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Survey of *Mycobacterium* in Peoria, Illinois Anthropogenic Freshwater Sources

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ABSTRACT

Mycobacteria are ubiquitous and commonly found in water and soil. Non-tuberculosis *Mycobacteria* (NTM) can cause lung disease, specifically in immunocompromised individuals. Recent studies have shown evidence of person-to-person transmission of NTM lung disease. Surface waterways, such as rivers and creeks, are commonly used for recreational activities and drinking water. Identifying the presence of *Mycobacterium* in water systems furthers our understanding of environmental exposure to NTM. Water samples were collected from the Illinois River, Dry Run Creek, and tap water from various Peoria buildings. Samples were centrifuged, plated, and acid-fast stained. The presence of *Mycobacterium* was found in the Illinois River and Dry Run Creek. None were detected from the tap water. This would support that Peoria, IL water treatment plants are removing *Mycobacterium* from the Illinois River, therefore decreasing the risk of NTM disease for immunocompromised people. The potential presence of combined sewage outfalls and clusters of NTM outbreaks is discussed in urban areas near major rivers.

Keywords: Mycobacteria, freshwater, Non-tuberculosis *Mycobacteria*, NTM-LD, combined sewage outfalls

INTRODUCTION

Non-tuberculosis *Mycobacteria* (NTM), or environmental *Mycobacteria*, are present in water, soil, and food. While most non-tuberculosis mycobacteria (NTM) are not pathogenic, some can be pathogenic. It is estimated that 25 of 150 species in the Mycobacterial genus can elicit human disease (Saxena, Spaink, and Forn-Cuní 2021). Immunosuppressed patients are at a higher risk for NTM disease, pulmonary and extrapulmonary disease, otherwise known as NTM lung disease (NTM-LD). Roughly 180,000 people in the United States have NTM-LD (Covert et al. 1999). HIV patients infected with NTM have a 10% mortality rate (Delghandi et al. 2020). Some of the NTM species that can lead to this disease are *M. abscessus*, *M. chelonae*, *M. fortuitum*, and *M. haemophilum* (Honda et al. 2018). The most commonly isolated group of *Mycobacteria* is the *Mycobacterium avium complex* (MAC). Roughly 80% of NTM-LD in the United States is caused by MAC. MAC consists of slow-growing species such as *M. avium*, *M. intracellulare*, *M. chimaera*, *M. colombiense*, *M. marseillense*, *M. arosiense*, *M. timonense*, *M. bouchedurhonense*, *M. kansasii*, and *M. ituriense*. Subspecies of *M. avium* include *silvaticum*, *hominissuis*, and *paratuberculosis*. The *M. avium* complex is non-spore-forming, gram-positive, takes 10-20 days to grow, and grows best at 28-38°C. Some species within MAC have been hypothesized to cause Crohn's disease, type 1 diabetes mellitus, and multiple sclerosis. Aerosolized particles and ingestion of the bacteria are thought to be the mechanism of infection; however, the ecological niche has not been identified (Akram and Attia 2024). Infection by NTM is most commonly associated with host susceptibility (pre-existing lung pathology) and constant, prolonged exposure to the bacterium (Saxena, Spaink, and Forn-Cuní 2021).

A substantial increase in reports of NTM-LD has occurred in recent years, especially due to strains of *M. avium* complex, *M. kansasii*, and *M. abscessus*. This is especially alarming due to the strains' propensity for antibiotic resistance by both intrinsic (i.e. thick cell walls and biofilms) and acquired resistance (Saxena, Spaink, and Forn-Cuní 2021). Furthermore, nearly all NTM-LD infections are treated with a relatively narrow range of macrolide-based antibiotics, emphasizing the need for more treatment modalities (Saxena, Spaink, and Forn-Cuní 2021). NTM infections were initially thought to be restricted to environmental exposure, however, more recent literature has described person-to-person transmission of pathogenic NTM strains (Ratnatunga et al. 2020). Climate change also has a link with the NTM problem. NTM infections are associated with tropical environments; 40% of the world population lives in the tropics, and climate change is known to affect the expansion of the tropics (Ratnatunga et al. 2020). More treatment modalities, more effective measures to control environmental exposure, and a better representation of the highest-risk groups are needed to control NTM infection rates.

