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## COMPARATIVE ANALYSIS OF MICROORGANISMS IN POTABLE WATER: A STUDY IN BIKANER ZONE, INDIA

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**Abstract**– Pathogenic bacteria contaminating drinking water is a serious public health concern, particularly in underdeveloped nations. The objective of this research was to examine and contrast the microbiological quality of several sources of drinking water in the Bikaner area of Rajasthan, India. In both urban and rural regions, water samples were taken from borewells, canals, rivers, tube wells, and taps. Conventional microbiological techniques were used to evaluate total coliforms, fecal coliforms and specific pathogens. The results showed that all water sources had higher microbial counts than WHO guidelines, with well water and river water being most contaminated. Borewell water had the lowest microbial load, though still above recommended limits. Total coliforms ranged from 2 x 104 to 32 x 104 CFU/100 ml across samples. *Escherichia coli, Klebsiella, Citrobacter* and *Pseudomonas* species were frequently isolated. This indicates fecal contamination and health risks for consumers. Proper treatment and disinfection of all water supplies is essential. Regular monitoring, improved sanitation infrastructure and hygiene promotion among communities is urgently needed to provide potable water and reduce waterborne diseases. This study provides important baseline data for designing appropriate interventions.

#### INTRODUCTION

Contamination by pathogenic bacteria, viruses, protozoa and helminths in drinking water leads to enteric infections such as diarrhea, cholera, typhoid, hepatitis A, amoebiasis and giardiasis (Ashbolt, 2004).

In India, piped water supply is inconsistent in urban areas and largely unavailable in rural areas. Many households rely on untreated surface water and groundwater sources like rivers, ponds, wells and borewells for domestic needs (Khurana and Sen, 2008). Open defecation, lack of sanitation facilities and discharge of sewage into water bodies aggravates microbiological pollution. This renders water unsafe for drinking without adequate treatment and disinfection (Gerba and Britton, 1984).

Microbiological analysis of water quality involves detection of indicator organisms like total coliforms, fecal coliforms and specific pathogens. Coliforms are commonly found in faeces of humans and animals. Hence their presence indicates faecal contamination. *E. coli* is the most definitive faecal coliform indicator (Ashbolt *et al.*, 2001).

Many studies across India have reported high levels of bacterial contamination in ground and surface water sources, especially in rural areas (Baghel *et al.*, 2005; Ramesh *et al.*, 2010; Rodrigues *et al.*, 2011). This is a major concern for public health. Rajasthan is a water-stressed state with high dependence on groundwater. However, limited data is available on microbiological quality of potable water sources in this region.

Therefore, this study aimed to analyze and compare microbial water quality across various drinking water sources like taps, tube wells, canals, rivers and borewells in urban and rural areas of Bikaner zone. Conventional culture techniques were used to evaluate total coliforms, fecal coliforms and specific bacterial pathogens as health risk indicators. The findings would help determine the scale of contamination and design appropriate treatment interventions.

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#### LITERATURE REVIEW

#### Diseases and pathogens that are spread by water

Worldwide, water-related infections are the leading cause of disease and mortality, especially in economically distressed areas. (Bain *et al.*, 2012) estimated that this illness causes approximately 1.6 million deaths annually, with children under the age of five accounting for the majority of these deaths. Ash bolt (2004) states that the following illnesses are among the most serious: cholera, typhoid, hepatitis A, polio, and guinea worm sickness.

The presence of bacterial pathogens is the main reason for concern when it comes to the microbiological quality of drinking water. Unlike viruses and protozoa, which are shed in much lower proportions (107-109 per gramme), they are discovered in faeces in exceptionally huge levels (up to 1011 per gramme). They are present in excrement. Bacteria are more resilient than viruses to the effects of aquatic environments, according to study done in 2001 by Enriquez and colleagues. The four illnesses most frequently linked to bacterial infections in aquatic environments are cholera, typhoid fever, bacillary dysentery/shigellosis, and pathogenic *Escherichia coli* gastroenteritis.

Cholera is caused by the *Vibrio cholerae* bacteria, namely the O1 and O139 serogroups. Watery, severe, and regular diarrhoea are the condition's hallmarks. If left untreated, the diarrhoea can quickly lead to fatal dehydration. As to the findings of Ali *et al.* (2015), cholera causes about 120,000 fatalities annually worldwide.

