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Microbial Source Tracking: a tool for identifying sources of microbial contamination in the food chain

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Abstract

The ability to trace fecal indicators and food-borne pathogens to the point of origin has major ramifications for food industry, food regulatory agencies and public health. Such information would enable food producers and processors to better understand sources of contamination and thereby take corrective actions to prevent transmission. Microbial source tracking (MST), which currently is largely focused on determining sources of fecal contamination in waterways, is also providing the scientific community tools for tracking both fecal bacteria and food-borne pathogens contamination in the food chain. Approaches to MST are commonly classified as library-dependent methods (LDMs) or library-independent methods (LIMs). These tools will have widespread applications, including use for regulatory compliance, pollution remediation and risk assessment. These tools will reduce the incidence of illness associated with food and water. Our aim in this review is to highlight the use of molecular MST methods in application to understanding the source and transmission of food-borne pathogens. Moreover, the future directions of MST research are also discussed.

Keywords

Microbial source tracking (MST), food-borne pathogen, fecal pollution, library-dependent methods, library-independent methods, indicator bacteria**INTRODUCTION**

Fecal contamination of water and food is a common and enduring problem, resulting each year in closed beaches and shellfish beds, tainted food products, and polluted waterways worldwide

(Jenkins et al., 2009; Lu et al., 2005; Parker et al., 2010). As reported, contamination with fecal coliform bacteria may signal the presence of other potential pathogens, such as hepatitis A virus, *Vibrio* spp., *Salmonella* spp., *Campylobacter*, protozoan parasites etc., which can create both public health and economic concerns (Abdelzaher et al., 2010). To correctly assess and properly manage the human health risk associated with water and food contamination, it is necessary to have information regarding the source of fecal contamination. Traditional and alternative indicator microorganisms have been used for many years to predict the presence of fecal pollution in water (Scott et al., 2002); however, it cannot be a satisfactory technique to identify the origin of nonpoint source fecal pollution. Consequently, a set of approaches developed recently which are termed as Microbial Source Tracking (MST) seek to define a variety of phenotypic and genotypic methods using indicator microbes or pathogens to determine sources of fecal contamination (Scott et al., 2002; Stoeckel and Harwood, 2007). So far, the available MST methods, although still under development, have already been widely applied to the management of fecally contaminated water bodies in the developed countries (Gourmelon et al., 2010; Jiang et al., 2007; Kim et al., 2010; Lu et al., 2009). MST, which currently is largely focused on determining sources of fecal contamination in waterways, is also providing the scientific community tools that may be adapted to broader applications, especially the tracking of food-borne pathogens and food safety (Foley et al., 2009).

The primary sources of microbes in raw food commodities are soil and water, the intestinal tracts of animals, and animal hides. During and after processing, foods can become contaminated via food contact surfaces, food handlers, and air and dust. There are many similarities between the concepts and methods used to identify sources of microbial contamination of the food chain and those used to track the sources of biological pollution in natural waterways. The aim of MST in water resource management is to better elucidate the contribution of various nonpoint or diffuse origins of pollution such as runoff from agricultural sources, including manure from livestock and poultry, and fecal contamination from wildlife, as well as to separate the sources of indicator organisms and potential human pathogens from sewage and fecal contamination (Field and Samadpour, 2007). The above similar issues also need to elucidate in the food processing environment and different food products. In this case, microbial contamination can enter the food chain at several levels: (i) the production environment, represented by the farm, orchard, or fishery; (ii) the processing environment, represented by the slaughterhouse, cannery, or packing plant; (iii) the preparation environment, either in a kitchen or food preparation service (Havelaar et al., 2010). Thus, the use of MST approach will open the door for the precise quantification of the various sources of hazards and risks in food safety (Fig. 1).

This review article will provide an overview of types of MST methods referred to the major genotypic methods currently used, and the application of MST in tracking sources of microbial

contamination in the food chain. Based on this state-of-the-art, future directions in the field will be also discussed.

