

Original Research Article

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Bacteriological Analysis of Water Quality in Hospital and Residential Water Supply of a Tertiary Care Hospital of Northern India

Shilpi Srivastava^{1*}, Alisha Singh¹ and Nadeem Ahmed²

¹Department of Microbiology, ²Department of Community Medicine, Integral Institute of Medical Sciences, Lucknow, India

*Corresponding author

ABSTRACT

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Diseases due to consumption of water contaminated with microbes extract considerable morbidity and mortality. Monitoring of drinking water quality is thus of paramount importance. This study was done to assess water quality of hospital and residential water supply at our Medical Institute. Drinking water samples were collected from taps water coolers and submersible pumps and analyzed by multiple tube culture method to obtain Presumptive coliform count which is an indicator of recent faecal contamination. Most probable no (MPN) of the coliforms was used to determine if the sample was satisfactory. Out of a total of 42 samples 10 (26.3%) samples were unsatisfactory and not suitable for human consumption, 5 (11.90%) samples were of intermediate quality, 17(40.47%) samples were satisfactory and 10 (26.3%) samples were found to be excellent. From the unsatisfactory and intermediate quality samples, *E. coli* was cultured from 16.6%, *Klebsiella* from 4.2% and *Pseudomonas* from 14.2% of samples. Half of the municipal tap water supply samples were not fit for consumption. Ground water and water from UV filters (AquaGuard) was of good quality. Water coolers had a variable water quality.

Introduction

Background

Water is most important life sustaining force for the inhabitants of this world. But when contaminated it is also the vehicle for transmission of infection to humans across this world. Worldwide nearly a billion people use water that is not potable and 40 percent do not have living conditions that have proper sanitation. The result points to a grave public

health crisis: 4,500 per day is the mortality from waterborne diseases, surpassing collectively Malaria AIDS, and tuberculosis (Pulitzer Centre, 2015). The United Nations identified improving water quality as one of the eight Millennium Development Goals. Its target is to reduce the number of people without access to safe water by 50% by 2015 (WHO, 2011).

Additionally there is evidence that water systems can be vectors for waterborne

Hospital acquired infection (Anaisse *et al.*, 2002). Infections with waterborne organisms such as Legionella, mycobacteria, *Pseudomonas*, and others cause significant morbidity and mortality, particularly in hospitalized immunocompromised patients (Decker *et al.*, 2014). A 2003 CDC guideline highlighted practices to control waterborne HAIs (HICPAC, 2003).

Indicator organisms are commonly used to assess the microbiological quality of drinking or potable waters and fecal coliforms (FC) are the most commonly used bacterial indicator of recent fecal pollution. They may not always cause disease by themselves but are indicative of contamination by sewage which contains organisms like Hepatitis A virus, *Vibrio cholerae* dysentery causing bacteria like *Shigella* and *Campylobacter*, ova and cysts of intestinal parasites among others. Those at greatest risk of waterborne disease are infants and young children, people who are debilitated or living under unsanitary conditions and the elderly (WHO, Guideline of drinking water, 2011).

Thus Microbiological examinations have important role in the investigation of waterborne outbreaks. The aim of this study is to determine the bacteriological quality of drinking water sources and the extent of contamination at study area which will help in the intervention actions to be taken by the concerned bodies at our institute in district Lucknow (India).

Materials and Methods

Study design

This is a cross sectional study. The study proposal was approved by Institutional ethics committee and Institutional Research committee. This is a pilot study for this area as no such study has been done previously.

Samples and study area

Samples were collected from the hospital water supply and drinking water sources within University Campus and hospital for a period of six months from 1st January 2017 to 30th June 2017.

Collection and transport of samples

Heat sterilized screw capped bottles (200 ml capacity minimum) were taken for collection of water. At least 150 ml of water was collected. Water was collected from 3 different sources of drinking water namely

Tap water: Water was collected only after running it from tap for 2-3 minutes. The outlet was sterilized with the flame of a spirit lamp.

Aqua guard/Water cooler: Water was collected only after running it from machine for 2-3 minutes.

Water from hand pump: Stagnant water in the outlet was flushed out by operating the pump for a few minutes.

Inclusion criteria

Any active water source which was used for drinking purpose and was being used by a fairly good number of people as a water source for drinking purpose.

