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Screening of Coliform group of bacteria and waterborne pathogenic bacteria from ground- water samples from Vasai, Thane district

RAJESH C. PATIL[1], DEVANSHI GOHIL[1], UJWALA JADHAV[2], MADHAV V. UPADHYE[2] and ABHISHEK MULE[1]

[1]Department of Microbiology, Bhavan's College, Andheri, Mumbai - 400 058 (India).
[2]Department of Life Sciences, University of Mumbai, Santacruz(E), Mumbai - 400 098 (India).

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ABSTRACT

The present study was carried out to study the microbiological characteristics of ground water samples collected from wells and bore wells near Mumbai. Samples were screened for coliform bacteria and waterborne pathogenic bacteria. The results indicated that all the water samples are highly polluted and not potable.

Key words: Ground water quality, Coliform bacteria, MPN, waterborne pathogens.

INTRODUCTION

Water pollution is a major problem in the global context. Contaminated water can transmit a variety of infectious diseases. Thus, proper treatment and maintenance of water and regular water testing can minimize risk of waterborne infections. Water quality is the composition of water affected by natural processes and human activities. Industrial and municipal discharge of contaminated sewage waters and chemical wastes and by-products are the major sources of water pollution.

Ground water comes from the small percentage of precipitation that falls, infiltrates the ground, traveling downward, and fills the available pore spaces within rock, sand, gravel, and clay. Any substance that comes in contact with the ground water can affect water quality. Ground water pollution is much more difficult to abate than surface pollution because groundwater can move great distances through unseen aquifers. The present study was undertaken to investigate the microbial quality of ground water samples collected from wells and bore-wells from Vasai in Thane district.

MATERIAL AND METHODS

Collection of samples

Water samples were collected from six different wells and bore wells from Vasai in Thane district. Sample 1 was collected from a well, Sample 2 was also from a well, Sample 3 was from a bore well, sample 4 was collected from a bore well, sample 5 was taken from well water and sample no.6 was again taken from a well. Each sample of about 1liter to 2 liter was collected in plastic bottles (clean & dry) from the upper 15 cm exposed area of water (Sharma and Kaur, 1998). The collected samples were then stored at refrigeration at temperature of 10 – 12°C and the analysis was carried out within 48 hours.

Table 1: Colony characteristics of isolated bacteria on EMB agar

		Colour	Size	Shape	Elevation	Opacity	Surface	Margin	Gram's Nature
Sample	Colony 1	Bluish-purple	Pin - point	Circular	Flat	Opaque	Smooth	Entire	Gram –ve coccobacilli
1	Colony 2	Pink	3 - 5 mm	Irregular	Flat	Opaque	Smooth	Irregular	Gram –ve short rods
Sample	Colony 1	Black with metallic sheen	2-3 mm	Circular	Flat	Opaque	Smooth	Entire	Gram –ve coccobacilli
2	Colony 2	Pink	5 - 6 mm	Irregular	Flat	Opaque	Smooth	Irregular	Gram –ve coccobacilli
Sample	Colony 1	Bluish-purple with metallic sheen	2 – 3 mm	Circular	Flat	Opaque	Smooth	Entire	Gram –ve thick rods
3	Colony 2	Pink	4 - 5 mm	Circular	Flat	Opaque	Smooth	Irregular	Gram –ve thick rods
Sample	Colony 1	Yellow	5 - 6 mm	Circular	Flat	Opaque	Smooth	Entire	Gram –ve thick rods
4	Colony 2	Light pink to colourless	2 - 3 mm	Irregular	Flat	Opaque	Smooth	Irregular	Gram –ve thin rods
Sample	Colony 1	Bluish-purple with metallic sheen	2 – 3 mm	Circular	Flat	Opaque	Smooth	Entire	Gram –ve thick short rods
5	Colony 2	Pink	3 - 5 mm	Circular	Dome	Opaque	Smooth	Entire	Gram –ve coccobacilli
Sample	Colony 1	Bluish-purple with metallic sheen	2 - 3 mm	Circular	Flat	Opaque	Smooth	Entire	Gram –ve coccobacilli
6	Colony 2	Pink	5 - 6 mm	Circular	Dome	Opaque	Smooth	Entire	Gram –ve thick short rods