Typhoid fever is a disease that affects the entire body and can be caused by two different types of bacteria. Typhoid fever causes over 12 million infections and 128,000 deaths annually, according to Baker *et al.* (2011). Most often, a non-typhoidal strain of Salmonella, like *S. Typhimurium*, is the cause of a kind of gastroenteritis that clears up on its own. The fight against antibiotic resistance is still very much in its infancy.

Bloody diarrhoea is the hallmark sign of shigellosis, sometimes referred to as bacillary dysentery. Another name for shigellosis is bacillary dysentery. Certain *Shigella* species possess the capacity to penetrate the colon's lining epithelial cells, leading to the development of ulcers and intense inflammation. According to Kotloff *et al.* (2018), 90 million illnesses and 100,000 fatalities are attributed to shigellosis annually. Children under

the age of five make up the bulk of individuals afflicted by the disease.

Certain *Escherichia coli* pathotypes have the potential to produce both bloody and watery diarrhoea. The chemicals that EHEC produces are called shiga, and they can cause potentially fatal kidney problems in infants. Because ETEC produce toxins that are either heat-stable or heat-labile, it has been proposed that they are the cause of traveler's diarrhoea (Crocen and Finlay, 2010).

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These enteric bacterial illnesses can also spread through waterborne mechanisms, such as the consumption of contaminated drinking or recreational water, irrigated crops, or both. Most cholera and shigella epidemics are associated with periods of high rainfall and flooding, which can cause sewage to overflow into water systems. (Payment *et al.*, 1997; Swerdlow *et al.*, 1992; Mason, 2009); the 2000 *E. coli* outbreak in Walkerton, Canada, which claimed seven lives; and the 1993 cryptosporidiosis outbreak in Milwaukee, which impacted 400,000 people.

# A comparative analysis of the water's microbiological condition

The quantity, virulence, and infectiousness of the pathogenic bacteria that are common in water are some of the variables that determine the total risk to public health. However, it is technically challenging to identify every disease that is spread through water. As per the findings of Ashbolt *et al.* (2001), indicator organisms are employed to ascertain the degree of microbiological quality existing in water.

When complete coliforms-a kind of rod-shaped, Gram-negative bacteria-are present, lactose ferments at a temperature between 35 and 37 degrees Celsius. They exist on Earth as well. *E. coli* and other thermotolerant and faecal coliform organisms can only break down lactose at temperatures between 44 and 45 degrees Celsius. *E. coli* and other faeces coliforms are therefore suggestive of a recent faecal infection. The most dependable indicator of faecal contamination is generally agreed to be *E. coli*, according to research done in 2005 by Tallon and colleagues (Tallon *et al.*, 2005).

When total coliforms are found in treated water, it means that the disinfection process was not thorough enough and that contamination is still present. It's possible that the identification of *E. coli* will prove the existence of intestinal infections. The annual tests that are conducted using piped sources should yield no more than five percent positive results. A 10% improvement in results can be achieved by using superior sources, such as covered dug wells and borewells; nevertheless, quick remedial action is still necessary.

Membrane filtering techniques are commonly employed in the identification of coliform bacteria. The rapid detection of many targets is made possible by the use of readily available chromogenic material. Maheux *et al.* (2014) state that another factor that helps distinguish *E. coli* from other coliform cultures is the presence of enzyme substrates.

However, especially in tropical waters, there is no assurance that the existence of coliforms signifies the existence of illnesses. Tallon *et al.* (2005) state that wherever facilities allow for it, direct detection of bacterial, viral, and protozoan illnesses is also done. Culture, immunological testing, and molecular techniques like polymerase chain reaction (PCR) and biosensors are used in the procedure. However, this calls for a substantial investment of time and money.

#### The level of bacteria present in India's water

In 2005, the National Institute of Urban Affairs performed studies which revealed that in India, piped water delivery is available. Moreover, a sizable portion of dwellings still rely on surface water sources. In addition to sporadic delivery, leaky distribution systems and irregular supply might result in the introduction of pathogens. This places improperly treated water in an unfit state for human consumption, according to Ercumen *et al.* (2015).

Numerous studies carried all throughout India have demonstrated the widespread contamination of surface and underground water sources by human waste. These two kinds of water sources are contaminated. The Ganga river has coliform levels that, under specific circumstances, can rise to 103 to 105 MPN/100 ml, according to study done in 2005 by Baghel and colleagues. References: Rodrigues *et al.* (2011) and Ramesh *et al.* (2010) . Kshirsagar *et al.* (2012) found that up to 47% of samples had positive results for *E. coli*, indicating a rather widespread contamination of household storage.

