Original Research Article

16S rDNA based identification of culturable bacteria from highly polluted Varthur and Bellandur lakes of Bangalore, India

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Abstract

This study investigated the microbial population of highly polluted Varthur and Bellandur lakes, the largest freshwater lakes of Bangalore, Karnataka. A motivation for this study was the numerous reports about these polluted lakes and the occurrence of pathogenic microorganisms in drinking water and the associated diseases. Water samples were collected from both the lakes in July 2017. PCR assay of 16S rRNA genes showed that the bacterial compositions of both lakes are largely similar. The present study identified 36 bacterial strains which include highly pathogenic bacterial species like *Klebsiellapneumoniae, Escherichia coli, Staphylococcus aureus, Citrobacterfreundii, Enterobacteraerogenes,* and industrially important bacterial species such as *Pseudomonas aeruginosa, Micrococcus luteus, Bacillus amyloliquifaciens*and *Bacillus subtilis*. This is the first report of molecular study ofbacteria from these lakes. This study has clearly revealed that currently, the microbiological quality of Varthur and Bellandur lakes water makes them unfit for drinking and recreational activities due to contamination by bacteria. The water analyzed in this study has clearly shown that they are loaded with the contaminants indicator organisms which are the indication of feacal pollution. This study helps to assess its usefulness as portable/ recreational water and to recommend control measures where necessary.

Key Words: Bacterial identification, lake water, 16S rDNA sequencing, Varthur and Bellandur lakes, water pollution, pathogenic bacteria, lakeeutrophication

Abbreviations: Ribosomal DNA- rDNA, Polymerase chain reaction-PCR, Basic local alignment search tool-BLAST.

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INTRODUCTION

In India, eutrophication of fresh water resources in urban context, like lakes are currently on the rise and most lakes and fresh water sources located near urban areas are deteriorating at an alarming rate (Mahapatra *et al.*, 2010) Bellandur and Varthur lakes are the two largest lakes, of Bangalore in Karnataka that have been receiving the largest amount of wastewater. During rainy season the lake water has frothed due to the presence of toxic chemicals due to industrial pollution, dumping of toxic debris and solid wastes in the lakes, which are also lifelines of India's IT capital. During the pre-monsoon of 2015, due to high wind coupled with rainfall, massive froth and aerosol formation was noticed in the southern waste weir of Bellandur and Varthurlakes eventually overflowing onto the neighbouring road obstructing traffic. The biggest lakes in the city have turned into a sewage septic tank courtesy the apathy shown by civic authorities. That the lakes are heavily polluted is probably visible even to the naked eye (the black colour of the water) and sensible by the human nose (the stench). There have been adverse environmental and public health consequences also. Previous reports of water quality analysis (Sengupta and Dalwani, 2008) showed that both

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lakes are heavily polluted/enriched with nutrients with high organic load, increased decomposition of organic matter, depletion of oxygen levels and macrophytes cover (Karnataka State Gazetteer, 1990). Nevertheless, several studies over several years have provided detailed information on the extent and nature of the degradation of the lake ecosystems. A motivation for this study was the numerous reports about the polluted lakes in Bangalore and the occurrence of pathogenic microorganisms in drinking water and the associated diseases. Since prokaryotes are a crucial group of organisms in the biosphere, the ecosystem function studies are largely based on bacterial communities. Therefore, bacterial community structure analysis should be a part of an integrated weight of evidence approach in pollution assessment. Several studies reported the ability of molecular techniques especially 16S rRNA gene sequencing to identify of pathogens directly from environmental samples makes a rapid identification tool and also to identify even new strains. Hence the aim of this study was to determine the bacterial communities of both Varthur and Bellandur lakes using 16S ribosomal DNA sequence analysis, with a view to assessing its usefulness as portable/ recreational water and to recommend control measures where necessary.

