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REVIEW

The looming tide of nontuberculous mycobacterial infections in Portugal and Brazil

Daniela Nunes-Costa ^a, Susana Alarico ^a, Margareth Pretti Dalcolmo ^b,
Margarida Correia-Neves ^{c,d}, Nuno Empadinhas ^{a,e,*}^a CNC – Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal^b Reference Center Helio Fraga, Fundação Oswaldo Cruz, FIOCRUZ, MoH, Rio de Janeiro, Brazil^c ICVS – Health and Life Sciences Research Institute, University of Minho, Braga, Portugal^d ICVS/3B's, PT Government Associate Laboratory, Braga/Guimarães, Portugal^e IIIUC – Institute for Interdisciplinary Research, University of Coimbra, Coimbra, Portugal

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SUMMARY

Nontuberculous mycobacteria (NTM) are widely disseminated in the environment and an emerging cause of infectious diseases worldwide. Their remarkable natural resistance to disinfectants and antibiotics and an ability to survive under low-nutrient conditions allows NTM to colonize and persist in man-made environments such as household and hospital water distribution systems. This overlap between human and NTM environments afforded new opportunities for human exposure, and for expression of their often neglected and underestimated pathogenic potential. Some risk factors predisposing to NTM disease have been identified and are mainly associated with immune fragilities of the human host. However, infections in apparently immunocompetent persons are also increasingly reported. The purpose of this review is to bring attention to this emerging health problem in Portugal and Brazil and to emphasize the urgent need for increased surveillance and more comprehensive epidemiological data in both countries, where such information is scarce and seriously thwarts the adoption of proper preventive strategies and therapeutic options.

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* Corresponding author. CNC – Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal.

E-mail address: numenius@cnc.uc.pt (N. Empadinhas).

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1. Introduction

The genus *Mycobacterium* is notorious for including the pathogens accountable for two of the most ancient diseases known to humankind, leprosy and tuberculosis (TB), responsible for countless deaths and affliction through human history [1,2]. However, since Hansen's discovery of *Mycobacterium leprae* in 1873 and Koch's discovery of *Mycobacterium tuberculosis* nine years later, the list of described mycobacterial species has grown and currently counts with 170 species and subspecies (www.bacterio.net/mycobacterium.html) (Figure 1). These less known and often neglected mycobacteria, collectively called nontuberculous mycobacteria (NTM), are ubiquitous environmental saprophytes and increasingly regarded as opportunistic human pathogens [6]. Although only a few NTM species are familiar to clinicians and commonly associated with human infection, many of the species described over the last two decades are regarded as potentially pathogenic (Figure 1) [7,8]. The success of NTM both as ubiquitous saprophytes and as opportunistic pathogens can in part be explained by their natural resilience to stress conditions such as desiccation, nutrient starvation, extreme temperatures and pH, and antimicrobials resistance [9,10]. Along with their biofilm-forming capabilities, these characteristics render NTM particularly well-suited for the colonization of artificial environments such as chlorinated water supply networks, where they can proliferate and gain advantage over their chlorine-sensitive competitors [9,11,12].

Mycobacteria are traditionally divided into slowly-growing mycobacteria (SGM) and rapidly-growing mycobacteria (RGM) based on growth rate and although historically the species within the SGM group are the ones that have almost always been associated with disease, the significant numbers of nosocomial infections caused by RGM have changed this perception [13]. In fact, convincing evidence indicates that the numbers of NTM infections caused by both SGM and RGM species are rising worldwide although the lack of systematic reporting in most countries still renders it difficult to establish definitive conclusions concerning their true global incidence [14–17]. Several hypotheses have been proposed to explain this rising trend, notably the increasing overlap of human and mycobacterial habitats (e.g., water distribution systems), increased numbers of NTM in drinking water due to changes in water quality and wider adoption of disinfection regimens that promote selection for these organisms, greater physician awareness of NTM as a possible cause of infections leading to more cases being diagnosed and reported, and the greater prevalence of some predisposing factors in the human population [18]. Risk factors for NTM disease that have been identified so far include: (i) lowered immunocompetence due to HIV infection, cancer, chemotherapy, use of immunosuppressive drugs following transplants or ageing-dependent immunosenescence; (ii) underlying lung conditions such as chronic obstructive pulmonary disease, cystic fibrosis, lung damage due to occupational exposure, to smoking, to prior tuberculosis or other lung disease; (iii) genetic factors, namely mutations in the cystic

fibrosis transmembrane conductance regulator gene or in the α -1-antitrypsin gene [19].

In this review, we will focus on the information available about the current status of NTM infections in Portugal and Brazil, two countries for which epidemiological data is still scarce and whose historically high TB burdens [20] have for many years overshadowed the role of NTM in human disease. In 2013, Portugal had a tuberculosis incidence rate of 26 cases per 100,000 population while in Brazil the incidence rate was 46 per 100,000 population [20]. In order to shed light into this emerging health problem, we conducted a survey of the available literature on NTM infections in Portugal and Brazil by searching PubMed, Web of Science, Google Scholar, SciELO and Lilacs databases between December 2014 and February 2015. The keywords mycobacter*, atypical or non-tuberculous or non-tuberculous, Portugal or Brazil, infection or outbreak or disease or isolation were used and the search was repeated in Portuguese. Reports in languages other than English or Portuguese and with ambiguous or absent geographical information were excluded, no other exclusion criteria were employed.

2. Pulmonary NTM infections

Pulmonary disease is a common manifestation of NTM infection and has been the focus of many recent reviews [21–24]. However, only a few studies have investigated the epidemiology of pulmonary NTM (PNTM) infections in Portugal and Brazil (Table 1) [25–44]. In addition many of these studies have important limitations as will be discussed later in this section. Two of these surveys, conducted in the Greater Lisbon Area, covering the period between 2005 and 2009, independently found NTM to account for approximately 12% of all isolates from patients diagnosed with mycobacterial disease, leaving the remaining 88% of the cases to species of the *Mycobacterium tuberculosis* complex [25,26]. In Brazil, the reported incidence rates differ significantly between different states, periods, and population samples. In the states of Bahia, Pará and Piauí, 8% of patients presenting symptoms compatible with pulmonary TB were actually found to be infected with NTM [39,43,44]. In the state of São Paulo, where most Brazilian studies have been conducted, the percentage of NTM infection among patients suspected of mycobacteriosis has been reported to be around 20% [32,33,37,34] and in the state of Rondônia one study also found a similar result [41]. Agertt and co-workers reported 33% of NTM in Rio Grande do Sul but the inclusion of non-clinically relevant samples of both pulmonary and extrapulmonary origin probably led to an overestimation and does not reflect the true incidence of PNTM disease [42].

