BIOINFORMATIC ANALYSIS OF QUINOLONE RESISTANCE IN BACTERIA

THESIS

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Department of Biotechnology and Bioinformatics

Jaypee University of Information Technology, Solan

In the partial fulfilment of the requirement for the degree of

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By

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Enrolment No. 161824

B. Tech Biotechnology [8th semester]

JUIT Solan (H.P)

Under the Supervision of

Dr. Jitendraa Vashistt, Assistant Professor (senior grade)
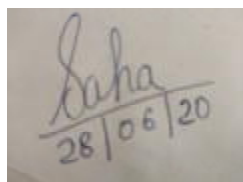
JUIT Solan (H.P)

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DECLARATION

I hereby declare that the work reported in the Bachelor of Technology thesis entitled **"**Bioinformatic analysis of quinolone resistance in bacteria**"** submitted at **Jaypee University of Information Technology, Waknaghat, India**, is an authentic record of work done by me (Shristi Saha) for the period of July 2019-May 2020 carried out under the supervision of **Dr. Jitendraa Vashistt, Assistant Professor (senior grade)**. I have not submitted this work elsewhere for any other degree or diploma.

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Jaypee University of Information Technology,

Waknaghat, India.

Date: 28/06/2020

SUPERVISOR'S CERTIFICATE

This is to certify that the work reported in the B. Tech thesis entitled **"**Bioinformatic analysis of quinolone resistance in bacteria**",** submitted by **Shristi Saha (161824) at Jaypee University of Information Technology, Waknaghat, India,** is a bonafide record of her original work carried out under my supervision, from July 2019 till May 2020. This work has not been submitted elsewhere for any other degree or diploma.

Supervisor

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Date: 28/06/2020

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Emotions cannot be adequately expressed in words but are transformed into more formalities. This acknowledgement is a profound expression of regard for all those who have made this work indelible.
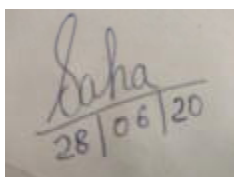
I am highly indebted to **Dr. Sudhir Kumar**, Head, Department of Biotechnology and Bioinformatics for giving me the golden opportunity and amenities required to carry out my project successfully.

I owe my gratitude and appreciation to my project supervisor **Dr. Jitendraa Vashistt** for his guidance and constant supervision as well as for providing me all the necessary information required for the completion of my project.

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I would like to express my sincere gratitude and appreciation to Mr. Baleshwar and Mrs. Mamta who gave their precious time in helping me and providing me all the chemicals and equipment's required during the project.

I bow my head before the **Almighty God** and **My Parents** whose blessing gave me the strength to make this successful venture and I dedicate my work and achievement to them.

Shristi Saha

Enrolment no. 161824

ABBEREVERATIONS

μg	-	Microgram
μl	-	Microliter
AST	-	Antibiotic susceptibility test
ATCC	-	American Type Culture Collection
BLAST	-	Basic local alignment search tool
DNA	-	Deoxyribonucleic acid
Gyr	-	Gyrase
LB	-	Luria broth
MDR	-	Multidrug resistance
MHA	-	mueller hinton agar
MIC	-	Minimum inhibitory concentration
min	-	Minutes
ml	-	Milli Litre
MSA	-	Multiple sequence alignment
NA	-	nalidixic acid
°C	-	degree Celsius
PCR	-	Polymerase chain reaction
QRDR	-	Quinolone resistance determining region
UTI	-	Urinary tract infections
V/V	-	Volume/Volume
vol	-	Volume

Abstract

Bacterial pathogens like *Acinetobacter baumannii*, *Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumonia,* cause common diseases like pneumonia associated with ventilator patients, urinary tract infections (UTIs), diarrhoea, cholecystitis, bloodstream infection, which are complicated and can be life-threatening. Previously these diseases were cured with the use of normal and simple drugs but nowadays with increased antibiotic resistance most of the infections are incurable.

Quinolones are the broad-spectrum antibacterial agents used to cure diverse bacterial infections. In many known bacteria, these have targets, DNA gyrase /topoisomerase IV. The structural and biochemical properties of such type of topoisomerase are extremely conserved, DNA gyrase induces negative supercoils and topo-IV unlinks the compounds and the chain of daughter chromosomes. The antibacterial activity of quinolones (Ciprofloxacin) is determined by how effectively it inhibits these two targets. Quinolones form a topoisomerase-quinolone complex and such kind of accumulation of complexes lead to the inhibition of DNA gyrase replications and generation of double-stranded breaks. My study was divided into two parts *in-vitro* and *in silico*. *In-vitro* experiments were carried out to see the minimal inhibitory concentration (MIC) and antibiotic susceptibility (AST) of different bacterial strains. For basic *in-silico* studies, text mining was done from the different written resources primarily from NCBI and related web sources (research and review papers). Different mutations in DNA gyrase of different bacteria were compared and analysed by MSA (Multiple sequence alignment). It was observed that different point mutations are present in the sequence of gyrase which may lead to altered binding with quinolone resulting in antibiotic resistance.

CHAPTER I

INTRODUCTION

Bacterial pathogens like *Acinetobacter baumannii*, *Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumonia* cause common diseases like pneumonia associated with ventilator patients, urinary tract infections (UTIs), diarrhoea, cholecystitis, and bloodstream infections which are complicated and can be life-threatening (Pachori *et al* 2019 and Chaudhry *et al* 2017). Previously these diseases were cured with the use of commonly used antibiotics but nowadays with increased antibiotic resistance most of the infections are incurable.

Quinolones are the broad-spectrum antibiotics used to cure diverse bacterial infections. In many known bacteria, they act on targets, DNA gyrase/ topoisomerase IV and inhibit bacterial replication (Hawkey *et al* 2003). The structural and biochemical properties of such type of topoisomerase are extremely conserved, DNA gyrase induces negative supercoils and topo-IV unlinks the compounds and the chain of daughter chromosomes.

Quinolones form a topoisomerase-quinolone complex and such kind of accumulation of complexes lead to the inhibition of DNA gyrase replications and generation of double-stranded breaks (Campoli *et al,* 1999).

Ciprofloxacin is one of the widely used quinolone given in the condition when other drugs don't cure the infection. However, from the past few years, it has been noticed that ciprofloxacin is not curing the disease. It is because of antibiotic resistance, which bacteria have developed against the drugs. Multidrug-resistant means when bacteria develop resistance against two or more than two drugs, and there can be a possibility that there can be a different mechanism to develop the resistance.

The mutation that is present in gyrase quinolone-resistant map to a segment is gyrA named as "quinolone resistance determining region (QRDR)" (Weigel *et al,* 1998).

Present study was carried out to study the effect of different changes in the sequence of gyrase of bacteria and their relation with quinolone resistance. Primary study was done *in-vitro* to check the resistant bacteria and antibiotic susceptible bacteria. The *in-silico* approach was done further for four types of

bacteria- *Acinetobacter baumannii*, *Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumonia.* Using a bioinformatics approach for these bacteria, I have analysed the mutations in each bacteria at the different amino acid position. BLAST- Basic local alignment search tool is used to analyse amino acid sequence of protein and DNA sequences nucleotide. (Korf *et al,* 2003) and Multiple sequence alignment was also used to compare different sequences in order to find the change in amino acid sites using Clustal Omega (Drilica *et al,* 1997).

CHAPTER II

REVIEW OF LITERATURE

Bacterial pathogens including *Acinetobacter baumannii*, *Pseudomonas aeruginosa, Klebsiella pneumonia,* and *Escherichia coli* are leading cause of the diseases like urinary tract infections, ventilator-associated pneumonia, bloodstream infection and diarrhoea. These above mentioned infections were easily curable with commonly used antibiotics before last two decades. But with increased antibiotic resistance among pathogens it is difficult to cure associated infections.

Quinolone Antibiotics

History of quinolones drug

Quinolones group of antibiotics were originated from nalidixic acid (NA) which was developed in the year 1962. Quinolone antibiotics have in general broad-spectrum bactericidal activity. These are used to treat the human as well as a veterinary bacterial infection. The 1st new quinolone, norfloxacin (NFLX) and it was patented in the year 1978(Yakushiganku *et al,* 2003).

Fluoroquinolones (quinolone) which contains a fluorine atom in the chemical structure and it is very much effective against both gram-negative and gram-Positive bacteria. Example: - Ciprofloxacin, worldwide used antibiotics. (Kelli *et al,* 2013)
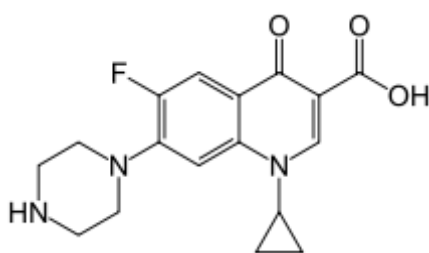
Fig 1:- Fluoroquinolone structure

Mechanism of action of Fluoroquinolones: Fluoroquinolones are bactericidal drugs, mainly used for clinical purposes. They protect by giving antibacterial effect. These antibiotics inhibit the bacterial type II topoisomerases (gyrase/topoisomerase IV) and hence stop DNA replication. Third and fourth-generation fluoroquinolones are more

selective for topoisomerase IV ligase domain, thus enhanced the Gram +ve bacteria coverage.

Cellular uptake: Fluoroquinolones can easily enter through pore forming porin proteins and are used to treat intracellular pathogen. For many gram-negative bacteria, DNA gyrase is targeted, and for topoisomerase IV is the target for many gram-positive bacteria (Turnidge *et al,* 1999). Table 1 shows the different generations of quinolones (Oliphant *et al,* 2003).

Table 1-Generations of the drugs

Generations	Drugs
1st Generation	Flumequine, Oxolinic acid
2nd Generation	Ciprofloxacin, fleroxacin, lomefloxacin,
3rd Generation	Levofloxacin, Sparfloxacin
4th Generation	Clinafloxacin, Besifloxacin

<u>Ciprofloxacin:</u> Ciprofloxacin is second-generation antibiotics of quinolone that are used to treat bacterial infections. It stops the multiplication of bacteria by inhibiting the reproduction and repair of their genetic (DNA) materials. In the year (October 1987) ciprofloxacin was 1st approved by FDA. It is used to treat the serious infection or the infection when other antibiotics stop working. It is used to treat infections, such as- chest Infections (including pneumonia) skin and bone infection, conjunctivitis, eye and ear infection, STIs (Campoili *et al,* 1988).

Multi-Drug resistance

Multidrug resistance (MDR) is an antimicrobial resistance shown by a species of a microorganism to at least one drug or more than one drug or its categories. Antimicrobial categories are classification of antimicrobial agents based on their mode of action and specific to target organism.

