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Microbial Source Tracking: Methods, Applications, and Case Studies

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Chapter 1
Overview

Charles Hagedorn, Valerie J. Harwood, and Anicet R. Blanch

Abstract Microbial source tracking (MST) is a still-new and emerging sub-discipline of Biology that allows practitioners to discriminate among the many possible sources of fecal pollution in environmental waters. MST's current and potential applications range from beach monitoring to total maximum daily load (TMDL) assessment of pollution sources, that in turn will mediate greater protection of public health and improvement of environmental water quality. This comprehensive book taps the expertise of many of the leading research scientists from an international assemblage, and contains chapters that range from China and developing nations (22) to New Zealand and Australia (21), plus the EU and USA. The book addresses subjects ranging from the fundamentals of performance criteria during method development (2), library-dependent (3) and library-independent (4) approaches with their pros and cons, and applications to case studies from agricultural (18), urban (19), and beach (20) watersheds. Separate chapters focus on viral (5), bacteriophage (6), protozoan (7), chemical (8), mitochondrial DNA (10), and community analysis (11) -based methods. Chapters that relate MST to the fecal indicator bacteria (15), determining when and where to use MST (16), and the environmental persistence of fecal bacteria (17) put MST in the context of environmental monitoring. Specialized topics include legal (13) and TMDL (14) -associated issues, public perceptions (12), statistical analysis (9), national security (23), risk assessment (24), food safety (25), and using MST in undergraduate education (26). We hope that this book will prove useful to new practitioners of MST as well as established researchers and scientists and that it will serve as a valuable reference for many years to come.

Keywords Source tracking methods • Case studies • Environmental persistence • Performance criteria • Monitoring and assessment • Water quality • Fecal indicator bacteria • Microbial tracers • Chemical tracers

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The progressive improvement of strategies for management of microbial quality of catchments during the last two centuries has played an essential role in the improvement of public health in human societies. The definition and implementation of microbial indicators to survey water quality and assess reductions in microbial pathogens of fecal origin have proven to be a practical and efficient measure for the protection and improvement of water resources. The citizens of developed countries are generally protected by legislation and regulations regarding water quality for many purposes, such as drinking, personal hygiene, recreational activities, agriculture watering, and food production. However, waterborne disease outbreaks remain an enormous burden in developing countries where management of water resources with the aim of reducing microbial contaminants is rare or nonexistent (Chap. 22).

It is important to understand that measurements of fecal indicator bacteria (FIB) for water quality do not provide information about the origin of fecal pollution, i.e., whether the host source is, for example, birds, dogs, cattle, or humans – or a combination of any of these. This limitation exists because the feces of most animals contain FIB concentrations that are great enough to affect water quality when many animals or their sewage impact a water body (Chap. 14). The detection of the origin of fecal pollution is assuming a prominent place in hazard identification related to host-specific pathogens (Chap. 24). Pathogens from infected animals or humans can be introduced into water resources through feces or sewage and can cause a human health risk. The identification of the fecal sources is important to protect the public from zoonotic pathogens that may be shed by animals such as wild birds, poultry, cattle, and pigs. The capability to detect human-source pollution is also crucial to management strategies, as sewage from human origin is generally expected to have a higher risk to public health than that of animal origin (Chap. 15). Consequently, understanding the origin of fecal pollution is essential in assessing potential human health risks as well as for determining the actions necessary to remediate the quality of waters contaminated by fecal matter.

The intensive research efforts directed at developing methods for detection of fecal pollution originated over the past few decades and have been grouped under the term microbial source tracking (MST). These studies began in the early 1980s (Geldreich 1976; Mara and Oragui 1981; Osawa et al. 1981; Mara and Oragui 1983), probably as a result of social and legal pressures. The term MST denotes procedures that use host-specific (found only in one host species or group) and host-associated (largely confined to one host species or group) microbial indicators to establish the origin of fecal pollution in water. From its inception, MST has experienced rapid growth in knowledge and technological capabilities, including PCR and quantitative PCR that have substantially augmented the established research field of water-quality microbiology.

