ISSN: 0975-833X

Available online at http://www.journalcra.com

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

International Journal of Current Research
Vol. 13, Issue, 12, pp.20063-20066, December, 2021

DOI: https://doi.org/10.24941/ijcr.41711.12.2021

RESEARCH ARTICLE

OCCURRENCE, DISTRIBUTION AND BIODIVERSITY OF NONTUBERCULOUS MYCOBACTERIA IN DRINKING WATER SYSTEMS IN UTTAR PRADESH, NORTH INDIA

Anjali Yadav[1,2], Ajay Vir Singh[1], Shweta Kushwah[1], Rajbala Yadav[1], Davuluri Kushma Sai[1], Rakesh Kumar Sharma[2] and Devendra Singh Chauhan[1*]

[1]Department of Microbiology and Molecular Biology, ICMR-National JALMA Institute for Leprosy and Other Mycobacterial Diseases, Agra-282004, Uttar Pradesh, India
[2]Department of Biotechnology and Life Sciences, Manglatayan University, Aligarh, Uttar Pradesh, India

ARTICLE INFO

Article History:
Received 27th September, 2021
Received in revised form
18th October, 2021
Accepted 15th November, 2021
Published online 29th December, 2021

Keywords:

Nontuberculous mycobacteria, Drinking water, reverse osmosis water, tap water

****Corresponding author:***
Devendra Singh Chauhan

ABSTRACT

Drinking water has been identified as an important source of various infectious diseases including opportunistic infections caused by Non-Tuberculous Mycobacteria (NTM) in humans. In this study the occurrence of mycobacterium species were examined in drinking water samples (n=198) collected from reverse osmosis water and tap water systems of hospital and household settings in Agra region of Uttar Pradesh using Ziehl-Neelsen staining and culture on Lowenstein–Jensen medium method. Acid-fast isolates were identified by PCR amplification and analysis of restriction endonuclease digestion fragments of the heat-shock protein 65 (*hsp65*) gene. Of the 198 drinking water samples, 22 (11.11%) samples were found positive for the presence of mycobacterium species. The presence of mycobacterium species was found higher in tap water (17.07%) as compare to reverse osmosis water (6.89%) systems and the difference was statically significant ($\chi 2$: 5.548, *p*-value: 0.018). The result of *hsp65*PCR revealed the presence of seventeen mycobacterium species predominated by *M.chelonae*, *M.flavescenes* and *M. intracellulare* in drinking water. Present study reported the occurrence, distribution and diversity of mycobacterium species in drinking water systems in Agra region of Uttar Pradesh and highlights the need of health policies aimed at mitigating the risk of NTM exposure through drinking water in India.

Citation: Anjali Yadav, Ajay Vir Singh, Shweta Kushwah, Rajbala Yadav, Davuluri Kushma Sai, Rakesh Kumar Sharma, Devendra Singh Chauhan. *"*Occurrence, distribution and biodiversity of nontuberculous mycobacteria in drinking water systems in Uttar Pradesh, North India*", 2021. International Journal of Current Research, 13, (12), 20063-20066.*

INTRODUCTION

Mycobacterial infections continue to plague mankind, with increasing worldwide mortality and morbidity. The genus mycobacterium contains more than 190 recognized species in the genus of Mycobacterium. Non-tuberculous mycobacteria (NTM) are opportunistic human pathogens, distributed ubiquitous in the environment. In recent years, infections caused by NTMs are emerging worldwide due to a higher prevalence of immunosuppressive illness and therapy (Moore *et al.,* 2010; Varghese *et al.,* 2017; Lin *et al.,* 2018). The increasing trend in the incidence of NTM diseases has also been reported in India (Narang *et al.,* 2005; Singh *et al.,* 2007; Jani *et al.,* 2011). In general, NTM are not supposed to be transmitted by the human to human route, but are instead thought to be transmitted from environmental sources. Among the environmental sources, water has been considered as primary source of NTM infections (Donohue *et al.,* 2015; Khosravi *et al.,* 2016). In India limited information is available on the presence of NTM in environmental samples and more information is required for the batter management of NTM infections in the country (Parashar *et al.,* 2004; Narang *et al.,* 2009). Therefore, present study was aimed to investigate the presence of NTM in reverse osmosis (RO) and tap drinking water from hospital and household settings in Agra region of Uttar Pradesh.