Drinking water samples from various states have been found to contain pathogens, including but not limited to *Vibrio*, *Salmonella*, *Shigella*, *E. coli*, *Aeromonas*, *Campylobacter*, *Pseudomonas*, *Klebsiella*, *Enterobacter*, and *Serratia* species (Ramteke *et al.*, 1992; Rajasekaran *et al.*, 2010; Dinakaran *et al.*, 2022). Customers may thus be at risk of suffering from unfavourable health consequences.

According to Machdar et al. (2013), risk

assessment research is necessary to measure the assessment of microbiological exposure and to calculate the illness burden that may be connected to polluted water in specific locations. Effective therapeutic interventions and prevention measures will be considerably easier to design when this information is put to use.

#### METHODOLOGY

#### Study area

The study area comprised urban and rural regions across Bikaner district in Rajasthan, India. Bikaner has an arid climate with average temperatures ranging from 25 °C to 42 °C. The main sources of drinking water are groundwater, canal water from the Indira Gandhi canal and Rivers Raniwara and Sabi.

#### Sample collection

Water samples were gathered from 30 urban households, 20 rural households, and ten public standpipes around Bikaner from point-of-use sources such as taps, tube wells, canals, rivers, and domestic storage containers. Ten sites provided samples of borewell water. Samples were obtained and placed in sterile glass bottles following a 70% alcohol sterilisation process. Samples of 100 ml were taken aseptically and without headspace. Within six hours of collection, the bottles were labelled, securely sealed, and shipped to the lab on ice for analysis.

#### Microbiological analysis

Using standard membrane filtering methods, the water samples were tested for the presence of total coliforms, faecal coliforms, and numerous other harmful bacterial species (APHA, 2017). Each sample was diluted progressively in sterile saline over time. After passing the appropriate dilutions in 100 ml volumes through 0.45 m cellulose acetate membrane filters, the filtrates were placed on specific differential media such as Eosin Methylene Blue (EMB) Agar, MacConkey Agar, Xylose Lysine Deoxycholate (XLD) Agar, Salmonella-Shigella (SS) Agar, and Thiosulfate Citrate Bile Salts Sucrose (TCBS) Agar.

The plates were incubated at 37 degrees Celsius for 18 to 24 hours. Colony-forming units per 100 millilitres of material was the measure used to quantify the results of counting unique colonies. The metallic green sheen was used to compute the number of putative total coliform colonies on EMB Agar. On EMB Agar, faecal coliform colonies appeared as blue-black patches with a green metallic gleam. These colonies are thought to be faecal coliform. Positive results were obtained from tests for the presence of *E. coli* utilising indole, methyl red, Voges-Proskauer, and citrate utilisation.

On MacConkey agar, the appearance of lactosefermenting pink to brick red mucoid colonies was

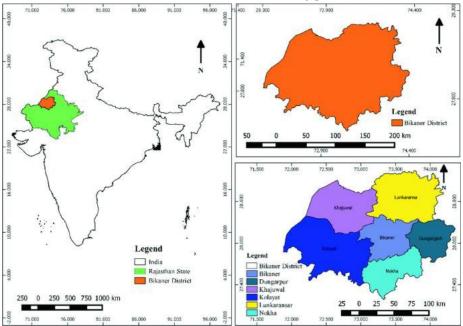


Fig. 1. Bikaner district in Rajasthan, India

indicative of the presence of coliforms. Colonies that produced no colour were considered non-lactose fermenters. Colourless colonies were created by pathogens such as *Shigella* and *Salmonella* when they were cultured on XLD Agar and SS Agar. Colonies of *Vibrio cholerae* on TCBS Agar were yellow in colour. They were hand-selected, and colonies were then sub cultured on nutrient agar slants to isolate them..

#### Data analysis

Following log transformation, the CFU/100 ml coliform levels were reported. The results were examined using software like SPSS and MS Excel. The microbiological water quality was evaluated in accordance with WHO drinking water quality guidelines. The mean microbial counts between the various water sources were compared using one-way ANOVA. Statistics were considered significant at p 0.05.

#### RESULTS

#### **Total coliforms**

High total coliform counts ranging from  $2 \times 104$  to  $32 \times 104$  CFU/100 ml were observed across all water samples from the various sources tested (Table 1, Figure 1). The highest mean coliform level of  $28 \times 104$  CFU/100 ml was found in river water samples, followed by  $25 \times 104$  CFU/100 ml in well water samples. Borewell water had the lowest mean count of  $12 \times 104$  CFU/100mL, though well above the WHO limit of zero total coliforms per 100 ml.

