The geographic diversity of nontuberculous mycobacteria isolated from pulmonary samples

An NTM-NET collaborative study

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ABSTRACT A significant knowledge gap exists concerning the geographical distribution of nontuberculous mycobacteria (NTM) isolation worldwide.

To provide a snapshot of NTM species distribution, global partners in the NTM-Network European Trials Group (NET) framework (www.ntm-net.org), a branch of the Tuberculosis Network European Trials Group (TB-NET), provided identification results of the total number of patients in 2008 in whom NTM were isolated from pulmonary samples. From these data, we visualised the relative distribution of the different NTM found per continent and per country.

We received species identification data for 20 182 patients, from 62 laboratories in 30 countries across six continents. 91 different NTM species were isolated. *Mycobacterium avium* complex (MAC) bacteria predominated in most countries, followed by *M. gordonae* and *M. xenopi*. Important differences in geographical distribution of MAC species as well as *M. xenopi, M. kansasii* and rapid-growing mycobacteria were observed.

This snapshot demonstrates that the species distribution among NTM isolates from pulmonary specimens in the year 2008 differed by continent and differed by country within these continents. These differences in species distribution may partly determine the frequency and manifestations of pulmonary NTM disease in each geographical location.

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Introduction

Disease caused by nontuberculous mycobacteria (NTM) has gained increased attention, in part because of an assumed increase in its incidence [1, 2]. With the emergence of case reports and series from diverse countries and regions, it has become clear that the distribution of NTM species that are isolated from clinical samples differs strongly by region [3]. Yet this geographic diversity has never been systemically studied. Increased understanding of this diversity is important, as it can provide important clues on the impact of geographical or climatic differences on NTM distribution and observed discrepancies in clinical relevance and treatment outcome [3, 4]. In this study we have collected pulmonary NTM isolation and identification results from laboratories in different regions in the world, collaborating within the NTM-Network European Trials Group (NET) network (www.ntm-net.org, a branch of the Tuberculosis Network European Trials Group (TB-NET)), from the same time period, to gain further insight on the geographical distribution of NTM species cultured from respiratory samples at a single time point.

Methods

Global partners in the NTM-NET framework were contacted and invited to provide data of the total number of patients from whom NTM were isolated from pulmonary samples in their hospital, regional or reference laboratory in the year 2008, as well as the species identification results and details of the identification methods used. Partners were eligible to contribute to the database if the number of patients with pulmonary NTM isolates per year exceeded 30, to ensure sufficient experience and interpretability of results; one isolate per species per patient was eligible for analysis. We have chosen pulmonary samples in an effort to minimise selection bias. Isolation of NTM from normally sterile sites such as blood or lymph nodes usually indicates definite disease and this may present a strong selection bias, as not all species are equally capable of causing such diseases.

We calculated total number of mycobacteria per continent, the relative contribution of the *Mycobacterium avium* complex for each continent or country and studied the differences in the relative contribution of other NTM between countries and continents by generating pie-charts. Data of the respondents were plotted on a world map. Since most NTM-NET members are located in Europe, data from European participants were assessed in greater detail, with a focus on north–south differences; we considered Denmark, Norway, Sweden, Finland, the Netherlands, Belgium, Germany, the UK, Ireland and Poland as Northern Europe; all countries to the south of these countries were considered Southern Europe.

Within this study we did not assess the clinical relevance of these isolates. Ethical approval was waived for this retrospective laboratory database study.

Results

Overview

62 centres from 30 countries across six continents participated in this study. 17 national reference laboratories provided data representative for their whole country. For other countries, data was provided by a certain number of laboratories not covering the whole country (see the online supplementary material for details). A total of 20 182 patients had NTM species cultured from pulmonary samples in these centres in 2008; 91.3% (n=18 418) of the isolates were identified to species/complex level; the remaining 1764 isolates (8.7%) were not identified beyond *Mycobacterium* species other than *M. tuberculosis* complex. A total of 91 different NTM species were encountered in this survey. The most commonly used identification assays were the GenoType CM/AS (n=28; Hain Lifescience, Nehren, Germany), AccuProbe assays (n=9; Gen-Probe, San Diego, CA, USA), *hsp65* PCR-restriction enzyme analysis (PRA) (n=6), Inno-LiPA Mycobacteria v2 (n=3; Innogenetics, Ghent, Belgium), in-house methods (n=6) or combinations thereof; mostly, these were supplemented by 16S rDNA sequencing. The six most frequently isolated NTM were *M. avium* complex (9421 isolates; 47%), *M. gordonae* (2170 isolates; 11%), *M. xenopi* (1605 isolates; 8%), *M. fortuitum* complex (1322 isolates; 7%), *M. abscessus* (664 isolates; 3%) and *M. kansasii* (720 isolates; 4%) (fig. 1). These six species accounted for 80% of all mycobacteria identified.

