Microbial analysis of water bodies near food industries

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Abstract— The quality of water and occurrence of water borne diseases are critical public health issues. The most serious public health risk associated with drinking water is microbiological contamination thereby making it a priority for water quality testing. The objective of the study was to assess the quality of drinking water by isolation and enumeration of organisms through microbiological examination. Water samples were collected from four different regions - North, South, East and Central Kolkata. Microbial and fermentation tests were performed in the collected water samples followed by confirmatory IMViC tests to detect the presence of microorganisms. Microbial estimations indicated the presence of coliforms in water which may lead to diseases like diarrhoea and gastrointestinal problems. The biochemical tests also displayed the occurrence of the members of the Enterobacteriaceae family including *E.coli*, *Enterobacter* sp and *Klebsiella sp*. These microorganisms deteriorate the quality of drinking water making it unfit for human consumption. Therefore, accurate testing of drinking water is crucial in maintaining the health and safety of inhabitants who rely on this resource.

Keywords— *E. coli*, *Enterobacter* sp, *Klebsiella* sp, Microbial analysis, Public health, Water quality

I. INTRODUCTION

21st century is century of pollution, global warming, insecurity and vulnerable health factors. Water pollution is one of the major concerns worldwide. This occurs when contaminants are discharged directly or indirectly into water bodies thereby affecting the aquatic flora and fauna. Moreover, human health is also influenced by polluted water [1]. While the total amount of water available in the world is constant and is generally said to be adequate to meet all the demands of mankind, its quality and distribution over different regions of the world is uneven leading to issues of scarcity and suitability. Additionally, the quantity of potable water is known to be limited [2]. Water of good drinking quality is of basic importance towards maintenance of proper human physiology and general wellbeing.

Industrial pollution is one of the leading causes of contamination and toxicity worldwide [3]. It not only affects the quality of drinking water but also has a deleterious impact on the soil microflora and aquatic ecosystems due to release of toxic effluents. Illegal discharges or accidental spillage has been shown to account for majority of the incidents of surface water pollution [4]. Food industries severely impact water quality. Systematic management of industrial run off is extremely essential for maintaining a balanced ecosystem [5]. Interestingly, pathogenic

microorganisms constitute a significant contaminant of food-processing wastewaters, particularly from meat, poultry and seafood processing facilities [6, 7]. Water contaminated by microorganisms may contribute to a wide range of health disorders. This has been documented to be responsible for more than 200 diseases, including typhoid fevers and cancers, which often lead to fatal outcomes especially in developing countries. Common foodborne pathogens have readily been found to produce biofilms on surfaces [8]. Most foodborne illnesses are limited to brief episodes of diarrhea, nausea, vomiting, weakness, chills, sore mouth, blurred vision, muscle paralysis and aches or other acute gastrointestinal problems. However, a small portion of foodborne diseases are indeed fatal, thereby enhancing the chances of food mediated death [4, 9].

Coliform bacteria including *E. coli, Enterobacter sp, Klebsiella* sp and *Salmonella sp* are members of the Enterobacteriaceae family and are known to be useful markers of fecal contamination. Coliform bacteria are often referred to as "indicator organisms" because they indicate the potential presence of disease-causing bacteria in water. The presence of these microorganisms in water not only guarantees the possibility of an associated illness but also indicates that a contamination pathway exists between a source of bacteria (surface water, septic system, animal waste, etc.) and the water supply. The existence of the above

has also been associated with numerous pathological outcomes. *E. coli* is known to produce potentially lethal toxins and cause food poisoning, the symptoms of which being abdominal pain, watery stool, nausea, vomiting and fatigue. *Enterobacter* is responsible for various ailments including urinary and respiratory tract infections [10]. Moreover, *Salmonella* and *Klebsiella* sp have also been established as agents of food borne diseases [11].

