International Journal of Science and Research (IJSR)
ISSN: 2319-7064
ResearchGate Impact Factor (2018): 0.28 | SJIF (2019): 7.583

Assessment on Bacteriological Contamination of River Water in the Urban Areas of Kozhikode, Kerala

Sreedevi N. Kutty[1], Sebastian C. D.[2]

[1]Department of Zoology, N. S. S. College, Nemmara, Palakkad, Kerala 678 508, India

[2]Division of Molecular Biology, Department of Zoology, University of Calicut, Kerala 673 635, India

Abstract: ***Water contamination refers to degradation of water quality from a public health or ecological view point. The pollution of municipal water by human and animal sources is the major threat to the public health in developing countries. The present study was conducted for detection, isolation and identification of human bacterial pathogens present in the river Mampuzha, near Kaduppini Bridge, Canoly Canal and Mampuzha tributary of Kallai River, Kozhikode which are sources for household and irrigational purposes. Standard MPN was used for detection of pathogens and 16rRNA gene was sequenced for pathogen identification. MPN results showed high rate of faecal contamination in the water source and molecular identification showed the presence of numerous opportunistic pathogens.***

Keywords: Pathogens, contamination, health, water quality

1. Introduction

Water has a profound influence on human health. The quality of water does have a great influence on public health; in particular the microbiological quality of water is important in preventing ill-health. Poor microbiological quality is likely to lead to outbreaks of infectious water-borne diseases and may cause serious epidemic outbreak. Water may act positively in the control of some diseases through its hygienic use, and may act as a source or vector for others where contact with water is required for disease transmission. Opportunistic pathogens are naturally present in the environment and normally present no risk to human health. They are able to cause disease in people with impaired local or general immune defences. Knowledge of the phenotypic, genotypic and biological characteristics of a microorganism is imperative in differentiating it from its pathogenic and/or toxigenic relatives or other microorganisms that are detrimental to the health of plants, animals, humans and the environment. Preliminary analysis in microbial identification often involves one or more phenotypic methods. Identification, using molecular methods, relies on the comparison of the nucleic acid sequences or protein profiles of a microorganism with documented data on known organisms. The molecular methods are considered sensitive enough to allow detection of low concentrations of viable or non-viable microorganisms in both pure cultures and complex samples. Gene amplification and sequencing of broad-range gene targets for bacteria and fungi have emerged as important tools to diagnose infections.

Many of the major problems that humanity is facing in the twenty-first century are related to water quantity and/or water quality issues [1]. These problems are going to be more aggravated in the future by climate change, resulting in higher water temperatures, melting of glaciers, and an intensification of the water cycle with potentially more floods and droughts [2], [3]. With respect to human health, the most direct and most severe impact is the lack of improved sanitation, and related to it is the lack of safe drinking water, which currently affects more than one third of the people in the world. Additional threats include, exposure to pathogens and chemical toxicants via the food chain (e.g., the result of irrigating plants with contaminated water and of bioaccumulation of toxic chemicals by aquatic organisms, including seafood and fish) or during recreation (e.g., swimming in polluted surface water). More than one-third of Earth's accessible renewable freshwater is consumptively used for agricultural, industrial, and domestic purposes [4].

Since it is not practical or technically feasible to monitor for all pathogens in drinking water, the microbiological quality of drinking water is evaluated based on indicator microorganisms, such as total coliforms and *E. coli* [5]. The coliform bacteria occur naturally in the environment and are not generally harmful themselves. However, their presence suggests that other types of disease-causing organisms may exist in the water. The coliform bacteria have been selected as an indicator for the bacterial quality of water in many nations. The presence of these bacteria in water may signify that it is defective, or that there may be problems with the water treatment, or the water distribution system. *Escherichia coli* or *E. coli* is a type of coliform bacteria commonly found in the intestines of humans and warm-blooded animals [6]. Most strains of *E. coli* do not cause illness in healthy humans and are actually beneficial to the synthesis of vitamins. Some strains, however, cause cramps and diarrhea in humans. Health organizations across the world have selected *E. coli* as the most reliable indicator for the bacteriological quality of water. The presence of *E. coli* in water is a strong indication of recent sewage or animal waste contamination. Sewage may contain many other types of disease-causing organisms.

