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Advanced preparedness for drinking water emergency: Ensuring safe microbial quality with rapid microbial source tracking and *E. coli* methods

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ABSTRACT

The increasing occurrence of water main breaks attributed to aging water infrastructure and other disaster-related events amplifies the risk of microbial contamination in drinking water. Conventional methods for assessing water quality after waterline disruptions involve using time-consuming fecal indicator culture-based techniques that fail to detect the sources of contamination. Thus, rapid emergency responses are critically needed to safeguard public health and swiftly resume community functioning. This novel study proposes a rapid emergency response method targeting *Escherichia coli* (*E. coli)* and microbial source tracking (MST, human (HF183) and universal (UniBac) fecal contamination) following water main breaks to evaluate water safety. Objectives were: develop a rapid method for quantifying fecal microbial contamination using droplet digital PCR (ddPCR), and compare rapid results to culture-based method. Results indicate zero *E. coli* presence using culture-methods. Conversely, results using rapid-ddPCR method show quantifiable positive detection of *E. coli* and MST markers (HF183 and UniBac) across various sites, suggesting potential widespread fecal contamination following the main break incident. Results highlight that a rapid molecular *E. coli* and MST techniques effectively detected fecal contamination in treated drinking water that culture-based methods failed to detect. Notably, the ddPCR method produced sensitive results 18 hours faster than culture-based method, highlighting that MST can be used to simultaneously determine water quality and fecal contamination sources. To our knowledge, this is the first pilot study employing highly specific and sensitive MST methodology in treated drinking water following distribution main break.

Introduction

Drinking water distribution systems play a crucial role in safeguarding the health and overall well-being of communities. Deteriorating water infrastructure and subsequent emergencies put water distribution systems at risk of microbial contamination, leading to detrimental impacts on water flow pressure, capacity, and quality (Allen et al., 2018). Between 2012 and 2018 in the United States (US) and Canada, water main breaks have increased by 27 % (Folkman, 2018). Approximately 28 % of water mains exceed 50 years old (the average age of failing water mains), 16 % are beyond their acceptable usage, and over 55 % of water utilities fail to conduct routine condition assessments and face financial restraints for replacement (Folkman, 2018). The American Society of Civil Engineers rated overall US water infrastructure with a "D+" grade, meaning poor quality and at-risk of failure (ASCE, 2017). The added stress of aging infrastructure with changing environmental conditions (e.g., temperature and soil) heighten the risk of water line breakages which ultimately threatens the water quality (Lai and Dzombak, 2021). Consequently, these types of unfavorable environmental conditions are expected to increase in their frequency and severity under climate change. Although attempts have been made to increase the urgency and funds to upgrade water infrastructure, pipe replacements are not progressing fast enough and current water utilities remain reactive with repairs rather than being proactive before water breaks occur. When a waterline breaks or pressure decrease due to power outages and/or natural disasters, microbial pathogens can enter the treated water by infiltrating the pipe's fractures and cracks (Gibson et al., 2019). Consequently, individuals downstream are at risk

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of exposure to the contaminated water and developing waterborne illnesses. Utility companies typically shut the waterline off to decrease exposure risk and the served population is placed under some form of a Boil Water Advisory (BWA) until water safety test results can ensure that no microbial contamination occurred following system repairs. People are instructed to refrain from using tap water which places the entire impacted community on hold; the daily lives of households, businesses, schools, restaurants, and hospitals are disrupted and resort to emergency functioning. For example, a recent and massive 120-inch water main break occurred on August 13th, 2022 in southeastern Michigan, nearly one million residents across seven communities were urged limited water usage under a BWA (https://www.michiganradio.org/transportation-infrastructure/2022-08-16/se-michigan-water-main-break-larger-than-thought-boil-water-advisories-expected-until-september). Nearly three months later, the water transmission was finally restored and water services resumed normal operations after subsequent water testing. Arguably, these water services could have resumed quicker if rapid emergency response methods were readily available. A significant problem of concern is the anticipated increase in water distribution emergencies, which pose a substantial risk on public health, especially in disadvantaged communities (Folkman, 2018).

