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# ASSESSMENT OF MICROBIAL POLLUTION IN DRINKING WATER IN AND AROUND PUNE CITY, MAHARASHTRA, INDIA

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**ABSTRACT**

**The quality of drinking water and treatment of waterborne diseases are crucial public health problems. Bacterial contamination of potable water resources is the most recurrent health hazard. The present investigation reveals bacteriological evaluation of drinking water sources in and around Pune City, Maharashtra, India. Residential areas of Pune get their drinking water supply from various sources like treated water from corporation purification plant, bore wells, hand pumps, wells etc. A total of 240 water samples were collected from different areas. The areas covered were from Kothrud to Aundh, Pashan to Katraj, Bhosari, Pimpri, Nigdi and the central Pune city. A questionnaire was prepared to collect information regarding source of drinking water, purification method performed, storage of water, and any infection experienced due to drinking water. About 80% samples showed presence of coliforms up to 1800 coliforms/100ml. Since the total coliforms test is a primary indicator of POTABILITY, i.e. suitability of drinking waterfor consumption, the results indicate that the drinking water available in and around Pune city is not at all safe for human health. 38% of the drinking water samples examined on a single water supply line showed presence of coliforms which indicate that the water has got contaminated somewhere in the piped drinking-water distribution system. Fecal coliforms indicated water contaminated with animal or human waste, i.e. feces in many samples. Since faecal coliforms have been examined in filtered drinking water samples efficiency of domestic filters is also questionable as regards killing of coliforms. Most of the samples taken from proper Pune city showed presence of less coliforms. So, in the present research it is concluded that the potable waterin Pune is contaminated with pathogenic microorganisms and unfit for drinking.**

**KEY WORDS** : Microbiological analysis, potable water, Coliforms, waterborne pathogens.

## INTRODUCTION

Drinking water is vital for human survival. World health organization (WHO, 1993) considers that "drinking-water" should be "suitable for human consumption and for all usual domestic purposes including personal hygiene."As per 2008 report of WHO approximately three out of five personsin developing countries do not have access to safe drinking water. In Pune city, drinking water supply system is incredibly old and it exists since 1750. Pune city received first piped drinking water supply from Katraj via Amboliodha up to Shaniwarwada. For Pimpri Chinchwad Municipal Corporation (PCMC), Pavana dam is a major source of drinking water, constructed in 1972 (Rode, 2009). Most common and widespread health risk associated with drinking water is contamination; directly or indirectly, by human or animal excreta, particularly faeces. The control of fecal contamination in drinking-water systems and sources, where it occurs, is of primary importance. Faecal coliforms areuniversally present in large numbers in the faeces of humans ,warm-blooded animals, readily detected by simple methods, do not grow in natural waters; andtheir persistence in water and removal by water treatment is similar to waterborne pathogens (Fewtrelland Bartram, 2001; Fricker, 1998). Diseases related to contamination of drinking-water comprise a major burden on human health.If

