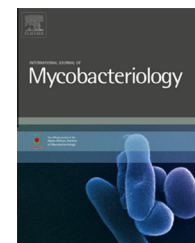


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Review

Review: Environmental mycobacteria as a cause of human infection

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ABSTRACT

Pulmonary infections with nontuberculous mycobacteria (NTM) are recognized as a problem in immunodeficient individuals and are increasingly common in older people with no known immune defects. NTM are found in soil and water, but factors influencing transmission from the environment to humans are mostly unknown. Studies of the epidemiology of NTM disease have matched some clinical isolates of NTM with isolates from the patient's local environment. Definitive matching requires strain level differentiation based on molecular analyses, including partial sequencing, PCR-restriction fragment length polymorphism (RFLP) analysis, random amplified polymorphic DNA (RAPD) PCR, repetitive element (rep-) PCR and pulsed field gel electrophoresis (PFGE) of large restriction fragments. These approaches have identified hospital and residential showers and faucets, hot-tubs and garden soil as sources of transmissible pathogenic NTM. However, gaps exist in the literature, with many clinical isolates remaining unidentified within environments that have been tested, and few studies investigating NTM transmission in developing countries. To understand the environmental reservoirs and transmission routes of pathogenic NTM, different environments, countries and climates must be investigated.

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Abbreviations: RFLP, restriction fragment length polymorphism; RAPD, random amplified polymorphic DNA; PFGE, pulsed field gel electrophoresis; RT-PCR, real time polymerase chain reaction; HPLC, high performance liquid chromatography; HP, hypersensitivity pneumonitis; Rep-PCR, repetitive polymerase chain reaction; AFLP, amplified fragment length polymorphism.

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Factors affecting the acquisition of pulmonary NTM

Pulmonary infections with nontuberculous mycobacteria (NTM) are an increasing problem in many countries. Symptoms include chronic cough, hemoptysis, weight loss, fever and progressive fatigue – similar to *Mycobacterium tuberculosis* (MTB) pulmonary infection. Diagnoses are made with chest radiography, detection of acid fast bacilli (AFB) in sputum and cultures from sputum and bronchoalveolar lavage (BAL) fluids [1]. The increasing prevalence may reflect an aging population, as symptomatic infections are most common in post-menopausal women and older men. NTM infections can also manifest as lymphadenitis, disseminated disease, or skin, tissue or bone disease. Disseminated NTM disease is associated with genetic defects in the Th1 pathway, and lymphadenitis is often seen in patients with advanced HIV disease, whilst skin, tissue and bone infections usually follow trauma at the site of infection. Pulmonary NTM infections in older individuals with no recognized immune defects carry a high morbidity and economic cost, as current treatments have multiple side effects and an intention-to-treat cure rate of <50%. Even in apparent cures, the relapse rate is 50% at 3 years [2]. It is important to determine the source and mode of transmission of pathogenic strains in the environment for the advancement of prophylactic measures, as subtle immune deficiencies are rarely evident prior to the diagnosis of NTM disease.

Pathogenic NTM comprise many species and strains found in water and soil. To elucidate NTM disease risk, clinical and environmental NTM isolates need to be matched. This will identify critical environmental reservoirs and routes of transmission. The following is a review of studies where clinical and environmental strains of mycobacteria were matched and a discussion of the limitations to the methodologies employed.

NTM are common in many environments

NTM have been isolated from drinking water pipelines [3,4], water tanks [5], hot tubs [6], residential faucets [7,8], hospital

faucets and ice machines [9,10], diagnostic laboratories [11], bottled and municipal water, commercial and hospital ice [12], potting soil [13], house dust [14,15], water damaged building materials [16], showerheads [17], shower aerosols [8], hot-tub aerosols [6], coniferous forest soils [18], brook waters [19], cigarettes [20], livestock [21], coastal mosses [22] and seawater [23]. Reports span multiple countries (including the USA, Australia, the UK, France, the Netherlands, Denmark, Czechoslovakia, Italy, Finland, Germany, Madagascar, Tanzania, Taiwan, Japan and Korea) and various climates. While many isolates were not directly associated with human disease, these reports show that NTM reside in a variety of natural and artificial environments.