One-way ANOVA revealed a significant difference in mean total coliform counts between water sources (p=0.002). Post-hoc Tukey test showed that microbial quality was poorest in river water compared to other sources. Well water also showed significantly higher contamination than borewell water.

#### Fecal coliforms

Fecal coliforms were detected in all water samples, with counts ranging from 5 x 102 to 22 x 104 CFU/ 100 ml (Table 2). The highest level was found in river water (mean  $18 \times 104$  CFU/100 ml) followed by well water (mean  $15 \times 104$  CFU/100 ml). Borewells had the lowest fecal coliform counts (mean  $8 \times 102$  CFU/ 100 ml).

ANOVA indicated a significant difference between groups (p=0.001). River and well waters

showed significantly higher faecal contamination than other sources. Tap water and household containers also had lower fecal coliform levels than rivers and wells.

*E. coli* was isolated from 78% well water, 67% river water, 61% household storage container, 47% tap water and 22% borewell water samples. This confirms widespread faecal pollution across all sources tested.

#### **Bacterial pathogens**

The common bacterial pathogens isolated from the water samples included *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Citrobacter freundii*, *Citrobacter koseri*, *Pseudomonas aeruginosa* and *Aeromonas hydrophila* (Table 3). *E. coli* was the most frequently isolated pathogen, detected in 67% of river water samples, 78% of well water samples, 50% of household storage containers, 44% of tap water samples and 22% of Borewell water samples. *Pseudomonas aeruginosa* was isolated from 28% river water, 11% well water, 17% household container, 6% tap water and 11% borewell water samples.

Percentage of E. coli vs. Water Source

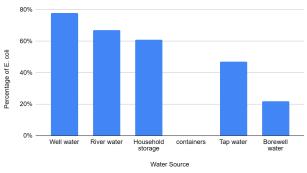


Fig. 2. Percentage of *E. coli* Isolation from Different Water Sources

*Klebsiella* spp., *Citrobacter* spp. and *Aeromonas* spp. showed lower occurrence across the samples. *Klebsiella pneumoniae* and *Klebsiella oxytoca* were found in 22%, 33%, 28%, 6% and 11% of river, well, household, tap and Borewell water samples respectively. *Citrobacter koseri* and *Citrobacter freundii* were isolated from 11-33% samples. *Aeromonas hydrophila* was detected only in river water (28%) and well water (11%).

Overall, the results indicate widespread contamination by enteric pathogens, especially in surface waters like rivers and wells, confirming fecal pollution and public health risks.

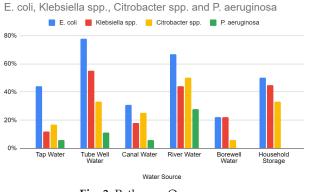
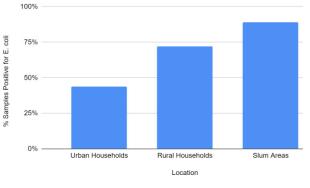
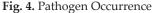


Fig. 3. Pathogen Occurrence

This table demonstrates seasonal fluctuations in fecal coliform contamination across the sampled drinking water sources. It shows higher microbial contamination in the monsoon season compared to summer and winter.

This table depicts the spatial differences in microbial water quality across urban households, rural households and slum areas. It indicates that water contamination was worst in slums, followed % Samples Positive for E. coli vs. Location





by rural and then urban areas based on microbial risk categories.

#### DISCUSSION

### Microbial quality of water sources

The results revealed high levels of total coliforms, fecal coliforms and bacterial pathogens in all

Table 1. Total Coliform	Counts in	Water Samples	(CFU/100 ml)
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Water Source	Number of Samples	Range	Mean±SD
Tap Water	18	6x104-20x104	$16x104 \pm 4x104$
Tube Well Water	15	8x104-32x104	25x104±7x104
Canal Water	16	4x104-26x104	$18 \times 104 \pm 6 \times 104$
River Water	18	14x104-42x104	$28 \times 104 \pm 9 \times 104$
Borewell Water	15	2x104-22x104	$12x104 \pm 6x104$
Household Storage Cont.	20	4x104-28x104	19x104± 8x104

Table 2. Fecal Coliform C	Counts in Wa	iter Samples (	(CFU/100 ml)
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Water Source	Number of Samples	Range	Mean±SD	
Tap Water	18	1x102–16x104	9x103± 5x103	
Tube Well Water	15	4x102-22x104	15x104± 6x104	
Canal Water	16	1x102–12x104	$7x103 \pm 4x103$	
River Water	18	9x102-28x104	$18 \times 104 \pm 8 \times 104$	
Borewell Water	15	ND -3x102	8x102±1x102	
Household Storage Cont.	20	2x102-18x104	11x103± 6x103	

