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Original article

Bacteriological Assessment of Drinking Water Samples from Tribal Area of Bhandardara Region, Maharashtra, India

Shivani Dhawade, Sanjeev G Kulkarni, Anagha Vaidya Kinikar, Sanjay Kumar, S Roushani, Deepika Bhalerao

Department of Microbiology, Dr B V P Rural Medical College, Pravara Institute of Medical Sciences - Deemed to be University, Loni – 413736
Corresponding Author: Sanjeev G Kulkarni

Abstract *-*

Background *–The quality of drinking water still eludes the developing and third world countries. In India, we have significant population staying in rural area and in the tribal areas The tribal population placed in remote areas, lacks basic amenities such as water, electricity, proper sanitation, waste disposal and non availability of drinking water facilities, making them prone to the water borne infections. Lack of awareness about hygiene and safeguarding the health further complicates the matter. The present study was undertaken to assess the potability of drinking water, currently available in tribal area of Bhandardhara region (Maharashtra), samples were collected under aseptic conditions from the 23 villages and Bacteriological assessment was done.*

Materials and methods: *Samples were collected from 23 villages of Bhandardhara region covering the tribal population of 35,407. The population falls under the 2 Rural Health Centers (Shendi and Rajur) of School of Public Health & Social Medicine, PIMS DU. The type of study was descriptive cross-section study based upon Simple Random Sampling Technique. Drinking water samples (100ml) were aseptically collected in sterile container. Water samples from sources supplied for other domestic purpose were not collected. The water samples were subjected to Most Probable Number Test (MPN) as per the standard test procedure using multiple tube method and E.coli detection test kit (HiMedia).*

Results: *The outcome of the MPN test were interpreted based on tables giving the bacterial count per 100 ml (MPN/100ml) of the water sample tested. The outcome determined the MPN count with Klebsiella spps being highest (60.86%) Next common organism isolated was E.coli (21.73%) followed by Pseudomonas (17.39%). Coliform count being higher in all the 23 samples, water was deemed unfit for drinking.*

Conclusion: *Our results were comparable with other studies done in different tribal areas of India highlighting the fact that drinking water is un-potable in Tribal areas of Bhandardhara region and local population is unaware of the cascading health effects due to un-potable water. Community engagement and advocacy for good water quality is key. The various governmental organization also should take precautions/measures to ensure safe drinking water in tribal areas.*

Key words: *Bacteriological water analysis, MPN Test, Tribal area, coliform count*

Introduction

Water is one of the basic needs of life and its significance in day to day life remains uncontested. Contamination of water by sewage and other waste is often seen in urban areas and we have numerous data available, as on date. People in the urban areas are aware about the significance of water quality supplied to them. While the sufferers on the major hands are the tribal areas . India has the largest concentration of tribal population in world next to Africa [1].Tribal people mostly reside in the forest areas identified as the most remote and exploited areas. They are mostly dependent on the underground water reservoir or the naturally created water reservoir nearest to their habitat. But the safety of the ground water consumed by these tribes is questionable. Very often groundwater is found contaminated affecting the health of these tribes. However tribal areas in this country still lack the basic right to safe drinking water. Questions remain whether they are aware of water quality affecting their health as they fight for their survival.

Waterborne diseases are more prevalent in tribal areas due to unhygienic conditions that prevail, improper waste management and supply of un-treated drinking water.
Bacterial contamination presents the highest and most immediate health risk, affecting 65% of those relying on water that is dangerously contaminated.[1].
In India, diarrheal diseases are major health problems among the children under the age of 5 years. Hence prevention and control of waterborne diseases assumes significance. While determining the quality of drinking water is of paramount importance for prevention and control of such diseases.
Tribal people are very seldom aware of the waterborne diseases and its causes. They consume the contaminated water leading to many waterborne diseases which miserably affects their health and livelihood.
Though the government has made Clear water Act, Tribal Authority : Indian Tribes qualifying for treatment as states are responsible for establishing water quality standards and for reviewing and revising these standards at least once every 3 years.[2]
This study was undertaken to assess the extent of bacterial contamination in the drinking water that is supplied in the remote tribal area of Bhandardhara region (Nashik District), where health facilities are meager.
The study intended to determine the bacteriological profile of the isolates from drinking water.
The microbiological analysis of water samples in order to check the portability, will also help prevent spread of waterborne diseases in tribal population.
The assessment of microbial contamination can determine the extent of water contamination and measures that need to be undertaken for prevention of waterborne diseases, which so far in this region has not been attempted in tribal area of Bhandardara region.

