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Article Information

Received: October 28, 2022

Accepted: November 6, 2022

Published: November 8, 2022

Keywords

Portable microbiology laboratory, Underground water, Rural Hyderabad.

Authors' Contribution

SU contributed in study design, manuscript writing, referencing by citation manager, analyzing results by SPSS; NS collected samples; AHN provided finance such as the materials used in study, cross study of techniques, finalizing discussion, approval of publication; MNI proofread and edited the manuscript.

How to cite

Urooj, S., Narejo, A.H., Sahar, N., Iqbal, M.N., 2022. Accessing the Fecal Pollution of the Aquifer Systems using Portable Microbiology Technique: An Affordable Approach to Enhance Coverage of Safely Managed Water Services. PSM Microbiol., 7(3): 73-81.

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Accessing the Fecal Pollution of the Aquifer Systems using Portable Microbiology Technique: An Affordable Approach to Enhance Coverage of Safely Managed Water Services

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Abstract:

The basic necessity of human life is the clean and potable water. Indus Basin is considered as chief source of water provision in Sindh, Pakistan. In this region only 10% of fresh ground water is available that present in the shallow aquifers. This study deployed community-based water testing using the Portable Microbiology Laboratory (PML) to assess the public health risk of spring wells (open wells whose water is from underground springs) and boreholes in a rural setting. This study used the PML to assess the level of disease risk linked to underground water sources in rural settings of Hyderabad, Sindh by testing for environmental coliform and *Escherichia coli* contamination. Overall, of the 78 underground water sources tested, 52 (67%) were spring wells while 26 (23%) were boreholes. Of the spring wells, 56% (29/52), 21% (11/52), 6% (3/52), and 17% (9/52) were classified as low, moderate, high, and as very high disease risk respectively. Of the borehole samples, 58% (15/26), 31% (8/26), 8% (2/26), and 4% (1/26) were categorized as low, moderate, high and very high disease risk respectively. All the underground water sources that posed high to very high disease risk to the community were situated with in less than 20 meters to human settlements. Community-based testing of underground water for public health safety using the PML enables access to safely managed water by off-grid rural communities in Hyderabad. Underground water sources located close to homesteads pose waterborne disease risk to user communities in rural settings. Community-based water microbiology using the PML will facilitate expansion of safe water services to rural settings in Hyderabad Sindh.

HIGHLIGHTS

- The Portable Microbiology Laboratory (PML) extends water microbiology to the rural communities in Hyderabad, Sindh Pakistan.
- Proximity of underground water sources to homesteads is associated with increased disease risk.
- Underground water sources carry varying disease risk to user communities in rural settings.



INTRODUCTION

Due to the inability of government to meet the ever-increasing water demand due to fast growing human populations, most communities resort to underground water sources as alternative to piped chlorinated water that is routinely monitored for safety. However, ground water sources are commonly vulnerable to microbial pollution (Urooj *et al.*, 2018; Ashraf *et al.*, 2019; Pang *et al.*, 2021). A major issue of concern is the growing contamination of sources of drinking water and its implications (Ashraf and Iqbal, 2020; Saleem *et al.*, 2020). Generally, ground water quality varies from place to place depending on seasonal changes, the soil type, and the rocks and surfaces through which it moves (Akhtar *et al.*, 2021; Hussain, 2022).

Besides that, human activities alter the natural composition of ground water through the disposal and dissemination of chemicals and microbial matter on the land surface and into ground water (State *et al.*, 2018; Hadyait *et al.*, 2020). Specifically, in Hyderabad, Sindh where the most common type of domestic fecal waste disposal system is the pit latrine, the deeply dug pits often connect to the underground water table and thus render the underground water a great microbial risk to user homesteads and communities (Hlongwane, 2021).

The most common and widespread public health risk associated with drinking water is microbial contamination (Fatima *et al.*, 2021a; Some *et al.*, 2021). Microbial contamination of major urban water systems has the potential of causing outbreaks of water borne diseases like cholera, typhoid fever, diarrhea, dysentery and amoebiasis (Ashraf and Iqbal, 2022; Obaideen *et al.*, 2022; Salehi, 2022). The use of contaminated water in food products may result in foodborne diseases and antibiotic resistance (Iqbal *et al.*, 2015, 2016). Maintaining appropriate water quality is critical to the health of the ecosystem (Iqbal and Ashraf, 2020; Echevarría, 2022).

