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ABSTRACT

Non-tuberculous mycobacteria (NTM) are ubiquitous dwellers of environmental niches and are an established cause of natural and nosocomial infections. The incidence of NTM infections is rising owing to a growing population of immunocompromised and vulnerable individuals, complex medical and surgical procedures, as well as increased awareness and diagnostic capabilities. The prevalence of different NTM varies between continents, regions, and countries. The true global burden of pulmonary and extrapulmonary disease is unknown and estimates are subject to under and/or over-estimation. Diagnosis requires confirmation by isolation of NTM along with clinical and radiological criteria, which may be suboptimal at all levels. Susceptibility testing is complex and clinical breakpoints are not available for many of the drugs. Frequently, NTM infections are not considered until late in the course of disease. Improved and rapid detection of tuberculosis cases in high-burden countries has, however, also brought NTM infections into the limelight, and has identified a need for research efforts towards rapid diagnostic tests and the identification of biomarkers to monitor the treatment response in patients with NTM infections.

Introduction

Non-tuberculous mycobacteria (NTM) are ubiquitous and widely found in water and soil, as well as residential environments. Survival in these ecological niches is supported by their ability to form biofilms (Esteban and Garcia-Coca, 2017). Until recently, NTM were considered transient colonizers in humans, although their association with disease is now well recognized and accepted as a growing problem (Prevots et al., 2017). The rise in NTM infections is likely to be multifactorial, including a growing cohort of at-risk populations coupled with perpetually improving diagnostic capacities.

More than 170 species of NTM are currently recognized, with the list ever growing (Forbes, 2017). NTM taxonomy is crowded and complicated, and different species may be more prevalent in specific geographic settings or milieus (Hoefsloot et al., 2013). NTM may cause pulmonary (nodular and fibrocavitary disease) as well as extrapulmonary disease (cutaneous, bone, disseminated), and frequently affect patients with predisposing conditions, e.g., cystic fibrosis (CF) and bronchiectasis/chronic obstructive pulmonary disease (COPD), as well as people living with HIV (Faverio et al., 2016).

Repeated exposure to environments inhabited by NTM is an accepted mode of transmission (Honda et al., 2018). Infection is frequently acquired through inhalation (i.e., aerosol, showers) or inoculation (trauma, plastic surgery, acupuncture). Outbreaks due to NTM are also well reported in the literature (Buser et al., 2019; Lyman et al., 2017).

Defining the problem

The global burden of NTM disease is largely unknown. Currently, the majority of NTM diseases are not notifiable, notable exceptions being *Mycobacterium chimaera* related to cardiac surgery and heater cooler units and *Mycobacterium ulcerans*. There

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are several challenges in describing the morbidity and mortality of NTM disease, its incidence and prevalence, trends, and the distribution of infections regionally, nationally, or globally. Moreover, certain countries may be able to enumerate NTM pulmonary cases through their tuberculosis (TB) programmes, but these will be an underestimate as they may only be cases de-notified from TB registries, whereas those not presenting to TB clinics may not be captured (Shah et al., 2016).

The classification of NTM disease is an integrated clinical–radiological–microbiological diagnosis, requiring the isolation of the same species of NTM from two separate sputa (or once only from bronchoalveolar fluid), evidence of clinical symptoms, and radiological signs of cavitary or nodular disease on X-ray or computed tomography (Griffith et al., 2007). Laboratory notifications on their own are not able to differentiate true disease from temporary colonization, and other approaches are needed to capture the full burden of NTM disease.

Given the situation, our current knowledge of NTM disease is based on fragments of information from different countries, regions, and multicentre laboratory studies. These suggest that NTM infections are becoming more prevalent; we do not however know whether this is due to our rapidly ageing populations, increasing prevalence of chronic lung diseases, cystic fibrosis cohorts, immunosuppressed patients, or to improved diagnostic methods, or most likely, a combination of these.

Diagnostic challenges

A major obstacle in determining the true burden of disease and identifying patients with NTM infections is limited diagnostic capacity (both clinical and laboratory) in many low and middle-income countries. The presentation of NTM pulmonary disease is usually non-specific, with radiological findings and disease sequelae being similar to those of TB. Most patients with NTM disease therefore end up receiving therapy for TB or other common infections for months or years. Even if the culture report shows growth of NTM, it may be ignored as a contaminant, and the patient may not be properly assessed for NTM disease (Varma-Basil and Bose, 2019). Extrapulmonary infections are frequently nosocomial: surgical site infections including post-laparoscopic surgeries, implant infections, central line-associated bloodstream infections, acupuncture needle-related infections, pedicure-related infections, hydrotherapy-associated infections, etc. (Shakoor et al., 2019). A mycobacterial aetiology is frequently not sought until the patient has failed the broadest of antibiotic coverage. When the infection is community-acquired, mycobacteria are much lower down the list of differentials unless there are concerted efforts to increase awareness, e.g., in the case of Buruli ulcer in Japan (Yotsu et al., 2012).