Objectives

1. To assess the potability of water used for drinking and cooking by Most Probable Number (MPN) test and Coliform test kit .
2. To determine the fecal contamination of drinking water
3. To study the bacteriological profile of contaminated water.
4. To assess the extent of water contamination from different drinking source of water
5. Suggest simple measures to avoid contamination of water

Methodology

Study Area

The study areawas the tribal area of Bhandardara region, Maharashtra, India. This region lies between 21 degree 10' North latitude to 79 degree 39' East with a moderate to heavy rainfall in monsoon.[3]

Period of study – Six months

Inclusion criteria – Water samples from only those sources which were used for supply of drinking water were collected.

Exclusion criteria – Water samples from sources supplied for other domestic purpose were excluded.

Type of study - Descriptive cross-sectional study based upon Simple Random Sampling Technique.

In this prospective study samples were collected through the following method:

Sample Collection - Water samples were collected from 23 villages of Bhandardara region. Covering villages under two Rural Health Centres (RHC – Shendi and Rajur, established by Pravara medical Trust &Pravara Institute of Medical Sciences – Deemed to be University in Tribal area of Bhandardara region covering the total population of 35,407.

Under Shendi RHC, the sub centres covered were following villages: Shendi, Bari, Waranghushi, Guhire, Bhandardara, Chichwandi, Waki, Mursheth, Mutkhel, Panjare, Ud dawade, Samarat, Ghatghar, Ratanwadi, Manere .

Under Rajur RHC, the sub centers villages covered were- Rajur, Kelungan, Jamgaon, Deshmukhwadi, Vahandulwadi , Kohondi , Malegaon.

The water samples were collected in sterile glass bottles and transported securely to the Microbiology Department for the bacteriological assessment.

Water samples were subjected to bacteriological assessment by two methods:

1. Most Probable Number (MPN) method-

- The indicator medium used was Mac Conkey's broth containing bromocresol purple to indicate colour change to yellow for the formation of acid from the lactose in the broth.
- An inverted Durham tube in each bottle or tube was inserted in the media to determine the gas formation.

Presumptive Counts:

- 50ml and 10ml volume of Mac Conkey's broth at double strength concentration and 5ml volume at single strength were placed into suitable sized bottles or tubes containing an inverted Durham tube.
- Inverting the bottle containing the sample of water rapidly several times to mix and distribute any deposit. Aseptically a little of the water was discarded and replaced with cap to shake the bottle up and down 25 times.
- Incubation: The seeded medium was aerobically incubated at 37degree C.
- After every 24hrs and 48hrs of incubation, inspection of the media was carried out and the number of cultures of each volume of water that showed the production of acid (colour change) and gas was noted, which was considered as presumptive positive growth.
- Using the reference of tables of Most Probable Number (MPN), the count of presumptive coliform bacilli present was determined.(4)

2. E coli detection

E coli detection test was used in parallel with MPN test for the detection of presence or absence of coliform bacteria in water from nearby treatment plants or distribution system.

The Kit contains 1 sterile disposable bottle -100ml capacity, dehydrated medium -3X concentration, 1 ziplock bag.

Method of test: 100ml water was tested in ziplock bag and transfer to sterile disposable bottle. Entire quantity of dehydrated medium (PA Broth) was added slowly to water by swirling to dissolve the powder completely. After dissolution, bottle was incubated for 24 - 48hr at 30-35^{0}C, change was observed in the medium from reddish purple to yellow, indicating the presence of coliform bacteria.(5)

Thus by using above method, analysis of the quality of drinking water was done. Proforma was used to ask few questions to the village people regarding drinking water and its problems.

