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BACTERIOLOGICAL ANALYSIS OF DRINKING WATER FROM 100 FAMILIES OF LAHORE BY MEMBRANE FILTRATION TECHNIQUE AND CHROMAGAR

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ABSTRACT

Introduction: Water borne diseases are caused by pathogenic microorganisms. In Pakistan, the availability of safe water is only 40% to 60%. Therefore it becomes imperative to determine the bacteriological status of drinking water. A few laboratories perform such an evaluation and, that too, by the old method technique i.e *Most Probable Number (MPN). We evaluated 100 samples of drinking water from some areas of Lahore by the Membrane Filtration Technique (MFT) using CHROMagar. Using this technique in one step a much large volume of water can be evaluated quantitatively in a short time and with ease. Use of CHROMagar straightaway confirms the presence of Escherichia coli which is accepted universally as the indicator of fecal contamination.*

Materials and Methods: It was a cross sectional study. A volume of 100 ml water was filtered under the vacuum pressure through Millipore membrane filters. After filtration, membrane filters were placed on CHROMagar and incubated at 35°C for 24 hr. Escherichia coli appeared as blue coloured colonies while coliforms yielded colonies of pink colour. Escherichia coli were further identified by API 20E and confirmed by Eijkman test.

Results: Escherichia coli was grown from 42% samples (all Eijkman positive). Coliform organisms were grown from 54% specimens.

Conclusion: It was alarming that 59% of drinking water was unsatisfactory for human consumption.

Keywords: Coliforms, Escherichia coli, Membrane filtration Technique, CHROMagar, IMViC, Eijkman.

INTRODUCTION

Public and environmental health protection requires safe drinking water. Present day water sources are being polluted largely by agricultural and industrial chemical waste disposals due to cross contamination with sewers, illegal connections, leakages and corrosions.[1] The diversity of waterborne diseases and their severity is more in underdeveloped countries especially in Pakistan, in rural as well as in urban areas the bacteriological contamination of drinking water has been reported to be one of the most serious problems.[2]

Water pollution causes a number of diseases like diarrhoea, dysentery, cholera, typhoid and infectious hepatitis.[3] World Health Organization (WHO) survey has revealed that 1.1 billion people all over the world do not have access to pure and safe drinking water. This situation is worst in Pakistan, as availability of safe water has been reduced from 60% to 40% due to the increased urbanization that has increased from 31% to 34%.[4] According to WHO biological contamination of water is responsible for 80% of all human illnesses in the developing world.[5] The problem has become manifold in our country, where studies have indicated widespread contamination of drinking water in main cities e.g. in Lahore 81.4%.[6]

A wide range of pathogenic microorganisms can be transmitted to humans via water contaminated with fecal material. Bacteriological quality of drinking water is primarily determined by using "indicator organisms", whose presence indicates fecal contamination.[7] Higher the level of indicator bacteria, higher the level of fecal contamination and greater risk of contracting disease.[8] Coliforms especially *Esch. coli* is the recommended indicator organism for portable water and indicator of direct or indirect fecal contamination.[9] It is found in large number in the intestinal flora of humans.[10] Fecal coliforms should not be present in 100 ml of drinking water especially *Esch. coli*.[11]

The Most Probable Number (MPN) and Membrane Filter Technique (MFT) are reference methods used for monitoring the quality of water. The MPN method provides results after 3 to 4 days and the interference by a high number of non coliform bacteria have been shown to alter the efficiency of the analysis whereas MFT requires only 1 day.[12]

CHROMagar simultaneously detects coliforms and *Esch. coli* on the basis of β-galactosidase and β-glucuronidase enzyme activity respectively.[13] CHROMagar contains Rose-Gal and X-Glu (chromogens) for the detection of coliforms and *Esch. coli* respectively. When β-galactosidase attacks Rose–Gal it produces pink coloured colonies (coliforms). *Esch. coli* a coliform has the ability to produce both enzymes β-galactosidase and β-glucuronidase. When these act on both substrates purple coloured colonies are formed.[14] The present study we also included the Heterotrophic Bacterial Count (HPC) / viable count. "Heterotrophic bacteria" includes all bacteria that use organic nutrients for growth. High levels of Heterotrophic bacterial count posses increased health risks.[15]

MATERIALS AND METHODS

Prior to the start of the study, approval was obtained from the Ethical Committee, University of Health Sciences, Lahore, Pakistan. It was a cross sectional study conducted at the Department of Microbiology, University of Health Sciences, Lahore.

