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## MICROBIAL SOURCE TRACKING: AN EMERGING TECHNOLOGY FOR MICROBIAL WATER QUALITY ASSESSMENT: A REVIEW

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### Abstract

*Microbial Source Tracking is a scientific approach that primarily aims to identify the sources of faecal contamination in water bodies. Microbial Source Tracking (MST) is a set of techniques employed to identify the origins of faecal contamination in water. The capacity to track faecal bacteria to their source is a crucial aspect of both public health and water quality management. The utilisation of information obtained from the method of MST would provide water quality managers with an enhanced comprehension of the origins of contamination, thus facilitating the implementation of remedial measures to impede transmission. Numerous studies have demonstrated that the gut-associated bacteria of the order Bacteroidales, specifically the Bacteroides genus, has a tendency to undergo co-evolution with the host, making it a highly viable option for MST applications. However, it is noteworthy that MST is also facilitating the scientific community with effective techniques for tracing faecal bacteria and pathogens in water sources. The methodologies utilised in MST are frequently categorised as either Library-Dependent Methods (LDMs) or Library-Independent Methods (LIMs). Microbial source tracking has been employed for diverse objectives, such as ensuring adherence to regulations, remediating pollution, and evaluating risk. The implementation of MST is expected to mitigate the prevalence of waterborne illnesses resulting from contamination. The implementation of MST has facilitated the ability to anticipate the probable origins of faecal contamination and the associated health hazards linked to compromised water systems. In addition to conventional faecal indicators, these methodologies are suggested as means to furnish supplementary insights into the origins of pollution, as well as the ecological and public health ramifications of animal-derived water contamination.*

**Key words:** Bacteriodes, Library-dependent, Library-independent, Microbial source tracking, Water quality.

### INTRODUCTION

Clean water is essential for human health and economic growth. Waterborne infections have plagued humanity for centuries, especially when transmission routes are unknown (Whitley *et al.*, 2019). Hlavsa *et al.* (2015) documented many drinking water and recreational waterborne illness outbreaks. Screening drinking water for dangerous microorganisms such bacteria, protozoa, and viruses reduces health risks. Polluted water usually contains many pathogenic microbes (Harwood *et al.*, 2014). Faecal indicator bacteria (FIB) in water bodies, such as *E. coli*, *Enterococcus spp.*, *Clostridium perfringens* spores, and coliphages, may indicate pathogens (Nappier *et al.*, 2019). Due to their simplicity of culture, simple enumeration, cost-effectiveness, and non-

pathogenicity, faecal indicator bacteria are important in pathogen detection. Campos *et al.* (2015) and Nappier *et al.* (2019) found that FIB outnumber germs in human and animal faeces. Identifying faecal indicator bacteria (FIB) in water quality monitoring provides advantages over identifying enteric pathogens, although it is more difficult. Korajkic *et al.* (2019) found that faecal indicator bacteria persist differently in various faeces. Zhang *et al.* (2015; 2019) stated that these microbes may survive in soils, sands, sediments, and aquatic vegetation. Faecal indicator bacteria (FIB) may be unreliable indicators of faecal contamination and pathogen prevalence if they proliferate or persist without faeces. Thus, many studies have found poor relationships between FIB and bacteria in sewage, recreational water, drinking water

reservoirs, stormwater (runoff), and groundwater (Zhang *et al.*, 2019).

Faecal indicator bacteria (FIB) monitoring cannot identify polluted sources. Without this knowledge, pathogen contributions and dangers vary, making it difficult to execute effective best management practises (BMPs) and remediation activities (Shenhav *et al.* 2019).