CLASSIFICATION OF MST METHODS

MST methods are commonly classified as library-dependent methods (LDMs) or library-independent methods (LIMs) (Scott et al., 2002). Both phenotypic and genotypic characteristics, which both can be formed as a library, have been used in LDMs studies. Tested phenotypic methods have included antibiotic resistance profiles (Carroll et al., 2009), carbon utilization profiles (Hagedorn et al., 2003), and whole-cell fatty acids (Duran et al., 2009). However, these methods have serious disadvantages, including unstable phenotypes, low sensitivity at the intraspecies level, and limited specificity. Tested genotypic methods are highly sensitive, rapid and easy to perform, and will be stated in further detail in the following section of this review.

Besides, analyses of certain chemicals associated with sewage, including fecal sterols, optical brighteners, and host mitochondrial DNA, have also been utilized for what can be more broadly termed fecal source tracking; however, in this review we compare the performance of only fecal source tracking studies in which the target(s) is microbial. For application, LDMs are relatively expensive and time consuming considering the number of isolates needed to develop robust libraries. An additional disadvantage of LDMs is that they are very often temporally and

spatially-specific thus they are not sufficiently applicable for routine use. Regardless of the limitations, LDMs have been widely used in the developed countries. LIMs offer a more efficient and cost-effective alternative but as yet do not individually offer adequate discriminatory power between animal sources (Stoeckel et al., 2007).

MOLECULAR METHODS FOR SOURCE TRACKING

A number of key features need to be evaluated when choosing an appropriate molecular method for a particular MST investigation. The techniques that will be introduced in this review include: ribotyping, pulsed-field gel electrophoresis (PFGE), amplified fragment length polymorphisms (AFLP), repetitive element PCR (rep-PCR), multilocus sequence typing (MLST) and host-specific molecular marker PCR, which are frequently used in most cases (Gourmelon et al., 2010; Lu et al., 2009; Lyautey et al., 2010; Nayak and Stewart-King, 2008; Scott et al., 2003). The applications, advantages and limitations of each method are also summarized in Table 1.

The Principle of Library-dependent Methods

Ribotyping. Ribotyping is a method of DNA fingerprinting whereby highly conserved rRNA genes are identified using oligonucleotide probes after treatment of genomic DNA with restriction endonucleases. With the technique, cellular DNA is cut with a frequent cutting restriction enzyme and the restriction fragments are separated by gel electrophoresis, transferred to a membrane, and incubated with a probe specific for a conserved region of the rRNA genes (Chisholm et al., 1999).

Differences in the number of rRNA genes and genetic variability in the regions flanking the rRNA genes leads to the production of distinct restriction fragment band profiles that can be used to discriminate between bacterial strains (Bouchet et al., 2008). Therefore, databases either may need to be extremely large and contain isolates from a very broad geographic region or must be designed exclusively for a specific environment with defined potential impacts. Although this method has been reported to effectively track human and nonhuman sources of pollution (Carson et al., 2001; Hartel et al., 2002; Parveen et al., 1999), it is expensive and labor-intensive, unless the procedure is streamlined and performed routinely.

PFGE. PFGE is a DNA fingerprinting method which is based on the restriction digestion of purified genomic DNA. It is currently considered the gold-standard method for subtyping food-borne pathogens. Briefly, bacteria are grown in broth or on solid medium and are combined with molten agarose. The resulting agarose plugs, containing whole bacteria, are then subjected to detergent-enzyme lysis and whole-genome digestion using a rare cutting restriction enzyme. The enzymatic digestion results in large DNA fragments (10 to 800 kb in length), which are electrophoresed under alternating electric currents, thereby producing a banding pattern or DNA fingerprint. PFGE forms the basis for PulseNet, a national molecular subtyping network that was established in 1996 by the CDC and is now utilized by various state public health laboratories and food safety laboratories at the FDA and USDA (Gerner-Smidt et al., 2006).

