Detection and occurrence of indicator organisms and pathogens

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Abstract
This review paper focuses on detection and quantification techniques of indicator organisms that can be used for water quality assessment. The environmental pathogens that are critical to understand and better evaluate water quality are also discussed in this paper. Several recent studies using culture-independent methods such as microbial source tracking, pulsed field gel electrophoresis, mitochondrial DNA, and next generation sequencing to assess various environmental samples and water bodies have been reviewed.

Practitioner points
- Various waterborne pathogens and cases of outbreak occurrences due to presence of pathogens are studied in this review paper.
- Recent studies for detecting major indicator organisms to evaluate the presence of pathogens in water bodies are reviewed.
- Culture-independent techniques as robust tools to detect and quantify waterborne pathogens are discussed in this review paper.

Key words
environmental health; indicator organisms; microbial source tracking; outbreak; pathogen

Pathogens
Human exposure to contaminated water environment for drinking, recreational as well as agricultural purposes such as crop irrigation and food processing can result in water-related disease. Waterborne pathogens and related diseases are a major public health concern. In addition to mortality related to waterborne diseases due to pathogens, their prevention and treatment cause a considerable cost to the community. Despite state-of-art technologies in water treatment processes, waterborne outbreaks are still a critical challenge globally. Therefore, proper detection of pathogens in water bodies and appropriate monitoring of water quality play key roles in decision-making regarding water supply, water treatment processes, and distribution systems' infrastructure.

Although most bacterial species are nonpathogen and even beneficial to environmental processes, there are about hundred bacterial species that cause infectious diseases in human. Pathogenic bacteria are capable of causing disease when enter into the body and can spread through water, air, soil, and physical contact. Although many bacterial pathogens causing diseases such as tuberculosis, typhoid, cholera, and dysentery have been controlled, many new bacterial pathogens have been recognized and many multidrug-resistant bacterial pathogens such as Staphylococcus aureus, Klebsiella pneumonia, and Mycobacterium tuberculosis have been reemerged. Waterborne bacterial pathogens of primary concern include species of Salmonella, Shigella, Clostridium, Legionella, Yersinia, and Mycobacterium families.

Viruses are also a major cause of water-related diseases. The most common viral pathogens causing gastrointestinal illnesses are rotavirus and norovirus (Scallan et
al., 2011). However, a wide variety of viruses may be found in human sewage. They can be categorized into single-strand RNA (ssRNA) such as enterovirus, hepatovirus, norovirus, sapovirus, and coronavirus; single-strand DNA (ssDNA) such as parvovirus and circovirus; and double-strand DNA (dsDNA) such as mastadenovirus and polyomavirus.

In addition to bacterial and viral pathogens, protozoan parasites have been one of the most frequently identified microbial agents involved in waterborne disease outbreak. According to Water Health Organization (WHO) report, waterborne parasitic protozoa are one of the main reasons for four billion cases of diarrhea, causing more than 1.6 million deaths each year. *Giardia* spp. and *Cryptosporidium* spp. are two main protozoan species contributing to waterborne infections causing diarrhea. Other parasitic protozoa transmitted through contaminated water that cause human infections are *Sarcocystis* spp., *Balantidium coli*, *Entamoeba histolytica*, and *Plasmodium* spp. causing malaria disease.

**Indicator Organisms**

Indicator organisms are microorganisms such as bacteria and viruses in water bodies, which are utilized as a surrogate to evaluate the presence of pathogens in that environment. These microorganisms are preferred to be nonpathogen, have no or minimal growth in water, and reliably detectable at low concentrations. The indicator organisms should be present in greater populations than the associated pathogen and ideally, they have similar survival rates compared to the pathogen. As discussed in this section, there are various indicator organisms that can be used in water quality monitoring, and the efficiency in predicting pathogens depends on their detection limit, their resistance to environmental stresses and other contaminations.

**Fecal Coliform**

Culturable fecal indicator bacteria (FIB) such as *Escherichia coli* (*E. coli*) is an indicator of the presence of fecal material from warm-blooded animals and can be used as a microbial surrogate for water quality monitoring. These bacterial species are native microflora colonizing in the intestinal tract of warm-blooded animals, and their existence in fresh and marine waters corresponds to the presence of bacterial pathogens (Meals, Harcum, & Dressing, 2013). Total coliform, fecal coliform, *E. coli*, and *Enterococcus* are four assessors of fecal contamination that have been developed. However, *E. coli* is considered a more specific fecal indicator bacterium than total and fecal coliforms as the test for total and fecal coliform can also detect thermotolerant nonfecal coliform bacteria (Francy, Myers, & Metzker, 1993).