These results illustrate the relevance of PNTM infections as a public health problem in Portugal and Brazil, but because PNTM disease cases are not of mandatory notification, the true incidence in both countries remains indeterminate due to lack of more comprehensive data. Even in countries with a high incidence of TB, it is important to be aware that a significant proportion of mycobacterial pulmonary disease cases are in fact caused by NTM,

Table 1

Summary of the characteristics and results of the studies concerning pulmonary nontuberculous mycobacterial (NTM) infections in Portugal and Brazil included in the present review.

Geographic range	Study period	Target population/Study characteristics	NTM frequency among total mycobacterial isolates (%)	No. NTM isolates	Most common species (frequency)	Less common species	Reference
Lisbon, Portugal	2008–2009	HIV-negative patients with lung infection/no confirmation of NTM infection after isolation	11.4	58	<i>M. avium</i> complex (22%), <i>M. fortuitum</i> (14%), <i>M. gordonae</i> (12%), <i>M. kansasii</i> (10%), <i>M. chelonae</i> (9%)	<i>M. peregrinum</i> , <i>M. abscessus</i> , <i>M. szulgai</i> , <i>M. triplex</i> , <i>M. mucogenicum</i> , <i>M. lentiflavum</i> , <i>M. simiae</i>	[25]
	2005–2007	Patients with presumptive active mycobacteriosis/no confirmation of NTM infection after isolation	12.6	149	<i>M. avium</i> complex (34%), <i>M. gordonae</i> (15%), <i>M. fortuitum</i> (9%), <i>M. kansasii</i> (9%), <i>M. chelonae</i> (7%), <i>M. xenopi</i> (6%)	<i>M. peregrinum</i> , <i>M. abscessus</i> , <i>M. mucogenicum</i> , <i>M. scrofulaceum</i> , <i>M. malmoense</i> , <i>M. simiae</i> , <i>M. lentiflavum</i> , <i>M. heckeshornense</i> , <i>M. celatum</i>	[26]
Northern Portugal	2008–2012	Patients with respiratory sample positive for NTM/confirmation of NTM infection	NA	378	<i>M. avium</i> complex (63%), <i>M. gordonae</i> (11%), <i>M. peregrinum</i> (9%), <i>M. chelonae</i> (7%), <i>M. kansasii</i> (3%), <i>M. abscessus</i> (3%)	<i>M. scrofulaceum</i> and others	[27]
		Subset of confirmed PNTM disease cases		68	<i>M. avium</i> complex (94%), <i>M. kansasii</i> (3%), <i>M. xenopi</i> (3%)	<i>M. scrofulaceum</i> and <i>M. abscessus</i>	
	2005–2011	Patients with confirmed PNTM disease	NA	11	<i>M. avium</i> complex (55%)	<i>M. kansasii</i> , <i>M. gordonae</i> , <i>M. fortuitum</i> , <i>M. peregrinum</i> , <i>M. scrofulaceum</i>	[28]
	2007–2010	Patients with respiratory sample positive for NTM/confirmation of NTM infection	NA	108	<i>M. avium</i> complex (40%), <i>M. gordonae</i> (34%)	Others	[29]
		Subset of confirmed PNTM disease cases		23	<i>M. avium</i> complex (73%), <i>M. kansasii</i> (9%), <i>M. gordonae</i> (9%), <i>M. xenopi</i> (9%), <i>M. genavense</i> (9%)	–	
1997–2004	HIV-negative patients with respiratory sample positive for NTM/confirmation of NTM infection	NA	102	<i>M. avium</i> complex (47%), <i>M. fortuitum</i> (21%), <i>M. gordonae</i> (19%), <i>M. kansasii</i> (7%), <i>M. chelonae</i> (2%), <i>M. peregrinum</i> (2%)	<i>M. abscessus</i> , <i>M. scrofulaceum</i> , <i>M. terrae</i>	[30]	
	Subset of confirmed PNTM disease cases		16	<i>M. avium</i> complex (75%), <i>M. kansasii</i> (13%), <i>M. fortuitum</i> (6%), <i>M. abscessus</i> (6%)	–		
Multistate, Brazil	1994–1999	Patients with presumptive mycobacteriosis/confirmation of NTM infection	NA	431	<i>M. avium</i> complex (38%), <i>M. kansasii</i> (14%), <i>M. fortuitum</i> (14%), <i>M. abscessus</i> (7%), <i>M. terrae</i> (4%)	<i>M. scrofulaceum</i> , <i>M. gordonae</i> , <i>M. peregrinum</i> , <i>M. szulgai</i> , <i>M. chelonae</i> , <i>M. flavescens</i> , <i>M. asiaticum</i> , <i>M. nonchromogenicum</i> , <i>M. mucogenicum</i> , <i>M. malmoense</i> , <i>M. marinum</i>	[31]
		Subset of confirmed PNTM disease cases	NA	64	<i>M. avium</i> complex (53%), <i>M. kansasii</i> (20%), <i>M. fortuitum</i> , <i>M. abscessus</i> (6%), <i>M. chelonae</i> (6%), <i>M. scrofulaceum</i> (4%), <i>M. szulgai</i> (3%)	–	
São Paulo state, Brazil	2009–2010	Patients with clinical sample positive for NTM/no confirmation of NTM infection	23	84	<i>M. avium</i> complex (45%), <i>M. fortuitum</i> (10%), <i>M. abscessus</i> (10%), <i>M. kansasii</i> (8%), <i>M. gordonae</i> (6%), <i>M. flavescens</i> (4%)	<i>M. xenopi</i> , <i>M. szulgai</i> , <i>M. parmense</i> , <i>M. chelonae</i> , <i>M. lentiflavum</i> , <i>M. simiae</i> , <i>M. sherrisii</i> , <i>M. mucogenicum</i> , <i>M. kubicae</i> , <i>M. alvei</i>	[32]
	2000–2004	HIV-positive patients with symptoms of pulmonary tuberculosis	24.2	42	<i>M. avium</i> complex (14%), <i>M. fortuitum</i> (14%), <i>M. kansasii</i> (12%)	<i>M. chelonae</i> , <i>M. shimodei</i> , <i>M. triviale</i> , <i>M. terrae</i> , <i>M. peregrinum</i>	[33]
	1996–2005	Patients with respiratory sample positive for NTM/confirmation of NTM infection	24.4	271	<i>M. avium</i> complex (56%), <i>M. gordonae</i> (12%), <i>M. fortuitum</i> (8%), <i>M. terrae</i> (3%), <i>M. chelonae</i> (3%)	<i>M. kansasii</i> , <i>M. abscessus</i> , <i>M. scrofulaceum</i> , <i>M. peregrinum</i> , <i>M. shimodei</i> , <i>M. malmoense</i> , <i>M. neoaurum</i> , <i>M. flavescens</i> , <i>M. interjectum</i>	[34]
		Subset of confirmed PNTM disease cases		35	<i>M. avium</i> complex (63%), <i>M. fortuitum</i> (11%), <i>M. gordonae</i> (9%), <i>M. chelonae</i> (6%)	<i>M. kansasii</i> , <i>M. abscessus</i> , <i>M. malmoense</i>	
	2000–2005	Patients with symptoms of pulmonary tuberculosis/confirmation of NTM infection	NA	194	<i>M. kansasii</i> (34%), <i>M. avium</i> complex (17%), <i>M. fortuitum</i> (13%), <i>M. peregrinum</i> (2%), <i>M. gordonae</i> (2%)	<i>M. nonchromogenicum</i> , <i>M. chelonae</i> , <i>M. terrae</i> , <i>M. triviale</i> , <i>M. flavescens</i> , <i>M. bohemicum</i> , <i>M. shimodei</i> , <i>M. lentiflavum</i>	[35]
	1991–1997	Patients with respiratory sample positive for NTM/no confirmation of NTM infection	NA	600	<i>M. avium</i> complex (51%), <i>M. kansasii</i> (21%), <i>M. gordonae</i> (12%), <i>M. fortuitum</i> (5%), <i>M. terrae</i> (3%)	<i>M. chelonae</i> , <i>M. xenopi</i> , <i>M. szulgai</i>	[36]