Currently, microbial water quality criteria use fecal indicator bacteria (FIB), such as total coliform, fecal coliform, *Enterococcus* spp., and *Escherichia coli,* to detect the potential presence of fecal contamination in water bodies since fecal contamination can pose a risk to human health (Wen et al., 2020). *E. coli* is regarded as the most common and sensitive indicator organism of fecal contamination in the natural environment, which is relatively specific to warm-blooded animals and is better correlated to gastrointestinal illness risk than other FIB in freshwater (Leonard et al., 2018; Marion et al., 2010). In addition, *E. coli* include specific strains (e. g. *E. coli* O157:H7) that are pathogenic to humans and can cause illness (Gizaw et al., 2022; Li et al., 2021). The presence of fecal indicator bacteria (FIB), such as *E. coli,* in water does not necessarily indicate the presence of pathogenic bacteria, but does indicate that fecal-borne microorganisms may be present in the water environment, indicating fecal pollution (Paruch and Mæhlum, 2012). Water utilities must adhere to the US environmental protection agency (USEPA) revised total coliform rule (RTCR) and ensure that the FIB(s) are not detected in drinking water (US EPA, 2015). After a waterline break, the USEPA Method 1603 is a reliable and commonly used procedure that measures the presence or absence of *E. coli* as a fecal indicator of water quality. The world health organization (WHO) also considers a non-detect of *E. coli* per 100 mL is safe for human consumption (WHO, 2004). However, this is a culture-based method that requires at least 24 hours of incubation which may take too long to restore clean drinking water to some communities. In addition, if the water is already clean and safe to use, the delayed result holds all water-related essential business, such as restaurants, hospitals, and schools, hospitals, on hold for an unnecessarily long period of time, which leads to a huge economic impact. It is evident that an improved rapid detection method is critically needed to produce timely results and protect the public from consuming contaminated water.

In addition to long incubation times and delayed response time to prevent harmful exposures, there are other limitations to using traditional FIB methods for microbial quality assessments. The reliability of FIB as indicators has been brought to question since FIBs have been documented to evolve across environments (Yang et al., 2020). Traditional culture methods only detect viable bacteria and may miss the presence of non-viable but potentially harmful microorganisms, which may underestimate the contamination level (Basili et al., 2023). Most importantly, traditional culture-based methods lack specificity and do not identify the potential source of the contamination. However, molecular advances such as microbial source tracking (MST), may serve as a novel solution to this issue. MST is an innovative method that is used precisely to identify major fecal pollution sources and whether they originate from human, wildlife, or livestock sources (McKee and Cruz, 2021). By identifying host-specific genetic markers indicative of fecal contamination, potential risks to human and ecosystem health can be assessed and effective actions can be taken to reduce the specific fecal pollution source. As a result, MST applications are diverse and commonly applied across society including the following fields, water quality management, industry (i.e., agriculture, wastewater), public health, environmental ecosystem impact assessments, and policy development (Barrios et al., 2018; Bernard et al., 2022; Frick et al., 2020; Fu and Li, 2014; Hsu et al., 2017; Lee et al., 2012; Nguyen et al., 2018; Weidhaas et al., 2010; Wu et al., 2023; Zhang et al., 2019). Remarkably, MST techniques help identify and mitigate the sources of microbial contamination, ultimately leading to safer and healthier communities and ecosystems more effectively. MST methods are constantly evolving in the scientific community to better identify and track sources of microbial contamination accurately (Holcomb and Stewart, 2020). Therefore, this study suggests advancing the field further by using current MST techniques as a rapid method to detect microbial contamination in a novel water matrix: treated drinking water.

This pilot study was developed in response to a recent and accidental water main break that occurred on a university campus in Ohio, United States. After the water disruption, the entire university campus was placed under a BWA; buildings were left with low water pressure, emergency services paused, classes were cancelled, food services were stopped, and hospital surgeries were postponed. The campus waited >24 hours until culture-based laboratory methodologies could confirm negative coliform tests from drinking water samples. Therefore, the purpose of this study was to develop a rapid-response method to target *E. coli* and MST gene markers (human-specific, universal fecal) in the aftermath of drinking water main breaks to evaluate the safety of water for minimizing the interrupted time. The first objective of this study was to develop a rapid emergency response method for quantifying the level of microbial contamination in drinking water using MST techniques and droplet digital PCR (ddPCR) with an efficient water concentrating method. The second objective of this study was to compare these rapid molecular-based results to traditional FIB culture-based techniques. As waterline breaks are expected to increase, this study emphasizes the critical need for developing rapid testing amidst drinking water emergencies to protect public health and resume daily functioning as quickly as possible. To our knowledge, this is the first proof-of-concept study on the application of highly specific and sensitive MST methodology in rapid drinking water testing as emergency preparedness.