The remarkable discriminatory power and reproducibility of PFGE has made it a widely applicable method for comparative typing of most bacterial species (Arthur et al., 2008; Chen et al., 2010; van Belkum et al., 2007). For the purpose of source tracking, Furukawa and colleagues used PFGE to estimate the source of faecal pollution in Aoshima Beach, Japan, and revealed the majority of *Enterococcus faecium* isolates as being from Oyodo River (Furukawa et al., 2011). However, this method is labor-intensive and often requires 2-4 days to perform the procedure and analyze the results. In addition, if a genetic 'event' does not affect the electrophoretic mobility of the enzyme-digested DNA fragment, then the change may not be identified as a separate genotype (Foley et al., 2009). Thus, the usefulness of PFGE for MST application needs to be further fully determined.

AFLP. AFLP analysis combines the beneficial traits of restriction digest analysis and PCR amplification for genotyping. Briefly, target DNA is digested with two different restriction enzymes, and adaptor oligonucleotides are ligated to the sticky-ended DNA fragments. A PCR method is then used to amplify a subset of these fragments, which are then separated and detected by an automated sequencer system. The restriction fragments analyzed are small, and even a single base mutation can be detected. The use of different sets of restriction enzymes or different primer pair combinations can generate large numbers of different AFLP fingerprints without prior knowledge of the genomic sequence. This technique has the high sensitivity of PFGE profiling and can be adapted to automatic analysis for higher throughput using fluorescent dye-labeled primers

(FAFLP) and an automated sequencer (Fry et al., 2009). AFLP systems for high-resolution genotyping of food-borne pathogens have been applied to outbreak investigation and source tracking of *Campylobacter* (Siemer et al., 2005), pathogenic *E. coli* (Tokunaga et al., 2007), *Salmonella* (Kober et al., 2011), etc.

Rep-PCR. Rep-PCR uses primers corresponding to interspersed repetitive DNA elements present in various locations within the prokaryotic genome to generate highly specific genomic fingerprints. Three methods of repetitive sequence analysis have been used, with each targeting a specific family of repetitive element. These methods include repetitive extragenic palindromic sequence PCR (REP-PCR), enterobacterial repetitive intergenic consensus sequence PCR (ERIC-PCR), and PCR with extragenic repeating elements (BOX-PCR). The repetitive units are considered to be highly conserved because rep sites are crucial protein-DNA interaction sites and/or these sequences may disseminate themselves as selfish DNA by gene conversion. Amplification of the distinct rep sites produces diverse-sized DNA fragments that can be separated by agarose gel electrophoresis, and the resulting banding patterns unique for specific bacterial strain can be compared. Most of the MST studies for identification of the source of *E. coli* have utilized the rep-PCR primers (Lyautey et al., 2010; Scott et al., 2002). However, successful identification of an unknown bacterial isolate requires that a reference database be established, and additional known isolates must be fingerprinted from a large geographic region in order to assess

the potential universal application of this method. Questions have also arisen as to the reproducibility of this method.

MLST. The fundamental basis of MLST is that the method uncovers genetic variation in multiple conserved genes, and this genetic variation is used to classify strains, identify clonal groups, and elucidate the history of divergence of the chromosomal background. The method relies on determining the nucleotide sequence of regions of approximately 500 base pairs from multiple genetic loci distributed around the genome of a particular bacterial species. The genes to be sequenced are amplified by specific PCR primers and the sequencing is generally performed using an automated sequencer.

When developing a new MLST scheme, an important consideration is the selection of gene targets. For MLST to be effective as an epidemiological tool, the selection and number of genes needs to be adequate to distinguish among closely related isolates. Housekeeping genes, with their low rate of genetic variability, provide a desirable sequencing target for global phylogenetic studies, but likely do not provide enough variability to distinguish among strains with more recent genetic divergence (Foley et al., 2009). Therefore, in order to provide better discriminatory power for studying food-borne pathogen, MLST can be performed on virulence genes and virulence-associated genes (MVLST), which is comparable to PFGE and provides greater discrimination than ribotyping (Lomonaco et al., 2008).

