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The fate and risk of nontuberculous mycobacteria in the water supply system: a review

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ABSTRACT

Nontuberculous mycobacteria (NTM) infection is estimated as the most serious waterborne infectious disease. NTM are ubiquitous in drinking water supply systems, which could be one of the possible exposure pathways for NTM disease, posing a serious concern to human health. Characteristics of NTM, such as exposure via inhalation, disinfectant resistance, survival in oligotrophic conditions, and association with amoebae, are largely different from those of *Escherichia coli* (*E. coli*) which has been traditionally regarded as a model bacterium causing gastrointestinal diseases in water safety. However, the fate of NTM in water supply systems from source water to the point of use has not been systematically revealed yet. Thus, this review proposes that NTM should be regarded as alternative model bacteria in water use by updating the current knowledge on the occurrence, removal efficiency, and regrowth of NTM in water supply systems. Moreover, we demonstrate the need to establish a comprehensive quantitative microbial risk assessment to identify the critical control point, which is indispensable to mitigate NTM risk in water use.

Key words: microbial risk assessment, nontuberculous mycobacteria, regrowth, water supply, water treatment, water use

HIGHLIGHTS

- Waterborne NTM are posing a significant health threat in many countries.
- NTM are persistent and can regrow in drinking water, which is so different from traditional model bacterium (*E. coli*).
- The fate of NTM from source to the point of use remains unclear.
- Comprehensive risk assessment is lacking for NTM in water supply systems.
- NTM can be new model bacteria to revisit the management of water supply systems.
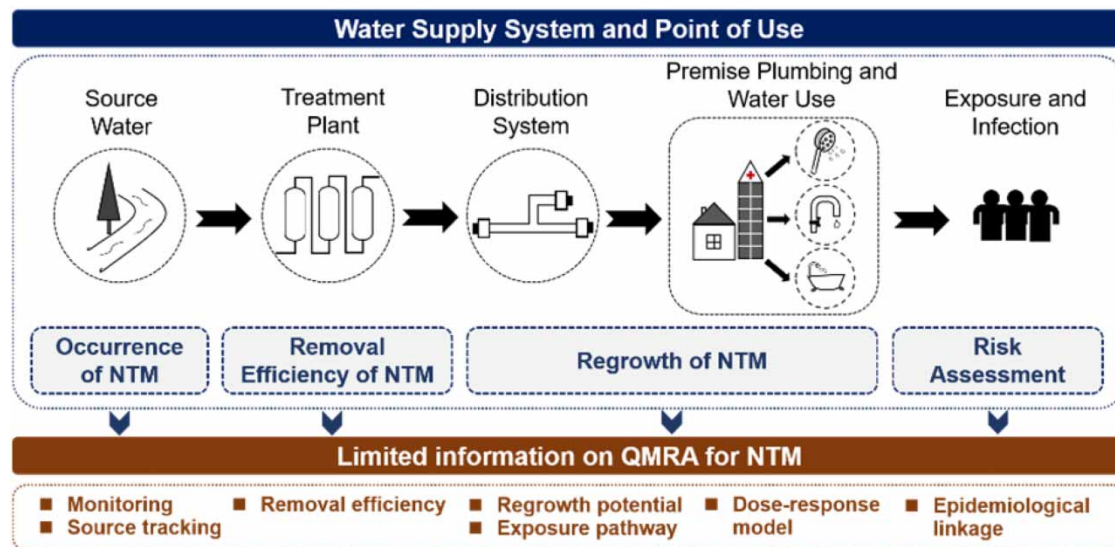
1. INTRODUCTION

The genus *Mycobacterium* in the phylum Actinobacteria contains over 200 species (Parte 2018). Nontuberculous mycobacteria (NTM) are defined as *Mycobacterium* species other than *Mycobacterium tuberculosis* complex (MTC) and *Mycobacterium leprae/lepromatosis* (Vera-Cabrera *et al.* 2011; Fedrizzi *et al.* 2017). *M. avium*, *M. intracellulare*, and *M. chimaera* are designated as *Mycobacterium avium* complex (MAC), which are related to human pulmonary infections (Busatto *et al.* 2019). NTM are ubiquitous in natural and human-associated environments (Falkinham 2015). As NTM can be persistent in drinking water supply system, they can transmit via water use including drinking (Thomson *et al.* 2013b; Zlojtro *et al.* 2015), showering (Gebert *et al.* 2018; Uwamino *et al.* 2020), spa (Nakanaga *et al.* 2011), and humidification (Utsugi *et al.* 2015). Exposure to pathogenic NTM through inhalation, oral ingestion, and dermal contact might result in pulmonary disease, disseminated infection, and skin/soft tissue infections, especially to immune-compromised people (Hamilton *et al.* 2017b; CDC 2019a). NTM infection is now one of the most severe waterborne illnesses in the United States, where the estimated number of deaths and direct healthcare cost of NTM infection is more extensive than that of other waterborne diseases (Collier *et al.* 2021). In parallel with an increase in the publications of overall NTM issues from 1996 to 2020 (Figure 1(a)), the number of papers on NTM related with topic for 'drinking water OR water supply OR water distribution OR water use' has also increased recently (Figure 1(b)). Diverse waterborne NTM species are prevalent in drinking water treatment trains (King *et al.* 2016; Wang *et al.* 2019a), drinking water distribution system (DWDS) (Gomez-Smith *et al.* 2015; Waak *et al.* 2019), premise plumbing (Feazel *et al.* 2009; Donohue *et al.* 2015), and point-of-use (POU) equipment (Falkinham *et al.* 2008; Gebert *et al.* 2018; Yoon *et al.* 2020). However, NTM are not yet routinely monitored, and little data is available to reveal the transmission route and infection source.

The characteristics of NTM are primarily different from those of *Escherichia coli* (*E. coli*) which has been studied as a model bacterium for gastrointestinal diseases in water supply systems (Edberg *et al.* 2000). The major exposure pathway of NTM is inhalation, while oral ingestion is a primary pathway of *E. coli* (Goslee & Wolinsky 1976). NTM are more resistant to disinfectants than *E. coli* (Taylor *et al.* 2000). Moreover, NTM can regrow in oligotrophic drinking water, while regrowth of *E. coli* is not considered (van der Wielen & van der Kooij 2013). Nevertheless, the fates of NTM in water supply systems are not fully revealed, resulting in uncertainties of quantitative microbial risk assessment (QMRA) of NTM.