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such contamination is recent, and if those responsible for it include carriers of communicable enteric disease, some of pathogenic microorganisms that cause these diseases may be present in water. The principle bacterial pathogens that have been shown to cause human intestinal disease associated with drinking water are *Salmonella typhi, Salmonella paratyphi-A*, other *Salmonella* species, *Shigella* sp., S. *flexneri*, and *S. sonnei*, *Vibrio cholerae*, *Escherichia coli* (specific strains) and *Pseudomonas aeruginosa*, etc. (National Research Council 1977). The pathogenic bacteria may cause diseases that vary in severity from mild gastroenteritis, nausea, vomiting to severe and sometimes fatal diseases like typhoid, hepatitis, which are widely distributed throughout the world. To eliminate the presence of such pathogens, chemical disinfectants are used. Chlorine is one such example and it is effective against bacteria and viruses (Water Quality Association, 2000). The qualityof water for drinking has deteriorated because of the problem of inadequate treatment plants, direct discharge of untreated sewage into rivers and inefficient management of the pipedwater distribution system (UNEP, 2016). As per World Health Organisation (WHO, 1993, WHO, 2003) standards, drinking water should not contain any microorganisms known to be pathogenic or any bacteria indicative of faecal pollution. Up to 80% of all sicknesses and diseases in the world are caused by inadequate sanitation and polluted water (Abera *et al.,* 2011, Sohani, 2012). Total and faecal coliform have been used as indicators for determining the sanitary quality of water sources. Detecting and counting of total coliforms and *E. coli* (faecal coliform) have traditionally been based either on the multiple-tube fermentation test (MTFT) i.e. most portable number (MPN) or membrane filtration (MF) methods. (Mengesha *et al.,* 2004; Tembekar *et al.,* 2008). Water borne outbreaks are the most obvious manifestation of waterborne disease. In Pune over 20% of the communicable diseases are due to poor environmental health conditions arising from unsafe and inadequate water supply. Rural water sources for drinking are still the traditional ones like dams, wells, rivers, streams and ponds which might harbour waterborne and vector-borne diseases (Zvidzai *et al.,* 2007). The provision of safe and quality water supply for the population has far reaching impacts on health, productivity, and value of life, as well as on the socioeconomic development of the nation. To get the maximum degree of protection, drinking water along with, distribution pipes, tanks, taps, as well as household storage vessels must be clean and intact (Robertson *et al*). The aim of this study was to analyse the microbiological quality of the potable water collected directly from various sources in and around Pune, Maharashtra, India, to check the drinking water quality and evaluate the awareness of people for maintaining cleanness and hygiene conditions for storage of drinking water.

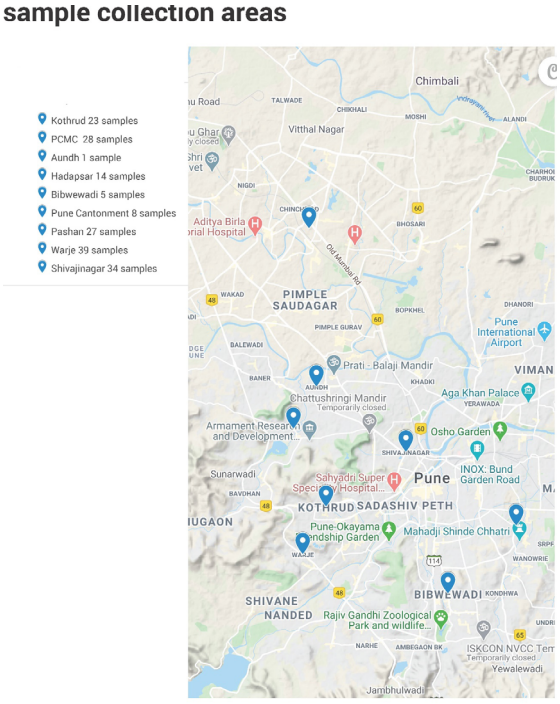
## MATERIALS AND METHODS

### Sample Collection

A variety of 240 samples (Table 1) were collected from various areas in and around Pune city. The areas covered were from Kothrud to Aundh, Pashan to Katraj, Bhosari, Pimpri, Chinchwad, Nigdi and the central Pune city (Shown in the map). Samples were collected from June to December. Water

**Table 1.** Sources of potable water samples

| Sr. No. | Source of water | Number of samples |
|---|---|---|
| 1 | Pune Municipal Corporation | 114 |
| 2 | Pimpri Chinchwad Municipal Corporation | 73 |
| 3 | Public Bore wells | 30 |
| 4 | Private bore wells | 23 |
| | Total Samples | 240 |



samples were collected in one-liter sterilized bottles and transported in icebox to the laboratory within 4 hours and then processed. Bacteriological analysis of drinking water samples was done within 48 hours of collection.

**Ethical concern**

Consent from Pune municipality for collection of public water source samples and consent from private water source (like wells) owners were obtained before water sample collection.

**Preparation of a questionnaire**

A questionnaire was prepared to collect information regarding source of drinking water, purification method performed, storage of water, and any infection experienced due to drinking water.

