Original Research Article

Microbial Quality of Water Used as Drinking Sources in Urban and Rural Households of Gujarat, India: A Cross-Sectional Study

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ABSTRACT

INTRODUCTION: Globally nearly 2 billion people have no access to safely managed drinking water services and over 1.7 billion lack adequate or basic sanitation facilities. India is a growing and developing nation and the quality of ground water and drinking water needs to be regularly monitored. There are several diseases in India that spread due to contaminated water. Annually about 37.7 million Indians are affected by waterborne diseases.
AIMS AND OBJECTIVES: This research has been undertaken with the Aim and Intent to study, test, assess, evaluate and understand the aspects of Microbial quality of water used as drinking sources in urban and rural households.
MATERIAL AND METHODS: The 40 numbers of drinking water samples were collected.20 water samples from the urban area of Vadodara city and 20 water samples from the rural areas on outskirts of Vadodara city. Multiple Tube Fermentation Technique was used for detecting presence of coliforms. The free residual Chlorine was measured by use of Chloroscope equipment
RESULTS: A total of about 17 water samples (11 rural samples of untreated water and 06 urban samples of treated water) showed a higher MPN. The growth of E. coli which indicates the fecal contamination rate was noted in 09 water samples which is about 22.50% (09 water samples - 06 rural samples and 03 urban samples). The absence of fecal streptococci, E. coli and total coliforms was found in only 6 samples which is about 15.00%.
CONCLUSION: Pathogens, coliforms and other microorganisms were found in water samples collected from both urban as well as rural areas. It is now important to educate and bring awareness among local people about quality of their water source, the importance of clean surrounding near water source, boiling of drinking water to eliminate contamination.

KEYWORDS: Fecal coliforms, E. coli, Chloroscope

INTRODUCTION

Globally nearly 2 billion people have no access to safely managed drinking water services and over 1.7 billion lack adequate or basic sanitation facilities. This leads to an estimated 0.9 million people dying every year from water and sanitation related diarrheal diseases. More than 30% of these are children under the age of 5 years, mostly in developing countries. [1]

Drinking water is a basic requirement and 10% of the global diseases can be prevented by improving the quality of drinking water supply from various water resources. [2] Water quality is largely affected by human & animal fecal matter, domestic sewerage, industrial waste, agricultural pesticide runoff, etc. Absent, inadequate or inappropriately managed water and sanitation services expose drinking water to contamination. [3]

India is a growing and developing nation and the quality of ground water and drinking water needs to be regularly monitored. There are several diseases in India that spread due to contaminated water. Reports & experts say that annually about 37.7 million Indians are affected by waterborne diseases. Thousands of children die each year of diarrhea and millions of working days are lost leading to a huge economic burden. [4]

The actual quality of water varies widely and quality assurance checks are lacking in many areas. What's passed off as drinking water often leaves much to be desired. Sharp geographic, socio-cultural and economic inequalities persist, not only between rural and urban areas but also within towns and cities where people living in low-income, informal or illegal settlements, slums, etc. usually have less access to improved sources of drinking- water than other residents.

Infectious diseases caused by parasites, viruses, pathogenic bacteria and microorganisms like *Escherichia coli, Salmonella spp, Cholera spp, fecal streptococci and Clostridium perfringens* are the most common health risk associated with drinking water.

The pathogenic microorganisms, their toxic exudates and other contaminants can cause various diseases and serious medical conditions.

The various methods [6] used for testing of water in order to study the quality of drinking water are:

i) Multiple Tube Fermentation Technique
ii) Statistical Method of Most Probable Number (MPN)
iii) Testing of water for free residual chlorine by Chloroscope.
iv) Antibiotic Susceptibility Testing

Microbial water analysis is an important procedure to analyze the quality of drinking water and to estimate the numbers of bacteria present and to allow for the recovery of microorganisms in order to identify them.