The history of MST research could be divided into several phases. Phase 1 was the initial phase, when defining new indicators (Brown 1993; Awad-El-Kariem et al. 1995; Hsu et al. 1995; Tartera et al. 1989; Bernhard and Field 2000; Nebra et al. 2003) and appropriate methods for source discrimination (Hagedorn et al. 1999; Wiggins 1996; Parveen et al. 1997; Whitlock et al. 2002; Harwood et al. 2000; Manero et al. 2002;

Wallis and Taylor 2003) were emphasized. In response to the emergence of MST as a potential regulatory strategy, Phase 2 saw three large multilaboratory method comparison studies (two in USA and one in Europe) plus numerous workshops, book chapters, and review articles dedicated to synthesizing information on the topic (Field et al. 2003; Harwood et al. 2003; Griffith et al. 2003; Myoda et al. 2003; Noble et al. 2003; Ritter et al. 2003; Blanch et al. 2004; Blanch et al. 2006). Furthermore, a federal (US EPA) guide document that described the uses and limitations of MST methods was published in 2005 (US Environmental Protection Agency 2005), and a book dedicated to MST as an emerging issue in food safety was published in 2007 (SantoDomingo and Sadowsky 2007). Over the past ten years, library-dependent MST methods (Chap. 3), which require a large assemblage of typed organisms from various host sources, have been largely supplanted by library-independent methods (Chap. 4) that rely on detection of a particular host-specific organism or gene.

To date, there has been no widespread consensus among researchers or any regulatory agency regarding the best indicators for MST. Many studies still focus exclusively on the development of new MST indicators and the improvement of their methods of detection and quantification (Chaps. 3–8 and 10). These documents cited above provide a collective body of literature on MST that, although frequently complementary, is at times conflicting, repetitious, and difficult to condense and interpret. In addition, they do not reflect the current diversity of MST approaches with different organisms, newer methodologies such as quantitative PCR and anthropogenic chemicals, nor do they reflect the scope of MST research being conducted around the world (Chaps. 21 and 22).

The goal of this book is to serve as a valuable reference for all those who are involved with water quality, whether they are students, researchers, managers, or regulators. This book also aims to be the first comprehensive source to present the MST spectrum at the international level and to act as a future guide for researchers who need to use, apply, and interpret MST in all manner of watershed environments. For that reason, the editors have intentionally sought out authors who collectively represent a comprehensive expertise and whose work reflects the rich diversity and truly international scope of MST.

The unifying theme throughout the book is the design of more standardized approaches to MST that include performance criteria, regardless of method or organism (Chap. 2), plus recommendations for field study design and MST implementation (Chaps. 14 and 16). The content is structured in four sections to facilitate the search of topics and practical reading. The first is a "Method Development" section that includes a wide spectrum of different fecal source indicators that have been or are being developed. Here, readers can find not only the current state of the science for these indicators but also the historical track, present challenges, and future perspectives.

Microbial indicators based on the detection of bacteria or their components, e.g., genes, are described in two chapters that are delineated by the method's dependence (Chap. 3) or independence (Chap. 4) on reference libraries composed of typed organisms from various host sources (library-dependent and library-independent methods). Different approaches are also discussed and compared, including requirements for cultivation and the dependence on a priori developed reference libraries.

Other proposed MST indicators are also considered in detail within this section, i.e., viruses (Chap. 5), bacteriophages (Chap. 6), and protozoa (Chap. 7). The advantages and challenges for these microbial groups are analyzed, and the potential for practical applications is also explained. Moreover, chemical and eukaryotic (mitochondrial) indicators that have been developed and evaluated for MST uses also have their respective chapters (Chaps. 8 and 10), where advantages and drawbacks are also identified, and new perspectives are indicated. This section also includes three chapters for specific topics that are essential to implement of MST indicators and to evaluate their feasibility for routine analyses. To that end, performance criteria (Chap. 2), statistical approaches and modeling (Chap. 9), and the development of community-analysis-based methods (Chap. 11) each have a dedicated chapter.