**Determination of pH**: The pH of all water samples was checked using pH meter (Systronics 361, India).

**Total viable count**

Total viable count (TVC), is a quantitative estimate of the concentration of microorganisms such as bacteria, yeast, or mold spores in a water sample. The count represents the number of colony forming units (cfu) per g (or per mL) of the water sample. It was achieved by plating serial tenfold dilutions of the water sample. The reported count was the number of colonies counted multiplied by the dilution used for the counted plate. A high TVC count indicates indicate poor quality for drinking water. TVC of bacteria for each water sample was performed after incubation at 37 °C for 24 hours.

**Membrane filter technique**: Membrane filters having porosity of 0.45µm were used. Water samples were passed through sterile membrane filters housed in filter apparatus contained in a suction flask. Following filtration, the filter disc containing the trapped microorganisms were aseptically transferred to a sterile petri dish with the suitable medium. The results were expressed as colony forming units per unit volume.

**Determination of Most Probable Number (MPN) of coliforms**

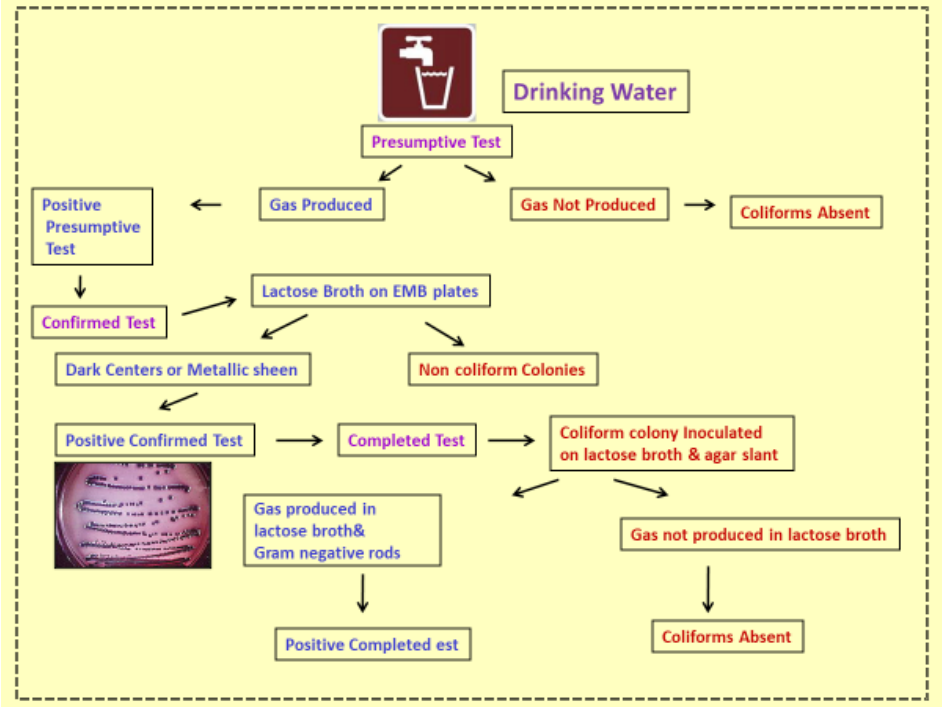
MPN of coliforms was determined for each drinking water sample with double strength MacConkey's broth and single strength MacConkey's broth (Cheesbrough, 2006, APHA-AWWA-WEF 1998). In the presumptive test for coliforms, five 10 mL, five 1mL, and five 0.1 mL volumes of the appropriate dilution of water samples were inoculated in respective fifteen fermentation tubes and inverted Durham tubes were also placed in MacConkey's broth todetect gas production. The inoculated testtubes were incubated for 48 h at 37°C. Positive MPN tubes were observed for colour change and gas production. The combination of positive tubes was referred to McCrady's Table (McCrady, 1915) and coliform count per 100 mL of water was determined. This was done for 240 water samples. For each set from a positive tube a loopful of water sample was transferred to EMB and Endo agar to confirm the presence of typical fecal coliforms (confirmed test) (APHA 1992). This allowed the differentiation of coliform (producing a green metallic sheen) from non-coliforms. The completed test was performed on well-isolated colonies to reaffirm gas production in lactose, and to determine the morphology and gram reaction (Gram negative rods) of the isolate from a nutrient agar slant (shown in the flowchart).

