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Research Article

MOLECULAR TYPING OF WATERBORNE *E. COLI* STRAINS USING REP-PCR

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ABSTRACT

Water borne bacteria, including *E. coli* as the top bacterial quality indicator, are over to be the vital concerns of some society. Typing of bacterial strains is used to confirm or reject epidemiologic evidence that a particular bacterium is the source of infection in a food type, water source or nosocomial infection. Molecular typing is a valued instrument for analysis of genetic relationship among the microbial strains. The purpose of the present study was to determine the molecular types of waterborne *E. coli* strains using REP-PCR. Present study comprised 100 waterborne *E. coli* strains isolated from different water sources in Karaj, Iran in 2013. Bacterial isolates were detected and identified *via* standard microbiological and biochemical exams. Genomic DNA was extracted *via* Genomic DNA Extraction Kit and genetic relationship among the strains was evaluated *via* REP-PCR using REP1 and REP2 primers. PCR amplicons were electrophoresed in 1.5% agarose gel and staining using ethidium bromide and visualized *via* a Gel DocTM XR+(BIORAD) and dendrogram was constructed based on Dice Comparison method and UPGMA Clustering. Using REP-PCR, all strains were typeable. Over 15 different bands ranging from 130 to 2300 bp were amplified in different profiles. Following digitizing (0 and 1) the positive genes and dendrogram construction by PAUP 4.0 software, dendrogram analysis showed the REP-PCR differentiated 100 environmental *E. coli* strains into 8 rep clusters however the r6 (28%) was the most prevalent cluster. Results of the present study indicated that *E. coli* strains isolated from different water sources in Karaj belong to diverse clones and different genotypes. Our finding also showed that REP-PCR is a powerful molecular tool with high performance and good discriminatory power for molecular typing of waterborne *E. coli.*

Keywords: *E. coli*; Molecular typing; Repetitive sequence-based PCR

Abbreviations: *E. coli*: *Escherichia coli*; UTIs: Urinary Tract Infections; ETEC: Enterotoxigenic; EHEC: Enterohemorrhagic; EIEC: Enteroinvasive.

INTRODUCTION

Waterborne diseases have a negative impact on public health in developing countries, where several people do not have access to a safe drinking water source and, therefore, several die of waterborne bacterial infections (Cabral, 2010; Ranjbar et al., 2016; Ranjbar et al., 2017a). The presence of *Escherichia coli* (*E. coli*) in drinking water is a significant concern for public health (Hunter, 2003; Tajbakhsh et al., 2015; Ranjbar et al., 2017 b). *Escherichia coli* is a commensal member of the intestinal flora of humans and many animal hosts. Several genotypes have acquired specific virulence factors and are capable of causing disease as gastrointestinal diseases, urinary tract infections (UTIs) and sepsis/meningitis (Nataro and Kaper, 1998; Kaper et al., 2004; Anvarinejad et al., 2012; Tajbakhsh et al., 2016). *E. coli* strains isolated from intestinal diseases have been grouped into at least 6 different chief groups, based on epidemiological sign, clinical features, phenotypic characters of the disease and specific virulence factors. Enterotoxigenic enterohemorrhagic (EHEC, namely O157), (ETEC, namely O148) and enteroinvasive serotypes (EIEC, namely O124) are of outstanding significance and can be transmitted over polluted water (Bettelheim, 2003; Scheutz and Strockbine, 2005; Torkan et al., 2016).

It is a well-known fact that *E. coli* happens primarily in the gastrointestinal tract of animals and humans. Therefore, it is also present in natural environment especially in water, soil, and on plants. The spread of *E. coli* in the environment is also affected by discharge of municipal sewage into surface water and soil (Watkinson et al., 2007; Hemmatinezhad et al., 2015; Jahandeh et al., 2015; Kheiri et al., 2016). The identification of *E. coli* in water is an implicit indicator of fresh fecal contamination and consequently of the hazard of cooccurrence of enteric pathogens that can cause infection in susceptible populaces (Yates, 2007). Several rules estimate and mandate agreement by recreational and drinking water quality standards on the basis of the large quantity and incidence of *E. coli* (Dufour, 1984; NRCC, 2004).

The spreading and fate of a species in a regular environment may possibly, in measure, be governed *via* range in the species;

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therefore, approximating this range is necessary. Some high-resolution molecular fingerprinting methods have been used to make public species and subspecies variety (Rademaker et al., 2000; Schloter et al., 2000; Vaneechoutte, 1996). As many as 291 and 94 rep-PCR genotypes were well-known in collections of 643 river isolates and 353 beach water *E. coli* isolates, respectively (McLellan, 2004). The incidence of pathogenic *E. coli* in environmental water creates a potential risk for infections in humans and animals especially since water is used for irrigation, a source of drinking water, and for recreational purposes (Hamelin et al., 2007; Kümmerer, 2009; Koczura et al., 2012). At this time, molecular biology-based techniques are used for epidemiological examinations for the control and of outbreaks and monitoring of the spread of potential pathogens (Rahimi et al., 2012; Raissy et al., 2014; Rahimi et al., 2017).