**Fecal Streptococci**

Fecal *streptococci* species is subgroup of the genus *Streptococcus* and comprises *Streptococcus faecalis*, *Streptococcus bovis*, *Streptococcus equinus*, and *Streptococcus avium*. There are significant number of these species inhabiting in the intestinal tract of human and other warm-blooded animals and can be used as an indicator organism. Fecal *streptococci* species do not grow and reproduce in water and other environmental systems, but they can survive for a long time, and therefore, they are considered as an indicator organism. In a case study conducted by Elkayam et al. (2017), *streptococci* along with other microbial indicators were used to evaluate the effectiveness of soil aquifer treatment in removing microbial contaminations. In another study, *streptococci* and other indicator bacteria, including lactobacilli and coliforms were quantified to assess the effect of anaerobic digestion on indicator microorganisms in swine and dairy manure (Costa et al., 2017).

**Bacteriophages**

Bacteriophages are viruses that infect bacteria and can be used as an alternative indicator organism due to their specificity to their host infection. Coliphages are viruses infecting *E. coli* as their host are proposed to serve as an efficient surrogate for water quality assessment. Somatic coliphage (*E. coli* CN-13 as host) and F-specific coliphage (*E. coli* F-amp as host) are two of the indicator organisms proposed by EPA.

Moreover, World Health Organization (WHO) has recognized somatic coliphages, male-specific RNA coliphages (F-RNA phages), and *Bacteroides*’ phages as indicators of water quality. Somatic phage group contains a big family of lytic phages with single or double-stranded DNA genomes (Hodgson, Torok, & Turnbull, 2017). These bacteriophages attach the coliform cell wall as the specific host, which may infect warm-blooded animals and duplicate in the gastrointestinal tract. Similar to the somatic coliphages, both F-RNA and *Bacteroides* phages replicate in the gastrointestinal tract of mammals. However, *Bacteroides* only survive and reproduce under anaerobic conditions with specific nutritional requirements in the gastrointestinal tract. Besides, F-RNA and *Bacteroides* have other different features. For instance, F-RNA phages are single-stranded RNA (ssRNA), while *Bacteroides* phages contain double-stranded DNA (dsDNA) genomes with long contractile tails. In general, F-RNA phages, also known as F-specific phages, are considered as effective indicators of enteric virus in most environments and treatment processes. Comparing somatic coliphage with F-specific coliphage, Jofre, Lucena, Blanch, and Muniesa (2016) studied various perspectives, including their population in different pollution sources, treatment methods, and persistence in different environments. Under most of the circumstances, somatic phages outnumbered the F-specific phages. Jofre and colleagues detected about $5 \times 10^6$ to $10^7$ plaque-forming units (PFUs) per 100 ml of somatic phages in the raw municipal wastewater, while F-specific phages were significantly lower with $10^3$ to $10^5$ PFUs per 100 ml. Furthermore, somatic phages were reported predominant in both surface water and groundwater. Besides, after the secondary treatment, both bacteriophages could be eliminated by 50%–99.9%, with $10^3$ to $10^5$ PFUs per 100 ml of somatic phages and $10^5$ to $5 \times 10^5$ PFUs per 100 ml of F-specific phages remaining in secondary effluent.

The relationships between coliphages and human viruses as well as its relationship to health risk were assessed in several
studies with inconsistent results. Some researchers (Jofre et al., 2016) considered coliphages correlated with human viruses and health risk, while others (McMinn, Ashbolt, & Korajkic, 2017) declined the statement. McMinn et al. (2017) considered Enterococci phages as potential fecal indicators, surviving in both fresh and marine water in tropical and subtropical regions. For Enterococci phages, both somatic phages and F-specific phages were detected in freshwater and marine water. Notably, somatic phages significantly outnumbered the F-specific phages. However, further investigation is essential to define Enterococci phages as indicator of fecal pollution due to the limited studied regions. They also indicated that somatic phages and F-specific phages had the highest concentrations than any other bacteriophages, such as Bacteroides and Enterococci phages. Moreover, they determined the removal rate in the wastewater treatment process and showed somatic phages, F-specific coliphages, and Bacteroides phages had similar reduction, while fecal coliform and E. coli as fecal indicator bacteria (FIB) showed significantly higher reduction than bacteriophages. Therefore, it is concluded that bacteriophages can act as a robust surrogate for evaluating human viral pathogen removal via wastewater treatment. Dias, Ebdon, and Taylor (2018) detected FIB, bacteriophages (somatic, F-RNA, and B. fragilis), and viral pathogens in activated sludge and trickling filter systems, and they also concluded that FIB was removed more efficiently than phages but noticed that FIB was less effective to indicate the removal of viral pathogens in the systems than phages. However, no correlation was found between indicator organisms and viral pathogens.