Thus, this review summarizes current knowledge on the occurrence, removal, and regrowth of NTM from source to POU in water supply systems, which is imperative to accurately identify critical control points and to mitigate potential health risks of waterborne NTM.
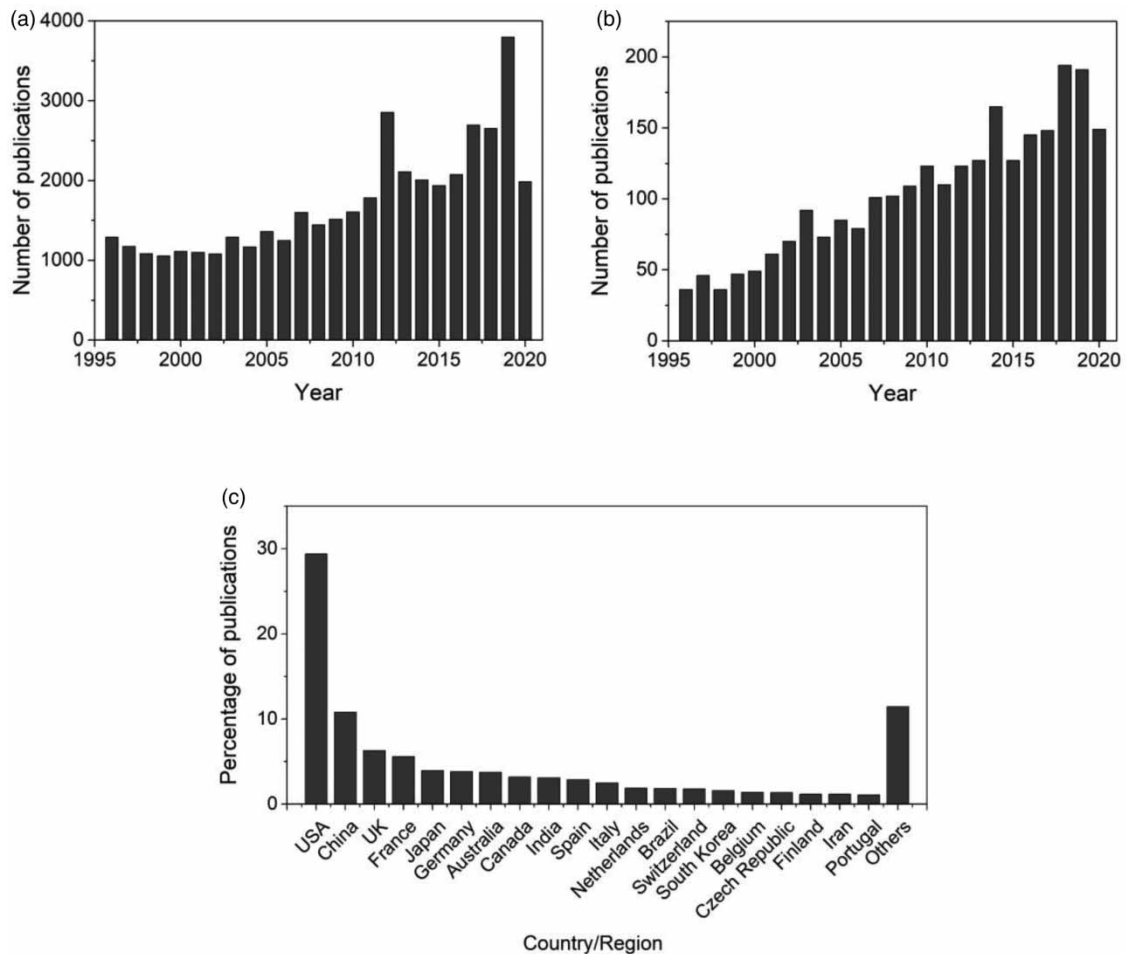
Figure 1 | Paper records from the Web of Science between 1996 and 2020. (a) The number of papers for topic 'mycobacterium OR mycobacteria' NOT 'mycobacterium *tuberculosis* OR mycobacterium *leprae* OR mycobacterium *lepromatosis*'. (b) the number of papers for topic 'mycobacterium OR mycobacteria' NOT 'mycobacterium *tuberculosis* OR mycobacterium *leprae* OR mycobacterium *lepromatosis*' AND 'drinking water OR water supply OR water distribution OR water use', and (c) the percentage of papers from different countries for topic 'mycobacterium OR mycobacteria' NOT 'mycobacterium *tuberculosis* OR mycobacterium *leprae* OR mycobacterium *lepromatosis*' AND 'drinking water OR water supply OR water distribution OR water use' within 1996–2020.

2. NTM INFECTION AND HEALTH IMPACT

2.1. NTM-related diseases

Although clinical studies have primarily focused on MAC and *M. leprae* (Mungroo *et al.* 2020; Goossens *et al.* 2021), some NTM are also potentially pathogenic to humans or animals (Falkinham 2016a). The pathogenic NTM can cause central nervous system disease, pulmonary, and skin/soft tissue infections in children, elderly individuals, especially patients with immunocompetent or immuno-compromised conditions, such as HIV-positive patients (Pedley *et al.* 2004; Adékambi 2009). NTM-related pulmonary infection is predominantly caused by MAC, *M. chelonae*, *M. fortuitum*, and *M. abscessus* (Kim & Shin 2017; Griffith & Daley 2021). Among the subspecies of *M. avium*, *M. avium* subsp. *hominissuis* is responsible for pulmonary infection, cervical lymphadenitis, and disseminated infection in humans (Busatto *et al.* 2019). *M. kansasii*, *M. malmoense*, *M. simiae*, *M. szulgai*, and *M. xenopi* can also be responsible for respiratory diseases (Lory 2014). On the other hand, *M. chelonae*, *M. abscessus*, *M. haemophilum*, *M. ulcerans*, *M. marinum*, *M. smegmatis*, and *M. fortuitum* can cause skin and soft tissue infections (Pedley *et al.* 2004; Lory 2014). In particular, *M. ulcerans* can cause a severe skin disease called Buruli Ulcer (BU) with the formation of ulcers on limbs (Maman *et al.* 2018). Some rapid-growing NTM can infect intravascular catheters and consequently cause bloodstream infections (El Helou *et al.* 2013). The prevalence of NTM disease in humans has been increasing around the world during the last decades (Henkle *et al.* 2015; Namkoong *et al.* 2016; Shah *et al.* 2016; Dakic *et al.* 2018; Greif *et al.* 2020; Harada

et al. 2020; Lin *et al.* 2020; Park *et al.* 2020; Thomson *et al.* 2020). Table 1 shows the increase in the incidence rate of NTM disease in many countries and regions. Besides, in Japan, the increase in the annual mortality rate of NTM disease was reported from 0.63/100,000 population in 1997 to 1.93/100,000 population in 2016, especially in the old female population (Harada *et al.* 2020). Although the increasing trend of NTM disease could be due to the recognition of NTM diseases and the improvement of diagnosis methods, elderly people in aging society might be more susceptible to a NTM disease (Namkoong *et al.* 2016; Harada *et al.* 2020).

2.2. Exposure routes of NTM infection

The most common waterborne exposure pathways leading to a mycobacterial infection include ingestion, inhalation, and dermal contact, especially after repeated exposure to aerosols while showering (WHO 2004; CDC 2019a). A few cases of person-to-person transmissions have also been reported in studies (Ricketts *et al.* 2014; Bryant *et al.* 2016). However, the transmission route from environmental sources is currently gaining more attention. The possible ecological sources of NTM include soils, especially acidic or coastal soils, natural waters, drinking water, and aerosols (Falkinham 2015).

