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Detection of Entamoeba in river water using multiplex-PCR

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ABSTRACT

In the last 20 years, molecular technique based on 16S rRNA gene and other genetic markers have been developed for bacteriological and analysis of lakes, soil. PCR based pathogen detection resolved some of the problems encountered using conventional methods. PCR has a detection limit of <10 copies of specific gene present in mixed sample. PCR reaction are designed to either amplify a single product or to use several primer pairs as part of multiplex PCR.

Keywords: multiplex PCR, multiplex PCR, Entamoeba

INTRODUCTION

Entamoeba is a genus of phylum Amoebozoa. It contains many species, six of which – *Entamoeba histolytica, Entamoeba dispar, Entamoeba moshkovskii, Entamoeba polecki, Entamoeba coli* and *Entamoeba hartmanni* resides in the human intestinal lumen. Among these the only species responsible for the pathological sequelae in humans is Entamoeba histolytica, others as non-pathogenic parasites. These parasites have a global distribution and the disease it causes is usually characterized by diarrhea. (Fotedar *et al*., 2007).

Though *E. histolytica*, *E. dispar* and *E. moshkovskii* are morphology identical, they can be differentiated at molecular level. (Tanyuksel and Petri 2003; Fotedar *et al*., 2007).

The morphological similarities among the pathogenic *E. histolytica*, non-pathogenic *E. dispar* and free-living *E. moshkovskii* has made the differential diagnosis of the three species by PCR necessary.

PCR – based approaches are the best method to achieve desired result for clinical and epidemiological studies in the developed countries. (Calderaro, *et al*., 2006; Hamzah, *et al*., 2006, Haque, *et al*., 2006 and Zaki, *et al*., 2002). PCR assay has been utilized to increase sensitivity and specificity of Entamoeba diagnosis in a variety of clinical specimens including fecal and liver abscess pus samples. (Anane and Khalid, 2005; Paul, *et al*., 2007; Kurt, *et al*., 2008).

PCR by amplifications of small subunit r RNA gene (SSU – rDNA) was reported to be 100 times more sensitive than ELISA for *E. histolytica* detection. (Mirelaman, *et al*., 1997; Fotedar, *et al*., 2007).

Small subunit r RNA gene (SSU – rDNA) is widely used as a target for the differential detection of Entamoeba species, as this target is present in multi copies when compared to DNA target present in single - copy gene, present on extra – chromosomal plasmids. (Bhattacharya, *et al*., 1989).

Several Molecular Diagnostic tests including Conventional nested and real – time PCR has been developed for the detection and differentiation of *Entamoeba histolytica, Entamoeba dispar*, *Entamoeba moshkovskii* in clinical samples. (Fotedar, *et al*., 2007).
Fode – Vaughan, *et al*., (2003) applied a simple and rapid Direct PCR (DPCR) approach for the detection of *E. coli* 0157: H7 in environmental water samples and milk using the untreated samples directly as a template in PCR and eliminating the steps of cell recovery or DNA extraction prior to PCR.

Santos, *et al*., (2010) developed a PCR procedure followed by the DNA sequencing analysis of specific regions of 18S r RNA gene to differentiate Entamoeba species commonly found in human's stools.

PCR BASED DETECTION OF *E. COLI*:

Escherichia coli are gram-negative coliform bacteria which grow abundantly in lower intestine of warm-blooded animals, expelled through feces and are easily culture-able (Winfield and Groisman, 2003).

E. coli is a clonal species including both commensal and pathogenic strains. As a pathogen *E. coli* is best known for its ability to cause intestinal diseases, Urinary Tract Infection in the brain stem.

Six classes or virotypes of *E. coli* that caused diarrheal diseases and now recognized and classified by the method of pathogenesis as:

1. Enteropathogenic *E. coli* (EPEC)
2. Enterotoxigenic *E. coli* (ETEC)
3. Enteroinvasive *E. coli* (EIEC)
4. Enterohaemorrhagic *E. coli* (EHEC)
5. Enteroaggregative *E. coli* (EAggEC)
6. Diffusely Adherent *E. coli* (DAEC)

Each class falls within a serological subgroup and manifests distinct features in pathogenesis. (Nataro and Kaper, 1998).

E. coli is a bacterium commonly found in the intestine of human and other mammals. Though most of the *E. coli* strains are harmless commensal, some strains can cause severe diseases. These pathogens are transmitted through consumption contaminated drinking water and foods such as raw or undercooked ground meat products, raw milk and even vegetables. (Kaper, *et al*., 2004).

