 2018)	clarithromycin and doxycycline (Kim et al., 2018).
Mycobacterium franklinii	DSM 45524T = ATCC BAA-2149T (Nogueira et al., 2015b; Simmon et al., 2011)	No data on inducement of clarithromycin resistance	Opportunist skin infection associated with tattoos (Stewart et al., 2017) chronic sinusitis and lower respiratory symptoms in patients with underlying pulmonary disease (Stewart et al., 2017; Tortoli, 2014)	MALDI-TOF will identify along with sequence identification using *hsp65, rpoB*, and *sodA* genes (Simmon et al., 2011; Stewart et al., 2017)	May carry an inducible *erm* gene (Simmon et al., 2011; Stewart et al., 2017)
Mycobacterium salmoniphilum	ATCC 13758T (Ross, 1960; Whipps et al., 2007)	No data on inducement of clarithromycin resistance	Fish pathogen (Whipps et al., 2007) Unknown pathogenicity in humans: no described human clinical isolates	HPLC analysis distinguishes from *M. fortuitum* (Whipps et al., 2007) but is similar to *M. chelonae;* Sequence analysis of the small subunit ribosomal (SSU) rRNA gene, *hsp65, rpoB*, and *ITS* regions (Whipps et al., 2007) is consistently distinct from other members of the *M. chelonae* complex.	One isolate susceptible to ciprofloxacin, clarithromycin, and amikacin; no documentation of inducible clarithromycin resistance (Chang and Whipps, 2015)
Mycobacterium saopaulense	EPM 10906T = UUCG 66554T (Nogueira et al., 2015a)	No data on inducement of clarithromycin resistance	Unknown; probably opportunist; 2 isolates obtained from corneal samples after keratopathy (Nogueira et al., 2015a)	HPLC analysis or biochemical testing does not distinguish this species from other members of the MC-AG (Nogueira et al., 2015a)	Susceptible only to clarithromycin; no data on inducible clarithromycin resistance (Nogueira et al., 2015a)
Mycobacterium stephanolepidis	NTB 0901T = JCM 31611T = KCTC 39843T (Fukano et al., 2017)	No data on inducement of clarithromycin resistance	Fish pathogen	Bruker MALDI-TOF probably can distinguish this species from other members of the MC-AG. Sequence ID of the *rpoB* and *hsp65* genes distinguishes from other members of the MC-AG (Nogueira et al., 2015a). Biochemical testing may separate out *M. salmoniphilum* and *M. chelonae*; MALDI-TOF was not useful. Five-gene sequence (16S rRNA, *rpoB, hsp65*, *recA*, and *sodA* genes) analysis confirmed that the isolates were unique but closely related to *M. chelonae* (Fukano et al., 2017)	Susceptible to clarithromycin (Fukano et al., 2017)
Mycobacterium immunogenum	ATCC 700505T = DSM 45595 = CIP 106684T (Wilson et al., 2001)	No functional *erm* gene (Brown-Elliott et al., 2015)	Most clinical isolates are considered contaminates; infections with this organism are rare (Kachhdiya et al., 2015; Wilson et al., 2001): Pulmonary infections Skin and soft tissue (Garcia-Zamora et al., 2017) Keratitis (Sampaio et al., 2006) Disseminated infections (Biggs et al., 2012) Possible cause of hypersensitivity pneumonitis (Shelton et al., 1999)	Biochemical testing can distinguish from *M. abscessus* and *M. chelonae* groups (Wilson et al., 2001) Sequencing has been used for the following target sites: *hsp65*, *ITS*, 16S rRNA, *sodA*, and *rpoB* (Sampaio et al., 2006)	Susceptible to clarithromycin and amikacin (Wilson et al., 2001); resistant to most other antibiotics

tolerance as several species of rapidly growing mycobacteria can tolerate chlorine levels found in drinking water (Covert et al., 1999; Le Dantec et al., 2002; Taylor et al., 2000). The ability of rapidly growing mycobacteria to survive in water is aided by several other characteristics including their growth rate which is normally slower than bacteria other than mycobacteria, thermoresistance (Schulze-Robbecke and Buchholtz, 1992), tolerance to a wide pH range (Le Dantec et al., 2002), impermeability of their cell walls (Falkinham III, 2002), and the ability to form biofilms. Biofilms are important habitats for rapidly growing mycobacteria (van Ingen et al., 2009); bacteria present in biofilms are more resistant to disinfectants than are organisms in a planktonic state (Donlan and Costerton, 2002).