Water quality management uses microbial source tracing (MST) to find faeces sources. MST tools for various targets and waterbodies have been created and applied recently (Nguyen *et al.*, 2018; Steele *et al.*, 2018). Microbial source tracking (MST) may identify faeces from humans, cattle, pigs, dogs, chickens, seagulls, possums, and other species. Green *et al.* (2014) and Stange and Tiehm (2020) showed that MST analysis using host-associated marker genes achieves this. Marker gene(s) analysis can accurately identify the source of faecal contamination in environmental water samples (Stange and Tiehm, 2020). Schramm *et al.* (2020) state that MST technologies have improved our ability to protect watersheds, particularly environmental water, and facilitate total maximum daily load (TMDLs). Due to pathogen concentration and type, faeces contamination's health risk varies (Hart, 2023). The association between contaminating sources, particularly MST data, and potential human health threats must be verified. Boehm *et al.* (2015) offer this validation process to confirm the theoretical potential of such a relationship. The above data helps regulatory bodies contextualise microbial source monitoring results in connection to human health hazards and develop suitable mitigation actions (Hughes *et al.*, 2017). This review will give an overview of the microbial source tracking methods that are currently used to predict and identify sources of faecal pollution in water. It will also give an idea of where the field is headed in the future.

#### **Microbial Source Tracking Methods**

Microbial source tracking (MST) refers to a set of techniques employed to distinguish between human and non-human origins of faecal pollution (Steele *et al.*, 2018). The techniques are classified as library dependent and library independent.

#### **Library dependent**

Library-dependent identification of microbes isolated from diverse water samples and faecal sources. The identification process involves a comparative analysis of the isolated strains with a pre-existing library of bacterial strains from known faecal sources. The utilisation of library dependent techniques necessitates the creation of biochemical (phenotypic) or molecular (genotypic) fingerprints for bacterial strains that

have been obtained from suspected faecal origins. The classification of fingerprints is accomplished by means of comparison to established libraries, as described by Knapp *et al.* (2020). The methodology of utilising faecal bacteria to identify the origin of faecal contamination is grounded on the premise that particular strains of faecal bacteria are linked with distinct host animals, and that the differentiation of strains from diverse host animals can be accomplished through the identification of phenotypic or genotypic markers (Yuknavage *et al.*, 2018). Procedures that rely on libraries tend to be costlier and more time-consuming, requiring the involvement of skilled personnel to conduct the analysis, owing to the time-intensive nature of library development. Moreover, a significant limitation of methodologies reliant on libraries is their tendency to exhibit temporal and geographic specificity, rendering them less universally applicable. Although this approach may have practical implications for a particular geographical area, its applicability on a broader watershed level or in addressing statewide issues is generally limited, as noted by Nimer *et al.* (2018).

#### **Library independent**

Library independent methods relies on the identification of a distinct genetic marker or gene target that is associated with the host, which is detected in the molecular material obtained from a water sample. The aforementioned techniques can facilitate the detection of bacterial sources by leveraging a discernible host-specific attribute (genetic marker) without resorting to or relying on a "library." A commonly employed method that is not reliant on library usage involves the utilisation of polymerase chain reaction (PCR) to amplify a gene target that is exclusively present in a host population. PCR offers the capacity to examine genetic material from bacteria, such as deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), that has been extracted from a water sample for a particular sequence or target within a brief duration (dela Peña *et al.*, 2021). The aforementioned techniques are not reliant on the direct isolation of DNA from the primary source. However, it is worth noting that certain methods may necessitate a pre-enrichment step to enhance the sensitivity of the approach, as stated by Bivins *et al.* (2020).

#### **Antibiotic Resistance Analysis (ARA)**

The phenotypic method known as antibiotic resistance analysis (ARA) is widely employed in various studies. Microorganisms acquire

resistance to antibiotics through repeated exposure resulting from the therapeutic administration or treatment of these drugs to human or animal hosts. Consequently, the utilisation of antibiotic resistance patterns of microbial isolates serves as a distinctive method employed by Antimicrobial Resistance Analysis (ARA) to differentiate and ascertain the origins of human and animal sources (Sun *et al.*, 2019). The analysis of antibiotic resistance necessitates the cultivation of a substantial quantity of isolates, followed by the screening of these isolates for antibiotic resistance across a range of doses or concentrations. Subsequently, discriminant analysis is employed to examine the fingerprints obtained from these screenings. The fingerprints are cross-referenced with a database of bacterial isolates obtained from established sources, serving as a point of reference. The analysis conducted by Sun *et al.* (2019) yields an average rate of accurate classification. The analysis of antibiotic resistance has primarily been applied to *E. coli* and *Enterococci*. In a study conducted by Harwood *et al.* (2014), ARA and discriminant analysis techniques were employed to differentiate between human and animal faecal isolates obtained from surface waters in Florida. According to Harwood *et al.* (2014), the mean rates of accurate categorization for faecal streptococci and faecal coliforms were 62.3% and 63.9%, respectively.