Water samples

One hundred drinking water samples were collected from various areas of Lahore (One hundred families). Sterile autoclave proof glass sample bottles of volume capacity 250 ml were used for water collection. Whereas, the water was boiled at 100°C for 1 – 2 minutes (according to the consumers) and then transferred to the sample bottle.

Transportation of water samples to the laboratory

An insulated cold box was used to transport the samples to the microbiology laboratory, UHS and processed within 6 hours after collection.

Physical appearance

All water samples were analysed physically by their appearance either clear or having contamination in the form of brown particles.

pH determination

The pH of all water samples was determined by using pH meter.

Membrane Filtration Technique

Membrane filtration technique (MFT) was used for the processing of all water samples by using Millipore membrane filtration system. Drinking water samples were filtered from the Millipore membrane filters (with pore size 0.45 µm and 47 mm in diameter) with a vacuum speed 5 to 15 mmHg. Organisms get concentrated on the surface of the membranes. These membrane filters were then placed on the CHROMagar surface and incubated at 35°C for 24 hours. The colonies formed on the surface of the CHROMagar were counted with the help of colony counter. Coliforms appeared in the form of pink coloured colonies and *Esch. coli* purple in colour (Fig. 1). They were represented in the form of Colony Forming Unit per 100 ml as CFU/ 100 ml, e.g.; 24 CFU of *Esch. coli* or coliforms / 100 ml.

Viable Count

A sterile spreader was used to spread the 10 µl water sample on MacConkey agar and then incubated at 37°C for 24 hours. After the incubation the colonies were counted.

Esch. coli identification

The representative purple coloured colonies of *Esch. coli* were subcultured on the MacConkey agar. Identification was done on the basis of cultural characteristics, gram staining, biochemical profile (IMViC), API 20E and Eijkman test.

Statistical Analysis

The data was analysed by computer software program SPSS 16.0. The frequencies and percentages were calculated. Chi sq and ANOVA was applied to evaluate statistical significance.

RESULTS

Drinking water samples collected from different areas of Lahore were distributed into the 9 towns of Lahore city (Table 1). Three types of water samples were collected; tap water (n = 71), filtered water (n = 17) and boiled water (n = 12).

Table 1: *Different areas of Lahore divided into 9 towns.*

Sr. No.	*Towns*	*Areas*
1.	Aziz Bhatti Town	5
2.	Data Gunj Bukhsh Town	4
3.	Gulberg Town	20
4.	Iqbal Town	34
5.	Nishter Town	12
6.	– Saman Abad Town	15
7.	Shalimar Town	5
8.	Wahga Town	5
9.	Ravi Town	0
	Total	100

The pH of the boiled water was observed to be more than the filtered water and tap water. The mean pH of boiled water, filtered water and tap water have significant difference (p = < 0.01). After applying the post Hoc test significant difference was observed (p = < 0.01) between the boiled water, filtered water and tap water. The pH of the boiled water was observed to be more than the filtered water and tap water; whereas no significant difference was observed between the pH of filtered and tap water (p = 0.470).

Table 2: *Esch. coli count grouping in different types of water samples.*

Sample type	*Esch. coli count groups*		*Total*
	Group 1	*Group 2*	
	0 CFU / 100 ml n (%)*	*≥ 1 CFU / 100 ml n* (%)*	
Boiled water	10 (83.3)	2 (16.6)	12
Filtered water	10 (58.8)	7 (41.2)	17
Tap water	38 (53.5)	33 (46.5)	71
Total	58	42	100

*n: Number of water samples

All the water samples were clear except 3% (n = 3). *Esch. coli* was grown from 42% (n = 42) of water samples (Table 2). Coliforms were grown from 8% (n = 8), 7% (n = 7) and 54% (n = 54) of water samples with a count of 1 – 3 CFU / 100 ml, 4 – 10 CFU / 100 ml and > 10 CFU / 100 ml respectively (Table 3).

Water quality was classified on the basis of count of *Esch. coli* and coliforms as mentioned by R. Cruickshank.[16] From the total drinking water samples (n = 100), 30% (n = 30) samples were found to be excellent, 7% (n = 7) were satisfactory, 4% (n = 4) were suspicious and 59% (n = 59) were unsatisfactory. Figure 2 shows the usage of different qualities of water in different towns of Lahore.