Biochemical mechanism of resistance

1) Mutational Alteration of the Target Protein

Bacteria make altered 30S ribosomes that do not bind to the drug or make altered penicillin-binding protein. Convert in a form of 50 S ribosomes so that it drug does not bind to them. It also makes alteration in DNA topoisomerase, polymerase and enzymes (Tenover *et al,* 2006).

2) Destroy Drug

Plasmid encodes enzymes that chemically alter the drug (e.g.) by acetylation or phosphorylation), thereby activating it. It also encodes beta-lactamase, which opens the beta-lactam ring inactivating it. (Alekshun *et al,* 2007)

3) Inhibits drug entry or remove the drug

Bacteria change the shape of the outer membrane porin proteins, preventing the drug from entering the cell. New membrane transport system prevents the drug from entering the cell and pumps the drug out of the cell (Nikaldo *et al*, 2009)

Table 2- Difference between Topoisomerase I and Topoisomerase II.

TOPOISOMERASE I	TOPOSIOMERASE II
The enzymes, which cut one of the two strands of double-stranded DNA relax the strand.	The enzymes, which cut both strands of the DNA helix simultaneously in order to manage DNA tangles and supercoils.
Generate single-strand breaks	Generates double-stranded breaks.
Does not required ATP hydrolysis	Requires ATP hydrolysis
Occurs in eukaryotes	Occurs in prokaryotes
Three subclasses Type IA topoisomerase Type II topoisomerase Type IC topoisomerase	Two subclasses:- Type IIA topoisomerase Type IIB topoisomerase

(Pommier *et al,* 1986)

Table 3- Difference between DNA Gyrase and Topoisomerase IV

DNA GYRASE	TOPOISOMERASE IV
It is the subunit of Type II topoisomerase.	It is also the subunit of Type II topoisomerase
DNA gyrase is supporting nascent chain elongation during replication.	During the terminal stage of DNA replication, topoisomerase IV separates the topologically linked daughter chromosomes
Genes encoded – gyrA ,gyrB	Gene encoded- ParC

(Drilica *et al,* 1997)

DNA Gyrase: DNA gyrase was first type II topoisomerase to be described. It was discovered in 1976. Among all the DNA gyrase is the only enzyme that is capable of actively unwinding that is negatively supercoiling the double helix.

In the DNA gyrase subunit, the C terminal domain is required for the enzyme to carry out these unique functions.

DNA gyrase plays an important role in the opening of DNA replication origins and removing positive supercoils that amass together in front of the replication fork and transcription complexes. DNA gyrase is a vital bacterial enzyme that catalyses negative supercoiling of plasmid and chromosomal DNA. Formation the reversible covalent protein- DNA complexes with the phosphodiester backbone, this reaction is carried out by topoisomerase. The supercoil levels vary with different optimal growth rates in different bacterial species. The potent antibiotic that blocks (ciprofloxacin) gyrase is activity have been developed to treat patients infected with an extensive range of pathogenic bacteria because the gyrase is not present in the eukaryotes (Reece *et al,* 1991).

The antibiotic that interferes with the structure of DNA and function

DNA gyrase (topoisomerase II) and the other topoisomerase (I, III and IV) plays an important role in maintaining the nucleoid structure and in the chromosome (compact supercoiled domains). These enzymes help with the winding and unwinding of the DNA that take place during replication and transcription. Drugs that affect the prokaryotic gyrase and topoisomerases affect replication, transcription, and the phosphate bonds are reform to repair DNA when the DNA strands are broken to introduce or reduce supercoiling.

Acinetobacter baumannii

Acinetobacter baumannii is a notorious pathogen. This is nosocomial acquisition (found in the hospital environment). It is typically short, round, and rod-shaped, Gram –ve bacteria. It was named after the bacteriologist Paul Baumann. There is a long history of multidrug-resistant *A. baumannii* infection occurred in the United States. In the year 1991-1992, an outbreak of Carbapenem-resistant was observed in the hospital of New York.

It is an opportunistic pathogen in human affecting people with their compromised immune system. It is exclusively isolated from the hospital environment occasionally. An important contribution of infection of *A.*

baumannii in the United States is the return of military personnel and soldiers who fought during the Iraq and Afghanistan war in March 2003, that's why it is also named as Iraqi bacteria.

Table 4- Known mutation in gyrA in *Acinetobacter baumannii*

Gene	Drug	Amino acid	Change in amino acid	Reference
gyrA	Ciprofloxacin and naladixic acid	Gly81	Val 81	Vila *et al,* 1995; Ardebili *et al,* 2015; Chien *et al,* 2009
gyrA	Ciprofloxacin and naladixic acid	Ser83	Leu83	Vila *et al,* 1995
gyrA	Ciprofloxacin and naladixic acid	Ala84	Pro84	Vila *et al,* 1995

Escherichia coli

Escherichia coli are a rod-shaped facultative anaerobic gram-negative bacterium found in the intestines and gut of certain animals. It is a widely studied prokaryotic organism especially in the field of microbiology and biotechnology because of its short doubling time of 20 minutes. It can easily be grown in Luria broth and on Luria agar plates. It was first discovered in 1885 by Theodor Escherichia. It is normally harmless but comprises of both pathogenic as well as non-pathogenic strains.

Mutations in the QRDR section of gyrA subunit of DNA gyrase are most common in quinolone-resistant clinical isolates.

Table no.5- Known mutations in gyrA in *Escherichia coli*

DNA Gyrase	Amino-acid	Mutated amino acid	References
gyrA	Tyr50	Phe50	Correia *et al*, 2017
gyrA	Ala51	Val51	Correia *et al*, 2017
gyrA	Ala67	Ser67	Correia *et al*, 2017
gyrA	Gly78	Cys78	Correia *et al*, 2017
gyrA	Ser80	Arg/Ile80	Correia *et al*, 2017
gyrA	Gly81	Asp81 Cys81	Correia *et al*, 2017
gyrA	Ser83	Ala83 Leu83 Ile 83 Trp 83 Tyr83 Val83	Varughese *et al*, 2018; Gruger *et al,* 2004 and Correia *et al,* 2017
gyrA	Ser83	Trp83	Gruger *et al,* 2004 and Correia *et al,* 2017
gyrA	Ala84	Pro84 Val84	Correia *et al*, 2017
gyrA	Asp87	Asn87 Glu87 Gly87 His87 Tyr87 Val87	Correia *et al*, 2017

gyrA	Gln106	Arg106 His106	Correia *et al*, 2017
gyrA	Ala119	Glu119	Correia *et al*, 2017
gyrA	Ala196	Glu196	Correia *et al*, 2017
gyrA	Arg237	His 237	Correia *et al*, 2017

Pseudomonas Aeruginosa

Pseudomonas aeruginosa is increasing worldwide because of the high rate of antibiotic resistance. It is a common gram-negative, rod-shaped, encapsulated bacterium that causes diseases in plant, animals and human-being. It is multidrug-resistant pathogen mainly recognized for its ubiquity, advanced antibiotic resistance mechanism, and it is associated with a severe illness that is hospital-acquired infection such as ventilator-associated pneumonia and many sepsis syndromes. It is an opportunistic pathogen and can cause serious disease cystic fibrosis and traumatic burn.

DNA gyrase (mutation) are commonly associated with antibiotic resistance. When grown in vitro condition designed to mimic the cystic fibrosis condition these genes mutate repeatedly.

Table 6-Known mutations in GyrA in *Pseudomonas Aeruginosa*

DNA Gyrase	Amino-acid	Mutated amino acid	Reference
GyrA	Glu-54	Lys54	Akasaka *et al*, 2001
GyrA	Ala-67	Ser 67	Akasaka *et al*, 2001
GyrA	Ser80	Ile80	Correia *et al*, 2017
GyrA	Ser83	Ile83 Leu83	Correia *et al*, 2017

		Phe83	
		Tyr83	
GyrA	Asp87	Asn87	Nouri R *et al,* 2016, Akasaka *et al,* 200, and Correia *et al,* 2017
		Ala87	
		Gln87	
		Glu87	
		Gly87	
		His87	
		Tyr87	
GyrA	Thr83	Ile83	Akasaka *et al,* 2001
		Ala83	
GyrA	Ser88	Ile88	Correia *et al,* 2017
		Phe88	
		Tyr88	

Klebsiella pneumoniae

Klebsiella pneumoniae is the most common bacteria which live on the feces and intestine of human being. It is gram negative bacteria, non-motile, encapsulated, lactose fermenting, facultative anaerobic, rod-shaped bacteria. When it is present in the intestine it is not harmful but when it reaches to other parts of the body than it can cause severe diseases. It can affect the brain, lungs, liver, bladder, eyes, and blood, wound. It spread person to person very quickly.

DNA gyrase (mutation) are commonly associated with antibiotic resistance. Fluoroquinolone resistance arises through specific mutation within the targeted protein of DNA gyrase, more specifically, within the region named as QRDR (quinolone- resistance determining region).

Table 7- Known mutations in GyrA in *Klebsiella pneumoniae*

DNA Gyrase	Drugs	Amino-acid	Mutated amino acid	Reference
gyrA	Ciprofloxacin	Ser83	Leu83	Fu Y Zhang *et al*, 2013
gyrA	Ciprofloxacin	Asp87	Asp87	Fu Y Zhang *et al*, 2013
gyrA	Ciprofloxacin	Ser83	Tyr83	Fu Y Zhang *et al*, 2013

CHAPTER 3

MATERIALS AND METHODS

3.1 Bacterial isolates and cultures media

Culture of *Acinetobacter baumannii* and *Escherichia coli* were collected from the Department of biotechnology, the Jaypee University of Information Technology. The cultures were confirmed by growing in selective media. Luria broth and MacConkey agar were used for routinely growth of bacterial cultures.

