ECRONICON OPEN ACCESS

MICROBIOLOGY
Review Article

Bacterial Source Tracking of Human Fecal Pollution Using Molecular Methodologies

Samendra Sherchan*

Department of Public Health, California State University-Fresno, USA

***Corresponding Author:** Samendra Sherchan, Department of Public Health, California State University-Fresno, Fresno, CA, 93740, USA.

Received: December 20, 2014; **Published:** March 31, 2015

Abstract

Considering the serious problem of fecal contamination of coastal waters, it is important to determine sources of fecal contamination in order to prevent diseases and assure water quality. Use of molecular techniques to determine human fecal pollution and water quality has become increasingly popular day by day. Detection of *Enterococci* and *Escherichia coli* are traditional methods of indicating fecal pollution. Both of them are naturally found in different types of animal intestinal tracts and feces and have a high survivability rate in the environment. Environmental Protection Agency (EPA) water quality criterion using membrane filtration for *Enterococci* is 33 colony forming units (CFU)/100 ml and for *E. coli* is 126 (CFU)/100 ml in freshwater systems. However, determination of the amount of *Enterococci* and *E. coli* in a water system just indicates fecal contamination and are time-consuming and will take days to be concluded; therefore, several molecular methods are developed to distinguish it from human fecal pollution or non-human. *Bifidobacteria* and *Bacteriodes* are two examples of bacterial indicators for host specific identification of human fecal pollution. *Bifidobacteria* are gram-positive rods and widely found in the intestinal microflora of humans and some species like *Bifidobacteria adolescentis* can be used as a genetic marker in Quantitative Polymerase Chain Reaction (PCR) technique allowing source tracking of human fecal contamination. This short review paper will discuss molecular methods available for microbial source tracking that can help to identify the source (human or non-human) of fecal contamination in the estuaries and rivers.

Keywords: *Fecal pollution; Water quality; PCR; Quantitative PCR*

Introduction

A clean water supply is crucial to the health of the human population, economic interests, and environmental concerns [1]. Water contaminated with feces impacts public health by introducing enteric pathogenic organisms. Recreational waters, shellfish farms, beaches, and drinking waters may be compromised by faulty sewage systems, dysfunctional septic tanks, agricultural runoff, and wildlife. Additionally, fecal pollution adversely affects aquatic ecosystems. Human waste is considered a greater public-health risk because it is more likely than animal feces to contain human pathogens [2]. The detection of fecal pollution and identification of its source is imperative in assessing the health risk and eliminating its source. Bacterial source tracking (BST) is important to protect water supplies and should correlate to epidemiology studies. It also aids in enforcing legality issues concerning responsibility [3]. Though fecal contamination can be quickly identified in waters, there is no standard method of identifying the source. Because there are no effective methods, coastal water pollution still persists.

New research and advances in molecular biology are thus helpful in BST. Until recently the presence of fecal pollution has been confirmed by detection and enumeration of certain bacterial indicators on selective growth media and traditional polymerase chain reaction. These indicators, fecal coliforms, are bacteria that are passed through the intestines of warm-blooded animals. Traditionally, these culture-dependent protocols are time consuming, labor-intensive, and their reliability is questionable [4].

EPA methods that use fecal coliforms, indicator bacteria, to determine fecal pollution still have problems related to sensitivity, specificity and time-consuming. The number of colony forming units (CFU) allowed per volume of water, is a standard set forth by the EPA to monitor water quality. The number of CFUs is calculated by filtering a given amount of water, usually 100 ml. The filter does not allow bacteria to pass through. The filter is then placed on a nutrient agar and incubated. Each viable bacterium CFU forms a visible colony. These colonies are then counted and the number of CFUs per 100 ml is calculated. These methods, total fecal coliform and fecal coliform counts, are currently the exclusive standard for determining the extent of fecal contamination [5]. US EPA membrane filtration method 1600 (MF 1600) and method 1603 are the most widely used and accepted method for enumerating *Enterococci* and *E. coli* in respective populations in marine environments.