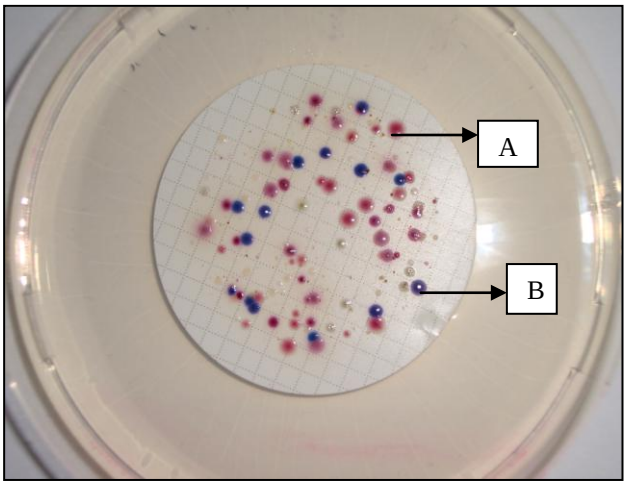

Fig. 1: *Colonies on CHROMagar with filter paper after 24hr incubation at 35°C.*

A: Shows the pink coloured colonies of coliforms.

B: Shows the purple coloured colonies of *Esch. coli.*

DISCUSSION

In Pakistan, the bacteriological contamination of drinking water has been reported to be one of the most serious problems. It can lead to water borne diseases.[17] In Punjab 90% of the people suffer from water borne diseases e.g. dysentery, typhoid, cholera and diarrhoea.[18] Analysis confirmed the presence of *Esch. coli* and coliforms. Therefore these samples were all unsatisfactory for human consumption. These samples may have cross contamination with sewerage pipelines. The MPN method gives only an estimated count qualified by a range of probable counts[19] while the MFT requires only 1 day and gives a precise count.[20]

In a total of 100 samples, 42% (n = 42) revealed growth of *Esch. coli* (table 2). A similar study conducted at Peshawar indicates that 43% samples were contaminated with *Esch. Coli*.[21] Coliforms with

Table 3: *Coliforms count grouping in different types of water samples.*

Sample type	*Coliforms count groups*				*Total*
	Group 1	*Group 2*	*Group 3*	*Group 4*	
	0 *CFU / 100 ml n (%)*	*1-3 CFU/ 100 ml n (%)*	*4-10 CFU/ 100 ml n (%)*	*> 10 CFU/ 100 ml n (%)*	
Boiled water	7 (58.3)	0	0	5 (41.6)	12
Filtered water	7 (41.2)	2 (11.7)	3 (17.6)	5 (29.4)	17
Tap water	17 (23.9)	6 (8.4)	4 (5.6)	44 (61.9)	71
Total	31	8	7	54	100