MATERIALS AND METHODS

Laboratory work of the present study was conducted at the Department of Microbiology and Molecular Biology, ICMR-National JALMA Institute for Leprosy and other

Mycobacterial Diseases, Tajganj, Agra, UP, India. During the period of January 2019 to June 2020, a total of 198 water samples were collected from different sources of Agra region of Uttar Pradesh and processed to enumerate the occurrence of NTM species in drinking water systems in study area. Sterile flasks were used to collect the water samples; each sample was collected after 1 min of free flow. Samples were transported to the laboratory and processed for the microbiological examination using microscopy and culture method. Water sample (about 50 ml) was collected from hospital drinking / tap water sources of Agra region of Uttar Pradesh and processed for the detection and isolation of mycobacterium species as per the method of Falkinham (1996). Briefly, water samples (20 ml) were centrifuged at 8,000×g for 15 min at room temperature and the supernatant was discarded. Pellets from water samples were re-suspended separately in 20 ml of 2% NaOH and incubated at room temperature (RT) for 30 min. After incubation the suspensions were centrifuged at 8,000×g for 15 min at RT, and the supernatants were again decanted. The pellets were washed twice with 20 ml of distilled water and finally re-suspended in 2 ml of distilled water. The slides were prepared from re-suspended material for the detection of acid fast bacilli. About 200 micro liter of re-suspended material was inoculated on Lowenstein- Jensen (LJ) media for the isolation of mycobacterium species. Once the growth was observed, it was sub-cultured and processed further for the identification as acid fast bacilli using ZN staining and species identification by *hsp65*PCR-REA method as per the method of Telenti *et al.,* (1993).

