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Original Article

Assessment of bacteriological quality of drinking water from various sources in Amritsar district of northern India

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Abstract

Introduction: Safe water is a precondition for health and development and is a basic human right, yet it is still denied to hundreds of millions of people throughout the developing world. Water-related diseases caused by insufficient safe water supplies, coupled with poor sanitation and hygiene, cause 3.4 million deaths a year, mostly in children.

Methodology: The present study was conducted on 1,317 drinking water samples from various water sources in Amritsar district in northern India. All the samples were analyzed to assess bacteriological quality of water for presumptive coliform count by the multiple tube test.

Results: A total of 42.9% (565/1,317) samples from various sources were found to be unfit for human consumption. Of the total 565 unsatisfactory samples, 253 were from submersible pumps, 197 were from taps of piped supply (domestic/public), 79 were from hand pumps, and 36 were from various other sources A significantly high level of contamination was observed in samples collected from submersible pumps (47.6%) and water tanks (47.3%), as these sources of water are more exposed and liable to contamination.

Conclusions: Despite continuous efforts by the government, civil society, and the international community, over a billion people still do not have access to improved water resources. Bacteriological assessment of all sources of drinking should be planned and conducted on regular basis to prevent waterborne dissemination of diseases.

Key words: water quality; coliforms; *Escherichia coli*; multiple tube method.

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Introduction

Sanitation and drinking water are universally accepted as essential for human life, dignity, and human development. Accessibility to and availability of fresh, clean water not only play a crucial role in economic development and social welfare, but are also essential elements in health, food production, and poverty reduction [1]. Water pollution is a global problem and poses a serious threat to human life. The World Health Organization estimated that there are four billion cases of diarrhea each year in addition to millions of other cases of illness associated with the lack of access to clean water. More than three million people in the world die of water-related diseases due to contaminated water each year, including 1.2 million children [2]. Outbreaks of waterborne diseases continue to occur throughout the world but are especially serious in developing countries. Around 37.7 million Indians are affected by waterborne diseases annually; 1.5 million children are estimated to die of diarrhea alone [3]. Almost 90 per cent of diarrhea cases are due to contaminated water. According to a World Health Organization/United Nations Children's Fund resource report, 70% of India's water supply is seriously polluted with sewage effluents, and it ranks 120th among the 122 nations in terms of quality of water available to its citizens [4].

It is well established that infectious diseases are transmitted primarily through water supplies contaminated with human and animal excreta, particularly feces. The human pathogens that present serious risk of disease whenever present in drinking water include *Salmonella* spp., *Shigella* spp., pathogenic *Escherichia coli*, *Vibrio cholerae*, *Yersinia entercolitica*, *Campylobacter* spp., various viruses such as hepatitis A, hepatitis E, rotavirus and parasites such as *Entamoeba histolytica* and *Giardia* spp. [5]. Public and environmental health protection require safe drinking water, which means that water must be free of all pathogenic bacteria.

As pollution, population, and environmental degradation increase, drinking water sources face increasing threat from contamination, both chemical and microbiological. A wide spectrum of pathogenic agents can be found in water, and monitoring for their presence on a routine basis is impractical.

Traditionally, microbial safety of drinking water has been confirmed by monitoring for absence of microorganisms of fecal origin [6]. Monitoring the microbiological quality of drinking water relies largely on examination of indicator organisms such as coliforms. *E. coli* is a member of the fecal coliform group and is a more specific indicator of fecal pollution than are other fecal coliforms. At present, *E. coli* appears to provide the best bacterial indication of fecal contamination in drinking water, based on the prevalence of *E. coli* in human and animal feces as compared to other coliforms and the availability of affordable, fast, sensitive, specific, and easier to perform detection [7].

Water supply from the source has to be regularly monitored by the authorities to ensure the delivery of pure and germ-free water to the general public. In this study, the bacteriological quality of water being supplied to different public places by various sources of water in and around Amritsar district of northern India was monitored and analyzed.

Methodology

Drinking water samples are received in the Department of Microbiology occasionally through government district health authorities for the assessment of bacteriological quality of water from various public places in the interest of public health. A total of 1,317 drinking water samples from various water sources in the district of Amritsar received between January 2013 and May 2014 were analyzed in the Department of Microbiology, Government Medical College & Hospital, Amritsar.