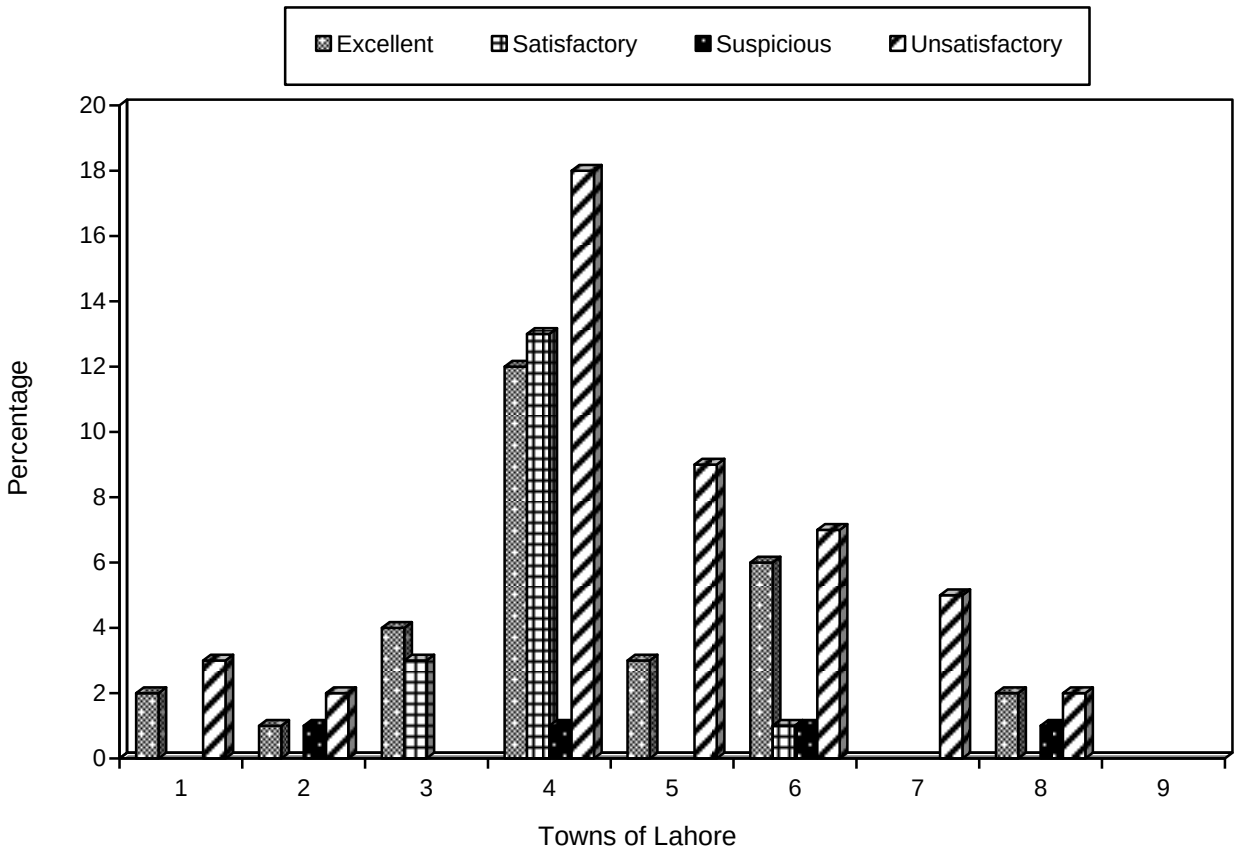

Fig. 2: *Graph of the percentage of different classes of water in different towns of Lahore.*
1: Aziz Bhatti Town 2: Data Gunj Bukhsh Town 3: Gulberg Town 4: Iqbal Town 5: Nishter Town 6: Samman Abad Town 7: Shalimar Town 8: Wahga Town 9: Ravi Town

the count of >10 CFU / 100 ml, 4–10 CFU / 100 ml, 1 – 3 CFU / 100 ml and 0 CFU / 100 ml were present in 54% (n = 54), 7% (n = 7), 8% (n = 8) and 31% (n = 31) of samples respectively (table 3).

Analysis of boiled water samples (n = 12) showed the growth of *Esch. coli* in 16.6% (n = 2) samples whereas coliforms were grown from 41.6% (n = 5) samples (table 2 and 3). Ideally all the boiled water should be sterile. Most probable cause of contamination might be the usage of contaminated vessel; direct dipping of bowls / glasses with unwashed hands by the family members. Inadequate boiling is one of the important causes as the water was boiled by the persons for only 1 – 2 minutes at 100°C. According to another Pakistani reports samples heating at 100°C for 5 – 10 minutes yielded no growth even after 48 hours of incubation.[22]

Among the filtered water samples (n = 17), 82% (n = 14) were from filters installed in the houses and 18% (n = 3) from filters installed by the government in the nearby locality. The filters installed by the government had less bacterial count as compared to the filters installed in houses. *Esch. coli* count was observed to be higher as compared to the tap water of the same house. It may be due to the usage of expired or clogged filters. Thus the organisms may grow in them and increase the bacterial count even in otherwise less contaminated water.[23]

Approximately, half of the tap waters (n = 71) had shown *Esch. coli* growth 46.5% (n = 33) (table 2) whereas coliforms were grown in 71.9% (n = 54) samples (table 3). These results correlate with another study by Shar *et al* (2008) in which the all the samples of tap water (100%) were found to be contaminated with coliforms and *Esch. coli*. This study also revealed that more contamination was found in consumer taps, followed by the distribution lines and reservoirs.[24]

On a much extended scale 0 – 3 total coliforms per 100 ml of drinking water is acceptable but without any *Esch. coli* which must not be present, at all, in 100 ml of drinking water.[25] The quality of water is categorized in to the four classes as mentioned by Cruickshank. Going by these standards, the situation is alarming; 59% (n = 59) of drinking water samples were found to be unsatisfactory for human consumption, 4% (n = 4) suspicious, 7% (n = 7) satisfactory and only 30% (n = 30) excellent. The dete-

ction of coliforms and *Esch. coli* in large numbers imply that the contaminated water may be responsible for increasing number of water borne diseases in the city. The present study supports that the quality of drinking water in Pakistan is not up to the WHO standard.

It is concluded that drinking water of different towns found to be unsatisfactory for the human consumption hence it is a health risk to use this water without purification.

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