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Identification of *Escherichia coli* from potable water sources of Durg-Bhilai, Chhattisgarh (India), using 16S rRNA gene sequence Analysis

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Abstract
Potable water sources mainly municipal water and ground water (from tube wells) were studied for the presence of multidrug resistant pathogenic *Escherichia coli*. A total of 11 isolates were obtained, eight from municipal water and three from tube well water. Antibiotic resistance analysis (ARA) showed that only two strains named EcPH2 and EcPul2 isolated from municipal tap waters were multidrug resistant (MDR). The strains were characterized using 16S ribosomal RNA gene sequencing. Phylogenetic analysis and similarity search showed isolate EcPH2 had 98% similarity and EcPul2 had 94% similarity to *E. coli.* The 16S rRNA sequences were submitted to Genbank with accession no. KC254645, KC254646 for isolate EcPH2 and isolate EcPul2 respectively.

Keywords: *E. coli,* Multidrug resistance, Potable water, 16S rRNA gene sequencing

INTRODUCTION

Diseases attributed to contaminated drinking water contribute a major burden on public health. Thus protecting source waters and implementing proper treatment plans is necessary for ensuring safety of drinking water supplies (WHO, 2004). Identification of contaminating agent is also extremely important in understanding the nature of threat posed by the source. The 16S rDNA sequencing has been used for rapid identification of bacteria from various environments and to determine taxonomy, phylogeny and diversity among the different organisms (Drancourt *et al*., 2000). In Durg district of Chhattisgarh, drinking water is supplied mainly by the Municipal Corporation to both rural and urban areas. The source of raw water is river Shivnath. Ground water is mainly developed in the form of public tube wells which also provide drinking water. This study aimed at isolating and identifying multidrug resistant *Escherichia coli* from both these drinking water sources using the 16S rDNA sequencing technique.

MATERIALS AND METHODS

Study Area and Collection of Samples

Water samples for microbiological analysis were collected randomly from Durg-Bhilai region. Municipal water samples (*n*=74) were collected from BSP Sector 10 (BSP), Supela (Sup), Power House (PH), Ruabandha (Ru), Borsi (Bor), Pulgaon (Pul) and Durg Station road (DSR), from taps situated along Municipal distribution lines. Ground water samples (*n*=30) were collected from tube wells from Chavni (Cha), Bhilai-3 (Bh) and Khursipar (Khu) areas. A volume of 100mL was collected for both municipal and ground waters. For chlorinated tap waters, samples were collected aseptically in sterile screw cap bottles containing sodium thiosulfate (0.1mL of 1.8% (W/V) solutions per 100mL of sample) to neutralize the residual chlorine (APHA, 1998).

Isolation and Identification *of E. coli*

The samples were filtered using membrane filters (Nylon66-Axiva Lab Filters, Delhi) of pore size 0.45µm. The filters were transferred to eosine methylene blue (EMB) agar plates (HiMedia Pvt. Ltd., Mumbai) and incubated at 30°C for 2 h and thereafter at 44.5°C for 20 h, for the selective isolation of thermotolerant *E. coli* (Feng *et al*., 2002).

Antibiotic Resistance Analysis

Disc diffusion method using HiMedia Mueller Hinton Agar (Andrews, 2007) was used to determine the antimicrobial resistance pattern of the isolates for the antibiotics viz., amoxicillin (Amx 30µg), chloramphenicol (C 10µg), co-trimoxazole (Cot 25µg), ceftriaxone (Ctr 30µg), ciprofloxacin (Cip 5µg), cephotaxime (Cfx 30µg), nalidixic acid (Na 30µg), gentamycin (Gen 10µg) and tetracycline (Te 30µg). The zone sizes were measured in millimetre and characterized as sensitive, intermediate and resistant according to standard interpretative chart (CLSI, 2007). Multidrug resistant (MDR) isolates were identified as those which showed resistance to three or more antibiotics.

DNA Sequencing

PCR Amplification of 16S rDNA

Pure culture of isolates was grown in Luria Bertani (LB) broth and genomic DNA was extracted following the protocol by Sambrook *et al.* (1989). PCR amplification of the 16S rRNA gene was performed using the universal primers 27F 5´-AGAGTTTGATCCTGGCTCAG-3´ (Lane, 1991) and 1492R 5´-GGTTACCTTGTTACGACTT-3´ (Turner *et al*., 1999) (Bioseve,

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Hyderabad). The reaction mixture (50µL) contained 30mM Tris (pH 8.4), 50mM KCl, 1.5mM $MgCl_2$, 50mM concentrations of each deoxynucleoside triphosphate, 10pmol of primer, and 1 U of *Taq* polymerase (Genei, Bangalore). PCR reaction conditions in MJ Research DNA Engine Tetrad were, 1 cycle of 95°C for 5min, 30 cycles of [94°C for 1min, 55°C for 1min, 72°C for 1min], 1 cycle of 72°C for 1min.

Purification of PCR Product and 16S rRNA Gene Sequencing

15µL of amplified DNA products were dissolved in 50µL of PCR cleanup solution mixed well and incubated at 55°C for 15min. The mixture was centrifuged at 12000 rpm for 15min to remove the supernatant. DNA pellet was precipitated by the addition of 600µL of 80% ethanol and centrifuged at 12,000rpm for 15min. Finally, the DNA pellets were dried and dissolved in 15µL of Milli Q water (Millipore, USA). Purified 16S rDNA product was extended using the primers 27F 5´AGAGTTTGATCCTGGCTCAG3´ and 1492R 5´GGTTACCTTGTTACGACTT3´. The extension products were purified by isopropanol precipitation. The purified extension products were separated using Big Dye chemistry in the ABI 3730xl DNA Analyzer (Applied Biosystems Inc.) by capillary electrophoresis. Sequence data analysis was done using Sequencing Analysis software.

