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# STUDY OF GENETIC VARIATIONS IN par *C* GENE IN QUINOLONE RESISTANT ISOLATES OF *Escheria coli* FROM URINARY TRACT INFECTIONS

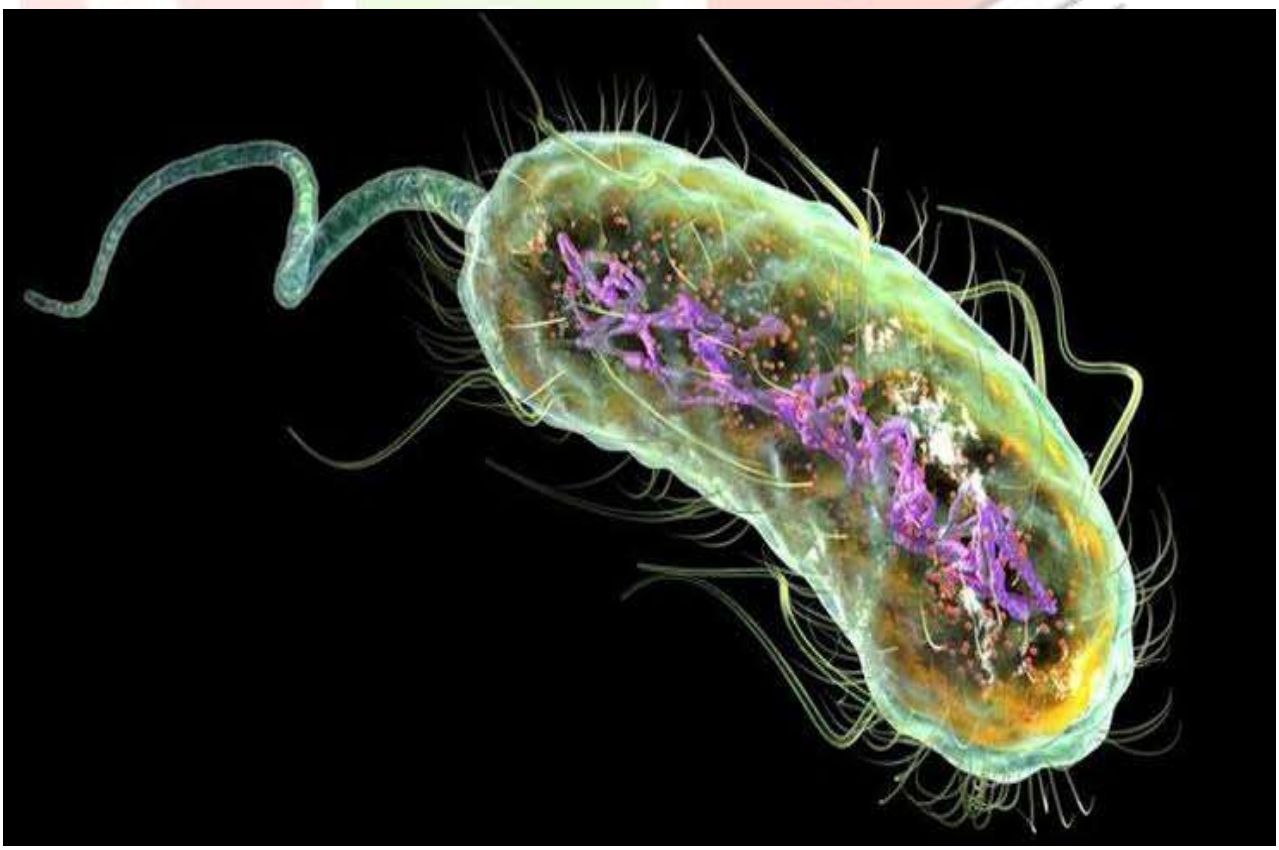
**Dini Chandran C S[1], Dr.Mangayarkarasi[2] and Dr.Parani[3]**