It was found over 78% of tested pond samples as *E. coli* contaminated. MPN of fecal coliform count was reported to

be around 28000 cfu/100 ml in Kakotibari Tea Estate and Sockieting Tea Estate of Assam [7]. They have reported over 0.7 % population affected by the diarrheal and dysentery disease. High coliform counts were the most common reason for the failure of potable water to meet acceptable standards. There reported the presence of faecal *Streptococci, Escherichia coli, Enterobacter* sp., *Aeromonas* sp. and *Vibrio* sp. in lake water of Tamil Nadu [8]. The detection of pathogenic enteric bacteria in different sources of water in this area also reveals the alarming situation for water borne diseases in this particular area. Water quality signifies that pollution of the water is increasing alarmingly and that it has created serious threat to human health and environment [2].

The historical method for performing this task is dependent on the comparison of an accurate morphologic and phenotypic description of type strains or typical strains with the accurate morphologic and phenotypic description of the isolate to be identified. The part of the DNA now most commonly used for taxonomic purposes for bacteria is the 16S rRNA gene [9]–[13].

The detection of pathogenic microbes in water typically involves three main steps: (1) recovery and concentration, (2) purification and separation, and (3) assay and characterization [14]. Bacteriological water analysis is a method of analyzing water to estimate the numbers of bacteria present and, if needed, to find out what sort of bacteria they are. It is a microbiological analytical procedure which uses samples of water and from these samples determines the concentration of bacteria. It is then possible to draw inferences about the suitability of the water for use from these concentrations [15].

The provision of safe drinking water has been one of humanity's most successful public health interventions and is a defining aspect of a developed country. Nonetheless, ignorance of the potential risks and inappropriate training of staff and managers working on drinking water systems still results in unnecessary waterborne disease outbreaks in affluent communities. Furthermore, re-introduction of once-controlled diseases, such as cholera, may rapidly spread during periods of disasters when sanitation systems are non-functional and drinking water treatment is inadequate.

Examination of water column for the presence of bacteria indicatory of the sanitary state and of pathogenic enteric bacteria may supply information on water quality in the nearest future that might be useful for an effective environment of state which is currently experiencing intense aquaculture, industrial and urbanization activities [16]. Assessment of the water and sediment quality by chemical analysis will possibly throw more light towards the microbial load in the samples and there by quarantine the site.

The present study focuses on the detection, isolation and identification of human pathogenic bacteria present in the water collected from the river Mampuzha, near Kaduppini Bridge, Canolli Canal and Mampuzha tributary of Kallai River, Kozhikode. The pollution assessment was carried out by standard MPN procedure. The pathogens isolated were identified by sequencing the 16S rRNA gene. Bacterial DNA was extracted, isolated and PCR amplification of 16S rRNA was done using suitable forward and reverse primers for accurate identification of the pathogenic strains. The water is already contaminated with plastic wastes, effluents from tiles factory, waste water from hospitals, etc. Water from this river is used for many purposes like irrigation, household needs etc.

2. Materials and Methods

2.1. The study area

Kozhikode, is situated in the northern part of Kerala at a latitude of 11°20' N and a longitude of 75° 52' E. The major rivers flowing through the Kozhikode district are Korappuzha, Chaliyar and the Kallai River and their tributaries. These three rivers join the Arabian Sea. Most of the people depend on homestead open wells for domestic purposes. There are a number of large and medium scale industries flourishing along the banks of rivers and near the coast line of Kozhikode district. These include textiles, detergent manufacturing industries, plywood industries, tiles and rayon factories. Besides these there are many hospitals, flat complexes and hotels constructed along these belts in the last three decades.

Kaduppini and Mampuzha rivers flow through the panchayats of Olavanna, Peruvayal and Perumanna of Kozhikode district. The Canoly (Conolly) Canal, running across the heart of the Kozhikode city, was constructed in 1848 by the then Collector of Malabar, Sir H.V. Conolly. It connects Korappuzha river estuary in the north and the Kallai river estuary in the south. It functions as a drain to reduce flooding in the city during the rainy season and as a navigation channel. Major contributor to pollution downstream of the Kallai River is the Canoly Canal. The accumulated solid waste and black silt mixed with sand obstruct the free flow of water. These three lotic water bodies issues such as weed infestation, discharge of untreated effluents from the city and suburban areas, dumping of hospital waste, siltation and unprotected embankment. Besides discharge of effluents from nearby workshops, hotels and garages and untreated waste from septic tanks add to the problem. The pollution of these waters has also resulted in the contamination of domestic open wells in the rural residential areas.

2.2. Sample collection

Water samples were collected from different sites of Kaduppini River (KR), Mampuzha River (MR) and Canoly Canal (CC) of Kozhikode in sterile bottles and transferred to the laboratory aseptically. The samples were inoculated to Lactose broth with Durham's tubes as a part of the standard MPN (Most Probable Number) procedure.