Environments of interest are defined

Inhalation of aerosols appears to be the primary transmission route of NTM causing pulmonary disease. This usually occurs in artificial water environments such as hot-tubs and showers, but may involve garden soil and house dust. Mycobacteria may aerosolise more readily than other bacteria as they have highly hydrophobic cell walls. NTM have been isolated from natural water environments in which aerosolisation increase the concentration of NTM in the air [24,25]. However, these studies will not be discussed here as the aerosols were not linked to NTM disease.

Species-level identification of NTM isolates is sufficient to determine the presence or absence in an environment, but strain level identification is required when matching clinical and environmental isolates. For species level NTM identification, cheap lower resolution techniques include phenotypic and biochemical typing, RT-PCR, species specific DNA probe kits, HPLC of mycolic acids and sequencing of select genes (namely 16S, *rpoB* and/or *hsp65*). Serotyping [5] and phage typing [26] have been utilized, but are now less popular as more species of mycobacteria have been recognized.

With low resolution techniques, environments of interest can be selected based on assessments of NTM presence. Demonstrations of transmission causing disease require higher resolution typing techniques such as RFLP, PFGE, or

Table 1 – Summary of molecular techniques described for NTM strain characterization.

Name	Method of characterization	Papers describing the method
Partial sequencing	Sequencing of particular genes and gene fragments (i.e., 16S rDNA). Can directly compare SNPs of genomic DNA	[47] [48]
Repetitive sequence PCR (rep-PCR)	PCR of genomic DNA with primers specific to multiple repetitive sequences. Separate amplicons via gel electrophoresis. Produces unique fingerprint patterns from multiple bands of varying intensity	[49]
Random amplification of polymorphic DNA (RAPD)	PCR amplification, primers of random sequence utilised. Polyacrylamide gel electrophoresis performed on amplicons. Unique fingerprint generated	[50]
Restriction fragment length polymorphism (RFLP)	Perform PCR with primer of interest. Digest amplicons with restriction enzymes and determination of fragment length via gel electrophoresis. Discriminates DNA strands based on the locations of restriction enzyme sites (as band patterns)	[51]
Pulsed field gel electrophoresis (PFGE)	Amplify Genomic DNA by culture, digest into large restriction fragments and separate large restriction fragments via pulsed-field gel electrophoresis (i.e., Electrophoresis where voltage periodically changes between three directions). Capable of accurate discrimination of sequences in long strands	[52,53]
Amplified fragment length polymorphism (AFLP)	Genomic DNA digested with restriction enzymes, adaptors ligated to restriction fragments, restriction fragments undergo PCR amplification, amplified fragments separated and visualized via polyacrylamide gel electrophoresis. Polymorphisms scored as present or absent from genome	[54]
Multiplex PCR	Multiple primer sets used in a single PCR reaction, targeting multiple genes. Amplicon sizes determined by gel electrophoresis	[55]
High performance liquid chromatography (HPLC)	Used to separate, identify, and quantify a mixture of components in liquid form. For NTM characterization, it is often used to fingerprint mycolic acid patterns	[10]
Identification kits	Pre-packaged kits designed to identify certain NTM species. Directions and techniques vary between kits and manufacturers	[49]

rep-PCR. Although commonly used for species identification, RT-PCR or gene sequencing provide strain level identification if optimized correctly (Table 1). These techniques are discussed further in Section ‘Molecular methods allow NTM from patients and their environment to be matched’. When clinical and environmental isolates are matched at the species level only, conclusions should be treated with caution. False-positive matches may occur when different strains of the same NTM species are present in different environmental locations [9].

Excessive exposure to NTM-containing aerosols can also result in hypersensitivity pneumonitis (HP) in people with no known immune deficiencies. This was documented in machine workers following repeated inhalation of aerosolised contaminated metal removal fluids [27]. However, the most frequently

reported cause of NTM HP is aerosolisation of contaminated hot-tub and swimming pool water, with multiple reports describing patients who developed NTM HP from these sources after frequent use [28–34]. Whilst patients with NTM HP can present acutely and be significantly unwell, most cases resolve after exposure to the contaminated aerosols is discontinued.