**Identification of Pathogens**

Detection of *Salmonella* and *Shigella* species were done by the enrichment of water samples on Selenite F broth, followed by isolation of the typical organism on selective medium, *Salmonella-Shigella* agar and Wilson-Blairs medium (Collee *et al.*, 1996). All colonies with different characteristics on selective media were sub-cultured on Nutrient agar (NA) for purification. Selective media like cetrimide agar for *Pseudomonas aeruginosa*, Mannitol salt agar for *Staphylococcus aureus* were used. Entericand other pathogenic bacteria isolated on respective selective or differential media were identified based on their colonial, morphological and Biochemical properties following Bergey's Manual ofDeterminative Bacteriology, 1994 (Buchanan, 1974).

## RESULTS AND DISCUSSION

The World Health Organization (WHO, 1993) estimated that every eight seconds a child died from a water-related disease and that each year more than five million people died from illnesses linked to unsafe drinking water or inadequate sanitation (Anon, 1996). A regular monitoring of the water quality for improvement not only prevents disease and hazards but also checks the water resources from going further polluted (Trivedy and Goel, 1986). Bacteriological pollution of drinking water supplies occurs either due to the failure of the disinfection of water at the treatment plant or due to




the infiltration of contaminated water (sewage) through cross connection or leakage points (Prasai *et al.*, 2007). So, the main intention of this study was evaluation of microbiologicalquality of drinking water from different sources in and around Pune.

**Total Viable count**

The TVCs for all the water samples were high. This exceeds the allowable limit of $10^2$ cfu/mL for drinking water. Public well water samples having an average valueof 18.7 x$10^5$cfu/mL showed the highest microbial load after 24h and 48h of incubation as compared to private bore wells showing an average TVC of 6.5x$10^4$ cfu/mL PMC and PCMC water samples showed average TVC values of 36.6x$10^4$cfu/mL and 42.5 x$10^4$cfu/mL, respectively.

**pH:** The pH for all drinking water samples ranged between 6.5 to 8.5. This value falls in the limits given by EPA. The probable reason why none of the samples resulted into any pH related disorders in consumers over a long period of time. Consuming excessively acidic or alkaline water is harmful, warns the Environmental Protection Agency (EPA). Drinking water must have a pH value of 6.5-8.5 to fall within EPA standards. While the ideal pH level of drinking water should be between 6-8.5, the human body maintains pH equilibrium on a constant basis and will not be affected by water consumption. For example, our stomachs have a naturally low pH level of 2 which is a beneficial acidity that helps us with food digestion.

**Most Probable Number (MPN) of coliforms**

In our investigation, 80% samples showed presence of coliforms ranging from 1 coliform/100 mL to more than 1800 coliforms/100 mL (Table 2, Fig 1). Ideally coliforms should be absent in any drinking water. In a bacteriological study of drinking water during epidemic of cholera in Delhi, 55% samples showed presence of coliforms with MPN value ranging from 10 to 1800+ per 100 mL (Baveja *et al.*,

**Fig. 1.** Determination of Most Probable Number (MPN) of coliforms

1989). In our study % samples showing presence of coliforms was found to be higher.

When borewell water samples without any other treatment were tested, showed coliform count in the range of 15-1800 coliforms/100 mL water (Fig. 2). Aundh and Belawade water samples showed more than 1800 coliforms/100 mL. Sinhagad road sample showed 350 coliforms/100 mL water.

