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ORIGINAL ARTICLES AND REVIEWS

Epidemiology and drug susceptibility of nontuberculous mycobacteria (NTM) in Italy in 2016-2020

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Abstract

Introduction. Nontuberculous mycobacteria (NTM) are environmental mycobacteria which may cause pulmonary and extrapulmonary diseases. These organisms are difficult to treat due to their intrinsic drug-resistance. In Italy, no major nationwide study on NTM epidemiology and drug susceptibility was performed.

Methods. Data on the epidemiology of 7,469 NTM clinical isolates identified in Italy in 2016-2020 and on the minimum inhibitory concentrations (MICs) of 1,506 of these strains were analysed.

Results. Overall, 63 species were identified in 42 hospital laboratories located in 16 out of 20 regions, with *Mycobacterium avium* complex (MAC) being the most frequently isolated, followed by *M. gordonae*, *M. xenopi*, *M. abscessus*. The MICs of 12 drugs for MAC, *M. xenopi*, *M. kansasii*, *M. abscessus*, *M. fortuitum* and *M. chelonae* were interpreted for clinical significance (susceptible, intermediate, resistant) based on the guidelines published by the Clinical and Laboratory Standards Institute in November 2018.

Conclusions. Our data are in line with other nationwide studies and may be of value for further update of microbiological and clinical guidelines.

Key words

- *Mycobacterium avium* complex
- *Mycobacterium abscessus*
- drug resistance, clarithromycin
- amikacin

INTRODUCTION

The nontuberculous mycobacteria (NTM) include approximately 200 species, some of which may cause diseases in humans by infecting pulmonary and extrapulmonary tissues [1-4]. Lungs are the most affected, however NTM species are phenotypically different, and generate a wide spectrum of clinical manifestations also in other organs. *Mycobacterium avium* complex (MAC), *M. xenopi*, *M. kansasii* and *M. abscessus* are the most frequent responsible of pulmonary diseases. *M. avium* complex may also cause disseminated infections, while *M. fortuitum*, *M. chelonae* and *M. marinum* are primarily responsible for skin and soft tissue infections initiated via surgery or accidental lesions.

For many years, the classification of Runyon differentiated NTM in two wide categories according to their growth rate, namely the rapidly growing mycobacteria (RGM) (e.g., *M. abscessus, M. fortuitum* and *M. chelonae*) and the slowly growing mycobacteria (SGM) (e.g., MAC, *M. xenopi, M. kansasii, M. simiae*), requiring ≤7 days and >7 days, respectively, to produce colonies on solid media [1-4]. With the increasing use of molecular methods of identification, this phenotypic classification was less used, however recent phylogenetic studies confirmed the separation of RGM and SGM species [5]. Among the RGM, the species of the *M. chelonae-abscessus* complex belonged to the most ancestral cluster, while members of the *M. terrae* complex appeared to be as the most ancestral SGM.

The NTM are highly abundant in the environment (soil, dust, water sources, shower-based aerosols), leading to high rates of human-mycobacterium contacts [2, 3]. Furthermore, host factors such as increasing age of the global population, pulmonary diseases (e.g., bronchiectasis and cystic fibrosis) and immunosuppression, contribute to the rise of NTM lung diseases, increasing worldwide health concern. In addition, mutations in the interferon-gamma-pathway and the use of tumor necrosis factor inhibitors for treatment of inflammatory diseases, increase the risk of MAC and *M. ascessus* infections. The growing availability of molecular tools also increases the detection of NTM infections, which are difficult to treat due to their intrinsic resistance to several antibiotics.

As to the geographical distribution of NTM, a study found that after examination of NTM data received

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from 30 countries across six continents, the species distribution among NTM isolates from pulmonary specimens differed by continent and by country within these continents [6]. Thus, differences in species distribution may partly determine the frequency and manifestations of pulmonary NTM disease in each geographical location. Other investigators found also fluctuations in NTM isolation and distribution. For instance, a systematic review and meta-analysis in mainland China, showed that *M. abscessus* was the prevalent species isolated in 2000-2014, while *M. intracellulare* was more prevalent in 2015-2019 [7, 8]. The geographic diversity of different species showed the effects of environmental and economic factors on NTM distribution. Overall, this epidemiological information can provide important clues on the discrepancies in clinical relevance and treatment outcome of NTM diseases [6].