The Principle of Library-independent Methods

Host-specific molecular marker PCR. Direct detection of host-specific genetic markers, belonged to library-independent methods, has been proposed as another means of identifying sources of fecal contamination in environmental waters. Proposed targets including a battery of specific toxin genes or additional host-specific genes are detected by PCR, and bacteria can be differentiated based on their pathogenic properties and the hosts they target. Recently, animal specific viruses have also used as host-specific indicators for animal fecal pollution (Fong et al., 2005; Hundesa et al., 2006). Fecal sources are determined based on the molecular detection of host-specific viral pathogens, such as polyomavirses and adenoviruses for humans (Bofill-Mas et al., 2006), canine parvovirus for dogs (Decaro et al., 2005), and bovine enterovirus for cattle (Ley et al., 2002). These approaches show promise for freeing practitioners of the resource commitment and complications inherent in building a representative library. However, in order to more accurately discriminate between different sources of fecal pollution, these library-independent methods will still need to be studied for identification of more host-specific markers.

MST METHODS APPLICATION FOR IDENTIFYING SOURCES OF BACTERIAL CONTAMINATION IN THE FOOD CHAIN

Waters for Agriculture and Aquaculture

Irrigation water, which can be a source of pathogenic microorganisms that potentially originated from feces, can ultimately contaminate agricultural products (Steele et al., 2005). A variety of

fecal contaminants and pathogens such as *E. coli, Salmonella* spp., *Listeria* spp., *Vibrio cholerae* and *Pseudomonas* spp. have been isolated from irrigation water and associated sediments (Mazari-Hiriart et al., 2008; Steele et al., 2005). *E. coli* O157:H7 contamination of produce can occur in the field by exposure to contaminated irrigation water in a major lettuce and spinach production region in California (Cooley et al., 2007). The problem is more significant in developing countries, where the use of sewage effluent for irrigation is common (Mazari-Hiriart et al., 2008) despite the fact that many countries outlaw the practice. Given the importance of food safety to modern society, the identification and mitigation of the possible sources of fecal contamination in these types of waters can make a significant impact on the production of microbiologically safe crops, fruits and vegetables. Cooley et al. (2007) investigated the sources, incidence, fate and transport of *E. coli* O157:H7 in waters by Multi-Locus Variable-number-tandem-repeat Analysis (MLVA) and PFGE methods near a production region, which may contribute to determination of the sources of pre-harvest contamination and potential risks for human illness. The results suggested that the sources of *E. coli* O157:H7 in the field could be from the contaminated low-flow rivers at multiple locations, and the incidence of O157 increased significantly when heavy rain caused an increased flow rate in the rivers. The genetic diversity of fecal *E. coli* in irrigation water has been also studied by PFGE to identify the potential contamination sources (Lu et al., 2004).

MST techniques may also provide a potentially important management tool for the shellfish growing waters and the regulation of the sanitary quality of shellfish. Shellfish may incidentally bioaccumulate pathogenic microbes to concentrations greater than present the water column. This creates a potential health risk for consumers ingesting raw or undercooked shellfish. Broadly, the microbial contaminants appeared in shellfish growing waters can be classified as either (i) being naturally occurring in the growing waters or (ii) related to fecal contamination of the waters. *Vibrio* spp. and *Aeromonas* spp. are generally indigenous to the marine environment. Their occurrence does not correlate well with indicator microorganisms associated with fecal pollution and, therefore, can not be managed by water quality control measures. However, fecal contamination of growing waters, which may signal the presence of potential human pathogens, such as hepatitis A virus, noroviruses, *Salmonella* spp., *Shigella* spp., and *Campylobacter* spp., can be controlled by source tracking strategies. For the first time to study the fecal pollution of coastal shellfish culture area of East China Sea, we have evaluated one of the library-dependent methods to differentiate the fecal indicator (*E. coli* isolates) from multiple sources mainly associated with domesticated animal wastes (Fig. 2). Our study indicated that *E. coli* in feces could spread from human sewage or domestic farms to the surrounding shellfish culture water, and potentially affect the quality of shellfish (Ma et al., 2011). In addition, our work also provides an important evidence of correlation between the presence of major zoonotic bacteria species and host-origin fecal pollution (Fu et al., 2011). Recently, the library-independent methods have been demonstrated to