Water

The most credible routes for pulmonary NTM infection involve inhalation of aerosols generated by hot-tubs and shower-heads. Indeed, the global increase in NTM infections may reflect the use of showers rather than bathing [35]. In a study of particular interest, Feazel et al. used quantitative

molecular techniques to show that the showerhead environment enriches biofilm-forming organisms, including mycobacteria [36]. NTM organisms are common in treated water. Their innate resistance to chlorine and other disinfectants provides a growth advantage. The disinfectant resistance of NTM was demonstrated in Australia in a study of *Mycobacterium avium* in hot-tubs [37]. Although the bacterial load excluding mycobacteria was kept at a low 1 CFU/ml by chlorination, the same samples yielded NTM numbering between 4.5×10^3 and 4.3×10^4 CFU/ml [37].

Although not affecting the lungs, NTM furunculosis has also been linked to salon foot-baths used by two patients in the USA [38]. The infections are attributed to the use of contaminated foot baths after shaving of the legs had breached the skin barrier.

NTM are also present in hospital environments. Earlier studies linking environmental and clinical NTM isolates found several NTM species were ubiquitous in hospital and residential faucets and showers in the UK, the Netherlands and the Czech Republic [26,39,7]. Diagnosis of NTM disease can be confounded by false-positive isolates due to contamination of diagnostic tests, as demonstrated in the USA [40] and Spain [41]. NTM contaminating hospital materials can cause nosocomial outbreaks and pseudo-outbreaks [42,43]. Prevention of nosocomial infections requires that medical instruments and patient wounds are not exposed to tap water [1].

Soil

Studies of NTM in soil are less numerous than those of water, perhaps because soils are considered a less likely/common source of nosocomial infection and contain more overgrowing contaminants, complicating NTM isolation from culture. However, NTM have been isolated from soil in multiple countries and climates, including Finland [18], Japan [44], the USA [45] and Uganda [46]. Some reports characterize isolates to the species level, but only one utilized molecular techniques to match clinical isolates with NTM isolated from garden soil [13]. Although the study matched 14 clinical isolates with isolates from the patient's own gardens, only three were true matches at the strain level. The authors isolated the organisms by generating aerosols with the soil samples, establishing that the isolates had the potential to be aerosolized to a respirable particle size.

House dust

Many NTM species have been isolated from house dust [14]. Although these studies used only species specific analyses for detection and characterization, they establish house dust as a source of NTM [15]. As dust particles are commonly suspended in the air, inhalation of mycobacteria associated with dust particles is a plausible source of pulmonary infections. An early study from Australia used serotyping (one of the first strain typing techniques described) to match house dust and patient isolates [47]. Approximately 50% of these house dust strains were *Mycobacterium intracellulare* matching the patient isolates. Further studies utilizing more powerful techniques are needed to confirm these findings.

Molecular methods allow NTM from patients and their environment to be matched

Within environments identified by low resolution methods, environmental NTM have been compared with isolates from patients using molecular techniques. These techniques (Table 1) have been used as standalone procedures, and as part of a multistep fingerprinting process. Whilst strain differentiation within some individual species of NTM has been demonstrated using a single technique, this is not the case for all. Strain level differentiation across multiple NTM species requires a combination of different techniques.

Molecular techniques used for characterization of NTM at the strain level include high performance liquid chromatography (HPLC), repetitive PCR (rep-PCR), random amplified polymorphic DNA (RAPD) PCR, pulsed-field gel electrophoresis (PFGE) of large restriction fragments, restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), partial gene sequencing, and multiplex PCR (i.e., using *hsp65*, 16S rDNA, *ropB*, etc.). Table 2 outlines studies in which these molecular techniques have been used to identify sources of clinical NTM isolates.

Many PCR-based analyses (e.g., 16S rDNA sequencing) do not distinguish mycobacterial taxa at the species level as the genes are conserved across the genus [48]. For example, *Mycobacterium kansasii*/*Mycobacterium gastri* and *Mycobacterium abscessus*/*Mycobacterium chelonae* share identical 16S rDNA sequences. Similar problems also affect NTM differentiation at the strain level and are avoided by the use of multiple analyses in tandem [49]. One study describes a comprehensive analyses of the discriminatory power of 16S rDNA, *hsp65*, and *ropB* gene sequencing, *hsp65* RFLP, rep-PCR, RAPD PCR, and PFGE of large restriction fragments for isolates of *Mycobacterium mucogenicum* and *Mycobacterium phocaicum*. PFGE analysis displayed the greatest differentiation power showing each environmental isolate as genetically unique [9]. However, PFGE cannot differentiate all NTM strains isolated [50] and should be used in combination with other techniques to achieve greater discrimination. It is also labor and time intensive.