Materials and methods

Water sample collection

On April 26, 2022, 16 hours after an Ohio university campus main water break, drinking water samples were collected across campus between 09:00 and 11:00 a.m. Usages from the public drinking water system includes consumption, recreation, irrigation, food preparation, and hygiene purposes. Seven sample sites (A-G) were selected to encompass central and boundaries of campus to reflect an accurate representation of drinking water contamination across the entire campus. At each sampling site, sink faucets were thoroughly flushed for 2-3 minutes until the water temperature stabilized and then grab samples (1L) were collected in sterile (Nalgene) bottles. Quenching of chlorine residuals was omitted due to similar fecal coliform levels in unchlorinated and chlorinated drinking water (Roberts et al., 2001). After collection, all samples were quickly transferred on ice to the Environmental Microbiology and One Health Laboratory (EMOHL), College of Public Health, The Ohio State University (Columbus, Ohio, United States). Samples were immediately processed following the two methodologies outlined below (Fig. 1).

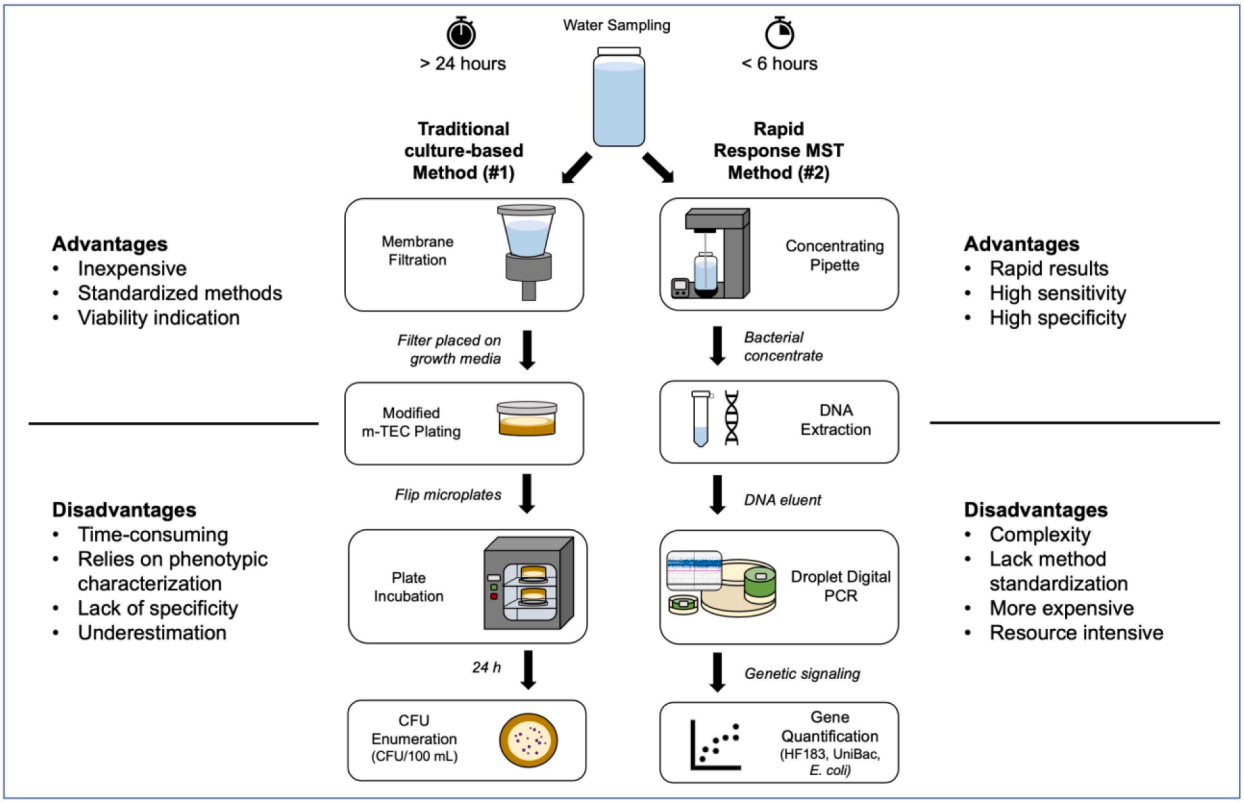


Fig. 1. The comparison of two methodologies used in this study.