2.3. DNA extraction and PCR of the isolates

The purified single colonies from nutrient agar medium were selected and inoculated into Luria–Bertani (LB) broth for

DNA extraction. DNA extraction was carried out by phenol-chloroform method [17].

The genomic DNA isolated from bacterial strain was amplified for 16S rRNA genes using the universal bacterial forward primer 27Fand reverse primer 1492R [18]. After ascertaining the PCR amplification of the corresponding 16S rRNA gene fragment, the PCR product was column purified using GeneJet PCR purification kit (Fermentas Life Sciences).

2.4. Sequencing of PCR product

The purified PCR product was sequenced from both ends using forward and reverse primers by Sanger's dideoxy chain termination sequencing method [19] at Agrigenom Labs Private Ltd., Cochin with ABI 3730XL automated sequencer. The forward and reverse sequences were trimmed for the primer sequences and then assembled by using ClustalW and the consensus was taken for the analysis [20]. Residue and pairwise distances were estimated using the Clustal W tool of MEGA6 software. The final sequences were searched for its similarity using BLAST programme of NCBI.

3. Results and Discussion

Most Probable Number of bacteria (MPN) in the case of water samples tested exceeded all the detectable limits (Table 1). Total and faecal coliforms were detected from these water samples indicating that the water is highly contaminated with faecal matter.

Table 1: Indication of potability of water samples collected from various river sites, Kozhikode

Water Sample	*MPN value per 100 ml*	*Portable /Non-potable*
CC 1.1	≥ 16000	Not potable
CC 1.2	≥ 16000	Not potable
CC 2.1	≥ 16000	Not potable
CC 2.2	≥ 16000	Not potable
KR 1.1	≥ 16000	Not Potable
KR 1.2	≥ 16000	Not Potable
KR 2.1	≥ 16000	Not Potable
KR 2.2	≥ 16000	Not Potable
MR 1.1	≥16000	Not potable
MR 1.2	≥16000	Not potable
MR 2.1	≥16000	Not potable
MR 2.2	≥16000	Not potable

The coliform positive culture tubes purified by quadrant streaking and single colonies obtained were selected for PCR amplification of 16S rRNA gene fragment for accurate identification. In the present study, only 12 purified colonies were subjected to PCR amplification studies, and the results are presented in Table 2.

All the isolates identified belong to those strains which cause nosocomial infections. The genus *Proteus* ranks third as the cause of these infections, particularly in hospital-acquired cases. As they are most resistant prompt diagnosis and early treatment is required to save the patient. *Proteus mirabilis* causes 90% of *Proteus* infections and can be considered a community-acquired infection. Patients with recurrent infections, those with structural abnormalities of the urinary tract, those who have had urethral instrumentation, and those whose infections were acquired in the hospital have an increased frequency of infection caused by *Proteus* and other organisms. *P. stutzeri* is widely distributed in the environment and rarely causes infections, but it has been isolated as an opportunistic pathogen in clinical conditions. It has been isolated as an opportunistic pathogen in immune-compromised hosts. Cases of *P. stutzeri* have been reported in the form of osteomyelitis, arthritis, endocarditis, meningitis, pneumonia, emphysema, skin infections, eye infections, urinary tract infections, and diverticulitis.

Providencia stuartii is the most frequently encountered human pathogen within the genus. The most frequent site of isolation is the urinary tract of chronically catheterized patients in hospitals and long-term care facilities. Among this patient population, *P. stuartii* is recognized as a persistent colonizer. Patients with burns are at a higher risk of *Providencia* wound infection. *P. stuartii* can directly adhere to urinary catheters, and adherence is associated with the presence of the mannose-resistant/ *Klebsiella*-like hem-agglutinatin, also known as MR/K fimbriae.

Pseudomonas hibiscicola is a common environmental contaminant. Multiple strains are often in circulation at any one time in a hospital unit.

Shewanella spp. is widely distributed in nature, with soil and water being their natural habitat. *Shewanella* infections occur in warm climates, especially warm summers. The infections commonly involve ears, skin and soft tissue, with or without bacteraemia. Most human infections are seen to be caused by *S. algae*. The increasing report of *Shewanella* infections in which the organism has been isolated in pure culture indicates the pathogenic potential of this genus.