Table 2: Colony characteristics of isolated bacteria on SS agar

		Colour	Size	Shape	Elevation	Opacity	Surface	Margin	Gram's Nature
Sample1	Colony 1	Pink with black center	2-3 mm	Circular	Flat	Opaque	Smooth	Entire	Gram –ve short rods
	Colony 2	Yellow	2-3 mm	Irregular	Flat	Opaque	Smooth	Irregular	Gram –ve thick short rods
Sample 2	Colony 1	Pink	3 - 5 mm	Circular	Dome	Opaque	Smooth	Entire	Gram –ve thick short rods
Sample 3	Colony 1	Pink	7 - 8 mm	Circular	Dome	Opaque	Smooth	Entire	Gram –ve rods
	Colony 2	Cream	4 - 5 mm	Circular	Dome	Opaque	Smooth	Entire	Gram –ve thick rods
Sample 4	Colony 1	Pink	7 - 8 mm	Circular	Dome	Opaque	Smooth	Entire	Gram –ve rods
Sample 5	Colony 1	Pink	3 - 5 mm	Circular	Dome	Opaque	Smooth	Entire	Gram –ve thick short rods
	Colony 2	Cream	2 - 3 mm	Circular	Dome	Opaque	Smooth	Entire	Gram –ve coccobacilli
Sample 6	Colony 1	Pink	2 - 3 mm	Circular	Flat	Opaque	Smooth	Entire	Gram –ve coccobacilli
	Colony 2	Cream	5 - 6 mm	Circular	Dome	Opaque	Smooth	Entire	Gram –ve thick short rods

Table 3: Colony Characteristics of isolated bacteria on TCBS agar

		Colour	Size	Shape	Elevation	Opacity	Surface	Margin	Gram's Nature
Sample 1	Colony 1	Green	2-4 mm	Circular	Dome	Opaque	Smooth	Entire	Gram –ve rods slightly curved
	Colony 2	Yellow	4-5 mm	Circular	Dome	Opaque	Smooth	Entire	Gram –ve coccobacilli
	Colony 3	Black	Pin - point	Circular	Flat	Opaque	Smooth	Entire	Gram –ve coccobacilli
Sample 2	Colony 1	Yellow	3 - 5 mm	Circular	Dome	Opaque	Smooth	Entire	Gram –ve short rods
	Colony 2	Black	1-2 mm	Circular	Dome	Opaque	Smooth	Entire	Gram –ve short rods
Sample 3	Colony 1	Green to blue	Pin point	Circular	Flat	Opaque	Smooth	Entire	Gram –ve short rods
Sample 4	Colony 1	Yellow	6 - 7 mm	Circular	Dome	Opaque	Smooth	Entire	Gram –ve short rods curved
	Colony 2	Black	1-2 mm	Circular	Flat	Opaque	Smooth	Entire	Gram –ve rods
Sample 5	Colony 1	Yellow	2 – 3 mm	Circular	Dome	Opaque	Smooth	Entire	Gram –ve short rods
Sample 6	Colony 1	Yellow	2 - 3 mm	Circular	Dome	Opaque	Smooth	Entire	Gram –ve thick short rods slightly curved
	Colony 2	Black	1-2 mm	Circular	Flat	Opaque	Smooth	Entire	Gram –ve rods

Table 4: Colony characteristics of isolated bacteria on Bile Esculin azide agar

		Colour	Size	Shape	Elevation	Opacity	Surface	Margin	Gram's Nature
Sample 1	No colonies found								
Sample 2	No colonies found								
Sample 3	No colonies found								
Sample 4	Colony 1	Off – white	Pin point	Circular	Flat	Opaque	Smooth	Entire	Gram + ve diplococci
Sample 5	No colonies found								
Sample 6	Colony 1	Off – white	Pin point	Circular	Flat	Opaque	Smooth	Entire	Gram + ve diplococci

Table 5: Result of most probale number and enrichment of pathogenic bacteria

	MPN: Number of tubes giving positive reaction out of			MPN Index per 100 ml	Gram's staining	Interfence	Alkaline peptone water Gram's staining	Azide dextrose broth with BCP Gram's staining	Mac conkey's broth's Gram's Staining
	3 of 10 ml	3 of 1 ml	3 of 0.1 ml						
Sample 1	3	3	3	2400	Gram –ve coccobacilli	Water sample is not potable	Gram –ve short rods and Gram + cocci	Artifacts	Gram –ve thick rods
Sample 2	3	3	3	2400	Gram –ve coccobacilli and artifacts	Water sample is not potable	Gram –ve thin short rods some slightly curved	Gram + cocci in chains	Gram –ve short rods
Sample 3	3	3	2	1100	Gram –ve coccobacilli & Gram – ve thin rods.	Water sample is not potable	Gram –ve thick rods	Gram + ve diplococci	Gram –ve thick rods
Sample 4	3	3	2	1100	Gram –ve coccobacilli and artifacts & Gram –ve thin rods	Water sample is not potable	Gram –ve thin short rods artifacts	Gram + cocci in chains some diplococci	Gram –ve short rods
Sample 5	3	3	3	2400	Gram –ve coccobacilli	Water sample is not potable	Gram –ve thick rods comma shaped	Gram + ve diplococci	Gram –ve long rods
Sample 6	3	3	3	2400	Gram –ve coccobacilli	Water sample is not potable	Gram –ve curved rods	Gram + ve diplococci	Gram –ve thin long rods