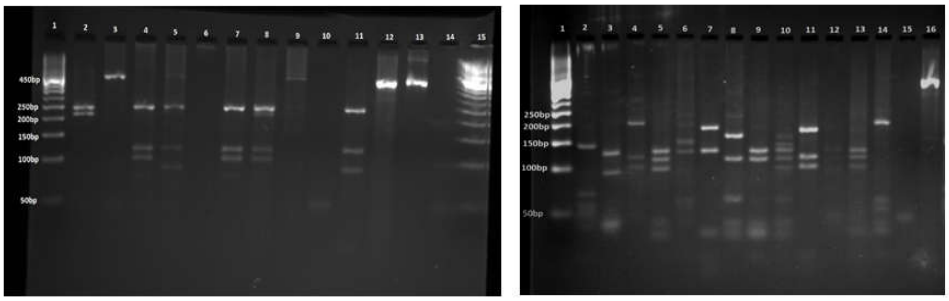
RESULTS AND DISCUSSION

In present study, a total 198 drinking water samples (Reverse osmosis water:116 & Municipal / ground water: 82) were collected from hospital and household settings of Agra region of Uttar Pradesh, North India, and screened for the presence of *Mycobacterium species* using ZN staining and culture methods. Of the 198 drinking water samples, none and 22 (11.11%) samples were found positive for the presence of acid fast bacilli and growth on LJ medium using ZN staining and culture method, respectively (Table 1). Similar to the present study, previous studies from different parts of the world have also reported the presence of mycobacteria in hospital water distribution systems and household tap water systems (Vaerewijck *et al.,* 2005; Sebakova *et al.,* 2008, Khosravi *et al.,* 2016). Covert et al., (1999) reported the presence of mycobacteria in 38% of public drinking water distribution systems in the USA. Mycobacteria were isolated from 21.3% and 72% of the samples from drinking water distribution systems in Patras (Greece) and Paris (France), respectively (Tsintzou *et al.,* 2000; Falkinham *et al.,* 2001). On the other hand, a number of authors failed to isolate NTM species from drinking water samples (Vaerewijck *et al.,* 2005), probably due to absence or temporary presence of NTMs in the piping, use of different culture medium or variation in decontamination techniques used (Parashar *et al.,* 2004). The missed detection of acid fast bacilli in water samples using ZN staining in present study can be attributed to the lower bacterial load. Since, high bacterial load (5,000-10,000 bacilli /mL) is required for detection of acid fast bacilli using ZN staining. In present study, the presence of mycobacterium species was found higher in tap water (17.07%) as compare to reverse osmosis water (6.89%) systems and the difference was statically significant ($\chi 2$: 5.548, *p*-value: 0.018). The higher presence of NTM in tap water (Municipal / ground water) may be due to increased frequency of mycobacterial colonisation and biofilm formation in pipes used for the supply of tap water, which may serve as a reservoir for these opportunistic pathogens. The reverse osmosis (RO) systems have been reported to reduce a variety of ions and metals as well as certain organic, inorganic and bacterial contaminants in drinking water. In present study, no significant difference ($\chi 2$: 0.528, *p*-value: 0.467) was found in occurrence of mycobacterium species in drinking water samples from household (11/83; 13.25%) and hospital (11/115; 9.56%) settings (Table 1). The findings of the present study highlight the relevance of hospital and household water as a source of NTM infection in Agra region of North India. In present study, species diversity of mycobacterium isolates was investigated using *hsp65*-PCR-restriction enzyme analysis (PRA) method (Fig 1). The *hsp65*-PRA method was first described by Telenti *et al.,* (1993) and has been frequently used for the species identification of mycobacteria from different sources including water (Chimara *et al.,* 2008; Tortone *et al.,* 2018). In present study, fifteen mycobacterium species (Table 1) were identified using *hsp65* PCR-REA and *M. chelonae*, *M. intracellulare*, *M. gordonae, M. flavescenes* were found as the most common species of NTM in drinking water samples of Agra region of Uttar Pradesh. Recovery of these species clearly demonstrated that these are the core of culturable microbial community living at different water distribution systems in Agra region of Uttar Pradesh, North India. The results of the present study supported the findings of previous studies reported *M. chelonae, M. intracellulare, M. gordonae* and as the most prevalent mycobacterial species in water supplies (Sebakova *et al.,* 2008, Shin *et al.,* 2008; Hussein *et al.,* 2009; Montanari *et al.,* 2009). Previous studies have reported the occurrence of other NTM species (i.e. *M. abscessus*, *M. aurum, M. brumae, M. chelonae, M. fortuitum, M. gadium, M. gastri, M. genavense, M. gilvum, M. gordonae, M. intracellulare, M. marinum, M. phlei, M. smegmatis, M. terrae, M. vaccae, M. xenopi*) in drinking water distribution systems (Vaerewijck *et al., 2005*). The results of present study do not mean the absence of other NTMs in drinking water systems in study areas. The absence of other NTMs in present study can be attributed to the geographical differences, variation in isolation methods and poor knowledge on the species distribution in place and time in the pipes of drinking water distribution systems. The occurrence of each NTM species at different points of water distribution systems remained to be investigated in future studies.

In India, the true burden of NTM diseases is largely unknown. Among the reports available, variable prevalence of NTM infections (0.5 to 8.6%) have been reported in different geographical regions of the country (Jani *et al.,* 2011). The recovery of several potential pathogenic NTM species from RO and tap drinking water of hospital and household settings in present study revealed the risk of acquiring NTM nosocomial infections and diseases through ingestion of contaminated drinking water. In case of severely ill or immunocompromised patients, other simple safety measures including sterilization of water before consumption can be suggested to reduce the risk of NTM associated infections. Present study highlight the need to enumerate the NTM species in drinking water systems and straitening of preventive measures for NTM derived opportunistic infections in India. The lack of environmental data (i.e. water quality parameters, pipe age/ condition, water age) and limited sample size are the major limitations of present study.

Table 1. Frequency of mycobacterium species recovered from household and hospital water distribution systems in Agra region of Uttar Pradesh.