The detection of bacterial indicators in drinking water can suggest the presence of pathogenic organisms that are source of waterborne diseases. Indicator micro-organisms survive better and longer with uniform and stable properties and may be easily detected using standard laboratory procedures. [5]

This research was carried out during mid-August to mid-October which included collection of samples during rainy season in Vadodara and its outskirts located in state of Gujarat, India. This research has been carried out over a period of prescribed 2 months.

Vadodara is a semi-arid region of Gujarat and there is rapid growth in urban areas of Vadodara and the demand of water has increased due to urbanization, industrialization and expansion in various agriculture related activities and the above factors can easily lead to depletion of water resources.

Due to above reasons, it was crucial to understand the influence and impact of various activities on quality of drinking water

in these areas. The analysis and determination of microbiological quality of drinking water is very essential. Thus a cross-sectional study was conducted to study and analyze the microbial quality of drinking water in urban and rural households of Vadodara located in state of Gujarat, India.

Aim:

Microbiological contamination of drinking water sources is a serious water-quality problem worldwide. Safe drinking water for human consumption should be free from pathogens such as bacteria, viruses and protozoan parasites.

Many a times, water supplies in developing countries are devoid of necessary treatments for safe drinking water and the communities have to make use of the most convenient supply.

Microbiological contamination of the water may occur at the source or during supply or between the collection point and the point-of-use making the drinking water a health risk.

The research and study have been carried out in and around the city of Vadodara, Gujarat, India. This research has been undertaken with the Aim and Intent to study, test, assess, evaluate and understand the aspects of Microbial quality of water used as drinking sources in urban and rural households.

Objectives:

The objectives to achieve the above aim in this research study are:

Primary Objectives:

1) To collect water samples used as drinking water from urban and rural households in order to study, assess and evaluate the microbiological quality of water.
2) To detect by way of laboratory tests the amount of bacterial contamination in drinking water.
3) To study the presence of various microorganisms like coliforms and others in drinking water and to detect the

possible drug resistance in common pathogens

Secondary Objectives:

The results obtained from this study may be used as a base for future researches to study the type and probable sources of contamination, to study measures that may be adopted for prevention of contamination and for disinfection of drinking water and thereby improving the quality of water supply and preventing outbreaks of water borne diseases in these rural and urban communities.

MATERIALS AND METHODS:

A. Study Design: -

a. Place of Study:

This study was conducted in the Microbiology laboratory on drinking water samples collected.

b. Source of data:

The 40 numbers of drinking water samples were collected:

20 water samples from the urban area of Vadodara city and

20 water samples from the rural areas on outskirts of Vadodara city.

c. Ethical Clearance:

The proposed research under reference does not include any sort of sample collection or testing on any human being or animal. Clearance and Approval from Institutional Ethics Committee has been obtained prior to initiation of this research work.

d. Duration of study:

The study of microbial quality of drinking water was carried out over a period of 2 months i.e. from mid-August 2022 to mid-Oct 2022 by collecting water samples from rural and urban households along with testing and lab analysis which were carried out in Microbiology Laboratory.

e. Selection Criteria:

i. Inclusion criteria: The present study investigated the quality of drinking and household water from these 40 samples collected in rural and urban communities. Informed verbal consent was obtained from the head of each household before collecting the water sample.

Wherever well water is being used as the main source of drinking and household water, the same has been included in the study.

ii. Exclusion criteria: Water from stored containers was not included in the collection of water samples and present analysis.

B. Materials and Equipment's used for the study: -

Apart from laboratory apparatus, the following principal materials and equipment's were used for the study under reference:

1) Sterilized sample collection bottles of 200ml each.
2) Durham glass test tubes 5ml, 10ml, 50ml.
3) MacConkey broth purple.
4) Bottles for broth.
5) Ortho-Tolidine, Chlorination testing reagent.
6) Choloroscope equipment.
7) Vitek 2 automated machine.

C. METHODOLOGY

Microbial water analysis is an important procedure to analyze the quality of drinking water and to estimate the numbers of microorganisms and bacteria present in the water sample and to allow for the recovery of microorganisms in order to identify them.