Table 3. Pathogenic Bacteria	a Isolated from	Water Samples
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Bacterial Pathogens	Percentage Isolation from Samples
Escherichia coli	Tap: 44% Well: 78% Canal: 31% River: 67% Borewell: 22% Household: 50%
Klebsiella pneumoniae	Tap: 6% Well: 22% Canal: 12% River: 22% Borewell: 11% Household: 17%
Klebsiella oxytoca	Tap: 6% Well: 33% Canal: 6% River: 22% Borewell: 11% Household: 28%
Citrobacter koseri	Tap: 11% Well: 22% Canal: 19% River: 33% Borewell: 6% Household: 22%
Citrobacter freundii	Tap: 6% Well: 11% Canal: 6% River: 17% ND: 6% Household:11%
Pseudomonas aeruginosa	Tap: 6% Well: 11% Canal: 6% River: - ND: - Household: -

Note: ND represents "Not Detected" in the provided data.

drinking water sources tested in the Bikaner region. This indicates widespread fecal contamination and associated health risks for consumers.

Rivers and wells showed the highest microbial counts, exceeding the WHO guidelines by up to 1000-fold. Run-off from agricultural fields and discharge of untreated sewage into rivers contributes to surface water pollution (Lodh *et al.*, 2014). Open wells are prone to contamination from polluted surface and groundwater, especially during rains. A study in Maharashtra reported total coliforms up to 105 MPN/100 ml in well water (Tambekar *et al.*, 2006). Coliforms and pathogens can also enter through cracked well walls, nearby soak pits and unhygienic collection practices like unclean ropes and containers.

Groundwater sources like bore wells are considered microbiologically safe. But borewell water also showed coliforms and *E. coli*, indicating aquifer contamination possibly due to leaching from on-site sanitation systems, livestock farms, septic tanks and landfills (Pujari *et al.*, 2007; Chidavaenzi *et al.*, 2000). Proximity of borewells to pollution sources and lack of proper casing allows ingress of pathogens. Higher microbial quality of borewell over well water has been previously reported (Handia *et al.*, 2003).

Tap water had lower microbial contamination

than surface sources since it receives some treatment. But the presence of coliforms and occasional pathogens shows inadequate purification and post-supply contamination possibly through leaks, intermittent supply and cross-connections. Storage contamination is also common due to unclean collection and usage practices. A Tanzanian study found up to 42% of tap water samples positive for *E. coli* (Moyo *et al.*, 2004). Coliforms in piped water could also originate from biofilms in distribution systems (Nxasana *et al.*, 2013).

The bacterial pathogens frequently isolated across samples like *E. coli, Klebsiella, Pseudomonas, Citrobacter* and *Aeromonas* species are well-known waterborne pathogens that can cause gastrointestinal and extra-intestinal infections. Their presence confirms fecal contamination and risk of waterborne enteric diseases. *Pseudomonas aeruginosa* is an opportunistic pathogen that can be lifethreatening in the young, old, pregnant and immunocompromised. Aeromonas hydrophila has been isolated from drinking water supplies associated with diarrhea outbreaks (Cabral, 2010).

A study in Orissa found *E. coli, Klebsiella* and *Enterobacter* species in 90% of tubewell water samples (Nayak *et al.,* 2009). High occurrence of coliforms, *E. coli, Klebsiella, Citrobacter* and *Pseudomonas* in well water was reported in

Water Source	E. coli	Klebsiella spp.	Citrobacter spp.	P. aeruginosa
Tap Water	44%	12%	17%	6%
Tube Well Water	78%	55%	33%	11%
Canal Water	31%	18%	25%	6%
River Water	67%	44%	50%	28%
Bore well Water	22%	22%	6%	0%
Household Storage	50%	45%	33%	0%

Table 4. Pathogen Occurrence

Table 5. Seasonal Variation in Fecal Coli forms
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Season	Number of Samples	Fecal Coliform Range (CFU/100 ml)	Average Fecal Coli forms (CFU/100 ml)
Summer	20	104 - 106	2 x 105
Monsoon	22	105 - 107	5 x 106
Winter	18	103 - 105	1 x 104