1995–1998	Patients with clinical sample positive for NTM/no confirmation of NTM infection	17.1	1604	<i>M. avium</i> complex (58%), <i>M. kansasii</i> (18%), <i>M. gordonae</i> (7%), <i>M. chelonae</i> (5%), <i>M. fortuitum</i> (4%)	<i>M. terrae</i> , <i>M. xenopi</i> , <i>M. marinum</i> , <i>M. genavense</i> , <i>M. szulgai</i> , <i>M. celatum</i>	[37]
2010–2011	Patients with presumptive mycobacteriosis/confirmation of NTM infection	NA	29	<i>M. massiliense</i> (45%), <i>M. avium</i> complex (21%), <i>M. simiae</i> complex (10%), <i>M. abscessus</i> (7%)	<i>M. boletii</i> , <i>M. morioikense</i> , <i>M. fortuitum</i> , <i>M. celatum</i> , <i>M. kansasii</i>	[38]
1999–2010	Patients with symptoms of pulmonary tuberculosis/confirmation of NTM infection	8	249	<i>M. avium</i> complex (36%), <i>M. simiae</i> complex (22%), <i>M. massiliense</i> (19%), <i>M. fortuitum</i> (10%), <i>M. boletii</i> (6%)	<i>M. abscessus</i> , <i>M. kansasii</i> , <i>M. scrofulaceum</i> , <i>M. parascrofulaceum</i> , <i>M. szulgai</i> , <i>M. terrae</i> , <i>M. smegmatis</i>	[39]
	Subset of confirmed PNTM disease cases		194	<i>M. avium</i> complex (36%), <i>M. simiae</i> complex (25%), <i>M. boletii</i> (7%), <i>M. abscessus</i> (4%), <i>M. fortuitum</i> (3%)	<i>M. kansasii</i> , <i>M. scrofulaceum</i> , <i>M. szulgai</i> , <i>M. terrae</i>	
1993–2011	Patients diagnosed with PNTM disease	NA	174	<i>M. kansasii</i> (34%), <i>M. avium</i> complex (30%), <i>M. abscessus</i> (13%), <i>M. fortuitum</i> (8%), <i>M. massiliense</i> (2%)	<i>M. peregrinum</i> , <i>M. asiaticum</i> , <i>M. simiae</i> , <i>M. lentiflavum</i> , <i>M. szulgai</i> , <i>M. celatum</i> , <i>M. terrae</i>	[40]
2008–2010	Patients with symptoms of pulmonary tuberculosis/confirmation of NTM infection	17	75	<i>M. abscessus</i> (32%), <i>M. avium</i> complex (23%), <i>M. fortuitum</i> (12%), <i>M. gordonae</i> (3%), <i>M. gilvum</i> (3%)	<i>M. asiaticum</i> , <i>M. szulgai</i> , <i>M. tusciae</i> , <i>M. phlei</i> , <i>M. simiae</i> , <i>M. novocastrense</i> , <i>M. porcinum</i> , <i>M. holsatticum</i>	[41]
2008–2010	Patients with clinical sample positive for NTM/no confirmation of NTM infection	33	64	Not identified to the species level		[42]
2007	Patients with symptoms of pulmonary tuberculosis/confirmation of NTM infection	8.7	9	<i>M. gordonae</i> (33%), <i>M. kansasii</i> (22%), <i>M. abscessus</i> (22%), <i>M. smegmatis</i> (11%), <i>M. flavescens</i> (11%)	–	[43]
1998–2003	Patients with symptoms of pulmonary tuberculosis/confirmation of NTM infection	8.2	19	<i>M. abscessus</i> / <i>M. chelonae</i> (58%), <i>M. avium</i> complex (16%), <i>M. kansasii</i> (16%), <i>M. fortuitum</i> (11%)	–	[44]

Society and the Infectious Diseases Society of America (ATS/IDSA) have issued diagnostic criteria to aid diagnosis of PNTM disease cases and distinguish them from simple colonization [45]. Clinical, radiographic and microbiological evidence are needed, as well as exclusion of other disorders such as TB. Unfortunately, for a great proportion of the published studies on PNTM these criteria and clinical relevance of NTM isolation were not assessed. However, some of these studies were relevant for the present review and were likewise included, although special care was taken to provide the accurate information to the reader. Furthermore, the population analyzed and type of clinical specimens included in each study varies widely, rendering comparisons between the few available studies difficult to draw. Nevertheless, some patterns emerge from a careful analysis of the literature, with some species being particularly prominent. Table 2 ranks the species isolated in Portugal and Brazil according to their frequency of isolation across all the studies included in this review, revealing *M. avium* complex, *Mycobacterium kansasii*, *Mycobacterium gordonae*, *Mycobacterium fortuitum* and *M. abscessus* as the most consistently frequent species when both countries are considered. Next, the NTM most frequently isolated from respiratory samples in Portugal and Brazil are presented and their clinical relevance briefly discussed.

