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Comparison of two methods for detection of fecal indicator bacteria used in water quality monitoring of the Three Gorges Reservoir

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ABSTRACT

Scientifically sound methods to rapidly measure fecal indicator bacteria are important to ensure safe water for drinking and recreational purposes. A total of 200 water samples obtained from the Three Gorges Reservoir during three successive one-year study periods (October 2009 to September 2012) were analyzed using multiple-tube fermentation (MTF) and most probable numbers combined with polymerase chain reaction (MPN–PCR). The MPN–PCR method was found to be significantly more sensitive than the MTF method for detecting *Escherichia coli* and *Enterococcus* spp., and of equal sensitivity for detecting total coliforms when all surface water samples were grouped together. The two analytical methods had a strong, significant relationship, but MPN–PCR took only 12–18 hr, compared with the 3–8 days needed using the MTF method. Bacterial concentrations varied per sampling site but were significantly lower in the mainstream of the Yangtze River than those in the backwater areas of tributaries. The water quality of 85.8% of water samples from the mainstream was suitable for use as a centralized potable water source, while the water quality of 52.5% of water samples from the backwater areas was unsuitable for recreational activities. Relationships between fecal indicator bacteria showed significant correlation ($r = 0.636–0.909$, $p < 0.01$, $n = 200$), while a weak but significant correlation was found between fecal indicators and water turbidity, water temperature, daily inflow, and total dissolved solids ($r = 0.237–0.532$, $p < 0.05$, $n = 200$). The study indicated that MPN–PCR is a rapid and easily performed deoxyribonucleic acid (DNA)-based method for quantitative detection of viable total coliforms, *E. coli*, and *Enterococcus* spp. in surface water.

Introduction

Thousands of microbial water quality decisions are made daily around the world using different methods to measure fecal indicator bacteria (FIB) (Sadowsky and Whitman, 2011). FIB are useful to indicate the potential occurrence of pathogens, because waterborne pathogens in waters are often sporadic and present in very low concentrations (Cabral, 2010). Quantitative detection of a wide array of pathogens in a given environmental sample is labor-intensive, expensive, time-consuming and impractical (McLellan and Eren, 2014). Ensuring the microbiological

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quality of different types of water to protect public health still relies on the detection of FIB as "indicators" of fecal contamination and possible associated enteric pathogens, because FIB can be detected and enumerated using much simpler, more practical and cost-effective methods (WHO, 2003; Tallon et al., 2005; Savichtcheva and Okabe, 2006; USEPA, 2012). FIB are usually commensal flora of the intestinal tract of warm-blooded animals and include total and fecal coliforms, *Escherichia coli*, *Enterococcus* spp., clostridia, and other bacteria. Of these, coliforms, *E. coli* and *Enterococcus* spp. are the most widely used as indicator organisms to determine microbial water quality (Tallon et al., 2005; Sadowsky and Whitman, 2011; Masters et al., 2011; Sidhu et al., 2012; Gilmore et al., 2014; Maheux et al., 2014).

Scientifically sound methods to rapidly measure FIB like coliforms, *E. coli* and *Enterococcus* spp. are vital in monitoring microbial water quality for drinking, recreation and food production (Sinigalliano et al., 2010; Wade et al., 2010; Sadowsky and Whitman, 2011). Test methods exist for coliforms, *E. coli* and *Enterococcus* spp. in formats ranging from presence–absence tests to most probable number (MPN), membrane filtration (MF), and polymerase chain reaction (PCR) assays (Sadowsky and Whitman, 2011). The MPN test (also called the multiple tube fermentation assay, MTF) and the MF method are extensively used as standard methods in drinking, source and recreational water quality assessment worldwide (Gronewold and Wolpert, 2008; Mesquita and Noble, 2013). However, MTF and MF methods for measuring coliforms, *E. coli* and *Enterococcus* spp. generally require two steps of presumptive and confirmative tests and take at least 72 hr. Compared with culture-based methods, PCR-based methods are faster, more sensitive and enable simultaneous detection of multiple bacteria, but it is impossible to determine concentrations of viable bacteria with PCR (Girones et al., 2010; Aghababaee et al., 2012; Xiao et al., 2013b). The MPN–PCR is a quantitative method to rapidly determine the density of viable organisms in a sample (Xiao et al., 2013b). The objectives of this study were to combine the MPN-method with duplex PCR and singleplex PCR for rapid quantitative detections of total coliforms, *E. coli*, and *Enterococci* used in water quality monitoring of the Three Gorges Reservoir, and to assess the consistency of results between the MTF and MPN–PCR methods.