A complete overview of all species isolated in all regions is provided in the online supplementary material.

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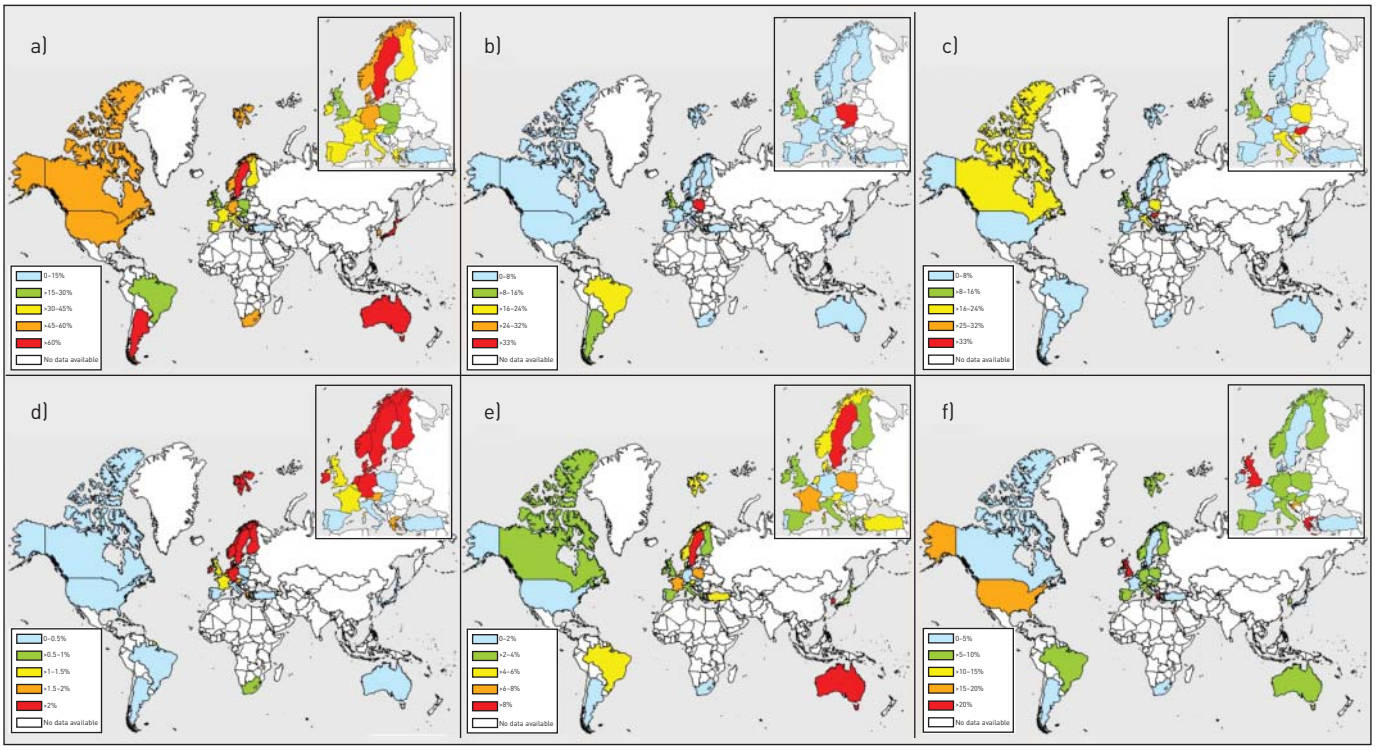

FIGURE 1 Worldwide distribution of different nontuberculous mycobacteria from pulmonary samples in 2008. a) *Mycobacterium avium* complex; b) *M. kansasii*; c) *M. xenopi*; d) *M. malmoense*; e) *M. abscessus*; and f) *M. fortuitum*. Note: the data presented in this figure are not *per se* representative for each country. Species diversity may differ per country. The exact, per-centre data can be found in the online supplementary material.