The samples were collected aseptically in sterilized containers and tested by trained health educators and a senior laboratory technician of the department, following guidelines of the WHO [8] and the Indian Council Medical Research (ICMR) [9]. Two hundred milliliters of water samples from each source were collected in sterile glass stopper bottles for microbiological examination. The samples were transported and stored strictly in accordance with the procedures and guidelines described in the WHO's guidelines for drinking water quality [8]. Water samples containing residual chlorine were neutralized by adding pre-sterilized 0.1 mL sodium thiosulphate (1.8% w/v) per 100 mL of water sample. The samples were stored at 2°C–8°C in a dark area to avoid changes in bacterial count until analysis. The total coliform count test was based on the multiple tube fermentation method to estimate the most probable number (MPN) of coliform organism in 100 mL of water for diagnosis of bacteriological contamination. The test was carried out by inoculation (for 48 hours at 35°C) of measured quantities of sample water (0.1, 1.0, 10, 50 mL) into tubes of double- and single-strength McConkey's lactose bile salt broth with bromocresol purple as an indicator. The tubes showing gas formation were considered to be presumptive coliform positive. The results of MPN were interpreted based on McCrady's probability tables from the number of tubes showing acid and gas (fermentation by coliform organisms) to define the sample as satisfactory or unsatisfactory [10]. Differential coliform count (Eijkman's test) was performed by incubating subcultures from the positive presumptive tests at 44°C and 37°C in lactose bile broth and the other subculture at 44°C in tryptophan broth. The presence of coliform bacilli was confirmed by the production of gas from lactose at 37°C, and that of *E. coli* was confirmed by the production of gas from lactose and indole from tryptophan at 44°C, followed by subculture on MacConkey agar [11]. All the media and reagents were procured from Himedia, Pv Ltd., Mumbai, India.

Results

A total of 551/1,317 samples from various sources were found to be unsatisfactory, and 21/1,317 were found to be suspicious after multiple tube fermentation tests for coliforms (Table 1). After subjecting the suspicious samples (21) to the Eijkman test, 14 were found to be unsatisfactory, growth of *E. coli* was obtained from 11 samples, and other coliforms were grown from 3 samples. A total of 565 samples were found to be unsatisfactory (551 detected by multiple

Table 1. Presumptive coliform count of total water samples tested

Grade of water sample	Presumptive coliform count/ 100 mL	Number (%) of water samples (n = 1,317)
Excellent	0	474 (35.9%)
Satisfactory	1–3	271 (20.6%)
Suspicious	4–10	21 (1.7%)*
Unsatisfactory	> 10	551 (41.8%)

*After subjecting the 21 suspicious samples to Eijkman test, 14 were found to be unsatisfactory while 7 were found to be satisfactory.

tube method, and 14 by the Eijkman test) and unfit for human consumption. Among the total samples, 451 were taken from schools (government and private), of which 215 were found to be unsatisfactory. Furthermore, 135/379 samples from religious places, 98/206 from domestic water supply, 33/79 from hospitals, followed by other public places (Table 2), were found to be unsatisfactory. Of the total 565 unsatisfactory samples, 253 were from submersible pumps, 197 were from taps of piped supply (domestic/public), 79 were from hand pumps, and 36 were from various other sources (Table 3).

Discussion

The potential of drinking water to transport microbial pathogens to great numbers of people, causing subsequent illness, is well documented in countries at all levels of economic development. It has been noted that most sporadic cases of waterborne intestinal illness will not be detected, or, if detected, may not be recognized to be water related. Several researchers have attempted to estimate the total burden of waterborne diseases, which might account for one-third of intestinal infections worldwide [12]. It has been estimated that water, sanitation, and hygiene are responsible for 40% of all deaths and 5.7% of the total disease burden occurring worldwide [13].

The provision of effective sanitation programs and access to safe drinking water have been a major problem for many developing countries. In the developing world, especially in remote rural areas and industrial areas, over three million deaths per year are attributed to waterborne diarrheal diseases, especially among infants and young children in poor communities [14]. A water survey in Pakistan revealed bacterial causes of water contamination to be 68%, giving rise to 100 million diarrheal cases seeking hospital admissions and an associated 40% mortality rate [15].

The microbiological quality of drinking water is a concern to consumers, water suppliers, regulators, and public health authorities alike. The number of outbreaks that have been reported throughout the world demonstrates that the transmission of pathogens by drinking water remains a significant cause of illness. However, estimation of illness based solely on detected outbreaks is likely to underestimate the problem. A significant proportion of waterborne illness is likely to go undetected by communicable disease surveillance and reporting systems. Pruss *et al.* estimated that water, sanitation, and hygiene were responsible for 4% of all deaths and 5.7% of the total disease burden occurring worldwide [13]. Hunter and Fewtrell estimated that waterborne diseases might account for one-third of the intestinal infections worldwide [12]. Moreover, patterns of infection change over time, and public health authorities can be seen faced with newly discovered or emerging species of pathogens including *E. coli* O157:H7, *Helicobacter* spp., *Mycobacterium avium* complex (MAC), protozoa

Table 2. Number of total and unsatisfactory samples collected from various places