The water supply system could also be a possible exposure site for people to get NTM infection. A study reported that healthcare-associated outbreaks were related with *M. abscessus* colonization in municipal water use, such as tap water and heater-cooler unit (HCU) (Baker *et al.* 2017). HCU contaminated with *M. chimaera* was also reported to be correlated with the global epidemic of a cardiac surgery-related *M. chimaera* disease (van Ingen *et al.* 2017). Aerators of hand-washing machine also caused pseudo-outbreak of *M. chimaera* (Nakamura *et al.* 2019). Another study reported a relationship between the relative abundance of pathogenic NTM (MAC and *M. abscessus*) in showerheads and the prevalence of a NTM disease across cystic fibrosis patients (Gebert *et al.* 2018). In Japan, the recent significant increase in the number of patients with a MAC lung disease was suggested to be correlated with the lifestyle tendency towards showering (Uwamino *et al.* 2020). In the United States, a positive association between NTM isolated from shower aerosols and the MAC pulmonary disease was observed for Washington and Oregon residents, indicating that shower aerosols could be the potential exposure source in the household (Tzou *et al.* 2020). A pseudo-outbreak of *M. gordonae* nosocomial infection and *M. gordonae* lung disease was correlated with the presence of *M. gordonae* in potable tap water and ultrasonic humidifier, respectively (Utsugi *et al.* 2015; Zlojtro *et al.* 2015).

Some reports revealed that NTM isolated from the patients were genetically identical to NTM in the household. *M. avium* isolated from patients with a NTM lung disease had a clonal relationship with the *M. avium* found from water in kitchen and bathroom and biofilm in showerhead in the patient's home (Falkinham *et al.* 2008; Yoon *et al.* 2020). *M. chimaera* recovered from indoor showerhead biofilms was generically similar to respiratory *M. chimaera* isolates (Virdi *et al.* 2021). *M. abscessus* isolates were found to be identical in patients and in potable

Table 1 | Increase in the incidence rate of NTM disease in different countries/regions

Country/Region	Incidence rate per 100,000 persons per year	Year	Reference
Japan	5.7	2007	Namkoong *et al.* (2016)
	14.7	2014	
Taiwan	5.3	2005	Lin *et al.* (2020)
	14.8	2013	
England, Wales, and Northern Ireland	5.6	2007	Shah *et al.* (2016)
	7.6	2012	
Oregon, USA	4.8	2007	Henkle *et al.* (2015)
	5.6	2012	
South Korea	1.2	2005	Park *et al.* (2020)
	4.8	2013	
Uruguay	0.33	2006	Greif *et al.* (2020)
	1.57	2018	
Serbia	0.18	2010	Dakic *et al.* (2018)
	0.48	2015	
Queensland, Australia	11.1	2001	Thomson *et al.* (2020)
	25.8	2016	

water (Thomson *et al.* 2013b). The isolates from patients with a chronic rhinosinusitis disease were clonally related to NTM isolates from household plumbing (Tichenor *et al.* 2012). These findings suggest that household plumbing and water use could be the possible route for NTM infection.

3. OCCURRENCE OF NTM IN SOURCE WATER

NTM are prevalent in natural water (Table 2). *Mycobacterium* sp. were detected from 94.28% of 70 samples in Kamp and Wulka Rivers in Australia (Delghandi *et al.* 2020). In the Han River in Korea, 59% of 76 surface water samples contained *Mycobacteria* spp., and *M. gordonae* was most frequently isolated (Lee *et al.* 2008). In China, 2.1×10^8–2.4×10^9 gene copies/L of *Mycobacterium* spp. and 3.5×10^4–2.3×10^5 gene copies/L of *M. avium* were quantified from source water for drinking water (Huang *et al.* 2021). In the United States, *M. avium* ($2.2\times10^4 \pm 3.1\times10^4$ gene copies/L) and *M. intracellulare* ($2.0\times10^3 \pm 1.8\times10^3$ gene copies/L) were detected from 25% (6/24) of surface water used for drinking water source (King *et al.* 2016). *M. gordonae* was observed more frequently in the distribution lines receiving treated surface water, while *M. nonchromogenicum* was frequently observed from the distribution system receiving groundwater with chlorine disinfection (Le Dantec *et al.* 2002b), indicating that water origins would affect the NTM species in the downstream system. However, the information on the prevalence of NTM in source water is limited. Thus, further study is necessary to reveal the impact of source water types and seasonal variation on the occurrence and diversity of NTM at the POU, which is informative for microbial risk assessment of source water for water supply.

4. THE FATES AND REMOVAL EFFICIENCY OF NTM IN DRINKING WATER TREATMENT PROCESSES

The occurrence of diverse NTM has been observed in drinking water treatment plant (DWTP) (Table 2); however, little is known about the removal efficiency of NTM in each treatment step.

In a DWTP in China, *Mycobacterium* spp. (2.2×10^7–3.6×10^8 gene copies/L) in raw water were removed to approximately 10^5 gene copies/L after sedimentation and ozonation (Wang *et al.* 2019a). While their abundances increased to around 10^6 gene copies/L after the subsequent granular activated carbon filtration, they were below 10^5 gene copies/L after sand filtration and chlorine disinfection (Wang *et al.* 2019a). However, in four DWTPs in China which treated surface water by coagulation/sedimentation, followed by combinations of sand filtration, ozonation, biological activated carbon (BAC) filtration, and disinfection (ClO_2 or $NaClO$), the removal efficiencies of *Mycobacterium* spp. and *M. avium* were 81.4–99.9% and 97.2–99.6%, respectively, which were similar to those of bacterial 16S rRNA genes (94.4–99.4%) (Huang *et al.* 2021). In these plants, sedimentation removed *Mycobacterium* spp. by 37–59% and *M. avium* by 1.7–79%. The removal of NTM by sedimentation could be related to the adherence of hydrophobic NTM to particulates (Falkinham 2016b). BAC filtration and/or sand filtration removed *M. avium* by 10–58%, while *Mycobacterium* spp. rather increased after the filtration process, suggesting that some NTM might proliferate in filter media (Huang *et al.* 2021). In the final step of a DWTP, ClO_2 inactivated *Mycobacterium* spp. and *M. avium* in the BAC filtration effluent by 83 and 97%, respectively (Huang *et al.* 2021).

NTM, such as *M. gordonae* and *M. nonchromogenicum*, were persistent even after ozonation (Lee & Deininger 2000). The relative abundance of 16S rRNA genes of *Mycobacterium* spp. increased after ozonation (Li *et al.* 2017), indicating that NTM could be relatively enriched after ozonation.