Species isolation worldwide

Figure 1 presents the distribution of the most common NTM on a world map. Table 1 and figure 2 show data for *M. avium* complex and other common NTM per continent.

M. avium complex

The *M. avium* complex (MAC) species accounted for 9421 (47%) of the 20182 isolates in the study. The highest relative contribution of MAC per continent was found in Australia (71%) and the lowest in South America (31%). Per country, this figure varied from 79% in Japan to 16% in Hungary. 59 out of 62 laboratories were able to identify MAC isolates to species level; the relative frequency of *M. intracellulare versus M. avium* in different parts of the world is shown in table 1. The most striking difference is the relative predominance, within the participating sites, of *M. avium* in North- and South America. In contrast, *M. intracellulare* was most frequent in Australia-Queensland (57% of all mycobacteria cultured and 80% of the MAC) and South Africa (40% of all mycobacteria cultured and 77.5% of the MAC). MAC isolates that were not identifiable to (sub)species level were common in participating laboratories in Asia and Europe (21% and 15% of all MAC isolates, respectively), but relatively rare in the participating laboratories in North America (8%).

M. gordonae

M. gordonae is the second most isolated NTM worldwide in this study, mainly due to a high isolation rate found in Europe, where *M. gordonae* was the second most isolated NTM. On all other continents, *M. gordonae* ranked third (North America, South America and Africa) or fourth (Asia and Australia).

M. xenopi

After MAC and *M. gordonae*, *M. xenopi* was the third most frequently isolated species in the survey, though its isolation was limited to distinct geographical regions; mainly in Europe and Ontario (eastern Canada) (but not Alberta, western Canada). In Hungary, *M. xenopi* is the predominant NTM isolate comprising 49% of all the NTM in this country. In Croatia, *M. xenopi* was the second most frequently isolated NTM after *M. gordonae*. Furthermore, *M. xenopi* was prevalent in the English Channel region, being the second most frequent NTM isolate in Belgium and south-east England and ranked third in France after MAC and *M. gordonae*. Differences within countries were also observed: in Spain, *M. xenopi* was the predominant

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TABLE 1 Distribution of respiratory nontuberculous mycobacteria (NTM) isolates

	Laboratories	Patients with NTM isolated	MAC isolates[#]
Europe	43	6803	2500 (36.9)
North America	4	4913	2553 (52.0)
South America	3	393	123 (31.3)
Australia (Queensland)	1	453	322 (71.1)
Asia	3	1974	1062 (53.8)
South Africa	2	5646	2849 (50.5)
Total	56	20 182	9421 (46.7)

Data are presented as n or n (%). MAC: *Mycobacterium avium* complex. [#]: percentage of all NTM.

NTM isolate in the Barcelona area but only ranked third after MAC and *M. fortuitum* in the Madrid area. *M. xenopi* was not isolated in the participating centres from Asia, Australia and South America.

M. kansasii

Overall, *M. kansasii* was the sixth most frequently isolated NTM. In South America *M. kansasii* was the second most isolated NTM after MAC, accounting for 19.8% of all NTM isolated. In Europe, Slovakia, Poland and the UK had the highest *M. kansasii* isolation rates of 36%, 35% and 11%, respectively, compared to a mean isolation rate of 5% in Europe. In the Paris region of France, *M. kansasii* was the third most isolated NTM after MAC. In the participating laboratory in Japan, *M. kansasii* ranked fourth after MAC, *M. gordonae* and *M. abscessus*. In South Africa, *M. kansasii* ranked sixth overall. However, in a centre with a large community of miners in the Johannesburg region of South Africa, *M. kansasii* was the second most frequent NTM isolated.

Rapid growers

M. abscessus and *M. fortuitum* were the most frequently isolated rapid-growing mycobacteria (RGM) worldwide. Other RGM were only isolated sporadically.

Among the RGM, important geographical differences were observed. RGM were highly prevalent isolates in the participating centres in East Asia, where they make up 27% of all NTM isolates in comparison to isolation frequencies of 17.9%, 16% and 14% in the participating centres in North America, South America and Europe, respectively. However, important differences in frequency of RGM isolation among countries within Asia were also noted. In Tokyo (Japan), rapid growers accounted for only 6.6% of all isolates, in contrast to the participating centres in Taiwan (50%) and South Korea (28.7%). Furthermore, in Taiwan, *M. fortuitum* and *M. abscessus* were the second and third most frequently isolated NTM species, while in South Korea *M. abscessus* was the second most frequently isolated NTM after MAC.