2.1. Pulmonary infections with *Mycobacterium avium* complex (MAC)

The *M. avium* complex harbors the species *Mycobacterium avium* (which itself includes the subspecies *M. avium* subsp. *avium*, '*M. avium* subsp. *hominissuis*', *M. avium* subsp. *paratuberculosis* and *M. avium* subsp. *silvaticum* [50,51]), *Mycobacterium intracellulare*, *Mycobacterium chimaera*, *Mycobacterium colombiense*, *Mycobacterium arosiense*, *Mycobacterium vulneris*, *Mycobacterium marseillense*, *Mycobacterium bouchodurhonense* and *Mycobacterium timonense* (Figure 1). In humans, '*M. avium* subsp. *hominissuis*', *M. intracellulare* and *M. chimaera* seem to be the most relevant opportunistic pathogens of the complex. These are the most frequently isolated mycobacteria in Portugal and Brazil, even though most studies do not attempt to distinguish between the different species and subspecies of MAC. The frequency of MAC among mycobacterial isolates shows a lot of variation between studies, but is often around 50%. In Portugal, it seems to be more common in the Northern region, with frequencies ranging from 55% to 75% of all NTM isolates [27–30], than in Lisbon, where it has been reported to be below 35% [25,26], although data are limited. One consistent finding across studies that attempt to distinguish disease cases within the sampled population is that MAC isolation, unlike isolation of other species such as *M. gordonae*, is significantly associated with NTM disease [29,31,34]. This highlights the clinical relevance of this documented and often deadly human pathogen [52]. MAC lung disease is known for its high treatment failure rates and poor outcome, mainly due to its relative resistance to chemotherapy and to a frustrating lack of correlation between *in vitro* susceptibility results and *in vivo* clinical responses to tested antibiotics [21]. This lack of correlation is also observed for other NTM species and has been attributed to innate resistance mechanisms, differences in the metabolic state of mycobacteria *in vitro* and *in vivo*, and to laboratory technical difficulties with drug susceptibility assays [46,47].

2.2. Pulmonary infections with *Mycobacterium kansasii*

Mycobacterium kansasii is the second NTM species most frequently isolated from respiratory samples in Brazil. In two studies conducted in São Paulo and Rio de Janeiro it was actually

Table 2
Rank order of the species of NTM isolated from clinical samples in Portugal and Brazil according to their frequency of isolation in all pulmonary NTM infection studies reviewed. A total of 806 NTM isolates in Portugal and 3781 in Brazil were considered.

Portugal		Brazil	
<i>M. avium</i> complex	49%	<i>M. avium</i> complex	48%
<i>M. gordonae</i>	16%	<i>M. kansasii</i>	17%
<i>M. fortuitum</i> , <i>M. peregrinum</i>	6%	<i>M. fortuitum</i>	7%
<i>M. kansasii</i> , <i>M. chelonae</i>	5%	<i>M. gordonae</i>	6%
<i>M. abscessus</i>	3%	<i>M. abscessus</i>	5%
<i>M. xenopi</i>	2%	<i>M. chelonae</i>	3%
<i>M. scrofulaceum</i>	1%	<i>M. terrae</i> complex, <i>M. simiae</i> complex	2%
<i>M. mucogenicum</i> , <i>M. szulgai</i> , <i>M. triplex</i> , <i>M. lentiflavum</i> , <i>M. simiae</i> complex, <i>M. genavense</i>	<1%	<i>M. scrofulaceum</i> , <i>M. szulgai</i> , <i>M. peregrinum</i> , <i>M. xenopi</i> , <i>M. flavescens</i> , <i>M. marinum</i>	<1%
<i>M. malmoense</i> , <i>M. heckeshornense</i> , <i>M. celatum</i> , <i>M. terrae</i> complex	≤0.1%	<i>M. shimodei</i> , <i>M. triviale</i> , <i>M. asiaticum</i> , <i>M. lentiflavum</i> , <i>M. malmoense</i> , <i>M. nonchromogenicum</i> , <i>M. celatum</i> , <i>M. smegmatis</i> , <i>M. parmense</i> , <i>M. gilvum</i> , <i>M. mucogenicum</i> , <i>M. genavense</i> , <i>M. parascrofulaceum</i> , <i>M. moriokaense</i> , <i>M. holsaticum</i> , <i>M. tuscicae</i> , <i>M. phlei</i> , <i>M. novocastrense</i> , <i>M. porcinum</i> , <i>M. bohemicum</i> , <i>M. interjectum</i> , <i>M. neoaurum</i> , <i>M. alvei</i> , <i>M. kubicae</i> , <i>M. sherrisii</i>	≤0.1%

the most prevalent species in a group of patients with symptoms of pulmonary disease [35,40]. A multi-country survey covering the period from 1991 to 1996 reported no *M. kansasii* isolates in Portugal, while in Brazil 12% of the NTM analyzed belonged to this species [53]. The scenario in Portugal seems to have changed since then, as all Portuguese studies considered in the present review report the isolation of *M. kansasii* strains, although still with much lower frequency when compared with Brazil. Despite being a clinically relevant NTM species and a common cause of pulmonary infection, *M. kansasii* is often considered the most easily treatable NTM pathogen, as it is generally susceptible to common antituberculous drugs and shows a good correlation between *in vitro* susceptibility tests and clinical response [21,45]. However, the possibility of emergence of additional or increasingly resistant strains should not be discarded. As an example, although *M. kansasii* is reportedly sensitive to ethambutol [45], Telles and co-workers found a strikingly high ethambutol-resistance frequency in clinical relevant isolates from São Paulo [54].

2.3. Pulmonary infections with *Mycobacterium abscessus*

The isolation of *Mycobacterium abscessus* from respiratory samples is generally not as frequent as MAC and *M. kansasii*, but the threat posed by an *M. abscessus* pulmonary infection is often more serious and deserves particular attention. *M. abscessus* comprises two different subspecies, namely *M. abscessus* subsp. *abscessus* and *M. abscessus* subsp. *bolletii*, which has also recently included *Mycobacterium massiliense* [55]. Members of this species are remarkably resistant to chemotherapy and have been referred to as an “antibiotic nightmare” [56] for which there are no reliable antibiotic regimens recommended [45]. Genomic analyses revealed unique features and genetic resources that render them one of the most divergent species of the genus, which also helps explaining and translates into particular physiological trends [10]. In Rio de Janeiro, Mello and co-workers followed 174 patients with PNTM disease and among 12 NTM species involved, the lowest cure rate was observed among *M. abscessus* infection cases, basically related to the severe patterns of resistance of this strain [40]. Another study conducted in Piauí in 2007 with 9 patients with NTM disease, revealed that two were infected with *M. abscessus*. Both suffered treatment failures and one died 5 years after the diagnosis [43].