Mycobacteria are resistant to pH changes, disinfectants, high temperatures, and low oxygen and are difficult to keep out of the water supply (Honda et al. 2017). Non-pathogenic and potentially pathogenic NTM have been found in household plumbing, especially shower heads and faucets, as well as major institutions, such as hospital systems (Honda et al. 2017). Most NTMs are susceptible to UV light. Though some of the pathogenic NTMs are resistant to UV, UV is a possible means to keep these microbes out of drinking water (Covert et al., 1999). As NTMs are common in surface water such as lakes and rivers, they can be aerosolized, especially in humid environments, and then inhaled, causing disease (Norton et al. 2020). A study

in Paris found that 72% of water samples from distribution centers in Paris were positive for *Mycobacteria* (Gebert et al. 2018). Many of the species isolated were consistent with the ones that may cause NTM disease. Shower heads are also a means of transmission. Since NTMs are chlorine-resistant, accumulation in the shower heads can occur, and *Mycobacteria* can become aerosolized and inhaled, which can result in disease. NTM disease is challenging to treat, and treatment includes a combination of antibiotics (Delghandi et al., 2020).

UV radiation is a novel way of treating drinking water sources that is currently being implemented in water treatment centers such as Peoria, Il. UV-C treatment induces damage to DNA and RNA that significantly slows or stops replication in bacterial and non-bacterial organisms (Maslowska et al. 2019). Recent experimental evidence highlighting the effectiveness of UV-C water treatment suggests as high as a 57% reduction in river water bacterial load and a 50% reduction in well water bacterial load. Additionally, *E. coli*, an indicator of fecal contamination, was reduced by 83.3% in well water after UV-C treatment (Adeniyi & Jimoh, 2024).

The Illinois American Water Company, located in Peoria, Illinois, services Peoria's drinking water supply. This company uses a three-step UV system along with other basic sedimentation removal. UV-C treatment was found to be effective in eliminating *M. abscessus*, *M. avium*, and *M. chimaera* (Covert et al. 1999). Determining whether *Mycobacteria* are present in Peoria waterways may promote awareness of NTM disease and the safety of public water. It can also show if water treatment is effective at removing these bacteria. This paper looks at the presence of *Mycobacteria* in surface water and treated water. We predict that *Mycobacterium* will be present in the surface water.

METHODS

Water samples were collected from the Illinois River (Figure 1), Dry Run Creek (Figure 1), and the Bradley University campus buildings in September 2022. Samples were collected on days with similar weather conditions. Samples were not collected on days when it was raining. Water collected from the Bradley University campus was at a similar temperature as that from the Illinois River and Dry Run Creek (~21°C). Faucets were run at Bradley University campus buildings for 30 seconds before collection, preventing bacterial contamination from previous usage of the faucets. While collecting water from Illinois River and Dry Run Creek, water was collected from approximately 0.5 m away from the water's edge, which is where people would most likely come into contact with the water. The water samples were collected using 15 ml sterile centrifuge tubes.

The water samples were centrifuged for 5 min at 5000 x g, to concentrate the infranatant, decanted, and then vortexed to be re-suspended in approximately 100 ul of the remaining supernatant. Each sample suspension was plated onto five different plates with Lowenstein-Jensen media (MacFarlane and Samaranayake 1989). The Lowenstein-Jensen media plates were then incubated at 30°C. After seven days of incubation, samples were Ziehl-Neelsen stained. The Ziehl-Neelson stained plates were examined under a light microscope using 1000x. Ziehl-Neelson staining is specific to *Mycobacterium* due to the unique mycolic acids found in *Mycobacterium* cell walls (Vilchèze and Kremer 2017). The microscopic view of the stained slides, as seen in Figures 2 and 3, shows the *Mycobacterium* cell walls stained red. The plates that had growth were then further subcultured onto the new Lowenstein Jensen media. Subculturing preserves the *Mycobacterium* cell lines and prevents other bacteria from colonizing the media (Metcalfe et al. 2017). The sub-cultured samples were incubated at 40°C with 5% CO_2 for seven days. The 5% CO_2 has been shown to result in earlier and increased growth of *Mycobacterium* (Realini et al. 1998). After seven days, the sub-cultured samples were Ziehl-Neelson stained and examined for *Mycobacterium* growth.