Surveillance of NTM for monitoring dominant species and their drug resistance profiles would be important to improve the management and treatment of these infections. Reporting of NTM disease to health authorities is not mandated in several countries. However, retrospective cohort studies or laboratory-based studies were recently reported in Germany [9], China [7, 8], United States of America (USA) [10], and other countries.

In Italy, no major information on the epidemiology and drug susceptibility of NTM infections is known [6, 11-13]. Here, we report nationwide data regarding NTM clinical strains isolated in our country in 2016-2020.

EPIDEMIOLOGY

In Italy, the monitoring of demographic and microbiological data on NTM species identification and minimum inhibitory concentrations (MICs) is coordinated by the Istituto Superiore di Sanità (Italian National Institute of Health, Rome, Italy), which operates in collaboration with a network of 42 hospital laboratories located in 16 out of 20 regions (Studio Multicentrico Italiano Micobatteri Non Tubercolari, IMS-NTM, Italian Multicentre Study on Nontuberculous Mycobacteria). The organisms are routinely identified by commercial methods [1] including line probe assay (LPA) (GenoType Mycobacterium CM, AS and NTM-DR; Hain Lifesciences, Nehren, Germany), matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS), and DNA sequencing. The MICs are determined by the broth microdilution method using the SLOMYCOI and RAPMYCOI Sensititre plates (ThermoFisher Scientific, Waltham, MA, USA) [1].

In 2016-2020, the IMS-NTM laboratories reported data on 7,469 nonduplicate NTM clinical isolates belonging to 63 species, including 6,319 SGM (37 species), and 1,150 RGM (26 species). *Figure 1* shows the 15 species representing 97.6% of all NTM isolates. Among the SGM, the MAC (*M. avium, M. intracellulare, M. chimaera*) accounted for 56.5% of all NTM isolated (28.6%, 19.8%, 8.1%, respectively), followed by *M. gordonae* (10.8%), *M. xenopi* (9.7%), *M. kansasii* (2.3%), and other 5 species. The most frequent RGM were *M. abscessus* spp. (6.6%, including the subspecies *M. abscessus ascessus, M. abscessus bolletii, M. abscessus massiliense*: 6.0%, 0.3%, 0.3%, respectively), *M. fortuitum* (4.4%), *M. chelonae* (2.9%), *M. mucogenicum* (0.7%). The methods used for identification were LPA (86%), DNA sequencing (11.2%) and MALDI-TOF MS (2.8%).

Overall, these observations showed that the species distribution in Italy in 2016-2020 was similar to that reported for NTM pulmonary samples in other European countries [6], with MAC organisms being the most frequently isolated, followed by *M. gordonae* and *M. xenopi*.

In 14 out of the 15 species shown in *Figure 1*, ≥64.5% of strains were isolated from pulmonary specimens, while 90.6% of *M. marinum* strains were isolated from extrapulmonary samples. This is in line with the knowledge that *M. marinum* is the causative agent of swimming pool or fish tank granulomas, as the result of finger, hand, arm or elbow soft tissue injuries [1].

Analysis of health-related and demographic characteristics showed that about half (50.3%) of NTM strains were isolated from male patients. In addition, 92.1% were isolated from Italian-born persons (IBP) and 7.9% from foreign-born persons (FBP), with mean ages of 65.1±19.9 and 45.5±19.3 years, respectively. In Italy, in 2019 the IBP and the FBP were 91.1% and 8.9% of resident population, respectively [14]. Thus, the risk of developing NTM infections was similar in IBP and FBP (mostly migrants), likely due to the knowledge that NTM are mostly of environmental origin worldwide. This conclusion is in keeping with a study carried out in Canada, in which NTM colonization risk was not so different between Canadian-born people and foreign-born people residing in Canada for at least 10 years [15].

