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### **Characterization of Microbial Quality of Well Water Sources from Rural Community in Allahabad, India: A Cross-Sectional Study**

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#### **ABSTRACT**

The objective of this study was to evaluate the microbial quality of the well water used for consumption in rural households of Allahabad district, Uttar Pradesh, India. A total of 30 well water samples from 5 different wells were analyzed at an interval of 24 hrs, 1 week, 2 week, 3 week and 4 week by subsequent treatment by the multiple fermentation tube method to determine the presumptive coliform count/most probable number of coliforms and the isolates were identified using standard procedures. Water samples from these wells showed presence of Fecal indicator organisms like coliforms (*Klebsiella* spp., *Enterobacter* spp. *E. coli* and *Proteus* spp.), *Enterococcus* spp. and saprophytic bacteria. All types of isolates were found to be significantly reduced by well treatment at 24 hrs, 1, 2 and 3 and 4 weeks post-treatment. *Proteus* spp., *Enterobacter* spp. and *Enterococcus* spp. were completely eliminated by treatment and no growth was seen at upto 3 weeks post-treatment in any sample. Regrowth of *Proteus* spp., *Enterobacter* spp. were noted on 4<sup>th</sup> week. Determination of presumptive coliform count in well water samples also suggested the significant effect of treatment. While MPN was high (>182) in water sample at pre-treatment time point, it is drastically reduced by treatment. Effect was significant till 3 week post-treatment. A comparison of pre and post treatment microbial quality of the water revealed significant differences among isolates which emphasizes the need for proper bacteriological surveillance in these water sources. It might be prudent to monitor the bacteriological quality of well water at the source in addition to resistance profiles of the isolates.

**KEYWORDS:** *Escherichia coli*, Fecal coliforms, Most probable number, Water quality

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## INTRODUCTION

Water pollution is a global public health problem and poses a threat to human life. Infectious diseases are the most common and prevalent health hazard allied with drinking water and this is caused by pathogenic bacteria, viruses, and parasites<sup>1,2</sup>. According to WHO, globally approximately 1.1 billion of population drink unsafe water and nearly 88% of diarrheal diseases are attributed to unsafe water, sanitation and hygiene<sup>3</sup>. Worldwide nearly four billion clinical cases of diarrhoea and more than 3 million deaths occur annually due to other water borne infections<sup>4</sup>. In India, 37.7 million people suffer due to waterborne diseases annually and nearly 1.5 million children die due to diarrhea alone<sup>5</sup>. WHO and UNCF reports suggested that global disease burden could be reduced to nearly one-tenth by improving the sanitation, hygiene, water supply, and management of water resources<sup>3,6</sup>.

Chief and adequate source of drinking water in India is ground water. Unfortunately this is only 0.61 % of the total available water on the Earth. Groundwater exploitation has been reached to extensive level and that resulted in lowering of water table in rural and urban areas of India. In rural areas, well water is still a main source of drinking and household purpose. Water is a good solvent and therefore picks up impurities easily. Ground water is also prone to get polluted easily by fecal matter, domestic sewage and agricultural and pasture runoff<sup>7</sup>. A lack of awareness and education among the users are additional factors for the well water contamination in villages. In addition to chemical contamination, microbial growth is commonly observed in groundwater. In crowded geographical areas in developing countries groundwater is preferably used for drinking purpose. In such places, contamination of groundwater has severe consequence for public health<sup>8</sup>. Microbiological pollution of groundwater sources exerted an immediate effect on large numbers of people if untreated or inadequately treated water is used for drinking<sup>8</sup>. Bacterial, viral as well as protozoan parasites serves as the causative agents for waterborne diseases including cholera, gastroenteritis, typhoid fever, hepatitis, and giardiasis<sup>9</sup>.

Recent research on water pollution is mostly focused on controlling water-borne pathogens in water resources. Presence of pathogenic organisms which cause waterborne diseases can be detected by analysis of bacterial indicators in drinking water<sup>11</sup>. Indicator microorganisms have better longevity than pathogens. Due to their uniform and stable properties, they may be easily detected by standard laboratory techniques<sup>10</sup>. Drinking water showed varying rates of contamination. This is with primarily of fecal coliforms and other heterotrophic bacteria as assessed by microbiological quality of water<sup>7, 11, 12, 13, 14, 15</sup>. Commonly observed indicator organisms in water are *Escherichia coli* (*E. coli*), thermotolerant (fecal) coliforms, fecal streptococci and *Clostridium perfringens*<sup>16</sup>. This paper analyzed and compared the microbiological quality of well water in Allahabad district of

Uttar Pradesh, India. Physical and chemical analysis of groundwater samples from this area suggested that the quality is doubtful and requires preventive measures be taken before supplying water to the rural people <sup>17</sup>.

