Original Article

A study of isolation and identification of bacteria from lake water in and around Udaipur, Rajasthan

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Abstract

Introduction: Water is an essential nutrient which plays an important role in digestion, absorption of food and elimination of waste products by urine. Udaipur also known as 'City of Lakes' or even the 'Venice of East' due to its unique and beautiful lake system has an exceptional importance at national and international levels. The aquatic systems are mostly dominated by bacteria and fungi and in the natural environments micro-organisms have very specific roles with regard to the recycling of materials and purification of water. Presence of coliform bacteria in the water indicates the fecal pollution of water. **Methods:** Water from all the three lakes were included for the assessment of their water quality parameters. Biological parameters like MPN of coliforms and presence of bacterial and cyanobacterial contaminants were read. The MPN (Most Probable Number) of Coliform was determined by the Presumptive Coliform Count (Multiple Tube Method) in 100 ml of sample water. **Conclusion:** The coliform bacteria is the primary bacterial indicator for fecal pollution in water. The purpose of study was to make people aware about the bacterial load in water bodies. Based on this, categorical representation of water was unacceptable for the purpose of swimming and bathing.

Keywords: Coliform, cyanobacterial, Fatehsagar, Pichhola, Swaroop sagar

Introduction

Udaipur also known as 'City of Lakes', or even the 'Venice of East, due to its unique and beautiful lake system, has an exceptional importance at national and international levels. These water bodies, which once considered as divine source of water are now increasingly being abused and severely polluted. Our fresh water bodies are contaminated with different kinds of pollutants resulting from increasing human population, urbanization and industrialization. In the context of international regulations, the contamination of water bodies by organic micro pollutants is the subject of constant interest and is always under investigation. Although the provision of safe drinking water has been one of humanity's most successful public health interventions. It is a defining aspect of a developing country's ignorance of the risks and inappropriate training of the staff and managers working on drinking water systems, which still results in unnecessary waterborne disease outbreaks in affluent communities. Disposal of domestic wastes in lakes is causing undesirable changes in physio-chemical and biological characteristics of these waters.[1] Microbes including bacteria, viruses and protozoa are the common cause of diseases in present aquatic ecosystems. Many major human diseases such as typhoid fever, cholera and other diarrheal diseases, poliomyelitis and viral hepatitis A and E are water borne. These pathogens reach water sources through fecal and sewage pollution. The aquatic systems are mostly dominated by bacteria and fungi and in the natural environments micro-organisms have very specific roles with regard to the recycling of materials and purification of water. *Salmonella, Acinetobacter, Chromobacterium, Alcaligens, Flavobacterium, Staphylococcus aureus*, *Pseudomonas aeruginosa, Clostridium botulinum, Vibrio cholerae* and *Escherichia coli* are the main human pathogens responsible for water contamination.[2] The presence of coliform a rod shaped, gram negative bacteria in the water indicates the

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Received: 19-11-2019 **Revised:** 21-01-2020
Accepted: 03-02-2020 **Published:** 28-02-2020

Access this article online

Quick Response Code:

Website: www.jfmpc.com

DOI: 10.4103/jfmpc.jfmpc_1032_19

How to cite this article: Bhumbla U, Majumdar S, Jain S, Dalal AS. A study of isolation and identification of bacteria from lake water in and around Udaipur, Rajasthan. J Family Med Prim Care 2020;9:751-4.

fecal pollution of water because they are invariably present in the feces of human beings and other warm-blooded animals in large numbers and can be easily detected in high dilutions. This confirms the presence of *E. coli* in the water bodies giving a definite proof of fecal pollution thus not being suitable for bathing and drinking.[3]

The intrusion of biological agents into water systems can pose serious public health risks as these agents cannot be easily detected and can remain hidden until a widespread contamination exists.

Review of Literature

South Rajasthan, also named as 'Mewar', is known for its beauty around lakes. Udaipur is well-known as 'City of lakes' and also for beautiful palaces in and around the city. Certain microorganisms including bacteria, viruses and parasites are well known water contaminants of which several may lead to waterborne disease and epidemics. Role of water in spreading communicable diseases is evident due to combined source of water, which is drinking as well as bathing. Contaminated water with faecal coliform severely affects the performance of humans. Contamination of water sources is counted when the man's action is adding or causing the addition of pollutants thus by altering its physical, chemical and biological characteristics to such an extent that it's utility for any reasonable purpose or its environmental value is demonstrably depreciated.

Disposal of domestic wastes in lakes of Udaipur is causing undesirable changes in physio- chemical and biological characteristics of these waters. An estimation of bacterial production is a crucial step in understanding quantitatively the function and contribution of bacteria in material cycling within given aquatic habits. Wild and domestic animals seeking drinking water can also contaminate the water through direct defecation and urination.[2] Various studies have evolved, wherein contamination of various water bodies across the country with different microorganisms is notified time and again.