Although not yet utilized in environmental and clinical isolate matching of NTM, it is becoming increasingly feasible to perform whole genome sequencing. Whilst offering definitive genomic differentiation of isolates, whole genome sequencing requires new stratagems in data management [50]. When more sequences become available, targeted sequencing for accurate species identification will reduce costs and increase accessibility. Whole or targeted genome sequencing will likely replace PFGE as the gold standard for bacterial strain typing.

Factors defining clinically important sources of NTM

Geographic location

Relevant studies have been conducted in different countries, with most from the USA and Australia (Table 2) and a few from the developing world. The countries best represented in the literature have a lower prevalence of MTB infection,

Table 2 – (a and b) Summary of studies utilizing molecular methods to differentiate and match clinical and environmental isolates of NTM. Relevant papers were identified using search engines (Google Scholar, PubMed, UQ Summon and UWA OneSearch) and citations from other papers. The databases were searched using the following keywords to screen for relevant studies: non-tuberculous mycobacteria, patient, environment, epidemiology, infection, water, dust, soil. All studies cited were available through the University of Queensland or University of Western Australia subscription services or publicly accessible. The absence of published data is represented by “NA”.

Country	Clinical samples	Environment investigated	Environmental samples containing NTM: Number of environmental samples taken	Matched clinical and environmental NTM isolates: total environmental samples	Species matched	Species characterization method	Strain characterization method	Source
(a)								
UK	Patients (n = 33) Isolates (n = 36)	Hospital water faucets (n = 97), tank (n = 5)	57:97 (faucet) 3:5 (tank)	36:102	<i>M. xenopi</i> (n = 36)	Culture Unspecified methods	NA	[39]
USA	AIDS patient stool, sputum and blood (n = 36)	Environmental and municipal water sources	10:NA	5:NA	<i>M. avium</i> (n = 5)	Culture DNA probe kits (SNAP, Gen-Probe)	PFGE-AseI	[60]
USA	Patient blood or bone marrow (n = 40)	Hospital water (n = 10) House water (n = 58) Reservoir water (n = 13)	10:10 (Hospitals) 54:58 (Houses) 12:13 (Reservoirs)	3:10 (Hospitals) 2:58 (Houses) 0:13 (Reservoirs)	<i>M. avium</i> (n = 5)	Culture Serotyping Species specific DNA probes (Syngene) Multilocus Enzyme Electrophoresis	NA	[61]
Canada	Patient sputum (n = 1)	Hot-tub water (n = 1)	1:1	1:1	<i>M. avium</i> (n = 1)	Culture Multilocus Enzyme Electrophoresis	RFLP (IS1245)	[30]
USA	Patients (n = 17) Isolates (n = 19)	Water reservoirs (n = 13) Residential water (n = 55) Commercial building water (n = 31) Hospital water (n = 15)	12:13 Water reservoirs 45:55 Residential water 31:31 Commercial building water 15:15 Hospital water	3:144	<i>M. avium</i> (n = 3)	Culture Biochemical analyses DNA probe kits (SNAP, AccuProbe) HPLC	PFGE-AseI	[62]
USA	AIDS and non-AIDS patients (n = 103 isolates)	Various vegetables (n = 121)	25:121	1:121	<i>M. avium</i> (n = 1)	Culture DNA probe kits (Accuprobe, SNAP) RFLP	RT-PCR (IS 1245 and IS1311) (<i>M. avium</i> only)	[63]
USA	Patient biopsy tissue (n = 2)	Nail salon whirlpool bath swabs (n = 7)	3:7	2:7	<i>M. mageritense</i> (n = 2)	Culture Biochemical analyses PCR-RFLP (<i>hsp65</i>) HPLC (mycolic acid esters)	rep-PCR PFGE-XbaI	[38]
USA	Patient sputum (n = 131) (n = 161 isolates)	Hospital hot water system isolates (n = 13)	13:13	85:13	<i>M. avium</i> (n = 85)	Culture PCR-RFLP (<i>hsp65</i>) Gen-probe DNA Probes	PFGE-AsnI, XbaI	[64]

Table 2 – (continued)