In most of the areas in and around Pune even

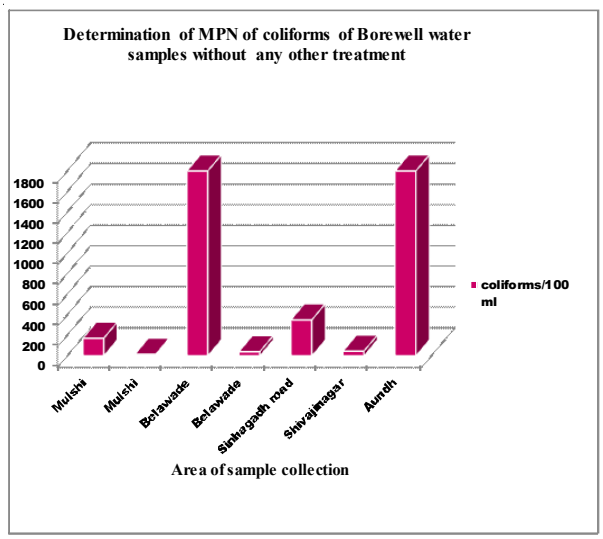



**Fig. 2.** Determination of Most Probable Number (MPN) of coliforms of borewell water samples without any other treatment

without using bacteriological filter or any other treatment method, corporation water samples contained more than 1400 coliforms/100 mL water tested (Table 3., Fig. 3). Most of the samples tested from central Pune city showed less number of coliforms.

According to the suggestions given by experts a single supply line was selected for analysis (Fig 4.). This was done to check if the coliform count was due to faulty distribution or due to individual

**Table 3.** MPN of coliforms in water samples of Corporation water without any treatment

| Sr No. | Area | MPN coliforms/100 mL |
|---|---|---|
| 1 | Aundh | 1600 |
| 2 | Aundh | 1800 |
| 3 | Balajinagar | 9 |
| 4 | Bhosari | 1800 |
| 5 | Bibwewadi | 1600 |
| 6 | Budhwarpeth | 0 |
| 7 | Budhwarpeth | 17 |
| 8 | Camp | 0 |
| 9 | Dhankawadi | 0 |
| 10 | Dhankwadi | 1 |
| 11 | Ganesh peth | 1800 |
| 12 | Guruwarpeth | 1800 |
| 13 | Guruwarpeth | 35 |
| 14 | Hadapsar | 1800 |
| 15 | Hadapsar | 12 |
| 16 | Hadapsar | 1800 |
| 17 | Karvenagar | 45 |
| 18 | Kasbapeth | 250 |
| 19 | Kasbapeth | 4 |
| 20 | Kasbapeth | 1 |
| 21 | Kasbapeth | 8 |
| 22 | Kothrud | 0 |
| 23 | Kothrud | 0 |
| 24 | Kothrud | 12 |
| 25 | Kothrud | 1600 |
| 26 | Laxminagar | 6 |
| 27 | Pashan road | 1800 |
| 28 | Pimpri-chinchwad | 35 |
| 29 | Rasta peth | 550 |
| 30 | Raviwarpeth | 1800 |
| 31 | Raviwarpeth | 20 |
| 32 | Salisbury Park | 1800 |
| 33 | Shaniwaarpeth | 225 |
| 34 | Shivajinagar | 1800 |
| 35 | Sinhagadh road | 1800 |
| 36 | Sinhagadh road | 11 |
| 37 | Somwarpeth | 12 |
| 38 | Somwarpeth | 1800 |
| 39 | Warje 0 | |