Escherichia coli, a member of the Enterobacteriaceae family is a facultative anaerobic, gram-negative, non-spore-forming, rod-shaped bacterium possessing the enzyme β-glucuronidase. *E. coli* occurs naturally in the intestines of humans and other warm blooded animals. In human and animal feces, *E. coli* is found at a concentration of about 10^9 per gram forming roughly 1% of the total biomass of the large intestine. In general terms, this microorganism survives for about 4-12 weeks in water containing a moderate microflora at a temperature of 15-18°C. The presence of *E.coli* in water is considered as a good indicator of fresh fecal contamination [12]. Furthermore, rapid rate of proliferation along with the known developmental stages of this bacterium makes it a suitable indicator of water contamination [13]. Most bacteria in the coliform group do not cause diseases. However, greater number of coliforms indicates that more disease-causing bacteria may be present in the same. Since coliform bacteria usually persist in water longer than most disease-causing organisms, the absence of coliform bacteria leads to the assumption that the water supply is microbiologically safe to drink [14].

Although water plays a significant role in maintaining human health, it is also known to be carrier of many diseases. Globally, drinking water has been established as a primary transmission pathway for diarrheal pathogens [15]. The microbial contamination of drinking water constitutes a major concern worldwide being an important source of infection and mortality. Waterborne infectious diseases are those in which the pathogens are present in water and ingested when that water is consumed. These diseases mostly originate from water contaminated with human feces. Each year, more than 800,000 children younger than 5 years of age die from diarrhea mostly in developing countries. This accounts for 11% of the total deaths globally. Similar to many other diarrheal diseases, ingestion of contaminated food and water can result in diarrhea due to Diarrheagenic *E. coli* (DEC). The occurrence of DEC has been widely established in areas possessing inadequate drinking water and poor sanitation. Different sources of waters including drinking water, rivers, swimming pools, surface waters, and lakes have been found to harbor these organisms. Transmission of these pathogens usually occurs while drinking, bathing, and/or using water for food preparation. According to the water-quality standards provided by WHO, the number of *E. coli* bacteria should be

nil in a 100 mL drinking water sample, although certain standards do allow the existence of ten coliforms/100mL potable water [16].

Drinking water pollution by fecal coliform bacteria harboring resistance genes is a water-quality affair of national importance. Coliform microorganisms can be considered as important vehicles for spread of resistance genes due to its abundance in such environments [16, 17]. The objective of this study was to determine the prevalence of microbial contaminants, especially the members of the Enterobacteriaceae family in water bodies of different regions of the city. The coliform group including *E. coli* and other bacteria were investigated in water bodies near industries which were being used as sources of drinking water. The study aims to interpret the microbiological safety of these water bodies for domestic uses and public health.

Rest of the paper is organized as follows: Section I contains the introduction of the topic, section II contains the related work, section III explains material and methods, section IV describes results and discussions, section V concludes the work carried out along with future directions.

II.   RELATED WORK

In a study published in the Procedia Environmental Sciences, the effluents of sugar and textile industry have been proved to have deleterious effects on the soil microflora, culminating in abnormal Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) [18]. As mentioned in the International Journal of Innovative Research in Computer and Communication Engineering, 1.8 million people have been estimated to die each year as a result of severe diarrhoea caused by drinking contaminated water. Contamination of water is a major cause for outbreak of enteric diseases. Furthermore, approximately 580 people die of water pollution related illnesses each day in India.

III.   METHODOLOGY

Selection of sample: Water samples were collected from aqueous bodies near food industries of North, South, East and Central Kolkata in containers previously autoclaved and sterilized at 121°C and 15psi (Table 1).

Table 1: Water samples analyzed

Different regions in Kolkata	Sample codes	Number of samples taken
North	N1 & N2	2
South	S1 & S2	2
East	E1 & E2	2
Central	C1 & C2	2
Total		8

Preparation of microbiological media, pour plating and spread plating: The samples collected and stored in

sterilized bottles were brought to room temperature and used for analysis. Bacteriological enumeration and characterization was carried out for indicator organisms' i.e. total and fecal coliform by plating on selective, differential as well as enrichment medias respectively. Nutrient agar medium was used as a general purpose, non selective media to culture all microbial cells. Eosine Methylene Blue agar medium (EMB) and MacConkey agar was used for the isolation, enumeration, and differentiation of Enterobacteriaceae. The inoculated plates were incubated at 37°C for 24 hours for observation of microbial growth. The results were expressed as Colony Forming Units per millilitre (CFU/ml).