2.4. Pulmonary infections with *Mycobacterium fortuitum*

Mycobacterium fortuitum was initially isolated in Brazil in 1938 and the subsequent isolates were later classified as *M. fortuitum*

subsp. *fortuitum* and *M. fortuitum* subsp. *acetamidolyticum* [57,58]. Commonly found in respiratory samples from Portugal and Brazil, the isolation of *M. fortuitum* does not seem to be as clinically significant as the isolation of MAC, *M. kansasii* and *M. abscessus*. Often, it is merely indicative of transient colonization in patients with an underlying lung disease and does not progress even without antibiotic therapy directed at *M. fortuitum* [59]. However, it can cause pulmonary disease in humans, particularly in patients with gastroesophageal disorders [45]. Among the studies conducted in Portugal, there is at least one described case of lung disease caused by *M. fortuitum* meeting the ATS/IDSA criteria in a patient who had to receive specific antibiotic treatment based on *in vitro* susceptibility tests [28]. In São Paulo, Pedro and co-workers found 4 cases of confirmed *M. fortuitum* lung infection [34]. Another study conducted in Rio de Janeiro identified 11 cases meeting the ATS criteria, of which only 5 were successfully cured [40].

2.5. Pulmonary infections with *Mycobacterium gordonae*

Ubiquitous in the environment and in tap water, *Mycobacterium gordonae* is isolated very frequently from respiratory samples but rarely associated with disease, being regarded more as a contaminant than as a pathogen [45,60]. Isolates of this species have been identified in all Portuguese and in most Brazilian studies dealing with NTM respiratory isolates, reaching frequencies of over 30% of all NTM isolated in some cases [29,43]. It is more frequently isolated in Portugal than in Brazil (Table 2). When clinical relevance is assessed, though, most *M. gordonae* isolates are found not to be associated with disease. Nevertheless, *M. gordonae* pulmonary disease is not unheard-of even in immunocompetent individuals [60] and some cases in Portugal and Brazil have been reported. In Portugal, Campaignha and co-workers identified 2 cases of pulmonary disease caused by *M. gordonae* [29]. In Brazil, 2 studies conducted in 2 different states reported 3 cases of *M. gordonae* lung disease each [43,34]. The 3 cases reported by Bona and co-workers were successfully treated with clarithromycin.

2.6. Pulmonary infections with other less common NTM species

In addition to the aforementioned NTM, 36 other species/complexes have been isolated from clinical samples in Portugal and Brazil, albeit less frequently (Table 2). Of these, only 15 are considered clinically relevant: *Mycobacterium asiaticum*, *Mycobacterium celatum*, *Mycobacterium chelonae*, *Mycobacterium flavescens*, *Mycobacterium genavense*, *Mycobacterium lentiflavum*, *Mycobacterium malmoense*, *Mycobacterium moriokaense*, *Mycobacterium*

peregrinum, *Mycobacterium scrofulaceum*, *Mycobacterium simiae* complex, *Mycobacterium smegmatis*, *Mycobacterium szulgai*, *Mycobacterium terrae* complex and *Mycobacterium xenopi* (Table 1). Two other NTM species deserve special mention because they were recently discovered and first described in respiratory specimens from Brazilian patients with lung disease. In 2013, Ramos and co-workers described *Mycobacterium fragae*, closely related to *M. celatum*, from 3 clinical isolates of a Ceará patient with lung infection [61]. Even more recently, Fusco da Costa and co-workers described a new species within the *M. simiae* complex, which appears to be particularly prevalent in the state of Pará and was denominated *Mycobacterium paraense* [62].

2.7. NTM pulmonary infection in cystic fibrosis patients

NTM are increasingly recognized as clinically relevant infectious agents in cystic fibrosis (CF) patients, colonizing and infecting their lungs and capable of significantly affecting morbidity and mortality [49,63,64]. Worldwide, NTM isolation in respiratory samples has been reported in 6–22% of patients with CF, with MAC and *M. abscessus* together accounting for over 90% of the cases [63]. It should be noted that in many cases a transient or persistent NTM colonization in CF patients does not progress to active pulmonary disease [64]. In Brazil, at least two studies have investigated this problem, one conducted in adults and the other in children. Paschoal and co-workers studied a population of adult CF patients with a less severe phenotype of the disease and found a colonization by NTM in 11% of the patients [65]. However, none of the patients were diagnosed with NTM disease and specific drug therapy was not prescribed. More recently, Cândido and co-workers reported a NTM frequency of 8% in CF children and performed species identification and antibiotic susceptibility tests with the isolated mycobacteria [66]. *M. abscessus* group species predominated followed by MAC and *M. fortuitum*. The antibiotic susceptibility profiles resulting from this study are alarming, with over 97% of the isolates being resistant to 5 or more drugs specifically used for mycobacterial infections, and one of the patients from whom one of these multidrug-resistant strains was isolated eventually developed PNTM disease without resolution to the date the article was elaborated [66].

3. HIV infection and NTM disseminated disease

The tight link between HIV infection and TB is widely known [67], but NTM also pose a serious threat and are a common cause of disease in AIDS patients [68]. NTM infection in AIDS patients may be present in the form of pulmonary disease, and it should be noted that many of the studies cited in section 2 include mixed samples of HIV-positive and HIV-negative persons. However, interpretation of NTM isolation from respiratory samples of HIV-positive patients requires additional considerations. Firstly, some species that are generally considered nonpathogenic and whose isolation from immunocompetent hosts is usually not clinically relevant have been associated with disease in HIV-infected persons [45]. Secondly, in the absence of evidence of pulmonary disease, isolation of NTM from respiratory samples of AIDS patients may indicate or foretell a case of NTM disseminated disease [45].

Diagnosis of NTM disseminated disease is usually performed through culture of clinical samples from sites presumed to be sterile of the patient, such as blood, bone marrow or lymph nodes [45]. In Portugal, the only data of NTM isolation from presumably sterile sites was reported by Couto and co-workers, who identified 14 MAC and one *M. simiae* isolates from blood cultures of patients

with symptoms of mycobacteriosis [26], although the HIV-status of the patients was not reported. MAC (specifically '*M. avium* subsp. *hominissuis*' in the vast majority of cases) is indeed the most frequently reported NTM associated with disseminated disease in HIV-positive patients, and the results from Brazilian studies are also consistent with this observation [69–71]. Other species isolated from presumed sterile sites of AIDS patients in these studies, and which were confirmed to be involved in disseminated disease, were *M. kansasii* and *M. scrofulaceum*. *M. lentiflavum* has also been isolated from blood of a patient with NTM disseminated disease in Brazil who was also co-infected with *M. avium* [72], and a fatal case of *M. simiae* disseminated disease has also been described [73].