Among the indicators of fecal contamination *E. coli* is considered the most reliable one, as its presence directly relates fecal contaminations with its implied threat of the occurrence of enteric disease. (Rice, *et al*., 1991).

Molecular methods such as "Polymerase Chain Reaction" (PCR) and molecular probes have been extensively used to detect *E. coli* involved in gastrointestinal infections. (Natro and Kaper, 1998).

Sandra (2004) used repetitive element anchored PCR to evaluate the genetic profile of *E. coli* isolated from surface water contaminated with urban storm water, sanitary sewage and gull feces to determine if strains found in environment samples reflect the strain composition of *E. coli* obtained from host sources.

Md. Shahedur Rahman, *et al*., (2011) explored the antimicrobial activity of extracts of natural spices on multiple drug resistant enteric pathogen like Escherichia coli isolated from drinking water and suggested the possibility of their use as new and effective herbal medicines to treat and prevent diarrheal diseases.

Diarrheagenic *E. coli* strains were among the first pathogens for which molecular diagnostic approaches were developed.

Molecular Approach like PCR showed ability to amplify and detect diarrheagenic *E. coli* and can be implemented in the diagnosis water, food-borne outbreaks related to *E. coli*. (Ahmed, *et al*., 2008).

Multiplex PCR (mPCR) has been developed for the detection of multiple bacterial species in a single test by amplification of multiple gene targets. A number of studies have reported on m PCR detection of multiple

waterborne pathogens in marine water samples. (Kong, *et al*., 1999; Kong, *et al*., 1995 and Kong, *et al*., 2002).

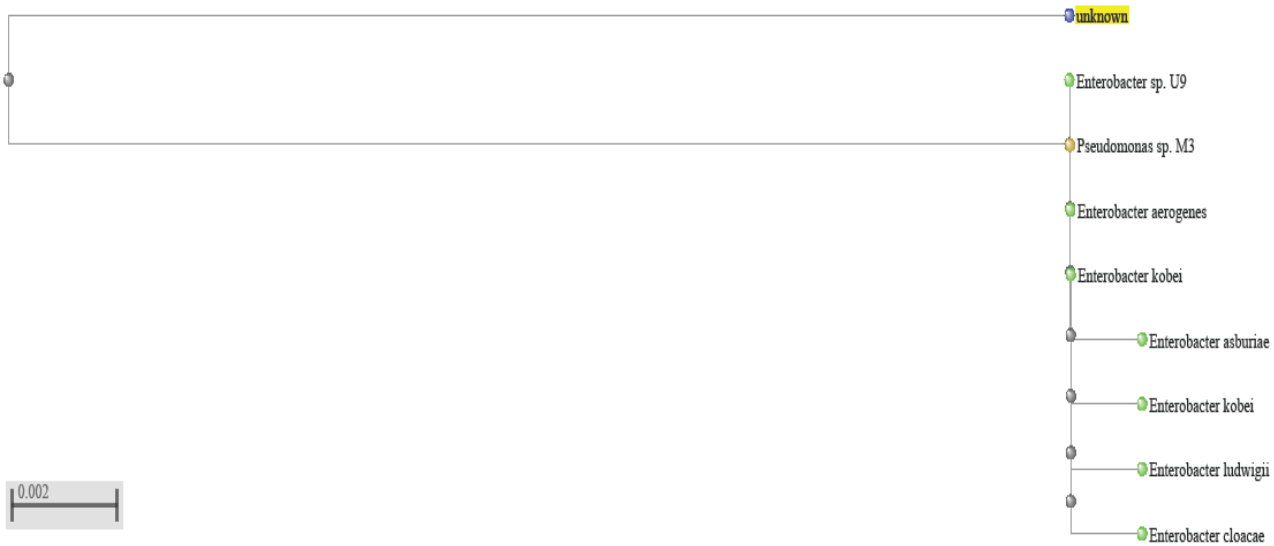
SEROTYPING OF *E. COLI*:

Serotyping with the 'O' antigen and the 'H' yields important epidemiological information.

Serotyping occupies a central place in the differentiation of pathogenic and non-pathogenic *E. coli* strains as specific Serogroups are associated with certain clinical syndromes. (Kaper, *et al*., 2004).

There are over 700 serotypes of *E. coli* found in humans, of which a small number of strains cause disease and are distinguished only by their 'O', 'H' and 'K' antigens. (Todar, 2002).