Sree Ramachandra Medical College, Kattankulathur, Chennai

## Introduction

Urinary tract infection (UTI) is a bacterial infection that affects any part of the urinary tract. Although urine contains a variety of fluids, salts, and waste products, it usually does not have bacteria in it. When bacteria get into the bladder or kidney and multiply in the urine, they cause a UTI. The most common type of UTI is a bladder infection which is also often called cystitis. Another kind of UTI is a kidney infection, known as pyelonephritis, and is much more serious. Although they cause discomfort, urinary tract infections can usually be quickly and easily treated when the patient sees a doctor promptly. A patient with dysuria and urinary frequency generally has a spot mid-stream urine sample sent for urinalysis, specifically the presence of nitrites, leukocytes or leukocyte esterase. If there is a high bacterial load without the presence of leukocytes, it is most likely due to contamination. The diagnosis of UTI is confirmed by a urine culture. If the urine culture is negative, symptoms of urethritis may point at *Chlamydia trachomatis* or Neisseria *gonorrheae* infection, Symptoms of cystitis may point at interstitial cystitis, in men, prostatitis may present with dysuria.In severe infection, characterized by fever, rigors or flank pain, urea and creatinine measurements may be performed to assess whether renal function has been affected. Most cases of lower urinary tract infections in females are benign and do not need exhaustive laboratory work-ups. However, UTI in young infants must receive some imaging study, typically a retrograde urethrogram, to ascertain the presence/absence of congenital urinary tract anomalies. Males too must be investigated further. Specific methods of investigation include x-ray, MRI and CAT scan technology. Most uncomplicated UTIS can be treated with oral antibiotics such as trimethoprim, cephalosporins, nitrofurantoin, or a fluoroquinolone (e.g. ciprofloxacin, levofloxacin). These are usually taken for 3 days in young adults, and 5 days in the elderly. Whilst co-trimoxazole was previously internationally used, the additional of the sulfonamide gave little additional benefit compared to the trimethoprim component alone, but was responsible for its high incidence of mild allergic reactions and rare but serious complications. If the patient has symptoms consistent with pyelonephritis, intravenous antibiotics may be indicated. Regimens

vary, usually Aminoglycosides (such as Gentamicin) are used in combination with a beta-lactam, such as Ampicillin or Ceftriaxone. These are continued for 48 hours after fever subsides. The patient may then be discharged home on oral antibiotics for a further 5 days. Escherichia coli is a bacterium that is commonly found in the lower intestine of warm-blooded animals. Most E. coli strains are harmless, but some, such as serotype O157:H7, can cause serious food poisoning in humans, and are occasionally responsible for costly product recalls, 112) The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin $K_2$, or by preventing the establishment of pathogenic bacteria within the intestine. E. coli are not always confined to the intestine, and their ability to survive for brief periods outside the body makes them an ideal indicator organism to test environmental samples for fecal contamination. (17 The bacteria can also be grown easily and its genetics are comparatively simple and easily-manipulated, making it one of the best-studied prokaryotic model organisms, and an important species in biotechnology. E. coli was discovered by German pediatrician and bacteriologist Theodor Escherich in 1885, 16) and is now classified part of the Enterobacteriaceae family of gamma as proteobacteria. Uropathogenic E. coli (UPEC) is responsible for approximately 90% of urinary tract infections (UTI) seen in individuals with ordinary anatomy. In ascending infections, fecal bacteria colonize the urethra and spread up the urinary tract to the bladder. Because women have a shorter urethra compared with men, they are 14-times more likely to suffer from an ascending UTI. Uropathogenic E. coli utilize P fimbriae (pyelonephritis-associated pili) to bind urinary tract endothelial cells and colonize the bladder. These adhesins specifically bind D-galactose-D-galactose moieties on the P blood group antigen of erythrocytes and uroepithelial cells.[13] Approximately 1% of the human population lacks this receptor, and its presence or absence dictates an individual's susceptibility to E. coli urinary tract infections.



