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Research Article

GENOTYPIC PROFILING OF VIRULENT *ESCHERICHIA COLI* ISOLATED FROM URINARY TRACT INFECTIONS IN COIMBATORE

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A B S T R A C T

A total of 139 urine samples including *E.coli* UTI and UTI suspected samples were collected from Coimbatore and surrounding districts of Tamil Nadu, India. The samples were assayed by various molecular biology tools like RAPD, ERIC-PCR, REP-PCR and 16SrRNA identification and compared with a standard strain MTCC 326. The RAPD, ERIC and REP profiles were done for the samples. The results showed sensitivity and accuracy in the molecular detection and estimation of *E.coli* from clinical samples. The methods were validated and optimized against conventional phenotypic identification methods due to high efficiency, accuracy and reproducibility within limited time duration.

INTRODUCTION

Escherichia coli is a common resident of the gastrointestinal tract of humans and animals (Pak *et al*., 2001). Usually, *E. coli* forms a beneficial symbiotic relationship with its host and plays important roles in promoting the stability of the luminal microbial flora and in maintaining normal intestinal homeostasis (Yan *et al*, 2004). As a commensal, *E. coli* rather remains harmlessly confined to the intestinal lumen and rarely causes a disease. However, in the debilitated or immuno suppressed host, or when the gastrointestinal barriers are violated, even nonpathogenic-commensal strains of *E. coli* can cause infection (Kaper*et al*., 2004). Some strains of *E. coli* can diverge from their commensal cohorts, taking on a more pathogenic nature (Zachary *et al*., 2015). These strains acquire specific virulence factors (via, DNA horizontal transfer of transposons, plasmids, bacteriophages, and pathogenicity islands) which confer an increased ability to adapt to new niches and allow the bacteria to increase the ability to cause a broad spectrum of diseases (Benny *et al*.,2018).

Urinary tract infections (UTIs) are considered the most disregarded diseases in the world and accountable for one fourth of the health care related infections. They are a most prevalent infectious disease causing serious irregular medications (Govindan *et al*.,2018).

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It has been calculated worldwide that about 150 million people suffer from asymptomatic and symptomatic UTIs each year (Moges *et al*.,2014). The diagnosis and treatment procedures are still a challenge since various infections are asymptomatic, and the association of related symptoms with the infections is uncertain (Hull *et al*., 2000). Most of the UTI producing organisms are enhancing the resistance against all the antibiotics (ampicillin, amoxicillin and third generation of cephalosporin) including fourth generation cephalosporin due to other co-expressed resistance mechanisms (Sabita *et al*., 2014); they lead to multi drug resistance (MDRs). Several studies show geographic variations in etiologic agents of UTIs and their resistance patterns to antibiotics (Nyah-tuku *et al*., 2016).

Current protocols of genotyping include restriction fragment length polymorphism identification (RFLPI) of genomic DNA, random amplified polymorphic detection (RAPD) of genomic DNA, increased fragment length polymorphism detection (AFLPD), polymerase chain reaction (PCR), DNA sequencing, allele-specific oligonucleotide (ASO) probes, and hybridization to DNA microarrays or beads (Navidinia *et al*., 2017). Genotyping is essential in research of genes and gene variants associated with the disease (Gillian A.M *et al*., 2018). RFLP, as a molecular marker, is particular to a single clone/restriction enzyme combination.Most RFLP markers are co-dominant (both alleles in the heterozygous sample will be detected) and highly locus-specific (Reshma *et al*., 2017). RADP markers are DNA fragments from PCR amplification of random segments of genomic DNA with a single primer of the arbitrary nucleotide sequence (Hafiz Muhammad *et al*., 2016). A PCR product will not be produced, if a mutation has been

observed in the template DNA at the site that was already complementary to the primer, resulting in various patterns of amplified DNA segments on the gel (Kavitha,*et al*., 2018). The 16S rRNA gene is a molecular marker used for identification of bacterial species, (Srinivasan *et al*., 2015). It is a new molecular approach to analyzing the genetic diversity of complex microbial populations (Eliana *et al*., 2018). This technique is based on the separation of polymerase chain reaction-amplified fragments of genes coding for 16S rRNA. Bacterial 16S rRNA is a usual target for taxonomic purposes, mainly due to the mosaic composition of phylogenetically preserved and variable regions within the gene. (Lenehan *et al*., 2017).

MATERIALS AND METHODS

Bacterial strain and isolates: A total of 139 samples of UTI and suspected UTI individuals A total of 139 urine samples were collected from the hospital attached laboratory and private clinical laboratory in and around Coimbatore, Erode, Tirupur, Kangeyam, Ooty and Salem districts during the period of November 2014 to May, 2015. Throughout the study the standard strain of *E.coli* MTCC 326 was used as the positive control.

Isolation of DNA*:* DNA extraction is a crucial first step in the experimental protocol of DNA Sequencing and Fragment analysis. The total quality, accuracy, and length of the DNA sequence read can be ideally affected by sample characteristics, and the method chosen for nucleic acid extraction. The Heat shock method was used in the preparation of DNA for all the samples in the study. The crude extract was used as the template DNA source in the study.