There are several potential issues that can arise in relation to ARA. One such concern is the transportation of antibiotic resistance genes by plasmids, which have the potential to be lost during the process of cultivation. The acquisition or loss of a plasmid has the potential to alter the antibiotic resistance profile of bacteria. Ahmed *et al.* (2018) stated that the process of antibiotic resistance analysis (ARA) involves the establishment of a comprehensive collection of phenotypic fingerprints obtained from bacteria that have been isolated from the faecal samples of identified human and animal origins. According to Karkman *et al.* (2019), it is imperative to ensure that the database is designed in a manner that incorporates an adequate representation of potential sources of contamination within a specific watershed. The optimal size of a representational library remains uncertain within academic discourse. The library possesses sufficient spatial capacity to encompass a wide-ranging geographical expanse. The correlation between antibiotic resistance patterns in a specific region and the source of faecal contamination in another region may not be conclusive. Furthermore, alterations in antibiotic usage can impact the antibiotic

resistance pattern of faecal bacteria. It is important to note that antibiotic resistance analysis may not be applicable to wildlife isolates, as wildlife may consume livestock feed, which can introduce confounding factors (Hendriksen *et al.*, 2019).

#### **Carbon Utilization Profiles (CUP)**

Bacterial isolates can be distinguished from one another by using the Carbon utilisation profiles (CUP) method, which takes into account variations in the utilisation of various carbon and nitrogen sources. 96-well microplates with 95 different carbon substrates are used in the BIOLOG system (Arias *et al.*, 2020). The substrates used by each isolate are recorded. A database of around 2,000 microorganisms called Biolog is then compared to the usage profile. This method was used to classify microorganisms in wastewater treatment plants. (Ahmed *et al.*, 2022). Detailed profiles of carbon utilisation were obtained for 365 *Enterococcus* isolates from 4 different locations. Comparing human and non-human two-way classification, a study found that the average rate of correct classification (ARCC) by source was 92.7%, while it was lower for three-way classification (Fujioka *et al.*, 2015). The use of a microplate reader is all that is needed to determine carbon source utilisation using CUP, which is one of its many advantages. The learning curve for this strategy is lower than that of ARA or genotypic approaches. In order to better categorise *E. coli* and *Enterococcus* strains, Biolog now provides microplates with various sources of Nitrogen, Phosphorus, and Sulphur (Fujioka *et al.*, 2015).

#### **Ribotyping**

Ribotyping is a molecular technique that involves the generation of fingerprint patterns using restriction fragment length polymorphisms (RFLP) of the genomic 16S rDNA. These patterns are essentially based on the variations in the sizes of DNA fragments that are associated with the presence of specific target sequences. In this technique, the entirety of genomic DNA is isolated from uncontaminated cultures, followed by enzymatic treatment of the DNA (Teaf *et al.*, 2018). The DNA fragments that have undergone digestion are initially separated using the technique of agarose gel electrophoresis and subsequently transferred onto nylon membranes. Subsequent to the aforementioned procedure, Southern blot hybridization analysis is conducted employing rDNA probes, yielding a discernible pattern consisting of a range of four to twelve bands. In order to enhance the discriminatory capability of this methodology, multiple restrictive enzymes can be employed separately in various analyses. Ribotyping methods have traditionally been employed in

epidemiological investigations, but they are currently being utilised in the context of microbial source tracking (MST) as well (Shanks *et al.*, 2016). Ribotyping has been employed as a method to differentiate between human-derived faecal *E. coli* and nonhuman-derived pooled faecal *E. coli* isolates. The present study observed an ARCC of 82% for both human and nonhuman isolates. The utilisation of *E. coli* ribotyping techniques has led to a substantial augmentation in the quantity of isolates, thereby enhancing the ability to evaluate the suitability of this approach for microbial source tracking (MST) (Ngugen *et al.*, 2018). An illustration of the utilisation of ribotyping can be observed in a study conducted by Ngugen *et al.* (2018), wherein isolates of *E. coli* obtained from cattle, horses, swine, and chickens situated in Idaho and Georgia were compared to demonstrate the presence of geographical variations.