Country	Clinical samples	Environment investigated	Environmental samples containing NTM: Number of environmental samples taken	Matched clinical and environmental NTM isolates: total environmental samples	Species matched	Species characterization method	Strain characterization method	Source
(a)								
Australia	Patient sputum (n = 4)	Spa water (n = 2)	2:2	3:2	<i>M. avium</i> (n = 3)	Culture Phenotypic characteristics	PFGE-AseI rep-PCR (between IS1245 and IS1311)	[37]
Korea	Patient blood culture (n = 12 isolates)	Hospital tap water n = 100	50:100	2:100	<i>M. mucogenicium</i> (n = 2)	Culture PCR-RFLP (<i>ropB</i>) – MspI, HaeIII	RAPD (10-mer primer)	[65]
USA	Patient sputum (n = 26)	Commercial potting soil aerosols (n = 2) Patient garden soil aerosols (n = 79)	21:21	3:79	<i>M. intracellulare</i> (n = 2) <i>M. avium</i> (n = 1)	Culture RFLP (<i>hsp65</i>) – HaeIII, BstEII Partial sequencing (16s and 23s rDNA)	PFGE-XbaI	[13]
Japan	Patient sputum (n = 30)	Patient residential bathrooms (showerhead water (n = 46), kitchen tap water (n = 48), bathtub tap (n = 48), showerhead scale (n = 37), drain slime (n = 49) and air conditioners (n = 45))	10:371 (all in bathrooms)	2:49	<i>M. avium</i> (n = 2)	Culture Phenotypic characteristics Partial sequencing (16s- 23s RNA ITS region)	Serotyping RFLP (IS 1245 and IS 1311) PFGE-XbaI, DraI	[66]
(b)								
USA	Patient sputum (n = 1)	Showerhead sediment (n = 6), hot (n = 6) and cold (n = 6) bathroom tap water	6:6 (Hot water) 6:6 (Cold water) 6:6 (Sediment)	1:18	<i>M. avium</i> (n = 1)	Culture Partial sequencing (16s rDNA)	RFLP (<i>hsp65</i> and IS 1245/IS 1311) - BstEII, HaeIII	[17]
USA	Patient blood (n = 5)	Texas hospital sinks, showers (n = 13) Municipal water tank swabs (n = 2) Municipal water plant tanks (n = 2) Hospital swabs (n = 10) Ice machine ice (n = 1)	12:27	1:27	<i>M. phocaicum</i> (n = 1)	Culture Partial sequencing (<i>hsp65</i> , 16S rRNA, and <i>rpoB</i>)	rep-PCR (Box A1R Primer) RAPD – PCR (RAPD1 primer) PFGE – AseI	[9]
USA	Patient isolates (n = 74)	Residential water supplying patients homes (n = 23)	23:23	1:23	<i>M. avium</i> (n = 1)	Culture Multilocus Enzyme Electrophoresis	PFGE-XbaI	[67]

Table 2 – (continued)