distinguish between human and animal contributions to contaminated water and shellfish (Gourmelon et al., 2010; Love et al., 2008; Wolf et al., 2008). These studies were performed with the host-specific marker PCR technique targeted *Bacteroidales* 16S rRNA gene, F^+ RNA bacteriophages or F^+ RNA coliphages. Moreover, the host-specific marker technique based on quantitative reverse transcription-PCR methods for human and animal enteroviruses detection have been also used as a means to trace fecal contamination sources in shellfish harvesting sites (Costantini et al., 2006; da Silva et al., 2007; Le Guyader et al., 2009).

Food Animals in Farming Environment

Food producing animals are often the primary source of zoonotic pathogenic agents associated with human infections. Pathogenic microorganisms can be transmitted from the infected or contaminated livestock and poultry at the farm to the animal-derived food products. Thus, source tracking and control of pathogen contamination at this pre-harvest stage is particularly important for the safety of animal food products and reducing the risk of human food-borne illness.

Beef cattle and dairy cows are the main reservoir for Shiga toxin-producing of *E. coli* (STEC) including *E. coli* O157:H7 (Berry and Wells, 2010). Arthur et al. (2008) conducted a tracking study of *E. coli* O157:H7 associated with beef cattle from the feedlot through processing, and found that the transfer of bacteria onto cattle hides that occurs in the lairage environments accounted for a large proportion of the hide and carcass contamination by PFGE analysis of the lairage environmental and cattle O157 isolates. Fecal and hide prevalence of *E. coli* O157:H7 were

also found to be correlated with carcass contamination at meat processing plants (Elder et al., 2000). *E. coli* O157:H7 strains isolated from feces, hides, and beef carcass were then subtyped by PFGE to investigate the potential that the same genotypes were being brought to the processing plant on live cattle.

Microbial subtyping and source tracking also have been used to investigate the transmission of other major zoonotic pathogens from pre- to post-harvest food animals. *Salmonella* have been associated with poultry meat and egg products, and these bacteria are capable of colonizing live poultry in their intestinal tracts. Source tracking and horizontal transmission pathways of *Salmonella* serovars was delineated in a turkey production environment (Nayak et al., 2008). The identical fingerprint profiles of *Salmonella* Heidelberg isolates were found in the birds at week 2 and birds within the same pen and other pens at weeks 10 and 18, suggesting possible horizontal transmission of this serovar across the production facility during the grow-out period.

Campylobacter is another widespread zoonotic pathogen that is carried by animals farmed for meat and poultry. The MLST analysis of *Campylobacter coli* and *Campylobacter jejuni* could lead potentially to more efficient source tracking in this species, and suggested that both sporadic and outbreak human clinical *Campylobacter* strains were primarily originated from food animal sources (cattle, chickens, swine and turkeys) (Miller et al., 2006; Wilson et al., 2008). In addition, pork and dairy products also have been identified as the source of human listeriosis. Both clinically diseased and healthy animals have been reported to excrete *L. monocytogenes* in their feces

(Mohammed et al., 2009; Yokoyama et al., 2005), which could eventually cause contamination of the bulk tank milk or pig carcasses. A source tracking study confirmed that the presence of *L. monocytogenes* in the pork production chain was followed from the farm to pork, since identical subtypes were found in the farm pigs and the cut meats from the same pig lots (Hellström et al., 2010). Besides, Latorre et al. (2009) reported that the potential sources of *L. monocytogenes* contamination were from bulk tank milk and in-line milk filters on a New York State dairy farm by MST study.