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

In the context of PFGE analysis, bacterial cells of pure culture are introduced into agarose plugs, within which the DNA undergoes digestion via various restriction enzymes. The digested plugs are subsequently embedded into specialised electrophoresis gels and subjected to electrophoresis for an extended duration (average 30 to 50 hours) with alternating currents from different directions utilising specialised equipment (Mathai *et al.*, 2020). Pulsed-field gel electrophoresis (PFGE) has been extensively employed in the field of clinical microbiology and is considered by certain clinical scientists as the preferred method. Nevertheless, pulsed-field gel electrophoresis (PFGE) is a time-consuming technique and has limitations in terms of the number of isolates that can be processed simultaneously. However, the utilisation of pulsed-field gel electrophoresis (PFGE) has been employed in the genotypic characterization and identification of *E. coli* and *Enterococcus* isolates from clinical and environmental sources, with the aim of discerning the origins of contamination (Beer *et al.*, 2015).

According to Meier *et al.* (2022), the application of pulsed-field gel electrophoresis (PFGE) to identify the origin of faecal contamination was conducted at a beach located in Oita, Japan, which is situated along the coastline of the Seto Inland Sea. The *Sma*I restriction enzyme was employed to analyse a total of 155 strains of *E. faecium* and 138 strains of *E. faecalis* that were obtained from water samples. A comprehensive analysis of *E. faecium* strains collected from the beach was conducted using pulsed-field gel electrophoresis (PFGE). The results revealed

that a total of 12 distinct PFGE types were identified among the analysed strains. The predominant PFGE types of *E. faecium* observed on the beach, as reported by Meier *et al.* (2022), consisted of the Fm-Rb-3 type, which accounted for the majority of the 72 strains identified.

#### **Denaturing Gradient Gel Electrophoresis (DGGE)**

Differences in electrophoretic mobility due to melting features of DNA fragments allow the DGGE technique to distinguish between unique PCR products of similar size. Human and animal faeces and gastrointestinal bacterial populations have both been studied and characterised using DGGE (Gomi *et al.*, 2023), but typically as a direct sample rather than a complicated matrix component. Although DGGE has been found to be useful for detecting and differentiating *E. coli* populations in faecal-affected water samples, it has not yet been utilised to differentiate between sources (Hlavsa *et al.*, 2015). DGGE was used to differentiate ambient *E. coli* isolates from those that originated in cattle, chickens, or humans. Kirs *et al.* (2016) found that the DGGE patterns generated from the samples investigated could not be used to trace pollution back to a specific point in the watershed under study. The researchers only used 132 isolates in their analysis, therefore a more complex database would be needed to use this approach for source tracking. It is important to note that for DGGE analysis to be successful, the gene of interest must have sufficient sequence diversity among strains for the detection of nucleotide modifications that are unique to a bacterial strain inhabiting a particular host (Kirs *et al.*, 2016).

#### **Repetitive DNA Sequences (rep-PCR)**

Differentiating between strains of the same bacterial species was achieved by using conserved sequences in bacterial repetitive elements as PCR primers, as described by Hagedorn *et al.* (2020). This method of bacterial typing, also known as rep-PCR, has been used to study a wide range of bacteria, including those that cause disease in plants, humans, and animals (de la Pea *et al.*, 2021). Faecal bacterial strains isolated from various faecal contamination sources have also been studied using the rep-PCR technique. Bacterial fingerprints typically consist of the three most commonly repeated components, REP, ERIC, and BOX. The majority of Gram-negative bacteria investigated include these repeating components, however BOX primers have proven to be more effective for MST studies. Rep-PCR using the BOX A1R primer was used by Kheiri and Akhtari (2017) to distinguish between faeces from humans and those from six other animals