The most important aspect of analysis is therefore to determine whether fecal contamination is present. The coliforms present in water indicate the possible fecal contamination of water and presence of pathogens. The most common coliform i.e., E. coli are the most appropriate indicators of faecal pollution.

The principal techniques and methods largely used to detect and count microorganisms in water are: [6]

Multiple Tube Fermentation Technique

Statistical method of most probable number (MPN) Membrane Filter Technique

Further, chlorine treated water supplies can be tested for free residual chlorine (mg/l) to assess the effectiveness of disinfection in drinking water.

Of the above methods used for detecting presence of coliforms and for presence of free residual chlorine in drinking water, I have carried out the following tests as briefly described below:

a) Multiple Tube Fermentation Technique / MPN Technique:

I have collected treated as well as untreated water samples from rural areas and the procedure was carried out accordingly.

In this technique a 100 ml water sample is used for treated water and for untreated water 105 ml water sample is distributed (five parts of 1 ml, five parts of 10 ml and one part of 50 ml) in bottles of sterile broth.

After incubation, the number of bottles in which fermentation with acid and gas production has occurred was counted. The fermentation occurs by the coliforms in the water.

By the statistical method of MPN, the most probable number of coliforms in the water sample was estimated.

b. Testing for free residual Chlorine by use of Chloroscope:

To ensure complete disinfection, chlorine is usually added to the water. The free residual chlorine present in drinking water can be used as a measure of the effectiveness of chlorine as a disinfecting agent.

The free residual Chlorine was measured by use of Chloroscope equipment. The result also depends on the distance from source of water to distribution point and also on the

temperature and pH of the water. This test was carried out only on treated water samples from urban areas.

Size of Water Sample and Sample Collection Method with description:

Water samples for drinking water were collected from forty households i.e. 20 households each from rural area and urban area. The houses for sampling were selected randomly. The collection of water samples was done without contaminating the sample collection bottle and cap. Standard water sample collection procedure was followed.

Sterilized sample collection bottles of 200 ml capacity were used for the collection of drinking water samples. [12] The bottle was held by the base in one hand and the cover was removed with other hand to prevent contamination and the cover was re-placed after collecting the water sample. No external surfaces were touched to the screw thread of the bottle neck or the inside of the cap to avoid contamination of water.

In case of collecting the samples from a tap all external fittings from the tap such as additional nozzle or rubber tube, etc. were removed and the tap was cleaned. The tap was then turned on and the water was allowed to run for a minute to flush away the stagnant water in the pipe if any. The tap was then sterilized using the flame of a gas lighter.

After running the water for a few more seconds, the sample bottle was filled from a gentle flow of water. Additional upper level of water in the bottle was discarded and cap of the bottle was re-placed.

Leaking taps were avoided and water samples from leaking taps were not collected to avoid any sort of external contamination. [14]

In case of collecting the samples from well, a string with stone was attached to the bottle.

The sterilized bottle was then opened and lowered into the well to a depth of about 1 meter or till it was completely immersed in water without touching the sides or bottom of the well. When the air bubbles stopped rising to the surface, the bottle was raised out of the well and the cap was carefully re-placed.

In both the case types, additional upper level of water say approximately 20 ml of water was discarded and the bottles were kept partly empty to provide sufficient air space to allow proper shaking of water sample before using the water for analysis so as to achieve a homogenous dispersion of the bacteria.

After sample collection, each bottle was labelled with sample number along with other details which included:

a) sample area i.e. urban or rural
b) type of sample i.e. treated or untreated
c) source of water,
d) site address from where sample was collected along with the
e) date and time of collection of water sample.

Necessary standard procedure & precautions were followed and the water samples collected were packed in a labelled box and were sent to the laboratory in Department of Microbiology for carrying out requisite tests.

