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Bioinformatic Analysis of Gyrase Gene QRDR Region Coding Drug Resistance among *Escherichia coli*

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Abstract: The present study was mainly aimed at analyzing gyrase gene of *Escherichia coli* and modelling of their respective protein, which will be of great interest in designing of antibiotic using Gyrase-A coded protein as a potential target. The prediction of secondary structure of *Escherichia coli* gyrase coding gene was carried out using SOPMA tool. Results of the NCBI queries reveals significant identity with the *Escherichia coli Gyrase* B subunit for strain TR 32 and 33 and *Gyrase* A subunit for strain EC 1 confirming the sequences as *Gyrase* gene of *Escherichia coli*. The nucleotide sequence alignment reveals 73 conserved regions with varying degree of transition and transversion while the amino acid sequences of the nucleotides reveals 6 conserved sites among all the sequences. The template that showed high percentage of identity with all the isolates were B,D,F and H fragments of crystal structure of gyrase bound to its inhibitor YACG. The isolates contain comparatively higher alpha helix than the Beta turn which is present in less percentages ranging from 5.21-10%. In conclusion, this study confirmed the conservation of QRDR coding regions in prokaryotes and the possibility of targeting the alpha helix in case of drug development.

Key words: Bioinformatics · Gyrase gene · QRDR Region · Drug resistance · *Escherichia coli*

INTRODUCTION

Escherichia coli is a common constituent of the gastrointestinal flora of most vertebrates, including humans and may be isolated from a variety of environmental sources [1]. Most strains of these organisms are nonpathogenic while certain ones can cause a variety of intestinal and extraintestinal infections as a result of gene-encoding virulence factors (VFs) such as adhesins, toxins and polysaccharide surface coatings [2]. This organism is the primary cause of urinary tract infections in humans [3] and is the most frequent nosocomial and community-acquired pathogen in all regions [4]. Resistance to fluoroquinolones develops more rapidly in *E. coli* than in other members of the Enterobacteriaceae [5]. Two mechanisms of resistance have been found to determine resistance to fluoroquinolones. The most important of these mechanisms in Gram negative bacilli is the accumulation of mutations in the bacterial enzymes targeted by fluoroquinolones; DNA gyrase and DNA topoisomerase IV [6].

The other described mechanism of resistance operates by decreasing intracellular drug accumulation by up regulation of native efflux pumps [7, 8] either alone or together with decreased expression of outer membrane porins [9, 10]. Chromosomal resistance to fluoroquinolones (FQs) due to amino acid substitutions in the quinolone resistance-determining regions (QRDRs) of DNA gyrase (GyrA) and/or topoisomerase IV (Par C) has been reported [11], while higher levels of quinolone resistance arose readily by mutation [12, 13]. The two genes encoding DNA *Gyrase* are present next to each other in the genome with *gyrB* upstream of *gyrA* genes. Majority of the fluroquinolone resistant isolates have amino acid substitution in the regions of DNA *Gyrase*

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subunit homologous to a conserved fluoroquinolone resistance determining regions [14]. Therefore, this study is mainly aimed at analyzing quinolone resistance-determining regions (QRDRs) of *Gyrase* gene sequences of *Escherichia coli isolates* for their sequence similarity, protein structure prediction and conserved regions.

MATERIALS AND METHODS

In Silico: The sequences AB823545.1, KJ920434.1 and KJ 920435.1 were obtained using nucleotide BLAST (http:// blast.ncbi.nlm.nih.gov/ Blast.cgi) [15] and subjected to evolutionary analysis using the MEGA explorer [14]. Pairwise distances were calculated using Kimura 2 parameter. In MEGA explorer, translate option was used for converting the gene sequence into amino acid sequence. Sequence similarity search with BLASTP [16] and best homologous protein was found using the multiple sequence alignment. Secondary structure of the protein sequences were predicted using SOPMA tool [17].