Schematic representation of *E.coli* DNA

Uropathogenic E. coli produce alpha- and beta-hemolysins, which cause lysis of urinary tract cells.UPEC can evade the body's innate immune defenses (e.g. the complement system) by invading superficial umbrella cells to form intracellular bacterial communities (IBCs). They also have the ability to form K antigen, capsular polysaccharides that contribute to biofilm formation. Biofilm producing E. coli are recalcitrant to immune factors and antibiotic therapy and are often responsible for chronic urinary tract infections.135 K antigen-producing E. coli infections are commonly found in the upper urinary tract.Descending infections, though relatively rare, occur when E. coli cells enter the upper urinary tract organs (kidneys, bladder or Ureters) from the blood stream. Enter pathogenic E. coli (EPEC) and Enterotoxaemia E coli (ETEC) - UTI or GIT infections in infants are caused by EPEC which presents as watery diarrhea, meaning that PNM's will not be observed in the stool neither with ethylene blue nor Gram stain. First off, G- vet, rods, with no particular arrangement are seen in Gram stain. Then, either McCone agar or EMB agar (or both) are inoculated with the stool. On McCone agar, deep red colonies are produced as the organism is lactose positive, and this utilization will cause the medium's pH to drop leading to darkening of the medium. Growth on Levine EMB agar would show black colonies with greenish black metallic sheen. This is diagnostic of E. coli. The organism is lysine positive, and grows on TSI slant with a (A/A/g+/$H_2S$-) profile. Also, lambic is ++ for E. coli, as its idol positive (red ring) and methyl red positive (bright red), but VP negative (no change colorless) and citrate negative (no change-green color). Serology is done using the SSS Co agglutination test.Antibiotic therapy and résistance, Bacterial infections are usually treated with antibiotics. However, the antibiotic sensitivities of different strains of E. coli vary widely. As Gram-negative organisms, *E. coli* are resistant to many antibiotics that are effective against Gram-positive organisms. Antibiotics which may be used to treat *E. coli* infection include amoxicillin as well as other semi-synthetic penicillins, many cephalosporins, carbapenems, aztreonam, trimethoprim-sulfamethoxazole, ciprofloxacin, nitrofurantoin and the aminoglycosides.Antibiotic resistance is a growing problem. Some of this is due to overuse of antibiotics in humans, but some of it is probably due to the use of antibiotics as growth promoters in food of animals. A study published in the journal Science in August 2007 found that the rate of adaptative mutations in E. coli is "on the order of $10^3$ per genome per generation, which is 1,000 times as high as previous estimates," a finding which may have significance for the study and management of bacterial antibiotic resistance. Antibiotic resistant E. coli may also pass on the genes responsible for antibiotic resistance to other species of bacteria, such as *Staphylococcus aureus. E. coli* often carry multidrug resistant plasmids and under stress readily transfer those plasmids to other species. Indeed, E. coli is a frequent member of biofilms, where many species of bacteria exist in close proximity to each other. This mixing of species allows the *E.coli* strains that are piliated to accept and transfer plasmids from and to other bacteria. Thus the *E.coli* and the other enterobacteria are important reservoirs of transferable antibiotic resistance. The quinolones are a family of broad-spectrum antibiotics. The parent of the group is nalidixic acid. The majority of quinolones in clinical use belong to the subset of fluoroquinolones, which have a fluoro group attached the central ring system, typically at the 6-position.Mechanism, Quinolones and fluoroquinolones are bactericidal drugs. Actively killing bacteria. Quinolones inhibit the bacterial DNA gyrase or the topoisomerase IV enzyme, thereby inhibiting DNA replication and transcription. Quinolones can enter cells easily via porins and therefore are often used to treat intracellular pathogens such as Legionella pneumophila and Mycoplasma

pneumoniae. For many gram-negative bacteria DNA gyrase is the target, whereas topoisomerase IV is the target for many gram-positive bacteria. Eukaryotic cells do not contain DNA gyrase or topoisomerase IV. Adverse effects, Quinolone antibiotics were once considered relatively safe, but several side-effects have become evident with experience. For example, numerous case reports have implicated their use since 1965 in spontaneous tendon ruptures or damage, especially with the concurrent use of a systemic corticosteroid. In the fall of 2004, the Food and Drug Administration upgraded the warnings found within the package inserts for all drugs within this class regarding such serious adverse reactions. It is important to note, however, that the incidence of this is quite rare, with occurrences at less than one per ten thousand person-years. The main objective of the present study, "Study of genetic variations in par C gene in Quinolone resistant isolates of E.coli from urinary tract infections ",was to find out the prevalence rate of quinolone resistant isolates of E.coli from the patients suffering from urinary tract infections in patients attending from SRM Medical college hospital and Research centre, Kattankulathur, Chennai.

