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Bacteriological assessment of drinking water of Islamabad Capital Territory, Pakistan

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ABSTRACT

This study was conducted to evaluate the bacteriological quality of drinking water of tube wells, filtration plants, and water supplies from various sectors of Islamabad Capital Territory (ICT), Pakistan. Fifty-five water samples were collected from 18 filtration plants, 26 tube wells, and 11 water supplies and analyzed for total heterotrophic bacterial count (THBC) by spread plate count method, total coliform (TC), and fecal coliform including *Escherichia coli* by most probable number technique along with physicochemical analysis. Results showed that 21 (38%) out of 55 samples were found contaminated with heterotrophic bacteria. Among the 55 sites tested in triplicates, 15% ($n = 8$) were contaminated with TC and 13% ($n = 7$) were contaminated with *E. coli* (EC). Different bacterial strains were isolated and identified by using API 20E method. According to this study, 14.5% of the water sources of ICT did not meet the safe limits for drinking water as described in the WHO guidelines.

Keywords: Bacteriological analysis of water; Drinking water; Water supply of Islamabad; Waterborne bacteria; Water quality

1. Introduction

Clean drinking water is essential to sustain life and is a matter of concern for all countries, especially for the developing countries. Nearly one billion people without access to adequate sanitation live in South Asia [1]. The amount of fecal coliform bacteria (associated with fecal matter) detected in Asia's rivers is 50 times the WHO guidelines, indicating a high level of dangerous microbial contaminants [2]. In Asia, and in countries around the world, these pathogenic microbes can invade drinking water from unsafe or inadequate water treatment, leading to a wide range of serious health threats. Some 30% of the world's freshwater stocks are found underground, supplying drinking water for an estimated two billion people and irrigation for an estimated 40% of the world's food production. Many groundwater systems act to filter and attenuate pollutants, especially microbial contaminants [3].

Pakistan ranks at number 80 among 122 nations regarding drinking water quality. Drinking water sources, both surface and groundwater, are contaminated

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with coliforms, toxic metals, and pesticides throughout the country. Various drinking water quality parameters set by WHO are frequently violated [4]. Previous studies showed significant problems associated with untreated drinking water which was found to be bacteriologically contaminated in major cities of Pakistan [4–6]. Drinking water assessment in Southern Sindh, Pakistan, revealed that coliform counts in different water bodies were between 77 and 96% higher than the WHO guideline values. Deterioration of water quality has occurred due to the dumping of industrial and urban wastes and the use of agrochemicals, limiting the fresh water resources [7].

Different studies conducted in the past by independent researchers, government, and non-government organizations all come to the same conclusion regarding the poor quality of drinking water throughout Pakistan including Islamabad Capital Territory (ICT). Bacteriological study of drinking water supplied to the eight hospitals in Islamabad was assessed by Akhtar et al. [8], and all the samples were found to be contaminated. This study further revealed that out of 16 samples, three samples were contaminated with *Klebsiella pneumoniae* and five samples were contaminated with *Salmonella typhi*. Analysis of bacteriological water quality of ground drinking water of Islamabad city carried out by Kanwal et al. [9] revealed that bacteriological water quality of tube wells was satisfactory and only a few tube wells were found to be contaminated. Monitoring bacteriological water quality of different dams (Simly, Khanpur, and Rawal dams) in Rawalpindi and Islamabad was also carried out by a number of independent researchers and organizations [9–12]. Monitoring different stations of Rawal dam filtration plant by Farooq et al. [10] revealed that most of the samples were contaminated with coliform. They suggested ensuring the quantity of disinfectant both at the end-user and at the distribution levels. Analysis of bacteriological water quality of Rawal, Simly, and Khanpur dams of Rawalpindi and Islamabad was carried out by Kanwal et al. [9], and samples collected from Rawal dam were found unfit for drinking purpose. According to a survey conducted by Pakistan Council of Research in Water Resources, all these dams were found to be contaminated with coliform [12].

