Detection and characterization of nontuberculous mycobacteria from selected water sources in the Central Region of Ghana

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Abstract

Background: Nontuberculous mycobacteria (NTMs) are common saprophytes found in environmental niches. These mycobacteria species are not classified as members of the *Mycobacterium tuberculosis* complex (MTBC). There are very few data on the isolation and identification of NTM from water sources in Ghana. The main objective of this study was to isolate and classify NTM infections occurring in various sources of drinking water that are frequently used by inhabitants of the Central Region of Ghana. **Methods:** In total, 45 water samples were collected from river bodies, Ghana Water Company Limited treatment plants, community taps, wells and boreholes. Conventional acid-fast staining, culture on Lowestein-Jensen (L-J) slants, the Capilia® TB-Neo assay, PCR and PCR-Restriction Fragment Length Polymorphism (PCR-RFLP) analysis were also performed. The 360 bp fragment of the *rpo*B gene was amplified for isolates characterised as NTM, and the products were individually subjected to digestion with the restriction endonucleases *Msp*I and *Hae*III. **Results:** Thirty-one (31; 68.9%) of the isolates obtained from the samples were confirmed by the Capilia TB-Neo assay to be NTM. The *rpo*B gene was detected in seventeen (17; 54.8%) of the samples. PCR-restriction fragment length polymorphism analysis revealed *Mycobacterium*

celatum, *Mycobacterium gordonae* type II, *Mycobacterium simae*, *Mycobacterium genavense*, *Mycobacterium kansasii type* V, *Mycobacterium kansasii type* I, and *Mycobacterium phlei* in the water samples. **Conclusion:** Hopefully, the findings from this study will add to the baseline information on the occurrence and characterisation of NTMs in various water sources (in the Central Region of Ghana).

Key words: NTM; Public Health; Drinking Water; Mycobacteria, PCR-RFLP.

INTRODUCTION

The genus *Mycobacterium* consists of several species that can be divided into three broad groups: the *Mycobacterium tuberculosis* complex (MTBC), *Mycobacterium leprae* and atypical or nontuberculous mycobacteria (NTMs) [1]. Nontuberculous mycobacteria (NTM) are mostly found in water bodies, soil, animals, birds and humans [2, 3, 4]. They can survive across a varied range of temperatures and pH values, making different water sources, such as treated water, ground water, surface water (particularly from riverine sources), waste water and recreational water (swimming pools and water bodies adapted for sports), suitable environments for survival for long periods of time [5, 6]. The most frequently found or isolated species are *M. avium, M. chelonae, M. fortuitum, M. gordonae, M. kansasii, M. abscessus, M. marinum* and *M. xenopi* [7, 8]. Nontuberculous mycobacteria (NTM) have also been isolated from meat, unpasteurised milk, and harvested fruits and vegetables [9]. These organisms were formerly not considered clinically significant until recently when they were found to be implicated in some human infections [10]. As of 2011, 172 different species of NTM had been classified, and the potential pathogenic species were *M. avium, M. intracellulare, M. kansasii, M. xenopi, and M. abscessus,* which can cause pulmonary disease [11]. Recent studies have identified more than 200 species of NTM via genomic data [12, 13, 14].

Infections caused by MTBC and NTM exhibit similar clinical and radiological features. Additionally, as with all Mycobacteria, both MTBC and NTM are acid-fast bacteria; therefore, it is not possible to differentiate them through acid-fast microscopy. The discovery of the opportunistic nature of these bacteria in human infections, particularly in persons with underlying conditions such as immune dysfunctions such as HIV, has propelled NTM as a mycobacterial threat of great importance for public health worldwide, especially because of its resistance to conventional antimycobacterial drugs such as anti-TB drugs [15]. However, developed countries have a well-documented prevalence of NTM disease [16], in contrast to less developed countries, where only a small amount of data is readily available on the occurrence of these organisms and their clinical manifestations [17]. Data and knowledge about the

distribution of NTMs in the environment, particularly in aquatic environments (water distribution systems in association with biofilm matrices and other aquatic ecosystems), are well known in developed countries where NTMs have been well established and used to design effective control measures.

In developing countries such as Ghana, there is a paucity of data on the occurrence and distribution of these organisms. The presence of NTM in human samples has been established, and NTM is gaining importance as a pathogen of public health interest [13]. The transmission of NTM from human to human is rare [18], but rather, human diseases are thought to be acquired from environmental sources such as soil, dust and water. Human infection with NTM through ingestion, inhalation and inoculation has been clearly attributed to environmental sources [19]. Therefore, isolating and identifying NTM are highly important for laying the foundation for understanding their epidemiology and for designing and implementing public health policies to help control and prevent their infection and transmission. Differentiations of strains or isolates have been performed by employing Restriction Fragment Length Polymorphism (RFLP) to characterize isolates below species or subspecies levels. The application of molecular biology techniques in bacterial typing to generate isolate specific genetic fingerprints has proven to be suitable in assessing epidemiological relatedness of organisms [20]. The main aim of this study was to recover and identify NTM in water samples obtained from the water supply chain in parts of the Central Region of Ghana using microbiological and molecular methods.