## Materials and methods

The study was conducted in V phases as follows. Phase I includes the case identification by verifying proforma like age,sex,physical conditions. Phase II includes Primary isolation of urine samples.Phase III includes biochemical conformations of the urinary samples. Phase IV includes isolation of DNA from samples and amplification of par C genes using PCR.Phase V included the sequencing of specified DNA samples. Cled agar is used for the primary isolation, enumeration and presumptive identification of microorganisms from urine. The result is obtained as growth formation with yellow colour colonies.Biochemical conformations included the TSI test,Citrate test,Mac Conkey Agar test, EMB agar test and antibiotic sensitivity test .Citrate, EMB agar and Mac Conkey tests are negative for *E.coli*.TSI test and antibiotic tests were obtained as positive for *E.coli*.Standardised PCR protocol for par C gene. Denaturation temperature: 95°C for 3 minutes,: 95 °C for 1 minute. Anealing temperature: 52 °C for 1 minute,Primer extention temperature : 72°C for 2 minutes, 72°C for 1 minutes 16°C for ∞ . Sample requirements PCR product to be sequenced.This mixture was first spinned down using a micro centrifuge and then vortexed and again centrifuged to ensure proper mixing**.** Post sequencing clean up Reaction -Sample Requirement,Sequencing PCR product.Sanger's method, which is also referred to as dideoxy sequencing or chain termination is based on the use of (ddNTP's)in addition to the normal (NTPs) found in DNA.Dideoxynucleotides are essentially the same as nucleotides except they contain a hydrogen group on the 3 carbon instead of a hydroxyl group(OH).These modified nucleotides, when integrated into a sequence, prevent the addition of further nucleotides. (Speed, 1992).This occurs because a phosphodiester bond cannot form between the dideoxynucleotide and the next incoming nucleotide, and the DNA chain is terminated.