**Table 2.** Determination of Most Probable Number (MPN) of coliforms:

| Total Samples 240 | MPN Count/100 mL (% water samples) | | | | | |
|---|---|---|---|---|---|---|
| Area of collection | 0 count | 1-50 count | 50-100 count | 100-500 | 500-1000 | 1000-2000 |
| Pune City (42) | 8 (19.04) | 16 (38.09) | 0(0) | 6(14.28) | 8(19.04) | 4(9.52) |
| Surrounding village (28) | 0(0) | 6(21.42) | 0(0) | 2(7.14) | 4(14.28) | 16(57.14) |
| Aundh, PashanRoad (34) | 8(23.52) | 2(5.88) | 0(0) | 0(0) | 6(17.64) | 18(52.94) |
| Katraj (26) | 6(23.07) | 4(15.38) | 0(0) | 0(0) | 4(15.38) | 12(46.15) |
| Hadapsar (30) | 2(6.66) | 8(26.66) | 0(0) | 0(0) | 6(20) | 14(46.66) |
| Sinhagad Road (24) | 0(0) | 2(8.33) | 0(0) | 2(8.33) | 2(8.33) | 18(75) |
| Kothrud &Shivajinagar (24) | 8(33.33) | 6(25) | 0(0) | 2(8.33) | 2(8.33) | 6(25) |
| Pimpri Chinchwad (32) | 24(75) | 4(12.5) | 0(0) | 0(0) | 2(6.25) | 2(6.25) |

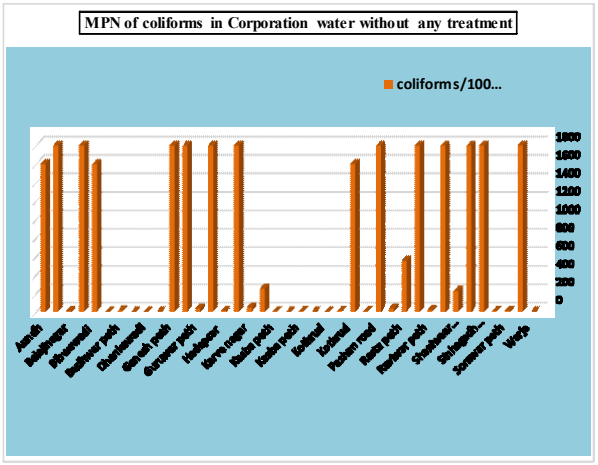



**Fig. 3.** MPN of coliforms in corporation water without any treatment

storage and distribution system 38% of the drinking water samples examined on the following single corporation water supply line showed presence of coliforms ranging from 1 per 100 mL to more than 1800 per 100 mL. In most of the cases presence of the coliforms has not revealed any intestinal disturbances. It may be because of gradual resistance towards coliforms.

Only 3 samples showed high number of coliforms where the corporation water was filtered (Fig.5., Table 4). Pashan road, Koregaon Park and Urali Kanchan area water samples demonstrated 1600 or high number of coliforms. In most of the potable water samples coliforms were absent or present in less numbers in the range of 1-225 coliforms/100 mL.

Total coliform bacteria are a collection of relatively harmless microorganisms that live in large numbers in the intestines of man and warmand cold-blooded animals. They aid in the digestion of food. A specific subgroup of this collection is the fecal coliform bacteria, the most common member being *Escherichia coli*. These organisms may be

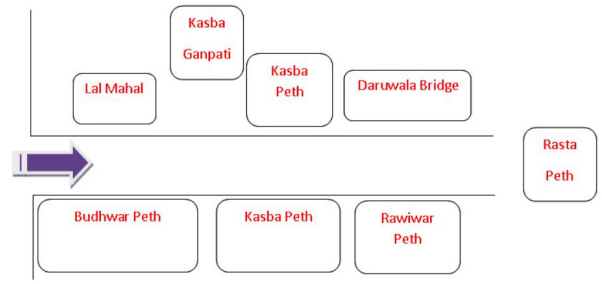



**Fig. 4.** Water analysis from a single corporation water supply line

**Table 4.** MPN of coliforms in corporation water with filtration

| Sr No. | Area | MPN[coliforms/100ml] |
|---|---|---|
| 1 | Wanwadi | 10 |
| 2 | Shivajinagar | 0 |
| 3 | Pashan road | 1800 |
| 4 | Dapodi | 0 |
| 5 | Bibwewadi | 0 |
| 6 | AB chowk | 0 |
| 7 | Sadashiv peth | 180 |
| 8 | Ganeshkhind | 25 |
| 9 | Mangalwarpeth | 0 |
| 10 | Sahakarnagar | 0 |
| 11 | Katraj | 0 |
| 12 | Kothrud | 140 |
| 13 | Bhosari | 14 |
| 14 | Dhankwadi | 0 |
| 15 | Statim | 45 |
| 16 | Koregaon park | 1600 |
| 17 | Baneshwar | 1 |
| 18 | Urli-kanchan | 1800 |
| 19 | Yerwada | 12 |
| 20 | SalunkeVihar | 225 |
| 21 | Salisbury Park | 15 |
| 22 | Bhawani peth | 8 |