Indicators, the methods used to detect and/or quantify them, and the appropriate performance characteristics need to be applied, understood, and properly interpreted by scientists, managers, and regulators who work on catchment management. The second section of the book covers "Use, Interpretation, and Application" and includes chapters on the public understanding of MST (Chap. 12), legal challenges (Chap. 13), and the use of MST indicators on the determination of the total load of fecal pollution that could support a catchment (i.e., TMDL) based primarily on the development of models for this purpose (Chap. 14). The relationship of MST indicators with respect to other standardized and routine microbiological parameters (i.e., microbial indicators and pathogens) that are used for water-quality management is also described in a specific chapter (Chap. 15). Designing representative sampling schemes and a decision-based matrix for when to use, or not use, MST are also included (Chap. 16). Lastly, this section includes a chapter on the persistence of indicator organisms in aquatic environments and sediments and sands, a very timely emerging issue (Chap. 17).

The third section is dedicated to "MST Case Studies." Field studies on agricultural and rural watersheds from different geographical areas are described, and implications for catchment management are discussed (Chap. 18). Many practical aspects of MST conducted in different geographic regions are described. Some are related to agricultural and rural watersheds (surface and karstic groundwater resources) but others to urban and suburban watersheds (Chap. 19). There is a chapter committed to the rationale for using microbial source tracking (MST) methods at beach sites and coastal water bodies (Chap. 20) and the use of MST methods for evaluating waters impacted by nonpoint sources of pollution. This chapter also describes the most common traditional and alternative MST markers used at beach sites. Lastly, this section contains two chapters outlining experiences and case studies on the application of MST methods in waterways in Australia and New Zealand (Chap. 21), and in China and developing countries (Chap. 22). The vast differences in the use of MST between developed and developing nations are readily apparent in these two final chapters of Sect. 3.

Finally, the fourth section is dedicated to the "Future Needs and Perspectives for MST Development." including more widespread application of MST on water management decisions. Issues and aspects of MST as related to national security (Chap. 23), quantitative risk assessment (Chap. 24), and food safety (Chap. 25) are

all presented. Lastly, a chapter on education presents some available training resources for future scientists and technical staff and demonstrates how MST can be a component of undergraduate education in both the four-year and community college settings (Chap. 26).

We hope that this book will prove useful to new practitioners of MST as well as established researchers and scientists and that it will serve as a starting point into this fascinating area of MST that merges basic and applied science, field work and laboratory studies, theory and practicality, as well as any scientific endeavor in modern biology. We trust that this book will need substantial revision at some point as the field of MST continues to grow and that it will serve as a valuable reference for many years to come.

We are grateful to Andrea Macaluso (editor at Springer-US), who first proposed to us the idea of an interdisciplinary MST book. We especially acknowledge all the authors for their dedication and contribution and their efforts to relate the different chapters to each other. This greatly simplified the always-complex process of editing a book with many highly qualified authors who are experts in the wide range of topics covered in this book.

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Chapter 2
Performance Criteria

Valerie J. Harwood and Donald M. Stoeckel

Abstract The establishment of rigorous, consistent performance criteria for microbial source tracking (MST) methods is essential for their usefulness and widespread acceptance as research and regulatory tools. In this chapter, we focus on performance criteria for library-independent methods, although many aspects of the discussion are applicable to both library-independent and library-dependent methods. We separate these criteria into three levels for ease of discussion: (1) the intrinsic characteristics of the "marker" (target), (2) protocols for generating laboratory data, and (3) field applications. By ensuring that a consistent set of metrics for characteristics such as accuracy and precision be applied to field studies and published works, we can begin to circumscribe the set of MST tools that will be most useful for discriminating among fecal pollution sources in environmental waters.