Fig No.1 Cled test

Fig No.2 Antibiotic sensitivity test

Positive conformation tests of *E.coli*

Fig No.3 Citrate test


Fig No.4 Mac Conkey test

Fig No.5 EMB test

Negative conformation test of *E.coli*

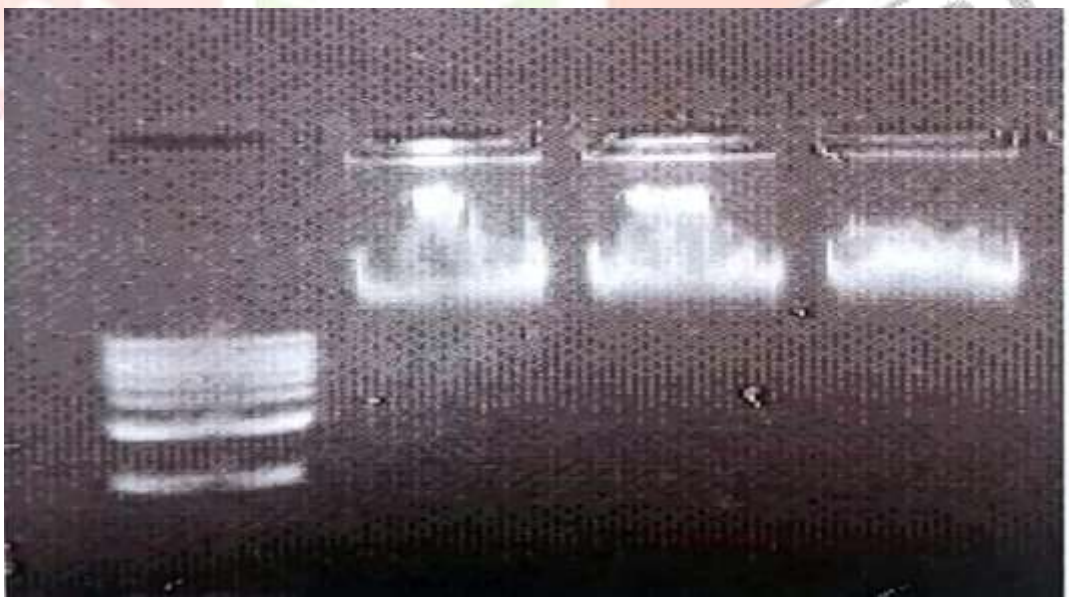


Fig No.7 Isolated DNA samples

**E.coli parC gene**

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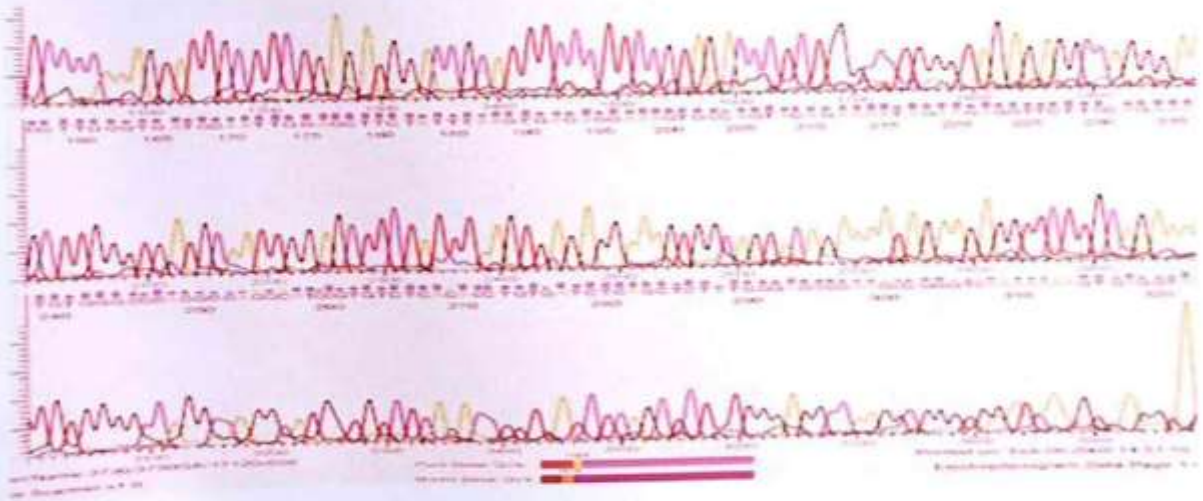
1    gcgaataagt tgaggaatca gaattaatga gcgatatggc agagcgcctt gcgtacatga
61   atttacggaa aacgcctact taaactactc catgtacgtg atcatggacc gtgcgttgcc
121  gtttattggt gatggtctga aacctgttca gcgccgcatt gtgtatgcga tgtctgaact
181  gggcctgaat gccagcgcca aatttaaaaa atcggcccgt accgtcggtg acgtactggg
241  taaataccat ccgcacggcg atagcgcctg ttatgaagcg atggtcctga tggcgcaacc
301  gttctcttac cgttatccgc tggttgatgg tcagggggaac tggggcgcgc cggacgatcc
361  gaaatcgttc gcggcaatgc gttacaccga atcccggttg tcgaaatatt ccgagctgct
421  attgagcgag ctggggcagg ggacggctga ctgggtgcca aacttcgacg gcactttgca
481  ggagccgaaa atgctacctg cccgtctgcc aaacatttgg cttaacggca ccaccggtat
541  tgccgtcggc atggcgaccg atattccacc gcataacctg cgtgaagtgg ctcaggcggc
601  aatcgcatta atcgaccagc cgaaaaccac gctcgatcag ctgctggata tcgtgcaggg
661  gccggattat ccgactgaag cggaaattat cacttcgcgc gccgagatcc gtaaaatcta
721  cgagaacgga cgtggttcag tgcgtatgcg cgcggtgtgg aagaaagaag atggcgcggt
781  ggttatcagc gcattgccgc atcaggtttc aggtgcgcgc gtactggagc aaattgctgc
841  gcaaatgcgc aacaaaaagc tgccgatggt tgacgatctg cgcgatgaat ctgaccacga
901  gaacccgacc cgcctggtga ttgtgccgcg ttccaaccgc gtggatatgg atcaggtgat
961  gaaccacctc ttcgctacca ccgatctgga aaagagctat cgtattaacc ttaatatgat
1021 cggtctggat ggtcgtccgg cggtgaaaaa cctgctggaa atcctctccg aatggctggt
1081 gttccgccgc gataccgtgc gccgccgact gaactatcgt ctggagaaag tcctcaagcg
1141 cctgcatatc ctcgaaggtt tgctggtggc gtttctcaat atcgacgaag tgattgagat
1201 cattcgtaat gaagatgaac cgaaaccggc gctgatgtcg cggtttggcc ttacggaaac
1261 ccaggcggaa gcgatcctcg aactgaaact gcgtcatctt gccaaactgg aagagatgaa
1321 gattcgcggt gagcagagtg aactggaaaa agagcgcgac cagttgcagg gcattttggc
1381 ttccgagcgt aaaatgaata acctgctgaa gaaagaactg caggcagacg cgcaagccta