**F + Coliphage**

F + coliphage is another new approach to detect and quantify pathogens and fecal pollutions in water bodies. Griffith et al. (2016) developed a regression model between F + coliphage, measured by EPA method, and gastrointestinal illness among swimmers in three California beaches. Comparing with Enterococcus population, F + coliphage was discovered to be better associated with gastrointestinal illness. Further, it was shown that F + RNA coliphage Genotype II was significantly related to gastrointestinal illness in one of the water bodies. Yamahara, Sassoubre, Goodwin, and Boehm (2012) evaluated the decay rate of F + coliphage, which indicated relatively higher than FIB or other pathogen indicators. They investigated the occurrence and persistence of FIB and pathogens from 53 California beaches by using qPCR as a culture-independent method. From the collections of dry sand samples, F + coliphages showed a first-order decay rate of 0.42/day, while other FIB and pathogens indicator resulted in much lower decay rate. Vijayavel et al. (2014) focused on monitoring the quality of recreation water and wastewater in Great Lakes region by comparing new Enterococcus host strains with F + coliphage and concluded that their new designed Enterococcus host strains (ENT-49 and ENT-55) have similar detection level compared to F + coliphage. In general, F + coliphage and Enterococcus were present at higher concentrations in wastewater, while low at rivers and harbors.

In another study by Lee, Lee, Cho, Hur, and Ko (2011), F + coliphage was detected in 71 groundwater and five surface water samples in Korea. While these samples were associated with chicken and human feces contamination in groundwater, the surface water contained F + coliphage correlating with cows. According to these case studies, F + coliphage could be applied either independently or along with other fecal indicator bacteria to detect fecal pollutions.

**Occurrence of Waterborne Pathogens**

Waterborne pathogens are introduced to water bodies from various means including inefficient treatment processes, defective water distribution system, and water contamination with sewage spill. A case study in 2015 reported that 188 patients experienced acute gastrointestinal symptoms after school camp in Korea, wherein these symptoms were caused by diarrheagenic E. coli (DEC) in drinking water. After investigation, the research team indicated that damaged pipeline and inefficient water purification system for bacteria and virus were two primary reasons (Park et al., 2018). Another outbreak of norovirus gastroenteritis in Italy was associated with improper drinking water system caused by an old chlorination device and close proximity to suspected illegal sewage dumping (Giammanco et al., 2018). In another recent study conducted by McClung et al. (2018), outbreaks associated with environmental as well as undetermined exposures to water were studied. They detected that legionella and Giardia were primary involved in human-made system and natural water system, respectively and have suggested a water management program on controlling legionella in human-made system and proper treatment processes must be applied to the raw water to prevent Giardia.

Water recycling and reused water in direct and indirect potable and nonpotable applications continue to expand over the last few years across the United States and globally due to limited access to freshwater. However, it is very critical to ensure the water quality in the reclaimed water. In the last few years, some of the research studies have focused on microbial quality characterization and detection of pathogens in reclaimed water. Zhu et al. (2019) investigated the fecal indicators and pathogenic bacteria in both reclaimed water and return flow (i.e., surface and subsurface water that leaves the field following the application of irrigation water) and detected E. coli, total coliform and enterococci as the fecal indicator in the reclaimed water. On the other hand, irrigation return flows contained a significant amount of bacterial indicators, which were significantly higher than EPA criteria measured at 126 colony-forming unit (CFU)/100 ml. In addition, Salmonella as the pathogenic bacteria was found in the return flows with the highest 4.6 most probable number (MPN)/L. However, they did not find a significant relationship between the fecal indicator and Salmonella concentration.