Stenotrophomonas maltophilia is not highly virulent but several factors may promote its ability to colonize the respiratory tract and plastic surfaces, such as catheters and endotracheal tubes. *S. maltophilia* is a common environmental contaminant. The rising incidence of *Stenotrophomonas maltophilia* infection has caused a lot of concern in the medical community because the bacterium is resistant the majority of broad-spectrum antibiotics and as a result, it is very difficult to treat.

Water is essential to life, but many people do not have access to clean and safe drinking water and many die of waterborne bacterial infections. In recreational waters a MPN for total coliforms below 1000 is generally acceptable, but for drinking water no level of fecal coliform is acceptable. According to the results obtained the total coliforms are 16000/100 ml, which is far beyond the desirable limits. The results obtained in this study shows a high MPN of faecal coliforms which are far beyond the recommended limits. So it is confirmed that there is a serious risk of disease outbreak in the near future if the pollution in this area has not been taken seriously in to account. If a large number of fecal coliform bacteria (over 200 colonies/100 milliliters (ml) of water sample) are found in water, it is possible that pathogenic (disease- or illness-

causing) organisms are also present in the water. Fecal coliform are not pathogenic; but they are indicator organisms, which means they may indicate the presence of other pathogenic bacteria. Here as the count of faecal coliforms are too high, it is confirmed that the presence of pathogenic microbes will also be in detectable limits. Pathogens are typically present in such small amounts that it is impractical to monitor them directly. But according to current results, there is a possibility of presence of pathogenic organisms in a quantifiable limit and a potential risk of disease outbreak persists.

Table 2: The bacterial strains isolated in the present study with their GenBank accession and nearest BLAST similarity

Sl. No	*Identification*	*GenBank Accession*	*Nearest neighbour by BLAST search*	*% of similarity*
1	*P. hauseri*	KY072916	NR104767	100
2	*P. mirabilis*	KY072883	CP051260	99.93
3	*P. mirabilis*	KY072886	MT276305	99.92
4	*P. mirabilis*	KY072888	MT276300	99.92
5	*P. vulgaris*	KY077144	LR590468	100
6	*P. vulgaris*	KY077680	MN326680	100
7	*P. stuartii*	KY072927	CP031508	99.83
8	*P. stuartii*	KY072885	CP044076	99.93
9	*P. hibiscicola*	KY072887	NR024709	100
10	*P. stutzeri*	KY072890	NR116489	100
11	*S. algae*	KY072884	MN960080	99.85
12	*S. maltophilia*	KY072928	MN732977	99.55

All the pathogens encountered in this study throw light to the fact that the area is highly contaminated with those bacteria which cause hospital acquired infections. So they may be present in the sample from a point source. The pathogens are virulent to the extent that they can colonize hospital equipments and even water treatment plants. The presence of such pathogens may be due to the discharge of waste materials from hospitals in the nearby area, which cause a potential threat to people who access the water from this canal for different purposes. All the pathogens cause urinary tract infections which are usually less noticed by public. But the long term infection may lead to other pathogenic colonization and deterioration of health of the individual finally making the person immuno compromised.

4. Conclusion

In this study, the main target was to assess the degree of water pollution in the study areas of Kozhikode district, Kerala together with the isolation and identification of human pathogenic bacteria through genetic markers. The water in the study area was turbid, foul smelling and its MPN value $\geq$16000 which indicates high faecal contamination. It cannot be used for irrigation, recreational as well as household purposes at this level of contamination. The overwhelming dependence of residents on private wells showed the gross inadequacy of public water supply in the study area. Lack of protection control over management of water sources is an indication of contamination risks. Wells in this area are constantly exposed to contamination from human activities.

The study provides evidence that the local communities are suffering from a variety of health problems. By using of river water for washing and bathing purposes, many waterborne diseases may outbreak. Skin problems, allergic conditions, itching and other skin lesions which are contact type diseases were widespread in the study area. Adequate measure has to be taken for the treatment of water that is used for various activities and also the discharge of sewage and other wastes has to be done only after appropriate treatment. Anthropogenic activities are responsible for water pollution, and it may disrupt human life to a great extent. The house hold water, the water that flows through the city drainage has to be treated properly. Laws and legislation relating to pollution should be strictly followed. Sanitation system must be improved. Ponds, lakes and wells meant for human use should be routinely cleaned and treated, so that it remains fit for human use. Public awareness has to be given and serious action has to be taken regarding the treatment of canal water. The microbes obtained in this study can even flourish in chlorine treated water, which is the general treatment procedure in our water distribution systems in urban areas. And hence the water disinfection procedures need to be analyzed and new methods has to be introduced for treatment of water distribution systems. Now a day there is an increasing concern of faecal contamination of rivers and canals throughout Kerala, which has to be given primary attention to prevent a possibility of epidemic outbreak.