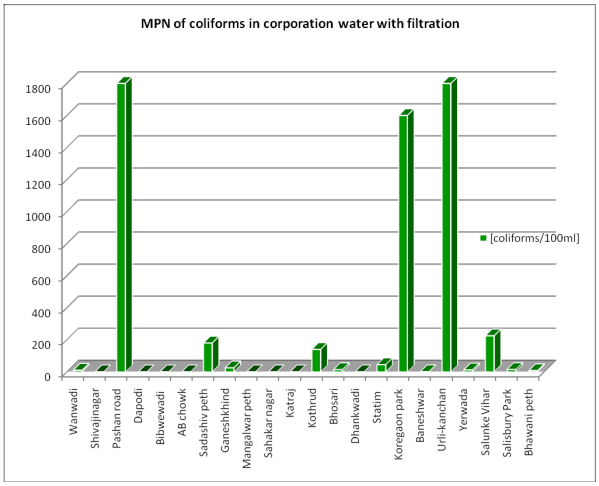



**Fig. 5.** MPN of coliforms in corporation water with filtration

separated from the total coliform group by their ability to grow at elevated temperatures and are associated only with the fecal material of warm-blooded animals.Since fecal coliforms have been examined in filtered drinking water samples efficiency of domestic filters is also questionable as regards killing of coliforms. Fecal Coliform bacteria indicate the presence of sewage contamination of a waterway and the possible presence of other pathogenic organisms.

**Identification of Pathogens**

Pune district gets drinking watermainly from Khadakwasladam constructed on the Mutha River. In our study several pathogens such as *E. coli, Salmonella typhi, Salmonella paratyphi A, Salmonella paratyphi B, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Shigella dysenteriae* were isolated and identified from the water samples tested (Table 5, Fig. 6.). *Staphylococcus aureus* was found in well water samples. *Salmonella Paratyphi* B and *Vibrio* were isolated from well water samples from coastal areas of Kanyakumari District, Tamil Nadu examined by Rajendra *et al.* (2006). Research was carried out by Nagpal *et al.,* (2011) to explore the presence of harmful microbes in the drinking water and the villages affected by reservoirs of dams at Narmada River where the samples were positive with *Vibrio* species and *Salmonella*. In our study *Vibrio* was not found. There are reports on isolation of *S. aureus* from rural well water samples (LeChevallier and Seidler, 1980. For pathogens transmitted by the faecal–oral route, drinking-water is only onevehicle of transmission. According to study carried out by Antony and Renuga, 2012, Tamilnadu potable water samples had pathogenic bacteria *Escherichia coli, Pseudomonas aeruginosa, Shewanellaputrefaciens, Klebsiella pneumoniae, Citrobacter freundii, Proteus mirabilis.* Bacteriological quality of drinking water in Nyala, Sudan demonstrated Enteric bacteria *Escherichia coli* (22.5%), *Enterococcus faecalis* (20.42%), *Klebsiella* (15.00%), *Citrobacter* (2.1%) andother*Enterobacter* (3.33%) (Amira and Yassir, 2010). The heterogeneous coliform group, which is present in human and animal faeces, as well as insoil, decomposing plant matter, is the most universal, non-specific indicator of faecal pollution, or inadequate disinfection, or post-treatment contamination of a water supply (Kravitz *et al.,* 1999). Quantitative microbial risk assessment (QMRA) is increasingly applied to estimate drinking watersafety. where the risk of infection is calculated from pathogen concentrations in drinkingwater (Smeets *et al.,* 2008).

