New RAPMYCOI Sensititre™ Antimicrobial Susceptibility Test for Atypical Rapidly Growing Mycobacteria (RGM)

Anna Borek *, Anna Zabost , Agnieszka Glogowska , Dorota Filipczak and Ewa Augustynowicz-Kopeć

Department of Microbiology National Tuberculosis and Lung Diseases Research Institute, 01-138 Warsaw, Poland

* Correspondence: a.borek@igichp.edu.pl

Abstract: Rapidly growing mycobacteria (RGM) cause an increasing international concern, mainly due to their natural resistance to many antibiotics. The aim of this study was to conduct species identification and determine the antimicrobial susceptibility profiles of RGM isolated in Poland. Antimicrobial susceptibility was tested using broth microdilution and the RAPMYCOI panel. A total of 60 strains were analysed, including the following species: M. fortuitum complex (30), M. abscessus subsp. abscessus (16), M. abscessus subsp. massiliense (7), M. chelonae (5), and M. mucogenicum (2). For 12 M. abscessus subsp. abscessus strains, the presence of the erm 41T28 genotype associated with inducible macrolide resistance and a functional erm gene was confirmed. A MUT2 mutation in the rrl gene (constitutive resistance) was identified for two strains from the subtype M. abscessus subsp. massiliense. Among the 15 tested antibiotics, amikacin and linezolid had the strongest antimycobacterial activity. Most of the tested strains were resistant to doxycycline and trimethoprim/sulfamethoxazole. Tigecycline MICs were low for all tested strains. Findings from our study highlight the importance of correct identification of clinical isolates and antimicrobial susceptibility testing.

Keywords: rapidly growing mycobacteria; antimicrobial resistance; broth microdilution; minimal inhibitory concentration (MIC)

1. Introduction

Nontuberculous mycobacteria (NTM), also known as mycobacteria other than tuberculosis (MOTT), are ubiquitous environmental microorganisms [1]. Currently, more than 150 species are known worldwide and many of them are increasingly recognized as important human pathogens [2,3]. Based on their growth rate, NTM species are divided into slowly growing mycobacteria (SGM) and rapidly growing mycobacteria (RGM) [4]. To date, more than 75 RGM species have been identified, which represents approximately 50% of all known mycobacterial species [5]. Due to advances in molecular research, the number of newly discovered species continues to increase.

RGM species are classified into six main taxonomic groups, distinguished based on genetic relatedness and the presence of pigment. These are:

1. M. fortuitum (M. fortuitum, M. peregrinum, M. sengalense, M. porcinum, M. neworleansense, M. boenickei, M. houstonense, M. brisbanense, M. septicum, and M. setense),
2. M. chelonae/M. abscessus complex (M. chelonae, M. immunogenum, M. franklinii, M. salmoniphilum, M. abscessus subsp. abscessus, M. abscessus subsp. massiliense, M. abscessus subsp. bolletii),
3. M. smegmatis (M. smegmatis and M. goodii),
4. M. mucogenicum (M. mucogenicum, M. phocaicum, and M. aubagnense),
5. M. mageritense/M. wolinskyi, and
6. pigmented RGM species (M. neoaurum, M. canariasense, M. cosmeticum, M. monacense, and M. bacteremicum) [6–12].

Tuberculosis caused by Mycobacterium tuberculosis complex remains a serious global health problem in developing countries [13]. However, in recent decades, the number of
reported cases of mycobacteriosis, a disease caused by atypical mycobacteria, has increased significantly [14]. Factors contributing to the increased incidence of NTM infections include: demographic changes, ageing of the population, increased incidence of comorbidities, and immunosuppression [15]. However, the epidemiology of NTM infections remains unknown as the reporting of mycobacterial cases to public health authorities is not mandatory in most countries [16]. Undoubtedly, the diagnosis of the disease is facilitated by recently improved testing methods [17].

Epidemiological statistics indicate that people living in Asia are particularly susceptible to NTM infections. In 2014, the incidence of NTM in the Japanese population was estimated at 14.7/100,000 [18–20]. In Great Britain, the incidence of NTM infection increased from 0.9 to 2.9/100,000 between 1995 and 2006 [21]. Studies from North America and Australia revealed that the annual incidence of NTM in these regions in 1997–2010 was 3.2–9.8/100,000 [14]. In Denmark, the incidence of NTM increased between 2003 and 2008 from 0.6 to 1.5/100,000 [22]. In Poland, statistics published by the National Institute of Public Health (PZH) and the Chief Sanitary Inspectorate show that the incidence rate of mycobacteriosis was 0.69 in 2017, 0.63 in 2018, and 0.61/100,000 in 2019 [23].

Atypical mycobacteria are opportunistic pathogens, ubiquitous in the environment, and are found in fresh and marine water, soil, and on biofilms [24]. Infections mainly concern the population of high-risk patients, which includes patients with cystic fibrosis (CF), bronchiectasis, emphysema, chronic obstructive pulmonary disease (COPD), and immunoincompentence (human immunodeficiency virus (HIV) infection, organ transplant, diabetes mellitus, renal failure). Among rapidly growing mycobacteria, the highly pathogenic non-pigmented species include M. fortuitum, M. abscessus, and M. chelonae, which are responsible for more than 80% of all clinical cases [25].

The clinical manifestations of RGM infections are very diverse. They most often concern the respiratory tract, skin, soft tissues, bones and joints, lymphadenitis, or disseminated infections [26]. Chronic lung infections are usually caused by M. abscessus subsp. abscessus and M. abscessus subsp. massiliense [25,27]. In patients with cystic fibrosis, these pulmonary infections are associated with a very high mortality. M. fortuitum complex is most frequently isolated from infected skin after accidental injuries, cosmetic procedures, and laser surgery. Reportedly, M. abscessus is responsible for 90% of respiratory diseases caused by RGM, and M. fortuitum is responsible for 60–80% of postsurgical and catheter-related infections [26]. The most common symptoms of infection caused by M. chelonae are diseases of the skin, bones, and soft tissues as well as ophthalmic infections, including keratitis. Rapidly growing mycobacteria are also isolated from patients with catheter-related bloodstream infection. In this case, the causative pathogens are M. mucogenicum and M. fortuitum, but also M. neoaurum and M. bacteremicum [28–31].

To determine the etiological factors of mycobacteriosis, it is necessary to correctly identify atypical mycobacteria to the species level. This is due to the different antimicrobial susceptibilities of mycobacteria. The management of a wide spectrum of NTM infections is a serious challenge worldwide. The selection of the appropriate antibiotic therapy for the patient should be based on the results of in vitro antimicrobial susceptibility testing. However, the suitability of antimicrobial susceptibility testing in the treatment of patients with mycobacteriosis remains controversial due to the discrepancy between test results and clinical response [27,32]. Good correlations demonstrated in the studies carried out to date have been shown for two groups of antibiotics: macrolides and aminoglycosides. Macrolides (clarithromycin and azithromycin) are among the basic antibiotics used in the treatment of mycobacteriosis. All macrolides bind to the V domain in 23S rRNA on the 50S ribosome subunit [33]. Two mechanisms of resistance to this class of drugs have been identified so far among atypical mycobacteria. The first mechanism is the constitutive resistance associated with a point mutation at either the A2058 or A2059 position of the 23S rRNA (rrl gene). The second mechanism, defined as inducible macrolide resistance, is associated with functional erm genes encoding ribosomal methyltransferase. The erm genes have been identified in the following species: erm (41) in M. abscessus subsp.
abcessus (serovars I, VI, VII (80% of isolates)) and M. abscessus subsp. bolletii; erm (39) in M. fortuitum, M. houstonense, M. porcinum, and M. neworleansense; erm (38) in M. smegmatis and M. goodie; erm (40) in M. mageritense and M. wolinsky. Clarithromycin-sensitive strains lack or have damaged *erm* genes. This group includes the following species: *M. abscessus* subsp. *abcessus* serovar II (Mab30), *M. abscessus* subsp. *massiliense*, *M. chelonae*, *M. immunogenenum*, *M. mucogenicum* group, *M. peregrinum*, and *M. senegalense* [13,34,35].

