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Original Research Article

PRESUMPTIVE COLIFORM COUNT AND DIFFERENTIAL COLIFORM COUNT OF THE WATER SAMPLES FROM AN URBAN SLUM AREA IN LUCKNOW

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ABSTRACT

Background: Faecal contamination in water sources is still found worldwide, particularly in urban slum areas of mid-low-income countries. Faecal infection might increase the risk of waterborne diseases in developing countries and in turn suggests poor hand hygiene and sanitation. This study aimed to assess quality of all water sources and to estimate the presumptive coliform count and differential coliform count of the water samples from an urban slum area in Lucknow. **Materials and Methods:** A cross-sectional survey design was conducted during the period of January to June 2019. Samples were collected from all the taps, hand pumps which are currently in use, along with potable water stored in households of Sarvodya Nagar (an urban slum area), Lucknow and were processed in Microbiology department of IIMS&R, Lucknow. **Results:** A total of 47.6% samples from various sources were found to be unsatisfactory, and 25.0% were found to be intermediate after multiple tube fermentation tests for coliforms.The highest value of MPN was found to be >180 both in Submersible and Public supply water.The most common isolates obtained was Pseudomonas sp. fallowed by E.coli and Klebsiella Spp. Among 40 unsatisfactory samples 38 (95%) showed growth of E.coli or Thermotolerent E.coli which is an indicator of the recent fecal contamination of the water sources. **Conclusions:** The most contaminated sources of water were found to be the Public supply.MPN is a good indicator for analysis of water quality for drinking purpose. Bacteriological valuation of all sources of drinking should be intentional and conducted on consistent basis to prevent waterborne diseases.

Key words: Bacteriological, water; coliforms, Escherichia coli,

INTRODUCTION

Water used for sanitation and drinking are very important for human life, dignity, and human growth. Accessibility to and availability of fresh, clean water even play a crucial role in economic growth and social welfare, but are also vital elements in health, food production, and poverty reduction **(1)**. Water pollution is a worldwide problem and poses an important threat to human life. The World Health Organization projected that there are 4 billion cases of diarrhoea each year in count to millions of other cases of illness related with the lack of access to hygienic water. More than 3 million individuals in the world die of water associated diseases due to polluted water each year, including 1.2 million children **(2)**. Water-borne diseases are serious in developing countries. Approximately 37.7 million Indians are affected by waterborne diseases annually; 1.5 million children are projected to die of diarrhoea alone **(3)**. About 90 per cent of diarrhoea cases are due to contaminated water. World Health Organization/United Nations Children's Fund reported that the, 70% of India's water supply is

seriously contaminated with sewage effluents, and it ranks 120th among the 122 nations in terms of superiority of water available to its citizens **(4)**. It is well well-known that infectious diseases are spread primarily through water supplies polluted with human and animal excreta, particularly feces. The human pathogens that present important risk of disease whenever present in drinking water contain Salmonella spp., Shigella spp., pathogenic Escherichia coli, Yersinia entercolitica ,Vibrio cholerae, Campylobacter spp., various viruses such as hepatitis E, hepatitis A, rotavirus and parasites such as Entamoeba histolytica and Giardia spp. **(5)**. Environmental and Public health protection require safe drinking water, that means that water surely free of all pathogenic bacteria. As pollution, population, and environmental poverty increase, drinking water sources face increasing threat from pollution. A wide range of pathogenic agents can be found in water, and monitoring for their occurrence on a routine basis is unfeasible. Usually, microbial safety of drinking water has been confirmed by monitoring for absence of microorganisms of faecal origin **(6)**. Monitoring the microbiological quality of drinking water depend on largely on investigation of indicator organisms such as coliforms. E. coli is a part of the faecal coli-form group and is a more precise indicator of faecal pollution than are other faecal coli-forms. Availability of reasonable, fast, sensitive, specific and easier to perform detection **(7)**. Water supply from the source has to be frequently monitored by the authorities to ensure the delivery of clean and germ-free water to the general public. This study aimed to assess quality of all water sources and to estimate the presumptive coliform count and differential coliform count of the water samples from an urban slum area in Lucknow

MATERIAL AND METHODS

Study Design: Observational cross sectional study design.