MATERIALS AND METHODS

Study Area

The main sources of potable drinking water in Ghana are designed, supervised and operated by the Ghana Water Company Limited (GWCL). The majority of the human population in the Central Region of Ghana accesses drinking water from GWCL supply points for their daily essential needs and activities. A total of eight (8) treatment plants (headworks) in the region namely, Sekyere Hemang, Brimsu Old, Brimsu New, Baifikrom, Essakyir, Winneba, Kwanyako Old and Kwanyako New were sampled as well as the feeder rivers to these plants. These plants use surface water (i.e., from rivers) and then apply conventional treatment methods (alum flocculation, sedimentation, rapid sand filtration and chlorination) for water

purification [21]. Treated water from the water distribution chain serves approximately 80% of the inhabitants of the region [22].

Fig 1Insert adopted central region map

Sample Collection

This was a cross-sectional study conducted within a five-week period (May to June 2018) from the feeder sources to the treatment plants, treated water at the GWCL treatment plants, reservoirs, taps in the community (i.e., point-of-use), community boreholes and open wells, which are being used by inhabitants of these towns. A total of 45 samples were obtained from thirty-nine (39) sources: twelve (12) from rivers, sixteen (16) from GWCL treatment plants, four (4) from reservoirs, five (5) from boreholes and two (2) from wells. Approximately 0.5 L of water was collected into labelled sterile plastic bottles. This sample was subsequently transported to the Cellular and Clinical Research Centre (CCRC) within the Radiological and Medical Sciences Research Institute at the Ghana Atomic Energy Commission (GAEC) on ice for analysis within 48 hours of collection. The parameters of the water samples, such as odour, appearance, colour, temperature, pH and residual chlorine content, were measured according to the procedures employed by GWCL in routine water quality assessments. A Multi-Parameter PCSTestr 35 (Oakton Instruments, Vernon Hills, IL 60061-USA) was used to measure the temperature and pH of all the samples. The residual chlorine concentrations were measured with a Hach portable digital colorimeter (DR/820 Hach Company, Loveland, Colorado, U.S.A.) following the manufacturer's instructions.

Identification of strains using phenotypic and genotypic tests

The isolation of strains included macroscopic and microscopic morphological characteristics. Approximately 200 ml of each water sample was decontaminated using 4% sodium hydroxide (NaOH) solution and centrifuged (IEC Refrigerated Centrifuge, IEC Centra GP8R, IEC International Equipment Company, USA) at 3000×g for 20 minutes at room temperature (25-27°C). The pellets were re-dissolved in sterile double-distilled water. Approximately 100 µL of the suspension was inoculated on slants of Lowestein-Jensen **(**LJ) media in replicates. These replicate slants were divided into two groups, incubated at 30°C and 37°C due to suitable environmental conditions, and observed routinely for eight (8) weeks. The observed slants with growths (visible colonies) were subsequently harvested and subjected to Ziehl–Neelsen (ZN) staining microscopy to confirm their acid-fastness. Colonies that were identified as AFBs were selected for categorisation as MTBC or NTM by employing the rapid diagnostic Capilia® TB-Neo assay (TAUNS Laboratories, Inc., Japan) following the manufacturer's instructions. All identified NTM isolates were selected for DNA analysis.

DNA extraction, PCR (rpoB gene amplification) and RFLP

For the extraction of DNA, the simple boiling method was applied and described by [23]. In brief, a loopful (1 µm) of each colony on the LJ medium was suspended in one ml of sterile distilled water. This suspension was heated in a heating block at 100°C for 20 minutes to lyse the mycobacteria to obtain DNA, which was subsequently centrifuged at 14000×g for 10 minutes. The supernatant containing DNA was transferred into a fresh sterile 1.5 ml capped tubes and stored at -20°C until further analysis.

For the identification of NTM, the *rpoB* gene-based PCR was performed using the primers RPO5' (5'-TCAAGGAGAAGCGCTACGA-3') and RPO3' (5'-GGATGTTGATCAGGG TCTGC-3') to amplify a 360 bp fragment (171 bp of variable region and 189 bp of conserved region) in the mycobacterial genome. Amplification was performed in a 25 µL reaction volume with each reaction mixture containing 15.1 µL sterilised distilled water, 2.5 µL of 10x reaction buffer, 0.4 µL of $MgCl_2$, 0.8 µL of dNTPs, 0.2 µL of 1 unit Hot Start *Taq* polymerase, 0.5 µL each of forward and reverse primers and 5 µL of DNA template. The PCR amplification cycle was performed under the following conditions: 95°C for 5 minutes; 35 cycles of 94°C for 1 minute, 58°C for 1 minute, and 72°C for 1 minute; and a final extension at 72°C for 7 minutes on a PCR machine (BIO RAD PTC0220, Bio-Rad Laboratories, Inc., USA). The amplified PCR products were

resolved by electrophoresis for 1 hour on a 1.5% SeaKem LE agarose gel (Lonza, Rockland, ME, USA) stained with ethidium bromide in a 1× TBE buffer (Tris base, boric acid, EDTA: pH 8.3; Sigma–Aldrich, Inc., USA) at 80 mV and 70 mA using a horizontal gel electrophoresis apparatus (Minicell Primo EC135). The DNA fragments were visualized with an Alpha DigiDoc Ease FC software system coupled to an Olympus digital camera (Model AT126 D) from Alpha Innotech Corp., USA. Band sizes were determined by mapping with positive controls and a standard 100-base pair DNA ladder (Metabion GmbH, Germany).