Disinfection in the treatment process is effective for inactivating pathogens. However, NTM have relatively high resistance to disinfection (Chiao *et al.* 2014). *M. avium* is generally 1,000-fold more resistant to chlorine than *E. coli* (Taylor *et al.* 2000). To achieve three log inactivation of *M. chelonae* and *M. fortuitum*, concentration–time (Ct) values of 100 and 135 mg·min/L of free chlorine were required in a laboratory-scale experiment, respectively, which are higher than the Ct value (60 mg·min/L) in water treatment lines (Le Dantec *et al.* 2002a). In the DWTP, *M. mucogenicum*, *M. fortuitum*, *M. lentiflavum*, *M. triplex*, and *M. phocaicum* were cultured from chlorinated or chloraminated water samples (12–80 CFU/L) (King *et al.* 2016). *M. avium* ($2.1\times10^3 \pm 3.3\times10^3$ gene copies/L) and *M. intracellulare* ($8.0\times10^2 \pm 1.4\times10^3$ gene copies/L) were detected from 24% of treated water by qPCR (King *et al.* 2016). Based on amplicon sequencing of 16S rRNA gene, the relative abundance of *Mycobacterium* spp. increased from 0.91 to 16.89% after chlorination (Guo *et al.* 2021). In a DWTP in Hubei province, China, the abundance of *Mycobacterium* spp. decreased in clear water tanks (free chlorine >0.1 mg/L after 30 min contact time), while their abundance in biofilm increased

Table 2 | Occurrence of different NTM species from source water to the POU

		MAC[a]	*M. avium*[a]	*M. intracellulare*[a]	*M. fortuitum*[a]	*M. gordonae*[a]	*M. mucogenicum*[a]	*M. lentiflavum*[a]	*M. chelonae*[a]	*M. arupense*[a]
Water source		–	King *et al.* (2016)	King *et al.* (2016) Lee *et al.* (2008)	King *et al.* (2016)	Lee *et al.* (2008)	–	Lee *et al.* (2008)	–	Lee *et al.* (2008)
DWTP	Sedimentation	–	Huang *et al.* (2021)	–	–	–	–	–	–	–
	Ozonation	–	–	–	–	Le Dantec *et al.* (2002b)	–	–	–	–
	Filtration	–	Huang *et al.* (2021)	–	–	–	–	–	–	–
	Disinfection	–	King *et al.* (2016)[b,c] Huang *et al.* (2021)[b,d]	King *et al.* (2016)[b,c]	King *et al.* (2016)[b,c]	–	King *et al.* (2016)[b,c]	King *et al.* (2016)[b,c]	–	–
DWDS		Whiley *et al.* (2014)[b,c]	van der Wielen *et al.* (2013a)[e]	Le Dantec *et al.* (2002b)[b]	Le Dantec *et al.* (2002b)[b]	Le Dantec *et al.* (2002b)[b] Liu *et al.* (2012)[b] Waak *et al.* (2019)[c] van der Wielen *et al.* (2013a)[e]	–	Gomez-Smith *et al.* (2015)[c]	Le Dantec *et al.* (2002b)[b]	Liu *et al.* (2012)[b]
Premise plumbing	Tap	–	Donohue *et al.* (2015)[-b-e] Perez-Martinez *et al.* (2013)[b] Huang *et al.* (2021)[b,d] Wallace *et al.* (2013)	Donohue *et al.* (2015)[b-e] Haig *et al.* (2018)[c]	Donohue *et al.* (2015)[b-e] Perez-Martinez *et al.* (2013)[b]	Donohue *et al.* (2015)[b-e] Perez-Martinez *et al.* (2013)[b] Haig *et al.* (2018)[c] Zlojtro *et al.* (2015)	Donohue *et al.* (2015)[b-e] Perez-Martinez *et al.* (2013)[b] Haig *et al.* (2018)[c]	Donohue *et al.* (2015)[b-e] Lee *et al.* (2008)[b]	Donohue *et al.* (2015)[b-e] Haig *et al.* (2018)[c]	Donohue *et al.* (2015)[b-e]
POU	Showerhead	Gebert *et al.* (2018)[b]	Feazel *et al.* (2009) Falkinham *et al.* (2008) Wallace *et al.* (2013)	Falkinham *et al.* (2008)	Gebert *et al.* (2018)[b]	Feazel *et al.* (2009) Gebert *et al.* (2018)[b]	–	Gebert *et al.* (2018)[b]	Virdi *et al.* (2021) Gebert *et al.* (2018)	–
	Swimming pool	–	–	–	Briancesco *et al.* (2014a)[b]	–	Briancesco *et al.* (2014a)[b]	–	–	–
	Spa	Lumb *et al.* (2004)	–	–	–	–	Briancesco *et al.* (2014a)[b]	Briancesco *et al.* (2014a)[b]	–	–
	Humidifier	–	–	–	–	Utsugi *et al.* (2015)	–	–	Edens *et al.* (2015)	–

		M. nonchromogenicum[a]	*M. immunogenum*[a]	*M. chimaera*[a]	*M. porcinum*[a]	*M. abscessus*[a]	*M. septicum*[a]	*M. peregrinum*[a]	*M. aurum*[f]	*M. chubuense*[f]
Water source		–	–	–	–	–	–	Lee *et al.* (2008)	–	Lee *et al.* (2008)
DWTP	Sedimentation	–	–	–	–	–	–	–	–	–
	Ozonation	Le Dantec *et al.* (2002b)	–	–	–	–	–	–	–	–
	Filtration	King *et al.* (2016)	–	–	–	–	King *et al.* (2016)	–	–	–
	Disinfection	–	–	–	–	–	–	–	–	–
DWDS		Le Dantec *et al.* (2002b)[b]	–	–	–	–	–	Le Dantec *et al.* (2002b)[b]	Le Dantec *et al.* (2002b)[b] Gomez-Smith *et al.* (2015)[c]	Le Dantec *et al.* (2002b)[b]
Premise plumbing	Tap	–	Donohue *et al.* (2015)[b-e]	Wallace *et al.* (2013)	Donohue *et al.* (2015)[b-e] Perez-Martinez *et al.* (2013)[b]	Donohue *et al.* (2015)[b-e] Haig *et al.* (2018)[c]	Donohue *et al.* (2015)[b-e]	Donohue *et al.* (2015)[b-e]	–	–
POU	Showerhead	–	Gebert *et al.* (2018)[b]	Wallace *et al.* (2013) Virdi *et al.* (2021)	Virdi *et al.* (2021)	Virdi *et al.* (2021) Gebert *et al.* (2018)[b]	–	–	–	–
	Swimming pool	–	Briancesco *et al.* (2014a)[b]	–	–	–	–	–	–	–
	Spa	–	–	–	–	–	–	–	–	–
	Humidifier	–	–	–	–	–	–	–	–	–

[a]NTM with human pathogenicity.
[b]Chlorinated water.
[c]Chloraminated water.
[d]Chlorine dioxide treated water.
[e]Untreated water.
[f]NTM without human pathogenicity.

from around 3.2×10^7 to 7.9×10^8 gene copies/g (Lin *et al.* 2014), indicating that NTM were more persistent in biofilm state (Steed & Falkinham 2006; Sousa *et al.* 2015). Higher pH could deteriorate the disinfection efficiency of hypochlorite as the proportion of dissociated ClO^- increases, which could not penetrate to mycobacterial cell due to repulsion (Wang *et al.* 2019b).