3.2 Antimicrobial susceptibility of bacterial isolates for different quinolones

Antibiotic susceptibility of *E. coli* and *A. baumannii* isolates were determined for different quinolones including ciprofloxacin, nalidixic acid and norfloxacin using Kirby-Bauer disc diffusion method. The procedure used for determining antibiotic susceptibility is explained in figure 2.
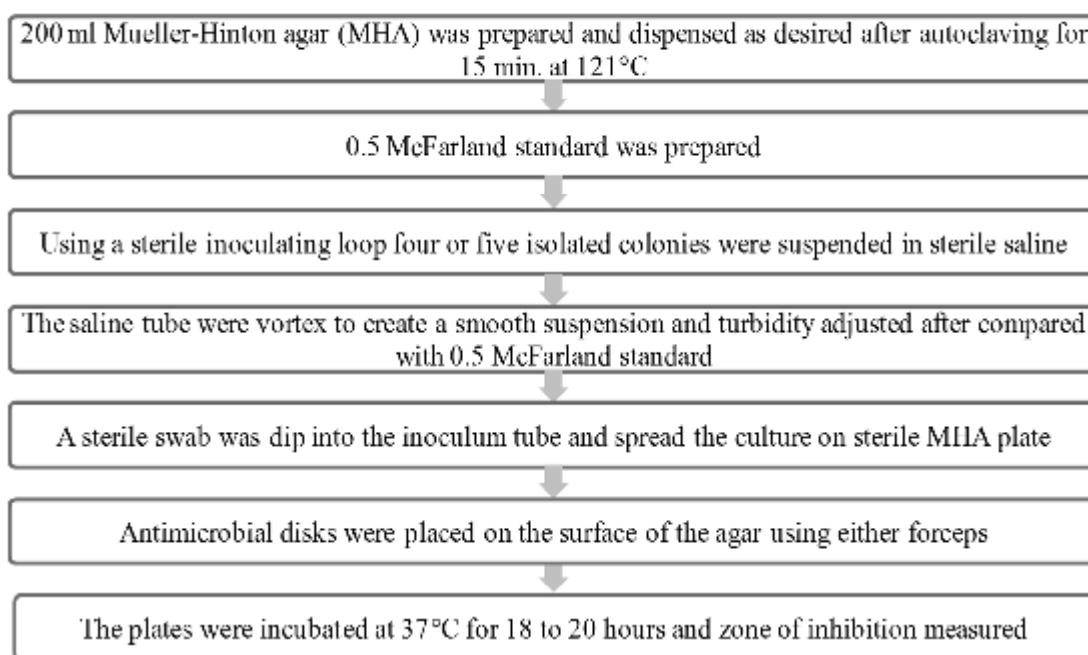
Fig 2:- A flowchart presenting procedure used for antibiotic susceptibility of bacterial isolates for different quinolones (Hudzicki *et al* 2009)

3.3 Text Data Mining

Text mining was done by extracting the information from different search engines like Google scholar, and PubMed. Key words used for literature search includes "quinolones resistance AND mutations in *Acinetobacter baumannii*", "quinolones resistance AND mutations in *Escherichia coli*", "quinolones resistance AND mutations in *Pseudomonas aeruginosa*", and "quinolones resistance and mutations in

Klebsiella pneumoniae". Research paper and review articles were downloaded from the website and relevant information was extracted and compiled as per the requirements of the study.

3.4 Protein FASTA Sequence of gyrA of different bacteria

3.4.1 *Acinetobacter baumannii*

Protein FASTA sequence for parental and the mutated gyrA sequences of *A. baumannii* were retrieved from Uniprot as mentioned in table 8.

gyrA parental sequence of *Acinetobacter baumannii*

>PJZ12373.1 DNA gyrase subunit A [*Acinetobacter baumannii* ATCC 19606 = CIP 70.34 = JCM 6841]
MSVSEIRPIAIEDELKHSYLDYAMSVIVSRALPDVRDGLKPVHRRVLYAMHELGNDYNKAYKKSARVVGDVIG
KYHPHGDSAVYETIVRMAQDFSLRYLLVDGQGNFGSIDGDSAAAMRYTEVRMTKLAHELLADLEKDTVDWE
DNYDGSERIPEVLPTRVPNLLINGAAGIAVGMATNMAPHNMTEVVNACLAYADNPNISIEGLMEYITGPDFPTG
GIIYGKSGIVDAYRTGKGRLHIRGKYHFEEDEKTGRTTIVFTEIPYQVNKARVIERIAELVKEKKLEGISELRDES
DKEGMRIAIDLKRGENAEVVVNNLFLNTQLENSFSINMVCLDNGQPKLMNLKDIIAAFIRHRQEVVTRRTMFEL
RKARERGHILEGLTVALANIDEIIETIKTSANPAEARERLLAGEWAGGGVVALLEKAGAISVRPDEIEGEDPNRP
FGLSDSIYRLSPTQVGAILELRLHRLTGLEQDKLHAEYTEILGQIAELTAILNDFNLLMGVIREELAQVLQQYGD
ARRTEIVESRVDFCREDLIPEEQVVLTVSQTGYAKTQPLSDYQAQRRGGRGKSATSMKDDDFIQHLIVASNHAT
VLCFTNVGKVYRLKVFEVPQASRGAKGRPIVNLLPLDATETVTAILPLTEFPENHYVFMATASGTVKRVELEQF
ANIRSNGLRAIELNEEDTLIGVAITDGNQQIMLFSNEGKAIRFAETDVRAMGRTAKGVRGMRVSFASSTLSEED
ADVENDDSDDNDDSADSSLVSRIVSLVVVPETGEVLCASANGYGKRTPVNDFPTKKRGGKGVIAIKTSERNGE
LVGAVSIDETKELLLISDGGTLVRTRAAEVAMTGRNAQGVRLIRLSEEETLVGVVSIEAVEDEEELLEGEVDTT
ETDSEEAVSNNEDTSEE

Table 8- Mutations in gyrA sequence of *A. baumannii* are listed in table

Protein FASTA Sequence	Accession IDs
MSVSEIRPIAIEDELKHSYLDYAMSVIVSRALPDVRDGLKPVHRRVLYAMHELGNDYNK AYKKSARVVGDVIGKYHPHGDLAVYKTIVRMAQDFSLRYLLVDGQGNFGSIDGDSAAA MRYTEVRMTKLAHELLADLEKDTVDWEDNYDGSERIPEVLPTRVPNLLINGAAGIAVGM ATNMAPHNMTEVVNACLAYADNPNISIEGLMEYITGPDFPTGGIIYGKSGIVDAYRTGKG RLHIRGKYHFEEDEKTGRTTIVFTEIPYQVNKARVIERIAELVKEKKLEGISELRDESDKEG MRIAIDLKRGENAEVVVNNLFLNTQLENSFSINMVCLDNGQPKLMNLKDIIAAFIRHRQE VVTRRTMFELRKARERGHILEGLTVALANIDEIIETIKTSANPAEARERLLAGEWAGGGVV ALLEKAGAISVRPDEIEGEDPNRPFGLSDSIYRLSPTQVGAILELRLHRLTGLEQDKLHAEY TEILGQIAELTAILNDFNLLMGVIREELAQVLQQYGDARRTEIVESRVDFCREDLIPEEQVV LTVSQTGYAKTQPLSDYQAQRRGGRGKSATSMKDDDFIQHLIVASNHATVLCFTNVGKV YRLKVFEVPQASRGAKGRPIVNLLPLDATETVTAILPLTEFPENHYVFMATASGTVKRVE LEQFANIRSNGLRAIELNEEDTLIGVAITDGNQQIMLFSNEGKAIRFAETDVRAMGRTAKG VRGMRVSFASSTLSEEDADVENDDSDDNDDSADSSLVSRIVSLVVVPETGEVLCASANGY GKRTPVNDFPTKKRGGKGVIAIKTSERNGELVGAVSIDETKELLLISDGGTLVRTRAAEVA MTGRNAQGVRLIRLSEEETLVGVVSIEAVEDEEELLEGEVDTTETDSEEAVSNNEDTSEE	WP_060853637.1
MSVSEIRPIAIEDELKHSYLDYAMSVIVSRALPDVRDGLKPVHRRVLYAMHKLGNDYNK AYKKSARVVGDVIGKYHPHGDLAVYETIVRMAQDFSLRYLLVDGQGNFGSIDGDSAAA MRYTEVRMTKLAHELLADLEKDTVDWEDNYDGSERIPEVLPTRVPNLLINGAAGIAVGM ATNMAPHNMTEVVNACLAYADNPNISIEGLMEYITGPDFPTGGIIYGKSGIVDAYRTGKG RLHIRGKYHFEEDEKTGRTTIVFTEIPYQVNKARVIERIAELVKEKKLEGISELRDESDKEG MRIAIDLKRGENAEVVVNNLFLNTQLENSFSINMVCLDNGQPKLMNLKDIIAAFIRHRQE VVTRRTMFELRKARERGHILEGLTVALANIDEIIETIKTSANPAEARERLLAGEWAGGGVV ALLEKAGAISVRPDEIEGEDPNRPFGLSDSIYRLSPTQVGAILELRLHRLTGLEQDKLHAEY TEILGQIAELTAILNDFNLLMGVIREELAQVLQQYGDARRTEIVESRVDFCREDLIPEEQVV LTVSQTGYAKTQPLSDYQAQRRGGRGKSATSMKDDDFIQHLIVASNHATVLCFTNVGKV YRLKVFEVPQASRGAKGRPIVNLLPLDATETVTAILPLTEFPENHYVFMATASGTVKRVE LEQFANIRSNGLRAIELNEEDTLIGVAITDGNQQIMLFSNEGKAIRFAETDVRAMGRTAKG VRGMRVSFASSTLSEEDADVENDDSDDNDDSADSSLVSRIVSLVVVPETGEVLCASANGY GKRTPVNDFPTKKRGGKGVIAIKTSERNGELVGAVSIDETKELLLISDGGTLVRTRAAEVA MTGRNAQGVRLIRLSEEETLVGVVSIEAVEDEEELLEGEVDTTETDSEEAVSNNEDTSEE	WP_057694568.1
MSVSEIRPIAIEDELKHSYLDYAMSVIVSRALPDVRDGLKPVHRRVLYAMHELGNDYNK AYKKSARVVGDVIGKYHPHGDSAVYETIVRMAQDFSLRYLLVDGQGNFGSIDGDSAAA MRYTEVRMTKLAHELLADLEKDTVDWEDNYDGSERIPEVLPTRVPNLLINGAAGIAVGM	WP_043041342.1