4. Infections

The *M. abscessus* group can produce 5 of the 6 major clinical syndromes including chronic respiratory disease, skin and soft tissue, skeletal, disseminated infections, and catheter-related infections. Lymphadenitis is rarely seen. The respiratory tract is the most common site for the *M. abscessus* group to infect.

In the United States, pulmonary infections caused by the MC-AG are second to the more common *Mycobacterium avium* complex infections, comprising 2.6–13% of all mycobacterial infections across various study sites (Lee et al., 2015). The MC-AG also accounts for 80% of rapidly growing mycobacteria respiratory isolates (Jarand et al., 2011). Infection arises in immunosuppressed patients and those with underlying disease but also affects immunocompetent individuals. In other parts of the world, *M. abscessus* group infection rates may vary. Because of limitations in correct and detailed species-level identification and lack of required reporting to public health authorities, the extent of disease is still likely underestimated.

The signs and symptoms for nontuberculous mycobacteria (NTM) lung disease are variable and often nonspecific, making it difficult to diagnose without positive respiratory cultures. Presenting symptoms are frequently a nagging cough and throat clearing with little or no productive secretions. The disease is progressive with declining pulmonary function and decrease in quality of life. In some cases, the disease can follow a more fulminant course with acute respiratory failure.

The 2007 guidelines published by the American Thoracic Society (ATS) and the Infectious Diseases Society of America outline the process for evaluation and diagnosis of NTM pulmonary infection. The minimum evaluation should include a chest radiograph or, in the absence of cavitation, a high-resolution computed tomography (HRCT) scan; 3 or more sputum specimens for acid-fast bacilli analysis; and exclusion of other disorders, such as *Mycobacterium tuberculosis* (MTB). Clinical, radiographic, and microbiologic criteria are equally important to make a diagnosis of NTM lung disease (Griffith et al., 2007).

Common radiographic findings of *M. abscessus* group pulmonary infections include bronchiolitis, bronchiectasis, nodules, and consolidation; less frequently seen are cavitary lesions. In a retrospective observational study of 107 patients who met ATS criteria, HRCT showed bronchiectasis and nodular opacities in 98% of patients and cavities in 44% (Jarand et al., 2011).

The largest group of patients with this lung disease is white, nonsmoking females older than 60 years, with no predisposing conditions or previously recognized lung disease. Lady Windermere syndrome was the eponym applied to these patients. The most common organisms isolated are *M. avium* complex and *M. abscessus* group (Wu and Holland, 2015). Pulmonary infection occurs primarily in patients with underlying lung disease (cystic fibrosis, bronchiectasis, chronic obstructive pulmonary disease, tuberculosis sequelae, cancer, or sarcoidosis) (Mougari et al., 2016). Individuals with a predisposing, underlying lung disease generally develop disease with *M. abscessus* group at a younger age, usually younger than 50 years of age, and almost all patients younger than 40 years have 1 of the predisposing disorders (Griffith et al., 1993).

Prior to the 1990s, isolating rapidly growing mycobacteria from patients with cystic fibrosis (CF) was rare. More recently, data from the United States showed that NTM represents 13% of lung infections in this population. *Mycobacterium avium* complex was the most prevalent, while *M. abscessus* group accounted for 72% of rapidly growing mycobacteria isolates (Olivier et al., 2014). A diagnosis of *M. abscessus* group pulmonary infection in a CF patient depends on a set of clinical, radiological, and microbiological findings. A single positive culture is not necessarily diagnostic for active infection in these patients. It has become clear that NTM can also transiently, intermittently, or permanently reside within the lungs of individuals with CF without causing disease, thus representing asymptomatic infection and creating difficulties in deciding how best to screen for and diagnose NTM active infection. In a multicenter study in patients with CF in France (mean age 18.9 years), approximately one half of the 104 isolates of NTM recovered were identified as *M. abscessus* subsp. *abscessus* and *M. abscessus* subsp. *bolletii* (Roux et al., 2009). Age also seems to play a factor in individuals with CF and the prevalence of certain organisms. *M. abscessus* group is isolated from all age groups but peaks between 11 and 15 years of age (Floto et al., 2016). A recent finding in *Science* revealed that the majority of *M. abscessus* group infections in individuals with CF are caused by genetically clustered isolates, suggesting recent transmission via fomite spread or aerosols as opposed to the acquisition of unrelated environmental organisms (Bryant et al., 2016).