Kataria *et al.*[4] (1997) investigated coliform count in the drinking water sources of Bhopal. Maximum probable number (MPN) in the study area exceeded the WHO limit at different sampling stations, as these were located in low-lying areas. Higher values in summer and Monsoon indicated a higher degree of pollution in the water bodies of that area.

Sinha *et al.*[5] stated that coliform count of water in monsoon season is at peak wherein its results in poor water quality of lake water. Chatterjee *et al.*[6] (2007) stated that the total coliforms fluctuated between 265 and 1753 MPN/100 ml. The peak value was observed in monsoon months, which was most probably due to the flushing of faecal contaminated water from the surrounding drains and bank sides. The increasing level of MPN of coliforms was observed in summer.

Sengupta *et al.*[7] (2008) studied that their microbiological studies included, MPN 542 to 2400/100 ml, wherein total coliform colonies were 27.5×10^3 to 84.17×10^3/ml. Fecal coliform ranged between 109 to 2400/100 ml for lake Pichhola, Fatehsagar, Swaroopsagar and Udaisagar. Higher values of microbial parameters give clear indication of very poor water quality.

Rajiv *et al.*[8] (2012) studied the river water from different parts of western Tamil Nadu, India. Samples were collected and microbial analysis was done. It stated that the number of bacterial colonies were 100-120 CFU/ml and number of fungal colonies were 30-45 CFU/ml.

Objective

The primary objective of the present study was to study the prevalence of bacterial contaminants from different water bodies in and around areas of Udaipur. Water from Pichhola, Fatehsagar and Swaroop Sagar lakes was studied along with their risk factors which were associated to it by observing various microbiological parameters.

Material and Methods

Ethical clearance

Ethical clearance was taken from the Institutional Ethical Committee before the commencement of the study.

Sample size

150 ml of water samples were collected from-

- Fatehsagar lake (geographical location and dimensions: coordinates; 24.6°N 73.67°E, catchment area of 54 km^2, max. length- 2.4 km, max. width- 1.6 km, surface area- 4 km^2, average depth- 5.4 m, max. depth- 13.4 m, surface elevation- 578 m).
- Lake Pichhola (geographical location and dimensions: coordinates; 24.572°N 73.679°E, catchment area of 55 km^2, max. length- 4 km, max. width- 3 km, surface area- 6.96 km^2, average depth- 4.32 m, max. depth- 8.5 m).
- Swaroop Sagar lake (geographical location and dimensions: coordinates; 24.6°N 73.67°E, catchment area of 10.5 km^2, max. length- 4 km, max. width- 2.5 km, surface area- 10.5 km^2, max. Depth 9 m).

Design of the study

Cross-sectional study.

Sample collection

Water from Lake Pichhola, Fatehsagar and Swaroop Sagar lakes was included in the present study for the assessment of their water quality parameters. Samples were collected from pre-monsoon season in a sterilised wide mouth bottles of at least 200 ml holding capacity. The bottles were opened and immersed at a depth of 30 cm with its mouth facing the current and the samples were brought to the Microbiology department

of Geetanjali Medical College and Hospital, Udaipur, for analysis. The water quality assessment included physical parameters like temperature and biological parameters like MPN of coliforms and presence of bacterial and cyanobacterial contaminants.[9]

Methodology

The MPN (Most Probable Number) of Coliform is determined by the Presumptive Coliform Count (Multiple Tube Method) in 100 ml of sample water.

Medium: MacConkey purple broth (double strength and single strength) in tubes were the standard medium of choice. Durham's tube was used to detect gas production and bromocresol purple was used as indicator.

Procedure: Measured amount of water samples were added to tubes containing MacConkey purple broth by sterile graduated pipettes as under:

- 50 ml of water - added to one bottle of 50 ml double strength medium
- 10 ml of water - added to one bottle of 50 ml double strength medium
- 10 ml of water each - added to 5 tubes of 10 ml double strength medium
- 1 ml of water each - added to 5 tubes of 5 ml single strength medium.

Tubes were incubated at 37 degree C for 48 hrs. Positive test was indicated by a colour change in medium from purple to yellow and gas collected in Durham's tube[10,11]

Estimate of coliform count per 100 ml of water was calculated from the tubes showing acid and gas production using the McCrady's probability table. It gave us the Presumptive coliform count (most probable number).[10-12] [Table 1].