ATNMAPHNMTEVVNACLAYADNPNISIEGLMEYITGPDFPTGGIIYGKSGIVDAYRTGKG RLHIRGKYHFEEDEKTGRTTIVFTEIPIYQVNKARVIERIAELVKEKKLEGISELRDESDKEG MRIAIDLKRGENAEVVVNNLFLNTQLENSFSINMVCLDNGQPKLMNLKDIIAAFIRHRQE VVTRRTMFELRKARERGHILEGLTVALANIDEIIETIKTSANPAEARERLLAGEWAGGGVV ALLEKAGAISVRPDEIEGEDPNRPFGLSDSIYRLSPTQVGAILELRLHRLTGLEQDKLHAEY TEILGQIAELTAILNDFNLLMGVIREELAQVLQQYGDARRTEIVESRVDFCREDLIPEEQVV LTVSQTGYAKTQPLSDYQAQRRGGRGKSATSMKDDDFIQHLIVASNHATVLCFTNVGKV YRLKVFEVPQASRGANGRPIVNLLPLDATETVTAILPLTEFPENHYVFMATASGTVKRVE LEQFANIRSNGLRAIELNEEDTLIGVAITDGNQQIMLFSNEGKAIRFAETDVRAMGRTAKG VRGMRVSFASSTLSEEDADVENDDSDDNDDSADRSIVSLVVVPETGEVLCASANGY GKRTPVNDFPTKKRGGKGVIAIKTSERNGELVGAVSIDETKELLLISDGGTLVRTRAAEVA MTGRNAQGVRLIRLSEEETLVGVVSIEAVEDEEELLEGEVDTTETDSEEAVSNNEDTSEE	
MSVSEIRPIAIEDELKHSYLDYAMSVIVSRALPDVRDGLKPVHRRVLYAMHELGNDYNK AYKKSARVVGDVIGKYHPHGDLAVYETIVRMAQDFSLRYLLVDGQGNFGSIDGDSAAA MRYTEVRMTKLAHELLADLEKDTVDWEDNYDGSERIPEVLPTRVPNLLINGAAGIAVGM ATNMAPHNMTEVVNACLAYADNPNISIEGLMEYITGPDFPTGGIIYGKSGIVDAYRTGKG RLHIRGKYHFEEDEKTGRTTIVFTEIPYQVNKARVIERIAELVKEKKLEGISELRDESDKEG MRIAIDLKRGENAEVVVNNLFLNTQLENSFSINMVCLDNGQPKLMNLKDIIAAFIRHRQE VVTRRTMFELRKARERGHILEGLTVALANIDEIIETIKTSANPAEARERLLAGEWAGGGVV ALLEKAGAISVRPDEIEGEDPNRPFGLSDSIYRLSPTQVGAILELRLHRLTGLEQDKLHAEY TEILGQIAELTAILNDFNLLMGVIREELAQVLQQYGDARRTEIVESRVDFCREDLIPEEQVV LTVSQTGYAKTQPLSDYQAQRRGGRGKSATSMKDDDFIQHLIVASNHATVLCFTNVGKV YRLKVFEVPQASRGAKGRPIVNLLPLDATETVTAILPLTEFPENHYVFMATASGTVKRVE LEQFANIRSNGLRAIELNEEDTLIGVAITDGNQQIMLFSNEGKAIRFAETDVRAMCRTAKG VRGMRVSFASSTLSEEDADVENDDSDDNDDSADSSLVSRIVSLVVVPETGEVLCASANGY GKRTPVNDFPTKKRGGKGVIAIKTSERNGELVGAVSIDETKELLLISDGGTLVRTRAAEVA MTGRNAQGVRLIRLSEEETLVGVVSIEAVEDEEELLEGEVDTTETDSEEAVSNNEDTSEE	WP_042760707. 1
MSVSEIRPIAIEDELKHSYLDYAMSVIVSRALPDVRDGLKPVHRRVLYAMHELGNDYNK AYKKSARVVGDVIGKYHPHGDLAVYETIVRMAQDFSLRYLLVDGQGNFGSIDGDSAAA MRYTEVRMTKLAHELLADLEKDTVDWEDNYDGSERIPEVLPTRVPNLLINGAAGIAVGM ATNMAPHNMTEVVNACLAYADNPNISIEGLMEYITGPDFPTGGIIYGKSGIVDAYRTGKG RLHIRGKYHFEEDEKTGRTTIVFTEIPYQVNKARVIERIAELVKEKKLEGISELRDESDKEG MRIAIDLKRGENAEVVVNNLFLNTQLENSFSINMVCLDNGQPKLMNLKDIIAAFIRHRQE VVTRRTMFELRKARERGHILEGLTVALANIDEIIETIKTSANPAEARERLLAGEWAGGGVV ALLEKAGAISVRPDEIEGEDPNRPFGLSDSIYHLSPTQVGAILELRLHRLTGLEQDKLHAEY TEILGQIAELTAILNDFNLLMGVIREELAQVLQQYGDARRTEIVESRVDFCREDLIPEEQVV LTVSQTGYAKTQPLSDYQAQRRGGRGKSATSMKDDDFIQHLIVASNHATVLCFTNVGKV YRLKVFEVPQASRGAKGRPIVNLLPLDATETVTAILPLTEFPENHYVFMATASGTVKRVE LEQFANIRSNGLRAIELNEEDTLIGVAITDGNQQIMLFSNEGKAIRFAETDVRAMGRTAKG VRGMRVSFASSTLSEEDADVENDDSDDNDDSADSSLVSRIVSLVVVPETGEVLCASANGY GKRTPVNDFPTKKRGGKGVIAIKTSERNGELVGAVSIDETKELLLISDGGTLVRTRAAEVA MTGRNAQGVRLIRLSEEETLVGVVSIEAVEDEEELLEGEVDTTETDSEEAVSNNEDTSEE	WP_038350115. 1
MSVSEIRPIAIEDELKHSYLDYAMSVIVSRALPDVRDGLKPVHRRVLYAMHELGNDYNK AYKKSARVVGDVIGKYHPHGDLAVYETIVRMAQDFSLRYLLVDGQGNFGSIDGDSAAA MRYTEVRMTKLAHELLADLEKDTVDWEDNYDGSERIPEVLPTRVPNLLINGAAGIAVGM ATNMAPHNMTEVVNACLAYADNPNISIEGLMEYITGPDFPTGGIIYGKSGIVDAYRTGKG RLHIRGKYHFEEDEKTGRTTIVFTEIPYQVNKARVIERIAELVKEKKLEGISELRDESDKEG MRIAIDLKRGENAEVVVNNLFLNTQLENSFSINMVCLDNGQPKLMNLKDIIAAFIRHRQE VVTRRTMFELRKARERGHILEGLTVALANIDEIIETIKTSANPAEARERLLAGEWAGGGVV ALLEKAGAISVRPDEIEGEDPNRPFGLSDSIYRLSPTQVGAILELRLHRLTGFEQDKLHAEY TEILGQIAELTAILNDFNLLMGVIREELAQVLQQYGDARRTEIVESRVDFCREDLIPEEQVV LTVSQTGYAKTQPLSDYQAQRRGGRGKSATSMKDDDFIQHLIVASNHATVLCFTNVGKV YRLKVFEVPQASRGAKGRPIVNLLPLDATETVTAILPLTEFPENHYVFMATASGTVKRVE LEQFANIRSNGLRAIELNEEDTLIGVAITDGNQQIMLFSNEGKAIRFAETDVRAMGRTAKG VRGMRVSFASSTLSEEDADVENDDSDDNDDSADSSLVSRIVSLVVVPETGEVLCASANGY GKRTPVNDFPTKKRGGKGVIAIKTSERNGELVGAVSIDETKELLLISDGGTLVRTRAAEVA MTGRNAQGVRLIRLSEEETLVGVVSIEAVEDEEELLEGEVDTTETDSEEAVSNNEDTSEE	WP_038344622. 1
MSVSEIRPIAIEDELKHSYLDYAMSVIVSRALPDVRDGLKPVHRRVLYAMHELGNDYNK AYKKSARVVGDVIGKYHPHGDLAVYETIVRMAQDFSLRYLLVDGQGNFGSIDGDSAAA MRYTEVRMTKLAHELLADLEKDTVDWEDNYDGSERIPEVLPTRVPNLLINGAAGIAVGM ATNMAPHNMTEVVNACLAYADNPNISIEGLMEYITGPDFPTGGIIYGKSGIVDAYRTGKG RLHIRGKYHFEEDEKTGRTTIVFTEIPYQVNKARVIERIAELVKEKKLEGISELRDESDKEG MRIAIDLKRGENAEVVVNNLFLNTQLENSFSINMVCLDNGQPKLMNLKDIIAAFIRHRQE VVTRRTMFELRKARERGHILEGLTVALANIDEIIETIKTSANPAEARERLLAGEWAGGGVV ALLEKAGAISVRPDEIEGEDPNRPFGLSDSFYRLSPTQVGAILELRLHRLTGLEQDKLHAE YTEILGQIAELTAILNDFNLLMGVIREELAQVLQQYGDARRTEIVESRVDFCREDLIPEEQV VLTVSQTGYAKTQPLSDYQAQRRGGRGKSATSMKDDDFIQHLIVASNHATVLCFTNVGK VYRLKVFEVPQASRGAKGRPIVNLLPLDATETVTAILPLTEFPENHYVFMATASGTVKRV ELEQFANIRSNGLRAIELNEEDTLIGVAITDGNQQIMLFSNEGKAIRFAETDVRAMGRTAK GVRGMRVSFASSTLSEEDADVENDDSDDNDDSTDSSLVSRIVSLVVVPETGEVLCASANG YGKRTPVNDFPTKKRGGKGVIAIKTSERNGELVGAVSIDETKELLLISDGGTLVRTRAAEV AMTGRNAQGVRLIRLSEEETLVGVVSIEAVEDEEELLEGEVDTTETDSEEAVSNNEDTSEE	CAM85817.1
MSVSEIRPIAIEDELKHSYLDYAMSVIVSRALPDVRDGLKPVHRRVLYAMHELGNDYNK AYKKSARVVGDVIGKYHPHGDLAVYETIVRMAQDFSLRYLLVDGQGNFGSIDGDSAAA MRYTEVRMTKLAHELLADLEKDTVDWEDNYDGSERIPEVLPTRVPNLLINGAAGIAVGM	CAJ77862.1