Test I - Multiple Tube Fermentation Technique & MPN: -

As briefly explained above, depending on the basis of type of water sample i.e. treated water sample or untreated water sample, the multiple tube fermentation technique and most probable number (MPN) test [14] was carried out in following manner:

Procedure & Testing Method:

Table A: (for Treated water samples)				
Type of sample	Number of Durham tubes	Quantity of broth in ml.	Strength of broth	Water sample in ml.
Treated water samples	1	50	Double	50
	5	10	Double	10

Table B: (for Untreated water samples)				
Type of sample	Number of Durham tubes	Quantity of broth in ml.	Strength of broth	Water sample in ml.
Untreated water samples	1	50	Double	50
	5	10	Double	10
	5	5	Single	1

Single strength broth was made by using quantity of MacConkey broth purple powder as instructed by the manufacturer. Double strength broth was made by using twice the normal quantity of MacConkey broth purple powder.

The following standard procedure was followed in the microbiology laboratory:

Each bottle of broth contained an inverted Durham tube for the collection of gas. The tube was added before the broth was sterilized. If any air bubble was found trapped in the tube, the bottle of medium was inverted and the bubble was allowed to rise out of the tube.

Thus it was made sure that the inverted tube was filed with broth and that there is no air bubble inside the tube.

Each sample of water was mixed thoroughly by shaking and inverting the sample collection bottle. The cap was removed and the bottles of sterile broth were inoculated as follows:

50 ml of water was added to the tube containing 50 ml of broth and this was done by pouring it directly into the bottle of broth up to a 100 ml mark previously marked on the bottle. Using a sterile pipette, 10 ml of water was added to each of the five bottles containing 10 ml of broth. *(Refer Table A)*

Thus, a total of 100 ml volume of each treated water sample was inoculated

As instructed by my guide, sufficient care was taken to not mouth pipette the water samples. Further for all untreated water samples, using a pipette filler 1ml of water was added into each of five bottles containing 5 ml of broth. *(Refer Table B)*

Thus, a total of 105 ml volume of each untreated water sample was inoculated.

The inoculated broths were incubated with the bottles loosely capped in an incubator at 37 ℃ for 24 to 48 hours. The fermentation then occurred by coliforms. After incubation, the number of bottles in which fermentation has occurred was counted.

Each bottle and durham tube were examined and it was noted and counted which sample had produced both acid and gas. Acid production was noted by a change in colour of the MacConkey broth from purple to yellow, and the production of gas was noted by collection of a bubble in the durham tube.

To determine the presumptive coliform count / most probable number of coliforms, suspensions from positive bottles were sub-cultured on MacConkey broth and isolate were identified by Vitek instrument and the resulting colonies were identified following standard operating procedures [16].

The water samples were processed using this method to determine the presumptive coliform count / most probable number (MPN) of coliforms.

The data collected and test results obtained were entered in an excel sheet for better assessment and comparison.

Test II - Testing for free residual Chlorine by use of Chloroscope:

Chlorine is usually added to the water to ensure complete disinfection. Thus the free residual chlorine present in 20 numbers of drinking water samples collected from urban areas was measured with the help of Chloroscope equipment.

Procedure & Testing Method:

Chloroscope is equipment used to check the residual chlorine in drinking water. In this test, a chlorinated water sample was taken in a glass tube. To this water in test tube, around 5 to 6 drops of Ortho-Tolidine chemical reagent were added. The colour of water

started changing and the colour formed was observed after 5 minutes.

Test tube was then inserted in right side of the chloroscope and the changed colour of the water sample in test tube was matched with the standard colour shades of yellow shown on left side of chloroscope. The same was verified in day light source.

The formation of very light yellow to yellow color indicates the presence of chlorine in the water. The more yellow the color, the greater is the chlorine residual in the water. The amount of residual chlorine was ascertained by comparing the colour developed in the glass tube with the standard readings of residual chlorine per ml mentioned on the Chloroscope.

As per standards, the amount of free residual chlorine in the water should normally be minimum 0.2 mg/l and less than 1 mg/l. This amount may vary based on the distance from source to distribution point and also on the basis of the pH of water.

Both the tests on the water samples were performed according to standard operating procedures which were strictly followed in the pre-analytical, analytical and post-analytical phases. Quality control measures were followed.

The observations & readings of both tests and further growth of microorganisms were noted and data collected along with test results obtained were entered in an excel sheet for better assessment and comparison.