Rare and geographically restricted NTM species

M. malmoense was found more often in Northern Europe (80 (2.6%) out of 3107 isolates); in comparison with Southern Europe (21 (0.6%) out of 3696 isolates). In South Africa, 43 *M. malmoense* isolates were found (out of 5646; 0.76%). Five *M. malmoense* isolates from the participating laboratories from North America were reported (five (0.1%) out of 4913 isolates). *M. malmoense* was not found in Queensland-Australia, Asia and South America. *M. simiae* was found worldwide except in Asia. A total of 97 patients were found in this study with a *M. simiae* isolate (0.5% of all 20182 NTM isolated). *M. scrofulaceum* was the second most isolated NTM in South Africa (383 isolates (6.8%) out of 5646). *M. lentiflavum* was found in several laboratories worldwide (generally <1% of all NTM isolated per laboratory), but was found more often in Portugal (6%) and Finland (5.8%). The majority of the 91 different NTM species in this study were infrequently isolated, *e.g. M. smegmatis* (n=5; 0.03%) and *M. interjectum* (n=36; 0.2%) (online supplementary material).

Focus on Europe

Because of a substantial number of participating countries in Europe, this continent was studied in more detail, with a focus on north–south differences. We collected data of 3107 isolates from Northern Europe and 3696 from Southern Europe. In figure 3, the pie-charts of species diversity in all participating countries in Europe are shown. MAC was isolated more frequently in Northern Europe (44% of all mycobacteria)