Noticeably, the pattern of NTM infections is different from that seen in tuberculosis (TB) patients, in which FBP often move from countries with endemic disease to countries with low TB levels, in the search for better living conditions and quality of life. Indeed, in a

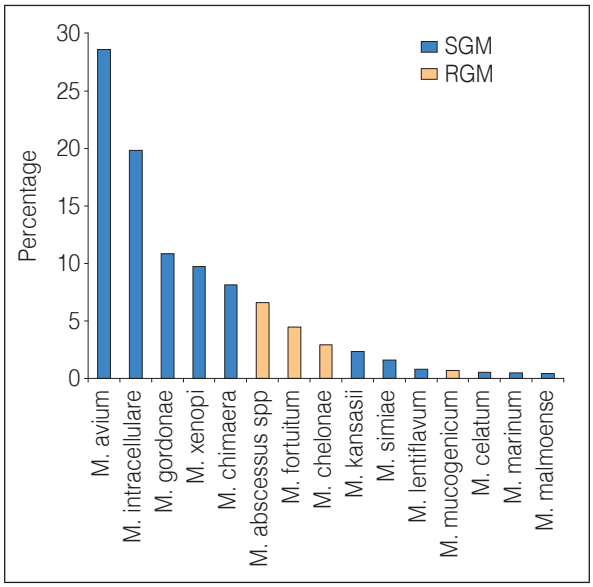

Figure 1
Species distribution among slowly growing mycobacteria (SGM) and rapidly growing mycobacteria (RGM).

recent paper of our group on the extent of TB in Italy in 2011-2020, we showed that 65% of cases were related to young FBP, mostly arrived from Romania, Morocco, Pakistan, Senegal, India [16]. For comparison, the FBP with NTM infections arrived in Italy from 83 countries, with the top six being Morocco (10.9%), Romania (7.8%), Albania (6.2%), Pakistan (5.6%), Nigeria and Senegal (4.9% each), corresponding to most of the countries of FBP with TB. In 2016-2020, the FBP arrived from these six countries represented 43.9% of migrants in Italy [17].

DRUG SUSCEPTIBILITY

The NTM are intrinsically resistant to several drugs, including anti-TB agents, and need to be treated with combinations of antibiotics, based on susceptibility testing. This poses major challenges for new drugs discovery and for therapy of pulmonary and extrapulmonary infections caused by these organisms [18, 19].

Drug resistance of NTM can be intrinsic (natural) or acquired [19]. During the evolution, several mechanisms of intrinsic resistance developed, including decreased permeability of the cell envelope, increased efflux systems and other mechanisms (e.g., drug degradation and target changes), or NTM presence in biofilms and granulomas, which effectively decreased drug uptake. Instead, acquired resistance refers to cases in which, often due to prolonged antibiotic treatments, a resistant strain emerges from a previously drug-susceptible population. Acquired resistance is particularly severe if the target protein is encoded by a single gene copy, increasing the possibility of acquiring mutations after single-drug treatments [19].

The guideline for antimycobacterial susceptibility testing (AST) set in 2011 by the Clinical and Laboratory Standards Institute (CLSI) was updated and expanded in November 2018 [20]. Both guidelines contained recommendations for NTM AST based on clinical data, comparative breakpoints, population distribution and the experience of panel of experts in the field of mycobacteriology [21].

The NTM may colonize the respiratory tract and other sites, thus it is not easy to correlate their isolation with clinical diseases. To this end, specific guidelines for establishing the clinical significance of NTM in patient specimens were published by the American Thoracic Society (ATS) and other scientific organizations in 1990, 1997, 2007 [22], and then updated in July 2020 [23].

Overall, the IMS-NTM laboratories tested 1,506 strains (291 RGM and 1,215 SGM) including 1,275 isolates originated from respiratory samples (84.7%), 201 from non-respiratory specimens (13.3%) and 30 from unknown sources (2%). MICs were interpreted as susceptible (S), intermediate (I), resistant (R), according to the CLSI breakpoints [20]. *Table 1* shows the antimicrobial susceptibility profiles of 12 drugs for 8 species (≥20 MIC values for each drug), including modal MIC, MIC_{50} and MIC_{90}.