Water distribution systems	Source	Number of sample	Number of positive samples on LJ culture medium	NTM species identified by *hsp*65 PCR-REA (no. of isolates)
Reverse osmosis (RO) water system	Hospital	73	5 (6.84%)	*M.fortuitum (1), M.gordonae (1), M.malmoense (1), M.nonchromogenicum (1), M. tuberculosis* complex
	Household	43	3 (6.97%)	*M.triviale, M.szulgai, M.gordonae*
	Sub-total	116	8 (6.89)	
Tap water (Municipal / ground water)	Hospital	42	6 (14.2%)	*M. chelonae* (2), *M. intracellulare* (2), *M. aurum (1)*
	Household	40	8 (20.0%)	*M.simiae* (1), *M.triviale* (1), *M.flavescenes (2), M.szuigai* (1), *M.kansasii* (1) and *M.avium* (1)
	Sub-total	82	14 (17.07%)	
Total		198	22 (11.11%)	



(A) (B)

Fig 1: Restriction band patterns of *hsp65* PCR products of NTM isolates using *BstEII (A)* and *HaeIII (B)* restriction enzymes. Lane 1: 50bp marker

CONCLUSION

Present study reported the presence of high diversity in NTM species in water distribution systems in Agra region of Uttar Pradesh, North India. The occurrence of potential pathogenic NTM species in drinking water raises the public health concern. Systematic large scale studies using advanced molecular tools with high discriminatory power and involving different geographical regions need to be carried out to determine geographical diversity of NTM distribution with confirmation of species and subspecies and delineate the role of drinking water systems in the distribution of NTMs in India.

ACKNOWLEDGMENT

Authors are thankful to Director, National JALMA Institute for Leprosy and Other Mycobacterial Diseases, Agra for extending necessary work facilities. Help extended by Ms. Damini Tiwari and Ms. Parul Maurya in the collection of water samples is duly acknowledged.

REFERENCES

Chimara, E., Ferrazoli, L., Ueky, S.Y., Martins, M.C., Durham, A.M., Arbeit, R.D., Leao, S.C. 2008. Reliable identification of mycobacterial species by PCR-restriction enzyme analysis (PRA)-hsp65 in a reference laboratory and elaboration of a sequence-based extended algorithm of PRA-hsp65 patterns. *BMC Microbiology* 8, 48.

Covert, T.C., Rodgers, M.R., Reyes, A.L. and Stelma Jr., G.N. 1999. Occurrence of nontuberculous mycobacteria in environmental samples. *Applied and Environmental Microbiology* 65, 2492–2496.

Donohue, M. J., Mistry, J. H., Donohue, J. M., O'Connell, K., King, D., Byran, J., Covert, T., Pfaller, S. 2015. Increased frequency of nontuberculous mycobacteria detection at potable water taps within the United States. *Environmental Science and Technology* 49,6127–6133.

Falkinham III, J.O. (1996). Epidemiology of infection by nontuberculous mycobacteria. *Clinical Microbiology Reviews* 9, 177–215.

Falkinham III, J.O., Norton, C.D., LeChevallier, M.W. 2001. Factors influencing numbers of *Mycobacterium avium*, *Mycobacterium intracellulare*, and other mycobacteria in drinking water distribution systems. *Applied and Environmental Microbiology* 67, 1225–1231.

Hussein Z., Landt O., Wirths B., Wellinghausen N. 2009. Detection of non-tuberculous mycobacteria in hospital water by culture and molecular methods. *International Journal of Medical Microbiology* 299, 281–290.

Jani MN, Rodrigues C, Mehta AP. 2011. The neglected and often ignored: nontuberculous Mycobacteria. *Journal of Global Infectious Diseases* 3, 94.

Khosravi, A. D., Hashemi Shahraki, A., Hashemzadeh, M., Sheini Mehrabzadeh, R., Teimoori, A. 2016. Prevalence of nontuberculous mycobacteria in hospital waters of major cities of Khuzestan Province, Iran. *Frontiers in Cellular and Infection Microbiology* 6, 42.