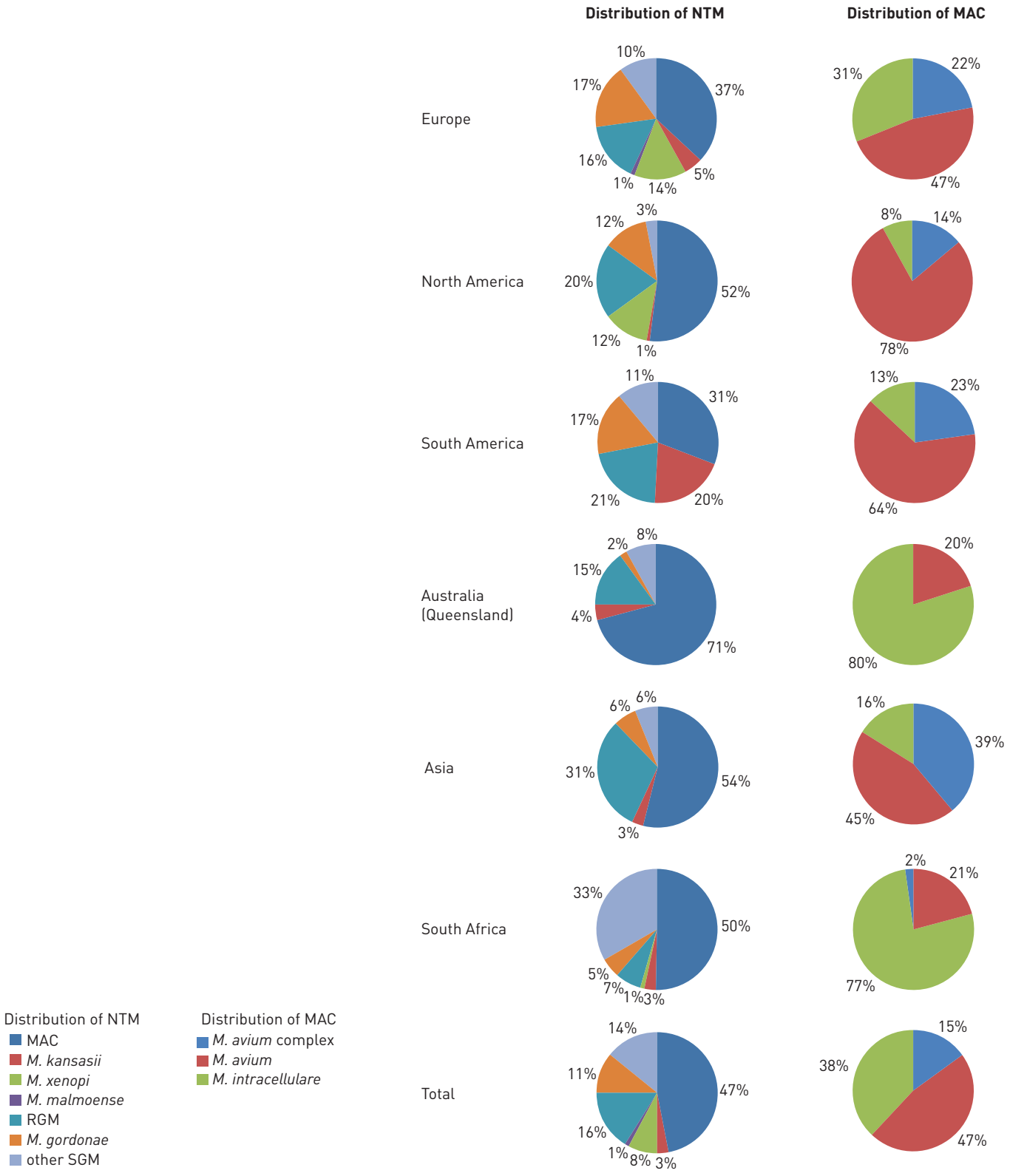

FIGURE 2 Distribution of respiratory nontuberculous mycobacteria (NTM) isolates. MAC: *Mycobacterium avium* complex; RGM: rapid-growing mycobacteria; SGM: slow-growing mycobacteria.

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than in Southern Europe (31%). In both Northern and Southern Europe, *M. avium* was the most frequent isolated subspecies of the MAC.

M. xenopi was more frequently isolated in Southern (778 (21%) out of 3697 isolates) compared to Northern Europe (190 (6%) out of 3107 isolates), partly due to substantial contribution of *M. xenopi* isolated in a single country (Hungary). In contrast, *M. bohemicum* was especially found in Northern Europe, mainly Finland, in contrast to only one isolate from Southern Europe and no isolate from any participating laboratory in other regions of the world.