RESULTS

The isolate EC1 showed maximum identity of 100% with the *Escherichia coli* gyrase A subunit. The sequence of the isolate TR32 showed 100% identity with the *Escherichia coli* TR33 *Gyrase* B subunit. The phylogenetic tree revealed evolutionary relation between the sequences while the nucleotide sequence alignment reveals 73 conserved regions with varying degree of transition and transversion. The amino acid sequences of the nucleotides reveal 6 conserved sites among all the sequences. The isolates EC1, ECTR32 and EC TR33 show 96, 140 and 133 amino acids residues respectively. These protein sequences however when queried against the protein databank database using BLASTp, the EC1 isolate with 96 amino acid produced significant alignment with the crystal structure of the N-terminal domain of GyrA, *Escherichia coli* GyrB domain insertion and the crystal structure of Gyrase bound to its inhibitor YACG. The EC TR32 isolate contains 140 residues and shares significant relationship with domain insertion in *E. coli* GyrB which adopts a novel fold that plays a critical role in gyrase function. It also shares significant relation with the B,D,F and H fragment of crystal structure of Gyrase bound to its inhibitor YACG and 3.5A Crystal structure of the catalytic core of *S.aureus* DNA gyrase. The EC TR33 isolate contains 133 aminoacid residues and it produced significant alignment with the Chain B fragment of *E. coli* Gyrase B, chain A fragment of 3.5A Crystal structure of the catalytic core of *S.aureus* DNA gyrase and the B, D, F and H fragment of crystal structure of Gyrase bound to its inhibitor YACG.

Secondary Structure Prediction: The secondary structure of the protein sequences was predicted from the raw sequence using the tool SOPMA.

10 20 30 40 50 60 70
| | | | | | |
LKPVHRRVLYAMNVLGNDWNKAYKKSARVVGDVIGKYHPHGDLAVYNTIVRMAQPFSLRYMLVDGQGNFG
ccccchhhheehhhhttchhhhhhhhhhhhhhheeccccccchhhhhhhhhhcctttteeeeeeccccee
SIDGDSAAAMRYTEIRLAKIAHELMA
eeccchhhhhhhhhhhhhhhhhhhh
Sequence length of *Escherichia coli* strain EC1: 96
SOPMA:
Alpha helix (Hh): 54 is 56.25%
3_{10} helix (Gg): 0 is 0.00%
Pi helix (Ii): 0 is 0.00%
Beta bridge (Bb): 0 is 0.00%
Extended strand (Ee): 14 is 14.58%
Beta turn (Tt): 5 is 5.21%
Bend region (Ss): 0 is 0.00%
Random coil (Cc): 23 is 23.96%
Ambiguous states (?): 0 is 0.00%
Other states: 0 is 0.00%

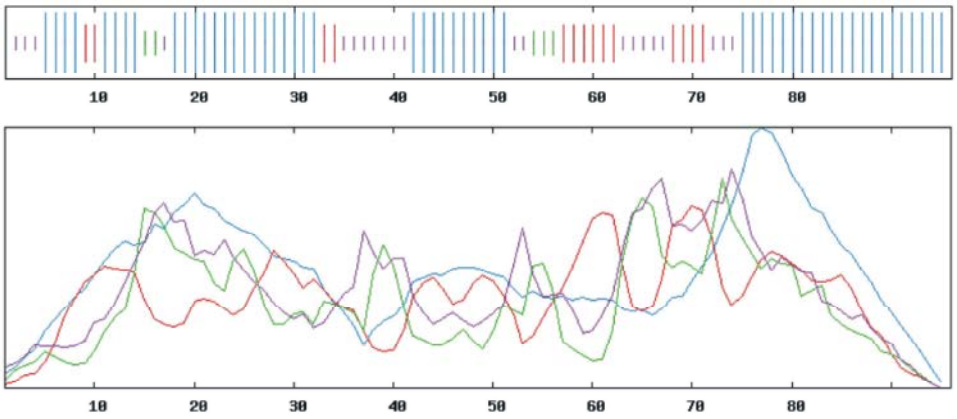

Fig. 1: Chromatographical representation of the secondary structure of *Escherichia coli* strain EC1