The aminoglycosides (amikacin and tobramycin) act by binding stably to the 30S ribosomal subunit in bacterial cells, leading to misreading of the genetic code and inhibition of protein synthesis and consequently to cell death. Resistance to aminoglycosides in atypical mycobacteria is associated with single-point mutations in the 16S rRNA (*rrs*) gene [36].

According to the Clinical and Laboratory Standards Institute (CLSI), the broth microdilution method is considered the gold standard for testing the drug sensitivity of atypical RGM strains. Antimicrobial susceptibility testing should include the following antibiotics: clarithromycin, amikacin, moxifloxacin, linezolid, imipenem, cefoxitin, ciprofloxacin, doxycycline, trimethoprim/sulfamethoxazole, and tobramycin (only for *M. chelonae*). It is also recommended to determine the minimal inhibitory concentration (MIC) value for tigecycline, but to date there are no consensus breakpoints or guidelines for the interpretation of results [34,37].

There is a commercially available RAPMYCOI test for RGM from Thermo Fisher Scientific (Waltham, MA, USA) that includes all the antibiotics recommended for the treatment of RGM infections. *M. fortuitum* complex, *M. abscessus* subsp. *abcessus*, *M. abscessus* subsp. *massiliense* and *M. chelonae* are the most common rapidly growing mycobacteria (RGM) isolated in Poland.

In the presented study, the susceptibility of 60 RGM strains to 15 antibiotics was determined using the RAPMYCOI panels. The obtained results were compared with data published worldwide, which made it possible to obtain a complete picture of the drug resistance in this group of mycobacteria.
Erm (41) gene was only detected in members of the M. abscessus complex. The above test detected mutations at position 28 of the erm (41) gene:

- If the strain had a genotype in which C was at position 28 it meant that the tested strain was sensitive to macrolides.
- If the strain had a genotype in which T was at position 28 it meant that the tested strain was resistant to macrolides.

In the Tables 1 and 2 below, the mutations detected by the applied test was shown.

Table 1. Mutations determining resistance to macrolides detected using the GenoType NTM-DR assay within the rrl gene.

| Absence of Wild-Type Band | Analysed Nucleic Acid Positions | Mutation Bands Present | Mutation | Phenotypic Resistance |
|---------------------------|---------------------------------|------------------------|----------|-----------------------|
| **rrl WT**                | 2058–2059                      | **rrl MUT1**           | A2058C   | macrolides            |
|                           |                                 | **rrl MUT2**           | A2058G   |                       |
|                           |                                 | **rrl MUT3**           | A2058T   |                       |
|                           |                                 | **rrl MUT4**           | A2059C   |                       |
|                           |                                 |                        | A2059G   |                       |
|                           |                                 |                        | A2059T   |                       |

Table 2. Mutations determining resistance to aminoglycosides detected using the GenoType NTM-DR assay within the rrs gene.

| Absence of Wild-Type Band | Analysed Nucleic Acid Positions | Mutation Bands Present | Mutation | Phenotypic Resistance |
|---------------------------|---------------------------------|------------------------|----------|-----------------------|
| **rrs WT**                | 1406–1409                      | **rrs MUT1**           | A1408G   | aminoglycosides       |
|                           |                                 |                        | T1406A   |                       |
|                           |                                 |                        | C1409T   |                       |

2.4. Phenotypic Determination of Antimicrobial Susceptibility Profile

Antimicrobial susceptibility was tested using broth microdilution. For this purpose, 96-well RAPMYCOI Sensititre™ titration plates (Thermo Fisher Scientific, Waltham, MA, USA) were used, which allow for the simultaneous determination of susceptibility to 15 antibiotics.

RAPMYCOI plates contain freeze-dried antibiotics in a range of concentrations (µg/mL). The plate design and the tested antibiotic concentrations are presented in Figure 1.

At the first stage of the test, an inoculum of a mycobacterial suspension at the optical density of 0.5 McFarland scale was prepared. A total of 50 µL of inoculum was transferred to 10 mL of CAMHB medium (cation-supplemented Mueller-Hinton broth and TES buffer) (Thermo Fisher Scientific, Waltham, MA, USA). The 100 µL suspension prepared according to this protocol was pipetted onto a 96-well titration plate and incubated at 30 °C ± 2 °C. Plates with RGM were incubated for 3 to 5 days. Only for clarithromycin, the incubation period was prolonged to 14 days in order to detect inducible resistance associated with the presence of the erm genes. If microbial growth in the positive control sample was sufficient, MICs were measured. In cases of difficulties with visual reading, 10 µL of Alamar Blue (BIO-RAD, Hercules, CA, USA) reagent and 25 µL of 5% Tween 80 (Fisher Scientific, Hampton, NH, USA) were added. A colour change from blue to pink indicated the growth of a strain. Measured MICs were interpreted and each strain was classified into one of three groups (sensitive (S), intermediate (I), and resistant (R)) in accordance with the CLSI guidelines (document M62, 1st edition) (37) (Table 3).
Antimicrobial agents and susceptibility breakpoints (MICs) for testing rapidly growing mycobacteria.

| Antimicrobial Agent | MIC (μg/mL) | Comment |
|---------------------|------------|---------|
| AMI                 | ≤16        | 32      | ≥64 | *M. abscessus* complex isolates with MIC of ≥64 μg/mL should be retested and/or the 16S rRNA gene sequenced to check for mutation |
| FOX                 | ≤16        | 32–64   | ≥128|
| CIP                 | ≤1         | 2       | ≥4  | Ciprofloxacin and levofloxacin are interchangeable, but both are less active than the newer B-methoxy-fluoroquinolones |
| CLA                 | ≤2         | 4       | ≥8  | See text for information on the erm gene; clarithromycin and azithromycin are interchangeable clinically |
| DOX                 | ≤1         | 2–4     | ≥8  |
| MIN                 | ≤1         | 2–4     | ≥8  |
| IMI                 | ≤4         | 8–16    | ≥32 |
| LZD                 | ≤8         | 16      | ≥32 |
| MXF                 | ≤1         | 2       | ≥4  |
| TMP-SMX             | ≤2/38      | ≥4/76   |     | MIC is 80% inhibition |
| TOB                 | ≤2         | 4       | ≥8  | Predominantly for *M. chelonae*; if MIC >4 μg/mL, the test should be repeated and/or the identification confirmed by *ropB* gene sequencing |
| TGC                 |            |         | POS |

3. Results

Table 4 below presents the percentage of strains that are sensitive, intermediate, and resistant to particular antibiotics. The classification was made on the basis of the obtained MIC values.
Table 4. Classification of analysed RGM species into groups: (S)-sensitive, (I)-intermediate, and (R)-resistant, based on the measured MIC values.

| Antibiotic Agent | M. abscessus subsp. abscessus n = 16 | M. abscessus subsp. massiliense n = 7 | M. chelonae n = 5 | M. mucogenicum n = 2 | M. fortuitum complex n = 30 |
|------------------|-------------------------------------|-------------------------------------|------------------|------------------|--------------------------|
| AMI              | 100 (S) 86 (S) 14 (R)              | 100 (S) 80 (S) 20 (R)              | 100 (S)         | 100 (S)         | 100 (S)                   |
| FOX              | 100 (I) 86 (I) 14 (S)              | 100 (S) 80 (S) 20 (R)              | 100 (S)         | 100 (S)         | 100 (S)                   |
| CIP              | 81 (R) 19 (I)                      | 100 (R) 80 (R) 20 (I)              | 100 (S)         | 100 (S)         | 100 (S)                   |
| CLA              | 75 (R) 25 (S)                      | 100 (S) 80 (R) 20 (I)              | 100 (S)         | 100 (S)         | 100 (S)                   |
| IMI              | 100 (I) 100 (I)                    | 80 (R) 20 (I) 50 (S) 50 (I)        | 63 (I) 27 (S) 10 (R) |
| LID              | 75 (R) 25 (S)                      | 100 (S) 100 (S)                    | 93 (S) 7 (I)    |
| DOX              | 100 (R)                            | 100 (R) 50 (S)                     | 56,6 (R) 43,3 (S) |
| MIN              | 100 (R)                            | 57 (S) 43 (R) 100 (R)              | 56,6 (R) 43,3 (S) |
| MXF              | 81 (R) 19 (I)                      | 80 (R) 20 (S) 100 (S)              | 100 (S)         |
| SXT              | 100 (R)                            | 100 (S) 100 (S)                    | 70 (R) 30 (S)   |

The data obtained in the performed antimycobacterial susceptibility test showed that amikacin and linezolid had the strongest antituberculotic activity against RGM. Most of the analysed strains were resistant to doxycycline and trimethoprim/sulfamethoxazole.