The amplified PCR products (360 bp products) were selected and digested with the restriction endonucleases *Msp*I and *Hae*III (New England Biolabs, Inc., USA) as described previously (21) following the manufacturer's instructions. The digestion was carried out for 2 hours at 37°C. *Msp*I digestion was stopped by the addition of 5 µL of (6X) loading buffer, while *Hae*III digestion was stopped by inactivation at 80°C for 20 minutes. The digested products were resolved on a 4% SeaKem LE Agarose gel (Lonza, Rockland, ME, USA) for analysis. Resolved fragments were analysed by the inclusion of positive and negative controls and molecular size markers (50bp and 100bp ladders). Species were identified upon comparison with an algorithm constructed by [24] for NTM species identification.

RESULTS AND DISCUSSION

Table 1: Chemical / Physical and Molecular parameters

	Source of Samples	Physical parameters			Culture on LJ media (n=45) Visible Colonies		Molecular Analysis (n=45)	
		Appearance	Odour	Colour	*rpo*B gene			
					30°C	37°C	30°C	37°C
Sample Source Categories	GWCL Treatment Plants	*Clear*	*Odourless*	*Colourless*	12.7	12.7	9.1	10
	GWCL Resevoirs	*Clear*	*Odourless*	*Colourless*	5.4	3.6	18.2	20
	River Sources	*Turbid*	*Earthy and fishy*	*Pale yellow/brown*	14.5	10.9	18.2	20
	Community Taps	*Clear*	*Odourless*	*Colourless*	18.1	16.4	36.3	40
	Boreholes/Wells	*Clear*	*Odourless*	*Colourless*	7.3	5.4	18.2	10
	General Characteristics							
	pH range	Residual Chlorine (mg/L)		Temp. (°C)				
	4.9 - 8.2	0.1 - 2.2		24.8 - 35.0				

Molecular analysis results

PCR-RFLP is one of the PCR-based techniques used for strain typing that focuses on studying the polymorphisms in one or a few genes for identification and differentiation. A gene is amplified, followed by restriction enzyme treatment of the PCR products and size separation of the resulting fragments. A total of 11 and 10 isolates realised after culture at 30°C and 37°C, respectively, were categorised as NTM by the Capilia® Neo-TB test for the two replicates. For the group of isolates obtained after culture, the *rpoB* gene was successfully amplified. The genetic variations (insertions, deletions, recombination, etc.) within the gene or locus in the restriction enzyme recognition sequence sites, are easily detected by agarose gel electrophoresis [25, 26, 27]. Figure 2 shows the resulting bands after PCR was run for eight samples.

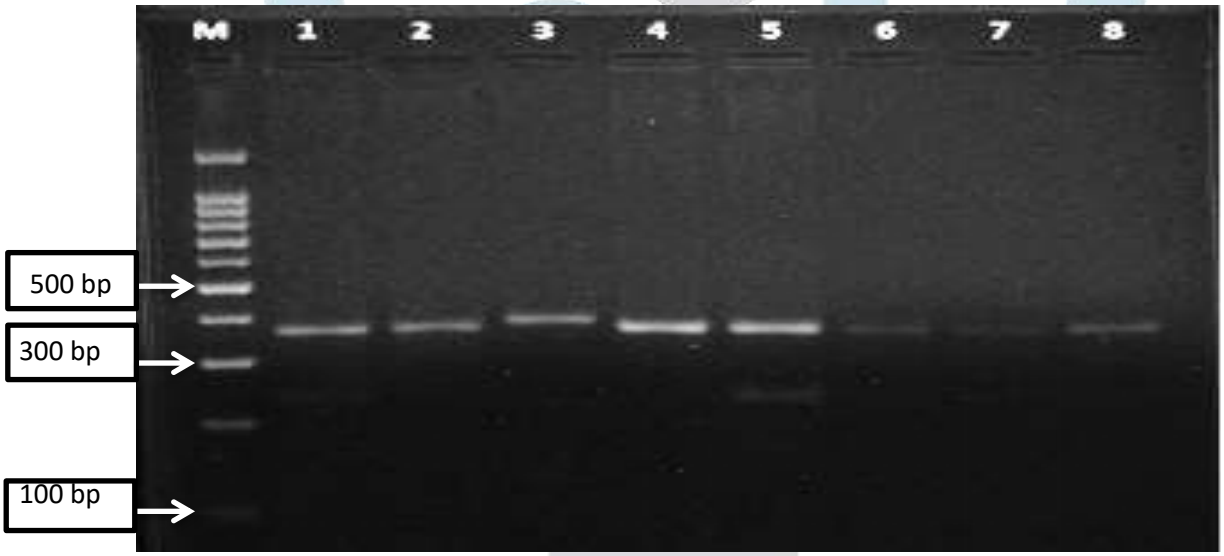

Figure 2: A representative electropherogram of amplicons obtained from samples after PCR of the rpoB gene.