MATERIALS AND METHODS

Collection of Water Samples: The water samples were collected during July 2017 from different sites of both the lakes in white plastic containers, which were previously rinsed with distilled water and sterilized by autoclaving. The samples were stored in a refrigerator at 4°C till further processing.

Isolation of Bacteria: Water samples obtained from different lakesites were serially diluted ten folds and then spread plate technique was followed for isolation of waterborne bacteria in the study, spreading 0.1ml inoculums from the serial dilution tubes on the Petri dishes containing Nutrient agar medium. The pH of the isolation medium was adjusted to 7.2 before sterilization. Two different techniques viz Serial dilution plate (Clesceri *et.al*., 1998) and Spread plate (Sharp and Lyles, 1969) were used for isolation of bacteria and the plates were incubated at a temperature of 37 °C for a period of 24 hours. After 24 hrs the colonies grown on the plates were subcultured to obtain a pure culture.

Identification by sequencing of 16S rDNA: Genomic DNA from the bacteria was isolated according to the protocol of Kumar et.al.,2004. Amplification of 16S rDNA was performed on Master Cycler Gradient Thermal Cycler (G-Storm 2, UK), with universal primer set 16S (FP) (5′-AGA GTT TGA TCC TGG CTC AG- 3′) and 16S (RP) (5′-AAG GAG GTG ATC CAG CCG CA- 3′) (Stackebrandt and Goebel, 1994) in 25 μl of reaction mixture containing 1X *Taq* buffer, 100 μmol l^{-1} dNTPs mix, 3 mmol l^{-1} $MgCl_2$, 10 μg BSA, 10 pMol each primer, 0·5 U of *Taq* DNA polymerase and 50 ngof template DNA. The thermal cycling conditions consisted of an initial denaturation at 94°C for 2 min, 35 amplification cycles of 94°C for 1 min 10 s, 48°C for 30 s, 72°C for 2 min 10 s and a final polymerization step of 72°C for 6 min 10 s. The final PCR product was resolved in 2% agarose gel, excised and purified within house elution kit. The cycle sequencing reaction was performed with 20–30 ng of purified amplicon using the ABI PRISM BigDye Terminators v1·1 cycle sequencing kit according to the manufacturer's instruction (Applied Biosystems, Foster city, CA). The purified products were sequenced bidirectionally to obtain complete coverage of the gene. The sequences were edited, and compared with GenBank sequences by BLAST analysis and accession number assigned. Nucleotide sequence similarities were determined using the NCBI or EMBL databases, and sequence identity *vis-à-vis* the bacterial identity was established by closest match (Altschul et.al., 1990).The 16s rDNA sequences were submitted in Gene Bank.

RESULTS

Lake water samples were analysed between August and September 2017. A total of 82 bacterial pure colonies were obtained. Genomic DNA was isolated from the prominent 36 bacterial strains out of the 82 of the colonies analysed and 16S rDNA was amplified. A 1500bp amplicon obtained for each bacterial strain was sequenced. Details of GenBank accession numbers and the bacterial identity are as follows

MF953247, MF953251, MF953252, MF953253, MF953254, MF953255, MF953255,MF953255,MF953256, MF953257, MF953258, MF953259, MF953260, MF953278, MF953279, MF953261, MF953263, MF953264, MF953265, MF953266, MF953267, MF953268, MF953269, MF953270, MF953271, MF953272, MF953273, MF953274, MF953277, MF953275, MF953250, MF953249, MF953248, MF953276, MF953280, MF953281, MF953282 and MF953262. Out of 36 strains, 25 different species were identified and few showed identity of uncultured bacterial clones. *Aeromonasaquariorum,, Citrobacterfreundii, Citrobacterfreundii, Klebsiella pneumonia, Aeromonashydrophila, Aeromonasveronii, Citrobacterfreundii, Aeromonasjandaei, Klebsiella pneumonia, Enterobacterkobei, Aeromonascaviae, Aeromonasveronii, Enterobacter cloacae, Escherichia coli, Enterobacter cloacae, Klebsiella pneumonia, Aeromonasaquariorum, Aeromonasveronii,*