The following tables (Tables 5–9) present the obtained results separately for each RGM species.

Table 5. Results of in vitro susceptibility testing for M. abscessus subsp. abscessus strains.

| AMI | FOX | CIP | CLA | DOX | IMI | LZD | MIN | MXF | TGC | SXT |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1   | 4 (S) | 32 (I) | 2 (I) | 0.5 (S) | >16 (R) | 16 (I) | 8 (S) | >8 (R) | 4 (R) | >8/152 (R) |
| 2   | 8 (S) | 32 (I) | 4 (R) | >16 (R) | 16 (R) | >16 (R) | >1 (S) | >8 (R) | 2 (I) | 0.5 | 8/152 (R) |
| 3   | 4 (S) | 32 (I) | 4 (R) | >16 (R) | 16 (R) | 16 (I) | 8 (S) | >8 (R) | 4 (R) | 0.5 | >8/152 (R) |
| 4   | 2 (S) | 32 (I) | 4 (R) | >16 (R) | 16 (R) | 16 (I) | 8 (S) | >8 (R) | 4 (R) | 0.5 | >8/152 (R) |
| 5   | 4 (S) | 32 (I) | 4 (R) | >16 (R) | 16 (R) | 16 (I) | 8 (S) | >8 (R) | 4 (R) | 0.12 | >8/152 (R) |
| 6   | 2 (S) | 32 (I) | >4 (R) | >16 (R) | >16 (R) | 8 (I) | 8 (S) | >8 (R) | 8 (R) | 0.5 | >8/152 (R) |
| 7   | 4 (S) | 32 (I) | 4 (R) | >16 (R) | >16 (R) | 16 (I) | 2 (S) | >8 (R) | 4 (R) | 0.06 | 8/152 (R) |
| 8   | 4 (S) | 32 (I) | 2 (I) | 2 (S) | >16 (R) | 16 (I) | 8 (S) | >8 (R) | 4 (R) | 1 | 8/152 (R) |
| 9   | 8 (S) | 32 (I) | 4 (R) | 1 (S) | >16 (R) | 16 (I) | 8 (I) | 4 (S) | >8 (R) | 4 (R) | 0.25 | >8/152 (R) |
| 10  | 4 (S) | 32 (I) | 4 (R) | >16 (R) | >16 (R) | 8 (I) | 8 (S) | >8 (R) | 4 (R) | 0.25 | >8/152 (R) |
| 11  | 4 (S) | 32 (I) | >4 (R) | >16 (R) | >16 (R) | 16 (I) | 16 (I) | >8 (R) | >8/152 (R) | 1 | >8/152 (R) |
| 12  | 8 (S) | 32 (I) | >4 (R) | >16 (R) | >16 (R) | 16 (I) | 16 (I) | >8 (R) | >8 (R) | 1 | >8/152 (R) |
| 13  | 4 (S) | 32 (I) | 2 (I) | >16 (R) | >16 (R) | 16 (I) | 2 (S) | >8 (R) | 2 (I) | 0.25 | 4/76 (R) |
| 14  | 4 (S) | 32 (I) | 4 (R) | >16 (R) | >16 (R) | 16 (I) | 4 (S) | >8 (R) | 2 (I) | 0.25 | >8/152 (R) |
| 15  | 4 (S) | 64 (I) | 4 (R) | >16 (R) | >16 (R) | 16 (I) | 8 (S) | >8 (R) | 8 (R) | 0.5 | >8/152 (R) |
| 16  | 4 (S) | 32 (I) | >4 (R) | 0.12 (S) | >16 (R) | 16 (I) | 4 (S) | >8 (R) | 4 (R) | 0.25 | >8/152 (R) |
Table 6. Results of in vitro susceptibility testing for *M. abscessus* subsp. *massiliense* strains.

| AMI | FOX | CIP | CLA | DOX | IMI | LZD | MIN | MXF | TGC | SXT |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1   | 4 (S) | 32 (I) | 4 (R) | 0.25 (S) | >16 (R) | 16 (I) | 8 (S) | >8 (R) | 8 (R) | 1 | >8/152 (R) |
| 2   | >64 (R) | 16 (S) | 4 (R) | ≤0.06 (S) | >16 (R) | 16 (I) | 2 (S) | >8 (R) | 2 (I) | 0.12 | 8/152 (R) |
| 3   | 4 (S) | 32 (I) | 4 (R) | 0.12 (S) | 8 (R) | 16 (I) | 8 (S) | 2 (S) | 4 (R) | 0.25 | >8/152 (R) |
| 4   | 8 (S) | 32 (I) | >4 (R) | 0.25 (S) | >16 (R) | 16 (I) | 8 (S) | >8 (R) | >8 (R) | 0.5 | >8/152 (R) |
| 5   | 4 (S) | 32 (I) | 4 (R) | >16 (R) | 16 (R) | 16 (I) | 8 (S) | 2 (S) | 8 (R) | 0.5 | 8/152 (R) |
| 6   | 4 (S) | 32 (I) | 4 (R) | >16 (R) | 16 (R) | 16 (I) | 8 (S) | 2 (S) | 8 (R) | 0.5 | 8/152 (R) |
| 7   | 8 (S) | 32 (I) | >4 (R) | 0.25 (S) | >16 (R) | 16 (I) | 8 (S) | 2 (S) | >8 (R) | 0.5 | >8/152 (R) |

Table 7. Results of in vitro susceptibility testing for *M. chelonae* strains.

| AMI | FOX | CIP | CLA | DOX | IMI | LZD | MIN | MXF | TGC | TOB | SXT |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1   | 16 (S) | >128 (R) | 4(R) | 0.5 (S) | >16 (R) | 16 (I) | 4 (S) | >8 (R) | 1 (S) | 0.5 | 2 (S) | 8/152 (R) |
| 2   | 8 (S) | 64 (I) | 4(R) | ≤0.06 (S) | >16 (R) | 32 (R) | 4 (S) | >8 (R) | 4(R) | 0.25 | ≤1 (S) | 8/152 (R) |
| 3   | 4 (S) | 64 (I) | 4(R) | 0.25 (S) | >16 (R) | 32 (R) | 4 (S) | >8 (R) | 4(R) | 0.5 | ≤1 (S) | >8/152 (R) |
| 4   | 4 (S) | 64 (I) | 4(R) | 0.25 (S) | >16 (R) | 32 (R) | 4 (S) | >8 (R) | 4(R) | 0.5 | ≤1 (S) | >8/152 (R) |
| 5   | 8 (S) | 64 (I) | 4(R) | ≤0.06 (S) | >12 (R) | 64(R) | 4 (S) | >8 (R) | 4(R) | 0.25 | ≤1 (S) | >8/152 (R) |

Table 8. Results of in vitro susceptibility testing for *M. mucogenicum* strains.