The higher resistance to disinfectants can be explained by several properties of NTM such as their specific cell wall, biofilm formation, and protection by free-living amoebae (FLA). The mycobacterial cell wall contains abundant mycolic acids, arabinogalactan, and peptidoglycan (Dulberger *et al.* 2020). The waxy outer membrane, rich in mycolic acids, is called mycomembrane, which is composed of lipids, glycolipids, and secreted proteins in the mycolic acid layer (Dulberger *et al.* 2020). The mycolic acids are composed of long carbon side chains (C_{60}–C_{90}) (Babalola 2015). The presence of the lipid- and wax-rich outer membrane allows NTM to adhere to surfaces and form biofilm (Falkinham 2016b) and protect NTM from physicochemical stresses including disinfection (Busatto *et al.* 2019; Wang *et al.* 2019b). NTM are capable of forming biofilm composed of extracellular matrix containing carbohydrates, lipids, proteins, and extracellular DNA (Sousa *et al.* 2015; Dokic *et al.* 2021). The biofilms can serve as a physical barrier, protecting NTM from disinfectants (Steed & Falkinham 2006). Planktonic *M. avium*, *M. intracellular*, *M. gordonae*, and *M. chubuense* are more susceptible to chlorine than those in biofilm (Steed & Falkinham 2006). FLA, such as *Acanthamoeba* spp. and *Naegleria* spp., can serve as the hosts for NTM, protecting them from external stress (Delafont *et al.* 2014). Intracellular colonization of NTM in FLA is one of the factors for NTM to survive disinfection (Steinert *et al.* 1998; Delafont *et al.* 2014). The association between NTM and FLA does play an essential role in helping NTM regrowth and survival in water systems. In hospital potable water systems, the high replication rate of *M. avium* was observed when they were only associated with *Acanthamoeba lenticulata* (Ovrutsky *et al.* 2013).

Based on the above studies, each treatment step positively or negatively contributes to NTM removal and inactivation. However, the fluctuation of NTM removal efficiency of different treatments remains unknown, which is vital in identifying the critical control points. As the removal efficiencies of *E. coli* and NTM could be different, it is necessary to optimize the treatment conditions for NTM.

5. THE REGROWTH AND PERSISTENCE OF NTM IN WATER DISTRIBUTION AND PREMISE PLUMBING

5.1. Water distribution system

Various NTM species have been identified in the DWDS (Table 2). NTM can regrow in drinking water with very low levels (<50 µg/L) of biodegradable organic carbon (Norton *et al.* 2004; van der Wielen & van der Kooij 2013). The growth of NTM can be promoted with the presence of *Acanthamoeba*, since they could release extracellular metabolites utilized by NTM (Steinert *et al.* 1998).

NTM can be attached to pipe surfaces due to their hydrophobic cell wall (Falkinham 2016b). *Mycobacterium* spp. occupied 25–78% of total bacterial community in the water main surface biofilm in the chloraminated drinking water system, regardless of the type of water main (tuberculated unlined cast-iron, nontuberculated unlined cast-iron, and cement-lined cast-iron water mains) (Gomez-Smith *et al.* 2015). They were the predominant microbial group (88% frequency of detection, 324 cell equivalent L^{-1}) in a chlorinated DWDS in a major metropolitan area in the United States (Lu *et al.* 2016). In the DWDS in Paris, NTM abundances were 1–50 CFU/L for 78% of samples and 51–500 CFU/L for 21% of samples, where *M. gordonae* and *M. nonchromogenicum* were the most frequently isolated species (Le Dantec *et al.* 2002b). *M. gordonae* was also isolated from biofilm in a Beijing DWDS, while *M. arupense* was the major NTM species found in a Guangzhou DWDS (Liu *et al.* 2012).

Disinfection applied to the treated water could have a certain impact on the occurrence of NTM in the DWDS. MAC were detected at 10^6 gene copies/L in the DWDS receiving chlorinated water (0.1–1.3 mg/L), while they were detected at 10^7 gene copies/L in the DWDS receiving the chloraminated water (<0.05–3.8 mg/L) (Whiley *et al.* 2014). In a DWDS with residual chloramine (3.8 ± 0.1 mg/L), biofilm on water main consisted of *M. gordonae* (Waak *et al.* 2019). In another DWDS with chloramine (3.5 ± 0.2 mg/L), *M. frederiksbergense* was abundant in biofilm on water main (Gomez-Smith *et al.* 2015). The impact of other features of DWDS on NTM remains unclarified, including pipe material and water retention time.

In the Netherlands, disinfectant residual is not maintained in drinking water. Even under such condition, mycobacterial 16S rRNA gene copy numbers in the DWDS were 6–38 times higher than those in finished water, indicating that NTM can multiply in an unchlorinated DWDS (van der Wielen & van der Kooij 2013).

Based on *hsp65* gene sequencing, NTM in an unchlorinated DWDS were composed of *M. avium*, *M. genavense*, *M. salmoniphilum*, *M. llatzerense*, and *M. gordonae* (van der Wielen *et al.* 2013a).

5.2. Presence of NTM in premise plumbing and POU equipment

In premise plumbing with a relatively higher surface-to-volume ratio, water stagnation, and residual disinfectant decay, temperature elevation can occur frequently (WHO 2011; Prest *et al.* 2016). NTM have the potential to regrow in the premise plumbing systems (Haig *et al.* 2020) and have been suggested as an indicator of biofilm in a premise pipe (Lu *et al.* 2017). NTM were the most abundant taxon accounting for 85% of the Actinobacteria phylum in the tap water with 0.22–3.63 mg/L chlorine residuals in 16 different cities in the United States (Holinger *et al.* 2014). NTM regrowth was also observed at faucets after overnight water stagnation with simultaneous decline of residual chlorine (Rahmatika *et al.* 2022). Different NTM species can be persistent in the premise plumbing (Table 2). *M. mucogenicum* (percentage of culture-positive taps: 52%), *M. avium* (30%), *M. gordonae* (25%), *M. intracellulare* (20%), and *M. kansasii* (18%) were found from the potable water tap in the United States (Donohue *et al.* 2015). *M. chimaera* (73%) and *M. avium* (7%) were identified from bathroom and kitchen faucet and showerhead water in the United States by Internal Transcribed Spacer (ITS) sequencing (Wallace *et al.* 2013). Besides, *M. abscessus*, *M. avium* subsp. *avium*, *M. chelonae*, *M. gordanae*, *M. intracellulare*, and *M. mucogenicum* were also detected from chloraminated kitchen faucet water (an average of 2.04 mg/L as Cl$_2$) in Michigan, the United States (Haig *et al.* 2018). In Mexico City, *M. avium*, *M. mucogenicum*, *M. porcinum*, *M. gordonae*, *M. fortuitum*, and *M. cosmeticum* were collected from household potable water with chlorine concentration of 0.2–1.5 mg/L (Perez-Martinez *et al.* 2013). In a northern city in China, NTM were detected from 100% of samples at an average abundance of 4.9×10^5 gene copies/L from tap water in washroom and kitchen with an average chlorine concentration of 0.09–0.14 mg/L in different buildings (Liu *et al.* 2019). In an eastern city in China, *Mycobacterium* spp. (7.4×10^7 gene copies/L) and *M. avium* (4.2×10^4 gene copies/L) were detected from tap water with ClO$_2$ or hypochlorite residual (ClO$^-$) of 0.17–0.39 mg/L (Huang *et al.* 2021). NTM (1–6×10^2 CFU/L) were detected from tap water in private buildings, schools, and hospitals in Rome, Italy (Briancesco *et al.* 2014b). In Seoul, Korea, 0–6 CFU/L of mycobacteria, such as *M. lentiflavun* and *M. triplex*, were detected from 91% of tap water samples with 0.64 mg/L free chlorine (Lee *et al.* 2008). Thus, household water could be the environmental niche for NTM.

