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Occurrence of Hemolytic Coliform Bacteria in Drinking Water Samples of Lahore

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Abstract.- Incidence of water borne bacterial infectious diseases is resurging recently due to scarce supplies of potable water. Inadequately chlorinated and untreated drinking waters are involved in the spread of enteric diseases. In this study, five drinking water samples collected randomly from different areas of Lahore were processed for the detection and enumeration of coliforms. All the samples were found to harbour coliform bacteria, with indication of *E. coli* in four of the samples. Six of the sixteen isolates were found hemolytic on blood agar. The present results indicate fecal pollution of water reservoirs as one of the spread routes of the enteric pathogens. Most of the water samples are not recommended for direct drinking and recreational purposes. They must be treated in some forms such as boiling or filtration before use.

Key words: Coliforms and drinking water, *E. coli* in water samples, pathogenecity of coliforms.

INTRODUCTION

Drinking water of most communities and municipalities is obtained from surface sources, the rivers, streams and lakes. Such natural water supplies, particularly streams and river, are likely to be polluted with domestic and industrial wastes, as the used water of a community is ultimately disposed off in these natural drains. It is not the mere number of microbes that affects water quality, as water containing large number of harmless bacteria may yet be safe for drinking, but it is the kind of specific microorganisms that is determinative (Benson, 1994; Farnleitner *et al*., 2001; Said *et al*., 2004).

The second water source originates from deep wells and subterranean springs and is relatively free from bacteria because of filtering action of earth through which the water has penetrated. However, even the ground water gets contaminated through the seepage as it flows into the soil (Borchardt *et al.,* 2004). Moreover, outbreaks of waterborne infections usually result from defects in one or more treatment processes or because there has been ingress of pathogens as a result of activities causing damage to some part of the distribution system. Defects in plumbing, cross connections, backflow and pressure fluctuations can cause contamination of distribution systems and can result in the transmission of organisms causing infections. Qureshi and Qazi (2000) have described that the seventeen drinking water samples collected from different areas of Lahore harboured coliform bacteria and that the water storage and water pipe lines were not being managed properly.

It has been established that routine examination of water for the presence of intestinal pathogens would be a tedious and difficult task, while it is much easier to demonstrate the presence of some non pathogenic intestinal types such as *Escherichia coli* or *Streptococcus faecalis,* since these organisms are always found in the intestine and are normally not present in soil or in water. Thus it can be assumed that their presence in water indicates that fecal material has contaminated the water supply. These bacteria also survive a little longer than the enteric pathogens (Pelczar *et al.*, 1986; Benson, 1994; Muneer *et al.*, 2001; Tantawiwat *et al*., 2005).

Water supply to urban areas of Lahore is managed from ground water source. The water is chlorinated routinely. However, the treated water is stored variously in overhead municipal as well as domestic tanks. The household level tanks are of various kinds. Owing to persistent and increasing trends in enteric infections in Pakistan, the drinking

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water facilities must be kept continuously under observation for their microbial content. Unfortunately, the public health authorities in this country are not making such efforts. Therefore, the present study was intended to detect and enumerate colifrom bacteria in drinking water from direct supplies, overhead tanks and underground points of different areas of Lahore. The results indicate that the water source, its storage or the pipelines are not being managed properly.

MATERIALS AND METHODS

Samples of drinking water were collected from direct supply lines, household overhead and underground tanks by taking about 100 ml of water in a sterile glass bottle. They were soon brought to the laboratory and inoculated on Eosin Methylene Blue (EMB) agar for the detection, isolation and enumeration of coliform bacteria. The medium was prepared according to Merck (1996-1997). Two dilutions of each sample were made by taking one ml in 99 ml of autoclaved water in a sterile container. This was subsequently diluted similarly one time more. Then 0.5 and 0.1ml of original sample as well as of both dilutions were spread on separate the EMB agar plates. The seventh plate was kept as control to verify the sterile nature of the medium and the working conditions. All the plates were incubated at 37°C up to 48 hours.