Molecular identification of E.coli

RAPD Amplification

The OPA2 primer (5'-TGC CGA GCT G-3') obtained from Eurofins mwg, Bangalore, were selected for RAPD-PCR amplification. The RAPD-PCR reaction mixture (25 µl), obtained from Chromus biotech Bangalore, constituted of 2.5 µl of 10X PCR buffer, 0.75 µl of 10 mM dNTPs, 2.5 µl of 25 mM $MgCl_2$, 0.33 µl of 1U/µl of *Taq* polymerase, 2.5 µl of primer and 2.5 µl of DNA template and added distilled water to make 25 µl. The RAPD-PCR amplification was carried out with a Thermal cycler (Eppendorf master cycler). The cycling parameters were 5 min at 94 oC for initial denaturation; 35 cycles each of 45 sec at 94 ^{o}C for denaturation, 1 min at 35 ^{o}C for annealing, 1 min at 72 ^{o}C for extension and a final extension at 72 oC for 5 min.

The amplified products with 3 µl Loading dye were separated on 1.5 % agarose gel containing ethidium bromide (1mg/ml) at 100 volts using 1X TAE buffer. 100 bp DNA Ladder and 1 Kb ladder were used as DNA molecular weight markers. The gels were photographed using Gel Documentation system (UPV GelDoc - IT 300 Imaging System, Cambridge, UK).

Enterobacterial Repetitive Intergenic Consensus (ERIC) PCR

The primers ERIC-1 (5'-ATG TAA GCT CCT GGG GAT TCA C-3') and ERIC-2
(5'-AAG TAA GTG ACT GGG GTG AGC G-3') Obtained from Eurofins mwg, Bangalore were used for ERIC-PCR study. The reaction mixture (25 µl), obtained from Chromus biotech Bangalore, consisted of 2.5 µl of 10X PCR buffer, 0.75 µl of 10 mM dNTPs, 2.5 µl of 25 mM MgCl2, 0.33 µl of 1U/µl of Taq polymerase, 2.5 µl of primer (1.25 µl each of forward primer and reverse primer) and 2.5 µl of DNA template and added distilled water to make 25 µl. The ERIC-PCR amplification was performed in a Thermal cycler (Eppendorf master cycler personal) with different temperatures, Initial denaturation at 95 oC for 5 min; 30 cycles each of 1 min at 94 oC for denaturation, 1 min at 50 oC for annealing, 8 min at 68 oC for extension and a final extension at 68 oC for 16 min. The amplified products with 3 µl Loading dye were separated on 1.5 % agarose gel containing ethidium bromide (1mg/ml) at 100 volts using 1X TAE buffer. The 100 bp DNA ladder and 1 Kb ladder were used as DNA molecular weight markers. The gels were photographed using Gel Documentation system (UPV GelDoc - IT 300 Imaging System, Cambridge, UK).

Repetetitive Extragenic Palidromic Sequence (REP) PCR

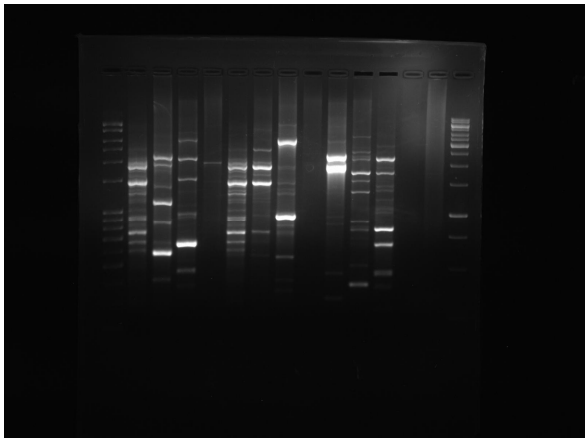
Both the primers REP-1 (5'-IIIICGICATCIGGC-3') and REP-2 (5'-ICGICTTATCIGGCCTA-3') were obtained from Eurofins mwg, Bangalore and used for the REP-PCR study. The reaction mixture (25 µl), obtained from Chromus biotech Bangalore, consisted of 2.5 µl of 10X PCR buffer, 0.75 µl of 10 mM dNTPs, 2.5 µl of 25 mM MgCl2, 0.33 µl of 1U/µl of Taq polymerase, 2.5 µl of primer (1.25 µl each of forward primer and reverse primer) and 2.5 µl of DNA template and added distilled water to make 25 µl.

The PCR reaction conditions were as follows: an initial denaturation (94 ºC, 7 min) followed by 30 cycles of denaturation (90 ºC, 30 sec), annealing (40 ºC, 1 min), and extension (72 ºC, 8 min) with a single final extension (72 ºC, 15 min). The size of the amplified fragments was observed by electrophoresis in submersed agarose gel (1.5%) using 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.