Study place: Samples were collected from all the taps, hand pumps which are currently in use, along with potable water stored in households of Sarvodaya Nagar (an urban slum area), Lucknow and were processed in Microbiology department of IIMS&R, Lucknow, Uttar Pradesh, India

Study period: Six months (from 1st January2019 – 30th June2019)

Collection of samples:

Sterile glass stoppered bottles (250mL) were used to collect water samples. Using a cotton wool soaked in 70% ethanol, outlet of the tap/hand-pump were sterilized. Tap were turned on, for maximum flow for two minutes and then 200ml water was collected under medium flow, cap was replaced and sample was kept within cool-packs and brought to the laboratory for processing within 6 hours. Water samples were properly labelled with full details of the source, time, place and date of collection. Questionnaires were administered to the 84 selected households to obtain data on the type of toilet facility used, major source of domestic water, method of human waste disposal, whether drinking water was boiled, and the perceptions of likely sources of water contamination in the area. For the method of excreta discarding and water source, the main method and source were considered in instances where there was more than one method or source, respectively. The distance between the pit latrines used by the households and the wells (in cases where they used wells) was estimated. We also observed sanitation practices.

Laboratory Examination of Drinking Water Samples:-

Bacteriological examination: It was done by two methods-

- 1: Presumptive coliform count
- 2: Differential coliform count

Presumptive coliform count (Multiple tube method):

Multiple tube method was used for the estimation of presumptive coliform count, which was expressed as the most probable number (MPN) of coliform organism per 100 mL of water.

Medium: Mac Conkey purple broth (double strength and single strength) in bottles or tubes.

- Durham's tube was used to detect gas production.
- Bromo-cresol purple was used as indicator.

Procedure: Exact quantity of water samples were added to tubes containing MacConkey purplebroth by sterile graduated pipettes as under:

- Fifty millilitre of water-added to one bottle of 50 millilitre double strength medium.
- Ten millilitre of water each-added to 5 tubes of 10 millilitre double strength medium.
- One millilitre of water each-added to 5 tubes of 5 millilitre single strength medium.

The vaccinated tubes were covered at 37°C for 48 hours. Positive test was indicated by (1) a color change in the medium from purple to yellow (due to lactose fermentation) and/or (2) gas **collected in the Durham's tube.**

Interpretation: The interpretation of presumptive coliform count was as follows:

Presumptive coliform count (Most probable number): An estimate of coliform count per 100mL of water was calculated from the tubes showing acid and gas production using the McCardy's probable table **(8)**.

Table 1:Presumptive coliform count.

Class	Grade of water	Presumptive coliform count/ 100 mL
I	Excellent	0
II	Satisfactory	1-3
III	Intermediate	4-9
IV	Unsatisfactory	≥10

Differential coliform count (Eijkman test)

The Eijkman test was done to confirm that the coliform bacilli detected in the presumptive test were faecal E coli. This were done by-

- Sub culturing the positive tubes (of the previously done presumptive coliform test) on lactose containing medium such as MacConkey agar.
- Demonstrating positive indole test at 44°C.

Count of faecal Streptococci.

When presumptive coliforms are present but E.coli is absent, a demonstration of the presence of faecal streptococci was confirm the faecal origin of the coliform bacilli.

1. Incubate tubes containing 5 mL sterile glucose azide broth in a water-bath thermostatically controlled at 44-45 °C.
2. When the tubes were warmed to the incubation temperature, they were seeded with heavy inocula from all the tubes in the presumptive coliform test that showed the formation of either acid and gas, or acid only. Immediately re-incubate at 44-45 °C.
3. After incubation for 48 hours, the cultures were inspected for acid production shown by a yellow colour change in the bromocresol purple in the medium. Those producing acid contain faecal streptococci.
4. Confirming the presence of faecal streptococci by sub-culturing each positive glucose azide culture on to a tube of bile aesculin azide agar incubated at 44-45°C.
5. Examining the plates after a few hours' incubation for a brown-black coloration around the inoculum, which were evidence of hydrolysis of the aesculin and confirms the presence of faecal streptococci **(9)**.