## **MATERIALS AND METHODS**

### ***Sites***

One well each from rural areas was selected through simple random sampling. Five domestic wells from five villages (Jhusi, Nibi Kala, Munshipurwa, Sonauti and Patelnagar) of Allahabad district of Uttar Pradesh, India were selected for investigation in the present study. Well water sources (dug wells) used as the main source of drinking and household water were included in the study, and wells that were not in use or wells that were declared unfit for use were excluded from the study. All of the wells screened were used by the population there. Municipal water sources or water from stored containers was not included in the analysis.

### ***Well treatment and sample collection***

Wells were treated by Double Pot Method <sup>18</sup>. Informed consent was obtained from the head of each village before the well treatment and water sample collection. Water samples were collected over a period of eight months between November 2017 and June 2018 for bacteriological analysis. During the day time water samples were collected (9:00 hrs and 13:00 hrs) aseptically in sterile containers. Samples were kept at room temperature and transported to the laboratory for analysis within 2 hours from collection. The samples were preserved at 4 °C when immediate analyses were not possible. Samples were collected prior to treatment and at different time points post-treatment; 24 hrs, 1 week, 2 week and 4 week by the multiple fermentation tube method. Following WHO guidelines<sup>19</sup>, clean, heat-sterilized bottles of 200 ml capacity were used for the water collection. Water is collected without touching the sides of the well and without hitting the bottom or disturbing any sediment. Approximately 20-30 ml of water was discarded to provide sufficient airspace in the bottle. This method is adapted to allow shaking to achieve a homogenous dispersion of the bacteria before subjecting water sample for analysis. All bottles were labeled with complete details after collection. This includes the source of the water, the sample site, the address, and the date and time of collection, and delivered (within 2 h) to the laboratory in a light-proof insulated box containing ice packs. Aqueous sodium thiosulphate solution (100 g/l) was added (4-5 drops) to the sampling bottles to neutralize any residual chlorine before sampling of well water. This is performed since a complete history of chlorination (quantity, time since last chlorination) could not be elicited. This is despite the prior chlorination status.

### ***Method of analysis***

The multiple fermentation tube method was used to determine the presumptive coliform count/most probable number (MPN) of coliforms in the water samples as described previously<sup>20</sup>. Suspensions from positive tubes were subcultured on MacConkey agar and incubated at 37 °C for 24-48 h and the resulting colonies were identified<sup>21</sup>. The microbial quality of the water samples was evaluated based on WHO guidelines<sup>19</sup>. We strictly followed the standard operating procedures while testing the water samples for all pre-analytical, analytical and post-analytical phases. Analytical quality control measures, including duplicate sample testing, were performed. The culture media were subjected to sterility and performance evaluations before the samples were inoculated.

### ***Statistical analysis***

The results of pre-treatment and post-treatment well water samples of rural areas were statistically compared using the One-way ANOVA followed by Dunnett's post hoc test.

## **RESULTS**

### ***Fecal contamination rate in wells from villages***

The present study investigated and compared the quality of pre and post-treated well water. We collected water from one well of 5 villages at prior to treatment and at different time points following treatment such as 24 hrs, 1, 2, 3 and 4 weeks. We counted the fecal and total coliforms in the water samples from these wells at the given time points and studied the effect of treatment on coliforms.

The indicator bacteria isolated from well water represented fecal contamination rate. We found that fecal coliforms like *E. coli* and *Enterococcus* spp. were present in 4 wells and 3 wells respectively in the pre-treatment water sample. Total coliforms were detected in water samples from all 5 wells. Following treatment, fecal streptococci were completely abolished from water samples and not detected at 24h, 1, 2 and 3 week time points. The water sample from only one well showed the presence of this bacteria at 4-week post-treatment (Table I).

Level of *E. coli* coliform was also found to be decreased by treatment. Wherein, water samples from only 1 well showed the presence of *E. coli* at 24 hrs, 1 and 2 week time points post-treatment. Up to 4 weeks following treatment, *E. coli* were detected in 4 wells as that of pre-treatment.