(cows, pigs, sheep, chickens, geese, and ducks). Using Jaccard similarity coefficients and Jackknife analysis, they correctly identified all 154 isolates; in particular, they were able to determine the species of all 78 chicken and cow isolates and 90% of the other isolates. In conclusion, Rep-PCR was demonstrated to be an efficient tool for distinguishing between and categorising *E. coli* isolates from both animals and humans. In a study comparing ribotyping with Rep-PCR on eight host classes (human, cow, pig, horse, dog, chicken, turkey, and goose), Carson *et al.* (2023) discovered that ribotyping had an ARCC of 73, whereas Rep-PCR had an ARCC of 88%. Rep-PCR was found to be more precise, repeatable, and productive than ribotyping. However, they only utilised one restriction enzyme (HindIII) for ribotyping in their comparative analysis, rather than the two that Garabetian *et al.* (2020) suggests. Raza *et al.* (2021) employed Rep-PCR to identify the animal host type of 91 *E. coli* and 68 *Enterococcus faecalis* strains from human, bovine, swine, and poultry faeces, similar to the procedures described by Garabetian *et al.* (2020). While Garabetian *et al.* (2020) and Carson *et al.* (2023) found that *E. coli* and *E. faecalis* strains clustered together based on host species, Raza *et al.* (2021) found no such correlation. While conducting his research, Raza *et al.* (2021) acknowledged that he may have sampled too few samples. Finally, Raza *et al.* (2021) determined that this method is not mature enough or trustworthy enough to identify the origin of feculent contamination in water without a very high sample size. The method has a shorter turnaround time and costs less than PFGE (Gomi *et al.*, 2023) does. However, Gomi *et al.* (2023) report that the power of rep-discriminatory PCR is less than that of PFGE. As stated by Tang *et al.* (2019), this method is challenging to apply for subtyping because to its limited repeatability and accuracy in predicting serovars.

#### **Length Heterogeneity-PCR (LH-PCR) and Terminal Restriction Fragment Length Polymorphism (T-RFLP).**

Detection of fluorescently tagged 16S rDNA PCR products using an automated DNA sequence is the basis of two recently proposed methods, LH-PCR and T-RFLP (Ahmed *et al.*, 2016). These techniques are used to analyse the effects of insertions and deletions on the length of gene fragments, and once an acceptable target sequence has been identified, it may be easily followed by the automated process. They offer advantages over other molecular and biochemical approaches since they don't need the construction of complex culture-based

libraries or the cultivation of bacteria from environmental samples. These methods have also been used to find rDNA sequences unique to anaerobic faecal bacteria found in the gastrointestinal tracts of animals (Rothenheber, 2017), which is an additional benefit. This is crucial since there are far more anaerobic bacteria than conventional indicator bacteria in animal faeces. Moreover, the presence of faecal anaerobes indicates recent faeces contamination events because these organisms cannot survive in the environment for long. Accurate targeting of *Bifidobacterium* and *Bacteroides* spp. relatives has been achieved by the use of LH-PCR and T-RFLP (Akoumianaki *et al.*, 2018). Using these methods, several important findings have been recorded. First, a large number of *Bacteroides-Prevotella* 16S rDNA sequences that have not been seen before have been discovered in the faeces of animals. Due to the difficulty in consistently identifying markers for *Bifidobacterium* in bovine faeces or in coastal waters associated with faecal pollution, it has been suggested that the *Bacteroides-Prevotella* group is a more reliable sign of source tracing in marine waters. Finally, a *Bacteroides-Prevotella* fingerprint could differentiate between bovine and human excrement. A more extensive test including other potential sources of contamination was carried out by Soller *et al.* (2018), although so far these processes have only been evaluated on a small number of animals faecal samples.

#### **Host-specific 16S rDNA**

Numerous quantitative polymerase chain reaction (qPCR) strategies have been developed to target the 16S rRNA gene of human-associated *Bacteroidales*, several of these primers overlap in the brief 600-bp region targeted by most assays; and the vast majority of primers and probes published exhibit 100% similarity to parts of the *B. dorei* 16S rRNA gene. Techniques for determining whether or whether a given sequence belongs to the genus *Bacteroides* and, if so, which species. *Bacteroidales* is an order used to classify organisms that are not particularly linked to one another or have clear boundaries (Feng and McLellan, 2019). Primer and probe location that varies from other species in this genus can affect a test's sensitivity or specificity. It was observed that qPCR is 10,000 times more sensitive than end point PCR using the same primer set (c. 107 vs. 105 gene copies L1) (Hinojosa *et al.*, 2020). A different strategy for eliminating human-associated *Bacteroides* was not as successful. Cross-reactivity with all species was proven by a TaqMan qPCR method (called HuBac), which showed 100% sensitivity for human poo samples