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ATNMAPHNMTEVVNACLAYADNPNISIEGLMEYITGPDFPTGGIIYGKSGIVDAYRTGKG RLHIRGKYHFEEDEKTGRTTIVFTEIPYQVNKARVIERIAELVKEKKLEGISELRDESDKEG MRIAIDLKRGENAEVVVNNLFLNTQLENSFSINMVCLDNGQPKLMNLKDIIAAFIRHRQE VVTRRTMFELRKARERGHILEGLTVALANIDEIIETIKTSANPAEARERLLAGEWAGGGVV ALLEKAGAISVRPDEIEGEDPNRPFGLSDSFYRLSPTQVGAILELRLHRLTGLEQDKLHAE YTEILGQIAELTAILNDFNLLMGVIREELAQVLQQYGDARRTEIVESRVDFCREDLIPEEQV VLTVSQTGYAKTQPLSDYQAQRGGRGKSATSMKDDDFIQHLIVASNHATVLCFTNVGK VYRLKVFEVPQASRGAKGRPIVNLLPLDATETVTAILPLTEFPENHYVFMATASGTVKRV ELEQFANIRSNGLRAIELNEEDTLIGVAITDGNQQIMLFSNEGKAIRFAETDVRAMGRTAK GVRGMRVSFASSTLSEEDADVENDDSDDNDDSTDSSLVSRIVSLVVVPETGEVLCASANG YGKRTPVNDFPTKKRGGKGVIAIKTSERNGELVGAVSIDETKELLLISDGGTLVRTRAAEV AMTGRNAQGVRLIRLSEEETLVGVVSIEAVEDEEELLEGEVDTTETDSEEAVSNNEDTSEE	
MSVSEIRPIAIEDELKHSYLDYAMSVIVSRALPDVRDGLKPVHRRVLYAMHELGNDYNK AYKKSARVVGDVIGKYHPGDLAVYETIVRMAQDFSLRYLLVDGQGNFGSIDGDSAAA MRYTEVRMTKLAHELLADLEKDTVDWEDNYDGSERIPEVLPTRVPNLLINGAAGIAVGM ATNMAPHNMTEVVNACLAYADNPNISIEGLMEYITGPDFPTGGIIYGKSGIVDAYRTGKG RLHIRGKYHFEEDEKTGRTTIVFTEIPYQVNKARVIERIAELVKEKKLEGISELRDESDKEG MRIAIDLKRGENAEVVVNNLFLNTQLENSFSINMVCLDNGQPKLMNLKDIIAAFIRHRQE VVTRRTMFELRKARERGHILEGLTVALANIDEIIETIKTSANPAEARERLLAGEWAGGGVV ALLEKAGAISVRPDEIEGEDPNRPFGLSDSFYRLSPTQVGAILELRLHRLTGLEQDKLHAE YTEILGQIAELTAILNDFNLLMGVIREELAQVLQQYGDARRTEIVESRVDFCREDLIPEEQV VLTVSQTGYAKTQPLSDYQAQRGGRGKSATSMKDDDFIQHLIVASNHATVLCFTNVGK VYRLKVFEVPQASRGAKGRPIVNLLPLDATETVTAILPLTEFPENHYVFMATASGTVKRV ELEQFANIRSNGLRAIELNEEDTLIGVAITDGNQQIMLFSNEGKAIRFAETDVRAMGRTAK GVRGMRVSFASSTLSEEDADVENDDSDDNDDSTDSSLVSRIVSLVVVPETGEVLCASANG YGKRTPVNDFPTKKRGGKGVIAIKTSERNGELVGAVSIDETKELLLISDGGTLVRTRAAEV AMTGRNAQGVRLIRLSEEETLVGVVSIEAVEDEEELLEGEVDTTETDSEEAVSNNEDTSEE	WP_000116442. 1

3.4.2 *Escherichia coli*

Protein FASTA sequences for parental and mutated gyrA protein of *E. coli* were retrieved from Uniprot as mentioned in table 9.

gyrA parental sequence of *Escherichia coli*

>EOR52871.1 DNA gyrase subunit A [*Escherichia coli* ATCC 25922]
MSDLAREITPVNIEEELKSSYLDYAMSVIVGRALPDVRDGLKPVHRRVLYAMNVLGNDWNKAYKKSARVVG
DVIGKYHPGDSAVYDTIVRMAQPFSLRYMLVDGQGNFGSIDGDSAAAMRYTEIRLAKIAHELMADLEKETV
DFVDNYDGTEKIPDVMPTKIPNLLVNGSSGIAVGMATNIPPHNLTEVINGCLAYIDDEDISIEGLMEHIPGPDFPT
AAIINGRRGIEEAYRTGRGKVYIRARAEVEVDAKTGRETIIVHEIPYQVNKARLIEKIAELVKEKRVEGISALRDE
SDKDGMRIVIEVKRDAVGEVVLNNLYSQTQLQVSFGINMVALHHGQPKIMNLKDIIAAFVRHRREVVTRRTIFE
LRKARDRAHILEALAVALANIDPIIELIRHAPTPAEAKTALVANPWQLGNVAAMLERAGDDAARPEWLEPEFG
VRDGLYYLTEQQAQAILDLRLQKLTGLEHEKLLDEYKELLDQIAELLRILGSADRLMEVIREELELVREQFGDK
RRTEITANSADINLEDLITQEDVVVTLSHQGYVKYQPLSEYEAQRRGGKGKSAARIKEEDFIDRLLVANTHDHI
LCFSSRGRVYSMKVYQLPEATRGARGRPIVNLLPLEQDERITAILPVTEFEEGVKVFMATANGTVKKTVLTEFN
RLRTAGKVAIKLVEGDELIGVDLTSGEDEVMLFSAEGKVVRFKESSVRAMGCNTTGVRGIRLGEGDKVVSLIV
PRGDGAILTATQNGYGKRTAVAEYPTKSRATKGVISIKVTERNGLVVGAVQVDDCDQIMMITDAGTLVRTRVS
EISIVGRNTQGVILIRTSEDENVVGLQRVAEPVDEEDLDTIDGSAAEGDDEIAPEVDVDDEPEEE

Table 9- Mutations in gyrA sequence of *E. coli* are listed in the table

Protein FASTA Sequence	Accession IDs
MSDLAREITPVNIEEELKSSYLDYAMSVIVGRALPDVRDGLKPVHRRVLYAMNVLGND WNKAYKKSARVVGDVIGKYHPGDSAVYDTIVRMAQPFSLRYMLVDGQGNFGSIDG DSAAAMRYTEIRLAKIAHELMADLEKETVDFVDNYDGTEKIPDVMPTKIPNLLVNGSS GIAVGMATNIPPHNLTEVINGCLAYIDDEDISIEGLMEHIPGPDFPTAAIINGRRGIEEAYR TGRGKVYIRARAEVEVDAKTGRETIIVHEIPYQVNKARLIEKIAELVKEKRVEGISALRD ESDKDGMRIVIEVKRDAVGEVVLNNLYSQTQLQVSFGINMVALHHGQPKIMNLKDIIA SFVRHRREVVTRRTIFELRKARDRAHILEALAVALANIDPIIELIRHAPTPAEAKTALVA NPWQLGNVAAMLERAGDDAARPEWLEPEFGVRDGLYYLTEQQAQAILDLRLQKLTG LEHEKLLDEYKELLDQIAELLRILGSADRLMEVIREELELVREQFGDKRRTEITANSADI NLEDLITQEDVVVTLSHQGYVKYQPLSEYEAQRRGGKGKSAARIKEEDFIDRLLVANT	EFX34722.1