Unlike to fecal coliform, total coliform was not altered with treatment. We detected total coliform at all time points in all 5 wells sample following treatment.

### ***Effect of treatment on presumptive coliform count in well water***

We later counted the presumptive coliform per 100 ml of water sample from all five well at pre-treatment and at different time points following treatment. For each well we statistically compared the MPN count at different time points post-treatment against pre-treatment count (Fig. 1A, B, C, D, E). Unanimously we found that MPN were significantly decreased in water samples when tested at 24 hrs, 1, 2 and 3 week time points as compared to pre-treatment ( $p < 0.001$ ). At 4 week post-treatment, MPN was found to be restored towards pre-treatment and it was not statistically significant as compared to pre-treatment ( $p > 0.05$ ). Mean of MPN from all five wells at different time points also revealed similar results (Insets in Fig. 1).

### ***Identification of bacterial isolates in well water and effect of treatment***

In pre-treated well water samples a total of 60 bacterial isolates were obtained. This includes coliforms (38, 63.3%), Enterococcus spp. (3, 5%) and saprophytic bacteria (19, 31.6%). The coliforms isolated showed presence of Klebsiella spp. (16, 26.6%), Enterobacter spp. (9, 15%), E. coli (10, 16.6%) and Proteus spp. (3, 5%). Treatment of wells reduced the number of coliform bacteria. Enterobacter spp. and Proteus spp. were not detected in any of the previously suspected sample on 24 hrs, 1, 2 and 3 week post-treatment. However, 3 isolate of Enterobacter spp. and 1 of Proteus spp. were detected at 4 weeks. While at prior to treatment 10 isolates of E. coli were found, treatment reduced this to 4, 1, 1, 2 and 7 isolates at 24 hrs, 1, 2, 3 and 4 weeks, respectively. Similarly, only 1 isolate of Klebsiella spp. were detected at 24 hrs, 2 and 3 week and number slightly increased to 4 isolates at 4 weeks as compared to 16 in pre-treatment. 3 isolates of Enterococcus spp. were found in pre-treatment samples out of 30 tested. Treatment resulted in complete elimination of these microbes and no growth was seen at post-treatment time point for any sample. The environmental saprophyte Pseudomonas spp. (19, 31.6%) were also isolated from a significant number of samples. Following treatment, number of isolates of saprophytic bacteria *Pseudomonas* reduced to 12 at 24 hrs, 7 at 1 week, 3 at 2 week, 4 at 3 week and 15 at 4 weeks (Table II).

## **DISCUSSION**

The determination of fecal indicator bacteria in water serves as an insightful method of quality assessment. We performed the microbial analysis of drinking water from wells of five villages from Allahabad district of Uttar Pradesh, India. For this geographical region, this study seems to be the first of its type. We highlighted the need for drinking water source monitoring for the presence of fecal contamination for better health in the community. In our study, *E. coli* was found to be present in drinking water sources at very high level. Presence of this flora provides evidence of

recent fecal pollution that requires immediate attention. Four out of 5 wells included in the study showed significant level of *E. Coli* before the treatment of well. After treatment, *E. Coli* were detected only in one well. *E. coli* contamination in the water sources were also reported by number of previous reports. This level ranged from 11.7% to 100%<sup>12,13</sup>. *Enterococcus* spp. were detected in three wells (60%) samples. Presence of fecal streptococci in water sources is more persistent than *E. coli* and coliform bacteria. In addition, they are highly resistant to drying which provides valuable measure for detecting pollution by surface run-off to groundwater or surface waters<sup>16</sup>.

The total coliforms, including *E. coli*, were found in all well water samples (100%). Various studies reporting microbial analysis of different water sources have revealed total coliform contamination rates ranging from 0% to 100%<sup>7,11,12</sup>. The presence of coliform bacteria indicated the inadequate treatment or post-treatment contamination<sup>7</sup>. Since we did not elicit the complete history of chlorination including quantity of disinfectant used and time since last chlorination, we could not comment on the reason behind the inadequacy of disinfectant treatment or post-treatment contamination with coliform bacteria. Coliform test did not directly correlate with fecal contamination or pathogens in drinking water. However, the coliform test is still useful for monitoring the microbial quality of public water supplies which is attributed largely to the fact that coliforms are easy to detect and count in water<sup>16</sup>. Coliform counts  $\geq 10$  per 100 ml often requires repeated sampling from that water sources and should be further investigated for the source of the pollution. Drainage from sewage and swamps, and watershed erosion are major cause of the bacterial pollution of well water<sup>22</sup>. The geographical region considered in this study is having dense population, colonies are crowded and with a lack of a proper drainage network. This might have contaminated the well water sources. In the present investigation, we also detected the presence of saprophytes, including *Pseudomonas* spp. and *P. aeruginosa* in the water. The detection of *P. aeruginosa* has been advocated as a method of assessing the hygienic quality of drinking water<sup>16</sup>.

