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Analytical comparison of nine PCR primer sets designed to detect the presence of *Escherichia coli/Shigella* in water samples

Andrée F. Maheux[a,b], *François J. Picard*[a,b], *Maurice Boissinot*[a,b], *Luc Bissonnette*[a,b], *Sonia Paradis*[a,b], *Michel G. Bergeron*[a,b,*]

[a]*Centre de recherche en infectiologie de l'Université Laval, Centre hospitalier universitaire de Québec (Pavillon CHUL), 2705 Laurier Blvd., Suite RC-709, Québec (Québec), Canada G1V 4G2*
[b]*Département de biologie médicale (microbiologie), Faculté de médecine, Université Laval, Québec (Québec), Canada*

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ABSTRACT

The analytical performance of 9 different PCR primer sets designed to detect *Escherichia coli* and *Shigella* in water has been evaluated in terms of ubiquity, specificity, and analytical detection limit. Of the 9 PCR primer sets tested, only 3 of the 5 primer sets targeting *uidA* gene and the primer set targeting *tuf* gene amplified DNA from all *E. coli* strains tested. However, of those 4 primer sets, only the primer set targeting the *tuf* gene also amplified DNA from all *Shigella* strains tested. For the specificity, only the primer sets targeting the *uidA* gene were 100% specific although the primer sets targeting 16S rRNA, *phoE*, and *tuf* genes only amplified *Escherichia fergusonii* as non-specific target. Finally, the primer set targeting the 16S-ITS-23S gene region, was not specific as it amplified DNA from many other *Enterobacteriaceae* species. In summary, only the assay targeting the *tuf* gene detected all *E. coli/Shigella* strains tested in this study. However, if it becomes important to discriminate between *E. coli* and *E. fergusonii*, assays targeting the *uidA* gene would represent a good choice although none of them were totally ubiquitous to detect of the presence of *Shigella* strains.

1. Introduction

In 1986, *Escherichia coli* has been established as the most reliable indicator of human fecal contamination to predict the microbiological quality of potable water. Since then, many PCR assays have been proposed to supplement or serve as a substitute to conventional recommended culture-based methods to monitor its presence (APHA/AWWA/WEF, 2005). Published tests used specific PCR primer sets targeting genes such as *uidA* (Bej et al., 1990, 1991a; Heijnen and Medema, 2006; Tantawiwat et al., 2005), *phoE* (Spierings et al., 1993), 16S rDNA (Tsen et al., 1998) as well as the gene region comprising the conserved flanking regions of the 16S rRNA gene, the internal transcribed spacer region and the 23S rRNA gene (16S-ITS-23S gene region; Khan et al., 2007). Other *E. coli*-specific PCR primer sets were also used in combination with probes (Bej et al., 1991a,b; Cleuziat and Robert-Baudouy, 1990; McDaniels et al., 1996), in multiplex PCR assays (Horakova et al., 2008), in nested PCR (Juck et al., 1996), or incorporated into real-time PCR assays (Frahm and Obst, 2003; Sandhya et al., 2008).

* *Corresponding author at*: Centre de recherche en Infectiologie de l'Université Laval, Centre hospitalier universitaire de Québec (Pavillon CHUL), 2705 Laurier Blvd., Suite RC-709, Québec (Québec), Canada G1V 4G2. Tel.: +1 418 654 2705; fax: +1 418 654 2715.
E-mail address: michel.g.bergeron@crchul.ulaval.ca (M.G. Bergeron).
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Traditionally, *E. coli* and *Shigella* are classified in different genera on the basis of biochemical and pathogenicity tests. For water quality testing, conventional culture-based methods to detect *E. coli* are based on β-glucuronidase detection, where the majority of the *Shigella* strains will not express a functional β-glucuronidase on specific *E. coli* media. However, DNA-relatedness studies have demonstrated that *E. coli* and *Shigella* should be considered as a single genetic species (Brenner et al., 1972, 1973; Lawrence et al., 1991; Paradis et al., 2005) and according to Bergey's Manual of Systematic Bacteriology (Brenner, 1984) they belong to the same species. Thus, it is practically impossible to distinguish *E. coli* and *Shigella* on the basis of their DNA sequences. Interestingly, most of the studies that proposed an *E. coli*-specific PCR assay did not address this problematic. Therefore, the detection of *Shigella* must be addressed in analytical evaluation of every *E. coli*-specific PCR assay.

The evaluation of PCR assays should be based on their rapidity, specificity (ability to target only the desired species), ubiquity (ability to detect all strains of the targeted species; Boissinot and Bergeron, 2002), and analytical detection limit. Considering that PCR assays are generally validated using less stringent criteria, a complete performance comparison of *E. coli/Shigella* primer sets available in the literature requires an extended study with a large collection of phylogenetically diverse bacterial species as well as *E. coli/Shigella* strains from various geographic origins.

In this study, 9 PCR primer sets designed to detect *E. coli/Shigella* from water samples, were compared in terms of specificity, ubiquity, and analytical detection limit using genomic DNA extracted from an extensive panel of strains (79 *E. coli*, 11 *Shigella*, and 192 non-*E. coli/Shigella* strains).