Fermentation Tests: Lactose and sucrose fermentation tests were performed in the samples to test for the presence of coliforms. The samples were inoculated in Durhams tube and incubated for 24 hours at 37°C. Phenol red was used as an indicator. A yellow colour post addition of indicator along with appearance of gas bubbles indicated a positive fermentation test.

IMViC Tests: IMViC, a series of four tests namely Indole, Methyl red, Voges Proskauer and Citrate utilization test were carried out for the confirmation of coliform types. The tests were conducted based on protocols mentioned by V. Hemraj et al.[17]

Statistical Analysis: Results were quantified as mean±s.d.and expressed as CFU/ml. p values were estimated using the GraphPad software (California, USA). 5 % level of significance and 90 % (***), 95 % (**) and 99 % (*) confidence intervals have been considered to be statistically significant.

IV. RESULTS AND DISCUSSION

Enumeration of microorganisms in water

A variety of microorganisms were observed on nutrient agar medium since it supports the growth of all kinds of microorganisms. Therefore, specific agars were used to examine the species of microorganisms in water. Two different medias, EMB agar and MacConkey agar were used to differentiate the microorganisms in water. EMB agar was used because it is a selective and differential agar medium used for isolation of coliforms. MacConkey agar was used as another media for selective isolation of gram negative bacteria and differentiation of the members of *Enterobacteriaceae* as lactose fermenters or non fermenters. Table 2 shows the number of colonies in nutrient agar, EMB and MacConkey agar which are expressed as mean±SD.

Table 2: Number of colonies in Nutrient Agar, EMB and MacConkey Agar

Number of samples	Number of colonies (CFU/ml)		
	Nutrient Agar	**EMB Agar**	**MacConkey Agar**
North	392±1.41**	68±0**	28±2.21**
South	280±1.41**	32±2.12**	27±1.41**
East	272±1.41**	56±2.12**	38±0.70**
Central	52±2.82*	17±2.12**	13±0.70**

Result expressed as (mean±SD) CFU/ml
* P<0.05 considered as statistical significant

Nutrient agar displayed the presence of microorganisms in all the water samples analyzed. As shown in Table 2, the number of colonies observed in EMB agar in samples from North Kolkata was found to be 68, 32 in samples from South, 56 in samples from East and 17 in samples from Central Kolkata. Appearance of dark purple coloured colonies in EMB agar indicated the presence of lactose fermenters, *Klebsiella* sp and *E.coli* whereas appearance of colourless colonies indicated the presence of non lactose fermenter including *Salmonella, Pseudomonas aeruginosa* and *Shigella*.

The number of colonies observed in MacConkey agar was found to be 28 in samples from North Kolkata, 27 in samples from South, 38 in samples from East and 13 in samples from Central Kolkata. Red and pink colonies in MacConkey agar are consistent with the characteristics displayed by *E. coli, Klebsiella pneumonia, Enterobacter aerogenes, Staphylococcus* sp and *Enterococcus* sp whereas colourless colonies indicated the presence of *Pseudomonas aeruginosa* in the water samples.

Fermentation tests

Fermentation test is the most common biochemical analysis used for detection of Enterobacteriaceae family, especially coliforms. This tests the bacteria's ability to ferment lactose and sucrose and produce acidic end products. A yellow colour post addition of indicator along with appearance of gas bubbles in the tube indicates a positive test for lactose and sucrose fermentation. A negative result shows no colour change. Table 3 shows the ability of the bacteria to ferment both lactose and sucrose. Positive lactose fermentation indicated the presence of *Klebsiella* sp and *E. coli* and negative test signified the presence of *Enterobacter* sp. Also, negative result for sucrose fermentation designated the presence of *Klebsiella* sp and *Salmonella* sp. Furthermore, gas bubbles were also observed in the Durham's tubes which indicated gas production as the result of fermentation.

Table 3: Fermentation analysis

Sample	Lactose fermentation	Sucrose fermentation
North	Positive	Negative
South	Positive	Negative
East	Positive	Negative
Central	Negative	Negative

IMVic Analysis

Following presumptive isolation of coliforms, further testing is required for confirmation of the type of coliform bacteria. Since bacterial organisms are similar in morphological and cultural characteristics, IMViC tests were used as a confirmatory test to differentiate between these bacteria.