| AMI | FOX | CIP | CLA | DOX | IMI | LZD | MIN | MXF | TGC | SXT |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1   | 2 (S) | 16 (S) | 0.25 (S) | 0.25 (S) | >16 (R) | 8 (I) | 2 (S) | >8 (R) | ≤0.25 (S) | 0.25 | 1/19 (S) |
| 2   | ≤1 (S) | 8 (S) | 0.5 (S) | 0.12 (S) | ≤0.12 (S) | 4 (S) | 2 (S) | ≤1 (S) | 0.5 (S) | 0.12 | 0.5/9.5 (S) |

3.1. *Mycobacterium abscessus* subsp. *abscessus*

All strains representing *Mycobacterium abscessus* subsp. *abscessus* were sensitive only to amikacin. Of the 16 strains, 12 (75%) were also sensitive to linezolid. However, they were all resistant to minocycline, trimethoprim/sulfamethoxazole, and doxycycline. Of the 16 strains, 12 (75%) were ciprofloxacin- and moxifloxacin-resistant. Among the 16 strains from this subtype, 12 (75%) were clarithromycin-resistant (MIC > 16 µg/mL) (Table 5). The GenoType NTM-DR assay confirmed the presence of the functional *erm* (41) gene in these strains, associated with inducible macrolide resistance (*erm*41T28 genotype). Another four strains were sensitive to clarithromycin (*erm*41C28 genotype).

3.2. *Mycobacterium abscessus* subsp. *massiliense*

Strains representing *Mycobacterium abscessus* subsp. *massiliense* were sensitive to linezolid (100%) and amikacin (86%). They were all resistant to trimethoprim/sulfamethoxazole, doxycycline, and ciprofloxacin. Of the 7 strains, 2 (29%) representing the above subtype were resistant to clarithromycin (Table 6). The GenoType NTM-DR assay revealed the presence of the MUT2 mutation in the *rrl* gene (constitutive resistance) (Table 1). One strain representing *Mycobacterium abscessus* subsp. *massiliense* and sensitive to clarithromycin, with the MUT1 mutation in the *rrs* gene, was resistant to amikacin (MIC > 64 µg/mL) (Table 2).

3.3. *Mycobacterium chelonae*

All *Mycobacterium chelonae* strains (5) were sensitive to amikacin, clarithromycin, linezolid, and tobramycin, but resistant to trimethoprim/sulfamethoxazole, ciprofloxacin, and doxycycline (Table 7).
### Table 9. Results of in vitro susceptibility testing for *M. fortuitum* complex strains.

| AMI | FOX | CIP | CLA | DOX | IMI | LZD | MIN | MXF | TGC | SXT |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1.≤1 (S) | 16 (S) | 0.5 (S) | 0.5 (S) | ≤2 (S) | 4 (S) | ≤1 (S) | ≤0.25 (S) | 0.12 | 2/38 (S) |
| 2.≤1 (S) | 16 (S) | 0.12 (S) | 0.12 (S) | >16 (R) | ≤2 (S) | 2 (S) | >8 (R) | 0.25 | 0.5 | 4/76 (R) |
| 3.≤1 (S) | 32 (I) | 0.12 (S) | >16 (R) | >16 (R) | 4 (S) | 2 (S) | >8 (R) | 0.25 | 0.25 | 0.5/9.5 (S) |
| 4.≤1 (S) | 16 (S) | 0.12 (S) | 0.12 (S) | >16 (R) | 4 (S) | ≤1 (S) | >8 (R) | 0.25 | 0.25 | 1/19 (S) |
| 5.≤1 (S) | 16 (S) | 0.12 (S) | >16 (R) | ≤0.12 (S) | 8 (I) | ≤1 (S) | ≤1 (S) | ≤0.25 | 0.25 | 2/38 (S) |
| 6.≤1 (S) | 32 (I) | 0.12 (S) | >16 (R) | >16 (R) | 8 (I) | 16 (I) | >8 (R) | 0.25 | 0.5 | 8/152 (R) |
| 7.≤1 (S) | 16 (S) | 0.25 (S) | >16 (R) | 0.5 (S) | 4 (S) | ≤1 (S) | ≤1 (S) | ≤0.25 | 0.25 | 1/19 (S) |
| 8.≤1 (S) | 16 (S) | 0.12 (S) | 0.25 (S) | >16 (R) | 4 (S) | ≤1 (S) | >8 (R) | 0.25 | 0.25 | 1/19 (S) |
| 9.≤1 (S) | 64 (I) | 2 (I) | >16 (R) | 0.5 (S) | 64(R) | 8 (S) | >8 (R) | 1 (S) | 1 | >8/152 (R) |
| 10.≤1 (S) | 64 (I) | 0.25 (S) | >16 (R) | >16 (R) | 16 (I) | 8 (S) | >8 (R) | 0.25 | 0.25 | >8/152 (R) |
| 11.2 (S) | 64 (I) | 0.25 (S) | >16 (R) | >16 (R) | 16 (I) | 8 (S) | >8 (R) | 0.5 | 0.25 | 4/76 (R) |
| 12.4 (S) | 64 (I) | 0.25 (S) | >16 (R) | 0.25 (S) | 64 (R) | 4 (S) | ≤1 (S) | ≤0.25 | 0.25 | 0.25 | 4/76 (R) |
| 13.≤1 (S) | 32 (I) | ≤0.12 (S) | ≤0.12 (S) | 4 (S) | 4 (S) | ≤1 (S) | ≤0.25 | 0.25 | 0.25 | 1/19 (S) |
| 14.≤1 (S) | 32 (I) | 0.25 (S) | >16 (R) | >16 (R) | 8 (I) | 8 (S) | >8 (R) | 0.25 | 0.25 | 8/152 (R) |
| 15.≤1 (S) | 32 (I) | ≤0.12 (S) | >16 (R) | 8 (I) | 8 (S) | >8 (R) | 0.25 | 0.25 | 0.25 | 8/152 (R) |
| 16.≤1 (S) | 8 (S) | ≤0.12 (S) | >16 (R) | 8 (R) | 4 (S) | 2 (S) | ≤1 (S) | 0.25 | 0.25 | 4/76 (R) |
| 17.≤1 (S) | 32 (I) | 0.25 (S) | >16 (R) | >16 (R) | 8 (I) | 8 (S) | >8 (R) | 0.25 | 0.25 | >8/152(R) |
| 18.≤1 (S) | 32 (I) | 0.25 (S) | 16 (R) | >16 (R) | 8 (I) | 8 (S) | >8 (R) | 0.25 | 0.25 | >8/152(R) |
| 19.≤1 (S) | 32 (I) | 0.25 (S) | >16 (R) | >16 (R) | 8 (I) | 8 (S) | >8 (R) | 0.25 | 0.25 | >8/152(R) |
| 20.≤1 (S) | 32 (I) | ≤0.12 (S) | >16 (R) | 0.25 (S) | 16 (I) | 4 (S) | ≤1 (S) | ≤0.25 | 0.25 | 0.25 | 4/76 (R) |
| 21.≤1 (S) | 32 (I) | ≤0.12 (S) | >16 (R) | >16 (R) | 8 (I) | 2 (S) | >8 (R) | 0.25 | 0.25 | 1/19 (S) |
| 22.≤1 (S) | 32 (I) | ≤0.12 (S) | >16 (R) | 0.5 (S) | 8 (I) | 8 (S) | ≤1 (S) | ≤0.25 | 0.25 | 4/76 (R) |
| 23.≤1 (S) | 32 (I) | ≤0.12 (S) | >16 (R) | 0.12 (S) | 8 (I) | 4 (S) | ≤1 (S) | ≤0.25 | 0.25 | 4/76 (R) |
| 24.≤1 (S) | 32 (I) | ≤0.12 (S) | >16 (R) | 0.25 (S) | 8 (I) | 4 (S) | ≤1 (S) | ≤0.25 | 0.25 | 4/76 (R) |
| 25.≤1 (S) | 16 (S) | 0.5 (S) | 1 (S) | 0.25 (S) | 8 (I) | 8 (S) | ≤1 (S) | ≤0.25 | 0.25 | 4/76 (R) |
| 26.≤1 (S) | 32 (I) | ≤0.12 (S) | >16 (R) | >16 (R) | 16 (I) | 16 (I) | >8 (R) | 0.25 | 0.25 | 4/76 (R) |
| 27.≤1 (S) | 32 (I) | 0.5 (S) | 0.25 (S) | 0.25 (S) | 32 R | 8 (S) | ≤1 (S) | ≤0.25 | 0.25 | 4/76 (R) |
| 28.≤1 (S) | 16 (S) | ≤0.12 (S) | >16 (R) | 0.25 (S) | 16 (I) | 4 (S) | ≤1 (S) | ≤0.25 | 0.25 | 8/152 (R) |
| 29.≤1 (S) | 16 (S) | ≤0.12 (S) | 0.12 (S) | 16 (R) | 8 (I) | 8 (S) | 8 (R) | 0.25 | 0.25 | 1/19 (S) |
| 30.≤1 (S) | 32 (I) | ≤0.12 (S) | >16 (R) | >16 (R) | 16 (I) | 8 (S) | >8 (R) | 0.25 | 0.25 | >8/152 (R) |