Enrichment of coliform group of bacteria (*E. coli)*

Presumptive test

Enrichment cultures were prepared in lauryl tryptose broth of single strength (6 tubes) and double strength (3 tubes) according to the size of water sample i.e. 0.1 ml & 1ml of sample in 5 ml single strength broth and 10ml of sample in 10 ml double strength broth.0.1 ml water sample was dispensed in each tube 1, 2, 3 and 1 ml in each tube 4, 5, 6 and 10 ml in 7, 8, 9. All the tubes were incubated at 35°C for 24-48 hours for the gas production. After incubation, the most probable number (MPN) of coliforms i.e MPN index per 100 ml was found out (Dubey and Maheshwari, 2002).

Confirmed test

For confirmation of a positive result in the presumptive test 0.2 ml of broth is inoculated into Brilliant green lactose bile broth (BGLB) tube. If gas is seen in the BGLB tube it indicates that the gas in the presumptive test tubes was indeed produced by the coliforms.

Enrichment of pathogenic bacteria

Salmonellae and Shigellae

Enrichment cultures were prepared by adding 5 ml of water sample to 100 ml of flask containing 50 ml of Selentine broth. Incubation was at room temperature under static condition. Bacterial growth was monitored every 2 days.

Vibrio

Enrichment cultures were prepared by adding 5 ml of water sample to 100 ml of flask containing 50 ml of Alkaline peptone water. Incubation was at room temperature under static condition. Bacterial growth was monitored every 2 days.

Streptococcus faecalis

Enrichment cultures were prepared by adding 5 ml of water sample to 100 ml of flask containing 50 ml of Azide dextrose broth with BCP. Incubation was at room temperature under static condition. Bacterial growth was monitored every 2 days.

Isolation and characterization of coliform & pathogenic bacteria isolation

Bacteria from each enriched broth were isolated on selective plates according to the type of bacteria under study. Isolation was carried out in duplicates. EMB agar was used for *E. coli*, SS agar for *Salmonella* and *Shigella* spp., TCBS agar was used for *Vibrio* spp. Whereas Bile Esculin Azide agar was employed for isolating *Streptococcus faecalis*. The plates were incubated at 37°C for 24 to 48 hours and the number of colonies on each plate was noted (Hi Media, 1998).

Characterization and identification of the bacteria

The morphological features such as colour, size, margin, elevation and gram's nature of each colony were noted. The isolated organisms were then maintained on nutrient agar slant overlayed with paraffin oil. The bacterial isolates were identified on the basis of Bergey's Manual of Systematic Bacteriology (Brenner *et. al*., 2005).

RESULTS AND DISCUSSION

It is not practical to test water directly for every possible disease-causing bacterium, virus, and protozoan, so the water is tested instead for a group of indicator bacteria, which measure the sanitary protection of the well and water system. This group of common bacteria, called the "total coliform group," is a good indicator of sanitary protection. Most coliform bacteria do not themselves usually cause disease, but they can indicate that surface contamination has somehow gotten into the water, and disease organisms may also be present. Pathogenic organisms found in water may be discharged by human beings who are carriers of a particular disease. These organisms are highly infectious and are responsible for many thousands of deaths each year. Hence screening of coliform bacteria and pathogens was carried out to analyse the potability of the water samples.

As seen in table 1, all the water samples were found to contain coliform bacteria *E. coli* which were selectively isolated on EMB agar.

Isolation of coliform and pathogenic bacteria on SS agar indicated that all the six water samples contained *Salmonella* and *Shigella* spp. (Table 2).

TCBS agar was employed for isolation of *Vibrio cholerae* and agar plates after incubation showed presence of *Vibrio cholerae* in all the water samples under study (Table 3).

Table 4 shows that isolation on Bile Esculin azide agar suggests that sample 4 and sample 6 contained *Streptococcus faecalis*.

The results of the present study indicate that water samples under study contained pathogenic bacteria such as *Vibrio cholerae* , *Streptococcus faecalis* and coliform group of bacteria such as *E. coli* indicating that all the water samples are highly contaminated and not potable which was confirmed by Most Probable Number and isolation, enrichment and characterisation of coliform and pathogenic bacteria on selective media (Table 5). If such water samples are consumed for industrial and house hold work, then they can be quite harmful to human health. Thus, it is advocated that prior to their use for human consumption, such ground water samples from wells and bore wells must be microbiologically anlysed to prevent various infectious diseases.

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