HDHILCFSSRGRVYSMKVYQLPEATRGARGRPIVNLLPLEQDERITAILPVTEFEEGVKV FMATANGTVKKTVLTEFNRLRTAGKVAIKLVEGDELIGVDLTSGEDEVMLFSAEGKVV RFKESSVRAMGCNTTGVRGIRLGEGDKVVSLIVPRGDGAILTATQNGYGKRTAVAEYP TKSRATKGVISIKVTERNGLVVGAVQVDDCDQIMMITDAGTLVRTRVSEISIVGRNTQG VILIRTAEDENVVGLQRVAEPVDEEDLDTIDGSAAEGDDEIAPEVDVDDEPEEE	
MSDLAREITPVNIEEELKSSYLDYAMSVIVGRALPDVRDGLKPVHRRVLYAMNVLGND WNKAYKKSARVVGDVIGKYHPHGDSAVYDTIVRMAQPFSLRYMLVDGQGNFGSIDG DSAAAMRYTEIRLAKIAHELMADLEKETVDFVDNYDGTEKIPDVMPTKIPNLLVNGSS GIAVGMATNIPPHNLTEVINGCLAYIDDEDISIEGLMEHIPGPDFPTAAIINGRRGIEEAYR TGRGKVYIRARAEVEVDAKTGRETIIVHEIPYQVNKARLIEKIAELVKEKRVEGISALRD ESDKDGMRIVIEVKRDAVGEVVLNNLYSQTQLQVSFGINMVALHHGQPKIMNLKDIIA AFVRHRREVVTRRTIFELRKARDRAHILEALAVALANIDPIIELIRHAPTPAEAKTALVA NPWQLGNVAAMLERAGDDAARPEWLEPEFGVRDGLYYLTEQQAQAILDLRLQKLTG LEHEKLLDEYKELLDQISELLRILGSADRLMEVIREELELVREQFGDKRRTEITANSADI NLEDLITQEDVVVTLSHQGYVKYQPLSEYEAQRRGGKGKSAARIKEEDFIDRLLVANT HDHILCFSSRGRVYSMKVYQLPEATRGARGRPIVNLLPLEQDERITAILPVTEFEEGVKV FMATANGTVKKTVLTEFNRLRTAGKVAIKLVDGDELIGVDLTSGEDEVMLFSAEGKV VRFKESSVRAMGCNTTGVRGIRLGEGDKVVSLIVPRGDGAILTATQNGYGKRTAVAEY PTKSRATKGVISIKVTERNGLVVGAVQVDDCDQIMMITDAGTLVRTRVSEISIVGRNTQ GVILIRTAEDENVVGLQRVAEPVDEEDLDTIDGSAAEGDDEIAPEVDVDDEPEEE	KIH25564.1
MSDLAREITPVNIEEELKSSYLDYAMSVIVGRALPDVRDGLKPVHRRVLYAMNVLGND WNKAYKKSARVVGDVIGKYHPHGDLAVYDTIVRMAQPFSLRYMLVDGQGNFGSIDG DSAAAMRYTEIRLAKIAHELMADLEKETVDFVDNYDGTEKIPDVMPTKIPNLLVNGSS GIAVGMATNIPPHNLTEVINGCLAYIDDEDISIEGLMEHIPGPDFPTAAIINGRRGIEEAYR TGRGKVYIRARAEVEVDAKTGRETIIVHEIPYQVNKARLIEKIAELVKEKRVEGISALRD ESDKDGMRIVIEVKRDAVGEVVLNNLYSQTQLQVSFGINMVALHHGQPKIMNLKDIIA AFVRHRREVVTRRTIFELRKARDRAHILEALAVALANIDPIIELIRHAPTPAEAKTALVA NPWQLGNVAAMLERAGDDAARPEWLEPEFGVRDGMYYLTEQQAQAILDLRLQKLTG LEHEKLLDEYKELLDQIAELLRILGSADRLMEVIREELELVREQFGDKRRTEITANSADI NLEDLITQEDVVVTLSHQGYVKYQPLSEYEAQRRGGKGKSAARIKEEDFIDRLLVANT HDHILCFSSRGRVYSMKVYQLPEATRGARGRPIVNLLPLEQDERITAILPVTEFEEGVKV FMATANGTVKKTVLTEFNRLRTAGKVAIKLVDGDELIGVDLTSGEDEVMLFSAEGKV VRFKESSVRAMGCNTTGVRGIRLGEGDKVVSLIVPRGDGAILTATQNGYGKRTAVAEY PTKSRATKGVISIKVTERNGLVVGAVQVDDCDQIMMITDAGTLVRTRVSEISIVGRNTQ GVILIRTAEDENVVGLQRVAEPVDEEDLDTIDGSAAEGDDEIAPEVDVDDEPEEE	KHJ14714.1
MSDLAREITPVNIEEELKSSYLDYAMSVIVGRALPDVRDGLKPVHRRVLYAMNVLGND WNKAYKKSARVVGDVIGKYHPHGDSAVYDTIVRMAQPFSLRYMLVDGQGNFGSIDG DSAAAMRYTEIRLAKIAHELMADLEKETVDFVDNYDGTEKIPDVMPTKIPNLLVNGSS GIAVGMATNIPPHNLTEVINGCLAYIDDEDISIEGLMEHIPGPDFPTAAIINGRRGIEEAYR TGRGKVYIRARAEVEVDAKTGRETIIVHEIPYQVNKARLIEKIAELVKEKRVEGISALRE SDKDGMRIVIEVKRDAVGEVVLNNLYSQTQLQVSFGINMVALHHGQPKIMNLKDIIAA FVRHRREVVTRRTIFELRKARDRAHILEALAVALANIDPIIELIRHAPTPAEAKTALVAN PWQLGNVXAMLERAGDDAARPEWLEPEFGVRDGLYYLTEQQAQAILDLRLQKLTGLE HEKLLDEYKELLDQIAELLRILGSADRLMEVIREELELVREQFGDKRRTEITANSADINL EDLITQEDVVVTLSHXGYVKYQPLSEYEAQRRGGKGKSAARIKEEDFIDRLLVANTHD HILCFSSRGRVYSMKVYQLPEATRGARGRPIVNLLPLEQDERITAILPVTEFEEGVKVFM ATANGTVKKTVLTEFNRLRTAGKVAIKLVDGDELIGVDLTSGEDEVMLFSAE GKVVRFKESSVRAMGCNTTGVRGIRLGEGDKVVSLIVPRGDGAILTATQNGYGKRTA VAEYPTKSRATKGVISIKVTERNGLVVGAVQVDDCDQIMMITDAGTLVRTRVSEISIVG RNTQGVILIRTAEDENVVGLQRVAEPVDEEDLDTIDGSAAEGDDEIAPEVDVDDEPEEE	KFH81288.1
MSDLAREITPVNIEEELKSSYLDYAMSVIVGRALPDVRDGLKPVHRRVLYAMNVLGND WNKAYKKSARVVGDVIGKYHPHGDLAVYDTIVRMAQPFSLRYMLVDGQGNFGSIDG DSAAAMRYTEIRLAKIAHELMADLEKETVDFVDNYDGTEKIPDVMPTKIPNLLVNGSS GIAVGMATNIPPHNLTEVINGCLAYIDDEDISIEGLMEHIPGPDFPTAAIINGRRGIEEAYR TGRGKVYIRARAEVEVDAKTGRETIIVHEIPYQVNKARLIEKIAELVKEKRVEGISALRD ESDKDGMRIVIEVKRDAVGEVVLNNLYSQTQLQVSFGINMVALHHGQPKIMNLKDIIA AFVRHRREVVTRRTIFELRKARDRAHILEALAVALANIDPIIELIRHAPTPAEAKTALVA NPWQLGNVAAMLERAGDDAARPEWLEPEFGVRDGLYYLTEQQAQAILDLRLQKLTG LEHEKLLDEYKELLDQIAELLRILGSADRLMEVIREELELVREQFGDKRRTEITANSADI NLEDLITQEDVVVTLSHQXYVKYQPLSEYEAQRRGGKGKSAARIKEEDFIDRLLVANT HDHILCFSSRGRVYSMKVYQLPEASRGARGRPIVNLLPLEQDERITAILPVTEFEEGVKV FMATANGTVKKTVLTEFNRLRTAGKVAIKLVEGDELIGVDLTSGEDEVMLFSAEGKVV RFKESSVRAMGCNTTGVRGIRLGEGDKVVSLIVPRGDGAILTATQNGYGKRTAVAEYP TKSRATKGVISIKVTERNGLVVGAVQVDDCDQIMMITDAGTLVRTRVSEISIVGRNTQG VILIRTAEDENVVGLQRVAEPVDEEDLDTIDGSAAEGDDEIAPEVDVDDEPEEE	KFI00766.1
MSDLAREITPVNIEEELKSSYLDYAMSVIVGRALPDVRDGLKPVHRRVLYAMNVLGND WNKAYKKSARVVGDVIGKYHPHGDLAVYDTIVRMAQPFSLRYMLVDGQGNFGSIDG DSAAAMRYTEIRLAKIAHELMADLEKETVDFVDNYDGTEKIPDVMPTKIPNLLVNGSS GIAVGMATNIPPHNLTEVINGCLAYIDDEDISIEGLMEHIPGPDFPTAAIINGRRGIEEAYR TGRGKVYIRARAEVEVDAKTGRETIIVHEIPYQVNKARLIEKIAELVKEKRVEGISALRD ESDKDGMRIVIEVKRDAVGEVVLNNLYSQTQLQVSFGINMVALHHGQPKIMNLKDIIA AFVRHRREVVTRRTIFELRKARDRAHILEALAVALANIDSIIELIRHAPTPAEAKTALVA NPWQLGNVAAMLERAGDDAARPEWLEPEFGVRDGLYYLTEQQAQAILDLRLQKLTG LEHEKLLDEYKELLDQIAELLRILGSADRLMEVIREELELVREQFGDKRRTEITANSADI NLEDLITQEDVVVTLSHQGYVKYQPLSEYEAQRRGGKGKSAARIKEEDFIDRLLVANT	CTT19640.1