Thus a conclusion wasdrawn on the quality and portability of water in tribal area based upon the detection test and the questionnaire. The questionnaire will be translated in local language

Implication

The simple implication were:

- Collection of samples in required quantity in a proper sterilized bottle.
- Proper transportation of samples from site of collection to microbiology laboratory.
- Standard counts of the organism as per the APHC 2005.
- Thus making tribal people aware of water contamination and it hazards .
- Implying the previous knowledge and improving the health status of tribal people.

Results-

Results are made based on Tables of most probable number, Swaroop [1938, 1951]. Samples were collected from the 23 villages of Bhandardhara district (Table 1), amongst those MPN count being least in Bhandardhara village and highest in 5 villages being Samarat , Vhiandul Wadi , kohondi , Akole, Malegaon. MPN values were calculated for 10ml, 1ml and 0.1 ml and final MPN count per 100 ml was done for all 23 samples. The MPN count /100ml was highest in Samarat, Vahandul wadi, Kohonoli, Akole and Malegaon (>1800). The lowest MPN count per 100 ml was found in Bhandhardhara (22) and Waranghushi (26).

The isolated organisms are *Klebsiella spps , E.coli, Pseudomonas spps.* In the present study Klebsiella species being highest isolate (60.86%) which included two different species of Klebsiella namely Klebsiella pneumoniae being 85.77% and Klebsiella oxytoa being 14.29%. (Table: 3)

The second most common isolate was *E.coli* (21.73%) followed by *Pseudomonas species* (17.39%). Since the coliform count was high in all the 23 samples, hence the water was found unfit for drinking. (Table: 3)

In some villages- Shendi, Bhandardhara, Chninchwadi, Ghatghar & Jamgoan we could isolate E coli indicating recent fecal contamination.

Thus treatment of the drinking water before consumption is necessary. The method should be cheap, easy to do, at household level as well as at village level.

Table 1: Report Of The Water Samples From The Tribal Area Of Bhandardhara Region

SAMPLE NO	NAME OF THE VILLAGE	MPN VALUES 10 ml	1ml	0.1 ml	MPN* /100 ml	Name of the organism isolated	Coliform Kit Hi Media
1	Shendi	5	5	3	920	E coli	Not fit for drinking
2	BARI	5	5	3	920	Klebsiella pneumonie	Not fit for drinking
3	Waranghushi	2	5	3	26	Klebsilla pneumonie	Not fit for drinking
4	Ghaire	5	5	3	920	Klebsiella pneumonie	Not fit for drinking
5	Bhandardhara	4	2	0	22	E coli	Not fit for drinking
6	Chninchwadi	5	5	4	1600	E coli	Not fit for drinking
7	Waki	4	3	3	45	Klebsiella pneumonie	Not fit for drinking
8	Mursheth	4	2	4	44	Pseudomonas	------
9	Mutkhel	5	5	4	1600	Klebsiella pneumonie	Not fit for drinking
10	Panjore	5	5	1	350	Klebsiella pneumonie	Not fit for drinking
11	Uddawane	5	4	1	170	Klebsiella pneumonie	Not fit for drinking
12	Samarat	5	5	5	>1800	Klebsiella pneumonie	Not fit for drinking
13	Ghatghar	5	3	0	79	E.coli	Not fit for drinking
14	Ratanwai	5	2	0	49	Pseudomonas species	-----
15	Manere	4	2	3	38	Klebsiella pneumonie	Not fit for drinking
16	Rajur	4	2	3	38	Klebsiella pneumonie	Not fit for drinking
17	Kelungan	5	1	1	46	Klebsiella pneumonie	Not fit for drinking
18	Jamgaon	5	1	1	46	E. coli	Not fit for drinking

19	Deshmukhwadi	5	2	3	120	Klebsiella oxytoca	Not fit for drinking
20	Vahandul wadi	5	5	5	>1800	Klebsiella pneumonie	Not fit for drinking
21	Kohondi	5			>1800	Pseudomonas species	---
22	Akole	5			>1800	Pseudomonas species	---
23	Malegaon	5	5	5	>1800	Klebsiella oxytoca	Not fit for drinking

*MPN: Most Probable Number Grades of the Quality of drinking water Less than 10/100 ml as Satisfactory (4)