Garner et al. (2018) detected and compared five opportunistic pathogens including Acanthamoeba spp., Legionella spp., Mycobacterium spp., Naegleria fowleri, and Pseudomonas aeruginosa in both reclaimed (nonpotable
Mycobacterium spp. and Legionella infection could not inhibit the growth of species as well as interacting with their concentrations in the influent, while UV disinfection was observed an increasing population in the effluent, compared to reclaimed water systems. In fact, the cluster of species was reported to survive at 70°C temperature, low-temperature environment is more ideal for them to grow. According to a survey of 40 cooling towers in Japan, 73% of the towers contained Legionella spp. while fewer abundance of Legionella spp. was reported to survive at 70°C in the water heaters. Furthermore, Kulkarni et al. (2017) analyzed water samples by exploring total bacterial community in the water heaters. Furthermore, Kulkarni et al. (2017) analyzed water samples by exploring total bacterial community from conventional wastewater treatment plants, spray irrigation site with UV and open-air storage treatment. This study concluded that opportunistic pathogens such as Legionella and Mycobacterium were not able to be removed and regrew in reclaimed water systems. In fact, the cluster of Legionella spp. was observed an increasing population in the effluent, comparing with their concentrations in the influent, while UV disinfection could not inhibit the growth of Legionella spp. as well as Mycobacterium spp.

**Detection and Quantification Techniques of Waterborne Pathogens**

Culture-dependent methods such as heterotrophic plate count (HPC) for detection and quantification of waterborne pathogens is costly and time-consuming. In addition, the laboratory procedures in these methods require intensive training for an efficient quantification. Furthermore, there are a vast majority of pathogens that exist in a viable but nonculturable (VBNC) state, which culture-dependent methods may yield false-negative results. Therefore, in this review, we have only discussed culture-independent methods which are increasingly developing in the last few years. The culture-independent methods are typically based on the detection and quantification of specific segments of the DNA or RNA in the waterborne pathogen’s genome. Further, there are several groups of fecal aerobic and anaerobic bacteria that are proper candidates as alternative indicators for assessing water quality, which are discussed in this section.

**Microbial Source Tracking (MST)**

Microbial source tracking is a technique to identify the sources of fecal indicator bacterial contamination. Fecal contamination is not limited to sewage, and it can be caused by surface runoff from agricultural activities, cattle feedlots, domestic animals, and fecal discharge by wildlife. Microbial source tracking can contribute to classify microorganisms in the water bodies based on their phenotypic or genotypic fingerprints and unlike fecal indicator bacteria; it can discriminate between different sources of fecal contamination. Microbial source tracking can incorporate genetic sequences (called as markers) unique to specific bacterial or viral species from specific animal hosts, which conveys insight into sources of fecal contamination.

In a study conducted by Bradshaw et al. (2016), waterborne pathogens were detected in water and stream-bed sediments via a statistical model, which composed of FIB and MST markers. They applied culture-based methods and qPCR assays for FIB quantification. In addition, they were able to detect four primary pathogens, including Campylobacter spp., Listeria spp., Salmonella spp., and Shiga toxin-producing *E. coli* in the water samples. Although, both FIB and MST markers were related to pathogens recovered from the water, their study indicated that FIB could not independently predict the waterborne pathogens. In another study conducted by Li et al. (2015), custom MST microarray was more effective than qPCR and culture-based methods. They designed an MST microarray to detect pathogens and fecal contamination in the diverse environmental samples, including wastewater, cattle manure, swine manure, and poultry litter. Meanwhile, they compared their microarray with other methods, such as qPCR, culture-based methods, and next-generation sequencing (NGS). In addition to detection of waterborne pathogens with the microarray, it was shown that the microarray correlated with qPCR and culture-based methods. Microarray and qPCR had relatively high correlations in detecting *Enterococcus* spp. and *Bacteroidales* with extremely low false-positive results.

Although phenotypic methods such as antibiotic resistance analysis (ARA) and carbon utilization profiles (CUP) have shown some potentials (Moussa & Massengale, 2008; Schwaiger, Schmied, & Bauer, 2010) as a microbial source tracking approach, more studies have implemented genotypic analysis. In the recent years, several DNA fingerprinting approaches have been used for MST, including pulsed-field gel electrophoresis (PFGE), mitochondrial DNA, and next-generation sequencing (NGS), which are discussed in the following section.