NTM have been detected from other human-made water systems. NTM were the most dominant bacteria in showerhead biofilms across the United States (Feazel *et al.* 2009; Gebert *et al.* 2018) and Europe (Gebert *et al.* 2018), indicating that showerhead may offer the suitable environment for NTM to form biofilm. In Hawaii, *M. chimaera*, *M. chelonae*, *M. abscessus*, and *M. porcinum* were frequently detected from household showerhead biofilm (Virdi *et al.* 2021). In swimming pool water with free chlorine of 1.2 ± 0.2 mg/L, the abundances of NTM were 2.9×10–3.1×10^4 CFU/L (Briancesco *et al.* 2014b). *M. mucogenicum* and *M. immunogenum* represented the most frequently detected species from swimming pool water with free chlorine of 1.26 mg/L and biofilms (Briancesco *et al.* 2014a). In spa water with free chlorine of 1.66 ± 0.04 mg/L, 1.4×10^2 CFU/L NTM were observed, and the detected NTM species include *M. mucogenicum*, *M. duvali*, *M. confluentis*, *M. lentiflavum*, and *M. goodii* (Briancesco *et al.* 2014a). Spa waters also yielded MAC with concentrations of 4.3×10^7 and 4.5×10^6 CFU/L (Lumb *et al.* 2004).

NTM could easily adhere to air bubbles or aerosols due to hydrophobic cell property (Falkinham 2016b). The existence of NTM in the form of bioaerosol was reported by the surveillance of water and air (Glazer *et al.* 2007). About 10^4–10^6 CFU/L and 6–77 CFU/m^3 of NTM were observed from 13/18 public hot tubs and warm water therapy pools disinfected by chlorine or bromine (Glazer *et al.* 2007). In halogen-treated therapy pool with residual halogen of 5.55 mg/L, 10^4–2.5×10^5 CFU/L of waterborne NTM and 6 CFU/m^3 of airborne NTM were observed (Glazer *et al.* 2007). In heater-cooler devices, *M. chimaera* (67.4% of water samples) was the most common isolate from water, while *M. mucogenicum*, *M. fortuitum*, and *M. abscessus/chelonae* complex were recovered from air specimens (Kaelin *et al.* 2020). Since hot water systems could be good habitats for NTM, shower aerosols may be enriched with NTM (Pedley *et al.* 2004). *M. abscessus*, *M. gordonae*, *M. mucogenicum*, *M. kansasii*, *M. fortuitum* complex, and *M. wolinskyi* were isolated from household shower aerosols, while no *M. intracellulare* was isolated from shower aerosols (Thomson *et al.* 2013a). *M. chelonae* was isolated from the water reservoir of a misting humidifier in an eye clinic (Edens *et al.* 2015). A household ultrasonic humidifier filled with sterile tap water inoculated with *M. abscessus* or *M. avium* could aerosolize *M. abscessus* at 28.6 ± 16.1 CFU/m^3 and *M. avium* at 445 ± 221 CFU/m^3 (Hamilton & Falkinham 2018). These results imply

that various water equipment and facilities could be the ideal reservoir for NTM, posing serious infection threat to human.

Some factors, such as water age (Haig *et al.* 2018), water temperature (Lu *et al.* 2017), pipe material (Gebert *et al.* 2018), and disinfection condition (Donohue *et al.* 2015; Gebert *et al.* 2018), could impact the regrowth of NTM. In a chloraminated premise plumbing system in the United States, the diversity of NTM community decreased as water age increased, and *M. avium* subsp. *avium* became predominated when the water age was higher than 27.5 h (Haig *et al.* 2018). The hot water system could be the ideal habitat for NTM (Wang *et al.* 2017). NTM showed higher densities in hot than cold tap water (Lu *et al.* 2017). Based on 16S rRNA gene sequencing, the relative abundances of NTM were higher in metal showerheads (around 15%) than in plastic showerheads (around 7%) (Gebert *et al.* 2018). The biofilm of *M. avium* was more enriched on iron pipe surfaces than on copper surfaces (Norton *et al.* 2004). Disinfection conditions also contribute to selection, survival, and proliferation for NTM in premise plumbing and POU (Le Dantec *et al.* 2002a; Gebert *et al.* 2018). NTM abundances in showerheads receiving chlorinated municipal water were higher than those receiving well water (Gebert *et al.* 2018). The average CFU counts of NTM in chlorinated tap water (208 CFU/L) were lower than those in tap water with chloramine (538 CFU/L) (Donohue *et al.* 2015).

The premise plumbing and POU equipment could be the hot spots determining the exposure level of NTM. Interventions and practical measures have been proposed to reduce the risk of pathogens in healthcare facilities (CDC 2019b). However, the regrowth potential of NTM has not been considered well. Therefore, some control measures, such as effective treatments, maintenance of sufficient residual disinfectants, prevention of water stagnation, and selection of proper materials of pipe and POU, should be considered to reduce NTM regrowth.

6. RISK ASSESSMENT OF NTM IN WATER USE

6.1. Significant risk of NTM in water use

In drinking water management, *M. avium intracellulare* and *M. avium* have been listed on the United States Environmental Protection Agency's drinking water contaminant candidate list 1 (CCL1) and 3 (CCL3) since 1998 (U.S. Environmental Protection Agency 1998) and 2009 (U.S. Environmental Protection Agency 2009), respectively. The WHO alerted NTM in water safety in buildings (WHO 2011). Water management program for healthcare facilities has been proposed by the United States Centers for Disease Control and Prevention (CDC) to mitigate the risk of pathogens in premise plumbing, which pays attention to the pathogens, including NTM, gram-negative bacteria, non-fecal coliforms, other bacteria/actinomyces, fungi, and protozoa (CDC 2019b). Many different NTM species are included in the list, as shown in Table 3, indicating that NTM are considered as a vital risk target (CDC 2019b). In 2021, CDC reported the estimation of burden and healthcare cost of 17 infectious waterborne diseases in the United States (Collier *et al.* 2021). In this report, among 7.15 million waterborne illnesses in 2014, total cases of

Table 3 | NTM in premise plumbing included in a watch list of water management program of CDC (2019b)

Category	NTM in premise plumbing
NTM	• *M. abscessus* clade (*M. abscessus, M. bolettii, M. massiliense*)
	• *M. chelonae*
	• *M. mucogenicum* clade (*M. mucogenicum, M. phociacum*)
	• *M. fortuitum* clade (*M. fortuitum, M. cosmeticum, mageritiense, M. porcinum, M. septicum*)
	• *M. immunogenum*
	• *M. smegmatis* clade (*M. goodii, M. wolinskyi*)
	• *M. aurum*
	• *M. simiae*
	• *M. avium* complex (*M. avium, M. intracellulare, M. chimaera, M. avium* subsp. *hominissuis, M. columbiense*)
	• *M. scrofulacuem*
	• *M. parascrofulaceum*
	• *M. xenopi*
	• *M. arupense*
	• *M. kansasii*
	• *M. haemophilum*
	• *M. nonchromogenicum* clade (*M. nonchromogenicum, M. triviale, M. terrae*)
	• *M. gordonae* (only among patients with severe immune deficiency)