1441 cggtgacgat cgtcgttcgc cgttgcagga acgcgaagaa gcgaaagcga tgagcgagca
1501 cgacatgctg ccgtctgaac ctgtcaccat tgtgctgtcg cagatgggct gggtacgcag
1561 cgctaaaggc catgatatcg acgcgccggg cctgaattat aaagcgggtg atagcttcaa
1621 agcggcggtg aaaggtaaga gcaaccaacc ggtagtgttt gttgattcca ccggtcgtag
1681 ctatgccatt gacccgatta cgctgccgtc ggcgcgtggt cagggcgagc cgctcaccgg
1741 caaattaacg ttgccgcctg gggcgaccgt tgaccatatg ctgatggaaa gcgacgatca
1801 gaaactgctg atggcttccg atgcgggtta cggtttcgtc tgcaccttta acgatctggt
1861 ggcgcgtaac cgtgcaggta aggctttgat cacttaccg gaaaatgccc atgttatgcc
1921 gccggtggtg attgaagatg cttccgatat gctgctggca atcactcagg caggccgtat
1981 gttgatgttc ccggtaagtg atctgccgca gctgtcgaag ggcaaaggca acaagattat
2041 caacattcca tcggcagaag ccgcgcgtgg agaagatggt ctggcgcaat tgtacgttct
2101 gccgccgcaa agcacgctga ccattcatgt tgggaaacgc aaaattaaac tgcgcccgga
2161 agagttacag aaagtcactg gcgaacgtgg acgccgcggt acgttgatgc gcggtttgca
2281 gtaa
```

**Primer sequences:**

5'-AAACCTGTTCAGCGCCGCATT-3' and
5'-GTGGTGCCGTTAAGCAAA-3'

**Length of the fragment to be amplified: 395bp**

# SEQUENCE ANALYSIS DATA OF par C GENE IN *E coli*



## Result

From 500 samples of quinolone resistant E.coli isolates causing urinary tract infections, DNA was isolated. These isolated DNA samples were subjected to PCR for the amplification of specific target gene, par c encoding the resistively sequences of E.coli. These mutations are mainly determined by the sequencing of the target region. The primary investigations of patient samples, isolated DNA samples, PCR amplified products, Sequence data analysis, all are explained in respective figures.

## Discussion

In the study, we were able to find out the prevalence rate of quinolone resistant isolates of E.coli from the patients suffering from urinary tract infections in patients attending from SRM Medical college hospital and Research Centre, Kattankulathur, Chennai. From data's obtained from the sequence analysis it is clearly evident that the rate of mutation of Quinolone resistant isolates in E.coli causing urinary tract infections.

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