Statistical Analysis:

Analysis of data was generally descriptive, involving determination of frequencies. Microsoft excel was used to analyze the data.

RESULTS

We have taken 84 water samples from different water sources. 21(25%) samples were taken from Submersible pump, 53(63%) samples were taken from Public supply, 3(3.6%) samples were taken from RO/UV filter and 7(8.3%) samples were taken from Gravity based purifiers.

Table2. Presumptive coliform count of total water samples tested

Grade of water	Presumptive coliform count/ 100 mL	Number (%) of water samples (n = 84)
Excellent	0	15(17.9)
Satisfactory	1-3	8(9.5)
Intermediate	4-9	21(25.0)
Unsatisfactory	≥10	40(47.6)

Total of 40/84 samples from various sources were found to be unsatisfactory, and 21/84 were found to be intermediate after multiple tube fermentation tests for coliforms. Only 8/84 samples from various sources were found to be satisfactory (Table 2)

Table 3: Most probable number(MPN) of the unsatisfactory samples obtained from Submersible pump

Type of water	Total No. of samples	Out of Total No. of sample	MPN	Isolates Obtained
Submersible pump	4	1	35	Pseudomonas spp. E.coli Thermotolerent E.coli
		1	92	Pseudomonas spp.
		2	>180	Pseudomonas spp. E.coli Thermotolerent E.coli

The maximum MPN which was obtained were >180 and >180 for Submersible & Public supply. Out of four unsatisfactory sample, Pseudomonas spp. E.coli Thermotolerent .coli were isolates obtained in two samples. Whereas Pseudomonas spp. E.coli Thermotolerent E.coli and Pseudomonas spp. were isolates obtained in one sample each. (Table 3 and table 4)

Table 4: Most probable number (MPN) of the unsatisfactory samples obtained from Public supply.

Type of water	Total No. of samples	Out of Total No. of sample	MPN	Isolates Obtained
Public supply	36	5	11	Pseudomonas spp. E.coli Klebsiella spp.
		2	13	Pseudomonas spp. E.coli Klebsiella spp. Enterococcus
		1	17	Pseudomonas spp. E.coli
		1	22	Pseudomonas spp. Klebsiella spp.
		5	24	Pseudomonas spp. E.coli Klebsiella spp. Citrobacter spp.
		3	28	Pseudomonas spp. E.coli Klebsiella spp.
		3	35	Pseudomonas spp. E.coli Klebsiella spp.
		2	43	Pseudomonas spp. Klebsiella spp.
		3	92	Pseudomonas spp. E.coli
		3	161	Pseudomonas spp. E.coli Thermotolerent E.coli Klebsiella spp.
		8	>180	Pseudomonas spp. E.coli Thermotolerent E.coli Klebsiella spp.

