INT. J. BIOL. BIOTECH., 4 (2-3): 219-222, 2007.

QUALITATIVE ASSESSMENT OF BACTERIOLOGICAL QUALITY OF DRINKING WATER IN NEW CAMPUS, PUNJAB UNIVERSITY, LAHORE, PAKISTAN

Ruqia Suliman, Rukhsana Bajwa and Ghazala Nasim

Department of Mycology and Plant Pathology, University of the Punjab New Campus, Lahore. Pakistan.

ABSTRACT

The present work was designed to analyze the quality of drinking water of new campus Punjab University, Lahore. This site was further divided into three areas such as residential/colony area, hostels and departmental areas for sampling purpose. Bacteriological quality of drinking water was assessed through microbiological techniques and biochemical testing by MICROBACT ™ GNB 12A/B/E, 24E Identification kit. It was noticed that drinking water was fully contaminated with enumerable species of *Enterobacteriaceae.* Some of which are faecal coliform i.e. *Enterobacter. cloacae, Enterobacter. Sakazakii, Hafnia. alvei, Citrobacter sp10 A, Citrobacter sp10 B ,Enterobacter agglomerans A, Enterobacter agglomerans B, Enterobacter agglomerans C* and non faecal human pathogenic bacteria i.e. Enteric Gp59 ,*Escherchia coli*-inactive, *Yersinia ruckeri, Yersinia. pestis, Serratia rubidaea, Serratia. liquefaciens, Serratia. plymuthica , Serratia. marcescens biogp1 A, Serratia. marcescens biogp1B, , Acinetobacter haemolyticus, and Acinetobacter. baumannii A, Acinetobacter. baumannii B.* All of these are opportunistic human pathogens. The presence of *E.coli* ensure poor quality of water because it is considered to be a universal indicator of contamination in water and water resources.

Key words: Drinking water, faecal contamination, opportunistic human pathogens.

INTRODUCTION

Fecal contamination is a major causative factor for incidence of water-borne infectious diseases. Globally over 250 million cases of water borne diseases are reported each year, resulting in about 10 million deaths (Esrey *et al.,* 1985). Water pollution is considered as a major cause of deaths in developing countries (Baylis *et al*.,1997) .WHO recommend treatment of water to make it free from disease producing organisms and toxic chemicals before human consumption (WHO,1976). In Pakistan, such treatment is seldom done and no such international standards are followed for maintaining microbiological and chemical quality of drinking water. In rural areas, where about 70% population lives, no proper water supply schemes are in action. It is believed and reported that 62% of the Pakistani population, have no access to clean drinking water (UNICEF, 2002) and an estimated 60% deaths are associated with the use of contaminated water. In a study in Swat, all the drinking water samples except spring were found unfit for human consumption (Khurshid *et al*., 1991). In Abbottabad, 57.2% water samples were found to have faecal contamination (Rab *et al*., 1993). In the industrialized cities, water reservoirs are polluted with the waste discharge of effluents. Various industries operating in Karachi are polluting the Lyari and Malir rivers by discharging 94% of untreated water (Khaliq *et al.,* 1986). Similarly in Lahore, river Ravi is being polluted with 100 million gallons of industrial waste, pesticides and raw sewerage (Sami *et al*., 1966), and Rawal lake in Islamabad with domestic and poultry waste (Chandio, 1998). In Kasur more than 100 tanneries are disposing their waste effluents and polluting ground water (Khurshid *et al.,* 1991)

A study from Punjab has shown that 90% of the households were suffering from water borne diseases. The study revealed that 32% of the residents suffered from malaria, 17% from dysentery, 13% skin diseases, 11% typhoid, 9% cholera and 8% suffered from diarrhea in the study area (Jehangir *et al*., 2001).The present work was designed to check the quality of drinking water of new campus area, University of the Punjab, Lahore.

MATERIALS AND METHODS

A total of thirty samples of drinking water were collected of which ten each from residential colony, Departmental area and hostels area .Each sample consisted of ten ml of drinking water taken in sterilized screw caped test tubes aseptically from sampling areas. Two selective media MacConkey Agar (MA), Blood Agar (BA) were prepared for isolation of human pathogenic bacteria (Enterobacteriaceae). The composition of media used i.e. for blood agar thirty nine gm of Columbia agar base was dissolved in 950 ml of sterilized distilled water and autoclaved then cooled to 50ºC in water bath then 50 ml of sterile defibrinated Blood was added. MacConkey agar sold media dissolved per liter of sterilized distilled water. Twenty µl of water from sampled tubes was inoculated by spread plate method in four replicas of BA and four of MA respectively under sterile conditions. Similarly all samples were inoculated and plates were incubated at 37ºC for 24 hours in incubator. After 24 hours fresh cultures were obtained. Cultures were purified by streak plate method for studying morphological, physiological,