Amplified products of the *rpo*B gene were separately subjected to digestion with the restriction endonucleases *Hae*III and *Msp*I.

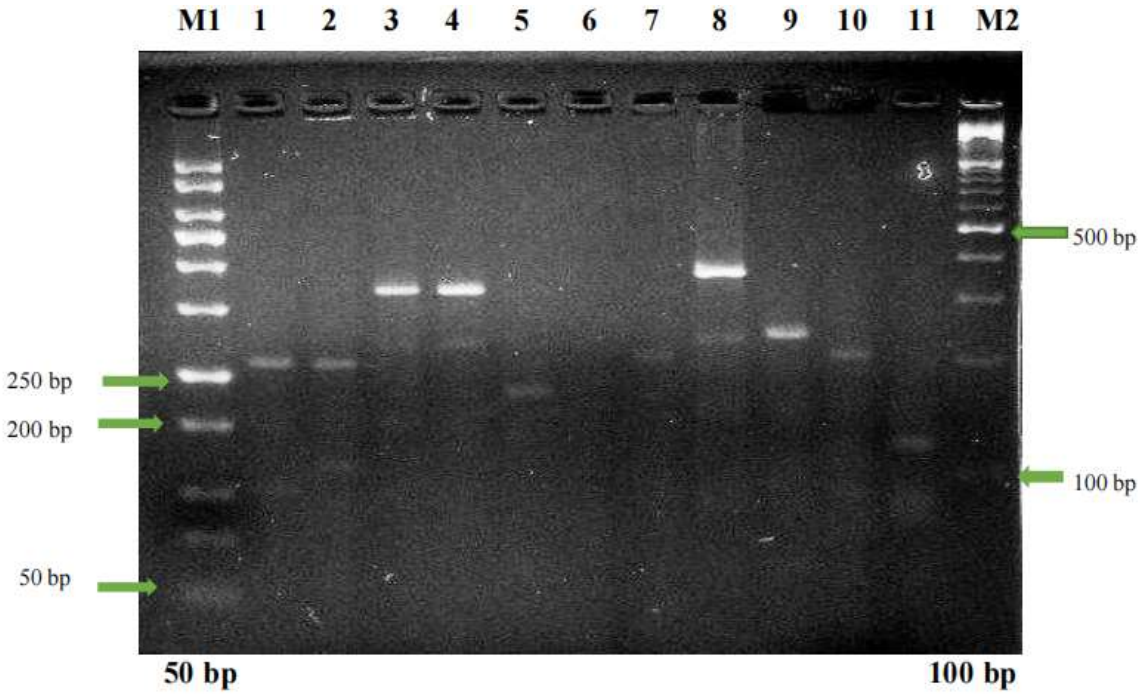

Figure 3: RFLP of PCR products with HaeIII restriction enzyme run on 4% agarose gel (AGE).

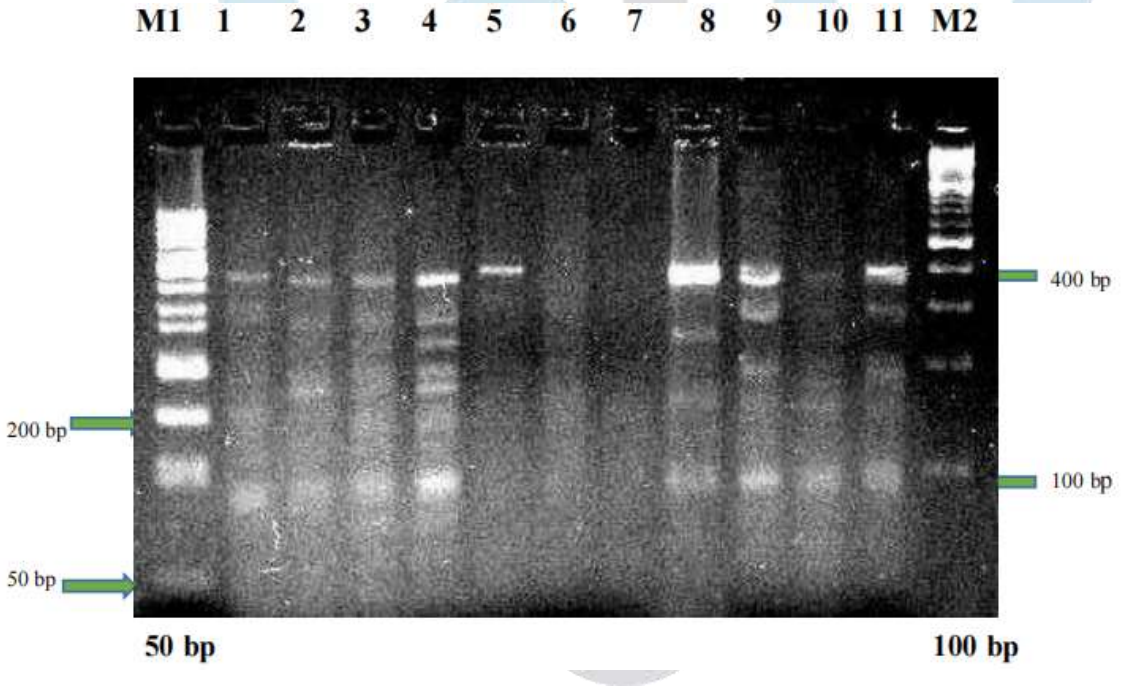

Figure 4: RFLP of PCR products with MspI restriction enzyme run on 4% agarose gel (AGE).