Food Products in Post-harvest Processing

In order to further prevent food-borne illnesses in humans, it is also important to determine where contaminating agents are entering the food supply. Bacteria can enter food processing facilities via raw products, and contamination of finished products can result from the presence and persistence of these bacteria in the processing environment of the plant. MST can add valuable information for discriminating these sources, in contrast to simply isolating and enumerating microorganisms at specific stages of processing.

Of particular concern in food processing plant is *L. monocytogenes*, as it has been shown to thrive in processing environments, and can form biofilms facilitating resistance to removal agents such as sanitizers. In studies of three cheese-processing facilities, multiple samples obtained over a 6-month period included finished product, food contact surfaces, and environmental samples (Kabuki et al., 2004). By ribotyping analysis, persistent contamination of a particular ribotype was

isolated from finished product as well as from the processing environment and food contact surfaces. In catfish and fresh fillet processing, the possible contamination sources of the *L. monocytogenes* isolates was investigated by a combination of PFGE and ERIC-PCR (Chen et al., 2010). The study suggested that *L. monocytogenes* contamination in the processed catfish fillets originated from the processing environment (mostly the fillet weighing table), rather than directly from catfish.

L. monocytogenes is particularly problematic for ready-to-eat (RTE) foods, as the consumer typically eats these foods without further heating or preparation. Contamination routes of *L. monocytogenes* were examined for 3 years in an Iberian pork-processing plant that produced high-quality RTE meat products (Ortiz et al., 2010). By PFGE analysis of isolates recovered from the environment and equipment, carcasses, raw products and dry-cured products, *L. monocytogenes* persistence appeared strongly linked to the intermediate manufactured products and the environment of the manufacturing area, and not to the raw material. Moreover, *L. monocytogenes* strains were also found in the retail environments, and a number of *L. monocytogenes* ribotypes isolated from environmental samples were identical to that from the food samples, indicating cross-contamination in retail and deli operations (Sauders et al., 2009). In addition, poor control of plant environment also facilitates *Salmonella* cross-contamination in pork products. In France, Giovannacci et al. (2001) found evidence for cross-contamination of pig

carcasses and the resulting pork cuts during slaughterhouse processing by utilizing PFGE to type *Salmonella* isolated from pigs, carcasses, meat cuts and the slaughterhouse environment.

CONCLUSIONS AND FUTURE DIRECTIONS

Microbial source tracking is an emerging field with a simple goal: to develop a tool(s) that discerns the hosts/sources of fecal indicators or food-borne pathogens found in environmental or food samples. The MST technology will allow us not only to detect fecal contamination using indicators as well as the food-borne pathogens contamination, but also to simultaneously determine the source of contamination in both water and food samples. These tools will likely have widespread applications, including use for regulatory compliance, pollution remediation, and risk assessments. These tools should facilitate a reduction in the incidence of illness associated with food and water and will improve public health and wellbeing. Potential applications of MST have been driving methods development, and we currently find ourselves with multiple library-dependent and library-independent approaches in various stages of development and validation (Harwood et al., 2009; Hassan et al., 2007; Lyautey et al., 2010). However, no one method has emerged as being superior enough to be adopted as a standard.