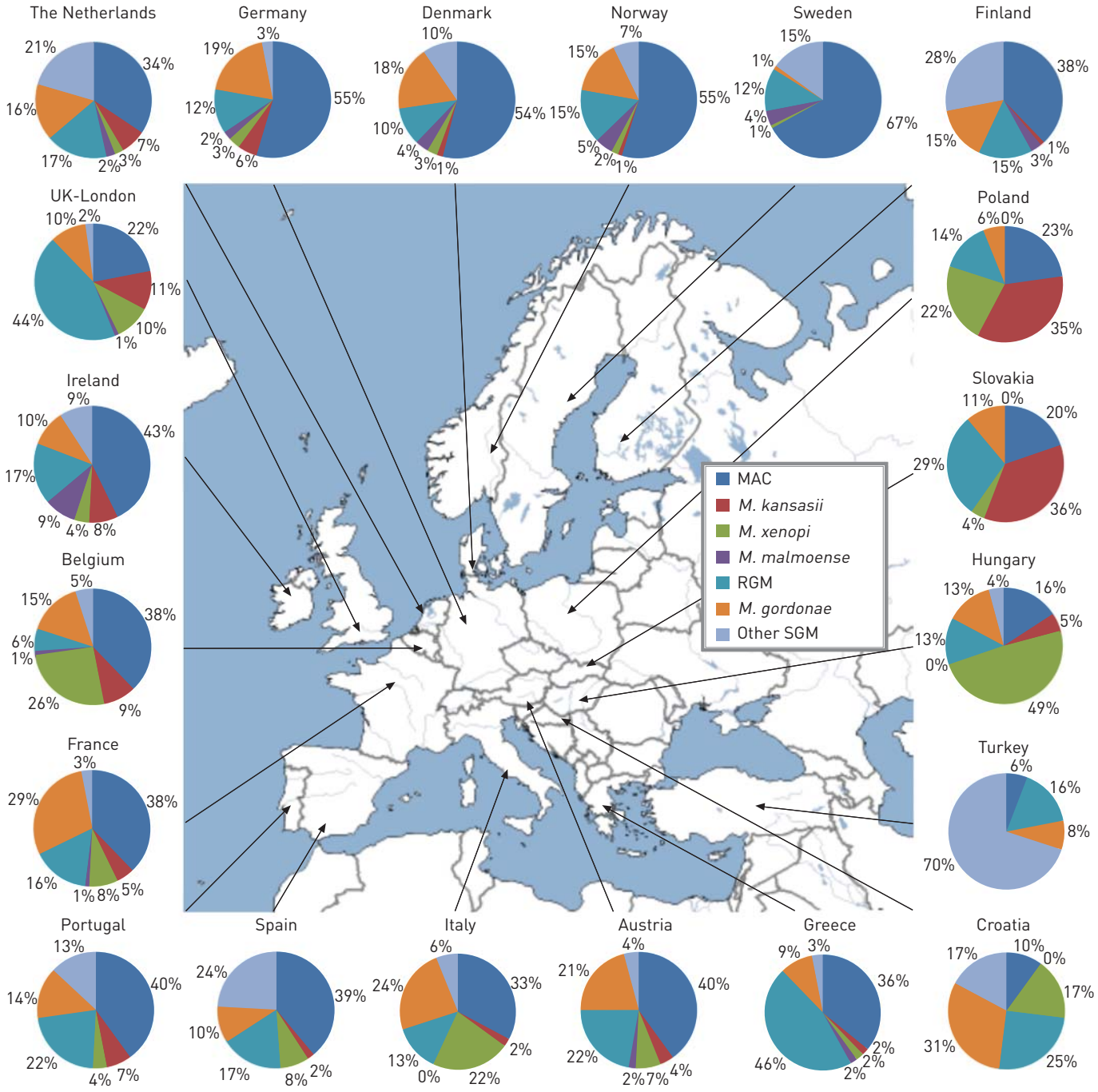

FIGURE 3 Distribution of different nontuberculous mycobacteria from pulmonary samples in 2008 in Europe. MAC: *Mycobacterium avium* complex; RGM: rapid-growing mycobacteria; SGM: slow-growing mycobacteria.

Discussion

For the first time, we provide a snapshot of the worldwide distribution of NTM species isolated from pulmonary samples. This snapshot illustrates that the species distribution among NTM isolated from pulmonary specimens in the year 2008 differs by region and differs by country within these regions. For many regions or countries that participated in this study, these are the first data covering this topic. The NTM species distribution in a country or region may have profound impact on the frequencies and manifestations of pulmonary NTM disease. It is now generally accepted that NTM species differ in their ability to cause lung disease in humans [3–6] and that the clinical relevance of a particular species can differ in different parts of the world [7, 8]. The species distribution presented in this study can help in identifying factors associated with human NTM infection, such as climate differences, population density or host factors. In the past, it has been suggested that differences in NTM species distribution may also affect the efficacy of bacille Calmette–Guérin vaccination [9].

The two studies that approached this subject were published by MARTÍN-CASABONA *et al.* in 2004 [2] and by MARRAS and DALEY in 2002 [3]; the former reported on NTM isolation over three decades, ending in 1996. The spread of molecular tools for identification in the years between 1996 and 2008 make the comparison of the two surveys difficult. The MARTÍN-CASABONA study only incorporated laboratories from Europe, Turkey, Iran and Brazil and sampling was not systematic as different laboratories produced data from different time periods. The review by MARRAS and DALEY adds more historical published data and is not a survey. Still, both showed the predominance of MAC isolation worldwide, together with the characteristic geographical distribution of *M. xenopi*, *M. kansasii* and *M. malmoense*. The review by MARRAS and DALEY [3] also showed that differences in species distribution may occur over time.

In this study, the members of the *M. avium* complex predominated in most regions, though *M. xenopi* predominated in Hungary and *M. kansasii* in Poland and Slovakia. The relative distribution of the various members of MAC again reveals geographic differences. While *M. avium* predominated in the participating centres in North and South America and Europe, *M. intracellulare* was most frequently isolated in South Africa and Australia. In Australia a significant increase in the isolation of this species has been reported before [5]. Few laboratories were able to reliably distinguish the novel MAC members, such as *M. colombiense* and *M. chimaera* [10, 11].

M. xenopi was particularly prevalent in the region covering Hungary, Croatia, Northern Italy, in Ontario-Canada and in the areas bordering the English Channel; the latter two are in line with earlier data [1, 2]. The frequently stated link to coastal areas [1, 2, 12] does not hold true in the light of the observed predominance in Hungary and its absence in coastal regions outside Europe. Nonetheless, the presence of a specific environmental niche for *M. xenopi* is a likely explanation. Other factors to be considered are differences in the potential to colonise or infect human airways between *M. xenopi* strains and host factors.