Following the incubation period, the plates having 30-300 colonies were selected for calculating colony forming units (CFU) of coliforms. The growth was also observed for the cultural characteristics viz., size, shape, color, margin, elevation, consistency and pigmentation of the colonies. While, calculated CFU in case when more than one values of CFU were found within the range *i.e*., 30-300/plate, only that one was calibrated as CFU/ml of original sample which was nearest to the figure 135 (the average of difference of 30 and 300). Each representative colony was streaked across the medium, surface in the quadrant manner. From the growth a well separated colony was picked by a sterilized transferring loop from the selective agar plate and streaked on a nutrient agar (Oxoid) plate. Colonial characteristics were also noted on this medium. Growth from the nutrient agar plate was restreaked on the selective agar plate (Black,1996). The pure cultures were preserved on nutrient agar slants. Bacterial isolates were then grown in nutrient broth and the growth was used for performance of various identification tests. The bacterial growth was processed for gram and endospore stainings, motility, catalase and oxidase tests according to Benson (1994), while, indole, methyl red and Voges Proskauer tests I and II were performed after Collins *et al.* (1995). Many of the isolates were identified generally upto the genus level on of the characteristics determined.

For Pathogenicity test blood agar base was prepared according to Merck (1996-1997). The medium was autoclaved routinely, cooled up to 48°C. Then sterile defibrinated (oxalated) rabbit blood was added in an amount of 60-70ml/L of the molten medium and mixed gently so as to avoid formation of bubbles. The blood containing medium was poured in pre-sterilized plates and allowed to solidify. Bacterial cultures were streaked on the blood agar plates and incubated in inverted position at 37°C for 24-48 hrs. The growth was then observed for the hemolytic activity of the bacteria. Greenish zone around the culture indicated α - hemolysis while clear transparent zone β- hemolysis (Benson, 1994).

RESULTS AND DISCUSSION

Some features of the drinking water samples are shown in Table I. As can be seen from this table all the samples indicated coliform bacteria. The highest numbers of coliforms CFU appeared for sample number 1 and 5 taken from overhead and underground tanks, respectively of the areas characterized by dense population and contaminated by animal manure due to still in use animals driven crafts. Infact house hold water storage facilities, especially the underground tanks are frequently contaminated by dust and lawn and garden origin organic materials. Such matter may not only carry coliform bacteria but they may also support growth of these organisms within water reservoirs. For example, Boualam *et al.* (2002) while studying relationship between coliform cultureability and organic matter in low nutritive waters found that coliform bacteria lost their cultureability more

rapidly in water samples with lower initial dissolved organic carbon levels. Thus it becomes suggestive that while measuring coliform content of water samples total dissolved organic contents should also be considered that may guide in tracing the reason of higher coliform content.

Table I.- Nature of the drinking water samples and corresponding colony forming units (CFU) on Eosin methylene blue agar medium

Sample No	Locality	Nature of Sample	CFU/ml of sample
1	Awan Town	Over head tank	23.4×10^4
2	Shalimar	Direct supply	72
3	Sabzazar	Underground tank	33
4	Lahore Cantt	Direct supply	18
5	Shadbagh	Underground tank	24×10^4

Various colonial and biochemical characteristics of the bacterial isolates have been shown in Table II. Not indicated in the table include margin of bacterial colonies, and result of indole test and endospore staining. Bacterial colonies of all isolates on EMB agar had smooth margins except the strains 1K2 and 4E2. These strains expressed wavy and raised margins, respectively. All the strains, except 4E1 and 4E2 gave -ve results for indole test. All the bacterial strains yielded negative results for endospore staining. Two strains of *E.coli,* while one of *Salmonella* were isolated from sample number 1. Colonial features on methylene blue agar and various biochemical characteristics of these isolates can be observed from Table II .The strain 1E2 of *E. coli* was found α-hemolytic. *Salmonella* isolate was found β-hemolytic. From this sample two strains of *Klebsiella* were also isolated. Of these isolates 1K2 expressed varied colonial features when grown on nutrient agar as compared to the characteristics following growth on EMB agar (Table II). On nutrient agar round amber colored colonies appeared having smooth margin and flat elevation. However, size of colonies remained same on the two media. From sample No. 2 again two isolates of *E. coli,* while one each of *Klebsiella* and *Shigella* were isolated. And of these the later was found β-hemolytic (Table II). Colonial characteristics of *Klebsiella* varied on the two media. The colonies appeared pink and blue with raised margin and convex elevation on nutrient agar. Two strains of *E. coli* and one of *Salmonella* were isolated from sample No 3. The *Salmonella* were found β-hemolytic. The 3E2 isolate of *E.coli* expressed round nucleated colonies with dull metallic sheen having diameter of 1mm, when grown on nutrient agar in contrast to its colonial features on EMB agar (Table II). The two strains of *E. coli* isolated sample No 4 were found β-hemolytic. While *Shigella,* was found non-hemolyitc. Apart from purplish pink colonies of 4E2 strain of *E.coli* on nutrient agar, the remaining colonial features appeared same on the two media (Table II). Only a *Klebsiella* strain was isolated from sample number 5. Colonies of the isolate had nucleated round configuration and dark/light pink color following growth on nutrient agar. The rest of the features appeared same on the two media (Table II).