Aeromonasaquariorum, Aeromonasenteropelogenes, Bacillus licheniformis, Bacillus subtilis, Aeromonasveronii, Aeromonasveronii, Escherichia coli, Pseudomonas sp., Pseudomonas sp., Aeromonashydrophila, Bacillus amyloliquefaciens, Aeromonasenteropelogenes, Uncultured bacterium, Uncultured bacterium, Uncultured bacteriumand *Bacillus amyloliquefaciens*

DISCUSSION

Lakes have been playing an important role as water conservation structures in Karanataka since ages. These served as sources of water for people by capturing rainfall and surface runoff. These structures are a good source of water and have proved useful in dry arid regions of the state. However in the past few decades one has seen many of these structures becoming dysfunctional. The usefulness of these structures still holds good and there has been initiatives across the city for revival of such systems. Identification of physiochemical, biological parameters are very important for the revival process. The objective of this study was to identify the bacteria from highly polluted Bellandur and Varthur Lakes of Bangalore in Karanataka, India. In this study, we used 16S based molecular identification for lake water bacterial isolates. It is already reported that microorganisms often play a major role in determining the extent of the pollution (Higgins and Burns, 1975)and the usefulness of 16S rRNA gene sequencing as a tool in microbial identification was already reported by Drancoutet.al.,2000.This study demonstrated the occurrence of total coliforms, faecal coliforms, heterotrophic bacteria, and Aeromonas and Pseudomonas in water samples analysed which indicated the incidence of water contamination as some of these species are indicators of faecal contamination. The presence of pathogenic organisms that can pose severe health risks to consumers in general and immunecompromised individuals. This study also could find opportunistic pathogens that may harbour multiple drug resistance determinants pose significant health hazards to consumers, especially those whose immune systems are compromised. Few bacteria identified are bacteria (heterotrophic bacteria) are generally harmless; but some may harbour pathogenic features which may cause potential health risks to humans and animals. Thus, the concern that we report here is the high levels of heterotrophic bacteria from water sources, particularlyin Ballandurlake. Further studies should be conducted to assess about the antibiotic resistant strains and the potential risks associated with human consumption of this polluted water. It is also very important that findings revealed that highly polluted lakes are abundant in various types of bacteria among which, *E. coli* as well as *K. pneumoniae, C. freundii, Aeromonasenteropelogene, Shigellasonnei*, *Enterobacterhormaecheiand P. aeruginosa*were predominant and municipal wastes and faecal matter colud be the main source of pathogenic bacteria. Moreover, Gram-positive bacteria includes *Bacillus spp, Pseudomonas sp.* which are washed out from the soil and get their entry into the water bodies during heavy rain falls also belong to the allochthonous bacteria. The results present in table 1 revealed that the 16S identity of bacteria. The role of air in water contamination is significant in densely populated areas of cities. The developmental activities and occupancy in the area is exerting pressure on the water body. Surface water in urban water bodies almost always contains some degree of contamination. This is due to exposure to animals, humans, aquatic life, etc. In addition to this, variety of other human activities resulted in increasing the bacterial concentration of lake water. The current study showed the presence of the most abundant facultative anaerobes Enterococci and *Enterobacteriaceae in lake water samples*. The main *Enterobacteriaceae*genera obtained are *Escherichia*, *Citrobacter*, *Klebsiella*and *Shigella*. It is already reported that *Enterobacter cloacae* subsp. *cloacae* (*E. cloacae*) occurs in the intestinal tracts of humans has been mostly isolated from sewage water and soil. *Citrobacterfreundii* is a member of the family *Enterobacteriaceae* and is often the cause of significant opportunistic infections and has also been associated with neonatal meningitis and brain abscess (Prince et.al., 1997). The mortality and morbidity rate of *Citrobacter* meningitis is unacceptably high. The fatality rate associated with neonatal meningitis is 25 to 50%; moreover, serious neurological sequelae result in 75% of survivors. The other identified bacteria, *Klebsiellapneumoniae* is an uncommon cause of community-acquired pneumonia except in alcoholics. Klebsiella may mimic pulmonary reactivation tuberculosis because it presents with hemoptysis and cavitating lesions. *Klebsiellapneumoniae* is a difficult infection to treat because of the organism's thick capsule. Perhaps, *Aeromonas*is the most prominent pathogen that can infect fish. Many *Aeromonas* species are responsible for several fish diseases including *A. hydrophilla.* Pathogenic bacteria cause an enormous economic loss to fisheries. They don't only cause mortality and cytotoxicity but also create uselessness of live fish as they are responsible for spots, lesions and scale loss on the infected ones. (Saikot*et al.*, 2013).Shigellosis, caused by members of the bacterial genus *Shigella*, is a severe and occasionally life-threatening diarrheal infection. Worldwide, *Shigella* spp. are the most common cause of