Undoubtedly, the clinical importance of NTM in public health has grown considerably in the last few decades [28, 29]. Water, in general, has been documented as one of the major sources of NTM infection, as well as birds, animals and soil [2, 3], but it is not the only source [30]**.** Nontuberculous Mycobacteria have also been isolated from meat, fish, dairy products (especially unpasteurised milk), fruits and vegetables [9]. They are normally found in natural environments.

Many pathogenic and potentially pathogenic NTMs are found in water sources, especially drinking water. Its isolation from portable water dates back to the early 1900s with the identification of several species, such as *M. kansasii, M. lentiflavum, M. avium, and M. intracellulare,* which are associated with human disease [31, 32, 33]. Further studies have expanded the list to include the *Mycobacterium avium complex, Mycobacterium gordonae, Mycobacterium kansasii, Mycobacterium fortuitum, Mycobacterium simiae, Mycobacterium senegalense, Mycobacterium chelonae and Mycobacterium xenopii* [30, 34, 35]. Nontuberculous mycobacteria (NTMs), which contaminate water distribution systems, are generally known to cause nosocomial infections in hospitals [36]. *Mycobacterium gordonae, M. chelonae and M. simiae* have been identified in dental cooling and spray syringe handpiece water samples [37].

Currently, very little data exist on the NTM species present, especially in water distribution systems, in Ghana. A study by [38] conducted in Ghana indicated that the prevalence and epidemiology of NTMs are largely unknown, while [39] focused on the isolation of NTM from smear–positive pulmonary TB cases in selected health facilities. No reported data on NTM isolated from direct water drinking sources have been reported. Most research on NTM has focused on its presence in human samples and water in the hospital environment. Some studies have failed to isolate and identify NTM from drinking water due to the isolation methods applied [40].

Suthienkul et al. analysed V. parahaemolyticus and the TDH and TRH genes of 137 V. parahaemolyticus using PCR-RFLP which indicated that TDH can divide into 5 types while TRH 4 types after digestion with restriction enzyme Hind III [41]. Klich et al. differentiated two related species A. flavus and A. oryzae using PCR-RFLP and stated their phylogenetic relationships [42]. The main techniques employed in this study were LJ culture (figure 1), the Capilia TB-Neo assay, the PCR restriction fragment length polymorphism (PCR-RFLP) with the *rpo*B gene (figure 2) and the restriction enzymes *Msp*I (figure 4) and *Hae*III (figure 3) for identification. The results obtained indicated that 38/45 (84.4%) of the samples had grown on LJ media. Of the isolates harvested from LJ slants, 31/38 (81.6%) were acid-fast, and all were confirmed to be NTM by the Capilia® TB-Neo assay. Of the 45 samples examined, 31/45 (68.9%) contained acid-fast bacilli according to ZN staining. This study revealed a high isolation rate of NTM from borehole water sources, similar to the findings of previous studies [43] in Zambia. This can be attributed to the high levels of organic matter in the boreholes, which encourages an increase in mycobacterial flora [30, 36]. The percentage of NTM isolated from community tap water after treatment was the lowest in their study but the

second highest in our study. This could be ascribed to old rusty pipes that have accumulated biofilms in the transmission line, as inferred or stated by [44] that, in Germany, 90% of biofilm samples from pipes of various water distribution systems contained mycobacteria, signifying the presence of mycobacterial biofilms in piping systems. The low mycobacterial isolation rate from water samples obtained from treated plants could be due to the lethal effect of chlorine during the treatment process.

Our study revealed the presence of *M. scrofulaceum, M. gordonae, M. genavense, M. celatum, M. kansasii* and *M. simiae,* which have been described as causing pulmonary or disseminated disease in immunocompromised patients in some studies [45, 46, 47]. Other studies isolated *M. kansasii, M. simiae* and *M. gordonae,* which are implicated in human disease and are capable of causing clinically significant diseases in both immune-competent and immune-compromised individuals [48, 49]. However, *M. gordonae* isolated from such sources has a limited need for nutrients and high tolerance for chlorine and is 330 times more resistant to chlorine than is *Escherichia coli* [5]. The prevalence of NTM isolated from drinking water sources in Australia, France, Germany and South America ranged from 57% - 72%, respectively [30, 44, 50], but our study recorded 82%. A previous study [51] indicated that 98% of mycobacteria can be cultured once from faucets in the U.S., while this study recorded an 84% growth rate.

