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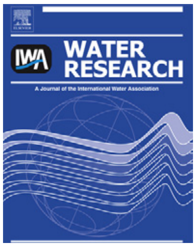

Molecular detection of pathogens in water – The pros and cons of molecular techniques

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ABSTRACT

Pollution of water by sewage and run-off from farms produces a serious public health problem in many countries. Viruses, along with bacteria and protozoa in the intestine or in urine are shed and transported through the sewer system. Even in highly industrialized countries, pathogens, including viruses, are prevalent throughout the environment. Molecular methods are used to monitor viral, bacterial, and protozoan pathogens, and to track pathogen- and source-specific markers in the environment. Molecular techniques, specifically polymerase chain reaction-based methods, provide sensitive, rapid, and quantitative analytical tools with which to study such pathogens, including new or emerging strains. These techniques are used to evaluate the microbiological quality of food and water, and to assess the efficiency of virus removal in drinking and wastewater treatment plants. The range of methods available for the application of molecular techniques has increased, and the costs involved have fallen. These developments have allowed the potential standardization and automation of certain techniques. In some cases they facilitate the identification, genotyping, enumeration, viability assessment, and source-tracking of human and animal contamination. Additionally, recent improvements in detection technologies have allowed the simultaneous detection of multiple targets in a single assay. However, the molecular techniques available today and those under development require further refinement in order to be standardized and applicable to a diversity of matrices. Water disinfection treatments may have an effect on the viability of pathogens and the numbers obtained by molecular techniques may overestimate the quantification of infectious microorganisms. The pros and cons of molecular techniques for the detection and quantification of pathogens in water are discussed.

Abbreviations: MST, microbial source-tracking; HAdV, human adenoviruses; HAV, hepatitis A virus; HEV, hepatitis E virus; JCPyV, human polyomavirus JC; BKPyV, human polyomavirus BK; PCR, polymerase chain reaction; qPCR, quantitative PCR; qRT-PCR, quantitative reverse transcriptase PCR; NASBA, acid sequence-based amplification; CFU, colony-forming units; mPCR, multiplex PCR; IFAs, immunofluorescent assays; IMS, immunomagnetic separation; RT-PCR, reverse transcriptase PCR; mRNA, messenger RNA; PAdV, porcine adenoviruses; BPyV, bovine polyomaviruses; EMA, ethidium monoazide; PMA, propidium monoazide; VBNC, viable non-culturable; PBS, phosphate buffered saline; nPCR, nested-PCR.

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Contents

1. Introduction

Significant numbers of human microbial pathogens are present in urban sewage and may be considered environmental contaminants. Although most pathogens can be removed by sewage treatment, many are discharged into the effluent and enter receiving waters. Point-source pollution enters the environment at distinct locations, through a direct route of discharge of treated or untreated sewage. Non-point sources of contamination are of significant concern with respect to the dissemination of pathogens and their indicators in the water systems. They are generally diffuse and intermittent and may be attributable to the run-off from urban and agricultural areas, leakage from sewers and septic systems, and sewer overflows (Stewart et al., 2008). Molecular methods are used to monitor viral, bacterial and protozoan pathogens, and to track pathogen- and source-specific markers in the environment.

Classic microbiological indicators such as faecal coliforms, *E. coli* and *Enterococci* are the indicators most commonly analyzed to evaluate the level of faecal contamination. They are also used to assess the efficiency of pathogen removal in water purification processes. However, whether these bacteria are suitable indicators of the occurrence and concentration of human viruses and protozoa cysts has been questioned (Lipp et al., 2001; Tree et al., 2003; Wéry et al., 2008). Indicator bacteria are more sensitive to inactivation through treatment processes and by sunlight than viral or protozoan pathogens (Hurst et al., 2002; Sinclair et al., 2009). Other limitations have been associated with their application: short survival compared to pathogens (McFeters et al., 1974), non-exclusive faecal source (Scott et al., 2002; Simpson et al., 2002), ability to multiply in some environments (Solo-Gabriele et al., 2000; Pote et al., 2009), inability to identify the source of faecal contamination (point and non-point) (Field et al., 2003) and low correlation with the presence of pathogens (Pina et al., 1998; Horman et al., 2004; Savichtcheva and Okabe, 2006). As a result, none of the bacterial indicators currently used meets all established criteria for water quality. Thus in certain cases, such as drinking or bathing water, direct analysis of specific pathogens of concern is considered to be a more suitable alternative.

Source water contamination by *Cryptosporidium* and *Giardia* presents a particular challenge to water-quality managers for several reasons. These include the ubiquity of protozoa in wastewater effluents (Carey et al., 2004), the widespread infection of domestic animals and wildlife (Fayer, 2004), the resistance of protozoans, especially *Cryptosporidium*, to traditional disinfection methods (Steiner et al., 1997), and the uncertain relationship between the presence of protozoans and faecal indicator bacteria typically used in water quality monitoring (Chauret et al., 1995; Cizek et al., 2008; Keeley and Faulkner, 2008).

Molecular techniques, specifically nucleic acid amplification procedures, provide sensitive, rapid and quantitative analytical tools for detecting specific pathogens, including new emergent strains and indicators. They are used to evaluate the microbiological quality of food and water, the efficiency of virus removal in drinking and wastewater treatment plants, and as microbial source-tracking (MST) tools (Albinana-Gimenez et al., 2009b; Field et al., 2003; Hundesa et al., 2006).

2. Pathogens in water

2.1. Viruses

The list of potentially pathogenic viruses present in urban sewage includes the DNA viruses, adenovirus and polyomavirus, and RNA viruses such as enterovirus, hepatitis A and E viruses, norovirus, rotavirus and astrovirus. Human