Mycobacterial culture or specific tests should be requested, and laboratories need to be equipped to identify mycobacteria beyond *Mycobacterium tuberculosis* complex (Varma-Basil and Bose, 2019). NTM are susceptible to harsh decontamination techniques and thus detection is difficult for fastidious species like *Mycobacterium haemophilum* and those NTM requiring incubation at 30 °C (e.g., *Mycobacterium marinum*) or 45 °C (e.g., *Mycobacterium xenopi*), as well as extremely slow-growing species (e.g., *M. ulcerans*) (Pfyffer, 2015).

The identification techniques, including commercial molecular systems that can identify clinically relevant mycobacteria, either directly in samples or from cultured isolates, have their own limitations: they are technically exacting, costly, and in cases have low accuracy (Pfyffer, 2015; Quan et al., 2018). Additionally, susceptibility testing is mostly limited to broth-based methods, which are difficult to perform and read. Furthermore, due to a paucity of data, clinical breakpoints for some drug–bug combinations are still not available (Clinical and Laboratory Standards Institute, 2018). More recently, another avenue being explored for clinical use is that of genotypic detection of resistance mutations (Huh et al., 2019).

Most importantly, maintaining tests to identify and report NTM is not cost-effective, especially where test volumes are low. Few centres in resource-limited settings therefore invest in such expertise, resulting in reduced access to reliable diagnostics.

Recognition of NTM as a global concern

According to recent studies, NTM disease is increasing in the developed world. A recent systematic review analysed 22 studies on NTM in North America, Europe, Asia, and Australasia reporting this trend (Brode et al., 2014; van der Werf et al., 2014). NTM lung disease appears to be more prevalent with older age, whereas NTM disease in children, HIV-infected patients, and the immunosuppressed is generally extrapulmonary with a predilection for lymphadenitis. Disseminated NTM disease is more likely in transplant patients or patients with a severe primary immunodeficiency, i.e., STAT1

Table 1
Clinical importance of common non-tuberculous mycobacteria (NTM).

NTM species	Comments
Mycobacterium avium–intracellulare complex (MAC)	Commonly found in most environments and became prominent during the HIV epidemics. Causes lymphadenitis and lung disease. Prevalence in the HIV population has been reduced dramatically by ART, but it can affect HIV non-infected people and elderly women (Lady Windermere syndrome).
Mycobacterium kansasii	Frequently associated with piped water, likely to affect middle-aged or older men with COPD, and can cause a pulmonary disease similar to TB.
Mycobacterium abscessus group	Frequently pathogenic and very challenging to treat, especially *M. abscessus* ssp. *abscessus*, given its resistance to macrolides; Other subspecies include *massiliense* and *bolletii*.
Mycobacterium fortuitum group	Not generally considered a pathogen unless nosocomial.
Mycobacterium marinum	Relatively rare, but is commonly associated with fish tanks and marine exposure, especially following skin breaks. Most likely manifestations are skin lesions, which may appear 2–4 weeks after exposure. A high index of suspicion and knowledge of occupational history and pets is required to make the diagnosis.
Mycobacterium ulcerans	Presents as a chronic skin infection and can be seen in, but is not limited to, Sub-Saharan Africa and the tropics. It is more prevalent following rains and flooding, frequently following trauma, and affects children/adolescents, producing painless nodules and ulcers due to the action of a toxin mycolactone.[a]
Mycobacterium scrofulaceum	Generally causes lymphadenitis in children.
Mycobacterium szulgai	Commonly reported as a cause of pulmonary disease and as complicating infection in post-TB patients.
Mycobacterium simiae	Does not always represent disease, but when it does, it can be very challenging to manage as it generally does not respond to treatment.

ART, antiretroviral therapy; COPD, chronic obstructive pulmonary disease; TB, tuberculosis.
[a] Reference: Sarfo et al., 2016.

deficiency, interferon-gamma receptor deficit, or recipients of anti-tumour necrosis factor alpha (anti-TNF-α). CF patients are likely to be colonized with NTM and may develop NTM disease. NTM can cause rapidly progressing fibrocavitary pulmonary disease or disseminated disease in the immunocompromised. Localized manifestations may be skin and soft tissue infection from direct inoculation and lymphadenitis, typically cervical. Table 1 shows the niches and importance of some common NTM species seen in clinical practice.