The main objective of this study was to assess the bacteriological water quality of the major sources for the provision of drinking water to ICT. These included tube wells, filtration plants, and water supplies of different sectors of Islamabad. The study was also aimed at isolating and identifying the waterborne pathogens persisting in water supplies after treatment.

2. Materials and methods

2.1. Study area and sampling frequency

The targeted area of current study included different residential sectors (densely populated areas) of Islamabad such as sectors F-5–F-10; G-5–G-11 H-8–H-10; I-8–I-10, Bari Imam, Margala Town, Rawal Town, and Bharakahu (Fig. 1). Drinking water is supplied to the residents of Islamabad through three main sources which are (i) tube wells, (ii) filtration plants, and (iii) direct water supplies from dams such as Rawal, Simly, and Khanpur. The 55 samples included 18 from filtration plants (FP = 33%), 26 from tube wells (TW = 47%), and 11 from water supplies (WS = 20%) as shown in Fig. 2.

2.2. Sampling methodology

All the 55 samples were collected in triplicates according to WHO standard procedures, and sampling sites were selected randomly covering a minimum of two and a maximum of three sub-sectors within a residential sector which covers an area of about 2 km^2. Sampling was performed between May and August 2011. Glass bottles (Pyrex; 500 ml) washed with detergents and rinsed with deionized water along with addition of dechlorinating agent (0.3 ml of 3% sodium thiosulfate) [13] were sterilized, using autoclave (at 121°C for 20 min), before sampling. Sodium thiosulfate neutralizes the residual chlorine effect in water so that results of analysis could be representative of the sample at the time of sampling. About 200–300 ml of water was sampled under controlled conditions (contamination free) from each site (tap water from filtration plants and water supplies) and labeled immediately with sample identification code and date. The precautionary measures were implemented while sampling to avoid cross-contamination. A pro forma was filled at the sampling point with the help of the concerned operator for necessary related information. Samples were transported under recommended conditions to the laboratory within four h of collection time and were processed on the same day.

2.3. Chemicals and culture media

All the chemicals and culture media like MacConkey broth, brilliant green lactose broth and nutrient agar (NA) used were of analytical and microbiological grade and purchased from Merck, UK.