In the future, additional development is needed to improve the discrimination of library-independent methods for fecal source identification in the food chain. Until individual MST methods are developed to the point of being accepted as both regulatory and management

tools, one way to overcome the limitations of any one method is to perform multiple methods concurrently. For example, Ballesté and colleagues (Ballesté et al., 2010) have recently developed the combined use of different molecular indicators be the best way of defining predictive models suitable for determining fecal pollution sources. Although the performance of multiple methods may lead to increased costs, linking the results of multiple methods may allow sources of contamination to be identified by a preponderance of the evidence. Moreover, it is necessary to look for the new MST targets/markers for the development of host-specific assays with no cross-amplification in nonspecific samples. Ideally these markers should be selectively maintained within the microorganism of interest and would avoid the use of cumbersome library-based methods and statistically intense schemes. The key question, however, is how do we identify these markers? One approach would be to use a rational selection process, such as using recognized virulence markers. Another approach is to attempt to correlate the presence of specific microbes that are only found in specific hosts. For example, Field and colleagues (Bernhard and Field, 2000; Dick and Field, 2004; Dick et al., 2005) have used terminal restriction fragment length polymorphism methods to identify 16S rRNA markers that appear to differentiate between organisms that are highly correlated with either humans or other animals. The obvious extension from these efforts is to screen a larger number of markers to identify gene fragments that are highly correlated with host origin for many bacterial species inhabiting the gastrointestinal tract. The practical way for this strategy to work is for a large number of markers to be screened

simultaneously, and the most efficient tool available for this purpose is DNA microarrays. For example, an approach of suppression subtraction hybridization (SSH)-based microarrays was used to generate goose- and duck-specific markers for *E. coli* that can be used in high-throughout MST studies (Hamilton et al., 2006). Advances in molecular methods and our understanding of the microbial ecology, microbial genetics, and microbial population dynamics of targeted species are likely to allow us to increase the accuracy and ease of microbial source tracking.

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Table 1 Example case studies of molecular methods for Microbial Source Tracking used in foods and the environment

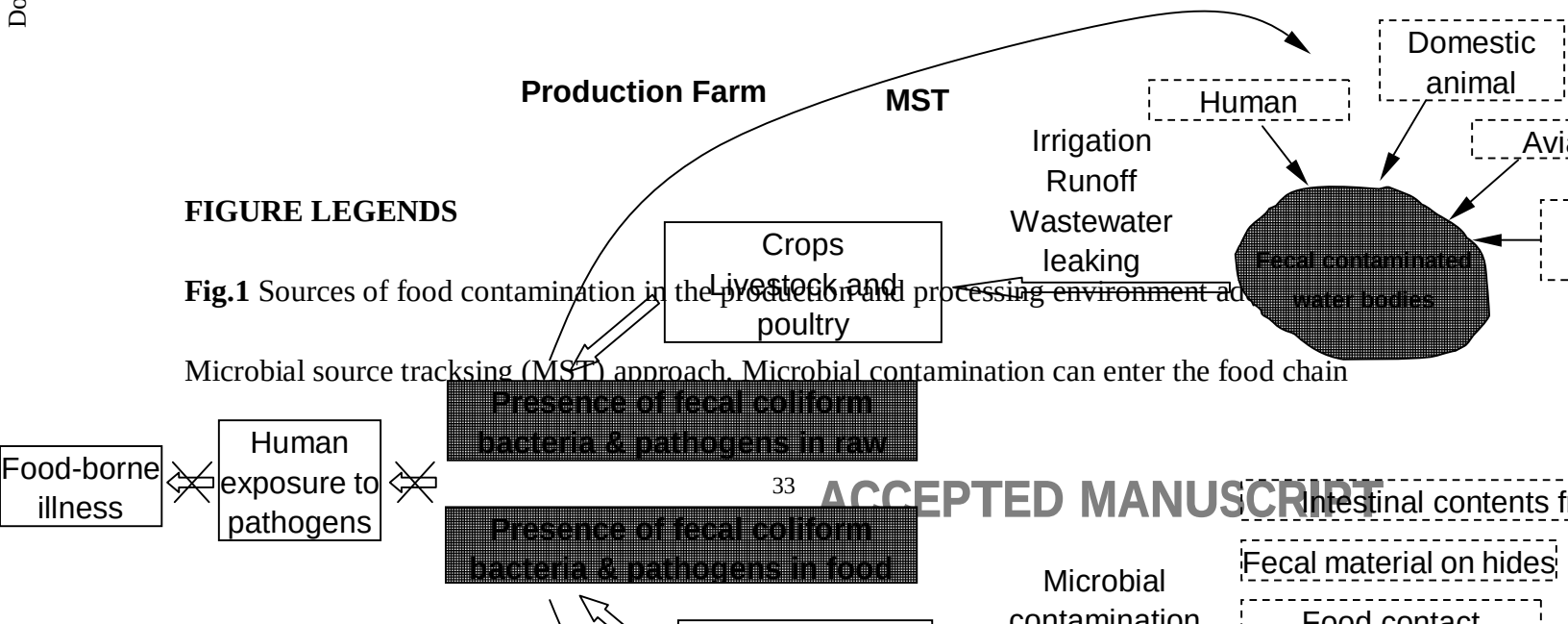
Indicator/Target	Application field	Advantages	Limitations	Referenc
E. coli *Listeria monocytogenes*	Waters (for harvesting seafood) Food chain (retail and deli operations)	(a) Highly reproducible (b) Automated fingerprinting systems available (c) Public databases available	(a) Labor-intensive (b) Reference database required (c) May be geographically	Scott et a Sauders e