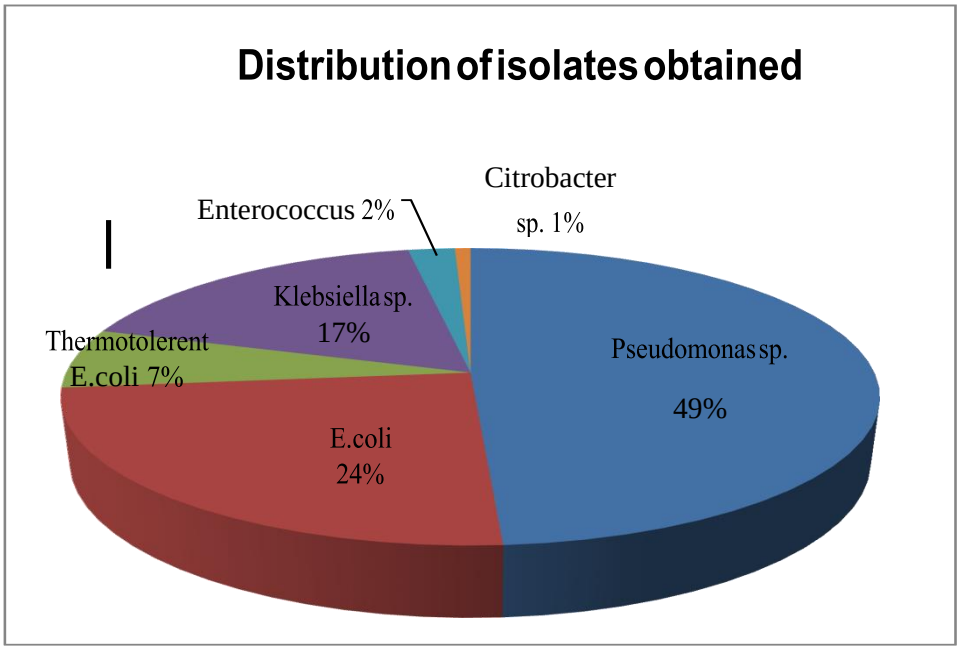

Figure 1:- Distribution of isolates obtained.

Pseudomonas spp. was found the most (49%) amongst all the isolates and Citrobacter spp. was the least in the list that is,1%. The most common isolates obtained was Pseudomonas sp. Followed by E.coli and Klebsiella Spp (Figure 1)

DISCUSSION

The probable of drinking water to passage of microbial pathogens to great numbers of individuals, causing subsequent illness, is well documented in countries at all levels of economic growth. It has been well-known that most sporadic cases of waterborne intestinal illness will not be noticed, 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 **(10)**. 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 **(11)**. The operative sanitation plans and access to safe drinking water have been a chief problem for several developing countries. Developing countries, especially in industrial areas and remote rural areas, over 3 million deaths per year are credited to waterborne diarrheal diseases, especially among infants and young children in poor communities **(12)**. 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 **(13)**. The present study evaluated the bacteriological quality of water in different drinking water sources in the district of Lucknow; 47.6% of the samples were found to be unsatisfactory and unhealthy for human consumption. The outcome are found to be similar with the various other studies shown in the same setting, which found 47.5% and 38.6% of samples, respectively, to be unsatisfactory **(14,15)**. Concordant findings were also reported from studies done in dissimilar settings of northern India **(12,16)**. A study reported that the MPN of all the water sources was higher in the rainy seasons as compared to winter and summer **(17)**. Similar finding by, Mohopatra et al., who reported that coliform counts in two water channels in Delhi had the lowest values in the month of December **(18)**. Jais et al. reported the highest coliform counts in the months of June and July **(19)** Kumar et al found that 63 out of a total of 116 drinking water samples were unsatisfactory with a very high MPN (≥ 180). The organisms were isolatedin total positive samples as Escherichia coli was (28%), Klebsiella spp. was (15%) and Pseudomonas spp. was (25%) **(20)**. Also Balaraman et al., performed bacteriological analysis of drinking water and reported that MPN index of water samples which tested satisfactory was < 2 per 100 ml and MPN index of water samples ,as unsatisfactory ranged from 38 to > 1600 per 100 ml. Coliforms isolated were Escherichia coli, Klebsiella spp. and Citrobacter freundii **(21)**. In addition, In

present study, the MPN of the unsatisfactory sample along with the type of sample the maximum MPN which was obtained were >180 and >180 for Submersible & Public supply, MPN of the Intermediate sample along with the type of sample the maximum MPN which was obtained were 5 & 8 for Submersible & Public supply. Such an terrifyingly high proportion of unsatisfactory water samples from places visited frequently by residents calls for public awareness, instant attention, and action by the authorities.

Limitations: The study focussed upon the pathogens present in the samples collected from the various water sources only. The antimicrobial sensitivity to various drugs shoud also been conducted. The study also didn't evaluate the water samples for micro nutrients and macronutrients as they also play an integral part in causation of cardiovascular diseases.

CONCLUSION

Highest value of MPN was found to be >180 both in Submersible and Public supply water. The most contaminated sources of water were found to be the Public supply. MPN is a good indicator for analysis of water quality for drinking purpose. A comprehensive development program must include a hands-on and cost-effective method to provide potable water and a more aggressive method to reduce the risk of water-related transmission of diseases.

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