The geographical distribution of *M. kansasii* has been the subject of previous studies. In our study, *M. kansasii* was frequently isolated in South America, Eastern Europe and the metropolitan centres of Paris, London and Tokyo and the Johannesburg region of South Africa. These findings match previously published data from those regions [2, 8]. Its isolation has been related to mining activities [13–15] as well as urbanisation and may be related to working and living conditions [16]. In Northern Europe, isolation frequencies of *M. kansasii* have been in decline for the past three decades [17], but the underlying causes are unknown. The high isolation frequency of *M. scrofulaceum* in South Africa is in accordance with the literature and probably related to mining activities [15].

M. simiae was traditionally thought to be confined to the Southern USA, Cuba and Israel [7], but in this study we demonstrate that this species has a global distribution (except in Asia), making up 0.67% of all isolates in this study, with the highest isolation of 1.1% found in Northern Europe. *M. malmoense* was found especially in Northern Europe, which is in accordance with the literature [8], but also in South Africa. *M. malmoense* isolation from clinical specimens from this continent has never been reported before, although its presence in soil has been reported [18].

RGM made up 10–20% of all NTM isolates worldwide, although they proved more prevalent in East Asia where they compose up to 50% (Taiwan) of all NTM isolated from pulmonary samples. The high isolation frequency of RGM, particularly *M. abscessus*, was also noted in previous studies focusing on this region [19–21]. The reason for the high isolation rate of RGM in Asian countries remains unclear, although geographical or climatic factors, host and laboratory factors have been suggested [20]. An important reason to perform studies on the geographical distribution of NTM is to help identify factors associated with differences in worldwide isolation patterns of specific species. Studies focusing on species-specific environmental niches and subsequent transmission to humans are not systematically performed. Yet these could offer important clues that would be of aid to prevent (re)infection of susceptible patients.

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In the current study, a multitude of molecular identification techniques was used. It is well known that these techniques can produce discrepant results [22, 23]. Yet this is likely to influence a minority of isolates and will not affect the snapshot of the geographic diversity in NTM species isolated from pulmonary samples, as presented in this study. The current study revealed the occasional presence of many rare species (online supplementary data). For many of these species no data other than reports of single cases of disease were available. Often such case reports or novel species descriptions seem to suggest a very limited geographic spread of the bacterium and, as they mostly concern cases of true disease, overestimate the clinical relevance of these rare species. We hope that the current study also provides a reference to clinicians and microbiologists faced with rare NTM species.

An important shortcoming of this study is the low number of participating laboratories, especially outside Europe. Inclusion of more data from different laboratories will probably reveal other isolation patterns.

Follow-up studies covering different time periods together with more participating laboratories may reveal additional clues on changing NTM pattern over time and geographic differences of NTM distribution on the national scale.

We have chosen pulmonary samples specifically because these best reflect the distribution of species in local environments. Nevertheless, presence and persistence within the human airways is also likely to represent a selection bias, as does the fact that we collected identification results of cultured isolates. The low isolation frequency of the majority of the 91 different NTM species may reflect an inability to persist in human airways or a scarce environmental presence.

In summary, this snapshot illustrates that the species distribution among NTM isolated from pulmonary specimens in the year 2008 differs by region and differs by country within these regions. MAC predominates in most settings, but the frequency of isolation of RGM, or slow growers like *M. xenopi*, *M. kansasii* and *M. malmoense* differs per setting. These differences in species distribution may determine, in part, the frequency and manifestations of pulmonary NTM disease in each region. Future studies need to address species-specific environmental niches as a cause of differences in worldwide isolation patterns.