4. Post-surgical infections with rapidly-growing mycobacteria

Infection by RGM following invasive procedures has been described worldwide and increasingly in Brazil [74–76] leading to its recognition as a serious health problem by the Brazilian National Agency for Sanitary Surveillance (Agência Nacional de Vigilância Sanitária - ANVISA) [76]. Inadequate decontamination of surgical equipment, emergence and dissemination of disinfectant-resistant strains and their presence in antimicrobial solutions as well as lack of appropriate infection-control measures are very often the source of severe post-surgical infection outbreaks [77]. Several studies have focused on this issue in Brazil and detailed descriptions of RGM surgical-site infection outbreaks can be found in the literature. Environmental investigation following major outbreaks is increasingly performed to identify the source of infection and inform the adoption of preventive measures, although in a few cases only after the clinics have adopted the adequate measures and changed practices to contain the outbreaks, including thorough cleaning and disinfection of the more obvious potential sources of contamination, resulting in the source of infection never being conclusively identified [78,79].

Unlike PNTM disease, NTM post-surgical infection cases are of compulsory notification in Brazil since 2009 due to the occurrence of outbreaks of unprecedented magnitude across the country, which led Brazilian health authorities to recognize these infections as a national health emergency [80]. In this section, the invasive health or cosmetic procedures most often associated with RGM post-surgical infections and outbreaks in Brazil over the last years are reviewed.

4.1. Infectious keratitis with NTM

Infectious mycobacterial keratitis is a rare complication of laser-assisted *in situ* keratomileusis (LASIK), a commonly used vision correction surgical procedure that enhances the refractive condition of the cornea [81]. Several cases and outbreaks of post-LASIK mycobacterial infection have been described in Brazil, mainly involving *M. chelonae*. In fact, the first detailed descriptions of RGM surgical infections in Brazil were associated with this procedure [82]. *M. chelonae* was responsible for the 2 largest reported outbreaks. The first occurred in 1999 at a private clinic in Rio de Janeiro, with 65 eyes diagnosed with infectious keratitis following LASIK, of which 16 were confirmed to be positive for *M. chelonae* [83]. One year later another *M. chelonae*-caused keratitis outbreak emerged at a private clinic in São Paulo, affecting 10 patients treated by the same surgeon. A possible source of infection was identified during the investigation that followed, with evidence pointing to the portable steamer used to clean the microkeratome, whose water was found to be contaminated with *M. chelonae* [84]. In 1998, two patients who underwent LASIK at a private clinic in Rio

de Janeiro developed surgical infections by *M. abscessus*. This mycobacteria was also responsible for 2 other cases at a clinic in São Paulo in 1999 and 2000 [83]. Although fluoroquinolones are often the antibiotics of choice for corneal infections, a subsequent investigation found that *M. abscessus* and *M. chelonae* isolates from these Brazilian outbreaks were highly resistant to this class of antibiotics, emphasizing the importance of early diagnosis of NTM-caused infections to expand therapeutic options [85]. *Mycobacterium immunogenum* was also implicated in an outbreak of keratitis in São Paulo involving 5 patients who underwent LASIK in 2003 [78].

4.2. NTM infection in prosthetic valve endocarditis

Prosthetic valve endocarditis (PVE) caused by NTM is a rare but documented complication of valve implant surgeries [86]. In São Paulo, a cluster of 13 implanted patients who developed PVE caused by *M. chelonae* has recently been reported [87]. All patients had received porcine valve implants between 1997 and 2002 and all the valves were from the same manufacturer. Investigation of 6 non-implanted and supposedly sterile valves revealed contamination with *M. chelonae*, which led the hospital to discontinue their use. These cases were not the first of *M. chelonae* PVE described in Brazil [88] and persistence of viable *M. chelonae* in porcine valves after chemical treatment for use as biological prosthetic valves had already been investigated and discussed in the Brazilian literature [89].

4.3. Post-mammoplasty NTM infections and the Campinas' outbreak

Between 2002 and 2004, an outbreak of post-mammoplasty surgical-site infections emerged in the city of Campinas, state of São Paulo, affecting 35 patients. All but one of the 12 isolates recovered were identified as *M. fortuitum*. During this period, 5 patients who underwent mammoplasty surgery in other cities and one patient who had abdominal wall plastic surgery in Campinas were also diagnosed with *M. fortuitum* surgical-site infections, although molecular typing results suggested that this was a polyclonal outbreak with no signs of clone dissemination between hospitals [90]. The source of infection was never conclusively identified, but the investigation that followed raised suspicions regarding the use of reusable sizers, commonly employed by surgeons to estimate the volume of the definitive implant prior to its application [91]. This led ANVISA to temporarily suspend the use of the device [92] until the manufacturers introduced the necessary improvements, one of the measures that likely contributed to the elimination of post-mammoplasty NTM infections in the affected hospitals the year following the outbreak [91]. In fact, *M. fortuitum* is the NTM most frequently associated with post-mammoplasty infections in Brazil, responsible for more than half of the cases reported to the health surveillance system until 2009, followed by *M. abscessus* [93]. Other NTM infectious agents have been implicated namely *Mycobacterium porcinum* which was responsible for one of the Campinas' outbreak cases [90]. In 2010 the first case of *Mycobacterium wolinskyi* breast infection in Brazil was reported [94]. Cases involving *M. peregrinum* and *M. smegmatis* have also been reported [93]. The most recent ANVISA report on RGM infections shows that post-mammoplasty mycobacterial infections are still an important health problem in Brazil despite the preventive measures enforced by ANVISA in the aftermath of the Campinas' outbreak, with 85 cases reported between 2010 and 2014, including an outbreak in a Bahia clinic in 2013 [76].