The secondary structure prediction of *Escherichia coli* strain TR32
10 20 30 40 50 60 70
| | | | | | |
KDKLVSSEVKSAVEQQMNELLAEYLLENPTDAKIVVGKIIDAARAREAARRAREMTRRKGALDLAGLPGK
cchhhhhhhhhhhhhhhhhhhhhhhhhccttcceeeehhhhhhhhhhhhhhhhhhhhhhtcceehttccth
LADCQERDPALSELYLVEGDSAGGSAKQGRNRKNQAILPLKGKILNVEKARFDKMLSSQEVATLITALGC
hhhhhcccthhhheeeeccccttccccccccttceeeeettceeeechhhhhhhhhhhhhhhhhhhhhtc
Sequence length: 140
SOPMA:
Alpha helix (Hh): 77 is 55.00%
3_{10} helix (Gg): 0 is 0.00%
Pi helix (Ii): 0 is 0.00%
Beta bridge (Bb): 0 is 0.00%
Extended strand (Ee): 19 is 13.57%
Beta turn (Tt): 14 is 10.00%
Bend region (Ss): 0 is 0.00%
Random coil (Cc): 30 is 21.43%
Ambiguous states (?): 0 is 0.00%
Other states: 0 is 0.00%

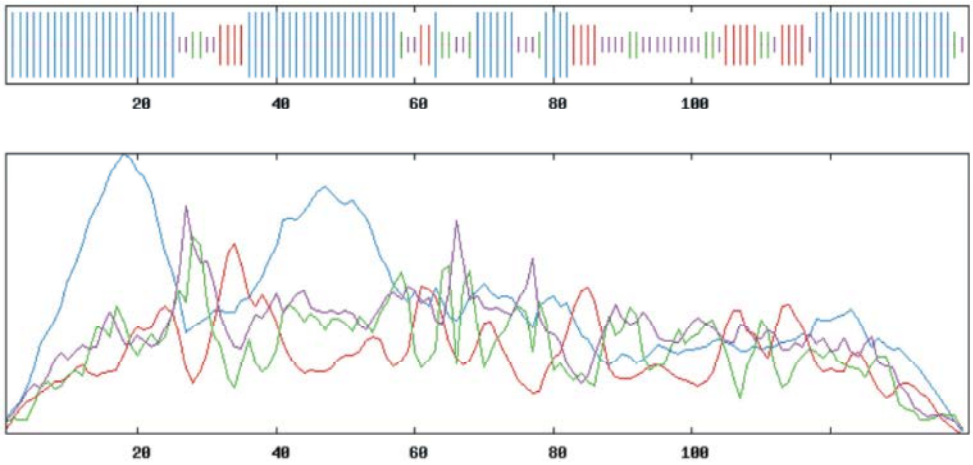

Fig. 2: Chromatographical representation of the secondary structure of *Escherichia coli* strain TR32.

The secondary structure of *Escherichia coli* strain TR33

10 20 30 40 50 60 70
| | | | | | |
KDKLVSSEVKSAVEQQMNELLAEYLLENPTDAKIVVGKIIDAARAREAARRAREMTRRKGALDLAGLPGK
cchhhhhhhhhhhhhhhhhhhhhhhhhcttcceeeehhhhhhhhhhhhhhhhhhhhhhhtcceehttccth
LADCQERDPALSELYLVEGDSAGGSAKQGRNRKNQAILPLKGKILNVEKARFDKMLSSQEVAT
hhhhhcccthhhheeeeccccttccccccccccttceeeeettceeeechhhhhhhhhhhhhhh
Sequence length : 133
SOPMA:
Alpha helix (Hh): 72 is 54.14%
3_{10} helix (Gg): 0 is 0.00%
Pi helix (Ii): 0 is 0.00%
Beta bridge (Bb): 0 is 0.00%
Extended strand (Ee): 19 is 14.29%
Beta turn (Tt) : 13 is 9.77%
Bend region (Ss): 0 is 0.00%
Random coil (Cc): 29 is 21.80%
Ambiguous states (?): 0 is 0.00%
Other states: 0 is 0.00%