biochemical, characteristics. Bacterial cultures were identified by studying morphology, physiological characteristics. Biochemical characterization accompanied by using MICROBACT ™ GNB 12A/B/E, 24E Identification kits for *Enterobacteriaceae*. For identification 18-24 hour pure culture of the organism were obtained on MacConkey agar (MA), Blood agar (BA) then 1-3 isolated colonies from cultures were emulsified in 2.5ml of sterile saline solution and mixed thoroughly to prepare a homogeneous suspension. Test strips were placed in holding tray and four drops of bacterial suspension were added by using a sterile Pasteur pipette. By using a sterile pipette, the substrates underlined were over layered on the holding tray with sterile mineral oil, i.e., wells 1, 2, 3 for 12A (12E) and well 8 for 12B. Inoculated rows were resealed with the adhesive seal. To record all positive result substrates were incubated at 35±2ºC for 18-24 hours. Strips or tray was removed from the incubator, peeled back the sealing tape. The reactions were evaluated as positive or negative by comparing them with the color chart.

RESULTS AND DISCUSSION

A total of twenty opportunistic pathogenic bacteria were isolated from all the three areas of new campus of the Punjab University, Lahore. The percentage incidence of some strains e.g., E. *agglomerans A, E. agglomerans C, E. cloacae, E. sakazakii,* Enteric Gp59, *S. plymuthica, A. haemolyticus* varied substantially whereas other strains occurred almost with the same frequency in three sampled areas (Table 1). *E.coli*-inactive showed maximum percentage of general incidence (83 ± 1.0) per 80 mm^2 followed by *E. agglomerans B* (81±1.0) and *E. cloacae* (69±10).

The presence of *Escherichia .coli*-inactive (Fig. 1) in large number of colonies counted indicated to the poor quality of drinking water of New campus sites of Punjab University. *Escherichia coli are* considered to be an indicator of faecal contamination and rendering the water quality unsafe for drinking (Dufour, 2003).

Table 1. Percentage incidence of human pathogenic bacteria in water samples collected from new campus, Punjab University, Lahore.

SERIAL NO.	BACTERIAL STRAINS	SAMPLE AREA		
		Residential/ colony area	Departmental area	Hostels area
		Number of Bacterial Colonies Per 80 mm^2		
1.	*E. agglomerans A	5.0 ± 1.1	3.0 ± 0.96	6.0 ± 0.38
2.	*E. agglomerans B	81.0 ± 3. 0	80.0 ± 0.24	82.0 ± 0.47
3.	*E. agglomerans C	8.0 ± 0.51	7.0 ±1.1	11.0 ± 0.86
4.	*E. cloacae	79.0 ±1.0	61.0 ± 0.88	68.0 ± 0.42
5.	*E. Sakazakii	62.0 ± 0.41	43.0 ± 0.61	59.0 ± 0.98
6.	∞H. alvei	2.0 ± 0.26	2.0 ± 0.47	2.0 ± 0.32
7.	*Citrobacter sp10 A*	2.0 ± 0.39	2.0 ± 0 .98	2.0 ± 0.61
8.	*Citrobacter sp10 B*	3.0 ±1.2	3.0 ± 0.35	3.0 ± 0.45
9.	Enteric Gp59	30.0 ± 0.73	21.0 ± 0.21	29.0 ±1.1
10.	$E.coli-inactive	83.0 ± 0.19	82.0± 0.12	84 ± 0.79
11.	*Y. ruckeri*	9.0 ± 0.68	9.0 ± 0.73	9.0 ± 0.83
12.	*Y.pestis*	7.0 ± 0.53	7.0 ± 0.89	7.0 ± 0.11
13.	*S. rubidaea*	20.0 ± 0.21	18.0 ±1.0	18.0 ± 0.38
14.	*S. liquefaciens*	42.0 ± 0.36	40.0 ± 0.33	40.0 ± 0.22
15.	*S. plymuthica*	6.0 ± 0.58	2.0 ± 0.86	5.0 ± 0.31
16.	*S. marcescens biogp1 A*	20.0 ±1.1	18.0 ± 0.27	18.0 ± 0.87
17.	*S. marcescens biogp1 B*	2.0 ± 0.83	2.0 ± 0.99	2.0 ± 0.13
18.	*A. haemolyticus*	10.0 ± 0.21	12.0 ± 0.11	15.0 ±1.1
19.	*A. baumannii A*	10.0 ± 0.57	11.0 ± 0.65	10.0 ± 0.52
20.	*A. baumannii B*	6.0 ± 0.78	7.0 ± 0.71	6.0 ± 0.48