There are many problems associated with fecal coliform counts. The number of colonies in a colony count does not account for the number of non-viable cells in a given water sample and no source is identifiable. The survival rate of fecal coliforms in the environment is ambiguous relative to time and environmental stress. No direct correlation exists between the number of fecal coliforms present in a sample and the number of accompanying pathogenic microbes. In the environment fecal coliform bacteria may multiply rapidly under favorable conditions or die in mass due to unaccommodating circumstances. These methods of identifying fecal coliforms do not give an indication of the origin of fecal contamination [4].

Recently the EPA has shifted the focus of their BST methods to use indicators other than *E. coli,* such as *Enterococci*, which however, still leave questions about the origin of pollution. Although *E. coli* and Enterococci enumeration can provide valuable data on the level of fecal pollution in the environment, the concentration of these fecal bacteria do not provide any indication of the source of the fecal pollution. Enterococci spp. and *E.coli* both live in a wide range of hosts and free in the environment. The high number of CFUs indicates only that there is a large number of viable indicator bacteria present, but gives no identification of their origin. These bacteria may have come from one or many sources, including but not limited to human and non-human feces. There are no perfect bacterial indicators of fecal pollution at present. The two indicators *Enterococci* and *E.coli* multiply independent of a host in an aqueous environment. Furthermore, they generally occur in higher numbers than their counterparts [5]. In contrast, *Bifidobacteria* spp. have stringent nutrient requirements and grow poorly outside of the animal gut, making this bacterial group a potentially useful indicator of recent fecal pollution. Some *Bifidobacteria* spp. are thought to be strictly of human origin while others have been suggested as exclusively associated with animal feces. Specifically, *Bifidobacteria dentium* and *Bifidobacteria adolescentis* have been suggested as useful for tracking human fecal sources in surface waters [6,7].

Group and species-specific 16S rRNA gene assays have been developed for *bifidobacterial* populations frequently isolated from human feces [8]. While the presence of these *Bifidobacteria* spp has been determined in human, their presence and diversity in non-human hosts is yet to be tested [9]. This is critical if these species are to be considered as useful markers for the specific detection of human fecal pollution sources in environmental waters.

Microbial techniques rely on phenotypic differences acquired by related strains living in the dissimilar gastrointestinal environments. Differences in internal gut conditions allow for the indirect identification of species-specific enteric bacteria. Some variables may include temperature, nutrients available, pH, and substrates present. Selective culture methods check for the utilization of specific substrates to differentiate host specificity. Surface anatomy such as flagella or cell surface antigens has been suggested to indicate a bacterium's the host of origin [4].

Bacteria living in the human gut and the gut of livestock to some extent have acquired multiple antibiotic resistance. Fecal bacteria retrieved from a water body confirmed to have a multiple antibiotic resistance could be assumed not to have originated from a wildlife source. Antibiotic resistance most likely originates in human sources [5]. Currently there is no perfect indicator organism or technique for assessing the concentration of fecal contamination in marine environments. Various molecular methods are currently under investigation as a means of source tracking of fecal contamination. Genetic methods, such as qPCR and DNA- DNA hybridization differentiate an organism's host of origin by utilizing genetic differences within separate bacterial lineages are also been used [7].

Using PCR along with genus and species specific primers, human and non-human fecal contamination can be distinguished in estuaries and coastal areas. However, traditional plate count techniques were used to assess the level of fecal bacteria concentration in the marine environments and PCR-dependent microbial source tracking methodologies were used to identify the likely source of the fecal contamination. A combination of quantitative data on fecal contamination with molecular source techniques has been shown to be very effective in assessing fecal pollution in the other environments [7]. Direct monitoring of human pathogens, enteric viruses, and parasites (for e.g. *Cryptosporidium* and *Giardia lamblia*) directly represents the human health risk associated with contamination. Seeking the presence of these direct health threats circumvents the need for indicator bacterial assays. Unfortunately, it is difficult to detect these pathogens in the environment because they are generally present at very low, although dangerous levels. Undetectable concentrations of pathogens are great health risks. The handling of these microbes in itself presents health risks for lab researchers [5,7].