acute, bloody diarrhea (dysentery) and are responsible for a significant proportion of the burden of morbidity and mortality associated with diarrheal disease. Finally, *Aeromonasenteropelogenes* is a Gram-negative, motile bacterium of the genus *Aeromonas* isolated from human stool in Varanasi, in India. Today, the genus *Aeromonas* is regarded not only as an important disease-causing pathogen of fish and other cold-blooded species but also as the etiologic agent responsible for a variety of infectious complications in both immunocompetent and immunocompromised persons. San Joaquin and Pickett, 1988 observed several complications related to Aeromonas intestinal infection. These included Gram-negative bacteremia, intussusception, internal hernia strangulation, hemolytic uremic syndrome and failure to thrive in patients with chronic diarrhea. Microbiology laboratories and research centers are encouraged to look for these organisms in clinical, food and water sources to attain a better understanding of the public health risks from these organisms in Arab countries. The organisms have been reported from diarrheal children, patients with cholera-like diarrhea, an outbreak of acute gastroenteritis and from different types of animals, foods and water source in several Arab countries in the Middle East (Ghenghesh *et.al*., 2015) and North Africa with predominance of *A. hydrophila, A. caviae and A. sobria*. Other important bacterial strains identified are members of Bacillus sp. *Bacillus licheniformis* and *Bacillus amyloliquefaciens*, organisms with great industrial potential and extensively used as genetically-manipulated microbes in industrial applications for substances such as enzymes, and antibiotics. Besides their economic importance, *B. licheniformis* is increasingly recognized as a human pathogen and causes serious infections, mainly in immunocompromised patients.

CONCLUSION

This study has clearly revealed that currently, the microbiological quality of Varthur and Bellandur lakes water makes them unfit for drinking and recreational activities due to contamination by bacteria. The water analyzed in this study has clearly shown that they are loaded with the contaminants indicator organisms which are the indication of feacal pollution and seem to have seeped into the water body via human activities and agricultural runoff. The bacteria identified in this study are known to present in all sorts of environment of human involvement, majority of them are pathogenic to human as well as animals and fishes. This is the first report of molecular identification of lake water using 16S rRNA gene sequences. This study reveals need of serious control activities around the lake to prevent microbial as well as harmful chemical contaminants which have public health implications. This study also suggests routine water quality survey and monitoring programs to estimate the pollution level, rate at which additional pollutants are getting added and the causes of pollution. It is concluded here that environmental status of these lakes with respect to microbial pollution is continuing to be deteriorated. The presence of pathogens such as *E. coli* in these lakes underpins the failure of the restoration efforts and especially the ineffectual operations of the wastewater treatment plants. It must be noted here that both lakes are important stop-over sanctuary for thousands of migratory bird species. The results of the present study also help the general public to be aware on dangers of contaminated water as well as prevention of indiscriminate dumping of domestic and industrial wastes into the lake. But until those implements, the city of lakes will soon be a history.

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