Data Analysis

16S rRNA gene sequences of isolates were compared to the non-redundant sequences database (GenBank, EMBL and DDBJ) using the BLASTn program in the National Centre for Biotechnology Information (NCBI) website (http://blast.ncbi.nlm.nih.gov/Blast.cgi.). Multiple sequence alignment was performed for homologous sequences and a phylogenetic tree was constructed using the neighbour joining method. The 16S rRNA gene sequences were also compared with database sequences at the Ribosomal Database Project (RDP) (Wang *et al.*, 2007) using the RDP classifier programme (http://rdp.cme.msu.edu/) for identification of the strains.

RESULTS AND DISCUSSION

A total of 8 *E. coli* isolates were identified from municipal tap water and 3 from tube well water using preliminary biochemical tests. Of the 11 isolates from both sites, resistance to one or more antibiotics was shown by 63.64% of isolates. Two MDR strains were isolated from municipal tap waters of Power House and Pulgaon which were named as EcPH2 and EcPul2 respectively. Isolate EcPH2 showed resistance to ciprofloxacin, gentamycin and tetracycline, while isolate EcPul2 was resistant to amoxicillin, gentamycin and tetracycline.

PCR amplification and sequencing of the 16S rRNA gene yielded 1467 bp sequence in isolate EcPH2 and 1465 bp sequence in EcPul2. The GenBank sequences obtained for isolates were KC254645 for isolate PH2 and KC254646 for isolate Pul2. The BLAST similarity search against database sequences revealed that the isolate EcPH2 had 98% similarity with a maximum score of 2464 bits, to *Escherichia coli* strain Y38 16S ribosomal RNA gene partial sequence (Accession no. AB480777.1). Isolate EcPul2 had 94% similarity with a maximum score of 2132 with *Escherichia coli* strain BEE15 16S ribosomal RNA gene partial sequence (Accession no. EF560791.1). A separate phylogenetic tree was constructed for both isolates using the homologous sequences from the BLAST search, showing relationship to *E. coli* strains (Fig.1 and Fig.2). Previous studies have also reported the use of 16S rRNA gene sequencing for identification of bacterial strains from water sources. Magray *et al.* (2011) have reported the identification of *E. coli* from Dal Lake, Srinagar, India, using 16S rRNA gene and 16S-23S rRNA internal transcribed spacer region sequences. Zwart *et al*. (1998) observed similarity in 16S rRNA sequences recovered from fresh water bacteria from lakes in North America and Europe.

RDP rank classifier programme for 16S rRNA sequence classified the isolates ECPH2 and ECPul2 as strains belonging to the genus *Escherichia coli* with a confidence level of 95%. The classification of isolate EcPH2 is:

KC254645,EcPH2
Root[100%]
Bacteria[100%]
"Proteobacteria"[100%]
Gammaproteobacteria[100%]
"Enterobacteriales"[100%]
Enterobacteriaceae[100%]
Escherichia/Shigella[100%]
The classification of isolate EcPul2 is:
KC254646,EcPul2
Root[100%]
Bacteria[100%]
"Proteobacteria"[100%]
Gammaproteobacteria[100%]
"Enterobacteriales"[100%]
Enterobacteriaceae[100%]
Escherichia/Shigella[66%]

In this study we used the 16S rDNA sequencing technique to identify two bacterial strains EcPH2 and EcPul2 isolated from drinking water sources of Durg-Bhilai in Chhattisgarh as *Escherichia coli* strains. The presence of multiple drug resistant pathogenic *E. coli* in drinking water poses great threat for both humans and animals consuming that water. Quick and accurate identification of microorganisms is essential for clinical as well as epidemiological studies during disease outbreaks. 16S rRNA gene sequencing can therefore be used in place of traditional culturing techniques for this purpose.

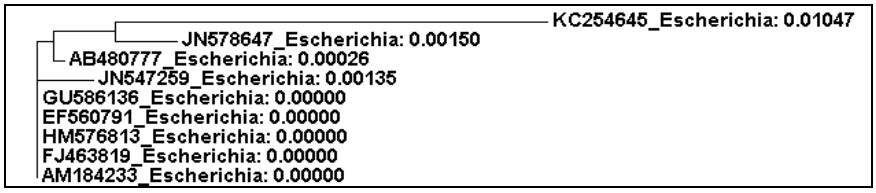

Fig 1. Phylogenetic tree with distance, of isolate EcPH2 (GenBank accession no. KC254645) created by BLAST similarity search of database sequences and using neighbour joining method.

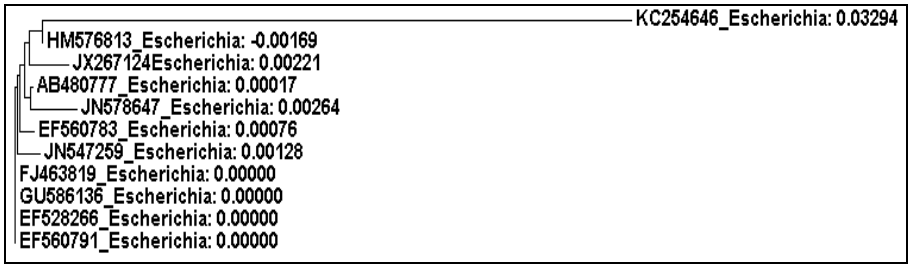

Fig 2. Phylogenetic tree with distance, of isolate EcPul2 (GenBank accession no. KC254646) created by BLAST similarity search of database sequences and using neighbour joining method.

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