The presence of certain chemicals in water can implicate human contamination. Caffeine is excreted whole in urine and is unique to human waste. Its presence is in such minute concentrations that it would be difficult to detect and it is not known how long caffeine survives in the environment. Sodium tripolyphosphate is a whitening agent found in laundry detergent and has been used with some success to identify sewage and septic leakage. Coprostanol is byproduct of the natural intestinal flora of warm-blooded animals. There is a higher level of coprostanol present in human waste. This chemical has been proposed as a chemical indicator of human or non-human fecal contamination. These methods may hold promise in the future, but for now detection of minute amounts of these chemicals in the environment is quite difficult [4].

Bifidobacteria spp. recently emerged as a bacterium that has the potential to track sources of fecal human pollution. This bacteria is difficult to culture, having strict growing conditions, but can be easily used in molecular source tracking. Certain species such as, *B. adolescentis* are found only in the intestines of humans. This makes *Bifidobacteria* a good candidate for distinguishing between human and non-human contamination of marine waters. Using *B. adolescentis* as a genetic marker in PCR it is easy to detect human fecal contamination in estuarine waters [6].

Proposed methods for Fecal Source Tracking in Water using molecular methodologies

Researchers are trying to develop several methods for source tracking of human fecal pollution. By targeting strain, genus, and species-species portions of bacteria genomes the presence of specific strains of bacteria can be confirmed. These techniques can be applied to environmental BST. Researchers are developing new strain, genus and species-specific primers for the detection of bacterial strains to advance research in BST, human intestinal ecology, and human health issues [4].

Bifidobacteria has positive effects on the health of its host and has been the subject of much clinical research recently. Clinical researchers and environmental scientists share interest in developing a rapid method of identifying strains of *Bifidobacteria* spp. in various environments. Evidence suggests that less than 25% of the microbial flora of the human intestines is known. Fewer than 60% of these known species have been cultured. This leaves a huge potential for future research [8]. An earlier study conducted by Peter Kaufmann and associates developed an identification protocol using genus specific 16S rRNA- targeted oligonucleotide probes, Im26 and Im3, that double as PCR primers. They targeted 30 different strains of Bifidobacteria successfully identified from food and fecal samples. Only two false positive reactions occurred but the band was faint. However, a 777bp band was viewed consistently when used with human fecal

isolated DNA [8] that means tracking *Bifidobacteria* using this protocol was hard to distinguish between human fecal pollution from non-human [10].

Currently, there is no consensus on which bacterial group or qPCR approach for detecting human fecal pollution is more reliable. Several studies have used the molecular detection of *B. adolescentis* to indicate the presence of human fecal pollution in environmental samples [7]. Matsuki., *et al*. [9], developed primers (ADO 1 and ADO 2) for the detection of *B. adolescentis* in human fecal samples. Bonjoch., *et al*. [3] and King., *et al*. [6] used a nested PCR approach that amplified the 16S rRNA gene fragments from the genus *Bifidobacteria* and then amplified *B. adolescentis* from this PCR product with the ADO primer pair to improve the sensitivity and reliability of detecting *B. adolescentis* in environmental samples. Lamendella., *et al*. reported that the one step PCR protocol of Matsuki., *et al*. [9], for detecting *B. adolescentis* in fecal samples, was not as reliable as a nested PCR protocol.

Bonjoch., *et al*. [3] was able to use a one-step nested PCR program. Their PCR mixture contained the four primers necessary to amplify *Bifidobacteria* spp. And *B. Adolescentis* in one PCR protocol, however, this protocol is found to be more fruitful if the PCR was split into two protocols. The first PCR amplified *Bifidobacteria* spp. whereas the second PCR amplified the species, *B. adolescentis*. In splitting the PCR into two separate protocols, the amount of *Bifidobacteria* template was increased for amplification with the species-specific primers [6]. This protocol led to more consistent results, and potentially reduced the risk of negative results [11].