Traditional culture-based method (#1)

Following the USEPA method 1603, membrane filtration was utilized to quantify the FIB, *E. coli*, across all locations (USEPA, 2009). *E. coli* analysis were conducted within 4 h of sample collection. For each site, 250 mL of undiluted water was filtered in duplicate through 0.45 μm sterile nitrocellulose membrane filters (Cat. No. HAWG047S6, Millipore Sigma, Burlington, MA, USA). This was repeated to produce four replicates for each site. Following filtration, sample filters were placed on modified m-TEC agar microplates (Difco, Detroit, MI, USA) and incubated in aerobic conditions for 2 hours at 35ºC and then 22 hours at 44.5ºC. After the 24-hour incubation period, the total number of colony forming units (CFU) were counted, and the results were enumerated as CFUs per 100 mL.

Rapid-response method (#2)

To evaluate the potential sources of fecal indicator bacteria, all samples across the 7 sites were processed for rapid and *Escherichia coli* (*E. coli*) and MST analyses of the following host markers, human-specific (HF183) and universal fecal (UniBac). For microbial filtration, each sample was processed in duplicate. 250 mL of water was concentrated using the Concentrating Pipette Select^{TM} (InnovaPrep, Drexel, MO, USA) with a 0.2 μm PS hollow fiber concentrating pipette tip (CC08022-10, InnovaPrep, Drexel, MO, USA). Notably, this concentrating step takes usually less than 3 minutes. 250 mL of sterile deionized water was also processed and concentrated in duplicate to generate a system control. Approximately 200-250 μL of concentrated bacterial eluent was obtained and stored in a sterile screw-cap microcentrifuge tube and stored at -20 °C until subsequent analysis within 1 week. Next, 200 μL of the bacterial eluent was input for DNA extraction using the DNEasy PowerSoil Pro Kit (Qiagen, Hilden, Germany) following manufacture protocol. The final DNA eluent produced was 100 μL per sample. A Quibit 3.0 fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) was used to measure quality and concentration of extracted DNA prior to further analyses. In order to identify and quantify the potential sources of drinking water contamination, MST methods were used to estimate fecal contamination from wastewater and other sources as a conservative measure for microbial contaminants. Droplet digital PCR was utilized for target gene quantification of UniBac (universal fecal contamination) and HF183 (human-specific fecal). UniBac and HF183 assays were modified from previous studies (Haugland et al., 2010; Kildare et al., 2007) and additional details can be found in our prior MST study, Lee et al., 2020. In this study, an additional ddPCR assay was developed to target the *E. coli* 23S rRNA gene based upon the Sivaganesan et al., 2019 EC23S857 qPCR assay, where primer and probe sequences can be found (Sivaganesan et al., 2019). Including the system controls, all three individual assays were conducted using the QX200 droplet digital PCR systems (Bio-Rad, Hercules, CA, USA). All reaction mixtures summated a total volume of 20 μL, containing 2 μL of DNA template, 10 μL ddPCR Supermix for Probe Mix (Cat No. 1863024, Bio-Rad, Hercules, CA, USA), 250 nM primers, 250 nM probe, and nuclease-free water. PCR reagents were mixed thoroughly, and the droplets were generated using the QX200 droplet generator (Bio-Rad, Hercules, CA, USA). Target genes were then amplified with a Bio-Rad C1000 Touch Thermal Cycler (Bio-Rad, Hercules, CA, USA) with the following conditions: initial cycle at 50°C for 2 min, 95°C for 10 min, 40 cycles of denaturation at 94°C for 30s, and finally, annealing the extraction under suitable conditions of reference conditions (Lee et al., 2020). Afterwards, target gene concentrations were determined using a QX200 droplet reader (Bio-Rad, Hercules, CA, USA) and QuantaSoft (V 1.7; Bio-Rad). The limit of quantification (LOQ) of all ddPCR reactions is 2 gene copies per reaction. For detected-but not-quantifiable measurements (DNQ), the results are recorded as one-half of the LOQ, thus the limit of detection (LOD) is 1 gene copy per reaction. For each ddPCR assay, two replicates were performed for each of the DNA eluent duplicates, producing a total of four replicates for each site. These total gene copy numbers were standardized via the system control to ensure the high accuracy and