HDHILCFSSRGRVYSMKVYQLPEATRGARGRPIVNLLPLEQDERITAILPVTEFEEGVKV FMATANGTVKKTVLTEFNRLRTAGKVAIKLVDGDELIGVDLTSGEDEVMLFSAEGKV VRFKESSVRAMGCNTTGVRGIRLGEGDKVVSLIVPRGDGAILTATQNGYGKRTAVAEY PTKSRATKGVISIKVTERNGLVVGAVQVDDCDQIMMITDAGTLVRTRVSEISIVGRNTQ GVILIRTAEDENVVGLQRVAEPVDEEDLDTIDGSAAEGDDEIAPEVDVDDEPEEE	
MSDLAREITPVNIEEELKSSYLDYAMSVIVGRALPDVRDGLKPVHRRVLYAMNVLGND WNKAYKKSARVVGDVIGKYHPHGDSAVYDTIVRMAQPFSLRYMLVDGQGNFGSIDG DSAAAMRYTEIRLAKIAHELMADLEKETVDFVDNYDGTEKIPDVMPTKIPNLLVNGSS GIAVGMATNIPPHNLTEVINGCLAYIDDEDISIEGLMEHIPGPDFPTAAIINGRRGIEEAYR TGRGKVYIRARAEVEVDAKTGRETIIVHEIPYQVNKARLIEKIAELVKEKRVEGISALRD ESDKDGMRIVIEVKRDAVGEVVLNNLYSQTQLQVSFGINMVALHHGQPKIMNLKDIIA AFVRHRREVVTRRTIFELRKARDRAHILEALAVALANIDPIIELIRHAPTPAEAKTALVA NPWQLGNVAAMLERAGDDAARPEWLEPEFGVRDGLYYLTEQQAQAILDLRLQKLTG LEHEKLLDEYKELLDQIAELLRILGSADRLMEVIREELELVREQFGDKRRTEITANSADI NLEDLITQEDVVVTLSHQGYVKYQPLSEYEAQRRGGKGKSAARIKEEDFIDRLLVANT HDHILCFSSRGRVYSMKVYQLPEATRGARGRPIVNLLPLEQDERITAILPVTEFEEGVKV FMATANGTVKKTVLTEFNRLRTAGKVAIKLVDGDELIGVDLTSGEDEVMLFSAEGKV VRFKESSVRAMGCNTTGVRGIRLGEGDKVVSLIVPRGDGAILTATQNGYGKRTAVAEY PTKSRATKGVISIKVTERNGLVVSAVQVDDCDQIMMITDAGTLVRTRVSEISIVGRNTQ GVILIRTAEDENVVGLQRVAEPVDEEDLDTIDGSAAEGDDEIAPEVDVDDEPEEE	CTW93136.1
MSDLAREITPVNIEEELKSSYLDYAMSVIVGRALPDVRDGLKPVHRRVLYAMNVLGND WNKAYKKSARVVGDVIGKYHPHGDSAVYDTIVRMAQPFSLRYMLVDGQGNFGSIDG DSAAAMRYTEIRLAKIAHELMADLEKETVDFVDNYDGTEKIPDVMPTKIPNLLVNGSS GIAVGMATNIPPHNLTEVINGCLAYIDDEDISIEGLMEHIPGPDFPTAAIINGRRGIEEAYR TGRGKVYIRARAEVEVDAKTGRETIIVHEIPYQVNKARLIEKIAELVKEKRVEGISALRD ESDKDGMRIVIEVKRDAVGEVVLNNLYSQTQLQVSFGINMVALHHGQPKIMNLKDIIA AFVRHRREVVTRRTIFELRKARDRAHILEALAVALANIDPIIELIRHAPTPAEAKTALVA NPWQLGNVAAMLERAGDDAARPEWLEPEFGVRDGLYYLTEQQAQAILDLRLQKLTG LEHEKLLDEYKELLDQIAELLRILGSADRLMEVIREELELVREQFGDKRRTEITANSADI NLEDLITQEDVVVTLSHQGYVKYQPLSEYEAQRRGGKGKSAARIKEEDFIDRLLVANT HDHILCFSSRGRVYSMKVYQLPEATRGARGRPIVNLLPLEQDERITAILPVTEFEEGVKV FMATANGTVKKTVLTEFNRLRTAGKVAIKLVDGDELIGVDLTSGEDEVMLFSAEGKV VRFKESSVRAMGCNTTGVRGIRLGEGDKVVSLIVPRGDGAILTATQNGYGKRTAVAEY PTKSRATKGVISIKVTERNGLVVSAVQVDDCDQIMMITDAGTLVRTRVSEISIVGRNTQ GVILIRTAEDENVVGLQRVAEPVDEEDLDTIDGSAAEGDDEIAPEVDVDDEPEEE	CTW43843.1
MSDLAREITPVNIEEELKSSYLDYAMSVIVGRALPDVRDGLKPVHRRVLYAMNVLGND WNKAYKKSARVVGDVIGKYHPHGDSAVYDTIVRMAQPFSLRYMLVDGQGNFGSIDG DSAAAMRYTEIRLAKIAHELMADLEKETVDFVDNYDGTEKIPDVMPTKIPNLLVNGSS GIAVGMATNIPPHNLTEVINGCLAYIDDEDISIEGLMEHIPGPDFPTAAIINGRRGIEEAYR TGRGKVYIRARAEVEVDAKTGRETIIVHEIPYQVNKARLIEKIAELVKEKRVEGISALRD ESDKDGMRIVIEVKRDAVGEVVLNNLYSQTQLQVSFGINMVALHHGQPKIMNLKDIIA AFVRHRREVVTRRTIFELRKARDRAHILEALAVALANIDSIIELIRHAPTPAEAKTALVA NPWQLGNVAAMLERAGDDAARPEWLEPEFGVRDGLYYLTEQQAQAILDLRLQKLTG LEHEKLLDEYKELLDQIAELLRILGSADRLMEVIREELELVREQFGDKRRTEITANSADI NLEDLITQEDVVVTLSHQGYVKYQPLSEYEAQRRGGKGKSAARIKEEDFIDRLLVANT HDHILCFSSRGRVYSMKVYQLPEATRGARGRPIVNLLPLEQDERITAILPVTEFEEGVKV FMATANGTVKKTVLTEFNRLRTAGKVAIKLVDGDELIGVDLTSGEDEVMLFSAEGKV VRFKESSVRAMGCNTTGVRGIRLGEGDKVVSLIVPRGDGAILTATQNGYGKRTAVAEY PTKSRATKGVISIKVTERNGLVVGAVQVDDCDQIMMITDAGTLVRTRVSEISIVGRNTQ GVILIRTAEDENVVGLQRVAEPVDEEDLDTIDGSAAEGDDEIAPEVDVDDEPEEE	CTU98127.1
MSDLAREITPVNIEEELKSSYLDYAMSVIVGRALPDVRDGLKPVHRRVLYAMNVLGND WNKAYKKSARVVGDVIGKYHPHGDSAVYDTIVRMAQPFSLRYMLVDGQGNFGSIDG DSAAAMRYTEIRLAKIAHELMADLEKETVDFVDNYDGTEKIPDVMPTKIPNLLVNGSS GIAVGMATNIPPHNLTEVINGCLAYIDDEDISIEGLMEHIPGPDFPTAAIINGRRGIEEAYR TGRGKVYIRARAEVEVDAKTGRETIIVHEIPYQVNKARLIEKIAELVKEKRVEGISALRD ESDKDGMRIVIEVKRDAVGEVVLNNLYSQTQLQVSFGINMVALHHGQPKIMNLKDIIA AFVRHRREVVTRRTIFELRKARDRAHILEALAVALANIDSIIELIRHAPTPAEAKTALVA NPWQLGNVAAMLERAGDDAARPEWLEPEFGVRDGLYYLTEQQAQAILDLRLQKLTG LEHEKLLDEYKELLDQIAELLRILGSADRLMEVIREELELVREQRRTEITANSADI NLEDLITQEDVVVTLSHQGYVKYQPLSEYEAQRRGGKGKSAARIKEEDFIDRLLVANT HDHILCFSSRGRVYSMKVYQLPEATRGARGRPIVNLLPLEQDERITAILPVTEFEEGVKV FMATANGTVKKTVLTEFNRLRTAGKVAIKLVDGDELIGVDLTSGEDEVMLFSAEGKV VRFKESSVRAMGCNTTGVRGIRLGEGDKVVSLIVPRGDGAILTATQNGYGKRTAVAEY PTKSRATKGVISIKVTERNGLVVGAVQVDDCDQIMMITDAGTLVRTRVSEISIVGRNTQ GVILIRTAEDENVVGLQRVAEPVDEEDLDTIDGSAAEGDDEIAPEVDVDDEPEEE	CTU78765.1
MSDLAREITPVNIEEELKSSYLDYAMSVIVGRALPDVRDGLKPVHRRVLYAMNVLGND WNKAYKKSARVVGDVIGKYHPHGDSAVYDTIVRMAQPFSLRYMLVDGQGNFGSIDG DSAAAMRYTEIRLAKIAHELMADLEKETVDFVDNYDGTEKIPDVMPTKIPNLLVNGSS GIAVGMATNIPPHNLTEVINGCLAYIDDEDISIEGLMEYIPGPDFPTAAIINGRRGIEEAYR TGRGKVYIRARAEVEVDAKTGRETIIVHEIPYQVNKARLIEKIAELVKEKRVEGISALRD ESDKDGMRIVIEVKRDAVGEVVLNNLYSQTQLQVSFGINMVALHHGQPKIMNLKDIIA AFVRHRREVVTRRTIFELRKARDRAHILEALAVALANIDPIIELIRHAPTPAEAKTALVA NPWQLGNVAAMLERAGDDAARPEWLEPEFGVRDGLYYLTEQQAQAILDLRLQKLTG LEHEKLLDEYKELLDQIAELLRILGSADRLMEVIREELELVREQFGDKRRTEITANSADI NLEDLITQEDVVVTLSHQGYVKYQPLSEYEAQRRGGKGKSAARIKEEDFIDRLLVANT	CTT63704.1

HDHILCFSSRGRVYSMKVYQLPEATRGARGRPIVNLLPLEQDERITAILPVTEFEEGVKV FMATANGTVKKTVLTEFNRLRTAGKVAIKLVEGDELIGVDLTSGEDEVMLFSAEGKVV RFKESSVRAMGCNTTGVRGIRLGEGDKVVSLIVPRGDGAILTATQNGYGKRTAVAEYP TKSRATKGVISIKVTERNGLVVGAVQVDDCDQIMMITDAGTLVRTRVSEISIVGRNTQG VILIRTAEDENVVGLQRVAEPVDEEDLDTIDGSAAEGDDEIAPEVDVDDEPEEE

3.4.3 *Pseudomonas aeruginosa*

Protein FASTA sequences for parental and mutated gyrA protein of *P. aeruginosa* were retrieved from Uniprot as mentioned in table 10.

gyrA parental sequence *Pseudomonas aeruginosa*

>EKA56174.1 DNA gyrase subunit A [*Pseudomonas aeruginosa* ATCC 25324]
MGELAKEILPVNIEDELKQSYLDYAMSVIVGRALPDARDGLKPVHRRVLYAMSELGNDWNKPYKKSARVVG
DVIGKYHPHGDTAVYDTIVRMAQPFSLRYMLVDGQGNFGSVDGDNAAAMRYTEVRMAKLAHELLADLEKE
TVDWVPNYDGTEQIPAVMPTKIPNLLVNGSSGIAVGMATNIPPHNLGEVIDGCLALMDNPDLTVDELMQYIPG
PDFPTAGIINGRAGIIEAYRTGRGRIYIRARAVVEEMEKGGGREQIIITELPYQLNKARLIEKIAELVKEKKIEGISE
LRDESDKDGMRVVIELRRGEVGEVVLNNLYAQTQLQSVFGINVVALVDGQPRTLNLKDMLEVFVRHRREVVT
RRTVYELRKARERGHILEGQAVALSNIDPVIELIKSSPTPAEAKERLIATAWESSAVEAMVERAGADACRPEDL
DPQYGLRDGKYYLSPEQAQAILELRLHRLTGLEHEKLLSEYQEILNLIGELIRILTNPARLMEVIREELEAVKAEF
GDARRTEIVASQVDLTIADLITEEDRVVTISHGGYAKSQPLAAYQAQRRGGKGKSATGMKDEDYIEHLLVANS
HATLLLFSSKGKVYWLRTFEIPEASRTARGRPLVNLLPLDEGERITAMLQIDLEALQQNGGADDDLDEAEGAV
LEGEVVEAAEVEEVEGETAELVAEPTGAYIFMATAFGTVKKTPLVQFSRPRSSGLIALKLEEGDTLIAAAITDGA
KEVMLFSSAGKVIRFAESVVRIMGRNARGVRGMRLGKGQQLISMLIPESGAQILTASERGFGKRTPLSKFPRRG
RGGQGVIAMVTNERNGALIAAVQVQEGEEIMLISDQGTLVRTRVDEVSLSGRNTQGVTLIKLASDEVLVGLER
VQEPSGGDDEDLPEGEEAAESLGESAESESEPAAEAEGNEE

Table 10-Mutations in gyrA sequence of *Pseudomonas aeruginosa* are listed in the table