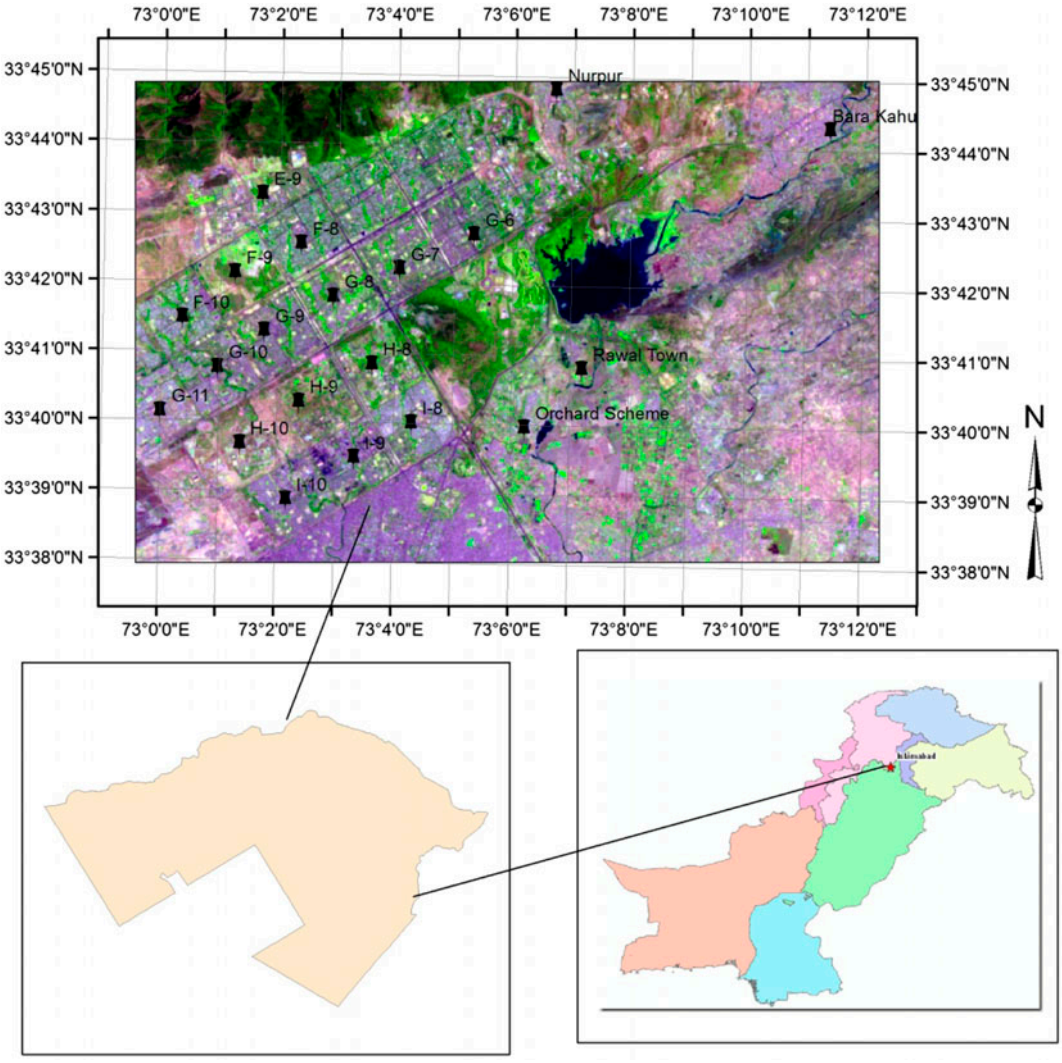

Fig. 1. Map of the study area showing sampling sites in Islamabad Capital Territory (ICT), Pakistan (modified from Google Maps https://www.google.com/maps/place/Islamabad).

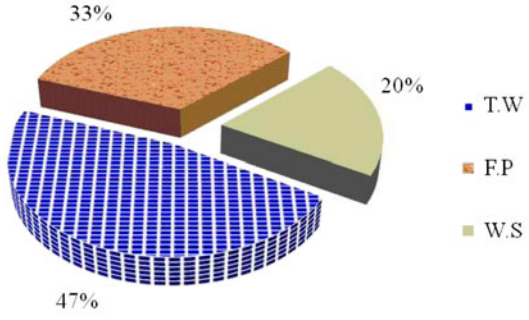

Fig. 2. Distribution of drinking water samples collected from different sources of Islamabad region.
Note: WS = water supply, TW = tube wells, FP = filtration plants.

2.4. Physicochemical and bacteriological analysis

Initial examination included aesthetical and physicochemical parameters. The pH of the water was examined at the site. Quantitative bacteriological analysis of water samples was carried out by heterotrophic bacterial count (THBC) method using spread plate technique. Plates containing 30–300 colonies were counted at 37°C after 24 h of incubation period. The mean total viable count of total aerobic heterotrophic bacteria on NA was expressed in log 10 values. Total coliform (TC) and fecal coliform including *Escherichia coli* were observed by using most probable number (MPN) technique [14]. Preliminary identification of bacterial isolates was performed by morphological and biochemical characterization (Gram's staining, indole test, catalase test, fermentation, and Simmon's

citrate test). *E. coli* presence was confirmed by EMB agar plate method along with other tests. Additional test such as oxidase was performed for *Pseudomonas aeruginosa* confirmation. Negative control was run with each test and bacterial species were confirmed with the help of API 20E Kit [15].