sensitivity of all assays. Final total gene copy numbers were converted to total gene copies per 100 mL (GC/100 mL) and normalized to $\log_{10}$ gene copies per 100 mL ($\log_{10}$ GC/100 mL). Data calculations were conducted in Microsoft Excel (V. 16.64) and data analyses were generated in RStudio (V. 2022.07.0).

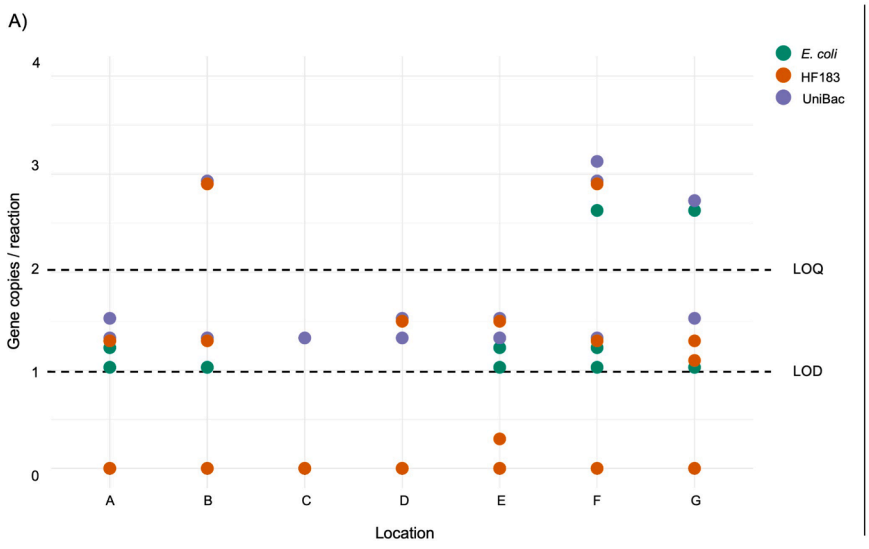
Results and discussion

Fecal indicator bacteria (E. coli)

For all seven sites using the traditional culture-based method, each replicate produced a zero-colony count (*E. coli* CFU/100 mL). In total, all 28 plates confirmed a negative detection of *E. coli*, indicating that these seven drinking water sites were not contaminated with the FIB. In comparison to the MST rapid molecular method, *E. coli* results show that the majority of the drinking water samples were negative for fecal contamination, with the exception of two replicates (Fig. 2). One sample replicate (1/4) for two sites (F and G) was detected above the LOQ (Fig. 3) and the remaining 26 sample replicates were either below the LOD or LOQ. Two sites (C and D) show no detection of *E. coli* contamination. The observed mean total *E. coli* concentration is 0.60 log GC/100 mL (Fig. 4). Notably, the quantifiable positive detection of *E. coli* in one replicate across two sites using rapid-ddPCR method contradicts the negative *E. coli* CFU/100 mL counts in the traditional culture-based method. This disagreement in results demonstrate potential *E. coli* contamination at these two sites. Discrepancy between traditional culture-based methods and molecular ddPCR methods for *E. coli* quantification may be attributed to sensitivity detection. The rapid-response molecular method has high sensitivity with the ability to detect very low levels of *E. coli* DNA, including non-viable cells, which may not be reflected in culture-based counts leading to an underestimation of *E. coli* CFU/100 mL. Notably, these results confirm that conventional culture-based techniques lack the speed, efficiency, and sensitivity required in emergency situations (Dorevitch et al., 2017). This proof-of-concept study confirms that the ddPCR rapid-response method produced highly sensitive results 18 hours faster than the traditional culture-based method. This rapid methodology can be effectively and quickly used for testing drinking water contamination after subsequent drinking water distribution system emergencies. This approach can be also adaptable to desirable stringency of water safety, making more conservative or less conservative, depending on the circumstances or the type of water. By drastically reducing the fecal microbial contamination testing time, this method ensures water quality and actively facilitates the protection of public health to resume speedier drinking water access to impacted communities.