5. Acknowledgements

The preliminary survey and sample collection done by Ms. Dhanusha, S., Ms. Gayathri, M. and Ms. Greeshma, M. as a part of their post graduate dissertation work under the supervision of corresponding author is gratefully acknowledged.

References

[1] UNESCO (United Nation's Education Science and Cultural Organization). The United Nations World Water Development Report 3: Water in a Changing World. Paris/New York: UNESCO/Berghahn Books, 2009

[2] Hinton JJ, Veiga MM, Veiga ATC. (2003). Clean artisanal gold mining: An utopian approach? *J. Clean. Prod.*, 11: 99–115.

[3] Oki T, Kanae S (2006). Global hydrological cycles and world water resources. *Science*, 313: 1068–1072.

[4] Schwarzenbach RP, Escher BI, Fenner K, Hofstetter TB, Johnson CA (2006). The challenge of micropollutants in aquatic systems. *Science*, 313: 1072–1077.

[5] Fenwick A (2006). Waterborne infectious diseases: Could they be consigned to history. *Science*, 313: 1077–1081.

[6] Sussman JI, Baron EJ, Tenenbaum MJ, Kaplan MH, Greenspan J, Facklam RR, Tyburski MB, Goldman MA, Kanzer BF, Pizzarello RA (1986). Viridans streptococcal endocarditis: Clinical, microbiological, and echocardiographic correlations. *J. Infect. Dis*. 154: 597–603.

[7] Borah M, Dutta J, Misra AK (2010). The Bacteriological quality of drinking water in Golaghat Sub-division of Golaghat District, Assam, India. *Int. J. Chem. Tech. Res*., 2: 1843-1851.

[8] Usha R, Ramalingam K, Rajan B (2006). Freshwater lakes - A potential source for aquaculture activities - A model study on Perumal lake, Cuddalore, Tamilnadu. *J. Environ. Biol.* 27: 713-722.
[9] Gruber N, Galloway JN (2008). An Earth-system perspective of the global nitrogen cycle. *Nature*, 451: 293– 296.
[10] Kern S, Fenner K, Singer HP, Schwarzenbach RP, Hollender J (2009). Identification of transformation products of organic contaminants in natural waters by computer-aided prediction and high-resolution mass spectrometry. *Environ. Sci. Technol*., 43: 7039–7046.
[11] Prevedouros K, Cousins IT, Buck RC, Korzeniowski SH (2006). Sources, fate and transport of perfluorocarboxylates. *Environ. Sci. Technol*., 40: 32–44.
[12] Gregoire C, Elsaesser D, Huguenot D, Lange J, Lebeau T (2009). Mitigation of agricultural nonpoint-source pesticide pollution in artificial wetland ecosystems. *Environ. Chem. Lett*., 7: 205–31.
[13] Swackhamer DL, Needham LL, Powell DE, Muir DC (2009). Use of measurement data in evaluating exposure of humans and wildlife to POPs/PBTs. *Integr. Environ. Assess. Manag*., 5: 638–661.
[14] Krieg NR, Holt JG (Ed.) (1984). Bergey's Manual of Systematic Bacteriology. The Williams & Wilkins Co., Baltimore, MD.
[15] Skipton SO, Dvorak BI, Niemeyer SM (2008). Drinking Water Treatment: Water Softening (Ion Exchange). University of Nebraska-Lincoln Extension.
[16] Ouseph PP, Prasanthan V, Abhilash PP, Udayakumar P (2009). Occurrence and distribution of some enteric bacteria along the southern coast of Kerala. *Ind. J. Mar. Sci.*, 38: 97-103.
[17] Sambrook J, Russel DW (2001). Molecular Cloning: A Laboratory Manual 3rd Edition. Cold Spring Harbor Laboratory Press, New York.
[18] Jiang H, Dong H, Zhang G, Yu B, Chapman LR, Fields MW (2006). Microbial diversity in water and sediment of Lake Chaka, an Athalassohaline Lake in Northwestern China. *Applied and Environmental Microbiology*, 72 (6): 3832 – 3845.
[19] Sanger F, Coulson AR (1975). A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. *Journal of Molecular Biology*, 94 (3): 441 – 448.
[20] Thompson JD, Higgins DG, Gibson TJ (1994). CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acid Research*, 11(22): 4673 – 4680.