S3 Phylogenetic Tree



S4 Phylogenetic Tree

>S3 Sequences

CACCGTGGCATTCTGATCCACGATTACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGACTACGACGCAC
TTTATGAGGTCCGCTTGCTCTCGCGAGGTCGCTTCTCTTTGTATGCGCCATTGTAGCACGTGTGTAGCCCTGGTCGTAAGGGCC
ATGATGACTTGACGTCATCCCCACCTTCCTCCAGTTTATCACTGGCAGTCTCCTTTGAGTTCCCGGCCGGACCGCTGGCAACAA
AGGATAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATTTCACAACACGAGCTGACGACAGCCATGCAGCACCTGTCTCACG
GTTCCCGAAGGCACATTCTCATCTCTGAAAACTTCCGTGGATGTCAAGACCAGGTAAGGTTCTTCGCGTTGCATCGAATTAAAC
CACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAACCTTGCGGCCGTACTCCCCAGGCGGTCGACTTAA
CGCGTTAGCTCCGGAAGCCACGCCTCAAGGGCACAACCTCCAAGTCGACATCGTTTACGGCGTGGACTACCAGGGTATCTAATC
CTGTTTGCTCCCCACGCTTTCGCACCTGAGCGTCAGTCTTCGTCCAGGGGGGCCGCCTTCGCCACCGGTATTCCTCCAGATCTCT
ACGCATTTCACCGCTACAC

>S4 Sequences

GCCCTCCCGAAGGTTAAGCTACCTACTTCTTTTGCAACCCACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGT
ATTCACCGTAGCATTCTGATCTACGATTACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTCCAATCCGGACTACGACG
CACTTTATGAGGTCCGCTTGCTCTCGCGAGGTCGCTTCTCTTTGTATGCGCCATTGTAGCACGTGTGTAGCCCTACTCGTAAGG
GCCATGATGACTTGACGTCATCCCCACCTTCCTCCAGTTTATCACTGGCAGTCTCCTTTGAGTTCCCGGCCTAACCGCTGGCAA
CAAAGGATAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACATTTCACAACACGAGCTGACGACAGCCATGCAGCACCTGTCTC
AGAGTTCCCGAAGGCACCACCGCATCTCTGATAAGTTCCGTGGATGTCAAGAATAGGTAAGGTTCTTCCCGTTGCATCCAATTA
AACCACATGGTCCACCGCTTGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAAACTTGCGGCCGTACTCCCCAAGCGGTCGACT
TAACGCGTTTGCTTCCGAAGCCACGGCTTCAGGGCACAACCTTCTAAGTCCACCTCGTTTACGGGGTGGACTAACAAGGTAACT
AATCCTGGTTGCTCCCCACGCTTTCCCACCTGAACGTTAATCTTTGTCCAGGGGGCCGCCTTC

When analyzed by serotyping, significant differences have been observed between fecal *E. coli* isolates from human and wildlife sources. (Parveen, *et al*., 2001).

Mohamed Hatha, *et al*., (2004), analyzed the prevalence of diarrheagenic serotypes of *E. coli* in the Cochin estuary, by serotyping the strains and worked out the seasonal variations in the prevalence levels of these organisms.

The following are the results of characterization of Pathogens from the positive samples (S3 & S4) and gene sequences.

CONCLUSION:

The deterioration of the quality of surface water is alarming and regular monitoring and preventive measures are required.

The present study warranted to safeguard ourselves against emerging water-borne pathogens.

During the study it was found that maximum number of physico-chemical parameters within the desirable limits (excepts alkalinity, total hardness and calcium), as suggested by WHO (1971) and BIS (1991).

As coliforms *E. coli* and Entamoeba histolytica were found in the water samples, hence precautions should be taken in usage of this water.

The results add to the increasing evidence of the occurrence of diarrheal pathogens in surface water bodies that may represents a threat to human beings exposed to water-borne pathogens and related consequences.

The present investigation may be worthwhile for the clinical laboratories of the surrounding locality to characterize *E. coli* serotypes isolated from the diarrheal patients, so as to get a clear picture of the emergence of pathogenic strains of *E. coli* in the study area.

Enumeration of culture able bacteria using count plates, isolated colonies with distinct morphologies from each plate were characterized by 16S r RNA gene sequencing using extracted genomic DNA from water samples.

PCR has been suggested as a sensitive and rapid alternative to traditional growth based methods for detection of bacteria in water.

PCR provides a useful molecular approach for the detection of diarrheal pathogens in environmental

samples. This approach is an alternative to the existing technique or may be used in conjugation with these methods. This can also be useful for the detection of other pathogens.

Serotyping is a common method used for the characterization of *E. coli* isolates and has a broad use in epidemiological and medical diagnosis. The existence association between serotype and pathotype makes this method a valuable tool for typing *E. coli* and other bacterial species.

Conflicts of interest: The authors stated that no conflicts of interest.

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