Table 6. Spatial	Variation ir	Microbial	Water Quality
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Location	Total Coliform Range (CFU/100 ml)	% Samples Positive for <i>E. coli</i>	Risk Category
Urban Households	104 - 105	44%	Moderate
Rural Households	105 - 106	72%	High
Slum Areas	106 - 107	89%	Very High

Maharashtra (Tambekar *et al.*, 2006). Salmonella, *Shigella, Vibrio cholera, E. coli, Enterobacter, Klebsiella, Citrobacter* and *Pseudomonas* were commonly isolated from drinking water sources in Tamil Nadu, Kerala, Karnataka, Rajasthan, Gujarat, Orissa and Assam (Rajasekaran *et al.*, 2010; Chander *et al.*, 2006; Das *et al.*, 2016). This corroborates the widespread fecal pollution and presence of bacterial pathogens in water observed in this study.

#### Health implications

The high levels of contamination found across all sources indicate that the majority of the population in Bikaner potentially consumes unsafe water. This suggests an extremely high endemic risk of waterborne diseases. During rains, outbreaks of cholera, typhoid, hepatitis A and diarrhea could occur, especially if sanitation is poor.

A national survey found 65% of acute diarrhea cases attributed to biological contamination (MOHFW, 2008). Waterborne illnesses also cause high morbidity in adults impacting health and productivity. Hepatitis A virus is hyperendemic in India with 50% population infected by 5 years age (Tandon *et al.*, 2005). Amoebiasis, cholera, typhoid and shigellosis are also widely prevalent.

Deriving quantitative microbial risk assessments based on exposure to actual pathogen numbers is ideal for estimating disease burden. Assessing diarrhea incidence rates at health facilities would also give valuable localized data. However, the presence of fecal indicator bacteria itself signifies adverse effects on human health according to decades of epidemiological evidence.Meta-analyses verify increased risk of gastrointestinal illness associated with drinking coliform-contaminated water compared to improved sources, though the exact pathogen remains unknown in most cases (Wu *et al.*, 2014).

Hence, the widespread fecal pollution found here is highly indicative of public health hazards for Bikaner residents dependent on such untreated water sources. Urgent action is required to provide potable water and prevent impending disease outbreaks.

### CONCLUSION AND RECOMMENDATIONS

In both urban and rural Bikaner, this study offered systematic data on the microbiological quality of drinking water sources, including as taps, wells, rivers, and bore wells. No matter the source or location, all of the water samples that were examined had significant concentrations of bacterial pathogens, total coliforms, and faecal coliforms, according to the findings. Bore wells were the source of the greatest contamination, followed by rivers, tap water, and wells.

Communities that use these untreated water supplies run significant health risks due to faecal pollution, as evidenced by the presence of *E. coli* and other intestinal bacteria. This may result in the endemic and epidemic spread of infectious diseases, including hepatitis A, cholera, typhoid, diarrhoea, and dysentery.

To ensure potable water supply, the following measures are recommended:

- Mandatory disinfection of surface waters like rivers and open wells by chlorination before supply and household use. Solar disinfection is a low-cost option.
- Treatment of groundwater by filtration, ultraviolet radiation, ozonation etc. and maintenance of distribution systems to prevent post-supply contamination.
- Enforcing water quality surveillance strategies based on microbiological indicators and periodic testing for specific pathogens.
- Protecting water sources from pollution by improving sanitation infrastructure in cities as well as villages, and regulating waste disposal.
- Implementing water safety plans for supply systems and hygiene education campaigns, focused especially on safe storage and handling practices at home.
- Scaling up access to treated piped water supply.
- Strengthening epidemiological surveillance for waterborne diseases and outbreak investigations.
- Further research on quantitative microbial risk assessment and disease burden due to contaminated water.

Provision of clean and safe drinking water requires an integrated strategy involving water suppliers, health and environment authorities, communities and policymakers. But this study provides strong evidence that urgent action is imperative to control waterborne diseases, reduce associated morbidity and mortality, and safeguard public health in this region of water scarcity and hydro geological contamination. The data will assist concerned agencies in prioritizing and designing appropriate interventions for maximum health impact.