Spain	Patients (n = 39) Patient urine (n = 23 isolates) Patient sputum (n = 19 isolates)	Hospital tap water Urine collection bottles	6:NA (Hospital tap water) 11:NA (Urine bottles)	21:NA (Patient urine) 5:NA (Patient sputum)	<i>M. avium</i> (n = 26)	Culture Phenotypic characteristics DNA Probe kit (AccuProbe MAC culture identification kit)	rep-PCR (IS1245 and IS901) Partial sequencing (<i>hsp65</i> , 16S rDNA) RFLP (IS 1311) – HinfI, MseI PFGE – XbaI	[41]
USA	Patients (n = 42)	Patient residential water systems (n = 37) Total samples taken (n = 394)	22:37 (houses) 109:394 (individual samples)	7:37	<i>M. avium</i> (n = 7)	Culture Partial sequencing (16S rDNA)	rep-PCR	[68]
USA	Patients (n = 24) Patient samples (n = 60)	Hospital water and ice machines (n = 139) Household water (n = 5 households)	112:139 (Hospital water and ice) 4:5 (Residential water)	5:139 (Hospital water and ice) 3:5 (Residential water)	<i>M. porcinum</i> (n = 8)	Culture HPLC of mycolic acids RFLP (<i>hsp65</i>)	PFGE – XbaI, AseI Partial sequencing (<i>hsp65</i> , <i>ropB</i> , <i>recA</i> and 16S rDNA)	[69]
Australia	Patients (n = 20)	Patient taps, swimming pools and rainwater tanks (n = 20)	19:20	7:20 (Species level) 1:20 (Strain level)	<i>M. abscessus</i> (n = 3), <i>M. avium</i> (n = 1), <i>M. gordonae</i> (n = 1), <i>M. lentiflavum</i> (n = 1), and <i>M. kansasii</i> (strain level match) (n = 1)	Culture RT-PCR and High Resolution Melt Curve Partial sequencing (16S rDNA) Multiplex PCR Hain Life Sciences GenoType Mycobacterium AS kit	rep-PCR	[8]
Australia	Patients (n = 68) Isolates (n = 72)	NTM patient residential water swabs and aerosols (n = 16 isolates) Water from main, reservoir and distribution points (n = 220)	n = 16 isolates (NTM Patient residential water swabs and aerosols) 49:220 (Water from main, reservoir and distribution points)	6:65 (environmental isolates)	<i>M. kansasii</i> (n = 6)	Culture Phenotypic characteristics Partial sequencing (<i>hsp65</i> , 16S rDNA) Multiplex PCR DNA probe kit (GenoType® Mycobacterium AS (additional species) kit)	RFLP (16S-23S ITS rDNA) rep-PCR	[50]
Australia	Patient samples (n = 74)	Municipal water Tap water Tank water Swimming pool water	15:NA	6:15 (water isolates)	<i>M. abscessus</i> (n = 6)	Culture Phenotypic characteristics Partial sequencing (16S rDNA, <i>hsp65</i> , <i>rpoB</i>) Multiplex PCR DNA probe kit (GenoType® Mycobacterium AS (additional species) kit)	rep-PCR	[3]

better diagnosis of NTM diseases and more funding available for research. The imbalance makes it difficult to address the role of climate and geographical location in the acquisition of NTM from water and soil. However, NTM does appear to be widespread in most regions, as is NTM disease [1,51]. It remains unclear why the species causing disease vary between countries, but there is potential for climate to influence the exposure of susceptible people through the prevalence of NTM in the environment [52].

Patient exposure

Many common activities expose individuals to pathogenic NTM. The most studied are hot-tub use [30,37,6], showering [8,9,17], faucet use [39,53,54] and gardening [13]. These sources have been investigated by studies matching NTM strains between the patient and the environment. NTM transmission has also been linked with exposure from commercial farming [55], aerosolised metalworking fluids [27] and tending of aquariums [56], but these routes have yet to be confirmed by matching clinical and environmental strains.

Sources of residential exposure to NTM have been investigated via questionnaire-based surveys [52,57,58]. Exposure to soil was identified as a risk factor in all three studies, and one showed more mixed and polyclonal NTM infections in patients regularly exposed to soil, linking environmental exposure with disease [57]. NTM disease did not correlate with exposure to specific foods or to pets [52,58].

Although useful data are provided, the use of surveys is flawed in that: (1) one exposure event may be enough for NTM colonization, making accounts of activity frequency less relevant; (2) isolates were not matched to ascertain that the source implicated was responsible for NTM disease; and (3) for a study of the general population, a cohort of 800 is insufficient in an area with an NTM infection rate of 1.2/100,000 [52]. Some studies have used sensitin skin testing as an indicator of NTM infection [52]. However, sensitin tests reflect both past and current infections, with a potential for false positives, and are poor markers of clinical disease.

The low ratio of successful matches between environmental and clinical isolates is striking. While some studies matched most clinical isolates with their reservoir [17,37], this is likely due to the low numbers of participating patients. Others achieved accurate matches with fewer than 20% of patient isolates, so any conclusions regarding relative environmental risk factors are broad extrapolations [9,13,53,54]. The reservoirs for most clinically infective NTM remain unknown, but many bacteria in the patient's immediate environment are never identified and characterized. The missing pathogens may have originated from a known environment of interest (e.g., a showerhead) that was not sampled for the study, or may have been transmitted via a novel route. The significant lag time between acquisition of infection and diagnosis of disease (1–10 years) makes it difficult to pinpoint the actual source.