However, despite the high incidence of waterborne diseases in this country, there are no published data on the determination of molecular types of waterborne *E. coli* strains using REP-PCR. Therefore, the purpose of this study was to determine the presence of waterborne *E. coli* strains and describe the strain diversity of an *E. coli* population retrieved from different water sources in Karaj by using a REP-PCR method. Therefore, our results will help to determine *E. coli* strains in water and to estimate the significance of these organisms for public health. Information obtained in this study will help to determine whether water of the examined reservoir contains a variety of *E. coli* genotypes and diversity strains of *E. coli* populations that can pose epidemiological threat.

MATERIALS AND METHODS

Present study contained within 100 waterborne *E. coli* strains isolated from different water sources in Karaj, Iran in 2013. Bacterial isolates were detected and identified by standard microbiological and biochemical tests. Genomic DNA was extracted through AccuPrep® Genomic DNA Extraction Kit.

REP-PCR conditions and primers

REP-PCR primer sequences: Rep1, 5'-IIIICGICATCIG-GC-3' and Rep 2. 5'-ICGICTTATCIGGCCTA-3' and the PCR reaction conditions were as described through Versalovic et al. (1991), in a final volume of 50 ml, by minor modifications as follows: an initial denaturation (94ºC, 7 min) followed *via* 30 cycles of denaturation (90ºC, 30 s), annealing (40ºC, 1 min), and extension (72ºC, 8 min) by a single final extension (72ºC, 15 min). The size of the amplified fragments was visualized through electrophoresis in submersed agarose gel (1.5%) by 100 bp and 1 kb DNA markers (Life Technologies) as standards. The PCR for each strain was performed in three separate experiments to confirm the pattern of amplified bands.

Agarose gel electrophoresis

Agarose (1.5%) gel electrophoresis was done as described *via* (Sambrook et al., 1989).

Fingerprint analyses

Rep-PCR DNA fingerprinting of amplified DNA fragments obtained through agarose gel electrophoresis were verified. The incidence of a given band was coded as 1 and the lack of a given band was coded as 0 in a data matrix and analyzed using the PAUP 4.0 software. Dendrograms of dissimilarity were constructed for separate case.

RESULTS

By Rep-PCR, all strains involved in this study were typeable. Over 15 different bands ranging from 130 to 2300 bp examined in this study were amplified in different profiles. Overall of 100 *E. coli* isolates were included in this study, of which majority of the isolates (28%) were categorized within 6 Rep clusters (Table 1). Following dendogram analysis (Figure 1), Rep-PCR could categorize the strains within 9 Rep clusters (Table 1).

Dendrogram (Figure 1), which showed the results of cluster analyses aiming at determining most closely related isolates, as well as verification of their potential relationship with the reference strain, were constructed based on the rep-PCR analyses. The figures show less significant diversity between *E. coli* isolates. Based on these dendrograms, it can be concluded that the examined isolates are characterized by small genetic diversity and they can be easily grouped into clusters.

DISCUSSION

Ensuring water safety is an ongoing challenge to public health providers. Evaluating the presence of fecal contamination indicators in water is important to defend public health from diseases caused by waterborne pathogens (Mendes Silva and Domingues, 2015). In the current study, overall of 100 *E. coli* isolates were recovered from different water sources. Overall, a relative high genotypic diversity was observed among the waterborne *E. coli* isolates. Small water systems that supply rural townships or camps have commonly been associated with waterborne outbreaks (Olsen et al., 2002).

The result of water borne strains with the similar genotypes, and the relationship of virulence gene profiles among strains, recommends that transmission of *E. coli* may happen among animals and humans or both host species are infected *via* a public source. Naturalized *E. coli* populaces have been identified in a range of environments, such as water or soil, and in tropical, temperate, or cold regions (Byappanahalli et al., 2006; Beversdorf et al., 2007; Byappanahalli et al., 2007; Ishii et al., 2007).

Table 1: Percentages of *E. coli* isolates.