1. Materials and methods

1.1. Site description and sampling time

The Three Gorges Reservoir is a 1084-km^2 artificial lake and a major water source in China (left map, Fig. 1). The Wanzhou watershed is located in the middle of the Three Gorges Reservoir and the Yangtze River runs across Wanzhou city, which is at about 800,000 population and is the largest city

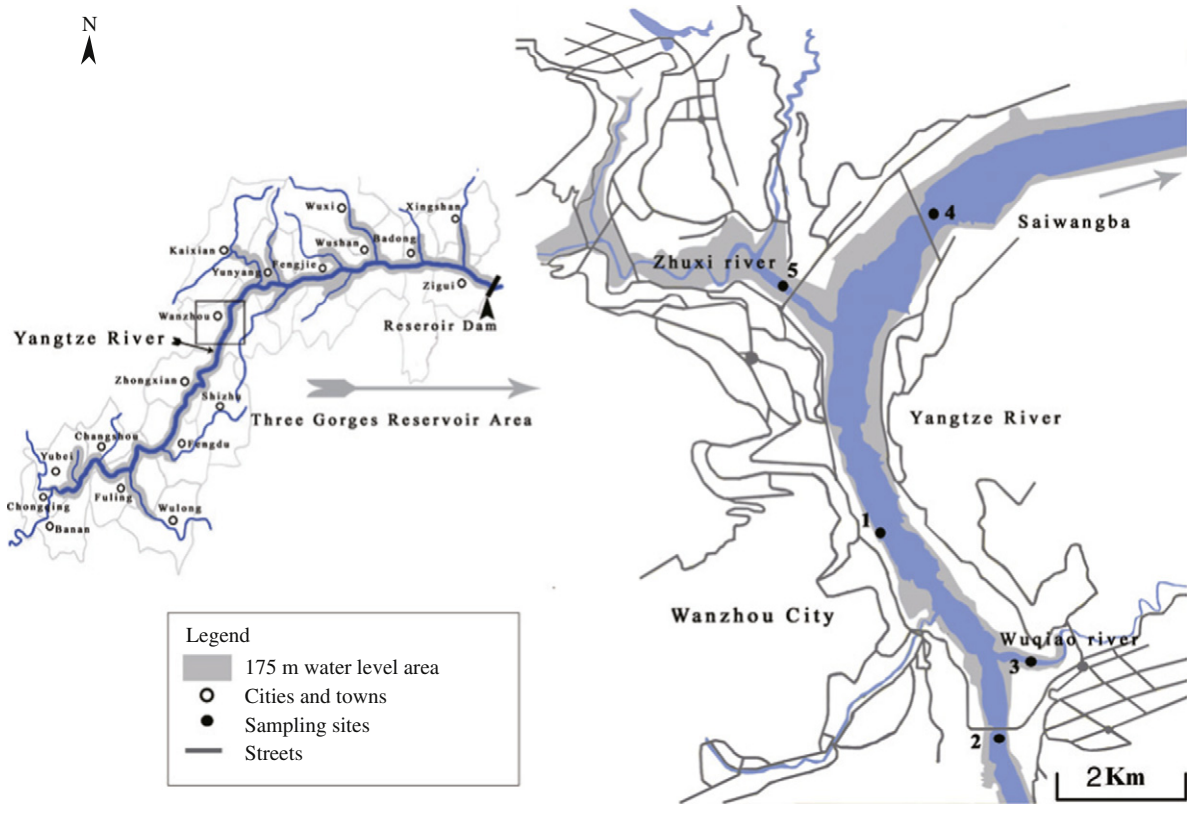

Fig. 1 – Regional (left) and local (right) maps of sampling sites. Intake of water plant (1), upstream (2), Wuqiao river (3), downstream (4), Zhuxi river (5) located in Wanzhou watershed of the Three Gorges Reservoir.