To see if our results correlate with other papers, we referred to other studies that were conducted looking at rates of infection of *Mycobacteria*, particularly in the neighboring state of Missouri. We then cross-referenced these survey results with data from the EPA to see where CSOs are prevalent in major waterways. Similar correlation comparisons have been done with *E. coli* and different types of water sources compared against infection rates (Coleman et al. 2013). Infection rates can be classified into different levels of cluster rates ranging from 1 (highest rate of infection) to 5 (lowest rate of infection) (Mejia-Chew et. al. 2023).

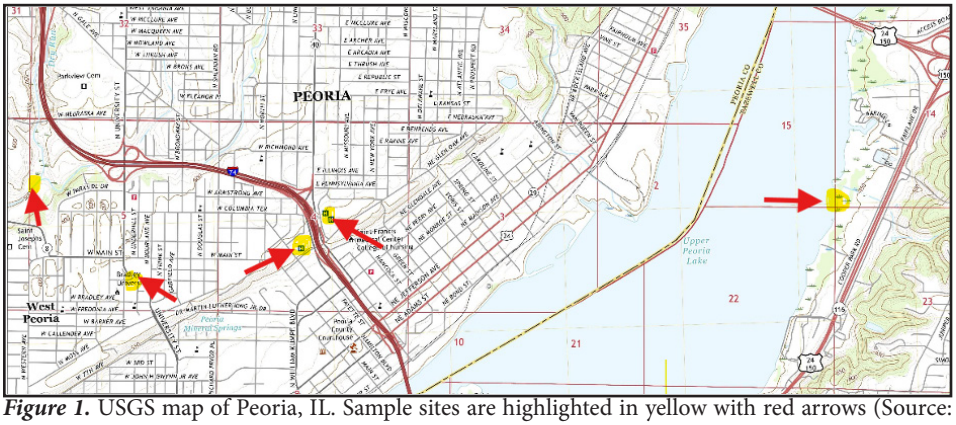

Figure 1. USGS map of Peoria, IL. Sample sites are highlighted in yellow with red arrows (Source: United States Geological Survey 2024 with additions of arrows and highlights from author).

RESULTS

After initial incubation at 30°C, *Mycobacterium* was present in the water samples collected from the Illinois River and Dry Run Creek (Figure 2 and Figure 3). After staining, *Mycobacteria*