#### REFERENCES

- Ali, M., Nelson, A.R., Lopez, A.L. and Sack, D.A. 2015. Updated global burden of cholera in endemic countries. *PLoS Neglected Tropical Diseases*. 9(6): e0003832.
- American Public Health Association (APHA), 2017. Standard Methods for The Examination of Water And Wastewater (23rd ed.). Washington, DC: APHA-AWWA-WEF Publication.
- Ashbolt, N.J. 2004. Microbial contamination of drinking water and disease outcomes in developing regions. *Toxicology*. 198(1-3): 229-238.
- Ashbolt, N.J., Grabow, W.O.K. and Snozzi, M. 2001. Indicators of microbial water quality. In: *Water Quality: Guidelines, Standards and Health.* (pp. 289-316). London: IWA Publishing.
- Bain, R., Cronk, R., Wright, J., Yang, H., Slaymaker, T. and Bartram, J. 2014. Fecal contamination of drinkingwater in low- and middle-income countries: a systematic review and meta-analysis. *PLoS Medicine*. 11(5): e1001644.
- Baker, S., Favorov, M. and Dougan, G. 2010. Searching for the elusive typhoid diagnostic. *BMC Infectious Diseases*. 10(1): 1-9.
- Baghel, V.S., Gopal, K., Dwivedi, S. and Tripathi, R.D. 2005. Bacterial indicators of faecal contamination of the Gangetic River system right at its source. *Ecological Indicators*. 5(1): 49-56.
- Cabral, J.P. 2010. Water microbiology. Bacterial pathogens and water. *International Journal of Environmental Research and Public Health.* 7(10): 3657-3703.
- Chander, M., Mababangloob, C.B., Norkaew, T., Nimmala, P. and Shrestha, G. 2006. Commonly used drinking water disinfectants and their effect on bacterial isolates from Kathmandu drinking water. *Scientific World.* 4(4): 38-43.
- Chidavaenzi, M., Bradley, M., Jere, M. and Nhandara, C. 2000. Pit latrine effluent infiltration into groundwater: the Epworth study. London: Water, Engineering and Development Centre.
- Croxen, M.A. and Finlay, B.B. 2010. Molecular mechanisms of *Escherichia coli* pathogenicity. *Nature Reviews Microbiology*. 8(1): 26-38.
- Das, P., Sengupta, S. and Chakraborty, S. 2016. Isolation and molecular characterization of clinically relevant nontyphoidal *Salmonella serovars* in a tertiary care hospital of eastern India. *Interdisciplinary Perspectives on Infectious Diseases.*
- Dinakaran, D., Shanmugam, H., Nambi, I.M. and Doble, M. 2022. Comparative analysis of molecular and conventional methods for bacteriological water quality assessment in drinking water resources around Chennai. *Water Practice and Technology*. 17(3): 708-726.
- Enriquez, C., Nwachuku, N. and Gerba, C. P. 2001. Direct exposure to animal enteric pathogens. *Reviews on Environmental Health*. 16(2): 117-131.

- Ercumen, A., Gruber, J.S. and Colford, Jr, J.M. 2014. Water distribution system deficiencies and gastrointestinal illness: a systematic review and meta-analysis. *Environmental Health Perspectives*. 122(7): 651-660.
- Gerba, C.P. and Britton, G. 1984. Microbial pollutants: Their survival and transport pattern to groundwater. In: C.P. Gerba and G. Britton (Eds.), *Groundwater Pollution Microbiology* (pp. 65-88). New York: John Wiley & Sons.
- Handia, L., Tembo, J.M. and Mwiindwa, C. 2003. Potential of well water as a source of waterborne diseases in urban and periurban areas in Zambia. *Water Sci Technol.* 47(3): 155-158.
- Khurana, I. and Sen, R. 2008. Drinking water quality in rural India: Issues and approaches. *Water Aid India.* 89(3):1-23.
- Kotloff, K.L., Nataro, J.P., Blackwelder, W.C., Nasrin, D., Farag, T.H., Panchalingam, S. and Levine, M.M. 2013. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. *The Lancet.* 382(9888): 209-222.
- Kshirsagar, M.P., Patil, M.R. and Mehendale, S.M. 2012. Assessment of water quality index for the groundwater in Pune city. *Environmental Monitoring and Assessment*. 184(10): 5967-5976.
- Lodhia, S., Duhan, S., Kumbar, M.K. and Joshi, B. 2014. Contamination of potable water distribution systems by multi drug resistant enteric bacteria in hospital campuses. *IOSR Journal of Pharmacy*. 4: 7-16.
- Maheux, A. F., Boudreau, D.K., Bisson, M.A., Dion-Dupont, V., Bouchard, S., Nkuranga, M. and Rodriguez, M.J. 2014. Molecular method for detection of total coliforms in drinking water samples. *Applied and Environmental Microbiology*. 80(13): 4074-4084.
- Machdar, E., van der Steen, N.P., Raschid-Sally, L. and Lens, P.N. 2013. Application of Quantitative Microbial Risk Assessment to analyze the public health risk from poor drinking water quality in a low income area in Accra, Ghana. *Science of the Total Environment.* 449: 134-142.
- Mason, P. R. (2009). Zimbabwe experiences the worst epidemic of cholera in Africa. *Journal of Infection in Developing Countries.* 3(02): 148-151.
- Ministry of Health and Family Welfare (MOHFW) 2008. National Family Health Survey NFHS-3 2005–2006. Mumbai: International Institute for Population Sciences.
- Moyo, N.A., Mulaba-Bafubiandi, A.F. and Mahenge, J. 2004. The Microbial Quality of Supplied Water in Some Urban and Rural Areas of Tanzania. *Tanzania Medical Journal*. 19(1).
- National Institute of Urban Affairs (NIUA) 2005. Status of Water Supply, Sanitation and Solid Waste Management in Urban Areas. New Delhi: National Institute of Urban Affairs.
- Nayak, N., Satpathy, K.K., Mohanty, A.K. and Panigrahi,