These studies have demonstrated that drinking water and its supply networks are common habitats for NTM, as stated by [52, 53]. The presence of these mycobacteria is a public health concern. Large numbers of NTM in the environment can cause serious outbreaks of pulmonary disease and disseminated disease with localised lesions, particularly in immunocompromised individuals, such as those with HIV/AIDS and persons receiving chemotherapy for neoplastic disease or steroid treatment [29, 54]. Individuals at high risk of NTM infection are those with a previous or present history of TB treatment, pre-existing pulmonary disease, chronic obstructive pulmonary disease, diabetes, or malignancy [28, 55].

CONCLUSION

This study highlights the emergence of NTM as a major public health threat in the public health sector in parts of the Central Region of Ghana through the isolation of NTM from different drinking water sources within the Cape Coast Municipality. Knowledge and management of NTM infection are major challenges because of a lack of data and information. The preliminary findings of this study will help construct data and information for further inquiry into the nature of NTM in the environment, particularly aquatic ecosystems. It is recommended that a wider study be conducted to increase the identification of many

additional NTM species and determine their distribution within aquatic ecosystems. Additional molecular studies are required to provide clearer genetic insight into NTM species isolated from water sources in Ghana.

Declarations

Data Availability

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

Conflicts of Interest

The authors declare that there is no conflicting interest regarding the publication of this paper.

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Authors' Contributions and Materials

OKG, KDB and YEQ conceived and designed the study. KDB and YEQ performed the experiments. KDB and OKG analysed the data. KDB and YEQ wrote the first draft of the manuscript. KDB, OKG, and FK critically read the manuscript. All the authors read and approved the manuscript.

Ethical Approval and Consent to participate

Permission was sort from Ghana Water Company Limited, Central Region, Ghana to sample water from their Water Treatment Plants (WTPs) as part of this study (Permission reference number: WO:224/11/SF.1/22)

References

[1] Park SC, Kang MJ, Han CH, et al., 'Prevalence, incidence, and mortality of nontuberculous mycobacterial infection in Korea: a nationwide population-based study,' BMC Pulmonary Medicine 2019; 19:140.

[2] Muwonge A, Kankya C, Johansen TB, et al., 'Non-tuberculous mycobacteria isolated from slaughter pigs in Mubende district, Uganda,' BMC Veterinary Research 2012; 8: Article ID 52(2012).

[3] Ricketts MW, O'Shaughnessy TC, van Ingen J. 'Human-to-human transmission of Mycobacterium kansasii or victims of a shared source,' European Respiratory Journal 2014; 2014: 1085–1087.

[4] Velayati AA, Farnia P, Mozafari M, et al., Molecular epidemiology of nontuberculous mycobacteria isolates from clinical and environmental sources of a metropolitan city,' PLoS ONE 2014; 9: no. 12.

[5] Le Dantec C, Duguet JP, Montiel A, Dumoutier N, Dubrou S, Vincent V. 'Chlorine disinfection of atypical mycobacteria isolated from a water distribution system,' Applied Environmental Microbiology 2002; 68(3): 1025–1032.

[6] Lee BY, Clemens DL, Horwitz MA. 'The metabolic activity of *Mycobacterium tuberculosis*, assessed by use of a novel inducible GFP expression system, correlates with its capacity to inhibit phagosomal maturation and acidification in human macrophages,' Molecular Microbiology 2008; 68(4):1047-1060.

[7] Beran V, Matlova L, Dvorska L, Svastova P, Pavlik I. 'Distribution of mycobacteria in clinically healthy ornamental fish and their aquarium environment,' Journal of Fish Diseases 2006; 29(7): 383-393.

[8] Gauthier DT, Rhodes MW. 'Mycobacteriosis in fishes: A review,' Veterinary Journal 2009; 180(1): 33-47.

[9] Konuk M, Korcan E, Dulgerbaki S, Altindis M. 'Isolation and identification of Mycobacteria from raw milk samples in Afyonkarahisar District of Turkey'. International Journal of Food Microbiology 2007; 115(3): 343–347.

[10] van Ingen J. 'Diagnosis of nontuberculous mycobacterial infections,' Seminars in Respiratory Critical Care Medicine 2013; 34(1):103-109.

[11] Kankya C, Muwonge A, Djønne B, et al., 'Isolation of non-tuberculous mycobacteria from pastoral ecosystems of Uganda: Public Health significance,' BMC Public Health 2011; 11(320).

[12] Matsumoto Y, Kinjo T, Motooka D, et al., 'Comprehensive subspecies identification of 175 non-tuberculous mycobacteria species based on 7547 genomic profiles,' Emerging Microbes and Infections 2019; 8(1): 1043-1053.

[13] Hamed KA, Tillotson G. 'A narrative review of nontuberculous mycobacterial pulmonary disease: microbiology, epidemiology, diagnosis, and management challenges,' Expert Review of Respiratory Medicine 2023; 12(24): 7751.

[14] Pascapurnama DN, Zavitri NG, Koesoemadinata RC, Cahyadi AI, Chaidir L.'Identification of Significant Pathogenic Nontuberculous Mycobacteria Species from Presumptive TB Patients Using Partial hsp65 Gene Sequencing,' Infection and Drug Resistance 2023; 2023(16): 6923-6930.