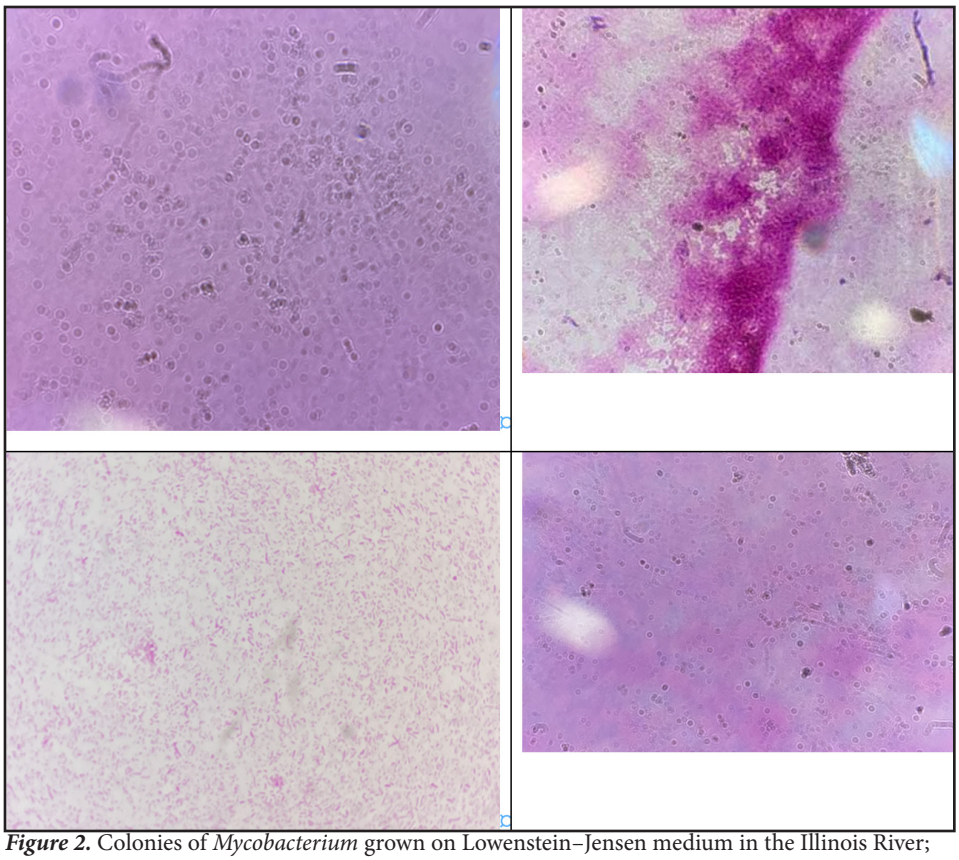

Figure 2. Colonies of *Mycobacterium* grown on Lowenstein–Jensen medium in the Illinois River; Ziehl–Neelsen positive staining of a suspected colony. Magnification 1000x (Source: Author-generated).

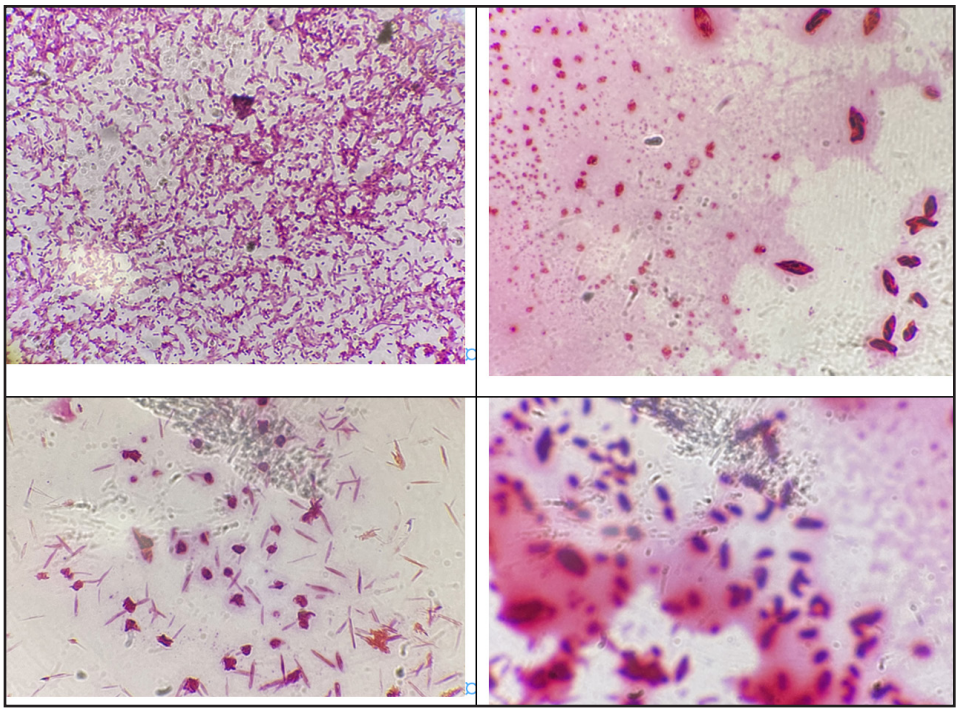

Figure 3. Colonies of *Mycobacterium* grown on Lowenstein–Jensen medium in the Bradley Creek; Ziehl–Neelsen positive staining of a suspected colony. Magnification 1000x (Source: Author-generated).

were observed, however, the species were unable to be determined. There was an increase in the growth of these bacteria when the samples were placed at 40°C in 5% CO_2.

Samples taken from the Peoria buildings did not show any bacterial growth.

