MICROBIOLOGICAL ANALYSIS OF DRINKING WATER QUALITY OF RATLAM, M.P. INDIA

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Abstract: The aim this research is find out the microbiological quality of drinking water and to analyze the factors involved in the knowledge of the public about the quality of drinking water in Ratlam city of western India.

A total of 90 water samples were collected from Dholwad dam and Morwani water treatment plant on daily basis for one month. Total coliforms, fecal coliform and fecal streptococci were counted using MPN (Most Probable Number method). An interview was conducted with 1200 residents using a questionnaire. Results indicate that total coliforms were detected in 4 (10%) of 40 samples from wells, 13 (32.5%) of 40 samples from tankers and 55 (68.8%) of 80 samples from roof tanks. Twenty (25%) and 8 (10%) samples from roof tanks were positive for *E. coli* and *Streptococcus faecalis*, respectively. Of the 200 inhabitants participating in the study, 55.5%, and 44.5% claimed that they depended on municipal water and well water respectively. The majority (95.5%) reported the use of roof water tanks as a source of water supply in their homes. Most people (80%) believed that drinking water transmitted diseases. Our results could help health authorities consider a proper regular monitoring program and a sustainable continuous assessment of the quality of well water. In addition, this study highlights the importance of the awareness and educational programs for residents on the effect of polluted water on public health.

Keywords: Coliforms, drinking water, fecal streptococci, *Pseudomonas aeruginosa*, well.

Introduction: Access to safe drinking-water and basic sanitation by 2015 is one and an important target of WHO Millennium Development Goals. But unfortunately, 783 million people still short of improved water supply and more than 2.5 billion people had no access to basic sanitation. [1] The quality and safety of drinking water remain as an important public health issue. Contamination of drinking water has often been blamed for the diffusion of infectious diseases that have caused serious illnesses with associated mortality worldwide. [1,2] An estimated 1.9 million deaths, mostly of children of under 5 age is due to inadequate sanitation and hygiene of drinking water.[3] According WHO improving water, sanitation, and hygiene could prevent approximately 9.1% of the global burden of water borne diseases deaths.[4,5]

Water may be contaminated by physical, bacterial, and chemical origins and many methods are available to deal the issue. [6] But, microbiological quality is the most important feature of drinking water with respect to waterborne diseases. Microbiological estimations, predominantly for total coliforms, fecal coliforms, and fecal streptococci are renowned as the major indicators of the presence of pathogenic enteric bacteria in water resources. [7]

Previously, the assessment of the microbiological quality of drinking water has been performed through the analysis of fecal pollution indicators in finished drinking water, which is anticipated to predict the possible presence of pathogenic microorganisms in the water.[8] However, earlier studies have reported cases in which the indicators have been present in the water when it was served to public.[2,4,8,9] For that reason, WHO has developed several guidelines for the exclusive purpose of examination the quality of finished drinking water.[10,11]

Ratlam city is the important tribal district of Malwa regions located on northwest part of Madhya Pradesh at latitude 230 05' and 230 52' North and between the meridians of longitude 740 31' and 740 41'. The district is bordered by Mandsaur district in the north, Jhabua and Dhar district in the south, Ujjain and Shajapur districts in the east, Banswara district of Rajasthan state in the west and Jhalawar district of Rajasthan state in the northeast.

Ratlam constituency falls under Ganga and Mahi river basins. The tributaries of Chambal River drain about 70 % geographical area of the district. Southwest part of the district is drained by the Mahi River and its tributaries. Dholwad reservoir is main water source of the town. Water is supplied from this dam to Morvani water treatment plant from where it supplied to the public. In order to compensate the water demand the municipal corporation has also provided many tube wells and hand pumps at several points of the city. People store the water in pits and overhead tanks. Till today, the city does not have complete underground sewerage system. Besides, many wells scattered throughout the region are used as the source of drinking water which may lead to cause many water borne diseases due to poor hygienic and improper drainage system. [12]