Microbial source tracking

To further investigate this potential fecal contamination, additional microbial source tracking ddPCR assays were conducted. It is acknowledged that using a single indicator, such as *E. coli*, to evaluate microbial quality in environmental waters has limitations and may withhold potential health hazards (Aw and Rose, 2012; Lee et al., 2016). FIB do not identify the source of the contamination; thus, MST was utilized to determine potential fecal pollution sources. As previously mentioned, MST has predominantly been used for diverse environmental applications ranging from surface water bodies to food quality (González-Fernández et al., 2021; Gyawali and Hewitt, 2020; Kongprajug et al., 2019). Notably as far as we know, this is the first study to apply MST to a water main break situation. Two relevant fecal host markers, human fecal (HF183) and universal fecal (UniBac), were selected in addition to *E. coli* ddPCR to determine the potential fecal contamination source or any presence of fecal contamination within the drinking water samples. For all three assays (HF183, UniBac, and *E. coli*) using ddPCR, the gene copy numbers per reaction in relation to the limit of detection (LOD) and limit of quantification (LOQ) can be seen in Fig. 2. ddPCR quantification in log scale (gene copies/100 mL) by site location and assay type are summarized in Fig. 3. For all ddPCR assays, only 10 of the 84 replicate samples (11.90 %) were detected and quantifiable (Fig. 2). However, 30 of the samples (35.71 %) were detected but below the limit of quantification, with most samples (52.38 %) being below the limit of detection (Fig. 2). For the HF183 assay, only one sample replicate for two sites (B and F) was detected above the LOQ (Fig. 2). The remaining 26 sample replicates were either below the LOD or LOQ. Site C is the only location with zero detection of the HF183 gene marker. For the UniBac assay, six sample replicates (across 3 sites) were positively detected above the LOQ, while the remaining 22 sample replicates were below the LOQ. (Fig. 2). Unlike the other two assays, the UniBac gene was detected at one site (F) with all four replicates being above the LOD. In addition, the UniBac target gene was the only assay with all seven sites detecting at least one replicate (Fig. 3). Overall, UniBac detection was the most frequent with a mean concentration of



B)

Threshold	Total Samples (n = 84)	ddPCR Assay	Samples by ddPCR Assay
> LOQ	10	*E. coli*	2
		UniBac	6
		HF183	2
< LOQ	74	*E. coli*	26
		UniBac	22
		HF183	26
LOQ < LOD	30	*E. coli*	10
		UniBac	12
		HF183	8
> LOD	40	*E. coli*	12
		UniBac	18
		HF183	10
< LOD	44	*E. coli*	16
		UniBac	10
		HF183	18

* LOQ = 2 GC/reaction
* LOD = 1 GC/reaction

Fig. 2. A) *E. coli* and MST gene marker (HF183, UniBac) raw gene copies per reaction (GC/reaction) by ddPCR assay and site location. Limit of quantification (LOQ) and limit of detection (LOD) are shown via dotted lines. B) Table showing the precise number of replicate samples above, between, and below the LOQ and LOD for each assay.