When comparing presence-absence of CSOs and NTM infection rates, results from Mejia-Chew et. al, 2023 found 14,203 mycobacterial cultures reported to MDHSS over 11 years resulting in a median rate of NTM infection as 68.04 (IQR 59.65–81.12)/100,000 persons for the study period, and compared with the 2008 baseline, yearly rate of infections had increased 5.7% by 2010 and 12.2% by 2019 (Table 1). When this is compared to the current listing of the EPA map of CSOs in Missouri, it is a positive relationship between areas of infection and the presence of CSOs (Table 2). Of the identified counties with infections, McDonald County was the only one without CSOs but also had the lowest infection rate of the counties, supporting the trend (Table 1 & 2). Cluster ratings were also not available for McDonald and Sullivan counties (Table 1).

DISCUSSION

Our study offers insight to the current diIn this study, we found the presence of *Mycobacterium* in the two Peoria surface waterways, but we failed to find any evidence of their presence in the water samples collected from Peoria city buildings. This indicated that the water treatment plants are effectively removing *Mycobacteria* from the water supplied to the city. Since Peoria's water treatment plants are utilizing a 3-stage UV system, it could be the reason why no *Mycobacterium* grew. Surface water has been shown to have an increased presence of *Mycobacterium* when compared to treated water. Water samples collected from water treatment plants around the United States were 36% positive for *Mycobacterium* (Le Dantec et al. 2002).

One consideration for the increased prevalence of *Mycobacterium* in surface

Table 1. Results from Mejia-Chew et al. 2023 of infection rates of different counties in Missouri per 100,000 persons and classification of cluster based on severity from 2008 to 2019 (Source: Mejia-Chew et al. 2023)

County	Buchanan	Cape Girardeau	St. Louis	Jackson	Sullivan	McDonald
Incidence per 100,000 persons	171.46	134.81	111.1	95.91	121.1	11.8
Cluster Rank	3	2	3	4	N/A	N/A

Table 2. Present-Absent data from the EPA on counties in Missouri that contain combined sewer overflow water systems.

County	Buchanan	Cape Girardeau	St. Louis	Jackson	Sullivan	McDonald
Presence of CSO	P	P	P	P	P	A

waters is contaminated wastewater. *Mycobacterium* Tuberculosis Complex (MTBC) has been isolated from wastewater (Mtetwa et al. 2022). Interestingly, a CDC epidemiological spatial analysis of Missouri indicated increased NTM infection rates in locations that experienced flooding (Mejia-Chew et al. 2023). Combined sewer overflows (CSO), such as the one found in Peoria, IL, are sewer systems that combine wastewater and rainwater and discharge it into rivers, lakes, and other surface waters. With our finding of the presence of *Mycobacterium* in Peoria's surface water, it could be that CSOs contributed to this presence. Missouri, as well as many states throughout the Midwest, have CSOs similar to Peoria, IL.

Looking at other locations, Missouri surveys have indicated a correlation between clustered cases of NTM and Missouri cities having CSOs (Mejia-Chew et al. 2023 and US EPA 2023). The cases in Missouri that showed high clusters of NTM infections are in St. Louis, Kansas City, Cape Girardeau, and St. Joseph (Mejia-Chew et al. 2023), which all are highly urbanized areas and located on rivers that receive CSO discharges. More evidence that links wastewater to these infection clusters is the lack of infection clusters in other cities such as Columbia, Jefferson City, and Springfield. Although the cities have significant human populations, are highly urbanized, and have much human activity, they lack river flow and, therefore, potential water contamination from CSOs. Our results showed NTM growth in Peoria's surface waters, including the Illinois River. Potentially linking these sources of contamination to infection rates is important for human health and should be further investigated. Flooding and human activity near sites having CSOs may increase the cases of NTM-LD from untreated water.