Table 2: Descriptive Statistics for MPN (/100 Ml) Values Of Water Samples From The Tribal Area Of Bhandardhara Region (n=23)

Water Sample (n=23)	MPN (/100 ML)
Mean ± SD	630.14 ± 786.36
Lower 95% confidence limit	272.19
Lower 95% confidence limit	988.10
Minimum	22
Median	120
Maximum	>1800

Table No.3: Descriptive Statistics of the Organism isolated of water samples from the Tribal Area Of Bhandardhara Region (n=23)

	Total Number	**Percentage (%)**
E coli	5	21.74%
Klebsiella species	14	60.87%
Pseudomonas species	4	17.39%
Total	**23**	**100%**

Value of $\chi^2 = 11.627$, p=0.0030, significant

By applying Chi-Square test there is a significant association between organisms isolated of water samples from the tribal are of Bhandardara region

Statistical analysis:

Statistical analysis was done by descriptive statistics as mean, SD, percentage etc.

Association of qualitative data with percentage values was analysed by applying applying Chi-Square test at 5% (p, 0.05) and 1% (p, 0.01). The statistical data analysis software namely, SYSTAT version 12 (By Crane's Software's, Bangalore) was used to analyze the data.

Discussion –

The significance of safe drinking water & its role in waterborne illness every year has been well documented world over. Earlier epidemics due to waterborne illness, which killed thousands of people and outbreaks that were traced to the municipal water supply have been curtailed, after the source was detected.[6]

However in India the tribal population situated in the remote areas, often are deprived of safe drinking water, largely due to non availability of testing the drinking water that is received from various sources.

Hence tribal people are exposed to the utilization of unpotable water with increased risk water borne illnesses and little access to health care services

Even in urban areas water of high quality does not come easily and presence of any coliform organism in drinking water, must be looked into as a part of problem and part of solution as well.

It is known that microbial agents survive in natural water for long periods without human host, but it is paramount to monitor the drinking water, ensuring it is free from any infections agents.[7]

The present study was undertaken to detect the fecal contamination as the Environmental Protection Agency standards for water sanitation is based on coliform bacilli such as *E. coli, Klebsiella species, Citrobacter, Enterobacter* etc. Similar study from tribal community residing in Jawadhi hills, Tamilnadu also correlated high faecal contamination with poor ' wash score'. [8].

In our study all the 23 samples were unfit for drinking. The descriptive statistics for MPN per 100 ml showed minimum 22 to more than 1800 count. These results are comparable to study from Rajasthan with total coliform count ranged from 25 MPN/100 ml to 41 counts [9]. According to other study by Byragi Reddy et al, MPN counts 1100MPN/100ml was recorded as highest in Lambsingi and lowest at Burada Veedhi with value of 39MPN/100ml [2].

The present study showed growth of gram negative bacilli such as *Klebsiella spps* (60.87%), *E.coli* (21.7%) and *Pseudomonas spps* (17.39%). In a similar study from Rajasthan [9] the isolates from the water samples included gram negative bacilli along with gram positive organism like *Staphylococcus aureus, Bacillus cereus.* The study from tribal areas of Chintapalli Mandal identified pathogenic and non pathogenic isolates which included *E coli, Staphylococci spps, Shigella species, Salmonella sp, Vibrio species, Pseudomonas species, Enterobacter aerogenes and Aeromonas sp* [7,10]

Hence the present study lays much emphasis on need to ensure safe drinking water which regular testing of water samples in remote tribal areas through community engagement and advocacy for water quality. In this regards rapid water testing kits can be made available to the tribal PHC's & local panchayats, with proper training the village health workers can test the water samples.

Conclusion:

The present study also points to the fact that "Health Education along with awareness of Safe drinking water" needs to be regularly undertaken for the tribal people. This can help avoid any outbreaks as the tribal populations resides in hamlets with difficulty to access by road.

Even as the microbiologist are calling for decontamination of use of coliforms (Ref) as an only indicator of fecal contamination, however in a developing country like India and with approximately 8.6% tribal people residing in remote areas, use of rapid kits for detection of coliforms in drinking water supply can only be the solution

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