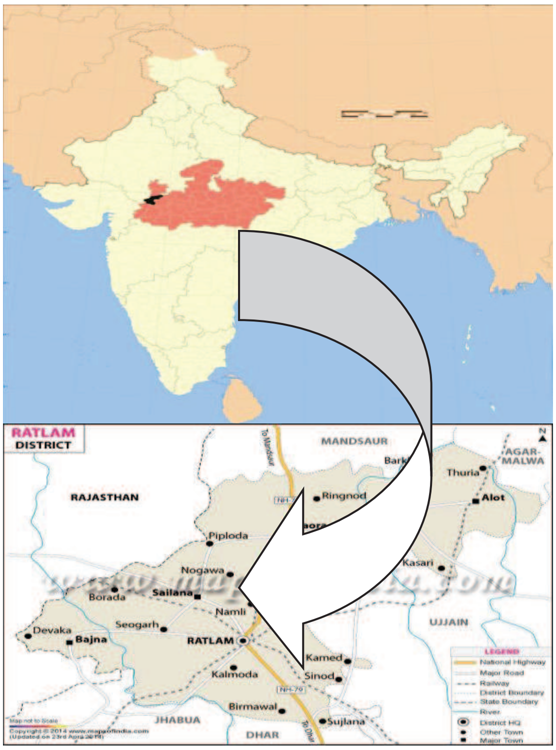

Fig.1. Map showing the location of Ratlam in India

Therefore we aimed to investigate the quality of drinking water from source (including wells, pits and overhead tanks) to the point-of-use in Ratlam region of western India, using bacteriological techniques. This study also aimed to explore the factors involved in public knowledge, self-described behaviors, and perception of the quality and risks of drinking water in the study area. The findings could be useful in deciding appropriate remedial measures for preventing contamination of drinking water and help as a basis for decisions on water health policy at different administrative levels in India.

Materials and Methods: Water samples were collected in accordance with the standard methods for the examination of water and wastewater.[13] A total of 90 water samples (30 samples from wells, 30 samples from pits and 30 samples from roof tanks) were collected from both urban and rural areas of the Ratlam town from December, 2015 to January, 2016. Samples were collected in 250 ml sterile glass bottles. They were kept in ice boxes and sent to the Microbiology Department of the Government PG Arts and Science College for bacteriological examination.

Total coliforms, fecal coliforms, and fecal streptococci were counted using Most Probable Number (MPN) method as previously described. [14]

MPN estimation:

MPN/100 mL = no. of positive tubes 100 √

mL sample in negative tubes mL sample in all tubes.

Coliform bacteria were determined by incubation of samples into tubes of lactose broth (Himedialabs, India) at 35°C for 48 hrs. Fecal coliforms were detected by subculture into brilliant green bile broth 2% (Himedialabs, India) and incubation at 37°C for 24–48 hrs. Positive samples were further inoculated into *Escherichia coli* broth (Himedialabs, India) and incubated at 44°C for 24–48 hrs. *E. coli* was identified by the standard biochemical tests. Fecal streptococci were detected by inoculation of water samples into azide dextrose broth (Himedialabs, India) and incubation at 37°C for 24–48 hrs.

The total viable bacteria (TVB) were enumerated using the TVB pour plate count method. [15] All water samples were first inoculated into water plate count agar media (Himedialabs). Two sets of plates were used for all samples. One set was incubated aerobically at 37°C for 48 hrs and the other set at 22°C for 72 hrs. All colonies were counted as colony forming unit per ml of the water sample. Then, the suspected colonies were sub-cultured into MacConkey and blood agar media and incubated at 37°C for 24 hrs. The organisms which were presumed to be pathogenic bacteria were identified at the genus and/or species level by Gram-staining, culture characters, and biochemical tests.