Detection of coliform bacteria does not always indicate fecal contamination, as it may have environmental contaminants too. Hence, it was further tested by differential coliform count to detect the fecal *E. Coli*.[13]

Samples were streaked for the growth of isolated colonies on Nutrient agar, Blood agar and MacCokey's agar. Culture plates were further incubated at 37 degree for 24-48 hrs for bacteria. Plates were examined for their morphology and same type of colonies were used for performing gram staining. Isolation of bacterial contaminants is performed by standard microbiological techniques and battery of biochemical reactions. Various biochemical reactions such as IMViC, Urease, Nitrate, Catalase, H_2S production, Sugar fermentation tests were identified for the identification of bacterial isolates.

Results

Water samples of 150 ml each were collected from Lake Pichhola, Fatehsagar and Swaroop Sagar Lake's water. It was observed that different bacterial colonies were isolated from the water of these three lakes. Colonies varied from circuar, irregular margins as well as rhizoidal and filamentous in shape. Different colonies obtained were subjected to gram staining and thus cocci, bacilli and coccobacilli forms were identified. It was found that maximum strains when subjected to microscopic examination revealed that gram negative bacilli were predominant followed by gram positive cocci and gram positive bacilli. The results are tabulated in Table 2**.**

Classification of the quality of water was based on certain bacteriological tests amongst which MPN count is major. In this study Most probable number (MPN) of water sample collected from all the 3 water bodies was estimated to be very high as much as 810 to >1600 in May.

Quality of water for drinking was UNSATISFACTORY with the coliform count elevated grossly. *Escherichia coli* per 100 ml of water sample was also more than 1, which further added to correspond the contamination level in these water bodies.

Discussion

The present study has clearly indicated that the areas of lake are highly contaminated with bacteria. Presence of bacteria is predominantly in all sorts of environment of human involvement, majority of them are human as well as animal pathogen. The water examined in the present study has clearly

Table 1: Classification of quality drinking water supply according to bacteriological tests[9]

Quality of drinking water supply		
Quality of drinking water supply	**Coliform count/100 ml**	**Thermotolerant E. coli count/100 ml**
Excellent	0	0
Satisfactory	1-3	0
Intermediate	4-9	0
Unsatisfactory	>=10	>=1

Table 2: Morphological and cultural characteristics of the organisms isolated from lake water samples

Samples	Gram staining	Motility	Colonies on Nutrient Agar
FatehSagar lake	Gram positive rod	Non motile	White rhizoidal
	Gram positive cocci	Non motile	Golden yellow
	Gram negative rod	Motile	Mucoid
Pichhola lake	Gram positive rod	Non motile	White rhizoidal
	Gram negative rod	Motile	Mucoid
	Gram negative coccobacilli	Motile	Pale
Swaroop sagar	Gram positive rod	Non motile	White rhizoidal
	Gram positive cocci	Non motile	Golden yellow
	Gram negative rod	Motile	Mucoid

demarcated that it's loaded with indicator organisms which are indication of fecal pollution and also human interference.[14]

Nowadays, lakes are contaminated with plastics also which further adds on to water pollution and leading to serious health hazards to the community. Although micro plastic exposure via ingestion or inhalation could occur, the human health effects are still unknown. If inhaled or ingested, limited data from animal studies suggest that micro plastics may accumulate and cause particle toxicity by inducing an immune response.[15,16]

The present study correlates with the study of Sengupta M[7] *et al.*, where in the bacterial load was as high as 542 as compared to 810 in summer season.

Most common bacteria isolated in the present study was gram negative bacilli and gram positive cocci which correlated with the study of Sengupta M,[7] wherein the commonest organisms were Gram positive cocci, gram positive bacilli and gram negative bacilli, respectively.

Escherichia coli was the predominant bacteria along with Klebsiella pneumoniae and Coagulase Negative Staphylococcus (CONS) respectively were isolated in the present study which correlated with the study of Paneerselvam A,[2] who isolated *Escherichia coli* and Klebsiella pneumoniae as the commonest organisms respectively.

Implications

The coliform bacteria is the primary bacterial indicator for fecal pollution in water. The purpose of this study was to make people aware about the bacterial load in our water bodies. Based on this the categorical representation of water as excellent, good, satisfactory, poor or unacceptable can be designated for the purpose of swimming and bathing.

As Government finished its 4 anniversary, to meet the standards of "Swachh Bharat Abhiyan", one really needs to monitor the microbial quality of water at regular intervals. To check the contamination of water with these pathogens, regular cleaning and proper sewage disposal plants should be implemented.

This present study has strongly implicated that the microbiological standards of lake water must be developed to a large extent to confirm the health standards. Also the slogan which states Green city -clean city, my dream city can only be achieved when clear cut evaluation is done to protect and use of these water bodies for various purposes.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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