### 3.4. Mycobacterium mucogenicum

Two tested *Mycobacterium mucogenicum* strains (100%) were sensitive to clarithromycin, amikacin, cefoxitin, ciprofloxacin, moxifloxacin, and trimethoprim/sulfamethoxazole. One strain was resistant to doxycycline (MIC >16 µg/mL) (Table 8).

### 3.5. Mycobacterium fortuitum Complex

All strains representing *Mycobacterium fortuitum* complex (30) were sensitive to amikacin and moxifloxacin, 29 out of 30 strains were also sensitive to ciprofloxacin, 23 (77%) were resistant to clarithromycin, 17 (57%) were resistant to doxycycline, and 21 (70%) were resistant to trimethoprim/sulfamethoxazole (Table 9).

In the analysed collection of RGM, all 60 (100%) strains had low MIC values (from 0.06 to 1 µg/mL) for tigecycline, an antibiotic considered as a potential therapeutic agent and a drug of last resort in the treatment of severe cases of mycobacteriosis.

### 4. Discussion

With advances in molecular techniques and genetic tools, including whole genome sequencing (WGS), knowledge about the genetic diversity of NTM species and genes determining resistance to antibiotics continues to grow. Long treatment (18 to 24 months on average) and the need to use a combination of antibiotics with multiple side effects increase the importance of drug resistance testing, especially in RGM strains naturally resistant to first-line antitubercuclotic drugs.
Guidelines on antimicrobial susceptibility testing (AST) of atypical mycobacteria were developed by the CLSI and last updated in December 2018. Currently, CLSI M24 (3rd edition) provides recommendations on AST for slowly growing non-tuberculous mycobacteria, including *M. avium* complex (MAC), *M. kansasii*, and *M. marinum*, as well as rapidly growing mycobacteria (RGM) [34]. Since atypical mycobacteria may colonize the respiratory tract, their isolation from clinical specimens does not always correlate with the identification of an etiological factor responsible for the observed changes. This primarily refers to single sputum cultures. A negative sputum smear indicates a small number of microorganisms that are unlikely to be clinically significant, i.e., insufficient to establish a diagnosis of NTM. Therefore, detailed criteria for the diagnosis of mycobacteriosis have been developed for clinically significant isolates from the respiratory tract [27,38]. These criteria include the following:

- at least two NTM culture-positive sputa or one bronchial wash or lavage sample,
- a transbronchial or lung biopsy specimen with supporting mycobacterial histopathology and a positive NTM culture.

According to the current CLSI recommendations, AST includes antimicrobial agents for RGM such as amikacin, cefoxitin, ciprofloxacin, clarithromycin, doxycycline (or minocycline), imipenem, linezolid, moxifloxacin, trimethoprim-sulfamethoxazole, and tobramycin (for *M. chelonae* only) (Table 3). Worth noting is the fact that there are insufficient data to establish MIC breakpoints for tigecycline and clofazimine, and therefore for these agents a MIC without interpretation should be given [34].

The results of AST with selected drugs may concern specific species of atypical mycobacteria. For this reason, CLSI and most experts in RGM recommend identifying RGM strains at the species or even subspecies level (Table 10), especially for the *M. abscessus* complex, before performing a new AST RAPMYCOI and initiating treatment [5,34,39,40].

**Table 10. Interpretation of AST results for *M. abscessus* complex and clarithromycin.**

| Sensitivity to Clarithromycin on Days 3–5 of Incubation | Sensitivity to Clarithromycin on Day 14 of Incubation | Genetic Mechanisms | Subspecies of *M. abscessus* | Phenotypic Sensitivity to Macrolides |
|--------------------------------------------------------|-------------------------------------------------------|--------------------|-------------------------------|-----------------------------------|
| sensitive                                               | sensitive                                             | non-functional *erm* gene (41) | *M. a. massiliense* | sensitive to macrolides           |
| sensitive                                               | resistant                                             | functional *erm* gene (41)     | *M. a. abscessus*           | inducible resistance to macrolides |
| resistant                                               | resistant                                             | 23S point mutation in rRNA    | any of the above listed     | high constitutive resistance to macrolides |

Because the incubation period for most RGM species ranges from 2 to 5 days, the final MIC reading in the RAPMYCOI test should be performed <5 days. This is mainly due to the instability of some drugs, including carbapenems and tetracyclines. There are only two exceptions where this incubation time should be extended when performing the RAPMYCOI test. The first case concerns strains representing *M. abscessus* complex isolated from patients who had a history of long-term treatment, including patients with cystic fibrosis. Mycobacterial strains isolated from this population of patients need a longer incubation period; therefore, in some cases it may be helpful to change the incubation temperature or to establish a culture in a shaking incubator. However, if the culture incubation period is longer than 5 days, results are only reliable for AST related to two drugs: clarithromycin and amikacin. The CLSI recommends a comment on the AST report such as: this NTM strain required extended incubation and results for only clarithromycin and amikacin are reliable after incubation for >5 days (Table 10) [34].

The second exception in the RAPMYCOI test to the incubation period longer than 5 days is clarithromycin. Phenotypical detection of inducible resistance to macrolides is achieved by extending the incubation of clarithromycin to 14 days unless the MIC is \( \geq 16 \, \mu\text{g/mL} \) at an earlier time point. If the clarithromycin MIC is 4 or 8 \( \mu\text{g/mL} \) after
14 days of incubation, the test should be repeated. If the MIC is 4 or 8 \( \mu g/mL \) in the retest, sequencing of the \( erm \) gene for the given strain is recommended.

Worth noting is the fact that several RGM species have a non-functional or absent \( erm \) gene and are naturally sensitive to clarithromycin [41]. Therefore, sensitivity to clarithromycin can be reported at the initial MIC reading as no prolonged incubation is required for these specific species [42].

In the presented study, we identified 60 rapidly growing mycobacterial strains and determined their antimicrobial susceptibility in accordance with CLSI guidelines. The most frequently isolated species was \emph{Mycobacterium fortuitum} complex, which accounted for 50% (30/60) of all identified strains. The tests demonstrated that among all RGM species this group is characterized by high sensitivity to antibiotics. Our study confirmed this thesis and showed that 100% (30/30) of the strains from this group were sensitive to amikacin and moxifloxacin, 97% (29/30) were also sensitive to ciprofloxacin, and 93% (28/30) were sensitive to linezolid. In contrast, tests with clarithromycin showed a high level of resistance for 77% (23/30) of the strains. This resistance is higher compared to that reported by Sriram et al. (100% of sensitive strains among 30 tested) and Bhalla et al. (94.1% of sensitive strains among 17 tested) [43,44].