Since the detection of many waterborne pathogens requires expensive and time-consuming techniques, that require a high level

of laboratory skillset and a high amount of investment in laboratory infrastructure. For resource-limited settings, other competing priorities and the lack of political will further diminish the prioritization of costly water microbiology infrastructure. For these reasons, national water quality testing programs have suggested to test for indicator organisms such as *E. coli* (Gibson *et al.*, 2021). One of the commonly used tests in Pakistan national water microbial quality testing programs is the 100ml membrane filtration technique which requires access to a well-built water microbiology laboratory (Sohail *et al.*, 2022).

Unlike the 100ml membrane filtration test, the cheap Portable Microbiology Laboratory (PML) was designed with portability and ease of use in mind, and it contains reliable tests for *E. coli* that the rural community can easily adopt. The PML aids the detection of coliforms in drinking water basing on specific enzymatic activity of β -D galactosidase (for total coliform) and β -D glucuronidase (for *E. coli*) (Maheux *et al.*, 2015). One of the components of the PML, the Petrifilm *E. coli* Count plate (PEC) 24h method, was found to be as good as or better than MPN method for the detection of *E. coli* and that it is more sensitive than the 9 tube MPN method for the detection of very low numbers of *E. coli* (Sohail *et al.*, 2022).

In Hyderabad, rural and peri-urban areas rely mostly on spring well, borehole, and roof top water for domestic use- these are not safely managed sources for drinking water. Water from these sources was found to hold *E. coli* counts higher than is recommended by the World Health Organization (WHO) and the PSQCA water standard (Imran *et al.*, 2020; Panhwar *et al.*, 2022; Khan *et al.*, 2018).

The Water and Sanitation Agency in Sindh, routinely tests piped distribution water for microbial safety, but its scope is largely limited to the urban areas. Despite the modern techniques for water purification, disinfection and sanitation that are used in the laboratories, underground water in the off-grid peri-urban and rural areas is not tested for microbial quality because the techniques available are time consuming and

expensive – a scenario experienced elsewhere under similar settings. In order to assess the level of disease risk associated with underground water sources in a rural setting, community-based water microbiology was done using the PML.

MATERIALS AND METHODS

A cross sectional study was carried in a rural area with no supply of chlorinated piped water in Hyderabad Sindh. Between July and December 2021, 78 underground water sources including spring wells and boreholes were investigated for their public health risk according to the World Health Organization (WHO) guidelines using the Portable Microbiology Laboratory (PML).

Study Setting

The study was carried out in Hyderabad, Sindh. The samples were collected from the administratively divided rural areas of Hyderabad such as: Hatri, Detha Goth, Masu Bhugri, Mossa Khatiyan, Haji Sawan Khan Gopang, Tando Qaiser, Tando Hyder, Husri, Moolan, Husri, Tando alam Mitri, Tando Fazal and Tando Jam (Figure 1). Approximately 70% of the citizens in these communities rely on underground water sources.



Fig. 1. Map showing the study area.

Sample Collection

The samples were collected using the depth-integrated-grab sampling technique at 5cm below the water surface for un built spring wells (the top of these wells is not covered) and the mid-stream sample collection method built spring wells (spring wells with a covered top) in sterile 100ml whirl Pak containers (Figure 2) and information such as location, distance from nearest homestead, and the date and time of collection was recorded before holding them at 4°C in a temperature monitored box for less than one hour prior to community-based water testing.



Fig. 2. Collection of water sample from a built water source into a zip-locked 100ml whirl Pak using sterile technique.

Community-based Testing Using the PML

Field-based testing was carried out by community members and included quantitative and qualitative (presence/absence) approaches. Of the 100ml of water sample collected, 10ml of sample were aspirated using a 1ml pipette into the Colilert® 10ml presence/absence test (IDEXX, Westbrook, ME) tube which was then inverted several times to form a homogenized mixture and incubated at 35 ± 2 °C. Interpretation of the results was based on the occurrence or absence of a color change and fluorescence when flashed with UV light from a battery-operated handheld lamp (Figure 3).