Table 4: IMViC tests of the water samples

Samples	Indole test	Methyl red test	Voges proskauver test	Citrate utilization test
N1	Positive	Positive	Negative	Positive
N2	Negative	Positive	Negative	Positive
S1	Positive	Positive	Negative	Positive
S2	Negative	Positive	Negative	Negative
E1	Positive	Positive	Positive	Positive
E2	Positive	Positive	Positive	Positive
C1	Negative	Positive	Negative	Positive
C2	Positive	Positive	Positive	Negative

IMViC is a series of four tests i.e. Indole, Methyl Red, Voges Prosekaeur, and Citrate Utilization tests which is carried out for identification of the members of Enterobacteriaceae family. This is done in accordance with the changes in colour of the medium on addition of the respective indicators. Majority of organisms showed Indole test positive, Methyl Red positive, Voges Proskauver negative and Citrate test positive, thereby positively indicating the presence of *E.coli* and *Klebsiella* sp in water samples (Table 4). Some organisms also displayed a negetive Indole and a positive Voges Proskauver test consistent with the characteristics of *Enterobacter sp* in the water samples.

Based on above microbial as well as biochemical analysis, it can be inferred that the water samples indicated the presence of various coliform microorganisms including *E.coli, Klebsiella sp and Enterobacter sp.* Therefore, the water samples tested are non portable and may lead to adverse health consequences if consumed directly.

Discussion

An adequate supply of safe drinking water is universally recognized as a basic human requirement. Previous reports have indeed documented that improving the microbial quality of drinking water by household treatment and safe storage reduces the development of diarrhea [15]. Yet millions of people in the developing world do not have ready access to an adequate and safe water supply owing to rapid urbanization, mostly in peri-urban and slum areas. The old water distribution and sewage disposal systems in addition to poor sanitation facilities generally facilitate the spread of fecal bacteria to surface and drinking water thereby contributing towards waterborne illnesses [16].

The bacteriological analysis of water determines the potability of water. According to Indian standard (BIS, 1981), 95% water samples are supposed to be devoid of any coliform organisms. However, bacteriological analysis of drinking water of several areas does exhibit contamination with members of the Enterobacteriaceae family along with other pathogens [18]. Presence of *E. coli* and other coliform bacteria reflect a poor quality of drinking water. Old water distribution networks, leakages in pipeline, inadequate sanitary condition and improper management of waste disposal have chiefly been identified as the main culprits of the above. Old pipes and leakages in pipes provide a passage to waste water and other pollutants thereby altering the quality and raising the probability of deleterious health outcomes in the consumers [19].

In the present study, results from the differential agars and microbial analysis revealed the contamination of water samples with microorganisms including *E.coli, Proteus sp, Klebsiella* sp, *Salmonella* sp and *Enterobacter* sp. Therefore, water samples analyzed were found to unfit for direct consumption. Contaminants in water can affect water quality and consequently human health. Also, the risk of acquiring a waterborne infection accelerates with the increase in the level of contamination by pathogenic microorganisms.

V. CONCLUSION AND FUTURE SCOPE

Clean water is poorly located and distributed, the result being that people continue to use a closer and contaminated source. Inadequate access to water has a serious impact on the wellbeing of the population. Contaminants in water can affect water quality and consequently human health. Also, the risk of acquiring a waterborne infection has been positively correlated with the amount of pathogenic microorganisms. Microbiological parameters are very important in water resources in terms of public health since they play a significant role in the transmission of enteric diseases. Thus, the present study determined the safety of water bodies near industries. The present study did display the presence of disease causing microorganisms in water. Therefore, direct consumption of the same without any decontamination measures may negatively impact the fitness of the consumers. Frequent quality analysis of drinking water is essential for maintenance of overall health. The current

analysis therefore attempts to contribute to the limited researches carried out for assessment of water safety in view of increasing public health awareness. Education of the population on simple domestic scale water purification in future may help in improving the general health status.

VI. ACKNOWLEDGMENT

CONFLICT OF INTEREST

The authors declare no conflict of interest.

SOURCE OF FUNDING

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