**Pulsed-Field Gel Electrophoresis (PFGE)**

Pulsed-field gel electrophoresis (PFGE) is an alternative method to detect waterborne pathogens. The principle of PFGE is to analyze the DNA fingerprinting or genetics of the samples and then to isolate them based on the protocol of Centers for Disease Control and Prevention (CDC). Previous studies have shown the effectiveness of PFGE in detecting pathogens such as *Salmonella* and *Listeria*. Donado-Godoy et al. (2015) applied PFGE and detected *Salmonella Paratyphi* and *Salmonella Heidelberg* from poultry farms, fecal samples, and retail chicken meat in Colombia. Total 82 *Salmonella Paratyphi* and 21 *Salmonella Heidelberg* patterns
were isolated, and both species resulted in over 85% similarity by comparing genotypes. However, they noticed that the capability of PFGE was decreasing to some serotypes such as Salmonella enteritidis. Hence, they suggested using other techniques including multilocus sequence typing (MLST), mutiple-locus variable-number tandem repeat analyses (MLVA), and repetitive element palindromic PCR (rep-PCR), along with PFGE can result in more reliable outcome. In two recent studies, PFGE technique has been combined with MLST (Kleta et al., 2017) and MLVA (Sharapov et al., 2016) to detect Listeria monocytogenes and E. coli O157:H7, respectively.

Mitochondrial DNA

Mitochondrial DNA represents very small fraction of an organism’s genome, but it can be used as a marker to track fecal contamination in the water. Mitochondrial DNA appears in multiple copies in the cell, and therefore, it is relatively easy to amplify and quantify. He et al. (2016) evaluated five microbial DNA (mDNA) and four mitochondrial DNA (mtDNA) markers from human and pig via polymerase chain reaction (PCR) method. In this study, sensitivities, specificities, concentrations, and decay rates of mDNA and mtDNA were evaluated and it showed that majority of mDNA had lower sensitivities than mtDNA. In addition, mtDNA was shown to have relatively higher specificities (98%) compared to mDNA specificities (92%). Furthermore, the decay rate of DNA markers was evaluated in various temperatures, which showed a significant increase in the decay rate in higher temperature. In another study, Villelmer, Imbeau, Vuong, Masson, and Payment (2015) extracted DNA from surface water samples of 82 watersheds to evaluate human, bovine and porcine mtDNA markers as well as Bacteroidales HF183 marker via PCR method. Based on their results, it was suggested that mitoHu (human mitochondrial DNA marker) and HF183 can be applied in source tracking. In another study, Pasha et al. (2018) also concluded that HF183 was correlated with coliforms with correlation coefficient of 0.79. They obtained water samples from a wastewater treatment plant in South Texas, and used BacHum and E. coli as fecal source tracking, along with human mtDNA as a direct marker. It was indicated that BacHum had correlations with coliforms at a correlation coefficient of 0.76. In addition to application of mtDNA as microbial source tracking marker in surface water and wastewater, mtDNA has indicated a potential as a microbial marker to detect fecal contaminations in stormwater or heavy rainfalls. Waso, Khan, and Khan (2018) successfully designed a novel mtDNA to target mtDNA Cytochrome b gene via mismatch amplification mutation assay (MAMA) with PCR that detected pigeon fecal contamination in the harvested rainfalls. The high host sensitivity (100%) and host specificity (96%) was obtained after screening all the fecal samples, which had an excellent performance based on the criteria of EPA. For newly designed MST markers, the standard established by EPA for host sensitivity and host specificity is 80%. In addition, they analyzed the concurrence of pigeon mtDNA marker with other indicator organisms, including heterotrophic bacteria, E. coli, total coliforms, and fecal coliforms. Their results demonstrated that pigeon mtDNA marker in this study could be applied as a supplemental tool along with other indicators due to relatively high concurrence frequency.

Bacteroides spp.