The MICs of clarithromycin (CLA), amikacin (AMI), moxifloxacin (MXF) and linezolid (LZD) were interpreted in terms of S, I or R rates. The MICs of *M. xenopi* and *M. kansasii* were interpreted also for RIF, rifabutin (RFB), ciprofloxacin (CIP), doxycycline (DOX), trimethoprim-sulfamethoxazole (SXT). Finally, the MICs of the RGM *M. abscessus* spp., *M. fortuitum* and *M. chelonae* were interpreted also for CIP, DOX, SXT, cefoxitin (FOX), imipenem (IMI) and tobramycin (TOB).

Assuming that a susceptibility rate ≥90% (highlighted in bold) represents high activity of a drug, CLA was highly active against 6 species/complex (MAC, *M. xenopi, M. kansasii, M. chelonae*), AMI against 3 species (*M. xenopi, M. kansasii, M. fortuitum*), MXF against 2 species (*M. xenopi, M. fortuitum*), LZD and RFB against 2 species (*M. xenopi, M. kansasii*), CIP against 1 species (*M. fortuitum*). Rifampicin, DOX, SXT, FOX, IMI and TOB showed susceptibilities <90%.

A comprehensive examination of the MICs of *Table 1*, and of their interpretation in light of the treatments recommended in the 2020 clinical practice guideline [23], is shown below.

MAC

Clarithromycin was the most effective drug against *M. avium, M. intracellulare* and *M. chimaera,* as shown by susceptibility rates of 95.3%, 95.1% and 98.3%, respectively, and modal MICs of 2 µg/mL for each species. *M. avium, M. intracellulare* and *M. chimaera*-resistant isolates were few (2.5%, 3.8% and 0%, respectively), as well as isolates with intermediate macrolide MICs (1.1-2.2%), which may indicate emerging resistance [20]. Higher susceptibility of *M. chimaera* to CLA was reflected also in its MIC_{90} (4 µg/mL), which was 1 dilution lower than those of *M. avium* and *M. intracellulare* (8 µg/mL). In keeping with the modal MICs shown here, CLA is considered a first-line agent for MAC [20]. CLA and AMI are drugs for which a clear correlation between *in vitro* and clinical activity was reported [21].

Amikacin (intravenous, or by inhaled liposomal formulation) is also considered a first-line agent for MAC [20, 21]. Here, the modal MICs were high (*M. avium* and *M. intracellulare,* 16 µg/mL; *M. chimaera*, 8 µg/mL). However, based on the CLSI interpretation [20], most MAC strains were susceptible to AMI (*M. avium,* 72.6%; *M. intracellulare,* 70.8%; *M. chimaera*, 88.4%), and resistance was not very high: *M. avium,* 6.6%; *M. intracellulare,* 4.7% *M. chimaera*, 1.9%. However, unlike what it was observed for CLA, several strains showed high levels of intermediate MICs: *M. avium,* 20.8%; *M. intracellulare,* 24.5%; *M. chimaera*, 9.7%, indicating probable emerging of resistance. Intrinsic resistance to aminoglycosides is due to modification of their hydroxyl and amino groups by specific enzymes, while acquired resistance may be related to their prolonged use in monotherapy, generating mutations in *rrs*, the 16S rRNA gene [2, 21, 24].

Overall, CLA and AMI were more active against *M. chimaera* than *M. avium* and *M. intracellulare*. MICs of CLA and AMI for these three organisms were very similar to those reported in a large European study which analysed a comparable number of MAC isolates [25].

Moxifloxacin and LZD were low active against *M. avium*, *M. intracellulare* and *M. chimaera* (susceptibility

≤33.6%). However, MXF showed modal MICs (2-4 µg/mL) and MIC_{90} (4 µg/mL) lower than those of LZD (16-32 µg/mL and ≥64 µg/mL, respectively). Overall, our data are in keeping with the CLSI indication that MXF and LZD are second-line agents against MAC [20].

According to the 2020 clinical guidelines [23], standard treatment of MAC infections includes ≥3-drug combinations containing the macrolides azithromycin (AZI) or CLA, the rifamycins RIF or RFB and ethambutol (EMB). If a more aggressive therapy is required (e.g., in cases of cavitary disease, extensive bronchiectatic disease, macrolide-resistant MAC), an injectable aminoglycoside (AMI or streptomycin), or liposomal AMI inhalation, may be added. Alternative drugs for patients who are intolerant of, or whose isolate is resistant to CLA and AMI, include MXF, LZD and clofazimine (CLO) [23].