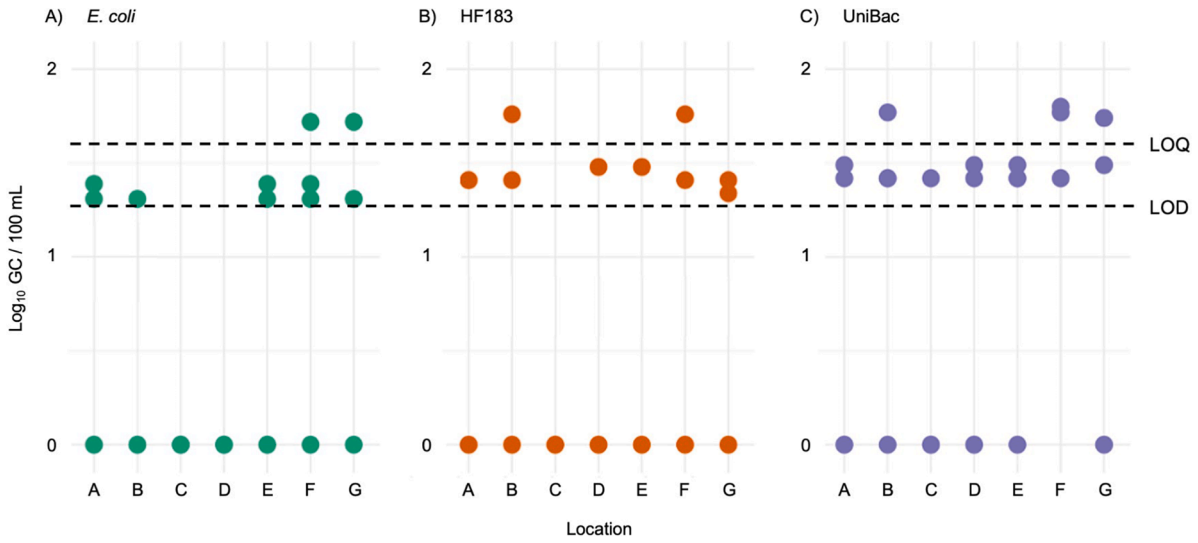


Fig. 3. *E. coli* and MST gene marker (UniBac, HF183) concentrations (log_{10} gene copies/100 mL) by ddPCR assay and site location. Limit of quantification (LOQ) and limit of detection (LOD) are shown via dotted lines.

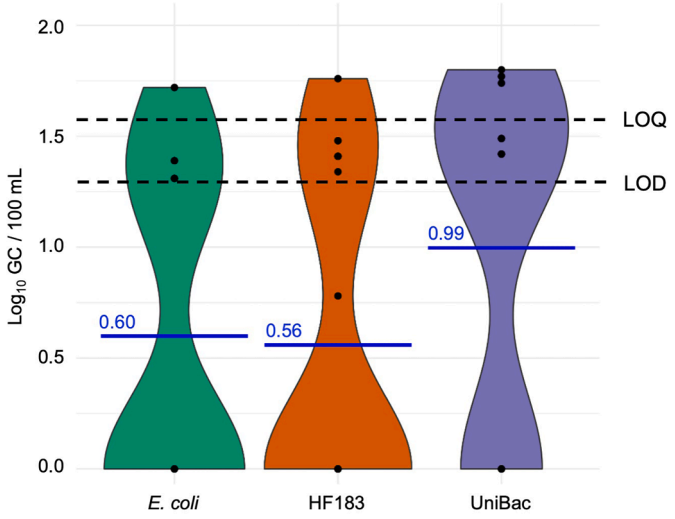


Fig. 4. *E. coli* and MST gene marker (HF183, UniBac) concentrations (log_{10} gene copies/100 mL) by ddPCR assay. Blue lines represent mean log_{10} GC/100 mL values. Limit of quantification (LOQ) and limit of detection (LOD) are shown via dotted lines.

log GC/100 mL of 0.99, *E. coli* mean log GC/100 mL of 0.60, and HF183 mean log GC/100 mL of 0.56 (Fig. 4).

As expected, results determined that UniBac has a higher gene concentration than HF183 which is congruent with previous environmental water studies in previous literature (Fig. 3) (Lee et al., 2020). UniBac is a comprehensive indicator of broader fecal contamination since it targets universal fecal *Bacteroidales* and HF183 targets a human-specific fecal bacterial marker (Kildare et al., 2007). For site F, three of the four replicates were positively detected and quantifiable for UniBac, indicating potential fecal contamination (Fig. 2). Similarly with the *E. coli* ddPCR results, one replicate (from two sites) detected and quantified the HF183 gene marker, indicating the site's drinking water is positive for human-fecal contamination. This fecal contamination may imply cross-contamination of a sanitary sewer or urban runoff infiltrating the drinking water main break (Steele et al., 2018). Overall, these quantifiable MST results demonstrate the potential fecal contamination of treated drinking water across three campus sites. Nonetheless, there are several challenges and limitations of this pilot study. It is acknowledged that the sample size is relatively small due to unforeseen study design restrictions, thus, future studies should include a larger sampling size when using these methodologies to evaluate water safety. Also, this pilot study omitted conducting more thorough statistical analyses since few samples (11.90 %) were above the LOQ for all MST assays and could not be analytically compared to the culture-based method, which produced zero *E. coli* counts. Likewise with molecular method techniques, the probability of PCR inhibition may be present in the water samples. While it was found that ddPCR assay is robust in PCR inhibition when tested with wastewater matrix in our previous study (data not published), future studies should ensure PCR inhibition is not producing inaccurate results when testing with quantitative PCR and follow DNA extraction and PCR reaction protocol to reduce inhibition in their assays. Future analyses must always include negative control samples to confirm no false positives within the sample matrix. Meanwhile, it is important to note that water samples were not collected prior to the accidental water break, therefore MST detection rate comparisons were not analyzed and fecal contamination is not guaranteed to be caused from the main water break. Future studies may include pre-break samples in addition to post-break sampling, though this may remain a limitation since the precise moment of water distribution system emergencies are unpredictable.