NTM infection were estimated to be 97,000, of which 68,900 cases were suspected as domestically acquired waterborne, which was higher than Legionnaires' disease (11,000) (Collier *et al.* 2021). About 6,630 deaths caused by domestically acquired waterborne illness were estimated, and 57% were attributed to NTM infection, indicating that NTM can be the largest waterborne risk in the United States (Collier *et al.* 2021). Domestic waterborne NTM infection also demonstrated the largest number of hospitalizations (51,400/118,000 hospitalizations) and the highest direct healthcare cost ($1.53 billion/$3.33 billion) in 2014 (Collier *et al.* 2021). This estimation clearly suggests that major waterborne illness in the United States has shifted from enteric illness to respiratory one and NTM are the most critical risk factor. In the Netherlands, NTM have been regarded to have high priority for further research based on the criteria of disease cases amount, epidemiology data, and occurrence possibility in drinking water (van der Wielen 2013a). Thus, risk assessment of NTM in the water supply system is urgent for appropriate interventions. However, the comprehensive risk assessment for NTM in water supply systems and water use is scarce.

6.2. Quantitative microbial risk assessment (QMRA)

QMRA is used to determine the potential risk of pathogens. QMRA consists of four steps, which are (1) problem formulation including hazards and hazardous event identification and exposure pathways, (2) exposure assessment, (3) health effect assessment using the dose–response model, and (4) risk characterization (Haas *et al.* 2014; WHO 2016). Nowadays, QMRA is increasingly applied to evaluate the risk of waterborne pathogens in drinking water and to estimate the required treatment efficiency. Generally, the most popular reference pathogens used for risk assessment are *Cryptosporidium*, *Giardia*, and *E. coli* (Owens *et al.* 2020). Some studies have also been devoted to revealing health risks of *Legionella* in water supply systems, which can be closely related to NTM risk (Hamilton & Haas 2016; Hamilton *et al.* 2019).

A few studies have investigated QMRA for NTM in water use (Table 4). QMRA for *M. avium* in tap water in eastern China was evaluated using the exponential model (Huang *et al.* 2021). The daily dose of *M. avium* was computed based on the product of daily oral ingestion volume (7–71 mL/person) of raw tap water, cell number determined by qPCR (gene copies), ratio of viable cells (3.92×10^{-4}), and human infectivity (0.1%). According to the calculation, the risk of *M. avium* in tap water was below the EPA risk benchmark (annual infection 10^{-4}) (Huang *et al.* 2021).

The risk of NTM in recreational water was also evaluated for Beijing Olympic Forest Park in China (Cui *et al.* 2017). The concentration of *M. avium* (10^{2-3} gene copies/100 mL) was determined by qPCR, and 95th percentile gene copies were used for QMRA. The oral ingestion was also assumed as exposure route, and the exponential dose–response model was used. The model was based on the oral route infection of *M. avium* subsp. *paratuberculosis* to red deer (O'Brien *et al.* 2006). Finally, the annual infection risks of *M. avium* (10^{-2}–10^{-0}) were greater than the EPA benchmark (10^{-4}) (Cui *et al.* 2017).

QMRA of MAC was applied for different uses of roof-harvested rainwater in Australia (Hamilton *et al.* 2017a). Various infection scenarios including both ingestion route (undisinfected drinking water with and without filtration, showering, garden hose use, car washing, toilet flushing, and so on) and inhalation route (showering, garden hose use, car washing, and toilet flushing), and different types of persons including children with different ages and immuno-compromised population were considered for MAC dose calculation in this study. In addition, disparate health endpoints, such as pulmonary in general population (inhalation route), cervical lymphadenitis in children (ingestion route), and disseminated infection in immuno-deficient individuals (ingestion route), were also considered for the choice of exponential and Beta-Poisson dose–response models for MAC (Hamilton *et al.* 2017a). For oral ingestion exposure route including undisinfected drinking water with and without filtration, the total annual risks of all the scenarios were above 10^{-4} benchmark. On the other hand, the total annual MAC risks for inhalation exposure were below 10^{-4} benchmark (Hamilton *et al.* 2017a). It turned out that drinking water (ingestion route) exhibited the most significant MAC risks for susceptible populations, including children and the immuno-compromised (Hamilton *et al.* 2017a).

The health risks of MAC in aerosolized water from toilet flushing and showering using rainwater without prior treatments were assessed (Kusumawardhana *et al.* 2021). The dose via inhalation was estimated by considering the concentration of MAC in water, partitioning coefficient (the ratio of water to aerosol), inhalation rate, exposure duration, fraction of respirable aerosol, the retention rate of aerosol, and the number of events per day (Kusumawardhana *et al.* 2021). The health endpoint for inhalation of NTM aerosols is pulmonary infection, which was referred to Hamilton's study (Hamilton *et al.* 2017b). Annual infection risks of MAC from toilet flushing and showering were estimated to be lower than 10^{-7} (Kusumawardhana *et al.* 2021).

Table 4 | Dose–response model and parameters for evaluating NTM risk in water use

NTM	Exposure route	Endpoint	Dose–response model[a,b,c]	Dose–response parameters	Exposure assessment parameters	Reference
MAC	Inhalation (showering)	Sub-clinical/moderate pulmonary infection	Modified exponential model	r follows lognormal distribution ($\mu = -13.742$, $\sigma = 0.208$) $C=500$	Breathing rate, shower duration, aerosol concentration, aerosol volume, deposition efficiency, recovery efficiency	Hamilton et al. (2017a)
MAC	Inhalation (showering/toilet flushing)	Clinical severity pulmonary infection	Exponential model	$r = 3.12 \times 10^{-9}$	Inhalation rate, exposure duration, partitioning coefficient, fraction of respirable aerosol, retention rate, number of events per day	Kusumawardhana et al. (2021)
MAC	Oral ingestion (drinking water)	Disseminated infection (immuno-compromised patients)	Beta-Poisson model	$\alpha = 0.201$, $\beta = 1.15 \times 10^6$	Intake volume, recovery efficiency	Hamilton et al. (2017a)
MAC	Oral ingestion (drinking water)	Cervical lymphadenitis in children	Exponential model	r follows lognormal distribution ($\mu = -19.006$, $\sigma = 1.008$)	Intake volume, recovery efficiency	Hamilton et al. (2017a)
M. avium	Oral ingestion (drinking water)	Pulmonary infection	Exponential model	$r = 6.93 \times 10^{-4}$	Ingestion volume, viable fraction, human infectivity	Huang et al. (2021)
M. avium	Oral ingestion (visiting fountain)	Pulmonary infection	Exponential model	$r = 6.93 \times 10^{-4}$	Exposure duration, ingestion frequency, droplet volume	Cui et al. (2017)
M. avium	Oral ingestion (boating)	Pulmonary infection	Exponential model	$r = 6.93 \times 10^{-4}$	Exposure duration, ingestion rate	Cui et al. (2017)
M. avium	Oral ingestion (playing with water)	Pulmonary infection	Exponential model	$r = 6.93 \times 10^{-4}$	Exposure duration, contact frequency, film thickness of water on hands, surface area of mouth-contacted hand	Cui et al. (2017)
M. avium	Oral ingestion (walking)	Pulmonary infection	Exponential model	$r = 6.93 \times 10^{-4}$	Exposure duration, ingestion rate	Cui et al. (2017)
M. avium	Oral ingestion (feeding fishes)	Pulmonary infection	Exponential model	$r = 6.93 \times 10^{-4}$	Exposure duration, ingestion rate	Cui et al. (2017)