A low rate of drug resistance according to CLSI was also found for \emph{Mycobacterium chelonae}. In our study, 100% of the strains (5/5) were sensitive to amikacin, clarithromycin, linezolid, and tobramycin. Our findings are consistent with those reported by Bhalla et al. In the cited study, no resistance to the four above-mentioned antibiotics was found for the three tested isolates [43].

\emph{Mycobacterium chelonae} and \emph{Mycobacterium mucogenicum} are classified into the group of species lacking functional \( erm \) genes. In our study, all strains of \emph{M. chelonae} (5/5) and \emph{M. mucogenicum} (2/2) were sensitive to clarithromycin and no \( erm \) genes were detected. However, Esteban et al. detected resistance to clarithromycin associated with the presence of \( erm \) genes in two strains of \emph{M. chelonae} [45]. In a study by Davalos et al., 100% (2/2) of \emph{M. chelonae} strains were sensitive to clarithromycin. However, one strain (25%) of \emph{M. mucogenicum} resistant to this antibiotic was detected [46]. In our study, two strains representing \emph{M. mucogenicum} were sensitive to most of the tested antibiotics. Only one strain was resistant to doxycycline and minocycline. A different antimicrobial susceptibility profile for this species was found by Faridah et al., who reported resistance to ciprofloxacin, doxycycline, clarithromycin, and tobramycin in a strain isolated from blood [47].

Isolates representing the \emph{Mycobacterium abscessus} complex accounted for 38% (23/60) of all identified strains and it was the second largest group. Most strains (16) represented \emph{M. abscessus} subsp. \emph{abscessus} subtype, while \emph{M. abscessus} subsp. \emph{massiliense} subtype was less frequently identified (seven strains). We did not identify \emph{M. abscessus} subsp. \emph{bolletii}. In our study, most strains of \emph{M. abscessus} subsp. \emph{abscessus} (75%) were resistant to clarithromycin (MIC >16 \( \mu g/mL \)). This resistance was associated with the presence of a functional \( erm \) gene. The situation was different for \emph{M. abscessus} subsp. \emph{massiliense}. Only 28% of strains representing this subtype were clarithromycin-resistant, and the MUT2 mutation in the \( rrl \) gene was responsible for the resistance mechanism. Our findings confirm the worldwide reports on the more frequent resistance of \emph{M. abscessus} subsp. \emph{abscessus} to clarithromycin compared to \emph{M. abscessus} subsp. \emph{massiliense} [48–50]. Considering amikacin, the vast majority of strains were sensitive to this antibiotic. Only one strain (4%) was resistant to amikacin (MIC > 64 \( \mu g/mL \)) and had the MUT1 mutation in the \( rrs \) gene. Similar findings were reported by Bhalla et al., who found 92.3% of sensitive strains [43].

Among the three tested tetracyclines (doxycycline, minocycline, and tigecycline), the lowest MICs (from 0.06 to 1 \( \mu g/mL \)) were found for tigecycline. If we assume the criteria for interpretation proposed by Wallace et al. (resistant strain when MIC \( \geq 8 \mu g/mL \)), all tested strains (60/60) were sensitive to tigecycline [51]. Similar relationships were observed by Pang et al.: sensitivity to tigecycline was found for 96% (53/55) of strains from the \emph{M. abscessus} complex, 91% (10/11) of \emph{M. fortuitum} strains, and 100% (3/3) of \emph{M. chelonae} strains [52]. Similarly, in a study by Comba et al., the MIC value was <0.25 \( \mu g/mL \).
for 45.7% of the strains (16/35), and from 0.25 µg/mL to 0.5 µg for 54.3% of the strains (19/35) [53]. According to worldwide reports, tigecycline is used in the treatment of the most severe infections with RGM mycobacteria, but to date there are no CLSI guidelines for the interpretation of MIC values in the AST.

5. Conclusions

The new RAPMYCOI test is a rapid tool for the determination of drug resistance profile in RGM. The obtained results are reliable and reproducible, and the test setup is not time-consuming. The broth microdilution method on which the test is based and the selection of antibiotics are consistent with the CLSI guidelines.

Taken together, the findings from the presented study highlight the importance of a correct identification of clinical isolates to the species and subtype level and the role of antimicrobial susceptibility testing, especially for highly resistant rapidly growing mycobacteria (RGM). The obtained results confirm previous assumptions published worldwide according to which there are predictable drug resistance profiles depending on the identified mycobacterial species. However, there are some exceptions to this rule, and therefore the drug resistance of individual strains should be tested as standard practice. The correlation between data obtained from AST with clinical findings proving the effectiveness of treatment will enable the development of new therapeutic regimens. As a result, effective drugs can be selected and the patient’s treatment optimized at an early stage.

Author Contributions: Conceptualization, A.B. and E.A.-K.; methodology, A.B., A.G. and D.F.; formal analysis, A.B.; writing—original draft preparation, A.B.; writing—review and editing, A.G., A.Z. and E.A.-K.; supervision, A.Z. and E.A.-K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the statutory activity of National Tuberculosis and Lung Diseases Research Institute, Task No 1.8/2019.

Institutional Review Board Statement: The study was approved by the Ethics Committee of the National Tuberculosis and Lung Diseases Research Institute (KB-64/2018, 14 December 2018) as a part of research on mycobacterial diseases in human.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data supporting reported results can be found in source data collected in National Tuberculosis and Lung Diseases Research Institute.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Daley, C.L.; Iaccarino, J.M.; Lange, C.; Cambau, E.; Wallace, R.J., Jr.; Andrejak, C.; Böttger, E.C.; Brozek, J.; Griffith, D.E.; Guglielmetti, L.; et al. Treatment of nontuberculous mycobacterial pulmonary disease: An official ATS/ERS/SCMID/IDSA clinical practice guideline. Eur. Respir. J. 2020, 56, 2000535. [CrossRef] [PubMed]
2. Heifets, L. Mycobacterial infections caused by nontuberculous mycobacteria. Semin. Respir. Crit. Care Med. 2004, 25, 283–295. [CrossRef] [PubMed]
3. Van Ingen, J. Diagnosis of nontuberculous mycobacterial infections. Semin. Respir. Crit. Care Med. 2013, 34, 103–109. [CrossRef] [PubMed]
4. Wen, S.; Gao, X.; Zhao, W.; Huo, F.; Jiang, G.; Dong, L.; Zhao, L.; Wang, F.; Yu, X.; Huang, H. Comparison of the in vitro activity of linezolid, tedizolid, sutezolid, and delpazolid against rapidly growing mycobacteria isolated in Beijing, China. Int. J. Infect. Dis. 2021, 109, 253–260. [CrossRef] [PubMed]
5. Brown-Elliott, B.A.; Philley, J.V. Rapidly Growing Mycobacteria. Microbiol. Spectr. 2017, 5, 703–723. [CrossRef] [PubMed]
6. Schinsky, M.F.; Morey, R.E.; Steigerwalt, A.G.; Douglas, M.P.; Wilson, R.W.; Floyd, M.M.; Butler, W.R.; Daneshvar, M.I.; Brown-Elliott, B.A.; Wallace, R.J.; et al. Taxonomic variation in the Mycobacterium fortuitum third biovariant complex: Description of Mycobacterium boenickei sp. nov., Mycobacterium houstonense sp. nov., Mycobacterium neoaurleansense sp. nov. and Mycobacterium brisbanense sp. nov. and recognition of Mycobacterium porcinum from human clinical isolates. Int. J. Syst. Evol. Microbiol. 2004, 54, 1653–1667. [CrossRef] [PubMed]
7. Adékambi, T.; Berger, P.; Raoul, D.; Drancourt, M. rpoB gene sequence-based characterization of emerging non-tuberculous mycobacteria with descriptions of Mycobacterium bolletii sp. nov., Mycobacterium phocacium sp. nov. and Mycobacterium aubagenense sp. nov. Int. J. Syst. Evol. Microbiol. 2006, 56, 133–143. [CrossRef] [PubMed]

8. Brown-Elliott, B.A.; Wallace, R.J., Jr. Mycobacterium: Clinical and Laboratory Characteristics of Rapidly Growing Mycobacteria. In Manual of Clinical Microbiology, 11th ed.; Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S., Warnock, D.W., Eds.; ASM Press: Washington, DC, USA, 2015; pp. 595–612.