NTM have recently been found to be putative causes of iatrogenic infections and outbreaks, and are now established as nosocomial pathogens (Desai and Hurtado, 2018). Outbreaks in CF patients have occurred, demonstrating the role of the environment in colonizing and infecting predisposed individuals (Martiniano et al., 2017), and these human-to-human transmission events have been proven as possible through whole genome sequencing (Bryant et al., 2013; Kapnadak et al., 2016). Therefore, efforts must be made to protect CF patients from the hospital environment, as well as to reduce the potential of spread of frequently drug-resistant nosocomial strains amongst patients; these include thorough cleaning between patients, environmental sampling, negative pressure rooms, and HEPA filters and UV lamps in outpatient spaces (Shakoor et al., 2019). Recently, the contamination of heater–cooler units produced by a specific manufacturer was implicated in a global outbreak of *M. chimaera* in post heart valve surgery patients, causing surgical site infections and/or endocarditis, characterized by high mortality rates. New models of the machines have rectified the problem; however, the infection can remain latent for many years. It is difficult to diagnose and treat, and therefore the extent of the problem is likely to be underestimated (Hasse et al., 2020; Scriven et al., 2018).

NTM as an emerging problem in Pakistan, a high TB burden country

Rapid diagnostics have not only improved TB detection, but the massive roll-out of Xpert MT/RIF in high-burden countries has also unmasked NTM infections masquerading as acid-fast bacillus (AFB) smear-positive TB that has failed to respond to several courses of anti-tubercular therapy: treatment failure or reactivation (Javaid et al., 2016; Tahseen et al., 2016; Velayati et al., 2015).

Data from the mycobacteriology section of the clinical laboratories of Aga Khan University (AKU), which is also the

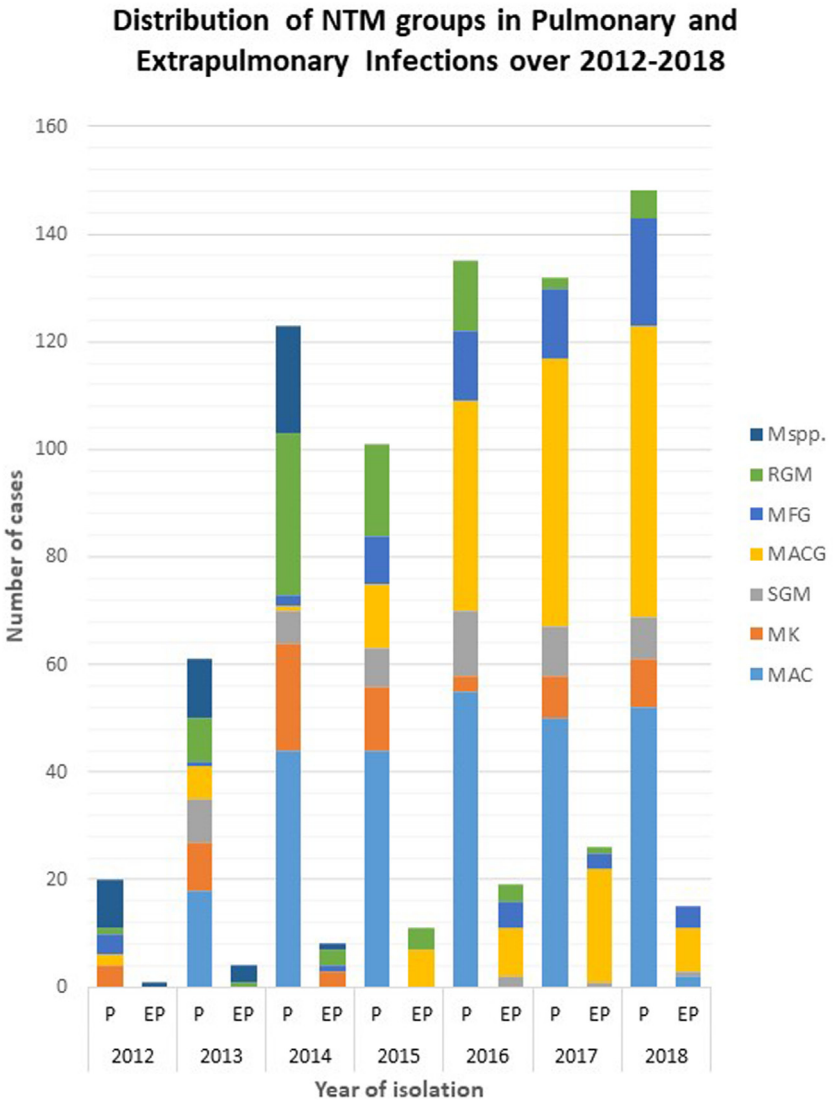

Figure 1. Distribution of the common groups of non-tuberculous mycobacteria (NTM) isolated from pulmonary and extrapulmonary samples with NTM infections over the period 2012 to 2018, from the Microbiology Laboratory of Aga Khan University Hospital, Karachi, Pakistan.
Note: MAC, *Mycobacterium avium–intracellulare* complex; MK, *Mycobacterium kansasii*; SGM, slow-growing mycobacteria; MACG, *Mycobacterium abscessus–chelonae* group; MFG, *Mycobacterium fortuitum* group; RGM, rapidly growing mycobacteria; M. spp., *Mycobacterium* species; P, pulmonary infections; EP, extrapulmonary infections.