NTM-LD increased from 3.13 to 4.73 per 100,000 people between 2008 and 2015 in the United States (Le Dantec et al. 2002). CSOs may not only increase the cases of NTM-LD but also contribute to antibiotic-resistant bacteria (Turns 2024). Antibiotics can enter surface waters through fecal content from CSO discharge (Turns 2024). These surface waters can then be sites of environmental exposure to humans. Many species of bacteria, including Mycobacterium, can gain antibiotic resistance from these antibiotic-containing waterways. With NTM-LD requiring a narrow range of antibiotics, it could become increasingly difficult to treat (Saxena, Spaink, and Forn-Cuní 2021). The Illinois American Water Company provides treated water to Peoria as well as 700 communities and 14 states (Illinois American Water Company 2022). In 2019, UV water treatment upgrades were started in Illinois (Winthrop et al. 2020). The lack of *Mycobacterium* presence in the Bradley University treated water could be due to the implementation of the UV water upgrades. We found *Mycobacteria* in Peoria surface waters but did not link that with NTM-LD incidence or prevalence in humans. Future studies could investigate that, as well as how NTM exposure is most affected.

CONCLUSION

While we acknowledge that this study did not seek to identify particular Mycobacterium species or pathogenic Mycobacterium, our study is relevant as it highlights that the surface water in Peoria contains *Mycobacteria* and could expose the public to pathogenic *Mycobacterium* species. Future studies could attempt to identify particular *Mycobacterium* species.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization, EA and TF.; Methodology, EA., and TF.; Validation, EA., and TF.; Investigation, EA., TF., JN., and TM.; resources, TF., and AD.; data curation, EA., and TF.; writing—original draft preparation, EA., and TF.; writing—review and editing, EA., TF., AD., JN., and TM.; supervision, EA, TF, and AD; project administration, EA, TF, and AD. All authors have read and agreed to the published version of the manuscript.

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LITERATURE CITED

Adeniyi, A. O. and M. O. Jimoh. (2024) Decontaimiation potential of Ultraviolet Type C Radiation in Water Treatment Systems: Targeting Microbial Inactivation. Water. 16:19, 2725. doi: https://doi.org/10.3390/w16192725

Akram, S. M., and Attia, F. N. (2024). "*Mycobacterium avium* Complex," in StatPearls, (Treasure Island (FL): StatPearls Publishing). Available at: http://www.ncbi.nlm.

nih.gov/books/NBK431110/ (Accessed April 7, 2024).

Coleman B. L., M. Louie, M. I. Salvadori, S. A. McEwen, N. Neumann K. Sibley, et al. (2013). Contamination of Canadian private drinking water sources with antimicrobial resistant *Escherichia coli*. Water Research. 47:9 3026-3036

Covert, T. C., Rodgers, M. R., Reyes, A. L., and Stelma, G. N. (1999). Occurrence of Nontuberculous *Mycobacteria* in Environmental Samples. Appl Environ Microbiol 65, 2492–2496.

Delghandi, M. R., Waldner, K., El-Matbouli, M., and Menanteau-Ledouble, S. (2020). Identification *Mycobacterium* spp. in the Natural Water of Two Austrian Rivers. Microorganisms 8, 1305. doi: 10.3390/microorganisms8091305

Gebert, M. J., Delgado-Baquerizo, M., Oliverio, A. M., Webster, T. M., Nichols, L. M., Honda, J. R., et al. (2018). Ecological Analyses of *Mycobacteria* in Showerhead Biofilms and Their Relevance to Human Health. mBio 9, e01614-18. doi: 10.1128/mBio.01614-18

Honda, J. R., Hasan, N. A., Davidson, R. M., Williams, M. D., Epperson, L. E., Reynolds, P. R., et al. (2016). Environmental Nontuberculous *Mycobacteria* in the Hawaiian Islands. PLoS Negl Trop Dis 10, e0005068. doi: 10.1371/journal.pntd.0005068

Honda, J. R., R. Virdi, and E. D. Chan. 2018. Global Environmental Nontuberculous *Mycobacteria* and Their Contemporaneous Man-Made and Natural Niches. Frontiers in Microbiology 9.

Illinois American Water Company Press ReleaseDetails.2022.https://www.amwater.com/press-room/press-releases/illinois/illinois-american-water-to-make-treatment-change-as-part-of-ultraviolet-disinfection-installation-at-water-treatment-plant.