The observations, readings and probability have been summarized to the best of my knowledge and understanding. They have been presented below in tabular format.

OBSERVATIONS AND RESULTS
The study checked the quality of household water from 40 samples collected in rural and urban houses. The microbial quality of the water samples was assessed based on standard guidelines.

Statistical Analysis method of Most Probable Number (MPN):
The MPN method is a statistical estimate of the number of coliform-group organisms per unit volume of sample water and was used to estimate the number of coliforms in water and to estimate the concentration of microorganism in the samples.

A total of about 17 water samples (11 rural samples of untreated water and 06 urban samples of treated water) showed a higher MPN.

The presumptive coliform count / most probable number (MPN) of coliforms are [15]:

Tables of Probability: For Estimation of MPN with respect to Faecal Coliform Bacteria

Table C: (for Treated water samples)				
Number of bottles used per water sample	**1**	**5**	**-**	
Volume of water sample in each bottle	**50 ml.**	**10 ml.**	**1 ml.**	**MPN / 100 ml.**
Number of Durham Tubes showing Positive result for presence of Coliform	0	0	-	0
	0	1	-	1
	0	2	-	2
	0	3	-	5
	0	4	-	7
	1	0	-	2
	1	1	-	3
	1	2	-	6
	1	4	-	16
	1	5	-	18

Table D: (for Untreated water samples)				
Number of bottles used per water sample	**1**	**5**	**5**	
Volume of water sample in each bottle	**50 ml.**	**10 ml.**	**1 ml.**	**MPN / 100 ml.**
Number of Durham Tubes showing Positive result for presence of Coliform	0	0	1	1
	0	0	2	2
	0	1	0	1
	0	2	1	3
	0	3	1	5
	1	0	1	3
	1	1	2	7
	1	2	1	7
	1	2	3	12
	1	3	2	14
	1	3	3	18
	1	4	1	17
	1	4	2	20
	1	4	3	30
	1	4	4	35
	1	5	1	25
	1	5	2	50
	1	5	3	90
	1	5	4	160
	1	5	5	180+

Identification & Analysis of Microorganisms in Water Samples:

Water samples were analyzed for the presence of various microorganisms like Escherichia coli, fecal streptococci, total coliforms, etc. in drinking water samples by multiple tube fermentation method.

The fecal contamination rate is prominently indicated by the growth of E. coli and bacterial isolates which were identified by use of VITEK 2 instrument as per standard procedures followed by susceptibility testing as described below. The microbial identification (ID) and antibiotic susceptibility testing (AST) was carried out.

The results of rural and urban areas are summarized in table below:

Table E: Indicator bacteria isolation rates from rural and urban water samples.				
Type of Bacteria	**Rural (from 20 water samples)**	**Urban (from 20 water samples)**	**Total no. of water samples (from total 40 water samples)**	**Percentage 100 x (Affected samples / Total samples)**
Escherichia coli	06	03	09	22.50%
Fecal streptococci	03	00	03	7.50%
Total Coliform bacteria	19	15	34	85.00%

From the above tables, the following observations were made:

The statistical method of most probable number (MPN) was used to estimate the number of coliforms in water and to estimate the concentration of microorganism in the samples.

A total of about 17 water samples (11 rural and 06 urban samples) showed a higher MPN. The growth of E. coli which indicates the fecal contamination rate was noted in 09 water samples which is about 22.50% (09 water samples - 06 rural samples and 03 urban samples).

Further total coliforms were noted in 34 water samples which is about 85.00 % (34 water samples - 19 rural samples and 15 urban samples).

The samples without E. coli were found to have lower coliform counts (<10 coliforms / ml.).

The absence of fecal streptococci, E. coli and total coliforms was found in only 6 samples which is about 15.00%.

Antibiotic Susceptibility Testing:

When it comes to fighting infectious organisms, microbial identification (ID) and antibiotic susceptibility testing (AST) are key to provide the right information and play a crucial role in providing information related to Multi-Drug Resistant Organisms (MDRO).