3. Results and discussion

3.1. Physicochemical analysis

All the water samples were found to be esthetically acceptable. Odor, cloudiness, visible color, and particulate material were absent. Average temperature values of samples from tube wells, filtration plants, and water supplies were 20˚C, 21˚C and 18.6˚C, respectively, which did not show much variation. Low temperatures along with other parameters suggest that water contains no waste material, because waste often raises the water temperatures [16]. Temperature is vital in determining the growth of micro-organisms, as high temperature enhances bacterial growth [17]. Low temperature reduces corrosion problems [18]. Temperature also affects dissolved oxygen level of water body that is critical for the survival of aquatic organisms.

Water samples obtained from tube wells showed pH values ranging from 6.8 to 7.6 with an average of 7.1, which is lower than that of the samples obtained from filtration plants and water supplies. Filtered water had a range of pH from 7.0 to 7.8 yielding an average pH of 7.3. Highest average pH of 7.4 was found for water supplies ranging from 6.8–8.0. All the water sources had pH values well within the desirable range of 6.5–8.5 [19]. Low pH water is likely to be corrosive and can affect the taste and appearance of water adversely. The pH of water must be controlled to minimize the corrosion of pipes and water systems, which is suggested in case the of water supplies and tube wells. Effective chlorination is required to keep the pH of water below 8 [20].

3.2. Disinfection measures

Data collected through the questionnaire revealed over half (54%) of the water supplies were not adopting any disinfection measure (chlorination), while only 36% were chlorinated. Data of remaining water supply were not available (10%). Tube wells were the most neglected ones with 65% non-chlorinated sites. Only 12% tube wells were chlorinated and the data of the remaining 23% tube wells were not available. It has been shown that people in Colombia drinking non-chlorinated water were at increased risk of cholera and diarrhea with prevalence odds ratios between 5.7 and 3.3, respectively [21]. Controlling the amount of chlorine is also important, as high dose of chlorine can cause cancer and other health risks [22,23].

3.3. Bacteriological analysis

Results showed that 21 out of 55 (38%) samples were positive (colony counts were in excess of the guideline values) for total heterotrophic bacterial count (THBC). This is not surprising as another study showed that 69% of the samples were positive for THBC in the drinking water of Rawalpindi and Islamabad [24].

Maximum average THBC was obtained for water supplies, 4,541 cfu/ml ($\log_{10} = 2.32$); followed by filtration plants; 622 cfu/ml ($\log_{10} = 1.56$); and tube wells, 550 cfu/ml ($\log_{10} = 1.50$) (Fig. 3). The excessive THBCs obtained from water supplies indicate that people utilizing water supplies directly are more exposed to contamination.

Kanwal et al. analyzed the water quality of tube wells of different sectors of Islamabad by performing heterotrophic bacterial count (THBC) by spread plate count method. They found that 2 out of 23 tube wells were contaminated [9].

3.4. TC and E. coli densities

Among the 55 sites tested in triplicates, 15% were contaminated with TC bacteria and 13% were contaminated with *E. coli* (EC). Aziz has reported that the poor bacteriological quality of drinking water has resulted in

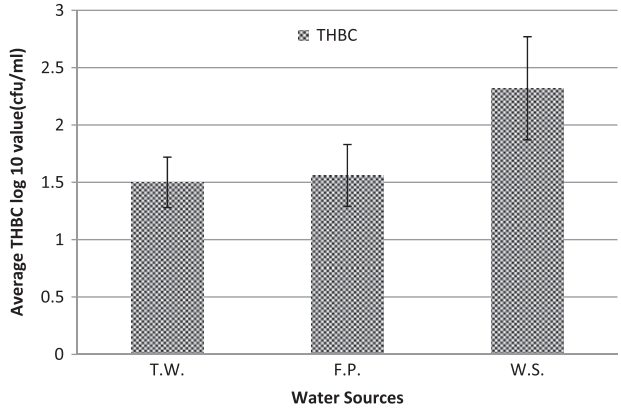

Fig. 3. THBC in different water samples of Islamabad region. Total number of samples (n) = 55.
Note: TW = tube wells, FP = filtration plants, WS = water supply.