Le Dantec, C., Duguet, J.-P., Montiel, A., Dumoutier, N., Dubrou, S., and Vincent, V. (2002). Occurrence of *Mycobacteria* in Water Treatment Lines and in Water Distribution Systems. Appl Environ Microbiol 68, 5318–5325. doi: 10.1128/AEM.68.11.5318-5325.2002

MacFarlane, T. W., and L. P. Samaranayake. 1989. Chapter 8 - Bacterial infections. Pages 103–121 in T. W. MacFarlane and L. P. Samaranayake, editors. Clinical Oral Microbiology. Butterworth-Heinemann

Maslowska, K. H., K. Makiela-Dzbensha, and I. J. Fijalkowska. (2018) The SOS system: A complex and tightly regulated response to DNA damage. Environmental and Molecular Mutagenesis. 60:4, 368-384. doi: https://doi.org/10.1002/em.22267

Mejia-Chew, C., Chavez, M. A., Lian, M., McKee, A., Garrett, L., Bailey, T. C., et al. (n.d.). Spatial Epidemiologic Analysis and Risk Factors for Nontuberculous *Mycobacteria* Infections, Missouri, USA, 2008–2019 - Volume 29, Number 8—August 2023 - Emerging Infectious Diseases journal - CDC. doi: 10.3201/eid2908.230378

Metcalfe, J. Z., E. Streicher, G. Theron, R. E. Colman, R. Penaloza, C. Allender, D. Lemmer, R. M. Warren, and D. M. Engelthaler. 2017. *Mycobacterium tuberculosis* Subculture Results in Loss of Potentially Clinically Relevant Heteroresistance. Antimicrobial Agents and Chemotherapy 61:e00888-17.

Mtetwa, H. N., Amoah, I. D., Kumari, S., Bux, F., and Reddy, P. 2022. The source and fate of *Mycobacterium tuberculosis* complex in wastewater and possible routes of transmission. BMC Public Health 22, 145. doi: 10.1186/s12889-022-12527-z

Nontuberculous *Mycobacteria* Infections in Immunosuppressed Hosts - PMC (n.d.). Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4710582/ (Accessed March 8, 2024).

Norton, G. J., M. Williams, J. O. Falkinham, and J. R. Honda. 2020. Physical Measures to Reduce Exposure to Tap Water–Associated Nontuberculous *Mycobacteria*. Frontiers in Public Health 8.

Ratnatunga, C. N., Lutzky, V. P., Kupz, A., Doolan, D. L., Reid, D. W., Field, M., et al. (2020). The Rise of Non-Tuberculosis Mycobacterial Lung Disease. Front. Immunol. 11. doi: 10.3389/fimmu.2020.00303

Realini, L., K. De Ridder, J.-C. Palomino, B. Hirschel, and F. Portaels. 1998. Microaerophilic Conditions Promote Growth of *Mycobacterium genavense*. Journal of Clinical Microbiology 36:2565–2570.

Saxena, S., Spaink, H. P., and Forn-Cuní, G. (2021). Drug Resistance in Nontuberculous *Mycobacteria*: Mechanisms and Models. Biology (Basel) 10, 96. doi: 10.3390/biology10020096

Turns, Anna. 2024. The superbugs lurking in seas and rivers. (n.d.). . https://www.bbc.com/future/article/20240709-why-cleaning-sewage-out-of-uk-waterways-could-stop-amr-superbugs.

US EPA, O. 2023. Where Combined Sewer Overflow Outfalls Are Located. Available at: https://www.epa.gov/npdes/where-combined-sewer-overflow-outfalls-are-located (Accessed April 7, 2024)

Vilchèze, C., and L. Kremer. 2017. Acid-Fast Positive and Acid-Fast Negative *Mycobacterium tuberculosis*: The Koch Paradox. Microbiology Spectrum 5:10.1128/microbiolspec.tbtb2-0003–2015.

Winthrop, K. L., Marras, T. K., Adjemian, J., Zhang, H., Wang, P., and Zhang, Q. 2020. Incidence and Prevalence of Nontuberculous Mycobacterial Lung Disease in a Large U.S. Managed Care Health Plan, 2008–2015. Annals ATS 17, 178–185. doi: 10.1513/AnnalsATS.201804-236OC