Keywords qPCR • Performance • Efficiency • Accuracy • Precision • Error

2.1 Introduction

The nascent field of microbial source tracking has relied upon both library-dependent and library-independent approaches (see Chaps. 3 and 4, respectively) to detect fecal contamination from particular hosts. In particular, the library-dependent approach experienced a high level of application in first five or so years of the 21st century, which included the introduction of statistical methods such as discriminant analysis (Wiggins 1996), principle components analysis (Dombek et al. 2000), or nearest-neighbor analysis (Albert et al. 2003; Ritter et al. 2003;

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Robinson et al. 2007) to evaluate complex patterns generated by antibiotic resistance analysis (Hagedorn et al. 1999; Harwood et al. 2000; Wiggins 1996), rep-PCR (Carson et al. 2003; Dombek et al. 2000; McLellan et al. 2003), pulsed-field gel electrophoresis (Myoda et al. 2003), ribotyping(Parveen et al. 1999), and other methods. The validity of results from these library-dependent methods began to be questioned following proficiency testing with blind samples (Griffith et al. 2003; Harwood et al. 2003; Stoeckel et al. 2004). Other pressing concerns with library-dependent methods include the size and scope required for a "representative" library and concerns about broad geographic applicability and temporal stability (Stoeckel and Harwood 2007; US Environmental Protection Agency 2005; Wiggins et al. 2003).

As a result of these findings and concerns, library-independent methods, many of which showed better accuracy in limited proficiency testing compared with the library-dependent methods (Griffith et al. 2003; Harwood et al. 2003; Myoda et al. 2003), began to be more intensively developed and used in field studies. As was done with library-dependent methods, as these methods and markers emerge they should be routinely validated for provision of accurate results. The purpose of this chapter is to outline a strategy for method validation and proficiency testing that is applicable to library-independent MST methods, many of which utilize PCR and/or quantitative PCR (qPCR) to detect a host-associated target organism or gene. By establishment of rigorous performance criteria and application of proficiency tests, MST methods will be evaluated within a consistent framework, paving the way for more confident use in regulatory and legal contexts.

This chapter considers performance of MST methods separately at three levels – the genetic target or "marker," since interpretation of MST data for fecal source indication is dependent upon marker characteristics (sensitivity and specificity within the target population); the protocol for generating laboratory data, since without confidence in the data results cannot be interpreted; and field application, since interpretation of data collected from uncontrolled settings poses additional challenges beyond basic laboratory quality control. In this chapter, we use "performance" when referring to inherent characteristics of the method, e.g., sensitivity, specificity, evenness; and "proficiency" when referring to testing that is specifically designed to evaluate the quality and reliability of laboratory-generated data.

The use of common performance measures and validation strategies in the many studies that are expected over the next decade should facilitate rapid progress in this area, as we continue to work toward availability of reliable analyses, classification approaches, and interpretation strategies for tracking fecal contamination to its sources by use of MST tools. Although we focus here on methods that target specific genes via PCR, the general strategies and most of the considerations discussed here apply in some measure to all of the methodologies discussed in this book (see Chaps. 3 and 9 for criteria that are more appropriate for library- and chemical-based methods, respectively).

2.2 Evaluation of Target (MST Marker) Performance and Suitability

The various markers used for library-independent MST detect the presence of host-associated microbial populations. Sensitivity, or completeness of marker representation in the host population, along with specificity, or exclusivity of the host–microbe association, are critically important parameters (Table 2.1) (Stoeckel and Harwood 2007). Relatively poor sensitivity, which is associated with low-prevalence markers such as those that detect some pathogenic viruses (Noble et al. 2003; Stoeckel and Harwood 2007), frequently causes false-negative results. Incomplete specificity, which is associated with many existing genetic markers

Table 2.1 Characteristics of an ideal vs. a useful MST marker (Harwood 2007; US Environmental Protection Agency 2005)