The target population was the residents of Ratlam region. The sample size was determined in order to have 95% confidence limits of 5% maximum error of the estimate. [16] The minimal sample size required for the study was calculated as 1090 residents to represent the entire population on a statistical basis. To avoid a no-response expectation, the sample size was increased to 1200 residents. The questionnaire included questions related to the following: Personal profile of the study population (age, occupation, and level of education), various aspects of domestic water supply for the people who live in the study area (source of drinking water and age of water network and the use of roof water tanks and information about them), situation of wastewater networks system (connection to sewage network, age of sewage network in the area and seasons of sewage flood) and knowledge of the study population of drinking water contamination.

Statistical Analysis: The entire study was performed in triplicates. The data were recorded as means ± standard deviation and analyzed using Microsoft excel. Student t-test was used to analyze the significant differences between their means. Differences between means at 5% significant level (P-value < 0.05) were considered.

Results: The results of present study were summarized in table 1 and 2. The conventional culture technique showed that 6 (15%) of 40 samples from wells, 12 (30%) of 40 samples from storage pits and 50 (62.5%) of 80 samples from roof tanks were positive with total coliforms. [Table 2].

In this study, the mean age of participants was 32.8 ± 8.9 years. More than half of those interviewed (57%) had a university degree, indicating a well-educated community. The response of the study population on various aspects of drinking water supply is summarized in Table 3. Only 120 (10%) participants said that they drank municipal water. However, 546 (45.5%) claimed that they depended on bottled water followed by 534 (44.5%) who depended on well water. Most people (95.5%) reported that the source of their water supply in their homes was roof water tanks and the majority (89.5%) of them used white plastic tanks. Although 60.7% of interviewees saw sediments in the tanks, 42% of people did not clean them. Most people (67.5%) said that their homes were not connected to the sewage network system. The majority (70%) of people did not know how old the sewage network was. Most people (75%) believed that drinking water transmitted diseases. However, about half (51%) of the people thought that the water in Ratlam was suitable for drinking [Table 2.].

Discussion: The occurrence of waterborne diseases in India is mainly due to contamination of drinking water with industrial and urban sewage and waste. It is hard with the current knowledge presently available to assess the risk to health presented by any particular level of pathogens in water, since this risk will depend equally on the ability of infestation, infection and invasiveness of the pathogen and immunity of the individuals consuming the water. It is only cautious to guess that no water in which pathogenic microorganisms can be detected can be regarded as safe. Moreover, only certain waterborne pathogens can be identified reliably and easily in water and some cannot be detected at all [15]. However, the presence of microbes in drinking water advocates the existence of pathogenic organisms that are the source of water borne diseases.[8] In the present investigation, the results of total coliform count showed that 28% of the samples from wells exceeded the standard values recommended by national (BIS) and international standards (WHO) of drinking water.[11].

In a recent study by Afshan, N et al [12] at Karachi, Pakistan, found that 60% samples were found highly contaminated. Another major study found the bacteriological characteristics of drinking water in Southwestern Saudi Arabia. They also detected fecal coliform, and fecal streptococci were detected in 87.9% and 57.6% of 33 well water samples. [13] In 2015, Chouhan et al [14] assessed the drinking water sold by roadside vendors of Delhi, India and all 36 samples were found to be contaminated with coliform organisms in the range of 14 to >1600 per 100 ml of sample. Our results were already expected since the wells are not given any chlorine disinfection treatment. The presence of coliform may be attributed to contamination of the houses used by farmers and livestock owners. [17] Earlier studies have pointed out that dust storms and livestock activity in the surrounding area of surface wells increase microbial levels and bacterial input. [18, 19, 20] Our results are troublesome yet well water is still the main source of drinking water in this area.