Various bacterial isolates were obtained out of which E.coli was most common & prominently found. AST of E.coli of was performed under guidance and was interpreted according to Clinical Laboratory Standards Institute (CLSI) guidelines. [17]

Antibiotic sensitivity pattern of E.coli is as follows:

Table F: Antibiotic sensitivity pattern	
Antibiotics / Drugs	**E-coli (in percentage)**
Gentamicin	75
Levofloxacin	75
Meropenem	50
Ceftazidime	25
Cefepime	25
Piperacillin and tazobactam	100

Result of Test for free residual Chlorine by use of Chloroscope:

The treated water samples turned light yellow on addition of drops of Ortho-Tolidine to water sample and each water sample was checked for amount of free residual chlorine by chloroscope as described earlier and all the readings were found to be between the range of

0.2 mg/l to 0.3 mg/l.

Multiple Tube Fermentation Technique for presumptive Coliform Count

Chloroscope & Ortho-Tolidine Reagent for Assessment of Chlorine level.

DISCUSSION

Microbial risk assessment and management of water quality is an important concern and focus of government policies throughout the world. Human impact on this category of contamination is significant and several human-related activities and also the population explosion have affected and are still affecting dramatically the aquatic environment.

This study was important for the region as this research supported the theory that analysis of water samples is very important. First and foremost, it is crucial to understand the microbial quality of drinking water used by people and thereafter the study helps in identifying various microorganisms like Escherichia coli, fecal streptococci, total coliforms, etc. affecting the quality of drinking water.

The goal of Antimicrobial susceptibility testing was to detect the possible drug resistance in common bacteria E.coli and the same was achieved. Many of the coliform strains were found to be susceptible to commonly used antibacterial agents but a significant resistance was observed among the isolates.

The various test results indicate that in order to monitor the microbiological quality of drinking water, the microbial analysis of water is required to be performed frequently at regular intervals. Contamination of water is often intermittent and samples are required to be tested over a longer period of time during both dry and wet seasons.

This study determines the various microbes causing water borne diseases / infections and can thus form a base for effective infection prevention & control (IPC). [9] It will further help in educating people to implement measures to prevent contamination of drinking water.

CONCLUSIONS

During the period of this study various tests were carried out and it has been found that there is contamination of water in untreated water as well as in treated piped water. The significant part of the study was detection of pathogens and microorganisms in water samples collected from both urban as well as rural areas.

The water samples were checked for free residual chlorine with the help of chloroscope and were found to be within the required range.

Pathogens, coliforms and other microorganisms were found in water samples collected from both urban as well as rural areas. Further by way of susceptibility testing, drug resistance to certain standard antibiotics

was noted and antibiotic sensitivity pattern for E.coli was checked.

Based on the study carried out during this research work, following further research and procedures will have an impact and are suggested:

i)   Environmental planning and sustainable management of water sources.
ii) Evaluation of the water supply system and drinking water collection methods.
iii) Continuous water testing to identify the sources and type of contamination.
iv) Antimicrobial susceptibility testing to detect drug resistance in common pathogens.

SUMMARY

Safe drinking water is a basic human right and if contaminated it has health related implications. Drinking water for human consumption should be free from pathogens, bacteria, etc. Human health can be protected by preventing microbial contamination of drinking water. The purpose of this study was to obtain information of microbiological quality of the drinking water in urban and rural houses and the same was achieved.

During this study, various tests were carried out by way of Multiple Tube Fermentation Technique, checking free residual Chlorine in water through Chloroscope and Statistical Analysis by way of MPN method was used to estimate the number of coliforms & concentration of microorganism in the water samples. The Microbial identification (ID) and Antibiotic Susceptibility Testing (AST) was performed with the help of automated equipment Vitek 2 in microbiology laboratory.

The Aim and Objectives of this research have been achieved. The research was important for people of this region so as to understand the microbiological quality of their drinking water.

It is now important to educate and bring awareness among local people about quality of their water source, the importance of clean surrounding near water source, boiling of drinking water to eliminate contamination and adopt measures to prevent contamination of ground water and drinking water.

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