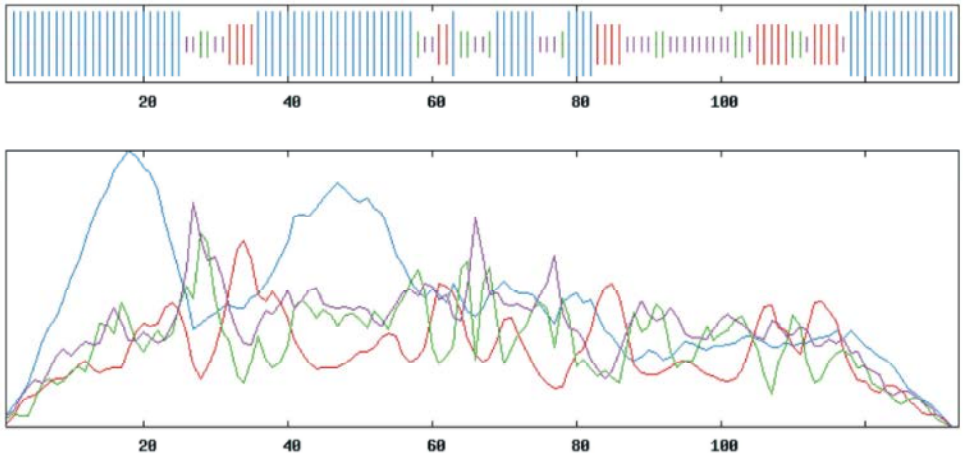

Fig. 3: Chromatographical representation of the secondary structure of *Escherichia coli* strain TR33

DISCUSSION AND CONCLUSION

Molecular identification of Gram negative organisms including *Escherichia coli* is gaining importance due to their rapid and accurate results in identifying the pathogens [18, 19]. In infections due to Gram negative bacteria including *Escherichia coli*, the PCR based amplification of target sequence is carried out mainly for identifying the resistance coding genes [20, 21]. The present study is aimed at analyzing Gyrase gene of *Escherichia coli* and modelling of the respective protein, which will be of great interest in designing of antibiotic using Gyrase-A and B- coded protein as a potential target. The sequences obtained were analyzed using bioinformatics tools and queried against the nucleotide database using the BLAST algorithm [22]. The BLAST analysis revealed homology with the available *Escherichia coli gyrA and B* gene sequence in the Genbank. The sequence similarity confirms the isolates as *Escherichia coli*. The first isolate produced 100% homology with *Escherichia coli* Gyrase A coding partial codons. The second isolate was 100% identical with the *Escherichia coli* TR32 gyrB coding genes. Alignment of the sequences of fragments defining the QRDR in the *gyr*

A genes showed high degree of homology while the protein sequences queried against the protein databank sequences also revealed significant similarities with EC1 producing significant alignments with the crystal structure of the N-terminal domain of GyrA , a domain insertion in *Escherichia coli* Gyr B and crystal structure of Gyrase bound to its inhibitor YACG. The EC TR32 isolate contains 140 amino acid residues and shares significant relationship with domain insertion in *E. coli* GyrB which adopts a novel fold that plays a critical role in gyrase function, 3.5A Crystal structure of the catalytic core of *S.aureus* DNA gyrase and B,D,F and H fragment of crystal structure of Gyrase bound to its inhibitor YACG. On the other hand, the EC TR33 isolate contains 133 amino acid residues and it produced significant alignment with the chain B fragment of *E. coli* Gyrase B,D,F and H fragment of crystal structure of Gyrase bound to its inhibitor YACG and chain A fragment of 3.5A Crystal structure of the catalytic core of *S. aureus* DNA gyrase. This significant relationship observed with the other gyrase enzymes may not be unconnected to the fact that the primary structures of the A and B subunits of DNA Gyrase are conserved among prokaryotes probably because of the essential function of Gyrase enzymes [23]. The templates that showed high percentage of identity with all the isolates were B, D, F and H fragment of crystal structure of Gyrase bound to its inhibitor YACG. This however, may be due to the conservation of the QRDR and its flanking region. The prediction of secondary structure of *Escherichia coli* Gyrase coding gene was carried out using SOPMA. The isolates contain comparatively higher alpha helix than Beta turn. Beta turns are present in less percentages of 5.21-10%. This knowledge is important as it helps us design low-molecular-weight synthetic agents that reproduce their essential features by using synthetic agents to mimic the helices. This also in particular, has immense interest in drug discovery because of the central role that the a-helical regions play in many biological processes. Again, information about the amino acid residues in the Gyrase QRDR determining region is needed to study the binding of target drugs in these regions. This is because changes in these residues results in the modification of drug binding sites. In conclusion, this study confirmed the conservation of QRDR coding regions in prokaryotes and the possibility of targeting the alpha helix in case of drug development.

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