Conclusion

PCR as a means of identifying fecal contamination have many advantages over culture-based methods. A nested PCR protocol is successfully developed for the detection of a genetic marker that indicates human fecal contamination. Since PCR is performed on DNA there need not be living cells within a sample. As long as there is intact DNA in a sample the target organism can be detected. Simple PCR is relatively inexpensive and simple compared to other molecular techniques such as real time PCR. Quantitative or real-time PCR requires a more expensive thermocycler and greater expertise, but allows for enumeration of sample bacteria per volume as well as identify specific targets. Special florescent tags such as SYBR Green are used and measured during each thermocycler cycle. This allows for the calculation of the number of organisms within a given sample size as well as detection. Detection limits are more sensitive while using real-time PCR [5]. However, real- time quantitative PCR is an ideal method of source tracking if expense is not an issue for a research project. Moreover, *Bifidobacteria* and other indicator bacteria show promise as a target for a simple, quick, and reliable PCR method for tracking the source of fecal contamination in water samples of the estuaries and rivers. To conclude, *Bifidobacteria adolescentis* is found to be a good candidate for discerning between human and non-human fecal contamination in water. It is not toxic, does not reproduce in the environment and is host specific. This method is the most reliable for identifying human fecal pollution in environmental water.

Bibliography

1. Amador JA., *et al*. "Tracking human fecal contamination in tropical reservoirs in Puerto Rico". *Lakes & Reservoirs: Research and Management* 13.4 (2008): 301-317.
2. Morrison CR., *et al*. "Quantification of enterococci and bifidobacteria in Georgia estuaries using conventional and molecular methods". *Water Research* 42.14 (2008): 4001-4009.
3. Bonjoch X., *et al*. "Multiplex PCR with 16S rRNA Gene-Targeted Primers of Bifidobacterium spp. to Identify Sources of Fecal Pollution". *Applied and Environmental Microbiology* 70.5 (2004): 3171-3175.
4. Field KG., *et al*. "A Comparative Study of Culture-Independent, Library-Dependent genotypic Methods of Fecal Source Tracking". *Journal of Water and Health* 1.4 (2003): 181-194.
5. Scott TM., *et al*. "Microbial Source Tracking: Current Methodologies and Future Directions". *Applied and Environmental Microbiology* 68.12 (2002): 5796-5803.
6. King EL., *et al*. "Rapid detection of human fecal contamination in estuarine environments by PCR targeting of Bifidobacterium adolescentis". *Journal of Microbiological Methods* 68.1 (2007): 76-81.

7. Harwood VJ., *et al*. "Microbial source tracking markers for detection of fecal contamination in environmental waters: relationships between pathogens and human health outcomes". *FEMS Microbiology Reviews* 38.1 (2003): 1-40.
8. Kaufmann P., *et al*. "Identification and Quantification of Bifidobacterium Species Isolated from Food with Genus-Specific 16S rRNA-Targeted probes by Colony Hybridization and PCR". *Applied and Environmental Microbiology* 63.4 (1997): 1268-1273.
9. Matsuki T., *et al*. "Distribution of bifidobacterial species in human intestinal micro flora examined with 16S rRNAgene- targeted species-specific primers". *Applied and Environmental Microbiology* 65.10 (1999): 4506-4512.
10. Bachoon DS., *et al*. "Assessment of fecal pollution and relative algal abundances in Lakes Oconee and Sinclair, Georgia, USA". *Lakes & Reservoirs: Research and Management* 14.2 (2009): 139-149.
11. Layton A., *et al*. "Development of Bacteroides 16S rRNA gene TaqMan-based real-time PCR assays for estimation of total, human, and bovine fecal pollution in water". *Applied and Environmental Microbiology* 72.6 (2006): 4214-4224.

Volume 1 Issue 2 March 2015