Skin and soft tissue infections are the second leading type of infection caused by *M. abscessus* group. Infections normally range from deep soft tissue to localized skin infections. The 2 main mechanisms for acquiring an infection with *M. abscessus* group are by 1) direct contact with contaminated material or water through traumatic injury, surgical wound, or environmental exposure and 2) secondary involvement of skin and soft tissue during disseminated disease. There are multiple reports of infection after trauma and surgical procedures. Other procedures associated with infection include acupuncture, tattooing, liposuction, silicone injection, breast implantation, intravenous catheter use, pacemaker placement, central nervous system (CNS) procedures, and subcutaneous and intramuscular injections (Galil et al., 1999; Lee et al., 2015; Liu et al., 2013; Uslan et al., 2006). Similar to *M. marinum* infections, *M. abscessus* group infections have been reported in fish handlers or individuals who have had exposure to salt water.

CNS infections with *M. abscessus* group are rare; until 2012, only 3 cases of CNS infection due to the *M. abscessus* group had been reported (Brown-Elliott and Philley, 2017; Lee et al., 2012, 2015). Infections with rapidly growing mycobacteria are seen with long-term intravenous catheters, or peritoneal or shunt catheters in adults and children (Apiwattankul et al., 2015; Brown-Elliott & Wallace, 2015a; Hibi et al., 2017; Mooren et al., 2017). Intravascular infections can lead to myocardial abscess, endocarditis, and implantable electronic device infections. When rapidly growing mycobacteria are isolated from the blood, they should be considered as true pathogens. Because these organisms are found in the environment, any breach in sterility could cause contamination of the blood, intravascular devices, or tissue specimens. Susceptibility to disseminated NTM infections may be attributed to systemic immune defects mostly involving the interleukin-12–interferon gamma pathway; localized NTM diseases may represent some impairment of local host defenses instead of a major immune defect.

5. Identification of MC-AG

Traditional biochemical testing to identify the MC-AG was the "gold standard" for most mycobacteriology labs before the advent of the newer identification methods described below. Biochemical testing for the MC-AG has been considered unreliable (Nogueira et al., 2015a), is up to 2.7 times more costly than 16S rRNA sequencing (Cook et al., 2003), and may potentially compromise patient care because of its long turnaround time. With the advances of new technologies (see

below), biochemical tests are rapidly disappearing from many mycobacteriology laboratories.

Numerous studies for identification of acid-fast bacilli by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF) have been published, and 1 review indicated only a 71% accuracy level among all tested acid-fast bacilli isolates using pooled results from 19 publications (Cao et al., 2018). In this summary paper, overall, only 78% of the *M. chelonae* group and only 82% of the *M. abscessus* group were identified correctly. However, other studies have showed almost a 100% accurate identification for members of the *M. chelonae* and *M. abscessus* groups (Body et al., 2018; Girard et al., 2016; Mather et al., 2014; Mediavilla-Gradolph et al., 2015). Factors that may account for these differences could be the system used (Bruker vs. Vitek MS), version of the database (including vendor only and/or use of homegrown databases), and time of isolate incubation before testing (Mather et al., 2014).

It is unclear whether MALDI-TOF will be able to identify the members of the *M. abscessus* group. The difficulty in distinguishing the 3 subspecies of *M. abscessus* by MALDI-TOF has been documented (Body et al., 2018; Girard et al., 2016; Kodana et al., 2016), though successful identification to subspecies among the *M. abscessus* group by MALDI-TOF has been reported (Fangous et al., 2014; Suzuki et al., 2015).

Molecular methods are currently the gold standard for the identification of acid-fast bacilli in the mycobacteriology laboratory. Many laboratories use the 16S rRNA gene (either by Sanger or pyrosequencing) as the target for the acid-fast bacilli identification. This target gene may also be used to detect and identify acid-fast bacilli, including the MC-AG from formalin-fixed paraffin-embedded tissues (Bao et al., 2018). 16S rRNA gene sequencing identifies most *Mycobacterium* species and *Nocardia* species but lacks the ability to further characterize individual species in the MC-AG. Other targets that can be used to further identify the MC-AG are the RNA polymerase (*rpoB*) gene, the heat shock protein (*hsp65*), superoxide dismutase *(sodA)* gene, and internal transcribed spacer *(ITS)* region genes. Using multiple targets may improve the final identification accuracy of the *M. abscessus* group (Macheras et al., 2009; Ringuet et al., 1999). Multiple target sequencing may be required to separate the *M. abscessus* group from *M. franklinii*. As of yet, no single gene target, including the *ITS*, has been shown to be sufficiently discriminatory to identify all rapidly growing mycobacteria to the species level. Line probe assays (Hain Lifescience GmbH and Fujirebio, formerly Innogenetics) for the identification of both MTB and the most common disease producing NTMs are used predominately outside of the United States. The Hain Lifescience GmbH AFB identification system distinguishes between the *M. chelonae* group and *M. abscessus* group and also identifies the 3 subspecies of *M. abscessus*. The Fujirebio kit does not distinguish between *M. chelonae* group and *M. abscessus* group.