2. Materials and methods

2.1. Bacterial strains

The ubiquity (*i.e.* the ability to detect all or most *E. coli* and *Shigella* strains; Boissinot and Bergeron, 2002) of the 9 *E. coli/Shigella* PCR primer sets was verified by using 79 *E. coli* strains of both clinical and environmental origins (Table 1). Nineteen (19) *E. coli* strains, obtained from the American Type Culture Collection (ATCC; Manassas, VA), were used in this study. Thirty-two (32) clinical isolates of *E. coli* obtained from various sources were also used. These strains were obtained from Huashan Hospital (Shanghai, China; $n = 1$), Hôpital Ambroise Paré (Boulogne, France; $n = 1$), Institut für Hygiene und Mikrobiologie der Universität Würzburg (Würzburg, Germany; $n = 10$), Laboratoire de Santé Publique de Québec (Sainte-Anne-de-Bellevue, Québec, Canada; $n = 15$), National Institute of Public Health (Warsaw, Poland; $n = 1$), Microbiology Laboratory of the Centre Hospitalier de l'Université Laval (Québec, Québec, Canada; $n = 3$), and Wyeth-Ayerst Research (Pearl River, NY; $n = 1$). Environmental isolates of *E. coli* ($n = 28$) were obtained from various sources and isolated by different methods including Colilert, MI, Chromocult, mFC agar, and modified mTEC agar. These environmental strains were isolated from (i) drinking water samples obtained from the Service d'analyse environmentale Bodycote (Québec, Québec, Canada) ($n = 9$), (ii) beach water samples from Bermuda ($n = 5$), (iii) river water samples across the province of Québec, Canada from the Centre d'expertise en analyse environnementale du Québec (Québec, Québec, Canada; $n = 14$). The ubiquity of the 9 *E. coli/Shigella*-specific PCR primer sets was also assessed by using 11 *Shigella* strains representing four species (Table 1). The specificity of each PCR assay was demonstrated by testing a battery of ATCC reference strains consisting of 139 non-*E. coli* Gram-negative and 53 Gram-positive bacterial species (Table 2).

The species identification of all strains used in this study was reconfirmed using an automated MicroScan Autoscan-4 system (Siemens Healthcare Diagnostic Inc., Newark, DE, USA) or with a Vitek 32 (bioMérieux SA, Marcy l'Étoile, France). Bacterial strains were grown from frozen stocks, kept at −80 °C in brain heart infusion (BHI) medium (Beckton, Dickinson and Company, Mississauga, Ontario, Canada) containing 10% glycerol, and cultured on sheep blood, chocolate or BCYE agar depending upon the specific growth requirement of each species.

2.2. PCR assays

The sequences of the PCR primers that were evaluated in this study to detect *E. coli/Shigella* are all shown in Table 3. The PCR primer set designed for this study was developed as the following. First, *tuf* gene sequences available from public databases were analysed with GCG programs (version 8.0; Accelrys, Madison, WI, USA). Based on a multiple sequence alignment and the Oligo primer analysis softwares (version 5.0; National Biosciences, Plymouth, MN, USA), PCR primers were designed from highly conserved regions of the *E. coli tuf* gene (Paradis et al., 2005). The chosen *E. coli*-specific PCR primers were TEcol553 (5′-TGGGAGCGAAAATCCTG-3′) and TEcol754 (5′-CAGTACAGGTAGACTTCTG-3′) which generated specific amplicons of 212 base pairs. Oligonucleotide primers were synthesized by Integrated DNA Technologies (Coralville, IA, USA).

PCR amplifications for specificity and ubiquity assessment were performed using a bacterial suspension adjusted to a 0.5 McFarland standard (Fisher Scientific Company, Ottawa, Ontario, Canada). The cells were lysed using the BD Diagnostics-GeneOhm Rapid Lysis kit as recommended by the manufacturer (BD Diagnostics-GeneOhm, Québec, Québec, Canada). PCR amplifications for analytical detection limit assessment were performed using bacterial DNA isolated from mid-log-phase cultures by using a BioSprint 15 DNA blood kit (Qiagen, Mississauga, Ontario, Canada) automated with a KingFisher mL instrument (Thermo Fisher Scientific, Walthman, MA, USA). Alternatively, the manual GNOME DNA kit (Qbiogene Inc., Carlbad, CA, USA) was used. DNA concentration has been determined by measuring A_{260}/A_{280} absorbance ratio. Agarose gel electrophoresis was also used to monitor the integrity of the genomic DNA.

Briefly, 1 mL of the standardized lysed bacterial suspension was transferred directly to a 19 mL PCR mixture containing 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.5 mM $MgCl_2$, 0.4 mM each *E. coli/Shigella*-specific primer, 200 mM each deoxyribonucleoside triphosphate (GE Healthcare Bio-Sciences Inc., Baie d'Urfé, Québec, Canada), 3.3 mg per mL