Remarkably, we found evidence of fecal pollution in treated drinking water following a watermain break. Result discrepancy between *E. coli* culture-based and molecular method indicates that that relying on one method of water quality assessment is not ideal for evaluating microbial fecal contamination in drinking water matrices. If rapid MST methods were not employed, culture-based techniques would fail to indicate fecal contamination following a water main break. In congruence with current literature, these significant results emphasize that future studies utilize a combination approach of traditional FIB methods with novel rapid MST techniques to provide a more comprehensive understanding of microbial water quality following drinking water emergencies (Basili et al., 2023; Pendergraph et al., 2021). Notably, this study design can be applied to additional water quality emergencies beyond drinking water main break scenarios. For example, wildfire intensity and frequency are increasing over time, especially in the Western region of the United States, which threaten the water quality of drinking water sources (Hohner et al., 2019). A recent case study reported widespread chemical contamination in a drinking water distribution system following wildfire incidents, which also suggests the simultaneous potential of microbial contamination (Proctor et al., 2020). Similar MST PCR methods used in this study can also be applied to wildfire or other disaster-related

emergencies to quickly and effectively evaluate the microbial water quality of impacted communities. In addition, there is a wide range of other MST genetic markers (human-, animal-, or sewage-specific) beyond UniBac and HF183 that can be used to detect suspected fecal sources in drinking water as needed (Chase et al., 2012; Mathai et al., 2020). These results, again, highlight that future studies should utilize a range of suspected MST markers with *E. coli* as broad indicators of drinking water quality rather than relying on one single indicator.

Conclusion

To the authors' knowledge, this is the first pilot study to utilize MST methods to quantitively identify potential fecal sources of contamination in treated drinking water, rather than drinking water sources. Results highlight that a rapid molecular MST technique effectively detected fecal contamination and its source in drinking water that traditional culture-based methods failed to detect. This proof-of-concept study highlights that MST via rapid ddPCR can be used to quickly assist public health officials in evaluating drinking water quality before returning to normalcy. Most importantly, these findings encourage the integration of both culture-based methods with molecular methods to enhance our understanding and efficiency of microbial risks in drinking water. This study confirms the emerging significance of using human-specific MST markers for drinking water quality management (García-Aljaro et al., 2019). As molecular methods become more widely used and reasonably priced, it may be more ideal to evaluate main break water safety with MST PCR methods rather than solely relying on a culture-based method. Detecting universal MST (e.g. UniBac) or equivalent would be a more conservative way to assess water safety rather than targeting human-specific MST or *E. coli*. Most notably unlike traditional FIB methods, this study's rapid-response methodology produces highly sensitive and quick results in less than 6 hours while also determining the source of fecal contamination. This study aids in protecting public health from a variety of potential contaminants and works to swiftly identify pollution sources for effective control strategies. Overall, this study confirms that MST fecal detection methods are instrumental in addressing water quality concerns and protecting public health by providing accurate and timely information about the sources and levels of contamination across diverse water matrices, including drinking water.

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CRediT authorship contribution statement

Emma Lancaster: Methodology, Investigation, Formal analysis, Visualization, Writing – original draft, Visualization. **Jiyoung Lee:** Conceptualization, Methodology, Resources, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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