but only 68% specificity when tested against excrement samples from domestic animals (Feng and McLellan, 2019). The BacHum-UCD TaqMan qPCR method was significantly more accurate than the HuBac technique. However, it was found to be 100% sensitive to raw sewage samples despite only being 67% sensitive to human faeces (Zhang *et al.*, 2020). Through a series of comparisons with established qPCR techniques, the authors of this work also found that the LOQ for the SYBR Green HF183 qPCR method described above was roughly 10-fold more sensitive than BacHum-UCD (three copies vs. thirty). Cross-reactivity to 25% and 14% of dog and cat faeces samples, respectively, lowered HF183's specificity compared to BacHum-UCD; nonetheless, HF183's specificity among all samples evaluated was still 95%. It was also established in this study (Nshimiyimana *et al.*, 2020) that the HuBac TaqMan approach has a rather low specificity (61%). BacH is an additional strategy for attacking the 16S rRNA gene of *Bacteroides* linked with humans. The method had an almost perfect sensitivity, with a specificity of over 99% (one false-positive cat sample), and comparable limits of detection (LOD) and quantification (LOQ) to earlier methods. Some of the research in this study was conducted in the field ('Selected Field Studies') (Feng and McLellan, 2019). Similar 16S rRNA gene sequences are compared in BacHuman qPCR. It was shown to be 100% sensitive to sewage, but only 81.5% specific since none of the indicators were entirely unique to humans and because the level and abundance of each marker varied among species. The HF183 marker functioned well because it was found in high concentrations in human faeces, in low concentrations in chicken and dog faeces (0.35 and 0.36 log<sub>10</sub> copy number per 1 ng total DNA, respectively), and in negligible concentrations in cow, pig, and cat waste (Nshimiyimana *et al.*, 2020).

An investigation into the available MST techniques and their relative importance for source tracking identification and characterization has been undertaken. While there has been substantial progress in recent years towards technique development, the body of review is difficult to interpret due to the heterogeneity across performance metrics and validity approaches in carrying out laboratory and field investigations. Multiple studies have shown that there is no one best approach. Therefore, there is no universally accepted approach to determining the origins of faecal pollution in the world's waterways. It is possible to distinguish between human and animal faeces contamination with the help of a reliable

method and indication (Symonds *et al.*, 2017). To better understand the land uses and environmental health risks connected with faecal pollution loading in a watershed, MST, which is based on the identification of specific molecular markers, can provide a more detailed picture than traditional indicators and approaches. Traditional culture-based methods just indicate the existence of faecal contamination, while MST approaches can identify "who" is responsible for pollution (Symonds *et al.*, 2017).

#### **Application of Microbial Source Tracking Water Quality Assessment**

The process of environmental water assessment encompasses the systematic observation and analysis of water resources in order to assess and appraise their quality. The utilisation of microbial source tracking (MST) is crucial in this procedure as it facilitates the discernment and distinction of microbial pollution originating from human, animal, and environmental origins (Rusiñol *et al.*, 2020). The identification of the sources of contamination through the use of microbial source tracking (MST) plays a crucial role in the development of specific approaches for the management and remediation of pollution (Serwecińska *et al.*, 2021)

#### **Environmental Studies**

The utilisation of MST algorithms exhibits a broad spectrum of applications within the field of environmental studies. The analysis facilitates comprehension of the origins and pathways through which microbial contaminants, including faecal pollution and pathogens, are disseminated (Ballesté *et al.*, 2020). The MST methods can be utilised by researchers to evaluate the effects of different land-use practises, agricultural activities, and urban development on water quality. This knowledge enables the application of evidence-based decision-making in the context of environmental planning and the development of policies (Teaf *et al.*, 2018).

#### **Total Maximum Daily Load (TMDL) Evaluations**

The term "total maximum daily load" (TMDL) describes a maximum loading profile for a pollutant that a water body can receive and still meet water quality standards (Adnan *et al.*, 2022). The evaluations TMDL encompass the process of ascertaining the uppermost permissible quantity of pollutants that a water body can accommodate without compromising its adherence to water quality standards (Goodwin *et al.*, 2017). The utilisation of microbial source tracking (MST) is of utmost importance in these assessments as it serves to identify and quantify the respective contributions of various pollution sources to the

overall microbial load. This information facilitates the establishment of attainable pollution reduction objectives, the implementation of efficient control strategies, and the enforcement of regulatory compliance (Tarek *et al.*, 2023).