4.4. Epidemic of *Mycobacterium massiliense* surgical-site infections in Brazil

Between 2004 and 2008, several Brazilian states faced nosocomial outbreaks involving a particular strain of *Mycobacterium massiliense* (recently reclassified as *M. abscessus* subsp. *bolletii*), often referred to as the BRA100 clone [55,95–97]. This nationwide epidemic began with the Belém outbreak in the state of Pará and involved 298 patients who had undergone laparoscopic surgeries in 16 different private hospitals in the city of Belém. A sample of 58 isolates were analyzed by *rpoB* sequencing and PFGE molecular typing and identified as a single clone of *M. massiliense* [98]. As soon as the Belém outbreak started to subside in the middle of 2005, an outbreak of surgical-site infections caused by the same strain of *M. massiliense* emerged in the state of Goiás, with 121 cases of patients diagnosed with NTM infection following laparoscopic and arthroscopic surgeries at 7 private hospitals in the city of Goiânia. Molecular typing and *rpoB* sequencing of 18 sampled isolates revealed the unexpected link with the epidemic isolates from the distant city of Belém [99].

In the state of Rio de Janeiro, this same strain caused the largest outbreak of RGM postsurgical infections recorded to date in Brazil, with over 1000 patients possibly affected and 63 public and private hospitals involved [95]. Between August 2006 and July 2007, 1051 possible cases were reported in this state of which 172 were confirmed by laboratory techniques. All patients had undergone video-assisted surgery. Randomly selected isolates were analyzed by *rpoB* and *hsp65* sequencing and PFGE fingerprinting, confirming their clonal identity with isolates from the Belém and Goiânia outbreaks [95]. Antimicrobial susceptibility tests revealed susceptibility to amikacin and clarithromycin and resistance to ciprofloxacin and doxycycline, as well as a high glutaraldehyde tolerance for BRA100 isolates [95,100]. This massive outbreak was followed by another in the state of Paraná, city of Curitiba, which lasted until January 2008 and involved 131 surgical patients from 7 private hospitals. Out of 39 isolates collected and analyzed, 36 were shown to belong to the *M. massiliense* BRA100 clone [96]. Other 3 outbreaks in the states of Espírito Santo, São Paulo, and Rio Grande do Sul were also found to be caused by this same strain [97].

All these outbreaks were associated with video-assisted surgeries involving the use of laparoscopic or arthroscopic equipment. At the time, all hospitals and clinics involved were using the same protocol for high-level disinfection of surgical equipment between surgeries: immersion in 2% glutaraldehyde for at least 30 min. While this procedure is effective in eliminating reference strains of RGM, the BRA100 clone was found to be resistant to immersion in 2% glutaraldehyde for as much as 10 h [100]. This led the Brazilian health surveillance agency to immediately suspend this practice [80]. The glutaraldehyde-resistant phenotype in mycobacteria has been associated with defects in porin expression [101] and could in part account for the Brazilian *M. massiliense* epidemic. Further research in the BRA100 clone also showed increased virulence and *in vivo* pathogenicity of this strain when compared with a glutaraldehyde-sensitive *M. massiliense* reference strain [102]. In addition to this, inspections carried out by public health authorities revealed that some of the hospitals involved were breaching important protocols of infection control and prevention [99,103], which may also have played a role in the epidemic.

A study conducted in Rio Grande do Sul in the aftermath of the epidemic found that the BRA100 clone persisted in several hospitals of the state after the major 2004–2008 outbreaks, causing postsurgical infections and small outbreaks across the state at least until 2011 [104]. Perhaps the most worrying finding of this study was the resistance to clarithromycin shown by a significant

proportion of the isolates involved in these more recent cases, something that had not yet been reported for the BRA100 clone.

Surprisingly, a recent study found remarkable genetic similarities between BRA100 epidemic isolates and *M. massiliense* isolates involved in an outbreak of respiratory infections in a cystic fibrosis center in the UK [105,106], raising the intriguing and alarming possibility of intercontinental dissemination of a particularly disinfectant-resistant and virulent mycobacterial strain.

4.5. Cutaneous NTM infections due to non-surgical invasive procedures

In addition to post-surgical infections by RGM, infections associated with the use of invasive procedures for cosmetic or medical purposes have also been described in Brazil and Portugal. In Brazil, there have been several reports of mycobacterial infections and outbreaks associated with mesotherapy, a popular treatment to reduce localized fat performed in aesthetic clinics that consists of intradermal or subcutaneous topical injections of pharmacological substances. In 2000, the largest described outbreak involving NTM infections and mesotherapy took place at a private clinic in São Paulo state, with 43 people diagnosed with cutaneous infections. The infectious agent was identified as *M. chelonae* [83]. In 2002 there was another outbreak in a cosmetic clinic in the same state, with 7 confirmed cases of *M. abscessus* skin infection. In both cases, multiple mesotherapy sessions were reportedly performed with solutions stored in multiuse vials, a factor that may have contributed to the outbreaks [83]. Concomitantly with the Belém *M. massiliense* outbreak mentioned in section 4.4, a smaller outbreak caused by another *M. abscessus* subsp. *bolletii* strain affected 14 patients who underwent mesotherapy at a private clinic also in Belém [98]. *M. fortuitum* has also been associated with mesotherapy-acquired skin infections in São Paulo and Bahia [107,108]. Recently disclosed data reveals that over the last 4 years (2010–2014) mesotherapy-associated RGM infections have remained a concerning public health problem. Over this period, 63 cases were reported in Brazil, all involving illegal subcutaneous injections of phosphatidylcholine in the form of Lipostabil® for cosmetic purposes [76]. Worldwide, mesotherapy-associated NTM infections have been traced to contaminated injectable solutions or skin antiseptics and to inappropriate cleaning of injection devices with nonsterile tap water, but no environmental source was identified in these Brazilian outbreaks [109,110].

Acupuncture is also a procedure for which association with mycobacterial infections has been described worldwide. In Brazil, Castro-Silva and co-workers described one case of an immunosuppressed kidney transplant patient who developed a cutaneous *Mycobacterium haemophilum* infection after being prescribed with acupuncture for pain relief [111]. Skin lesion sites matched needle insertion sites perfectly, strongly suggesting inoculation during the acupuncture sessions. The patient reported lack of infection control measures by the acupuncture practitioner, namely that his skin was not cleaned prior to needle insertion and that the same reusable needles were used multiple times during the session. In Portugal, a case of *M. fortuitum* skin infection following acupuncture has also been reported [112].

Although there are no reports of NTM infections associated with tattooing in Portugal and Brazil, many cases and outbreaks of tattoo-associated NTM skin infections have emerged over the last decade, with reports from the USA, France, Germany, Spain, Scotland, Switzerland, Australia, Israel, Serbia and Thailand [113–116]. In almost all cases the source of infection was traced to the tattoo ink or to tap water used for ink dilution, and the species involved were *M. chelonae*, *M. abscessus*, *M. haemophilum*, *M. fortuitum* and *M. immunogenum* [116].