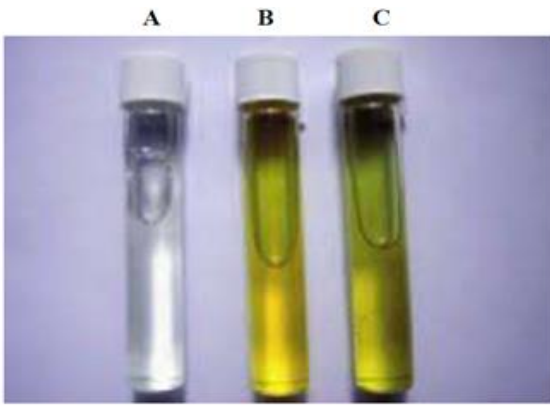


Fig. 3. Interpretation for the Colilert test after 12-18h incubation.

A clear tube showed no coliforms present (A), A yellow tube with no fluorescence under long wave UV light indicates coliform bacteria other than *E. coli* are present.

A yellow tube with blue fluorescence under long wave UV light showed that *E. coli* was present.

For the *E. coli*/ Coliform Count Petrifilm™ (3M, St Paul, MN), using a sterile 1ml pipette, 1ml of sample was placed into the center of the Petrifilm agar surface after folding back the plastic layer which was then carefully rolled back down to cover the agar surface without trapping air bubbles. Uniform spreading of the sample was done using a plastic spreader (3M, St Paul, MN). The Petrifilm was then sandwiched by two paper cardboard quadrants and fastened with an elastic rubber band before incubation at 35 ±2 °C. The results provided a disease risk assessment of water sources that correlated with the World Health Organization’s guide for Drinking Water quality (Table 1).

Table 1. Disease Risk Level as per the WHO guidelines for drinking water quality.

<i>E. coli</i> in sample	Colilert MUG+	<i>E. coli</i> colonies on Petrifilm	Disease Risk Level
<1/10ml	-	0	Low
1 -10/10ml	+	0	Moderate
1-10/ml	+	1-10	High
>10/ml	+	>10	Very High

*The Colilert and Petrifilm tests are specific for *E. coli* because they contain a substrate for the beta-glucuronidase enzyme that is produced by *E. coli* but not by the environmental coliform bacteria.

Quality Control

The pre-use Colilert tubes and the *E. coli* Coliform Count Petrifilm™ (3M, St Paul, MN) were kept away from light in a cardboard box. For each batch of test samples, a positive (Figure 4) and negative control was run along with the test samples. Sterile laboratory grade water was used as the negative control while an in-house positive control of water from a contaminated source was used as the positive control. Positive and negative controls were incubated under similar conditions as the test samples.



Fig. 4. Positive controls used for the Colilert presence/absence test and the *E. coli*/ Coliform count Petrifilm test.

RESULTS

Of the 78 water samples, 56.4% (44/78) posed a low disease risk, 24.4% (19/78) posed a moderate disease risk, 6.4% posed a high disease risk, and 12.8% posed a very high disease risk to the user communities.

All the wells that presented a low disease risk level to the communities were located further

from the homesteads regardless of the type of the underground water source. Overall, more of the water samples collected from the spring wells (9/52) were very highly contaminated compared to the those from bore holes (1/26) (p-value=0.036). All the samples that presented moderate to high to very high public health risk was situated closer to the homesteads.

Table 2. Comparative assessment of disease risk level in spring wells and boreholes.