#### **Legal System**

The utilisation of the MST methods demonstrates its pragmatic relevance in the legal domain, specifically in instances concerning infringements on water quality standards and disputes pertaining to contamination. The utilisation of scientific evidence regarding the origins of microbial pollution by Microbial forensics can be considered a relative newcomer to the legal arena, since such evidence was first produced in a criminal trial in 1998 (Koblentz and Tucker, 2016). A judge in that case concluded that the evidence satisfied necessary scientific and evidentiary criteria, as defined by what is known as the "Daubert Decision." In 2001, the techniques of microbial forensics and source tracking were employed in the scientific and legal investigations related to a bioterrorism event in which letters laced with *Bacillus anthracis* spores caused lethal cases of anthrax in several U.S. individuals (Shanks *et al.*, 2020). Microbial Source Tracking aids in facilitating legal investigations and attributing accountability. The enhancement of environmental regulations enforcement is facilitated, and the resolution of legal disputes is supported through the provision of impartial data (Shanks *et al.*, 2020).

#### **Bacteroides in Microbial Source Tracking**

*Bacteroides* are a type of commensal bacteria that inhabit the gastrointestinal tracts of warm-blooded animals, encompassing both humans and other animal species. *Bacteroides*, by virtue of their widespread presence and enduring presence in faecal matter, can be regarded as dependable markers for the detection of faecal contamination in aquatic environments. The effectiveness of MST can be enhanced by gaining a comprehensive understanding of the abundance and distribution patterns of the subject (Yoshida *et al.*, 2018). One of the primary benefits associated with the utilisation of *Bacteroides* in Microbial Source Tracking (MST) is their notable degree of host specificity. Various species or strains of *Bacteroides* exhibit associations with distinct animal origins, facilitating the discrimination between faecal contamination from humans and animals (Chen *et al.* 2017; Hjorth *et al.*, 2018).

The application of *Bacteroides* in Microbial Source Tracking (MST) presents numerous benefits. These tools establish a direct

correlation with sources of faecal pollution, facilitate prompt identification using molecular techniques, and enable quantitative evaluations of pollution levels. DNA-based techniques, such as polymerase chain reaction (PCR), have been employed to detect the presence of *Bacteroides*. This approach offers advantages in terms of efficiency and cost-effectiveness (Wexler and Goodman, 2017).

Although *Bacteroides* serve as valuable indicators, there are certain challenges associated with their application. The presence of variations in species composition across different hosts and geographic locations can pose challenges to the process of source tracking. Moreover, the occurrence of *Bacteroides* in non-faecal settings, such as natural habitats, could potentially lead to erroneous positive results. Additional investigation is required to effectively tackle these constraints and enhance the precision of Microbial Source Tracking (MST) employing *Bacteroides*, as suggested by Porter *et al.* (2020).

The utilisation of *Bacteroides* in microbial source tracking (MST) holds significant promise for the evaluation of water quality. Through the process of quantification and the utilisation of markers specific to each species, it becomes feasible to ascertain the origins of pollution, evaluate the efficacy of remediation endeavours, and observe the consequences of land-use practises on water contamination (Chan *et al.*, 2017; Tan *et al.*, 2019).

#### **Future Prospects of Microbial Source Tracking Methods.**

The potential for advancing our understanding of microbial contamination sources and enhancing water quality management practises is significant in the future prospects of microbial source tracking (MST). The following are significant domains of prospective advancement and potential applications for MST:

The ongoing advancement and increasing affordability of Next-Generation Sequencing (NGS) technologies present significant opportunities for augmenting Molecular Surveillance and Tracking (MST) capabilities. Next-generation sequencing (NGS) technology facilitates the concurrent examination of numerous microbial markers, thereby facilitating a comprehensive comprehension of sources of contamination and enabling more precise identification and tracking. The incorporation of Next-Generation Sequencing (NGS) in conjunction with Massively Parallel Sequencing (MST) methodologies will enhance the precision of analyses and streamline the

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detection of newly emerging pathogens (Goh *et al.*, 2021).