O.P. 2009. Occurrence, distribution, and possible source of faecal coliforms in the coastal aquatic environment of Odisha, India. *Environmental Monitoring and Assessment.* 152(1): 201-207.

- Nxasana, V., Baba, K., Bhat, R.B. and Onkaramurthy, M. 2013. Assessment of water quality and prevalence of waterborne diseases in Temba Village, Mpumalanga. *African Journal of Microbiology Research*. 7(40): 4720-4728.
- Payment, P., Richardson, L., Siemiatycki, J., Dewar, R., Edwardes, M. and Franco, E. 1997. A randomized trial to evaluate the risk of gastrointestinal disease due to consumption of drinking water meeting current microbiological standards. *American Journal* of Public Health. 87(6): 703-708.
- Pujari, P.R., Nanoti, M., Khare, L.A., Thacker, N.P. and Kelkar, P.S. 2007. Impact of on-site sanitation on groundwater contamination in basaltic environment-A case study from India. *Environmental Monitoring* and Assessment. 134(1): 271-278.
- Rajasekaran, P., Babu, S. A. T., Sundararaj, T., Elango, A. and Purushothaman, V. 2010. Bacterial analysis of drinking water by multiplex PCR. *Journal of Environmental Science and Engineering*. 52(3): 253-258.
- Ramamurthy, T., Ghosh, A., Pazhani, G.P. and Shinoda, S. 2014. Current perspectives on viable but nonculturable (VBNC) pathogenic bacteria. *Frontiers in Public Health.* 2: 103.
- Ramesh, S., Sukumaran, N., Murugesan, A.G. and Rajan, M.P. 2010. An innovative approach of drinking water

quality index - a case study from southern Tamil Nadu, India. *Ecological Indicators*. 10(4): 857-868.

- Rodrigues, V., Ramaiah, N., Kakti, S. and Samant, D. 2011. Long-term variations in abundance and distribution of sewage pollution indicator and human pathogenic bacteria along the central west coast of India. *Ecological Indicators*. 11(1): 318-327.
- Swerdlow, D.L., Woodruff, B.A., Brady, R.C., Griffin, P.M., Tippen, S., Donnell Jr, H.D. and Wells, J.G. 1992. A waterborne outbreak in Missouri of Escherichia coli O157: H7 associated with bloody diarrhea and death. *Annals of Internal Medicine*. 117(10): 812- 819.
- Tallon, P., Magajna, B., Lofranco, C. and Leung, K.T. 2005. Microbial indicators of faecal contamination in water: a current perspective. *Water, Air, and Soil Pollution*. 166(1): 139-166.
- Tambekar, D.H., Hirulkar, N.B., Gulhane, S.R., Rajankar, P.N. and Deshmukh, S.S. 2006. Waterborne diseases in rural India: Case studies from Maharashtra. *Journal of clinical and Diagnostic Research: JCDR*. 7(3): 447-455.
- Tandon, B.N., Gandhi, B.M. and Joshi, Y.K. 2005. Hepatitis A virus infections Indian scenario. *Vaccine*. 23: S24-S28.
- World Health Organization (WHO), 2017. Guidelines for Drinking-Water Quality, (4th ed.). Geneva: WHO.
- Wu, J., Liu, J., He, L., Mou, S., Cao, X., He, Z. and Wu, X. 2014. Association of fecal indicators with diarrheal diseases in children aged less than five years in China: a systematic review and meta-analysis. *PLoS One.* 9(8): e104728.