[15] van Ingen J, Boeree MJ, van Soolingen D, Iseman MD, Heifets LB, Daley CL. 'Are phylogenetic position, virulence, drug susceptibility and in vivo response to treatment in mycobacteria interrelated?' Infection Genetics and Evolution 2012; 12(4): 832-837.

[16] McCarthy KD, Cain KP, Winthrop KL, et al., 'Nontuberculous mycobacterial disease in patients with HIV in Southeast Asia,' American Journal of Respiratory and Critical Care Medicine 2012; 185(9): 981-988.

[17] Chanda-kapata P, Kapata N, Klinkenberg E, et al., 'Non-tuberculous mycobacteria (NTM) in Zambia : prevalence, clinical, radiological and microbiological characteristics,' BMC Infectious Diseases 2015; 15(500):1-7.

[18] Sousa S, Bandeira M, Carvalho PA, Duarte A, Jordao L. 'Nontuberculous mycobacteria pathogenesis and biofilm assembly' International Journal of Mycobacteriology 2015; 4(1): 36–43.

[19] Griffith DE, Aksamit T, B. Brown-Elliott A, et al., 'An official ATS/IDSA statement: Diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases' American Journal of Respiratory and Critical Care Medicine 2007; 175(4): 367-416.

[20] Foxman B, Riley L. Molecular epidemiology: focus on infection. American Journal of Epidemiology 2001; 153(12): 1135–1141.

[21] Addico GND, Hardege JD, Kohoutek J, deGraft-Johnson KAA, Babica P. 'Cyanobacteria and microcystin contamination in untreated and treated drinking water in Ghana,' Advances in Oceanography and Limnology 2017; 8(1): 92-106.

[22] Ghana Water Company Limited (GWCL), 'History of water supply in Ghana, Retrieved from http://www.gwcl.com.gh/pgs/history.php, 2016.

[23] Blackwood KS, He C, Gunton J, Turenne CY, Wolfe J, Kabani AM. Evaluation of recA sequences for identification of Mycobacterium species. Journal of Clinical Microbiology 2000; 38: 2846–2852.

[24] Lee H, Park HJ, Cho SN, Bai GH, Kim SJ. 'Species identification of mycobacteria by PCR-restriction fragment length polymorphism of the *rpoB* gene,' Journal of Clinical Microbiology 2000; 38(8): 296-297.

[25] Cloeckaert A, Verger J-M, Grayon M, Grépinet O. Restriction site polymorphism of the genes encoding the major 25 kDa and 36 kDa outermembrane proteins of Brucella. Microbiology 1995; 141(9): 2111-2121.

[26] Pourzand C, Cerutti P. Genotypic mutation analysis by RFLP/PCR. Mutation Research 1993; 288(1): 113-121.

[27] Wichelhaus TaMD, Hunfeld KPMD, Böddinghaus BMD, Kraiczy PP, Schäfer VMD, et al. Rapid molecular typing of methicillin resistant Staphylococcus aureus by PCR-RFLP. Infection Control and Hospital Epidemiology 2001; 22(5): 294-298.

[28] Suryawati B, Sari Y, Saptawati L. 'The Increase of *nontuberculous mycobacteria* Infection: Emphasis on Epidemiology, Risk Factors and Diagnostic Tools,' 1st International Conference on Health, Technology and Life Sciences, KnowledgeE Life Sciences 2019; 4(12): 139-146.

[29] Zulu M, Monde N, Nkhoma P, Malama S, Munyeme M, 'Nontuberculous Mycobacteria in Humans, Animals, and Water in Zambia: A Systematic Review,' Frontiers in Tropical Diseases 2021; 2(679501): 1-13.

[30] Thomson R, Tolson C, Carter R, Coulter C, Huygens F, Hargreaves M. 'Isolation of nontuberculous mycobacteria (NTM) from household water and shower aerosols in patients with pulmonary disease caused by NTM,' Journal of Clinical Microbiology 2013; 51(9): 3006–3011.

[31] Lewis Jr AG, Lasche EM, Armstrong AL, Dunbar FP. 'A clinical study of the chronic lung disease due to non-photochromogenic acid-fast bacilli,' Annals of Internal Medicine 1960; 53: 273–285.

[32] Eilertsen E. 'Atypical mycobacteria and reservoir in water,' Scandinavian Journal of Respiratory Disease Supplementum 1969; 69: 85–88.

[33] Collins CH, Grange JM, Yates MD. 'Mycobacteria in water,' Journal of Applied Bacteriology 1984; 57(2): 193–211.

[34] Falkinham JO III, Norton DC, LeChevallier MW. 'Factors influencing numbers of Mycobacterium avium, Mycobacterium intracellulare, and other Mycobacteria in drinking water distribution systems,' Applied and Environmental Microbiology 2001; 67(3):1225–1231.

[35] Primm TP, Lucero CA, Falkinham JO III. 'Health impacts of environmental mycobacteria.' Clinical Microbiology Reviews 2004; 17(1): 98-106.