M. xenopi

Clarithromycin, AMI, MXF, LZD and RFB were the most active against this species, as shown by

Table 1
Drug resistance profiles of 8 nontuberculous mycobacteria (NTM) species for 12 drugs

		Drugs											
Organisms	**Parameters**	**CLA**	**AMI**	**MXF**	**LZD**	**RIF**	**RFB**	**CIP**	**DOX**	**SXT**	**FOX**	**IMI**	**TOB**
M. avium	Strains (number)	588	481	562	563								
	Modal MIC (ug/ml)	2	16	2	32								
	MIC50 (ug/ml)	2	16	2	32								
	MIC90 (ug/ml)	8	32	4	≥64								
	Susceptible (%)	**95.3**	72.6	22.8	8.3								
	Intermediate (%)	2.2	20.8	39.1	12.3								
	Resistant (%)	2.5	6.6	38.1	79.4								
M. intracellulare	Strains (number)	367	318	354	352								
	Modal MIC (ug/ml)	2	16	2	32								
	MIC50 (ug/ml)	2	16	2	32								
	MIC90 (ug/ml)	8	32	4	≥64								
	Susceptible (%)	**95.1**	70.8	11.1	8.5								
	Intermediate (%)	1.1	24.5	43.2	10.5								
	Resistant (%)	3.8	4.7	45.7	81								
M. chimaera	Strains (number)	115	103	113	113								
	Modal MIC (ug/ml)	2	8	4	16								
	MIC50 (ug/ml)	2	8	4	32								
	MIC90 (ug/ml)	4	32	4	≥64								
	Susceptible (%)	**98.3**	88.4	18.5	33.6								
	Intermediate (%)	1.7	9.7	31	53.1								
	Resistant (%)	0	1.9	50.5	13.3								
M. xenopi	Strains (number)	88	82	87	87	87	85	87	65	71			
	Modal MIC (ug/ml)	≤0.06	4	0.25	4	1	≤0.25	1	≥16	0.5/9.5			
	MIC50 (ug/ml)	0.25	4	0.5	4	1	≤0.25	1	8	0.5/9.5			
	MIC90 (ug/ml)	1	16	1	8	4	1	2	16	8/152			
	Susceptible (%)	**97.8**	**96.3**	**90.8**	93	81.6	**97.7**	68.9	7.6	85.9			
	Intermediate (%)	1.1	0	6.9	1.2	-	-	23	33.9	-			
	Resistant (%)	1.1	3.7	2.3	5.8	18.4	2.3	8.1	58.5	14.1			
M. kansasii	Strains (number)	48	43	45	44	48	46	47	33	40			
	Modal MIC (ug/ml)	0.5	4	0.5	2	0.5	≤0.25	2	≥16	8/152			
	MIC50 (ug/ml)	0.5	4	0.5	4	0.5	0.5	4	16	8/152			
	MIC90 (ug/ml)	2	16	2	8	2	≤0.25	16	≥16	≥8/152			
	Susceptible (%)	**100**	**90.7**	77.8	**90.9**	87.5	**97.8**	23.4	12.1	37.5			
	Intermediate (%)	0	2.3	17.8	6.8	-	-	23.4	15.2	-			
	Resistant (%)	0	7	4.4	2.3	12.5	2.2	53.2	72.7	62.5			