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References

1 Marras TK, Chedore P, Ying AM, *et al.* Isolation prevalence of pulmonary non-tuberculous mycobacteria in Ontario, 1997–2003. *Thorax* 2007; 62: 661–666.
2 Martín-Casabona N, Bahrmand AR, Bennedsen J, *et al.* Non-tuberculous mycobacteria: patterns of isolation. A multi-country retrospective survey. *Int J Tuberc Lung Dis* 2004; 8: 1186–1193.
3 Marras TK, Daley CL. Epidemiology of human pulmonary infection with nontuberculous mycobacteria. *Clin Chest Med* 2002; 23: 553–567.
4 van Ingen J, Bendien SA, de Lange WC, *et al.* Clinical relevance of non-tuberculous mycobacteria isolated in the Nijmegen-Arnhem region, the Netherlands. *Thorax* 2009; 64: 502–506.
5 Thomson RM. Changing epidemiology of pulmonary nontuberculous mycobacteria infections. *Emerg Infect Dis* 2010; 16: 1576–1583.
6 The Mycobacteriosis Research Group of the Japanese National Chest Hospitals. Rapid increase of the incidence of lung disease due to *Mycobacterium kansasii* in Japan. *Chest* 1983; 83: 890–892.
7 Griffith DE, Aksamit T, Brown-Elliot BA, *et al.* An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *Am J Respir Crit Care Med* 2007; 175: 367–416.
8 Hoefsloot W, Boeree MJ, van Ingen J, *et al.* The rising incidence and clinical relevance of *Mycobacterium malmoense*. *Int J Tuberc Lung Dis* 2008; 12: 987–993.
9 Black GF, Dockrell HM, Crampin AC, *et al.* Patterns and implications of naturally acquired immune responses to environmental and tuberculous mycobacterial antigens in northern Malawi. *J Infect Dis* 2001; 184: 322–329.
10 Murcia MI, Tortoli E, Menendez MC, *et al. Mycobacterium colombiense* sp. nov., a novel member of the *Mycobacterium avium* complex and description of MAC-X as a new ITS genetic variant. *Int J Syst Evol Microbiol* 2006; 56: 2049–2054.
11 Tortoli E, Rindi L, Garcia MJ, *et al.* Proposal to elevate the genetic variant MAC-A, included in the *Mycobacterium avium* complex, to species rank as *Mycobacterium chimaera* sp. nov. *Int J Syst Evol Microbiol* 2004; 54: 1277–1285.
12 Dailloux M, Abalain ML, Laurain C, *et al.* Respiratory infections associated with nontuberculous mycobacteria in non-HIV patients. *Eur Respir J* 2006; 28: 1211–1215.
13 Corbett EL, Blumberg L, Churchyard GJ, *et al.* Nontuberculous mycobacteria: defining disease in a prospective cohort of South African miners. *Am J Respir Crit Care Med* 1999; 160: 15–21.
14 Chobot S, Malis J, Sebáková H, *et al.* Endemic analysis of infections caused by *Mycobacterium kansasii* in the Karviná district in 1968–1995: an analysis of epidemiological data. *Cent Eur J Public Health* 1997; 5: 164–173.
15 Corbett EL, Hay M, Churchyard GJ, *et al. Mycobacterium kansasii* and *M. scrofulaceum* isolates from HIV-negative South African gold miners: incidence, clinical significance and radiology. *Int J Tuberc Lung Dis* 1999; 3: 501–507.
16 Bloch KC, Zwerling L, Pletcher MJ, *et al.* Incidence and clinical implications of isolation of *Mycobacterium kansasii*: results of a 5-year, population-based study. *Ann Intern Med* 1998; 129: 698–704.
17 van Ingen J, Wagner D. Epidemiologie der nichttuberkulösen mykobakteriellen Erkrankungen in Deutschland und weltweit [The epidemiology of nontuberculous mycobacterial disease in Germany and worldwide]. *Pneumologe* 2011; 8: 396–403.
18 Portaels F, Larsson L, Jenkins PA. Isolation of *Mycobacterium malmoense* from the environment in Zaire. *Tuber Lung Dis* 1995; 76: 160–162.
19 Koh WJ, Kwon OJ, Jeon K, *et al.* Clinical significance of nontuberculous mycobacteria isolated from respiratory specimens in Korea. *Chest* 2006; 129: 341–348.
20 Simons SO, van Ingen J, Hsueh PR, *et al.* Nontuberculous mycobacteria in respiratory tract infections, eastern Asia. *Emerg Infect Dis* 2011; 17: 343–349.

 DOI: 10.1183/09031936.00149212

21 Chen CY, Chen HY, Chou CH, *et al*. Pulmonary infection caused by nontuberculous mycobacteria in a medical center in Taiwan, 2005–2008. *Diagn Microbiol Infect Dis* 2012; 72: 47–51.
22 Tortoli E, Bartoloni A, Böttger EC, *et al*. Burden of unidentifiable mycobacteria in a reference laboratory. *J Clin Microbiol* 2001; 39: 4058–4065.
23 van Ingen J, de Zwaan R, Enaimi M, *et al*. Re-analysis of 178 previously unidentifiable *Mycobacterium* isolates in the Netherlands in 1999–2007. *Clin Microbiol Infect* 2010; 16: 1470–1474.