Lin, C., Russell, C., Soll, B., Chow, D., Bamrah, S., Brostrom, R., Kim, W., Scott, J., Bankowski, M.J. 2018. Increasing prevalence of nontuberculous mycobacteria in respiratory specimens from US-Affiliated Pacific island jurisdictions. *Emerging Infectious Diseases* 24, 485–491.

Montanari, L.B., Sartori, F.G., Cardoso, M.J., Varo, S.D., Pires, R.H., Leite, C.Q., Prince, K., Carlos, H.G.M. 2009. Microbiological contamination of a hemodialysis

center water distribution system. *The Revista do Instituto de Medicina Tropical de São Paulo* 51, 37–43.

Moore, J.E., Kruijshaar, M.E., Ormerod, L.P., Drobniewski, F., Abubakar, I. 2010. Increasing reports of non-tuberculous mycobacteria in England, Wales and Northern Ireland, 1995-2006. *BMC Public Health* 10, 612.

Narang, P., Narang, R., Mendiratta, D.K., Roy, D., Deotale, V., Yakrus, M.A., Das, R., Katoch, V.M. 2005. Isolation of *Mycobacterium avium* complex and *M. simiae* from blood of AIDS patients from Sevagram, Maharashtra. *Indian Journal of Tuberculosis* 52, 21-26.

Narang, R., Narang, P., Mendiratta, D.K. 2009. Isolation and identification of non-tuberculous mycobacteria from water and soil in central India. *Indian Journal of Medical Microbiology* 47, 247–250.

Parashar, D., Chauhan, D.S., Sharma, V.D., Chauhan, A., Chauhan, S.V., Katoch, V.M. 2004. Optimization of procedures for isolation of Mycobacteria from soil and water samples obtained in Northern India. *Applied and Environmental Microbiology* 70, 3751.

Sebakova, H., Kozisek, F., Mudra, R., Kaustova, J., Fiedorova, M., Hanslikova, D. 2008. Incidence of nontuberculous mycobacteria in four hot water systems using various types of disinfection. *Canadian Journal of Microbiology* 54, 891–898.

Shin, J.H., Lee, H.K., Cho, E.J., Yu, J.Y., Kang, Y.H. 2008. Targeting the rpoB gene using nested PCR-restriction fragment length polymorphism for identification of nontuberculous mycobacteria in hospital tap water. *Journal of Microbiology* 46, 608–614.

Singh, S., Gopinath, K., Shahdad, S., Kaur, M., Singh, B., Sharma, P. 2007. Nontuberculous mycobacterial infections in Indian AIDS patients detected by a novel set of ESAT-6 polymerase chain reaction. *Japanese Journal of Infectious Diseases* 60,14-18.

Telenti, A., Marchesi, F., Balz, M., Bally, F., Bφttger, E.C., Bodmer, T. 1993. Rapid identification of mycobacteria to the species level by polymerase chain reaction and restriction enzyme analysis. *Journal of Clinical Microbiology* 31:175-178.

Tortone, C.A., Zumárraga, M.J., Gioffr, A.K., Oriani, D.S. 2018. Utilization of molecular and conventional methods for the identification of nontuberculous mycobacteria isolated from different water sources. *International Journal of Mycobacteriology* 7:53-60.

Tsintzou, A., Vantarakis, A., Pagonopoulou, O., Athanassiadou, A., Papapetropoulou, M. (2000). Environmental mycobacteria in drinking water before and after replacement of the water distribution network. *Water, Air, & Soil Pollution* 120, 273–282.

Vaerewijck, M.J., Huys, G., Palomino, J.C., Swings, J., Portaels, F. 2005. Mycobacteria in drinking water distribution systems: ecology and significance for human health. *FEMS Microbiology Reviews* 29, 911–934.

Varghese, B., Enani, M., Shoukri, M., Al-Thawadi, S., Al-Johani S, Al-Hajoj S. 2017. Emergence of rare species of nontuberculous mycobacteria as potential pathogens in Saudi Arabian clinical setting. PLoS Neglected Tropical Diseases11, e0005288.

****