Hospital associated water supply like taps in the OTs, water cooler installed in wards.

Exclusion criteria

A newly drilled hand pump or rarely used one was not sampled unless the facility had been pumped for more than 48 hours. Samples from toilets were not taken.

Although recommendations vary, the time between sample collection and analysis

should, in general, not exceed 6 hours, and 24 hours is considered the absolute maximum. The samples were immediately placed in a lightproof insulated box containing melting ice or ice-packs with water to ensure rapid cooling. If ice was not available, the transportation time was kept under 2 hours. It was made sure that samples were kept in the dark and that cooling was rapid. If these conditions were not met, the samples were discarded.

Sample analysis

Standard methods were used for the bacteriological examination of the samples (Britton and Greeson, 1987; American Public Health Association, 1998).

Presumptive coliform count

Multiple tube method was used for the estimation of presumptive coliform count, which is expressed as the Most Probable Number (MPN) of coliform specimen in 100 mL of water. Briefly MacConkey purple broth (double strength and single strength) in bottles or tubes was used. Durham's tube was used to detect production of gas. Bromocresol purple was used as indicator. Measured amount of water sample were added to tubes containing MacConkey purple broth by sterile graduated pipettes. About 50 mL of water was added to one bottle of 50 mL double strength medium; 10 mL of water was added to all 5 tubes of 10 mL double strength medium; 1 mL of water each - was added to 5 tubes of 5 mL single strength medium and 0.1 mL of water each - was added to 5 tubes of 5 mL single strength medium (Mackie and McCartney: Practical medical microbiology).

Interpretation

An estimate of presumptive coliform count per 100 mL of the water was calculated from the

number of tubes showing acid and gas production using McCarty's probability table.

Differential coliform count (Eijkman test)

It was done to confirm that the coliform bacilli which were detected in presumptive test were faecal *E. coli*.

Method

The positive tubes (of MPN) were subcultured on lactose containing medium - MacConkey agar. Lactose fermenting colonies which demonstrated positive indole test at 44°C were identified as faecal *E. coli*. Additional biochemical tests (Indole, MR, VP, Citrate utilization, Urease, Triple sugar iron test) were also set up to confirm bacteriological identity.

Demonstration of recent and remote contamination

Recent contamination

Faecal *Escherichia coli* is the most sensitive and best indicator of the recent human or animal faecal contamination of water as it does not survive in water for long time. It was demonstrated in water sample as it ferments lactose at 44°C with production of acid and gas and gives positive indole test at 44°C.

Remote contamination

Faecal *Streptococci*: The presence of Faecal *Streptococci* in water is an indicator of remote faecal contamination. It can be either *Enterococci* or non-enterococcal *Streptococci*. Subcultures were made from positive tubes which were obtained from presumptive coliform test into tubes containing 5mL of glucose azide broth and were incubated at 45°C for 48 hours. Presence of acid in the medium indicated faecal *Streptococci*.

Clostridium perfringens: Their spores can survive for longer time in water and their presence indicates remote contamination in the water. First of all, the water sample was heated to kill all the vegetative spores of *Clostridium perfringens*. Then, multiple tube test was performed by sub culturing it onto Robertson cooked meat broth.

Quality of water supply

It was determined by the presumptive coliform count (Table 1).

Results and Discussion

Total 42 water samples from different water sources were collected. About 16(38%) samples were taken from tap water, 15(36%) samples were taken from Water cooler

embedded with filters, 6(14%) samples were taken from Submersible pumps and 5(12%) samples were taken from Aqua guard embedded with UV filters. Out of the total samples (n=42) 10(26.3%) samples were unsatisfactory and not suitable for human consumption whereas, 5 (11.90%) samples were of intermediate quality, 17(40.47%) samples were satisfactory and 10(26.3%) samples were found to be excellent (Table 2 and Fig. 1). It is to be noted that the number of unsatisfactory sample is zero for Aqua guard and Submersible pumps whereas the tap water is most contaminated as 8 samples were found to be unsatisfactory. *E. coli*, *Klebsiella* and *Pseudomonas* were isolated on final analysis of the samples which were not satisfactory. Of the five intermediate quality samples, three samples grew *E. coli* and two *Pseudomonas* species (Table 3 and 4).