Coliform bacteria have been used to assess the quality of water and presence of pathogens. Although several of the coliform bacteria are not usually pathogenic themselves, but they serve as an indicator of potential bacterial pathogens contamination. Indeed many of the coliforms including strains of *E. coli* comprise entero-pathogenic serogroups and have been found to be hemolytic and verocytotoxigenic (Pelczar *et al.*, 1986; Welch *et al.*, 2000).All the samples except the sample number five, indicated the presence of *E. coli*. *E. coli* has been considered the most prominent fecal coliform. Its presence indicates that there might have been some contaminations from sewage and that *Salmonella* or other intestinal pathogens might be present (Momba and Kaleni, 2002; Okafo *et al*., 2003).This notion is verified by the present data that indicates that *E. coli* were accompanied in majority of the samples, by pathogenic strains of *Salmonella* and *Shigella.* It is known that the pathogens that gain entrance in the body of water arrive there from intestinal discharges of humans or other animals (Pelczar *et al*., 1986; Trevett *et al*., 2004). The most common pathogens transmitted in water are *Salmonella* sp., *Shigella* sp. and *E. coli*. These organism are responsible for water borne diseases (Alcamo, 1994; Muneer *et al*., 2001; Dorner *et al*., 2004). Thus the microbial content

Table II. Colonial and biochemical characterization of coliforms isolated from different water samples.

Sample No.	Bacterial isolates	Strains symbol	Growth on eosin methylene blue agar				Biochemical characterization*						
			Color	Configuration	Elevation	Size (mm)	A	B	C	D	E	F	Hemolytic activity
1.	*E. coli*	1E1	Metallic sheen	Round nucleated	Convex	2	-	+	+	+	-	-	-
		1E2	Blue	Round	Flat	2	-	+	+	-	-	-	α-hemolytic
	Klebsiella	1K1	Pink (milky)	Round	Flat	3	-	+	+	-	-	-	-
		1K2	Pink	Irregular	Hilly	1	-	+	+	-	+	-	-
	Salmonella	1S	Amber	Round	Flat	2	-	+	+	+	-	-	β-hemolytic
2.	*E. coli*	2E1	Metallic Sheen	Round Nucleated	Convex	2	-	+	-	+	+	+	-
		2E2	Blue	Round	Convex	1	-	-	+	+	-	-	-
	Klebsiella	2K	Pinkish	Round	Flat	2	-	+	+	+	+	-	-
	Shigella	2S	Amber	Round	Flat	1	-	+	-	-	+	+	β-hemolytic
3.	*E. coli*	3E1	Metallic Sheen	Round Nucleated	Convex	3	+	+	+	+	+	+	-
		3E2	Blue	Round	Convex	3	+	+	+	-	+	+	-
	Salmonella	3S	Amber	Round	Flat	2	+	+	+	+	+	+	β-hemolytic
4.	*E. coli*	4E1	Blue & Pink	Round	Convex	3	-	+	-	-	-	-	β-hemolytic
		4E2	Purplish Pink	Round	Flat	5	-	+	+	-	+	+	β-hemolytic
	Shigella	4S	Amber	Round	Flat	2	-	+	-	-	+	+	-
5.	*Klebsiella*	5K	Opaque Pink	Round	Convex	2	+	+	+	+	+	+	-

*A, Oxidase; B, Catalase; C, Motility; D, MR; E, VP-I; F, VP-II; -, negative; +, positive.

of present samples are warning from public health view point. Hanif (1991) has reported that city of Lahore generates about 240 million gallons of sewage per day which contains bacteria and toxic material. One of the factor influencing the total bacterial content of a water supply in Pakistan which increases the incidence of water borne infections is that in urban and rural areas, the drinking water lines and sewage lines are laid side by side resulting in frequent contamination of potable water, when the fresh water pipes erode. All the water samples reported here appear unsafe for drinking purpose and many gastrointestinal diseases might have been prevailing in these areas of Lahore. Provision of safe drinking water needs improvements in sanitation and excreta disposal.

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