**E= Enterobacter ∞H=Hafnia # E= Escherchia ∞Y= Yersinia *S=Serratia *A= Acinetobacter*

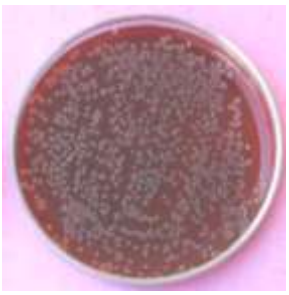

Fig 1: Blood Agar plate showing *E.coli*-inactive *colonies*

The high percentage of general incidence of *E. agglomerans B* (81±1.0), *E. cloacae* (69±10), *E. sakazakii* (54±10*), S. liquefaciens* (42±1.1*)* of new campus site also indicates that water is unfit for drinking purpose. *E. cloacae* are of faecal origin whereas *E. agglomerans B, E. sakazakii, S. liquefaciens* are of non-faecal origin but are opportunistic pathogens. According to Leclerc *et al*. (1981), the coliform species of faecal origin and their isolation frequency in faeces are as *E. coli*, 100%; *Citrobacter diversus*, 70%; *Citrobacter amalonaticus*, 70%; *Citrobacter freundii*, 70%; *Klebsiella pneumoniae*, 49%; *Klebsiella oxytoca*, 49%; *Enterobacter cloacae*, 9%; and *Enterobacter aerogenes*, 9%.

The distribution of all bacterial strains varied greatly from area to area, however, the percentage occurrence of *Y. pestis, H. alvei, Citrobacter* sp10 *A, Citrobacter* sp10 B, *Y. ruckeri, Y.pestis* remain the same in three areas of sampling (Table 1). The incidence *of A. baumannii* A, *A. baumannii* B, *S. marcescens* biogp1 A, *S. liquefaciens, and S. rubidaea varied* but little among the three sites.

The occurrence of *E. agglomerans* A, *E. agglomerans* B, *E. agglomerans* C, *Enteric* Gp59, *E.coli*-inactive, *S. rubidaea, S. plymuthica, E. cloacae, E. sakazakii*, was more in hostels and residential areas as compared to departmental area (Table 1). The significance of various coliform organisms in water is still an extensively studied subject (Caplenas, 1984). However, the specificity of faecal coliforms as indicator of faecal pollution varies considerably depending on the environmental conditions and source of contamination (APHA, 1995).

REFRENCES

APHA (American Public Health Association) (1995). *Standard methods for the examination of water and wastewater*, 19th ed. Washington, D.C: American Public Health Association.

Baylis, C. L. and S.B. Petitt (1997) . The significance of coliforms and *Escherichia coli* and the *Enterobacteriaceae* in raw and processed foods. In: *Coliforms and* E. *coli*: problem or solution? (Kay D, Fricker C. Kay, D, Fricker C. Eds.). Cambridge, United Kingdom: The Royal Society of Chemistry, pp. 49–53.

Brodsky, M. H. (1997). Abolition of the term "fecal coliforms" seconded. *ASM News.*, 63: 345–346.

Caplenas, N. R. and M.S. Kanarek (1984). Thermotolerant non-fecal source *Klebsiella pneumoniae*: validity of the fecal coliform test in recreational waters. *Am J Publ Health*.,74: 1273–1275.

Chandio, B.A. (1998). "*Keynote" National workshop on quality of drinking water*. Islamabad, Pakistan.

Dufour, (2003). *E. coli* and Public Health, Monitoring the Quality of Recreational Waters. *LakeLine*., 23(2): 14.

Esrey, S.A., R.G. Feachem and J.M. Hughes (1985). Interventions for the control of diarrhoeal diseases among young children; improving water supplies and excreta disposal facilities. *Bull*. 63:757.

Jehangir, W.A., W.V.D. Hoek and F. Konradsen (2001). Domestic uses of irrigation water and its impact on human and livestock health in Pakistan. *Pak J. Health.*, 38(1-2): 12-17.

Khaliq, M.A., S.P. Khan and J.A. Khan (1986). Bacteriological study of drinking water in Distt. Abbottabad . *PJMR*., 25(2) : 97-100.

Khurshid, S.J., et al., (1991). Detection of coliform organisms in drinking water. *JPMA.*, 41(7): 157-60.

Leclerc, H. and J.C. Moriamez (1980). Etude quantitative de la flore fécale de l'adulte et du nourrison alimenté artificielment. *Pathol Biol*., 28: 217–226.

Leclerc, H., F. Gavini and C. Oger (1981). Les indicateurs bacteriens dans le controle bacteriologique de l'eau: exigences et limites. *J Fr Hydrol*., 12: 213–228.

Mossel, D. A. A. (1997). Request for opinions on abolishing the term "fecal coliforms." *ASM News*, 63: 175.

Rab, F.E., T. Akhtar, Z. Ullah and T.U. Haq (1993). Effect of seasonal variation on quality of drinking water. *JMS*., 3(1):17-20.

Sami, Z., M.A. Khan and A. Ghafoor (1966). Bacteriological analysis of drinking water. *JPMA*, 38: 92.

UNICEF (2002). *Official Summary, The State of the World Children*. Page, 2.
WHO (1976). *Surveillance of drinking water quality*. Geneva: World Health Organization, (WHO monograph sr.no.63).
Witt, V.M. (1982). Developing and applying international water quality guide lines. *J. Am. Water Works Assoc*., 74: 178.
Zai, S. and T. Akhtar (1982). Bacteriological analysis of drinking water in Swat. *PJMR*, 21 (3 - 4): 93-97.

(*Accepted for publication June 2007*)