5.1. Antibiotic susceptibility testing

The Clinical and Laboratory Standards Institute (CLSI) guidelines for rapidly growing mycobacteria describe how to perform susceptibility testing using a standard broth dilution method; with the exception of clarithromycin, all drugs can be reported within 5 days (Clinical and Laboratory Standards Institute (CLSI), 2011). Currently, the only commercially available broth microdilution panel (Trek) is research-use only. CLSI does not recommend agar dilution most likely because *M. chelonae* preferentially grows in broth (Swenson et al., 1985). Both BioMerieux (Marcy-l'Étoile, France) and Liofilchem (Roseto degli Abruzzi (Te) Italy) offer gradient diffusion strips for rapidly growing mycobacteria.

CLSI guidelines include instructions to incubate clarithromycin-susceptible rapidly growing mycobacteria isolates for at least 14 days before reporting (Clinical and Laboratory Standards Institute (CLSI), 2011) due to the potential presence of the inducible erythromycin ribosomal methylase *(erm) 41* gene (Nash et al., 2009). Resistance may be reported at any time. Studies by microbroth dilution demonstrated increased clarithromycin MICs as early as 5 days and consistently at 9 days. Exposure to telithromycin, clindamycin, HMR3004, and quinupristin all induced markedly increased *erm (41)* RNA levels, and ketolides are also known to induce *erm (41)* (Nash et al., 2009). While there are no specific recommendations from the gradient diffusion strip manufacturers regarding extended incubation for clarithromycin, Bastian et al. demonstrated results comparable to a 14-day microdilution method by incubating Etest for 7 days (Bastian et al., 2011).

Functional *erm* genes encode transferases that specifically methylate nucleotides A2057-A2059, which confer macrolide resistance (Kim et al., 2010). There is a strong association between species and presence of the *erm (41)* gene (Kim et al., 2010), though there are reports of strains that do not follow the patterns (Shallom et al., 2013). *M. chelonae* lacks *erm (41)* and thus does not exhibit macrolide-induced resistance. *M. abscessus* subsp. *massiliense* usually contains 2 deletions in *erm (41)*, resulting in a nonfunctional *erm (41)* and susceptibility to macrolides (Hanson et al., 2014; Kim et al., 2010). In contrast, *M. abscessus* subsp. *abscessus* and *M. abscessus* subsp. *bolletii* possess the *erm (41)* gene and may develop resistance to clarithromycin during therapy. Noninducible *erm* (41)–susceptible isolates of both *M. abscessus* subsp. *abscessus and* subsp. *bolletiia*may develop after a thymidine-to-cytosine polymorphism at position 28. This results in a tryptophan-to-arginine amino acid change at codon 10 and a nonfunctional *erm (41)* gene (Bastian et al., 2011; Nash et al., 2009).

In addition to *erm (41)*, a mutation in the 23S rRNA gene which encodes the peptidyltransferase domain causes base changes at position 2057, 2058, or 2059 and confers acquired macrolide resistance in the MC-AG (Luo et al., 2015; Nash et al., 2009). These *rrl* mutations are detected within routine incubation periods (Hanson et al., 2014). Further, an alanine to glycine mutation at position 1408 of the 16S rRNA gene confers resistance to most aminoglycosides (Prammananan et al., 1998). The presence of the *erm (41)* gene can be detected by PCR or sequencing, and the results can be used in resistance determination, as well as aid in subspecies identification. A molecular test called the GenoType NTM-DR Ver1.0 is available in Europe (but not FDA approved) for the detection of resistance due to either *erm (41)* or *rrl*. The advantage of molecular tests over traditional methods is that the analytical time is measured in hours and not days.