[36] Edirisinghe EAR, Dissanayake DRA, Abayasekera CL, Arulkanthan A, 'Occurrence of nontuberculous mycobacteria in aquatic sources of Sri Lanka,' International Journal of Mycobacteriology 2014; 3(4): 242–246.

[37] Schulze-Robbecke R, Feldmann C, Fischeder R. Janning B, Exner M, Wahl G. 'Dental units: an environmental study of sources of potentially pathogenic mycobacteria,' Tubercle and Lung Disease 1995; 76(4): 318–323.

[38] Couto I, Machado D, Viveiros M, Rodrigues L, Amaral L, 'Identification of non-tuberculous mycobacteria in clinical samples using molecular methods: A 3-year study,' Clinical Microbiology and Infection 2010; 16(8): 1161-1164.

[39] Otchere ID, Asante-Poku A, Osei-Wusu S, Aboagye SY, Yeboah-Manu D, 'Isolation and characterization of non-tuberculous mycobacteria from patients with pulmonary tuberculosis in Ghana supplementary table,' International Journal of Mycobacteriology2017; 6(1): 70-75.

[40] Vaerewijck MJM, Huys G, Palomino JC, Swings J, Portaels F, 'Mycobacteria in drinking water distribution systems: ecology and significance for human health,' FEMS Microbiology Reviews 2005; 29(5): 911–934.

[41] Suthienkul O, Iida T, Park KS. Restriction Fragment Length Polymorphism of the tdh and trh Genes in Clinical Vibrio parahaemolyticus Strains. Journal of Clinical Microbiology 1996; 34(5): 1293-1295.

[42] Klich MA., Yu J, Chang PK, Mullaney EJ, Bhatnagar D, Cleveland TE. Hybridization of Genes Involved in Aflatoxin Biosynthesis to DNA of Aflatoxigenic and Non-Aflatoxigenic Aspergilli. Applied Microbiology and Biotechnology 1995; 44(3-4): 439-443.

[43] Monde N, Munyeme M, Muwonge A, Muma JB, Malama S. 'Characterization of non-tuberculous mycobacterium from humans and water in an Agro-pastoral area in Zambia, BMC Infectious Diseases 2018; 18(20).

[44] Le Dantec C, Duguet JP, Montiel A, Dumoutier N, Dubrou S, Vincent V. 'Occurrence of mycobacteria in water treatment lines and in water distribution systems,' Applied Environmental Microbiology 2002; 68(11): 5318–5325.

[45] Horsburgh Jr CR, Selik RM. "The epidemiology of disseminated nontuberculous mycobaterial infection in the acquired immunodeficiency syndrome (AIDS)," The American Review of Respiratory Disease 1989; 139(1): 4–7.

[46] Pate M, Žolnir-Dovč M, Kušar D. et al., 'The First Report of *Mycobacterium celatum* Isolation from Domestic Pig (*Sus scrofa domestica*) and Roe Deer (*Capreolus capreolus*) and an Overview of Human Infections in Slovenia,' Veterinary Medicine International 2011; 2011(43295).

[47] Faria S, Joao I, Jordao L. 'General Overview on Nontuberculous Mycobacteria, Biofilms, and Human Infection,' Journal of Pathogens 2015; 2015(809014).

[48] Wallace Jr RJ, Brown BA, Griffith DE, 'Nosocomial outbreaks/pseudo-outbreaks caused by nontuberculous mycobacteria,' Annual Review of Microbiology 1998; 52: 453–490.

[49] Mazumder A, Hicks, A, Norwood J. 'Mycobacterium gordonae pulmonary infection in an immunocompetent adult,' North American Journal of Medical Science 2010; 2(4): 205–207.

[50] Hussein Z, Landt O, Wirths B, Wellinghausen N, 'Detection of non-tuberculous mycobacteria in hospital water by culture and molecular methods,' International Journal of Medical Microbiology 2009; 299(4): 281–290.

[51] Donohue MJ, Mistry JH, Donohue JM, et al., 'Increased Frequency of Nontuberculous Mycobacteria Detection at Potable Water Taps within the United States,' Environmental Science & Technology 2015; 49(10): 6127–6133.

[52] Honda JR, Virdi R, Chan ED. 'Global Environmental Nontuberculous Mycobacteria and Their Contemporaneous Man-Made and Natural Niches,' Frontiers in Microbiology 2018; 9(2029).

[53] Loret JF, Dumoutier N. 'Non-tuberculous mycobacteria in drinking water systems: A review of prevalence data and control means,' International Journal of Hygiene and Environmental Health 2019; 222(4): 628–634.

[54] Buijtels PCAM, van der Sande MAB, de Graaff CS, et al., 'Nontuberculous Mycobacteria, Zambia,' Emerging Infectious Disease 2009; 15(2): 242–249.

[55] Saptawati L, Kusumo H, Suryawati B. 'Prevalence of Non-tuberculous Mycobacteria (NTM) in Surakarta, Indonesia: Higher Than Expected,' 1st International Conference on Health, Technology and Life Sciences, KnowledgeE Life Sciences 2019; 4(12):132-138.

List of Figures