Characteristic	Ideal marker	Useful marker
Specificity	Marker found only in target host species	Marker is differentially distributed among host species
Distribution in host population	Found in all members of all populations of target host species; contributes to sensitivity of method	Consistently found in host species whose feces could impact the target sites
Evenness	Quantity in the feces of individuals is similar	Quantity in aggregate sources, e.g., sewage influent; animal populations, is similar
Temporal stability in host	Frequency and concentration in host individuals and populations does not change over time	Despite variation in marker frequency and concentration in individuals, these characteristics are stable at the population level
Geographic range/ stability	The frequency and concentration in geographically separated host populations are similar	The marker can consistently be detected and quantified across the geographic area to be studied
Environmental persistence	Consistent decay rate in various matrices and habitats; no increase under any conditions; response to treatment processes and environmental insults is similar to that of pathogens	Predictable decay rate in various matrices and habitats; no increase under ambient conditions; response to treatment processes and environmental insults is characterized
Quantitative assessment	Can be accurately quantified	Accurately indicates presence/ absence of contamination source
Relevance to regulatory parameters	The marker is derived from an organism that is a regulatory tool	The marker is correlated with an organism that is a regulatory tool
Relevance to health risk	The marker is strongly correlated with risk of all types of waterborne disease, e.g., gastroenteritis, dermatitis, upper respiratory infections	The marker constitutes a health risk or is otherwise correlated with a subset of waterborne disease, e.g., viral gastroenteritis

(Harwood et al. 2009; Korajkic et al. 2009; Shanks et al. 2010), can cause false-positive results. The third major issue relevant to performance measurement for markers is evenness of marker distribution (in terms of prevalence and quantity), which applies both among populations and among individuals within a given host population. If the evenness of the marker is different from the evenness of fecal indicator bacteria or pathogens, then simple detection or even quantification of the marker may not be directly comparable to existing regulations or public health risk outcomes. These considerations are discussed in detail below.

2.2.1 Choosing the Tool(s) to Fit the Question

Potential applications of MST include (a) assessment of sources of fecal contamination in recreational or drinking source waters, (b) prioritization of impaired water bodies for total maximum daily load (TMDL) implementation or other interventions, (c) source apportionment for TMDL plans, and (d) forensic applications, i.e., assigning (or relieving) responsibility for pollution. The goals of a given study must be carefully considered when choosing or designing MST marker(s), and deciding whether conventional (presence/absence) PCR-based methods are sufficient or if quantitative PCR (qPCR) is required. For example, if one is most concerned about determining when and where contamination from human sources is present, a suite of human-specific markers may be chosen, and conventional PCR may be sufficient to achieve the study goals. If, however, one is attempting to apportion contributions from various fecal sources for TMDL applications, it would be necessary to use a suite of markers for the identified sources of fecal loading, and qPCR would be required.

Many authors have recommended toolbox or tiered approaches for MST study design, the first meaning that a group of MST methods is on hand and ready for deployment as the specific situation demands and the second meaning that lower cost methods that broadly measure contamination, such as conventional fecal indicator bacteria measurements, are used first, followed by more expensive, technically demanding methods such as PCR where they are needed to accomplish specific goals (Boehm et al. 2003; Lu et al. 2009; McQuaig et al. 2006; Noble et al. 2006; Vogel et al. 2007) (see also Chaps. 16 and 19). Another aspect of the toolbox approach is that multiple methods for detection of contamination from one source can be used to support one another (see below), alleviating the uncertainty that results from imperfections in all methods reported to date. On the contrary, the use of multiple tests increases the cost of a given study and can be unacceptably expensive for end users such as regulatory agencies. This situation can be a particular concern when multiple methods are used to identify one source.

One must also consider the performance characteristics of the methods and how they might affect interpretation of the results; for example, one could use a human-associated marker with high concentration in sewage but incomplete specificity to minimize the probability of false-negative results. Because use of such a marker could yield false-positive results, one might also use a highly human-specific marker that