5. High-risk environmental isolation of NTM

The ubiquity of NTM in the environment and the very limited evidence of human-to-human transmission seem to point to the surrounding environment as the most likely source of human NTM infection and colonization [19,106]. Tap water in particular seems to be an important reservoir of these environmental species of mycobacteria, which are highly resistant to chlorine disinfection and thrive in showerhead biofilms [11,12]. In Lisbon, the detection of *M. gordonae*, *M. intracellulare*, *M. kansasii* and *M. chelonae* in the municipal water distribution system has been reported [117]. Coincidentally, these four species are also among the most frequently isolated NTM in respiratory samples of Lisbon residents [25,26].

NTM isolation from hospital settings is also common and poses a particularly serious risk for hospitalized patients, who are frequently more vulnerable to these opportunistic pathogens. Recognizing this threat, Restrepo and co-workers investigated the presence of NTM in samples of tap water, antiseptic solutions and surgical gloves from a university hospital of Manaus and concluded that the hospital water distribution system was colonized by several species of NTM. *M. celatum* and *M. gordonae* were the most frequently isolated species, followed by *M. intracellulare*, *M. lentiflavum* and *Mycobacterium mucogenicum* [118]. In a similar study carried out at a São Paulo hemodialysis center, Sartori and co-workers isolated *M. lentiflavum*, *M. gordonae*, *M. kansasii*, *Mycobacterium gastri* and *M. szulgai* in its hydric system and suggested that NTM biofilms in the center's plumbing and inadequate water decontamination processes were likely the source of the contamination [119]. *M. lentiflavum* was particularly abundant and seemed to persist and thrive in the hemodialysis center water system. Also in São Paulo, Souza and co-workers isolated NTM from exposed surfaces in the clinical environment of 2 health institutions and found both potentially pathogenic and rarely pathogenic species, including *M. avium*, *M. fortuitum*, *M. kansasii*, *M. malmoense*, *M. lentiflavum*, *M. simiae*, *M. mucogenicum*, *M. smegmatis*, *M. flavescens*, *M. gastri*, *M. genavense*, *Mycobacterium vaccae* and, the most frequent, *M. gordonae* [120]. In Portugal the lack of this type of environmental investigation is even more conspicuous than in Brazil. In a preliminary assessment we have recently isolated several drug resistant NTM strains from surfaces of a Portuguese central hospital belonging to the species *M. gordonae*, *M. mucogenicum* and *Mycobacterium obuense* (our unpublished results). Alarming, susceptibility tests revealed multidrug-resistant phenotypes to multiple classes of antibiotics (>5 antibiotics recommended by Clinical & Laboratory Standards Institute (CLSI) standards in addition to the 5 antibiotics used for selective isolation of mycobacteria).

6. Future directions

The therapeutic challenges posed by opportunistic mycobacteria are a direct consequence of their unique physiology and ecology, and will not be met without intensifying efforts into understanding their traits and finding new strategies for prevention and treatment of NTM infections. Because tap water seems to be one of the main routes of human exposure to NTM, reducing everyday exposure to these pathogens is necessarily tied to reducing their numbers in household and hospital plumbing premises. Measures to achieve this have been proposed based on recent research, including raising water heater temperature to 55 °C and improving aeration [6]. Treating most NTM infections is currently an arduous task, limited by their intrinsic resistance to many known antibiotics. New drugs are urgently needed, and while attempts to test antibiotics developed for *M. tuberculosis* against NTM have produced disappointing results [121,122], the same avenues of research may be applied for the specific cases of NTM species. Some

of these approaches include revealing and exploring novel targets against which new drugs can be designed through combining atomic-level analyses with protein chemistry and cell biology, repurposing and re-engineering existing compounds, and finding innovative immune-based strategies such as autophagy manipulation [10,123,124].

7. Concluding remarks

The current status of NTM infections in Brazil is definitely alarming while in Portugal it is largely and worryingly unknown and severely underreported. In both countries, there is an urgent need for more and better-planned epidemiological surveys and for in-depth environmental investigations of NTM persistence in water and inanimate surfaces of high-risk sites, healthcare settings primarily. Even more critically, studies comparing environmental isolation of NTM with spatially and temporally related clinical cases of NTM infection are urgently required to better understand and manage the real risks of NTM exposure in different settings. The fact that the few existing studies already show the isolation of so many NTM strains from healthcare settings, some of which belonging to species often involved in serious human infections, is a matter of increasing concern and calls for the adoption of more effective and regular disinfection protocols. Our preliminary results obtained with a set of multi-drug resistant strains recently isolated from a Portuguese tertiary hospital indicate that they are susceptible to common disinfectants, reinforcing the importance of implementing proper and regular disinfection procedures in NTM infection prevention. If cost reduction policies continue to hinder the implementation of adequate preventive measures in Portuguese hospitals and other healthcare institutions, the situation can rapidly run out of control and reach massive proportions, as was the case with the *M. massiliense* epidemic in Brazil.

In view of the therapeutic challenges posed by these opportunistic mycobacteria, it is also essential to get a clearer picture of antibiotic resistance profiles of NTM strains circulating in Portuguese and Brazilian hospitals. The unusually high and multiple resistances to antibiotics and disinfectants are hallmarks of the *Mycobacterium* genus, to which both intrinsic and acquired mechanisms contribute. Intrinsic drug resistance has been primarily attributed to mycobacteria's lipid rich cell envelope which confers low permeability to antibiotics and other chemotherapeutic agents [125]. More recently, efflux mechanisms have also been recognized as an important factor in the natural resistance of mycobacteria as they play an important role in mediating the efflux of drugs such as tetracycline, fluoroquinolones and aminoglycosides, among others [126–128]. Acquired drug resistance is generally mediated by horizontal transfer of genetic elements through plasmids, transposons or integrons. In *M. tuberculosis*, which has been specifically studied in the context of drug resistance/susceptibility, this type of resistance is caused mainly by chromosomal spontaneous or induced mutations, favoring the selection of resistant strains during sub-optimal drug therapeutic regimens [129]. In some clinically relevant NTM species, such as *M. abscessus* and *M. fortuitum*, an inducible mechanism of macrolide resistance has been found to confer *in vivo* acquired resistance to this class of antibiotics in some strains without affecting *in vitro* MIC values, which further complicates the already difficult task of choosing appropriate therapeutic schemes [21]. With no reliable and clearly defined antibiotic regimens and with a constantly changing and increasingly complex scenario, NTM antibiotic resistance surveillance is therefore mandatory in order to comprehensively understand this emerging health threat and to guide increasingly difficult therapeutic decisions.

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