high incidence of waterborne diseases [25]. Another study showed high percentage (40%) of *E. coli* in drinking water from Rawalpindi and Islamabad [24]. Yet another study conducted to assess bacteriological contamination in and around Islamabad reported 46 (81%) untreated and 21 (38%) treated water samples as positive for coliforms [26]. Kanwal et al. assessed the water quality of tube wells of different sectors of Islamabad and reported the growth of *B. cereus* and *E. coli* in the contaminated water samples [9]. Therefore, with reference to results of this study, it can be concluded that drinking water of Islamabad is bacteriologically less contaminated than drinking water of Rawalpindi. The consumers benefitting from filtration plants are at higher risk of getting infected due to the presence of TCs and *E. coli* ranging from 11 to 170 MPN per 100 ml and from 8 to 34 MPN per 100 ml, respectively, (avg. MPN per 100 ml: TC = 10 and EC = 2.33) (Fig. 4). Water supplies showed greater contamination than filtration plants where total and fecal coliforms ranged from 2 to 130 MPN per 100 ml and from 2 to 34 MPN per 100 ml, respectively, (avg. MPN/100 ml: TC = 12.6 and EC = 3.45) (Fig. 4). Less number of tube wells were found unsafe for drinking purpose though the risk associated with contamination was low ranging from 2 to 26 MPN per 100 ml for both total and fecal coliforms *(E. coli)*, yielding an average MPN of 1.15 per 100 ml (Fig. 4). The samples tested to be positive for *E. coli* remained consistent in all trials, showing microbially degraded water. Recent studies from different areas of Pakistan also complement our results regarding drinking water contamination [27,28]. A study suggested that over 70% of stored water samples from self-reported boilers met the World Health Organization guidelines for safe drinking water (0 TTC/100 ml), and only 10.7% fell within the commonly accepted low-risk category (1–10 TTC/100 ml); hence, boiling is recommended as a control measure [29].

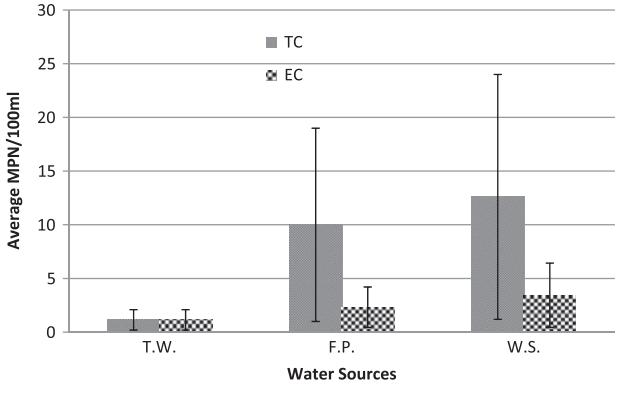

Fig. 4. Comparison of TCs and *E. coli* in different water sources of Islamabad region. Total number of samples (*n*) = 55.
Note: TW = tube wells, FP = filtration plants, WS = water supply.

3.5. Identification of bacterial isolates

Bacterial species were confirmed with the help of API 20E Kit. *E. coli*, *Enterobacter sakazakii*, *Aeromonas hydrophila*, *Klebsiella pneumonia*, *Enterobacter cloacae*, *Pantoea agglomerans*, *Serratia marcescens*, and *P. aeruginosa* were identified with the help of API 20E strips (Tables 1 and 2). *E. coli* was the most frequently encountered followed by *K. pneumonia*. According to WHO, the presence of *E. coli* has high health significance in water supplies, whereas the presence *P. aeruginosa* has moderate significance [30]. *E. cloacae* is considered as an important nosocomial pathogen responsible for various infections.