Alternatively, human transmission may be responsible for more infections than is generally suspected. Although this has rarely been observed, recent studies show links between isolates from multiple patients [59–61]. These results do not prove human-to-human transmission, as patients may have

acquired the pathogen from the same environmental reservoir. However, this route of NTM infection should not be dismissed without further study.

Species and strain infectivity

Species of NTM differ in habitat and in their potential for human infection. A summary compiled by the American Thoracic Society shows the relative risk of infection by different NTM species in the USA [1]. *M. kansasii* remains one of the most infective species whilst others (i.e., *Mycobacterium frederiksbergense*, *Mycobacterium flavescens*, *Mycobacterium moriokaense*) appear to be non-pathogenic to humans [1].

Moreover, pathogenicity may vary between strains of the same species. In a study of clinical isolates of *M. kansasii* ($n=191$), isolates from different subtypes differed in pathogenicity based on the percentage of patients infected [62]. Subtype 1 accounted for a majority of isolates and was considered pathogenic in 81% of cases, while subtype 3 was only pathogenic in 6% of cases and was considered a non-pathogen [63].

Antibiotics play a central role in the treatment of NTM disease, with individual NTM species and patients requiring different antibiotic cocktails. Difficulties prescribing the correct treatment arise from the limited ability of *in vitro* susceptibility tests to predict antimicrobial responses for all NTM species. For example, *M. kansasii* is susceptible to multiple antibiotics and *M. avium* is susceptible to the macrolide antimicrobials *in vivo*, but *M. abscessus subs abscessus* has inducible resistance to macrolides [1]. In rare cases, side effects of effective antibiotic treatment outweigh the consequences of the disease.

Strain typing of NTM is not routine clinical care, but could reveal patterns of pathogenicity and antibiotic resistance present in the population. For example, *M. abscessus* strains with a functional erythromycin ribosome methyltransferase (*erm*) gene are resistant to macrolide antibiotics. Strains lacking a functional *erm* gene are susceptible to macrolides *in vivo* and *in vitro* [64].

An understanding of NTM transmission will facilitate targeted prophylactic measures

NTM are ubiquitous in the environment and vary in infectivity, pathogenicity and susceptibility to antibiotics. Although most studies using high resolution molecular techniques are from the USA and Australia, NTM have been isolated from similar sources in other countries and climates. The risk of a particular environmental source transmitting pathogenic NTM is determined by three variables: (1) the capacity for pathogenic NTM growth in the environment; (2) the frequency of human exposure from the source in question; and (3) the method of patient exposure to pathogenic NTM from the source. Environments of interest are those with high NTM burdens in a form that is easily aerosolised, namely showers, hot-tubs, garden soil and house dust.

From studies in which environmental NTM have been matched by strain to pathogenic isolates, key sources appear to be hot-tubs, faucets and showerheads, with similar frequencies of isolation from hospitals and private residences.

However, most private residences sampled belonged to patients already infected with NTM. When private residences of uninfected individuals were sampled, NTM were recovered at a much lower frequency, suggesting the community at large has a low risk of infection from household water [53].

Whilst many studies confirm that NTM is transmitted from water, this may simply reflect the large number of water-based studies. *M. avium* has a longstanding link to water environments, as does *M. abscessus* [8]. Although *M. kansasii* can be found in a tap water, few strains from tap water matched disease isolates. *M. intracellulare* infections were also associated with water, but these waterborne *M. intracellulare* isolates have since been reidentified as a novel species, *Mycobacterium chimaera* [65]. Although *M. intracellulare* infection is still commonly reported [66], since this re-classification there remains no evidence linking water to *M. intracellulare* infection. Transmission may involve soil exposure, which correlates with NTM pulmonary disease in multiple studies [44,52,57,58] although it has only been confirmed as a transmission route via strain-matching in one [13].

Presently, prophylaxis of pulmonary NTM disease involves avoidance of exposure to possible NTM containing aerosols. Steps that can be made to reduce levels of NTM in water include: maintaining hot water system temperatures at above 60 °C, and the correct pH and chlorination of pools and spas. Nosocomial infections can be prevented by cleaning medical instruments using only sterile water and disinfectant before use, and use of personal protective equipment can reduce aerosol inhalation (e.g., when gardening). These precautions can be recommended to individuals perceived to be at a high risk of infection. A more extensive understanding of NTM transmission will allow more targeted prophylactic measures.

Conflict of interest

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