When water sample showed  $\geq 10$  coliforms per 100 ml, and such samples requires constant monitoring for the source of the pollution<sup>23</sup>. In the present study, statistical analysis by one-way ANOVA followed by Dunnett's post hoc test suggested significant effect of treatment on MPN as compared to basal level. Unifying results were observed in all five wells. As compared to pre-treatment (basal) level MPN was significantly reduced at 24 h, 1 week, 2 week, 3 week post-treatment ( $p < 0.001$  for all well and at all time points). However, at 4 week post-treatment MPN was found to be restored towards basal level ( $p > 0.05$ ). We also compared the mean of MPN of all five wells as different time points after treatment with the pre-treatment level. Similar to that observed in case of individual well, mean MPN at 24 h, 1 week, 2 week and 3 week post-treatment was significantly reduced ( $p < 0.001$ ) as compared to that of pre-treatment. MPN at 4 week was slightly

towards basal level and difference between them was not significant. This result suggests that well water from villages of Allahabad district showed high MPN count and treatment showed significant cleansing effect on water that reduced the MPN count.

Quality of drinking water is primary cause of good health in society. However, in rural areas in developing or poor countries, there is lack of water quality literacy. People need to get educated about the quality of their drinking water, its sources and methods of water purification. Literacy in the subject may help to understand the importance of clean and healthy surroundings near water sources and people by themselves can implement some measures to prevent the contamination of water sources in the community. To boil the water before drinking is common practice in urban as well as rural areas. This is advised to disinfect the water from pathogenic microorganisms and to confirm that the contamination is eliminated. It is widely recommended that well owners should test their well water for coliform bacteria at least once a year. In addition, frequent tests are advised in the well water who documented contamination previously. As a pilot study, the population in this work was limited to 5 villages, and the water quality was investigated for 2 months period; therefore, variations in the water quality with seasonal changes are likely to have been missed. In the present study we did not perform investigations on the source of coliform contamination. However, we suggest that such kind of analysis would be fruitful to implement control measures on drinking water.

## **CONCLUSION**

Our data suggested that drinking water from wells of Allahabad district contain bacterial coliforms and it can be reduced by proper treatment of wells. Further studies are required to elucidate the root cause of water pollution and to search the novel methods of proper preventive measures for drinking water health hazards. It might be prudent to monitor the bacteriological profile of well water at the source along with resistance profiles of the isolates.

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***Conflict of interest:*** None declared

***Ethical approval:*** The study was approved by Institutional Ethics Committee

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**Table I. Number of wells indicating isolation rate of indicator bacteria**

Type of Coliforms	Post-treatment time points					
	Pre-treatment	24 hours	1 week	2 week	3 week	4 week
Escherichia coli	4	1	1	1	2	4
Fecal Streptococci	3	0	0	0	0	1
Total Coliform Bacteria	5	5	5	5	5	5

Table II. Various indicator organisms and saprophytes isolated from well water samples

Type of isolates	Pre-treatment	Number of isolates				
		24 hours	1 week	2 week	3 week	4 week
<b>Coliform bacteria</b>						
E.coli	10	4	1	1	2	7
Klebsiella spp.	16	1	0	1	1	4
Proteus spp.	3	0	0	0	0	1
Enterobacter spp.	9	0	0	0	0	3
<b>Fecal Streptococci</b>						
Enterococcus spp	3	0	0	0	0	0
<b>Saprophytes</b>						
Pseudomonas spp	19	12	7	3	4	15
<b>Total</b>	<b>60</b>	<b>17</b>	<b>8</b>	<b>5</b>	<b>7</b>	<b>30</b>