Table.1 Grading of water Quality (Mackie and MacCarteny, 14th edition)

Grade of water sample	Presumptive coliform count (MPN)/100 ml	<i>E. coli</i> count/100 ml
Excellent	0	0
Satisfactory	1-3	0
Intermediate	4-9	0
Unsatisfactory	>10	>1

Table.2 Comparison of the quality of samples with the site of collection

Site of Sampling	Unsatisfactory No (%)	Intermediate No (%)	Satisfactory No (%)	Excellent No (%)	Total samples No
UV filter(Aqua guard)	0(0)	0(0)	1(20%)	4(80%)	5
Submersible pumps	0(0)	0(0)	0(0)	6(100%)	6
Water cooler fitted with filter	2(13.3)	4(26.6)	9(60)	0(0)	15
Tap water	8(50)	1(6.25)	7(43.7)	0(0)	16
Total	10(23.8)	5(11.9)	17(40)	10(23.8)	42

Table.3 MPN No. of the unsatisfactory samples obtained from TAP WATER along with the site of collection

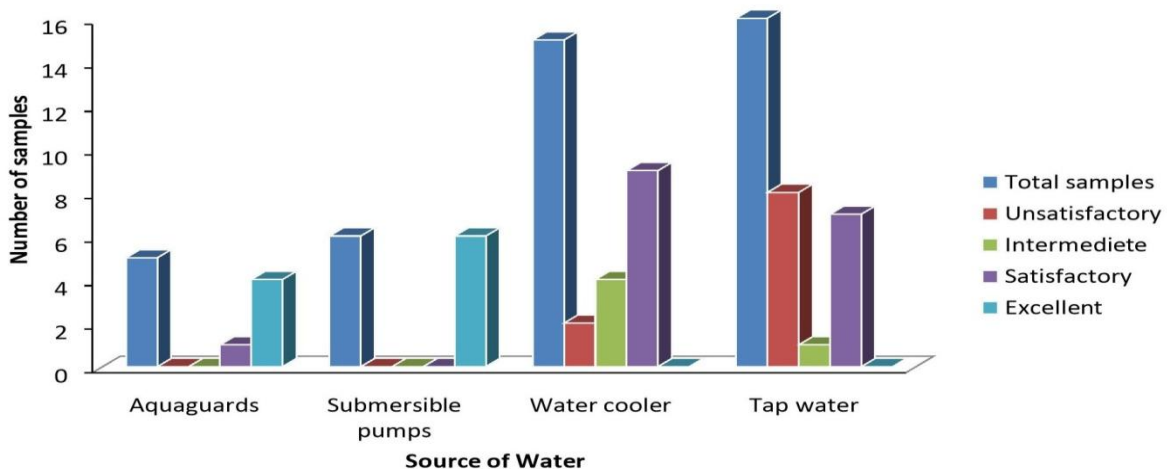
Site	Type	MPN
NICU	Tap Water	10
Academic block	Tap Water	14
Girls hostel	Tap Water	16
ICCU	Tap Water	14
MICU	Tap Water	19
Blood Bank	Tap Water	24
Emergency ward	Tap Water	38
Wash Basin(Hospital)	Tap Water	49

Table.4 Microbes isolated from the unsatisfactory and intermediate quality samples

Source of water sample	No. of sample collected No (%)	No. of Unsatisfactory samples No (%)	No of Intermediate quality samples No (%)	Organism Grown		
				<i>E. coli</i>	<i>Klebsiella sp.</i>	<i>Pseudomonas sp.</i>
				No	No	No
Submersible	6 (14.2%)	0(0)	0	-	-	-
Tap water	16 (38%)	8 (50%)	1	3	1	4+1*
Water cooler	15 (35.7%)	2 (13.33%)	4	1+3*	1	1*
UV filter(Aquaguard)	5 (11.9%)	0(0)	0	-	-	-
Total	42	10(23.8)	5(11.9)	7(16.6)	2(4.7)	6(14.2)

*numbers in blue are of intermediate quality samples

Fig.1 Bacteriological quality of the water samples



Many diseases affecting mankind are only due to consumption of contaminated water or raw food washed with dirty water. WHO (2010) reports that over 2.6 billion people lack access to clean water, which is responsible for about 2.2 million deaths annually, of which 1.4 million are in children. Improving water quality can reduce the global disease burden by approximately 4%. Monitoring the levels of indicator organisms (such as faecal coliforms, *E. coli*) is a common approach for quantifying the potential pathogen present in a source of drinking water. Studies have evaluated water quality by enumerating fecal coliforms and *E. coli* levels in rivers, lakes, estuaries, and coastal waters (Pandey *et al.*, 2012a; Pandey *et al.*, 2012b).