9. Brown, B.A.; Springer, B.; Steingrube, V.A.; Wilson, R.W.; Pfaffer, G.E.; Garcia, M.J.; Menendez, M.C.; Rodriguez-Salgado, B.; Jost, K.C.; Jr.; Chiu, S.H.; et al. Mycobacterium wolinskyi sp. nov. and Mycobacterium goodii sp. nov., two new rapidly growing species related to Mycobacterium smegmatis and associated with human wound infections. A cooperative study from the International Working Group on Mycobacterial Taxonomy. Int. J. Syst. Bacteriol. 1999, 49, 1493–1511. [CrossRef]

10. Wallace, R.J., Jr.; Brown-Elliott, B.A.; Wilson, R.W.; Mann, L.; Hall, L.; Zhang, Y.; Jost, K.C., Jr.; Brown, J.M.; Kabani, A.; Schinsky, M.F.; et al. Clinical and laboratory features of Mycobacterium porcinum. J. Clin. Microbiol. 2004, 42, 5689–5697. [CrossRef]

11. Jiménez, M.S.; Campos-Herrero, M.I.; García, D.; Luquin, M.; Herrera, L.; García, M.J. Mycobacterium caraniense sp. nov. Int. J. Syst. Evol. Microbiol. 2004, 54, 1729–1734. [CrossRef]

12. Whipp, C.M.; Butler, W.R.; Fourahmad, F.; Watral, V.G.; Kent, M.L. Molecular systematics support the revival of Mycobacterium salmoniphilum (ex Ross 1960) sp. nov., nom. rev., a species closely related to Mycobacterium chelonae. Int. J. Syst. Evol. Microbiol. 2007, 57, 2525–2531. [CrossRef]

13. Huh, H.J.; Kim, S.Y.; Jhun, B.W.; Shin, S.J.; Koh, W.J. Recent advances in molecular diagnostics and understanding mechanisms of drug resistance in nontuberculous mycobacterial diseases. Infect. Genet. Evol. 2019, 72, 169–182. [CrossRef]

14. Prevots, D.R.; Marras, T.K. Epidemiology of human pulmonary infection with nontuberculous mycobacteria: A review. Clin. Chest Med. 2015, 36, 13–34. [CrossRef]

15. Larsson, L.O.; Polverino, E.; Hoefsloot, W.; Codecasa, L.R.; Diel, R.; Jenkins, S.G.; Loebinger, M.R. Pulmonary disease by non-tuberculous mycobacteria-clinical management, unmet needs and future perspectives. Expert Rev. Respir. Med. 2017, 11, 977–989. [CrossRef]

16. Stout, J.E.; Koh, W.J.; Yew, W.W. Update on pulmonary disease due to non-tuberculous mycobacteria. Int. J. Infect. Dis. 2016, 45, 123–134. [CrossRef]

17. Park, S.C.; Kang, M.J.; Han, C.H.; Lee, S.M.; Kim, C.J.; Lee, J.M.; Kang, Y.A. Prevalence, incidence, and mortality of nontuberculous mycobacterial infection in Korea: A nationwide population-based study. BMC Pulm. Med. 2019, 19, 140. [CrossRef]

18. Namkoong, H.; Kurashima, A.; Morimoto, K.; Hoshino, Y.; Hasegawa, N.; Ato, M.; Mitarai, S. Epidemiology of Pulmonary Infections with Nontuberculous Mycobacteria in Japan. Emerg. Infect. Dis. 2016, 22, 1116–1117. [CrossRef]

19. Simons, S.; van Ingen, J.; Hsuue, P.R.; Van Hung, N.; Dekhuijzen, P.N.; Boeree, M.J.; van Soolingen, D. Nontuberculous mycobacteria in respiratory tract infections, eastern Asia. Emerg. Infect. Dis. 2011, 17, 343–349. [CrossRef]

20. Yang, S.C.; Hsuue, P.R.; Lai, H.C.; Teng, L.J.; Huang, L.M.; Chen, J.M.; Wang, S.K.; Shie, D.C.; Ho, S.W.; Luh, K.T. High prevalence of antimicrobial resistance in rapidly growing mycobacteria in Taiwan. Antimicrob. Agents Chemother. 2003, 47, 1958–1962. [CrossRef]

21. Moore, J.E.; Kruij shaar, M.E.; Ormerod, L.P.; Drobniewski, F.; Abubakar, I. Increasing reports of non-tuberculous mycobacteria in England, Wales and Northern Ireland, 1995–2006. BMC Public Health 2010, 10, 612. [CrossRef]

22. Benfield, T.L.; Duhaut, P.; Sørensen, H.T.; Les cure, F.X.; Thomsen, R.W. Nontuberculous pulmonary mycobacteriosis in Denmark: Incidence and prognostic factors. Am. J. Respir. Crit. Care Med. 2010, 181, 514–521. [CrossRef]

23. Czarkowski, M.P.; Cieleba, E.; Staszewska-Jakubik, E.K.B. Mycobacterium: Clinical and Laboratory Characteristics of Rapidly Growing Mycobacteria. In Manual of Clinical Microbiology, 11th ed.; Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S., Warnock, D.W., Eds.; ASM Press: Washington, DC, USA, 2015; pp. 595–612.

24. Jang, M.A.; Koh, W.J.; Huh, H.J.; Kim, S.Y.; Jeon, K.; Ki, C.S.; Lee, N.Y. Distribution of nontuberculous mycobacteria with descriptions of Mycobacterium bolletii sp. nov., Mycobacterium phocacium sp. nov. and Mycobacterium aubagenense sp. nov. Int. J. Syst. Evol. Microbiol. 2006, 56, 133–143. [CrossRef] [PubMed]

25. Griffith, D.E.; Aksamit, T.; Brown-Elliott, B.A.; Catanzaro, A.; Daley, C.; Gordin, F.; Holland, S.M.; Horsburgh, R.; Huit t, G.; Iademarco, M.F.; et al. ATS Mycobacterial Diseases Subcommittee; American Thoracic Society; Infectious Disease Society of America. An official ATS/IDSA statement: Diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. Am. J. Respir. Crit. Care Med. 2007, 175, 367–416. [CrossRef]

26. Brown-Elliott, B.A.; Wallace, R.J., Jr.; Pett i, C.A.; Mann, L.B.; McGlasson, M.; Chihara, S.; Smith, G.L.; Painter, P.; Hail, D.; Wilson, R.; et al. Mycobacterium neoaurum and Mycobacterium bacteremicum sp. nov. as causes of mycobacteremia. J. Clin. Microbiol. 2010, 48, 4377–4385. [CrossRef]

27. Brown-Elliott, B.A.; Wallace, R.J., Jr.; Pett i, C.A.; Mann, L.B.; McGlasson, M.; Chihara, S.; Smith, G.L.; Painter, P.; Hail, D.; Wilson, R.; et al. Mycobacterium neoaurum and Mycobacterium bacteremicum sp. nov. as causes of mycobacteremia. J. Clin. Microbiol. 2010, 48, 4377–4385. [CrossRef]

28. Washer, L.L.; Riddell, J., IV; Rider, J.; Chenoweth, C.E. Mycobacterium neoaurum bloodstream infection: Report of 4 cases and review of the literature. Clin. Infect. Dis. 2007, 45, e10–e13. [CrossRef]
31. Martínez López, A.B.; Álvarez Blanco, O.; Ruiz Serrano, M.J.; Morales San-José, M.D.; Luque de Pablos, A. Mycobacterium fortuitum as a cause of peritoneal dialysis catheter port infection. A clinical case and a review of the literature. *Nefrologia* **2015**, *35*, 384–386. [CrossRef]

32. Haworth, C.S.; Banks, J.; Capstick, T.; Fisher, A.J.; Gorsuch, T.; Laurenson, I.F.; Leitch, A.; Loebinger, M.R.; Millburn, H.J.; Nightingale, M.; et al. British Thoracic Society Guideline for the management of non-tuberculous mycobacterial pulmonary disease (NTM-PD). *BMJ Open Respir. Res.* **2017**, *4*, e000242. [CrossRef]

33. Markiewicz, Z.; Korsak, D.; Popowska, M. Antibiotics in the Era of Increasing Drug Resistance, 1st ed.; PWN: Warsaw, Poland, 2021; p. 43.