supranational reference TB laboratory, were analysed. The laboratory, situated in Karachi, receives specimens from most parts of the country through a network of specimen collection units; however, the data only represent a single laboratory experience from Pakistan. These showed a 7.7-fold increase in NTM infections, from 21 cases in 2012 to 163 in 2018 (Figure 1). The greatest increase in numbers was seen in pulmonary disease, contributing to 89.6% of the NTM cases, with most patients having a prior history of TB and evidence of bronchiectasis and fibrocystic changes in their lungs.

Extrapulmonary NTM infections are less common, but are increasingly recognized as important agents of nosocomial infections. Reports from Pakistan have already been published and a review of laboratory data from AKU revealed that 42% of extrapulmonary NTM infections were post-surgical, after caesarean section, laparoscopic surgeries, orthopaedic surgeries with and without implants, and mastectomies. The rates of these procedures are increasing in both the private and public sectors of developing countries like Pakistan, but the accompanying infection prevention strategies both during and after surgery are woefully inadequate (Shakoor et al., 2019). With a low index of suspicion and limited access to proper laboratory diagnosis, this documented rate of nosocomial NTM infections may very likely be an underestimate of the real situation.

As expected, with such different predisposing factors, the spectrum of NTM species causing pulmonary and extrapulmonary infections also differs. In our setting, a predominance of slow-growing mycobacteria, *Mycobacterium avium–intracellulare* complex (MAC) and *Mycobacterium kansasii*, was common in the group with pulmonary infections, but not in those with extrapulmonary disease. Rapidly growing mycobacteria (RGM), primarily from *Mycobacterium chelonae/Mycobacterium abscessus* group (MCAG) and *Mycobacterium fortuitum* group (MFG), while seen in both pulmonary and extrapulmonary infections, were associated with more than 80% of extrapulmonary NTM infections.

An earlier study evaluating the distribution of NTM species across Pakistan suggested geographical variation across different regions, every area having its own distribution spectrum (Ahmed et al., 2013). The differences were especially discernible between urban centres where access to bronchoscopy and more in-depth diagnostic efforts contribute to the detection of a larger number of NTM cases. The study further suggested clarithromycin as the mainstay of therapy for NTM infections in both slow-growing (SGM) and rapidly growing (RGM), with linezolid as a good alternative. Resistance to fluoroquinolones was, however, prevalent. Among injectable drugs, most RGMs were susceptible to amikacin, while limited imipenem activity was reported on isolates from Pakistan (Ahmed et al., 2013). The susceptibility profile suggested the use of antimicrobials in the area, nosocomial isolates being more resistant to injectables, but community-acquired isolates showing higher resistance to commonly used oral agents like fluoroquinolones.

Future directions

A low level of suspicion, limited knowledge, and paucity of specialized laboratories frequently lead to diagnostic delays. The median time to diagnosis may be as high as 2 years or longer. The delay frequently results in increased disease severity, and loss of functional capacity is not infrequent. The advent of molecular testing for TB is a surrogate test that allows the diagnosis of NTM through deduction in the case of AFB-positive, TB PCR-negative results. MALDI-TOF MS (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry) and sequencing has further reduced the time to diagnosis of NTM infections and has improved our capacity for speciation. These new techniques enable outbreak investigations to be performed with some certainty. Phenotypic sensitivities are important, especially in patients who have received macrolides as monotherapy for infective exacerbation of COPD or who have relapsed or non-responsive disease. Differentiating colonization from disease often requires more than one outpatient visit and several sputum samples or a bronchoscopy to isolate mycobacteria. In patients with a co-existing lung pathology, the NTM may be a relatively innocent bystander or be a co-infection in synergy with *Aspergillus* spp. or *Pseudomonas aeruginosa* (Geurts et al., 2019).

Simpler and more rapid tests would be ideal in making the diagnosis of NTM disease more quickly. However, the development of such tests is complicated by the large number of NTM species that can cause disease and by the criteria for disease, which require more than just the presence of the microorganism. As a result, NTM disease will require multiple diagnostic parameters, leading to delays and higher costs. More specific biomarkers to monitor the progress of treatment and test cure are lacking. The treatment of NTM remains lengthy, in many cases 18 months with a minimum 12 months after culture conversion in lung disease. In the future, more effective therapies, biomarkers, and/or imaging may help reduce the duration of treatment in a precise way.

Ethical approval

Not required.

Conflict of interest

None to declare.

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