In this study we found that there was evidence of recent faecal contamination in about 25% of samples which were unfit for human consumption. There was no evidence of any remote faecal contamination in the samples analysed. Over half of tap water samples were unsatisfactory and not fit for use as drinking water. No tap water sample was of excellent quality. Of the unsatisfactory quality samples 40% grew *E. coli*, 10% *Klebsiella* species and 40% samples were contaminated with *Pseudomonas* species. Of the five intermediate quality samples, three samples grew *E. coli* and two *Pseudomonas* species. This data shows that the municipal supply of water has fluctuating quality. Either some water pipelines are damaged and contaminated with sewage water or the filtration and chlorination treatment of water is not adequate. Similar findings have been reported by Shamsi *et al.*, (2015) from Fatehgarh (U.P). In about one hundred water samples collected from tap water, handpump and water coolers, the authors found that almost half of the samples were unsatisfactory. *E. coli* was cultured from 26% of samples and *Pseudomonas* from 20% of collected samples. In a study done in Merrut

on drinking water samples, over 69% tap water samples and 70% of cooler water were unsatisfactory. Organism identified were *Escherichia coli* (28%), *Klebsiella* sp. (15%) and *Pseudomonas* sp. (25%) in total positive samples (Kumar *et al.*, 2013). The MPN number of some samples was very high (≥ 180). In this study the MPN no ranged from 10 to 49 among the tap water samples.

Compared to tap water the untreated water of rivers springs and ponds is now highly contaminated. One study on such sources in field areas has reported MPN count more than 1600 in some cases (Goel *et al.*, 2007). Microbial contamination of ground water sources can be a problem in urban areas also. In a study done in Mardan, Pakistan on samples of wells and tube wells, faecal coliform were found in 90% samples and *E. coli* in 56% samples. The (MPN) ranged from 40 to as high as 2400 (Khan *et al.*, 2012). In contrast in our study water samples from the submersible pump accessing the ground water were of excellent quality. Also water filtered by standalone Aquaguard (UV light based) filter was mostly of excellent quality. All samples were fit for consumption.

In comparison water from water coolers fitted with aquaguard had variable quality with two out of fifteen samples showing unsatisfactory result and four were of intermediate quality. This data throws light on a very important but often missed parameter that is storage of potable water. Even though the water was filtered, it became contaminated due to deficient cleaning of storage tank or improper use of the faucets. There is still need of creating awareness for cleaning of storage vessels and tanks. Another factor to be noted was that the intermediate quality though had a low MPN, yet came positive for *E. coli* on culture.

Pseudomonas species was cultured from six hospital supply samples. *Pseudomonas* has

the propensity to form biofilms and may have colonized the faucets. This finding is significant as an association between *Pseudomonas aeruginosa* infections and water sources has been found (Cervia, 2012). *Pseudomonas aeruginosa* causes infections generalized by inflammation and sepsis, which can be fatal if colonization occurs in critical body organs, such as the lungs, urinary tract or kidneys. There is plenty of evidence associating *P. aeruginosa* infections and tap water used in the intensive care unit (ICU) and patient rooms (Trautman *et al.*, 2001; Reuter *et al.*, 2002). In a study using genetic-based epidemiological evidence association was found between *P. aeruginosa* and waterborne HAIs. Over a six-month period, 19 out of 38 patient infections were acquired via tap water or cross-transmission (Roques *et al.*, 2007). There has been a difference of opinion on the current indicator organisms and their ability to represent the source of faecal contamination as human vs animal excreta and bird droppings Microbial source tracking to trace the origin of faecal coliform is a new approach that holds promise. (Grave *et al.*, 2007; Dickerson *et al.*, 2007)

This study concludes that the tap water supply is not potable in about half of the samples. UV filters should be used at point source of drinking and hospital supply. The ground water is not contaminated in our area. We recommend that potable water supply for drinking purposes and for washing in hospitals and residences should be analysed bacteriologically at regular intervals so that feedback could be given to the concerned authorities.

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