		for some pathogens	specific	
Enterococcus spp. *Salmonella*	Waters (for drinking and recreation) Food chain (turkey production)	(a) Extremely sensitive to minute genetic differences (b) Differences accumulate fast enough for outbreak investigation	(a) Labor-intensive (b) Too sensitive to broadly discriminate for source tracking	Kim et al Nayak et
Shigella	Food chain (food source outbreaks)	(a) High sensitivity to genetic changes (b) Dfferences accumulate fast enough for outbreak investigation	(a) Genetic basis of fingerprint changes not immediately known (b) High expense per isolate	Sirisriro
E. coli *Salmonella*	Freshwater resources Food chain (food animal sources)	(a) Rapid (b) Easy to perform (c) No prior genomic knowledge is required	(a) Reproducibility a concern (b) Reference database required (c) May be geographically specific	Lyautey Foley et
Campylobacter coli *Bacillus cereus*	Food chain (food animal sources) Food chain (foodstuff)	(a) Highly reproducible and discriminable (b) Determines exact nucleotide differences for conserved loci (c) Data portable and internet accessible	(a) Requires prior knowledge of gene sequences (b) High expense per isolate	Miller et Cardazzo
Bacteroidales host-specific 16S rRNA gene & F-specific bacteriophage	Coastal areas (commercial and recreational shellfish areas)	(a) Host-specific PCR method is rapid, easy to perform (b) Library-independent	(a) More targets need to be identified (b) Some targeted genes have little to do with host-microbe interactions	Gourmel
Human and animal adenoviruses and polyomaviruses	Slaughterhouse sludge and wastewater		(c) Lack of molecular database	Hundesa

FIGURE LEGENDS

Fig.1 Sources of food contamination in the production and processing environment ad

Microbial source tracksing (MST) approach. Microbial contamination can enter the food chain



mainly at the production and processing levels: (i) The first level is the production environment, represented by the farm, orchard, or fishery. Contamination at this preharvest stage is particularly serious for foods that are consumed raw or undercooked. (ii) The second level associated with microbial contamination of the food chain is the processing environment represented by the slaughterhouse, cannery, or packing plant. Contamination during processing can come from a variety of sources, such as intestinal contents from carcasses, food contact surfaces, food handlers etc.

Fig.2 Use of rep-PCR to differentiate *E. coli* isolates from domesticated animal sources in the fecal contaminated shellfish growing waters of East China Sea. After isolation and characterization of the fecal indicator (*E. coli*), the DNA fingerprints of isolates were performed by rep-PCR. Cluster analysis and Multivariate analysis of variance (MANOVA) of rep-PCR DNA fingerprints revealed this method can differentiate fecal *E. coli* isolates from animal sources at the shellfish culture area of East China Sea.