Disease Risk Level	Spring wells (N=52)		Boreholes (N=26)	
	<50m from homesteads	>50m homes	from	<50m from homesteads
Low		29		15
Moderate	11		8	
High	3		2	
Very high	9		1	
Total	23	29	11	15

DISCUSSION

Groundwater accounts for 99% of all liquid freshwater on Earth but is poorly understood, undervalued, mismanaged, and abused but the vast potential of ground water can no longer be overlooked. As we pursue to achieve the SDG 6, putting emphasis on ground water and adopting simple technologies to monitor safety is a short cut to cost-effectively delivering safely managed water services to millions of rural inhabitants. Even though the quality of groundwater is generally good, different forms of land have led to its contamination with pathogens that lead to waterborne diarrheal diseases. Additionally, as a way of climate change adaptation, aquifer systems could be used to store seasonal or episodic surface water surpluses to improve year-round freshwater availability, as aquifers incur substantially lower evaporative losses than surface reservoirs. If safely managed, the improved access to clean water could lead to a reduction in occurrence of diarrheal disease. According to UN water, residents of rural area of Hyderabad have limited access to safely managed water and sanitation services and there are several contributing factors to this statistic including lack of infrastructure, political will, poverty, and lack of skilled personnel.

The National water and sanitation agencies of Hyderabad, routinely monitors the quality of chlorinated piped water using the 100ml membrane filtration test (Javaid *et al.*, 2022), but its scope is limited to mainly the urban areas. Off grid zones in rural and peri-urban areas with no access to piped water resort to the use of underground water sources such as spring wells and boreholes but these are not part of the testing scope of laboratories. Previous studies showed that underground water in both rural and urban areas in Hyderabad, was not fit for human consumption and carried high loads of *E. coli* and other fecal coliforms (Hussain *et al.*, 2021; Javaid *et al.*, 2022; Manoiu *et al.*, 2022; Nishad Khan *et al.*, 2021; Ramesh and Thiyagarajan, 2021). Another study that assessed the risk factors for underground water contamination found that there was a significant relationship between median level of contamination and rainfall, total sanitary score, and that population density was a confounding factor – findings that are in tandem with those of this study (Contreras *et al.*, 2022).

This study was conducted in a rural setting with varying levels of anthropogenic activity and found that construction of an underground water source close to human homesteads (within 50m)

significantly increased the level of disease risk associated with the water source. This finding is in resonance with an earlier study which revealed that in such settings with high levels of anthropogenic activity, the water table responded rapidly to short rains (48 h) due to the pervious and shallow vadose zone which was thin due to anthropogenic activities and had a limited contaminant attenuation capacity. Consequently, some diarrheal disease outbreaks have been associated with drinking unprotected well water contaminated with feces (Fatima *et al.*, 2021b; Saqib *et al.*, 2022), while others could not be linked to a single source .

Since the levels of underground water microbial contamination varied with seasons (Abiriga *et al.*, 2021), routine surveillance of underground water is of paramount relevance. One of the limitations to routine surveillance of underground water quality using the 100ml membrane filtration technique, is the expense and skill required to build and maintain a water microbiology laboratory to serve rural and remote areas with no access to piped chlorinated water. Currently, rural population has limited access to safely managed drinking water largely due to the limitations of the scope of water laboratory services and the associated logistics. In order for Hyderabad to timeously achieve SDG 6.1, there is need to alter water testing strategy in such a manner that testing can be done by the concerned rural communities. Access to safe drinking water in homes, healthcare facilities, schools and workplaces effectively reduces water-borne disease and malnutrition, which are leading causes of death among children under five (Aziz *et al.*, 2022; Bilal *et al.*, 2022; Chan *et al.*, 2021). The PML has already been used in the rural villages of Kenya – for example in Lower Nyakach which reported a 40-73% decrease in diarrhea case (Debes *et al.*, 2021; Osiemo *et al.*, 2019; Peletz *et al.*, 2016). Here, schools reported dramatic decrease in absenteeism due to diarrhea illness and that very little anti-diarrheal medications were being sold. Coupled with onsite chlorine dispensers, the use of the PML could potentially aid the control of waterborne disease in remote rural areas.

CONCLUSION

Fecal bacteria have become a potential source of illness in the community. All of the underground water sources that offered a high to extremely high risk of illness to the community were located less than 20 meters from populated areas. Off-grid rural areas in Hyderabad now have access to securely managed water attributable to community-based testing of underground water for public health safety utilizing the PML.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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