Source of collection	Total samples	Unsatisfactory samples
Schools	451	215 (47.6%)
Religious places	379	135 (35.6%)
Domestic supply	206	98 (47.5%)
Railway stations/bus stands	165	75 (45.4%)
Hospitals	79	33 (41.7%)
Others	37	9 (24.3%)

Table 3. Results of presumptive coliform count in relation to water source

Source	No. of samples analyzed (n = 1,317)	Excellent	Satisfactory	Unsatisfactory
Submersible pumps	531 (40.3%)	199 (37.4%)	78 (14.6%)	253 (47.6%)
Taps	477 (36.3%)	153 (32.0%)	124 (25.9%)	197 (41.2%)
Hand pumps	185 (14.0%)	75 (40.5%)	29 (15.6%)	79 (42.7%)
Tube wells	105 (7.9%)	45 (42.8%)	33 (31.4%)	27 (25.7%)
Water tanks	19 (1.5%)	2 (10.5%)	7 (36.8%)	9 (47.3%)

Cryptosporidium and *Cyclospora* spp. that may be able to overcome the barrier of water treatment and distribution systems [16].

The present study assessed the bacteriological quality of water in different drinking water sources in the district of Amritsar; 42.9% of the samples were found to be unsatisfactory and unfit for human consumption (Table 1). The results are found to be consistent with the various other studies conducted in the same setting, which found 47.5% and 38.6% of samples, respectively, to be unsatisfactory [17,18]. Concordant findings were also reported from studies done in different settings of northern India [14,19]. In our study, the sources of water were taps, hand pumps, water tanks, and underground water being dug out by motors and submersible pumps. A significantly high level of contamination was observed in samples collected from submersible pumps and water tanks (Table 3) as compared to hand-pump and taps; these sources of water are more exposed and liable to contamination by raw sewage overflow, septic tanks, leaking sewer lines, land application of sludge, and animal droppings/bird feces. Though the authorities are taking utmost care to supply treated (chlorinated) water, a large infusion of pathogens and organic matter (as often happens in depressurized water pipes in large cities of developing countries) routinely overwhelms the protection provided by residual chlorine. The increase in incidence of unsatisfactory samples in the present study could also be due to the rapid growth of the city, with a present population of about 2.5 million and reflecting an ailing sewer system, which has not been able to cope with the growth of this holy city.

A wide spectrum of pathogenic agents can be found in water, and monitoring for their presence on a routine basis is impractical. Our study supports the finding that coliforms has long been recognized as a suitable microbial indicator of drinking water quality largely because they are easy to detect and enumerate in water [7]. The WHO has identified *E. coli* to be the most discriminating marker for fecal contamination and therefore a microbiological indicator of choice for drinking water potability and safety, especially in developing countries with limited resources [20]. The indicator organisms, namely heterotrophic bacteria, and total and fecal coliform for ensuring water quality, were also taken as indicators of water quality in other studies [17,19]. It was observed in our study that more than 40% samples taken over a period of one-and-a-half years were found to be bacteriologically contaminated, which shows that surface water is highly contaminated with animal or human fecal matter. Sinha *et al*. also reported numbers of coliform bacteria, which varied from 600–1,600 per 100 mL from Susta pond and 1,200–2,000 per 100 mL from Madhaul pond of Muzzarpur, Bihar [21]. Similarly, 197 coliform species were isolated from drinking water in five rural areas of Lucknow [22].

It was further observed in our study that the MPN of all the water sources was higher in the rainy seasons as compared to winter and summer. These results were in concurrence with the findings of Mohopatra *et al*., who reported that coliform counts in two water channels in Delhi had the lowest values in the month of December [23]. Jais *et al*. reported the highest coliform counts in the months of June and July [24]. Finally, the population pressure cannot be ignored as an important source of contamination. A large proportion of the population in developing countries does not have access to sanitary toilets, and is thus forced to defecate in the open, leading to contamination of water resources. According to a recent World Bank report, the sanitation coverage in India is only 68%. Though India as an emerging economic superpower in the world, open defecation still remains a major public health concern, with 6% of its gross domestic product (GDP) wasted annually due to lost productivity, healthcare provision, and other consequences of poor sanitation [3].

A report quoting the WHO stated that more people would die from consuming unsafe drinking water and unsanitary conditions by the year 2020 than would die from AIDS if steps to improve water quality were not taken at this point in time [19]. As noted by LeChevallier *et al*. [6], knowledge is the first line of defense in providing safe drinking water.

Water quality is a growing concern, and availability of safe drinking water is still out of reach for the majority of the people in developing countries. Such an alarmingly high percentage of unsatisfactory water samples from places visited regularly by residents calls for public awareness, immediate attention, and action by the authorities. A comprehensive development program must include a practical and cost-effective approach to provide potable water and a more aggressive approach to reduce the risk of water-related transmission of diseases.

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