The current research endeavours to explore and establish novel microbial indicators with enhanced specificity and sensitivity for the purpose of source tracking. The identification of novel indicators will augment our capacity to distinguish between sources of pollution, encompassing distinct animal species and human populations. The endeavour to classify the microbiomes of various hosts and environments This information enables the development of focused treatment approaches, thereby mitigating the risk of introducing harmful pathogens into the environment. Moreover, microbial source tracking (MST) can be instrumental in the surveillance and control of microbial communities in resource recovery operations, such as the generation of biogas or nutrient-dense fertilisers from organic waste (McKee *et al.*, 2020).

The integration of MST into continuous monitoring systems can be facilitated by advancements in real-time monitoring technologies. The integration of MST with sensors and automated data collection platforms enables the detection and tracking of contamination sources in a nearly instantaneous manner. The integration of this system facilitates timely responses to instances of pollution, thereby enabling proactive measures to mitigate its effects and enhance the safeguarding of water resources and public health (Tiwari *et al.*, 2021).

The application of One Health approaches acknowledges the interdependence of human, animal, and environmental health. The utilisation of MST has the potential to make substantial contributions to One Health strategies through its ability to provide insights into the transmission dynamics of microbial contaminants across diverse ecosystems and host populations. This comprehension will facilitate the formulation of comprehensive approaches for the prevention and management of diseases, effectively tackling health issues that arise at the convergence of human, animal, and environmental health (Marti *et al.*, 2017). The utilisation of Decision Support Systems (DSS) in conjunction with policy implementation can yield significant benefits in the realm of water quality management. By leveraging the capabilities of DSS, valuable data can be obtained to inform the decision-making processes and facilitate the development of policies in this domain. The incorporation of MST data into decision support systems and modelling frameworks will facilitate the ability of stakeholders to effectively prioritise pollution

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will enhance the identification of novel microbial indicators that possess enhanced discriminatory capabilities (Gitter *et al.*, 2020). The utilisation of MST can be of significant importance in the enhancement of wastewater treatment procedures and the promotion of resource recovery. Microbial Source Tracking (MST) plays a crucial role in wastewater management by identifying the origins of microbial contaminants.

control measures, allocate resources in an optimal manner, and implement policies that are grounded in empirical evidence. The utilisation of the Modified Streamflow Trend (MST) methodology has the potential to augment the efficacy of evaluations pertaining to Total Maximum Daily Load (TMDL), watershed management plans, and regulatory frameworks aimed at safeguarding water quality (Tiwari *et al.*, 2021).

## CONCLUSION

The proliferation of new MST methods in recent years has been primarily driven by the necessity to tackle specific challenges, with a particular emphasis on human sewage and animal faeces. Despite ongoing efforts to find the optimal approach, it has been demonstrated that MST holds significant merit in the realm of water management. Through the consistent detection of microorganisms associated with humans or the identification of dominant sources of agricultural animals, MST facilitates the focused allocation of resources towards enhancing sanitation practises or managing agricultural waste, respectively. The application of MST, being a relatively nascent method, endeavours to cultivate methodologies for the discernment of origins of faecal indicator bacteria in water and environmental specimens. The aforementioned tools possess a broad spectrum of potential applications, encompassing areas such as regulatory compliance, pollution remediation, and risk assessments. The implementation of MST has the potential to significantly mitigate the prevalence of waterborne illnesses, thereby leading to notable enhancements in both public health and environmental well-being. The advancement and verification of MST techniques have resulted in the emergence of several library-dependent and library-independent approaches at different stages of progress. It is of utmost importance to acknowledge the significant potential of MST and to recognise the shared difficulties that arise in the process of evaluating and enhancing these techniques. Although this review has presented a concise overview of the present



state of scientific knowledge, it is anticipated that the field of MST will undergo rapid advancements, surpassing the capabilities discussed in this paper. The progress of MST exhibits considerable promise in tackling the challenges associated with water quality

management. The continuous development of MST techniques will persistently augment our comprehension of contamination origins, bolstering focused interventions, and ultimately resulting in enhanced water safety and public health consequences.

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