Clinical and Laboratory Standards Institute. *Susceptibility Testing of Mycobacteria, Nocardia spp., and Other Aerobic Actinomycetes*, 3rd ed.; CLSI Standard Document M24; Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2018.

35. Huang, W.C.; Yu, M.C.; Huang, Y.W. Identification and drug susceptibility testing for nontuberculous mycobacteria. *J. Formos. Med. Assoc.* **2020**, *119* (Suppl. S1), S32–S41. [CrossRef] [PubMed]

36. Kotra, L.P.; Haddad, J.; Mobashery, S. Aminoglycosides: Perspectives on mechanisms of action and resistance and strategies to counter resistance. *Antimicrob. Agents Chemother.* **2000**, *44*, 3249–3256. [CrossRef] [PubMed]

Clinical and Laboratory Standards Institute. *Performance Standards for Susceptibility Testing of Mycobacteria, Nocardia spp., and Other Aerobic Actinomycetes*, 1st ed.; CLSI Document M62; Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2018.

38. Clinical and Laboratory Standards Institute. *Laboratory Detection and Identification of Mycobacteria*; CLSI Guideline M48; Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2018.

39. Brown-Elliott, B.A.; Woods, G.L. Antimycobacterial Susceptibility Testing of Nontuberculous Mycobacteria. In *Antibiotics in the Era of Increasing Drug Resistance*, 1st ed.; PWN: Warsaw, Poland, 2021; pp. 15–59.

Brown-Elliott, B.A.; Vasireddy, S.; Vasireddy, R.; Iakhiaeva, E.; Howard, S.T.; Nash, K.A.; Parodi, N.; Strong, A.; Gee, M.; Smith, T.; et al. Utility of sequencing the erm(41) gene in isolates of Mycobacterium abscessus subsp. abscessus with low and intermediate clarithromycin MICs. *J. Clin. Microbiol.* **2016**, *53*, 1211–1215, Erratum in *J. Clin. Microbiol.* **2016**, *54*, 1172. [CrossRef] [PubMed]

40. Brown-Elliott, B.A. Laboratory diagnosis and antimicrobial susceptibility testing of nontuberculous mycobacteria. In *Nontuberculous Mycobacterial Disease*; Griffith, D.E., Ed.; Respiratory Medicine Humana Press: Cham, Switzerland, 2018; pp. 15–59.

Nash, K.A.; Brown-Elliott, B.A.; Wallace, R.J. Jr. A novel gene, erm(41), confers inducible macrolide resistance to clinical isolates of Mycobacterium abscessus but is absent from Mycobacterium chelonae. *Antimicrob. Agents Chemother.* **2009**, *53*, 1367–1376. [CrossRef] [PubMed]

41. Nash, K.A.; Brown-Elliott, B.A. Antimycobacterial Susceptibility Testing of Nontuberculous Mycobacteria. *J. Clin. Microbiol.* **2019**, *57*, e00834-19. [CrossRef] [PubMed]

42. Brown-Elliott, B.A.; Woods, G.L. Antimycobacterial Susceptibility Testing of Nontuberculous Mycobacteria. *J. Clin. Microbiol.* **2019**, *57*, e00834-19. [CrossRef] [PubMed]

43. Bhalla, G.S.; Grover, N.; Singh, L.; Sarao, M.S.; Kalra, D.; Pandey, C. RAPMYCO: Mitigating conventional broth microdilution woes. *J. Health Res. Rev.* **2018**, *5*, 93–97.

44. Sriram, R.; Sarangan, P. Antimicrobial susceptibility testing of rapidly growing mycobacteria isolated from cases of surgical site infections by microbroth dilution method at a Tertiary Care Center. *J. Mar. Med. Soc.* **2017**, *19*, 93–97. [CrossRef]

45. Esteban, J.; Martín-de-Hijas, N.Z.; Garcia-Almeida, D.; Bodas-Sánchez, A.; Gadea, I.; Fernández-Roblas, R. Prevalence of erm methylase genes in clinical isolates of non-pigmented, rapidly growing mycobacteria. *Clin. Microbiol. Infect.* **2009**, *15*, 919–923. [CrossRef] [PubMed]

46. Dávalos, A.F.; Garcia, P.K.; Montoya-Pachango, C.; Rengifo, A.; Guerrero, D.; Diaz-Ordoñez, L.; Díaz, G.; Ferro, B.E. Identification of Nontuberculous Mycobacteria in Drinking Water in Cali, Colombia. *Int. J. Environ. Res. Public Health* **2021**, *18*, 8451. [CrossRef]

47. Faridah, S.; Siti Asma’, H.; Zeti, N.S.; Tuan Noorokirina, T.K.; Intan Baiduri, B.; Azura, H. Fatal outcome of catheter-related bloodstream infection caused by Multidrug-Resistant Mycobacterium mucogenicum. *Med. J. Malays.* **2021**, *76*, 248–250.

48. Aono, A.; Morimoto, K.; Chikamatsu, K.; Yamada, H.; Igarashi, Y.; Murase, Y.; Takaki, A.; Mitarai, S. Antimicrobial susceptibility testing of Mycobacteroides (Mycobacterium) abscessus complex, Mycolicibacterium (Mycobacterium) fortuitum, and Mycobacteroides (Mycobacterium) chelonae. *J. Infect. Chemother.* **2019**, *25*, 117–123. [CrossRef]

49. Lee, S.H.; Yoo, H.K.; Kim, S.H.; Koh, W.J.; Kim, C.K.; Park, Y.K.; Kim, H.J. The drug resistance profile of Mycobacterium abscessus group strains from Korea. *Ann. Lab. Med.* **2014**, *34*, 31–37. [CrossRef]

50. Koh, W.J.; Jeon, K.; Lee, N.Y.; Kim, B.J.; Kook, Y.H.; Lee, S.H.; Park, Y.K.; Kim, C.K.; Shin, S.J.; Huitt, G.A.; et al. Clinical significance of differentiation of Mycobacterium massiliense from Mycobacterium abscessus. *Am. J. Respir. Crit. Care Med.* **2011**, *183*, 405–410. [CrossRef]

51. Wallace, R.J., Jr.; Brown-Elliott, B.A.; Crist, C.J.; Mann, L.; Wilson, R.W. Comparison of the in vitro activity of the glycylcycine tigecycline (formerly GAR-936) with those of tetracycline, minocycline, and doxycycline against isolates of nontuberculous mycobacteria. *Antimicrob. Agents Chemother.* **2002**, *46*, 3164–3167. [CrossRef]

52. Pang, H.; Li, G.; Zhao, X.; Liu, H.; Wan, K.; Yu, P. Drug Susceptibility Testing of 31 Antimicrobial Agents on Rapidly Growing Mycobacteria Isolates from China. *Biomed. Res. Int.* **2015**, *2015*, 419392. [CrossRef]

53. Comba, I.Y.; Tabaja, H.; Almeida, N.E.C.; Fida, M.; Saleh, O.A. Bloodstream infections with rapidly growing nontuberculous mycobacteria. *J. Clin. Tuberc. Other Mycobact. Dis.* **2021**, *25*, 100288. [CrossRef]