Previous studies have provided evidence that Bacteroidales group has a high degree of host specificity (Bernhard & Field, 2000), and therefore, this genus can be used as a microbial indicator. Development of PCR assays of 16S rRNA genes in Bacteroidales has proved that the V2 region can be used to differentiate Bacteroidales from different hosts (Mieszkin, Yala, Joubrel, & Gourmelon, 2010; Okabe, Okayama, Savichtcheva, & Ito, 2007). In a study by McLellan and Eren (2014), HF183 primer and ruminant markers indicated that Bacteroidales are present in ruminant hosts and other species. However, comparing Bacteroidales host genetic markers illustrated that human-specific markers were less stable than ruminant markers. In another study conducted by Kirs et al. (2016), Bacteroidales spp. (HF183 TaqMan) markers as the indicators were used to determine sewage contamination in Hawaii. They concluded that concentration of HF183 in human fecal samples was 1,000-fold more than the animal fecal samples. Also, Kirs and colleague compared the decay rate of HF183 with enterococci under sunlight and dark conditions and showed that the decay rate of HF183 was significantly lower than enterococci in sunlight, while the decay rate was very similar in the dark. Moreover, the sensitivity (80%) and specificity (78%) were detected relatively high for human samples, and therefore, Bacteroides can be applied to identify the origin of fecal pollution and waterborne pathogens. Marti et al. (2013) studied the relations among MST marker (Bacteroides), fecal bacteria indicators (FIB), and pathogens. They concluded that presence and absence of the Bacteroides marker were not correlated to FIB, but positively related to pathogens such as Cryptosporidium and Giardia. In another study conducted by Newton, VandeWalle, Borchardt, Gorelick, and McLellan (2011), Bacteroides as MST marker was used to evaluate chronic human sewage contamination in urban harbor. Their experimental results indicated that total Bacteroidales spp. were abundant in human fecal samples, which illustrated strong correlations with human fecal contamination. Besides, Bacteroidales spp. was detected frequently during combined-sewer overflow periods comparing with dry seasons.

Lachnospiraceae

Lachnospiraceae is another group of fecal anaerobic bacteria that occurs especially abundant in human sewage and has been explored as a microbial indicator for assessing water quality. McLellan and Eren (2014) investigated human fecal pollution indicators and showed over 75% of the fecal microbial community was associated with Lachnospiraceae. The comparison of sequence analysis of sewage samples in a study by Newton et al. (2011) resulted a human-associated phylotype within Lachnospiraceae family. This phylotype, termed as Lachno2, closely related to Blautia genus was found to be the second most abundant fecal bacterial phylotype and was
involved to assess chronic human sewage contamination in urban harbor. Close relationship between Bacteroides and Lachnno2 confirmed the feasibility of using this indicator as an excellent candidate for host-associated fecal indicator alternative to Bacteroides for determining human fecal pollution in surface water. In a recent study by Feng, Bootsma, and McLellan (2018), designated genetic markers, termed as Lachnno3 and Lachnno12, which dominate in V6 region, were evaluated to detect human and animal fecal pollution in urban water. These assays were assessed with fecal samples of cat, dog, pig, cow, and deer. Although both Lachnno3 and Lachnno12 markers were primary human-associated, Lachnno12 marker was detected at low levels in samples from dogs and cows, while Lachnno3 marker was detected only in human fecal contamination or sewage. It is suggested that combination of multiple complimentary markers such as Lachnno3 with lachnno12 or HF183 might improve identification of human fecal pollution sources.

**Next-Generation Sequencing (NGS)**

Next-generation sequencing (NGS) is characterized as a novel approach to detect pathogen indicator individually or along with FIB and pathogens in detection of human sewage and drinking water quality. Sequencing various regions in small subunit (SSU) rRNA as one of the major components in the microbial communities identifies signature microbial species that can serve as indicators for fecal or other pathogenic contamination, and therefore, NGS can provide high-quality screen for sequences to evaluate human fecal and sewage pollutions. In a study conducted by Tan et al. (2015), V1 and V2 hypervariable regions of SSU rRNA were sequenced to detect pathogens in urban and agricultural watersheds along Santa Ana River, CA. In another study, Shrestha et al. (2017) used 16S rRNA gene NGS and detected Acinetobacter, Arcobacter, and Clostridium as possible pathogenic bacteria. As the same sample did not show any fecal indicator bacteria, they suggested the limitation of using only fecal indicator bacteria in evaluating water quality contaminated with pathogens. The pathogen diversity was evaluated by amplicon NGS in a study conducted by Cui, Fang, Huang, Dong, and Wang (2017). Genomic DNA from various samples was sequenced targeting the V4 region of the 16S rRNA gene, and pathogenic species were identified by comparing the sequences with a reference database of human pathogenic bacteria.

**Conclusion**

Water contaminated with pathogens can result in water-related disease, which is considered a major public health concern. Therefore, detection and quantification of these pathogens in water bodies have become critical portions of water quality assessment. Microbial indicators have shown correlation with pathogens, and hence, they can be used as a surrogate to evaluate microbial quality in water bodies. However, many pathogens may not behave like fecal microbial indicators and no indicator has been developed for their presence. As database for these pathogens grows in future years, we may find microbial indicators specific to those pathogens of public health importance. Until then, combining various microbial indicators from different taxonomic groups may be required for better quantification and detecting co-occurrence of the pathogens.

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