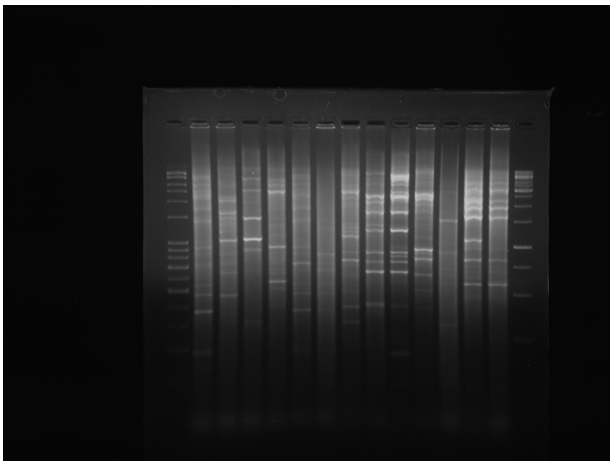
16S rRNA Sequencing

Full-length 16S rRNA sequences were amplified, 2 ml of extracted DNA was used as the base template in 20-µl reactions including 1 unit AmpliTaq polymerase (Applied Biosystems), 1x Buffer II, 1.5mM MgCl2, 0.02% Triton X-100, 20mM tetramethylammonium chloride, 5% DMSO, 100 nM of each dNTP, and 20 nM each of forward and reverse primers (Integrated DNA Technologies). The forward primer consists of 90% bacterial primer 8F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 10% 8F-Bif targeting Bifidobacteria (5′-AGGGTTCGATTCTGGCTCAG-3′); it was paired with the three domain reverse primer 1391R (5′-GACGGGCGGTGTGTRCA-3′). Thermocycling involved a 5-min denaturation at 95 °C followed by 15–22 cycles of 94 °C (30 s), 55 °C (30 s), and 72 °C (90 s), with a final extension at 72 °C for 8 min. The fewest possible cycles were used to produce a faint band of the expected size under UV illumination after electrophoresis of 5-µl reaction product in a 1% agarose minigel containing 1 mM ethidium bromide. Two 4-cycle, 50-µl reconditioning PCR reactions were performed per sample to eliminate heteroduplexes, with 5-µl aliquots of the initial PCR product mixture as the template and other PCR conditions unchanged. Products of the two reconditioning PCR reactions per sample were combined, purified using QIAquick PCR purification columns (Qiagen), were cloning and automated bidirectional dideoxy sequencing.

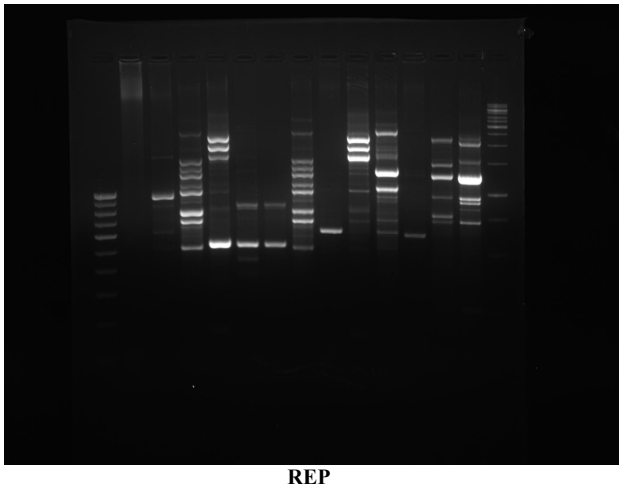
RESULTS AND DISCUSSION



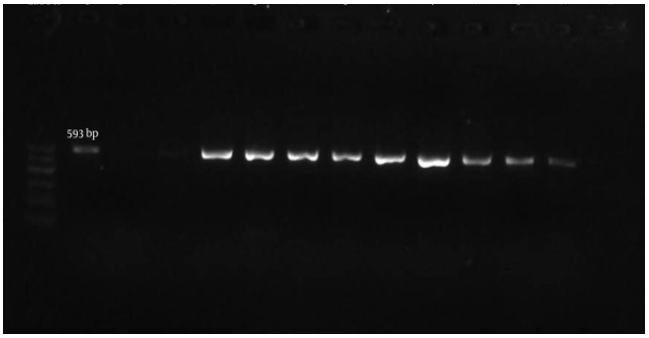
RAPD



ERIC



REP



16SrRNA

The molecular tools for identification and characterisation of *E.coli* among the samples collected were effective. The standard in each case was the MTCC strain of *E.coli.* The RAPD profile of the samples and standard MTCC was used to study the genetic variations among the samples. Further, establishing a phylogenetic relationship among the samples from the geographical region would give insights of possible genetic relationship among the UTI infection samples under study. The specific PCR methods of ERIC and REP for *E.coli* showed amplification of specific genes by the primers used in the study. The amplification was visualised in the gel with reference to the standards and markers. Further the *E.coli* specific 16srRNA gene amplification was done to confirm the molecular identification of E.coli among the samples. An amplified product of 593bp was obtained among the positive samples.

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