6. Treatment

Treatment of infections due to NTM remains difficult as they are resistant to many of the first-line antituberculosis medications and because there are so few other agents available for therapy. The correlation between in vitro sensitivity and clinical treatment outcomes for some drugs and NTM species has been poor; the role of antibiotic susceptibility testing in guiding treatment remains under debate (Cowman et al., 2016). *M. abscessus* group is one of the more antibiotic-resistant rapidly growing mycobacteria species, and only a few antibiotics are available for treatment. Clarithromycin became the drug of choice in the 1990s and remains part of the core strategy unless resistance has occurred due to either the *erm (41)* or *rrl* genes.

Untreated *M. abscessus* group isolates generally have low or intermediate MICs compared with achievable drug levels to clarithromycin (100%), amikacin (90%), and cefoxitin (70%) (Griffith et al., 2007). Most wild-type isolates of subspecies *massiliense* contain a nonfunctional *erm (41)* gene which makes the macrolides a viable treatment option. Therefore, the utility of a precise *M. abscessus* group subspecies identification could have value in drug selection for treatment (Mougari et al., 2016).

In the treatment of serious skin, soft tissue, and bone infections, clarithromycin should be combined with parenteral antibiotics (cefoxitin, amikacin, or imipenem). The macrolides are the most reliable oral agents that show in vitro activity to *M. abscessus* group. The most active of the parenteral agents is amikacin; a combination of amikacin and high-dose cefoxitin is recommended as an initial therapy

until clinical improvement is noted. Length of therapy should be from 4 months up to 6 months for more serious skin and bone infections (Brown-Elliott and Philley, 2017; Brown-Elliott and Wallace, 2015b; Griffith et al., 2007). Surgery is generally indicated with extensive disease, abscess, breast implants, and catheters when drug therapy may be difficult.

For pulmonary infections, the ATS guidelines recommend a goal of 12 months of negative sputum cultures while on therapy, but there is no medication strategy to reliably achieve this goal. Single-drug therapy with macrolides is not sufficient to produce microbiologic cure for *M. abscessus* group lung disease. Combination drug therapy with amikacin and cefoxitin or imipenem generally produces clinical and microbiologic improvement, but the cost and morbidity are significant factors interfering with a chance at a cure. A recent paper showed a wide range of strategies in the approach to *M. abscessus* group infections. Inhaled amikacin has been used successfully for difficult-to-treat NTM pulmonary disease, but randomized controlled trials are still needed to better evaluate risks and benefits (Olivier et al., 2014; Yagi et al., 2017). Liposomal amikacin was approved by the FDA in September 2018 for *Mycobacterium avium* complex disease; some clinicians may use this new product to treat refractory *M. abscessus* group respiratory infections.

Clofazamine is an antituberculous drug that has historically been used in the treatment of MTB and leprosy. There are some published in vitro studies that show >90% susceptibility (MIC ≤1 μg/mL); a combination of clofazamine and amikacin demonstrated significant synergistic activity against selected *M. abscessus* group isolates (van Ingen et al., 2012). In a retrospective review of 42 patients treated with clofazamine-containing regimens for *M. abscessus* group lung disease, the treatment response rate based on symptoms was 81%. However, only 24% of patients had converted to culture-negative sputum after therapy, and only 31% had a radiographic response (Yang et al., 2017).

Other drug classes that have shown some promise in the treatment of *M. abscessus* group infections include the oxazolidinones and glycylcyclines (Cowman et al., 2016; Griffith et al., 2007; Wallace et al., 2001, 2014). In 1 study, more than 60% of patients with MC-AG infections treated with tigecycline, including those with underlying cystic fibrosis, showed improvement despite failure of prior antibiotics. When bedaqualine has been used in salvage treatment, 60% (6 of 10) of patients demonstrated a microbiological response at 6 months, with 50% having 1 or more negative cultures (Philley et al., 2015).

7. Summary

In summary, infections due to the MC-AG are being increasingly recognized in the medical profession through greater awareness and better molecular identification methods. These organisms may be ubiquitous in some environments which may help facilitate transfer to humans and result in infections. Clinically significant isolates should be identified to the species level, at a minimum, to distinguish *M. abscessus* group from *M. chelonae* group given the differences in antibiotic resistance. It may become standard of care to identify to the subspecies level clinically important *M. abscessus* group isolates. Detecting the presence of or ruling out a functional *erm (41)* gene may be helpful in difficult-to-treat cases. When *erm (41)* gene testing is not available, it would be clinically valuable to identify *M. abscessus* group isolates to the subspecies level.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflicts of interest

There is no conflict of interest.

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