Continues

Table 1
Continued

Organisms	Parameters	Drugs											
		CLA	AMI	MXF	LZD	RIF	RFB	CIP	DOX	SXT	FOX	IMI	TOB
M. abscessus spp	Strains (number)	186	195	175	194			189	174	177	189	171	106
	Modal MIC (ug/ml)	≥16	16	≥8	≥32			≥4	≥16	≥8/152	64	≥64	≥16
	MIC50 (ug/ml)	2	16	8	16			4	≥16	≥8/152	64	16	16
	MIC90 (ug/ml)	≥16	32	≥8	≥32			≥4	≥16	≥8/152	≥128	≥64	≥16
	Susceptible (%)	52.7	76.4	9.3	47.4			11.6	3.5	11.9	22.2	14	13.7
	Intermediate (%)	7	13.3	14.9	22.2			11.1	3.4	-	52.9	42.7	12.7
	Resistant (%)	40.3	10.3	75.8	30.4			77.3	93.1	88.1	24.9	43.3	73.6
M. fortuitum	Strains (number)	44	44	43	43			44	42	42	43	42	22
	Modal MIC (ug/ml)	≥16	≤1	≤0.25	2			≤0.12	≥16	≥8/152	16	4	≥16
	MIC50 (ug/ml)	2	≥1	≤0.25	2			≤0,12	8	≥8/152	32	4	8
	MIC90 (ug/ml)	8	4	0.5	16			0.25	≥16	≥8/152	≥128	32	≥16
	Susceptible (%)	50.6	**100**	**100**	88.4			**100**	23.8	83.3	44.2	59.5	9.1
	Intermediate (%)	9.1	0	0	2.3			0	11.9	-	39.5	19.1	31.8
	Resistant (%)	40.3	0	0	9.3			0	64.3	16.7	16.3	21.4	59.1
M. chelonae	Strains (number)	37	36	23	22			35	36	20	36	35	34
	Modal MIC (ug/ml)	0.25	32	≥8	16			≥4	≥8	≥8/152	≥128	≥64	2
	MIC50 (ug/ml)	0.5	16	4	16			2	≥8	≥8/152	≥128	32	2
	MIC90 (ug/ml)	2	32	≥8	≥32			≥4	≥8	≥8/152	≥128	≥64	4
	Susceptible (%)	**94.6**	72.2	26.1	40.9			31.4	19.4	15	6.6	14.2	61.7
	Intermediate (%)	0	27.8	17.4	40.9			20	16.7	-	21.2	28.6	32.4
	Resistant (%)	5.4	0	56.5	18.2			48.6	63.9	85	72.2	57.2	5.9

CLA: clarithromycin; AMI: amikacin; MFX: moxifloxacin; LZD: linezolid; RIF: rifampicin; RFB: rifabutin; CIP: ciprofloxacin; DOX: doxycycline; SXT: trimethoprim-sulfamethoxazole; FOX: cefoxitin; IMI: imipenem; TOB: tobramycin; (-): MIC value not indicated in the Clinical and Laboratory Standards Institute (CLSI) M62 document [20]; empty spaces: MIC interpretation not reported in the M62 document; percentages of drug susceptibility ≥90% are highlighted in bold. MIC: minimum inhibitory concentration.

susceptibility of 90.8-97.8%, modal MICs of ≤0.06-4 µg/mL, and MIC_{90} of 1-16 µg/mL to these drugs. Based on susceptibility, RIF was less active than RFB, and CIP was less active than MXF. Trimethoprim-sulfamethoxazole was very active, while DOX was not active (susceptibility of 85.9% and 7.6%, respectively).

The 2020 guideline on the therapy of NTM pulmonary diseases [23], recommended regimens of ≥3 drugs including the macrolides AZI (or CLA) and/or MXF, RIF or RFB, EMB. As suggested for MAC infections, treatment of *M. xenopi*-infected patients with cavitary or advanced/severe bronchiectatic diseases, should include addition of parenteral AMI to above drugs, and obtaining expert consultation for the management of these complicated infections [23], Our data that *M. xenopi* isolates showed high susceptibility not only to CLA, AMI, MXF and RFB, but also to LZD, adds another drug to the therapeutic armamentarium for these patients, which should be treated aggressively given the high mortality of the disease. The high activity of LNZ against *M. xenopi* was previously reported by other investigators [26].