Presence of *E. coli* and *K. pneumonia* in drinking water poses a potential human health hazard. The level of coliform contamination in drinking water in capital city is considerably low (14.5%) as compared to other cities like Lahore where bacterial contamination is 37.2% [5], Karachi 36% [31], and alarmingly high in Khyber agency with 80% TCs and 66% *E. coli* [32]. A study of drinking water of Pakistan revealed that 67% sources were contaminated with total and fecal coliforms [33]. Therefore, preventive measures need to be adopted to ensure safe drinking water quality.

4. Conclusion

Majority of the water sources were found to be safe for drinking purpose in ICT and only 14.5% were above the limits as described in WHO guidelines for drinking water. Filtration plants were the minimum contaminated among all the water sources. An equal number of tube wells and water supplies were found unsafe for drinking purpose by this study. *E. coli*, *E. sakazakii*, *A. hydrophila*, *K. pneumonia*, *E. cloacae*, *P. agglomerans*, *S. marcescens*, and *P. aeruginosa* were isolated and identified during the survey of different water supplies. However, *E. coli* was the most frequently encountered followed by *K. pneumonia* which can pose serious health hazards. Maintenance of quality filters in filtration plants, disinfection with chlorine in water storage tanks, installation of functional UV-light system, and replacement of leaked and worn-out pipes should be worked out properly. Regular monitoring and treatment of water sources should be performed to prevent any outbreak in the capital city

Table 1
Biochemical characteristics of identified bacteria isolated from drinking water samples of Islamabad

S. #	Characteristics	*E. coli*	*E. sakazakii*	*K. pneumoniae*	*E. cloacae*	*P. agglomerans*	*P. aeruginosa*	*A. hydrophila*	*S. marcescens*
1	Morphology	Rods, single	Rods, single	Rods, single	Rods, single	Rods, single	Rods, single	Rods	Rods
2	Gram's staining	−	−	−	−	−	−	−	−
3	Ortho-nitrophenyl-β-galactosidase, (ONPG)	+	+	+	+	+	−	+	−
4	Arginine dihydrolase (ADH)	−	+	−	+	−	+	+	−
5	Lysine decarboxylase (LDC)	+	−	+	−	−	−	−	+
6	Ornithine decarboxylase (ODC)	+	−	−	+	−	−	−	+
7	Citrate test	−	+	+	+	+	+	−	+
8	H_2S production	−	−	−	−	−	−	−	−
9	Urease test	−	−	+	−	−	−	−	−
10	Tryptophan deaminase (TDA)	−	−	−	−	−	−	−	−
11	Indole test	+	−	−	−	+	−	−	−
12	Voges Proskauer	−	+	+	+	−	−	+	−
13	Gelatin hydrolysis	−	−	−	−	−	+	+	+

Note: +, positive test, −, negative test.

Table 2
Fermentation test of waterborne bacterial strains

Sugars fermented by bacteria										
S. #	Microorganisms	Glucose	Mannitol	Inositol	Sorbitol	Rhamnose	Sucrose	Melibiose	Amygdalin	Arabinose
1	*E. coli*	+	+	−	+	+	+	+	-	+
2	*E. sakazakii*	+	+	−	−	+	+	+	+	+
3	*K. pneumoniae*	+	+	+	+	+	+	+	+	+
4	*E. cloacae*	+	+	−	+	+	+	+	+	+
5	*P. agglomerans*	+	+	−	+	+	+	−	+	+
6	*P. aeruginosa*	−	−	−	−	−	−	−	−	+
7	*A. hydrophila*	+	+	−	−	−	+	−	+	+
8	*S. marcescens*	+	+	+	+	−	+	−	+	−

Note: +, positive test, −, negative test.

and to reduce health risks associated with contaminated drinking water. Drinking water supplies should adopt proper disinfection via chlorination or other suitable methods to ensure better health for the people.

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