Cluster	1r	2r	3r	4r	5r	6r	7r	8r
Number	14	2	7	19	9	28	10	11
Percent	14%	2%	7%	19%	9%	28%	10%	11%

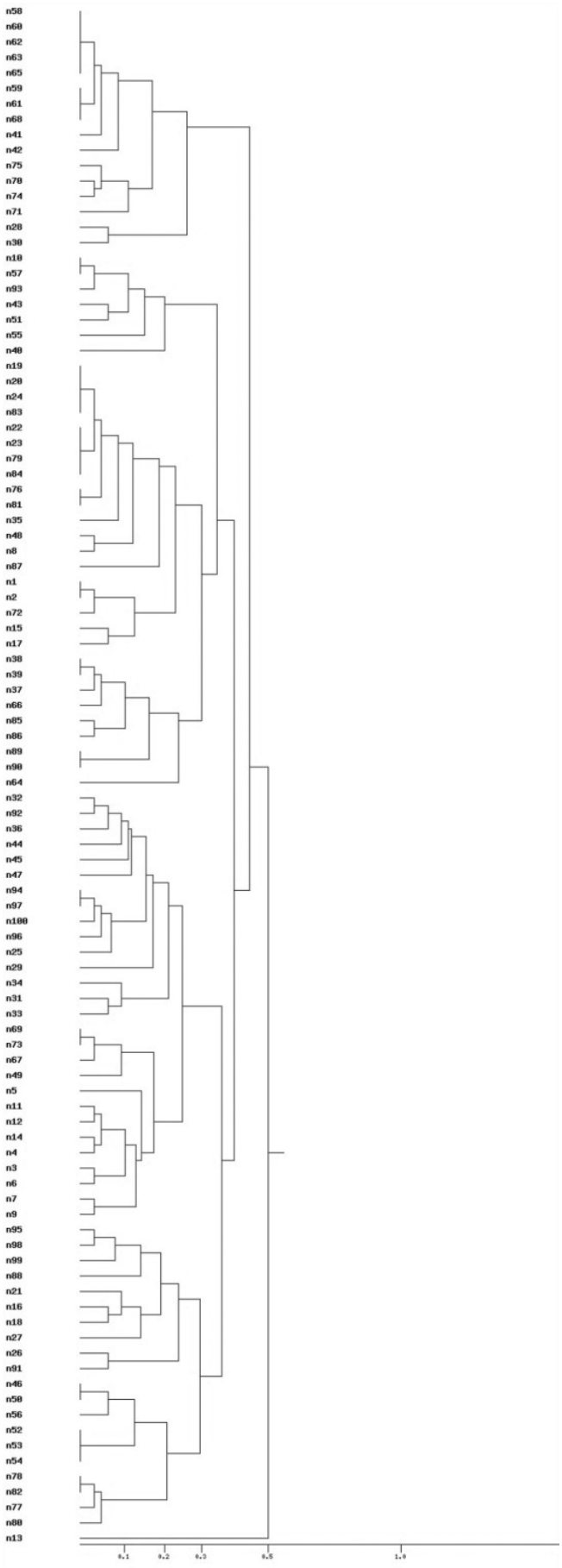

Figure 1: Dendrogram cluster analysis.

Escherichia coli genotypic and phenotypic diversity is believed to be extended (Ishii and Sadowsky, 2008), and it has been recommended that collections of as various as 40,000 isolates might be essential in order to capture all of the *E. Coli* diversity based on rep-PCR DNA fingerprinting (Johnson et al., 2004). The result of our study showed, Rep-PCR could categorize the strains within 9 Rep clusters. Several characteristics of this enormous diversity have significance for the valuation and organization of water quality. Rep-PCR is a microbial source tracking (MST) technique generally employed to elucidate the source of fecal contamination of surface water (Johnson et al., 2004; EPA, 2005; Edge and Schaefer, 2006).

REP-PCR was a number of as the molecular typing method for the *E. coli* isolates since it is rapid, reproducible, easy to perform, and highly discriminatory at the subspecies level (Olive and Bean, 1999), yielding results that compare well by pair wise DNA-DNA analyses (Rademaker et al., 2000). The present study presented, clusters were famous *via* host species nonetheless did depend powerfully on virulence gene profiles. Earlier studies that have used microbial source tracking (MST), PFGE, and whole genome sequencing to compare O26 strains from food animals and human have establish like results (Leomil et al., 2005; Ju et al., 2012).

In additional lake study showed on 11 sites over a 9-month period, repPCR genotyping of *E. coli* isolates showed that a little *E. coli* genotypes consistently dominated populations recovered from the area (Walk et al., 2007). As it has been described before, phages infecting distantly related bacterial hosts usually share slight or no nucleotide sequence similarity, even though phages infecting a specific bacterial host are additional similar (Hatfull, 2008).

Escherichia coli isolates examined in this study are characterized by small genetic diversity and they can be easily grouped into clusters. REP-PCR is a powerful molecular tool with high performance and good discriminatory power for molecular typing of waterborne *E. coli.* Therefore, the results show that it is urgent to evaluate the management of different water sources and their quality in Karaj to prevent the emergence of infectious outbreaks.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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