**Table 5.** Bacterial Pathogens in the water samples

| Sr. No. | Bacterial Pathogen | % water samples |
|---|---|---|
| 1 | *Staphylococcus aureus* | 17% |
| 2 | *Salmonella typhi* | 4% |
| 3 | Salmonella paratyphi A | 9% |
| 4 | *Salmonella paratyphi B* | 8% |
| 5 | *Shigella dysenteriae* | 4% |
| 6 | *Klebsiella pneumoniae* | 12% |
| 7 | *Pseudomonas aeruginosa* | 7% |
| 8 | *Esherichia coli* | 28% |

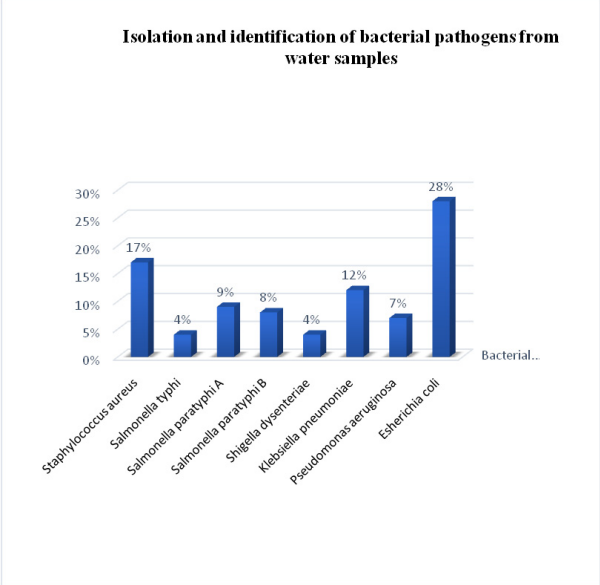



**Fig. 6.** Isolation and identification of bacterial pathogens from water samples

## CONCLUSION

Monitoring the quality of drinking water during distribution and storage play especially important role. This study determines the quality of drinking water sources and the extent of contamination at Pune water sources which will help in the intervention actions to be taken by the concerned bodies and will provide baseline information for further study. 80% drinking water samples showed presence of coliforms. Because the total coliforms bacterial test is a main indicator of POTABILITY," i.e. suitability for consumption of drinking water, drinking water available in and around Pune city is not at all safe for human health. According to our survey on a single supply line 38% of drinking water samples showed presence of coliforms. The presence of coliform in drinking water indicate that the water has got contaminated somewhere in the distribution system. The proportion of waterborne disease outbreaks associated with the distribution system failures has been increasing over the years (Kurup *et al.,* 2010). Presence of Fecalcoliforms, exemplified by *E. coli,* indicated water contaminated with animal or human waste, i.e. feces in many samples. The major concern is that periodic cleaning of overhead tanks decreased the coliform count so they should be

periodically and thoroughly cleaned. Since fecal coliforms have been observed in filtered drinking water samples efficiency of domestic filters is also questionable as regards killing of coliforms. Many pathogenic bacteria were also isolated from the potable water samples. According to Indian standard IS 1622 (1981), no sample should contain *E. coli* as well as other coli form organisms more than 10 per 100 mL. The present study indicated that all the Publicborewell water sources tested were of poor microbiological quality as compared to private bore wells. It indicated extremely high level of contamination of open well water. Municipal water contained fecal coliform mainly due to leakage of pipelines. On a global scale, mishandling of water within the home is likely to be the most significant source of contamination. Simultaneously, awareness among the people towards sanitation and hygienic conditions for storage of drinking water is necessary to promote the use of contamination free water.

The findings in this study points to the fact that the prevalence of waterborne disease is linked up with the quality of drinking water sources in and around Pune. This implied that the quality of available drinking water provides as a tool in determining the health condition of any community. Therefore, effort should be made by proper authorities to conduct quality assessment of water sources from time to time toconfirm that safe drinking water of good quality is available to everyone in Pune. In addition, distinct member of a community also has a role of safeguarding and maintaining good hygienic conditions in and around Punewater sources.

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