According to the responses to the questionnaire, 39% of interviewees depend on well water for drinking. In this study, 55% of the water samples from tankers had higher total coliform than national and international standard values. [11] Poor microbial quality of community tanker water in our study is likely to be due to contamination of dispensing devices. Contamination of water in the tanker by dust during transportation and the lack of or inadequate water treatment is also a major factor. [21] Moreover, water is stored in the tanker for long hours thus promoting bacterial growth and development. [22]

The present study revealed higher levels of bacterial indicators from roof tanks in many samples than the national and international standard values. [11]. Very recently Chandra, S et al [23] assessed the microbiological and physicochemical quality of drinking water supplied in Jaipur and found that many parameters except few were found to be within bureau of Indian standards safety limits. It also confirms the presence of pathogenic bacteria in drinking water. Earlier reports suggested that water contamination noticeably occurred during storage in house reservoirs, and was possibly implicated in the increased prevalence of water borne disease in the particular region[24] Likewise, our results indicated that the water gets more get worse at the point-of-use than at the source. This could be a result of biofilm growth in the household tanks. [11]. In the present study, 96% of candidates stated that roof tanks for water storage is a common practice in Ratlam region and 59% of people in this study stated that they had observed sediments in their roof tanks. The lack of cleaning as admitted by 56% of the interviewed people may contribute to water contamination. Earlier reports reveal that diarrhea and other water borne diseases were strongly linked with the cleaning of water tanks. [25,26]. Flooding the sewage in monsoon may be the cause of the penetration of wastewater which in turn may contribute to microbial contamination of water in the wells and house tanks.

In order to preserve the quality of drinking water in roof tanks as received from the source, it would be essential to implement effective awareness and educational programs among the public. Though many candidates in this study reported that drinking water and water from roof tanks transmitted diseases, but only few had attended any environmental awareness programs conducted by public health department.

Conclusions: Bacteriological contamination of water samples between the source and point of use in Ratlam region is extensive and highly upsetting. The local health officials should consider a proper regular checking program to constantly evaluate the quality of well water. Suitable methods should be found to prevent the worsening of the quality of well water and eliminate health problems. Public tankers should be treated with chlorine and thoroughly washed regularly. Safer household water storage and treatment is recommended to prevent post collection contamination.

Table.1. Standards of coliform in different water sources

Use	Total Coliform MPN per 100 ml	Faecal Coliform MPN per 100 ml	Agency / Country
Public water supply	0	0	WHO
Drinking water source, no conventional treatment, with disinfection	<50	No value	India
Drinking water source, with conventional treatment, and disinfection	5000	No value	India
Bathing, recreation water	5000 guide 10,000 mandatory	100 guide 2000 mandatory	Europe
Outdoor bathing (organized)	500	no value	India

Table 2. Bacteriological analysis of the different drinking water sources in Ratlam

Source	Turbidity (nephelometric turbidity units, NTU)	Source	Total coliforms%	Faecal coliforms
Wells	1.4±0.2	Wells	11±21.3	3.21± 0.5(NSPP)
Overhead Tankers	0.8 ±0.1	Overhead Tankers	3.1±0.8	O.91±0.11(NSPP)
Storage Pits	1.1±0.3	Storage Pits	4.11±1.2	11±5(NSPP)
Pipe lines	0.4±0.01	Pipe lines	1.1±0.2	0.1±0.01(NSPP)

*Not Suitable for Potable Purpose (NSPP)

Tab.2. Tab.2 Survey testing the knowledge of the population on various aspects of drinking water in

S.No.	Variable	N%
1.	Source of Drinking water Bottled water Well water Municipal water	 24 55 21
2.	Use of Roof tanks Yes No	 95.5 4.5
3.	Type of Tanks Plastic white Plastic black	 15 85
4.	Cleaning of water Tanks (Yearly) Yes No	 12 88
5.	Settlements Observed Yes No	 85 15
6.	Connected to network Yes No	 80 20
7.	Age of sewage network 1-3 years 3-5 years	 55 35

	Above 5years	10
8.	Do you think drinking water transmit diseases Yes No	 95 05
9.	Do you thank roof tank water transmit diseases Yes No	 75 25
10.	Do you think water in Ratlam is suitable for drinking Yes No	 51 49

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