[a]Modified exponential model: $P_d = 1 - e^{-rd/C}$, where P_d is the daily infection probability, d is the daily dose, r is the parameter, and C is the conversion factor.
[b]Exponential model: $P_d = 1 - e^{-rd}$, where P_d is the daily infection probability, d is the daily dose, and r is the parameter.
[c]Beta-Poisson model:, where P_d: Daily infection probability, d: daily dose, a and b: parameter.
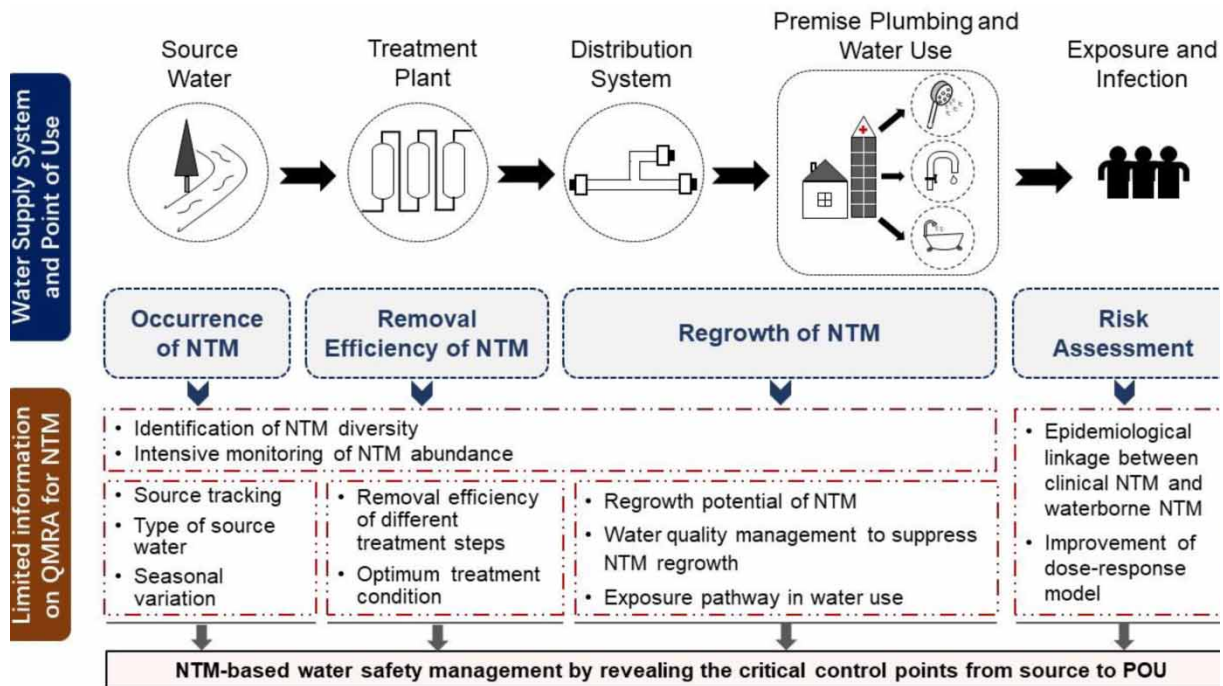
Figure 2 | Application of comprehensive QMRA to NTM in water supply and water use.

6.3. The limitations in QMRA for NTM

There are several limitations in application of QMRA to NTM in water supply systems and water use (Figure 2). For accurate estimation, abundances of NTM in water should be correctly quantified. The culture-based method can quantify viable bacteria, while it takes a longer time to enumerate slow-growing NTM and misses viable but nonculturable (VBNC) NTM (Hamilton *et al.* 2017a). While quantitative PCR is rapid, the conversion of gene copies to viable cells is sometimes overestimated (Kusumawardhana *et al.* 2021).

Inhalation or respiration of aerosol is a significant exposure pathway for NTM in water use (Goslee & Wolinsky 1976). However, accurate exposure estimation is complicated, which involves many parameters that have uncertainties (Table 4). Multiple assumptions might increase overall uncertainties. Besides, epidemiological evidence to connect human clinical NTM and waterborne NTM should be intensively confirmed.

Dose–response models for MAC were required with high priority to control opportunistic pathogens in premise plumbing (AWWA 1999). Seven new exponential dose–response models for human-relevant MAC have been proposed based on animal tests for different endpoints of NTM diseases (Hamilton *et al.* 2017b). Both the exponential model and the Beta-Poisson model were used for the oral ingestion dosage estimation of MAC or *M. avium*, while the exponential model was applied to inhalation of MAC (Table 4). However, differences in human susceptibilities to subspecies of MAC or other pathogenic NTM should be revealed for accurate evaluation (Casanova & Abel 2002).

In water engineering, the removal efficiency of NTM by water treatment has not been systematically studied. NTM are resistant to ozonation and disinfection, which does not allow us to simply apply an *E. coli* model to NTM. Moreover, the regrowth of NTM in water distribution and premise plumbing is highly dependent on environmental conditions, which increases uncertainties in risk assessment at the POU. The variation of NTM removal efficiency by each unit process and environmental conditions, which promote or mitigate their regrowth, should be incorporated to comprehensive QMRA to explore the critical control points in the water supply system.

7. OUTLOOKS

NTM are ubiquitous in water-related spots, including source waters, water treatment plants, distribution systems, and water use equipment. The frequent occurrence of pathogenic NTM, such as *M. avium*, *M. intracellulare*, *M. gordonae*, *M. lentiflavum*, and *M. fortuitum* in water supply systems, indicates that water could be a potential exposure route for NTM infection. NTM can survive the water treatment processes and rather proliferate in the

distribution system and premise plumbing. However, the fate of NTM in water supply systems from source to the POU is still not clear due to the lack of monitoring data.

The health burden of waterborne NTM is increasing in many countries. Although the microbial risk of gastro-intestinal diseases in water supply systems has been studied based on traditional model bacteria, such as *E. coli*, it is time to revisit the operation and management of water supply systems based on NTM. A comprehensive QMRA is necessary to clarify the current risk of NTM in water use and required interventions. Furthermore, cost-effectiveness analysis and/or cost–benefit analysis would be crucial to implement the identified interventions for monitoring/controlling NTM.

AUTHOR CONTRIBUTION

Y.G. and I.K. conceptualized the study, searched the literature, and wrote the original draft preparation. I.R., F.K., H.F., D.S., H.F., and Y.H. involved in writing the review and editing the manuscript. I.K. performed funding acquisition. I.K., F.K., and H.F. supervised the work.

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The authors state that ethical approval is not required because this is the review paper.

COMPETING INTERESTS

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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