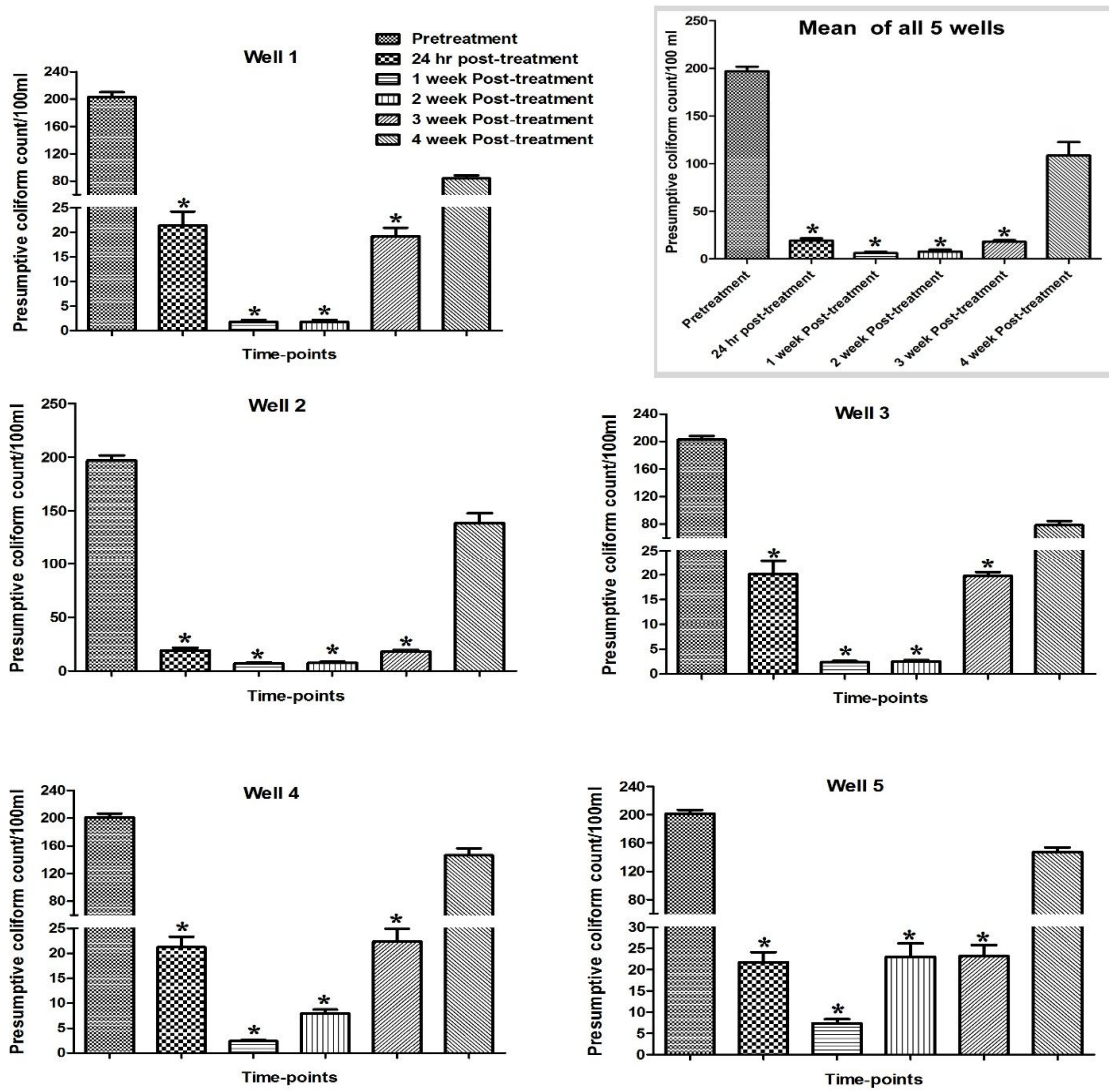


Fig. 1. Presumptive coliform count (PCC) per 100 ml of water sample from different wells selected from five villages. Water samples collected at pre-treatment and at different time points following well treatment. PCC/MPN was detected by multiple fermentation tube method. PCC at different post-treatment timer points were compared with pre-treatment count for each well (A, B, C, D, and E). Inset represents mean of MPN for all five wells at different time points and its comparison. One-way ANOVA followed by Dunnett's post-hoc test was used for statistical comparison. \* $p < 0.001$  vs pre-treatment MPN count.