M. kansasii

Similar to that seen with *M. xenopi,* CLA, AMI, LZD and RFB were the most active also against *M. kansasii* (susceptibility of 90.7-100%, modal MICs of ≤0.25-4 µg/mL, MIC_{90} of ≤0.25-16 µg/mL). Moxifloxacin was less active against *M. kansasii* than against *M. xenopi* (susceptibility of 77.8% and 90.8%, respectively). Susceptibilities to SXT, CIP and DOX were ≤37.5%. Clarithromycin and RIF are considered to be the first-line agents for *M. kansasii* infections [20]. Here, RIF was a bit less active than RFB (susceptibilities of 87.5% and 97.8%, respectively). The therapeutic regimen against *M. kansasii* recommended in the 2007 guideline contained isoniazid (INH), EMB and RIF [1, 22]. However, two subsequent studies demonstrated good treatment outcome when CLA was substituted for INH [23]. Thus, the guidelines updated and expanded in 2020 [23] recommended 3-drug oral regimens containing either INH or a macrolide (AZI or CLA) in combination with RIF (or RFB) and EMB. The parenteral use of AMI is not warranted unless it is impossible to use oral regimens, or severe disease is present [23]. As for *M. xenopi,* also *M. kansasii* showed high susceptibility to LZD [26].

M. abscessus spp.

None of the 12 drugs tested showed ≥90% susceptibility against this species. All modal MICs and MIC_{90}

were ≥4 µg/mL. Clarithromycin and AMI were the most active against these difficult-to-treat pathogens (susceptibility of 52.7% and 76.4%, respectively); all other drugs had susceptibilities ranging from 3.5% (DOX) to 47.4% (LZD). A large study on RGM showed a similar MIC pattern [27].

Acquired resistance to CLA may be due to mutations in the *rrl,* the 23S rRNA gene. Furthermore, adaptive resistance is related to over-expression of erm(41), a CLA-inducible enzyme methylating a nucleotide in the 23S rRNA [2, 21, 24]. Clarithromycin-resistant strains (40.3%) had MICs ≥8 µg/mL.

M. abscessus was quite susceptible to AMI (76.4%). However, the modal MIC was high (16 µg/mL) and 10.3% of isolates had MICs ≥64 mg/ml, likely due to mutations in the *rrs*, the 16S rRNA gene [2, 21, 24].

Thus, treatments of *M. abscessus* infections are related to the macrolide susceptibility pattern, including mutational resistance (*rrl* mutations) or inducible resistance (functional erm(41) gene: resistant; non-functional erm(41) gene: susceptible) [23]. They comprise surgical resection of lung tissue, and/or multidrug therapy with AZI (or CLA), AMI, FOX, IMI, LZD, tigecycline and CLO, a drug that may act synergistically with AMI and macrolides [23]. Overall, our MICs values are fully in line with the great difficulty to treat these infections.

M. fortuitum

Amikacin, MXF and CIP were the most active against this species (susceptibility of 100%). High susceptibility to MXF and AMI was also reported by other investigators [27, 28]. Linezolid and SXT showed susceptibilities of 88.4% and 83.3%, respectively, while CLA was active against 50.6% isolates. *M. fortuitum* infections are usually treated with two drugs, including AMI, LZD, sulfonamides, DOX [1]. Our observations that the fluoroquinolones MXF and CIP were very active *in vitro* may be useful for the management of these infections.

M. chelonae

Clarithromycin, AMI and TOB were the most active drugs against this species (susceptibilities of 94.6%, 72.2% and 61.7%, respectively) but TOB showed high intermediate MIC rates (32.4%). A similar pattern was shown by a large study on RGM [27]. All other drugs had susceptibilities ≤40.9%. Regimens for treatment of *M. chelonae* infections may include CLA, TOB or AMI, LZD, IMI or CLO [1].

CONCLUSIONS

The NTM species distribution in Italy is similar to that reported in other European countries [6]. Furthermore, the MIC values, being similar to those reported in other nationwide studies on SGM [25] and RGM [27], indicate a good quality of the performance of NTM DST in our country. The observation that LZD, despite adverse reactions observed during treatment with this drug in some cases [23], was highly active *in vitro* against *M. xenopi* and *M. kansasii* isolates, may be important to design new therapeutic regimens against these pathogens.

Authors' contributions

FG, AL, AI and LF conceived and wrote the study; the IMS-NTM laboratories collected the data and critically revised the manuscript.

Conflict of interest statement

None.

The Italian Multicentre Study on Nontuberculous Mycobacteria (IMS-NTM)

The members of the IMS-NTM laboratory network are:

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