Protein FASTA Sequence	Accession Ids
MGELAKEILPVNIEDELKQSYLDYAMSVIVGRALPDARDGLKPVHRRVLYAMSELGN DWNKPYKKSARVVGDVIGKYHPHGDTAVYDTIVRMAQPFSLRYMLVDGQGNFGSVD GDNAAAMRYTEVRMAKLAHELLADLEKETVDWVPNYDGTEQIPAVMPTKIPNLLVN GSSGIAVGMATNIPPHNLGEVIDGCLALMDNPDLTVDELMQYIPGPDFPTAGIINGRAGI IEAYRTGRGRIYIRARAVVEEMEKGGGREQIIITELPYQLNKARLIEKIAELVKEKKIEGI SELRDESDKDGMRVVIELRRGEVGEVVLNNLYAQTQLQSVFGINVVALVDGQPRTLNL KDMLEVFVRHRREVVTRRTVYELRKARERGHILEGQAVALSNIDPVIELIKSSPTPAEA KERLIATAWESSAVEAMVERAGADACRPEDLDPQYGLRDGKYYLSPEQAQAILELRLH RLTGLEHEKLLSEYQEILNLIGELIRILTNPARLMEVIREELEAVKAEFGDARRTEIVASQ VDLTIADLITEEDRVVTISHGGYAKSQPLAAYQAQRRGGKGKSATGMKDEDYIEHLLV ANSHATLLLFSSKGKVYWLRTFEIPEASRTARGRPLVNLLPLDEGERITAMLQIDLEAL QQNGGADDDLDEAEGAVLEGEVVEAAEVEEVEGETAELVAEPTGAYIFMATAFGTVK KTPLVQFSRPRSSGLIALKLEEGDTLIAAAITDGAKEVMLFSSAGKVIRFAESVVRIMGR NARGVRGMRLGKGQQLISMLIPESGAQILTASERGFGKRTPLSKFPRRGRGGQGVIAM VTNERNGALIAAVQVQEGEEIMLISDQGTLVRTRVDEVSLSGRNTQGVTLIKLASDEVL VGLERVQEPSGGDDEDLPEGEEAAESLGESAESESEPAAEAEGNEE	RFQ01914.1
MGELAKEILPVNIEDELKQSYLDYAMSVIVGRALPDARDGLKPVHRRVLYAMSELGN DWNKPYKKSARVVGDVIGKYHPHGDTAVYDTIVRMAQPFSLRYMLVDGQGNFGSVD GDNAAAMRYTEVRMAKLAHELLADLEKETVDWVPNYDGTEQIPAVMPTKIPNLLVN GSSGIAVGMATNIPPHNLGEVIDGCLALMDNPDLTVDELMQYIPGPDFPTAGIINGRAGI IEAYRTGRGRIYIRARAVVEEMEKGGGREQIIITELPYQLNKARLIEKIAELVKEKKIEGI SELRDESDKDGMRVVIELRRGEVGEVVLNNLYAQTQLQSVFGINVVALVDGQPRTLNL KDMLEVFVRHRREVVTRRTVYELRKARERGHILEGQAVALSNIDPVIELIKSSPTPAEA KERLIATAWESSAVEAMVERAGADACRPEDLDPQYGLRDGKYYLSPEQAQAILELRLH RLTGLEHEKLLSEYQEILNLIGELIRILTNPARLMEVIREELEAVKAEFGDARRTEIVASQ VDLTIADLITEEDRVVTISHGGYAKSQPLAAYQAQRRGGKGKSATGMKDEDYIEHLLV ANSHATLLLFSSKGKVYWLRTFEIPEASRTARGRPLVNLLPLDEGERITAMLQIDLEAL QQNGGADDDLDEAEGAVLEGEVVEAAEVEEVEGETAELVAEPTGAYIFMATAFGTVK KTPLVQFSRPRSSGLIALKLEEGDTLIAAAITDGAKEVMLFSSAGKVIRFAESVVRIMGR NARGVRGMRLGKGQQLISMLIPESGAQILTASERGFGKRTPLSKFPRRGRGGQGVIAM VTNERNGALIAAVQVQEGEEIMLISDQGTLVRTRVDEVSLSGRNTQGVTLIKLASDEVL VGLERVQEPSGGDDEDLPEGEEAAESLGESAESESEPAAEAEGNEE	TKW45649.1
MGELAKEILPVNIEDELKQSYLDYAMSVIVGRALPDARDGLKPVHRRVLYAMSELGN DWNKPYKKSARVVGDVIGKYHPHGDTAVYDTIVRMAQPFSLRYMLVDGQGNFGSVD	KGB86713.1

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GDNAAAMRYTEVRMAKLAHELLADLEKETVDWVPNYDGTEQIPAVMPTKIPNLLVN GSSGIAVGMATNIPPHNLGEVIDGCLALMDNPDLTVDELMQYIPGPDFPTAGIINGRAGI IEAYRTGRGRIYIRARAVVEEMEKGGGREQIIITELPYQLNKARLIEKIAELVKEKKIEGI SELRDESDKDGMRVVIELRRGEVGEVVLNNLYAQTQLQSVFGINVVALVDGQPRTLNL KDMLEVFVRHRREVVTRRTVYELRKARERGHILEGQAVALSNIDPVIELIKSSPTPAEA KERLIATAWESSAVEAMVERAGADACRPEDLDPQYGLRDGKYYLSPEQAQAILELRLH RLTGLEHEKLLSEYQEILNLIGELIRILTNPARLMEVIREELEAVKAEFGDARRTEIVASQ VDLTIADLITEEDRVVTISHGGYAKSQPLAAYQAQRRGGKGKSATGMKDEDYIEHLLV ANSHATLLLFSSKGKVYWLRTFEIPEASRTARGRPLVNLLPLDEGERITAMLQIDLEAL QQNGGADDDLDEAEGAVLEGEVVEAAEVEEVEGETAELVAEPTGAYIFMATAFGTVK KTPLVQFSRPRSSGLIALKLEEGDTLIAAAITDGAKEVMLFSSAGKVIRFAESVVRIMGR NARGVRGMRLGKGQQLISMLIPESGAQILTASERGFGKRTPLSKFPRRGRGGQGVIAM VTNERNGALIAAVQVQEGEEIMLISDQGTLVRTRVDEVSLSGRNTQGVTLIKLASDEVL VGLERVQEPSGGDDEDLPEGEEAAESLGESAESESEPAAEAEGNEE	
MGELAKEILPVNIEDELKQSYLDYAMSVIVGRALPDARDGLKPVHRRVLYAMSELGN DWNKPYKKSARVVGDVIGKYHPHGDTAVYDTIVRMAQPFSLRYMLVDGQGNFGSVD GDNAAAMRYTEVRMAKLAHELLADLEKETVDWVPNYDGTEQIPAVMPTKIPNLLVN GSSGIAVGMATNIPPHNLGEVIDGCLALMDNPDLTVDELMQYIPGPDFPTAGIINGRAGI IEAYRTGRGRIYIRARAVVEEMEKGGGREQIIITELPYQLNKARLIEKIAELVKEKKIEGI SELRDESDKDGMRVVIELRRGEVGEVVLNNLYAQTQLQSVFGINVVALVDGQPRTLNL KDMLEVFVRHRREVVTRRTVYELRKARERGHILEGQAVALSNIDPVIELIKSSPTPAEA KERLIATAWESSAVEAMVERAGADACRPEDLDPQYGLRDGKYYLSPEQAQAILELRLH RLTGLEHEKLLSEYQEILNLIGELIRILTNPARLMEVIREELEAVKAEFGDARRTEIVASQ VDLTIADLITEEDRVVTISHGGYAKSQPLAAYQAQRRGGKGKSATGMKDEDYIEHLLV ANSHATLLLFSSKGKVYWLRTFEIPEASRTARGRPLVNLLPLDEGERITAMLQIDLEAL QQNGGADDDLDEAEGAVLEGEVVEAAEIEEVEGETAELVAEPTGAYIFMATAFGTVK KTPLVQFSRPRSSGLIALKLEEGDTLIAAAITDGAKEVMLFSSAGKVIRFAESVVRIMGR NARGVRGMRLGKGQQLISMLIPESGAQILTASERGFGKRTPLSKFPRRGRGGQGVIAM VTNERNGALIAAVQVQEGEEIMLISDQGTLVRTRVDEVSLSSRNTQGVTLIKLASDEVL VGLERVQEPSGGDDEELPEGEEGAEALGESVESEPAAEAEGNEE	PHJ32334.1
MGELAKEILPVNIEDELKQSYLDYAMSVIVGRALPDARDGLKPVHRRVLYAMSELGN DWNKPYKKSARVVGDVIGKYHPHGDTAVYNTIVRMAQPFSLRYMLVDGQGNFGSVD GDNAAAMRYTEVRMAKLAHELLADLEKETVDWVPNYDGTEQIPAVMPTKIPNLLVN GSSGIAVGMATNIPPHNLGEVIDGCLALMDNPDLTVDELMQYIPGPDFPTAGIINGRAGI IEAYRTGRGRIYIRARAVVEEMEKGGGREQIIITELPYQLNKARLIEKIAELVKEKKIEGI SELRDESDKDGMRVVIELRRGEVGEVVLNNLYAQTQLQSVFGINVVALVDGQPRTLNL KDMLEVFVRHRREVVTRRTVYELRKARERGHILEGQAVALSNIDPVIELIKSSPTPAEA KERLIATAWESSAVEAMVERAGADACRPEDLDPQYGLRDGKYYLSPEQAQAILELRLH RLTGLEHEKLLSEYQEILNLIGELIRILTNPARLMEVIREELEAVKAEFGDARRTEIVASQ VDLTIADLITEEDRVVTISHGGYAKSQPLAAYQAQRRGGKGKSATGMKDEDYIEHLLV ANSHATLLLFSSKGKVYWLRTFEIPEASRTARGRPLVNLLPLDEGERITAMLQIDLEAL QQNGGADDDLDEAEGAVLEGEVVEAAEVEEVEGETAELVAEPTGAYIFMATAFGTVK KTPLVQFSRPRSSGLIALKLEEGDTLIAAAITDGAKEVMLFSSAGKVIRFAESVVRIMGR NARGVRGMRLGKGQQLISMLIPESGAQILTASERGFGKRTPLSKFPRRGRGGQGVIAM VTNERNGALIAAVQVQEGEEIMLISDQGTLVRTRVDEVSLSGRNTQGVTLIKLASDEVL VGLERVQEPSGGDDEDLPEGEEAAESLGESAESESEPAAEAEGNEE	OXR97249.1
MGELAKEILPVNIEDELKQSYLDYAMSVIVGRALPDARDGLKPVHRRVLYAMSELGN DWNKPYKKSARVVGDVIGKYHPHGDTAVYDTIVRMAQPFSLRYMLVDGQGNFGSVD GDNAAAMRYTEVRMAKLAHELLADLEKETVDWVPNYDGTEQIPAVMPTKIPNLLVN GSSGIAVGMATNIPPHNLGEVIDGCLALMDNPDLTVDELMQYIPGPDFPTAGIINGRAGI IEAYRTGRGRIYIRARAVVEEMEKGGGREQIIITELPYQLNKARLIEKIAELVKEKKIEGI SELRDESDKDGMRVVIELRRGEVGEVVLNNLYAQTQLQSVFGINVVALVDGQPRTLNL KDMLEVFVRHRREVVTRRTVYELRKARERGHILEGQAVALSNIDPVIELIKSSPTPAEA KERLIATAWESSAVEAMVERAGADACRPEDLDPQYGLRDGKYYLSPEQAQAILELRLH RLTGLEHEKLLSEYQEILNLIGELIRILTNPARLMEVIREELEAVKAEFGDARRTEIVASQ VDLTIADLITEEDRVVTISHGGYAKSQPLAAYQAQRRGGKGKSATGMKDEDYIEHLLV ANSHATLLLFSSKGKVYWLRTFEIPEASRTARGRPLVNLLPLDEGERITAMLQIDLEAL QQNGGADDDLDEAEGAVLEGEVVEAAEVEEVEGETAELVAEPTGAYIFMATAFGTVK KTPLVQFSRPRSSGLIALKLEEGDTLIAAAITDGAKEVMLFSSAGKVIRFAESVVRIMGR NARGVRGMRLGKGQQLISMLIPESGAQILTASERGFGKRTPLSKFPRRGRGGQGVIAM VTNERNGALIAAVQVQEGEEIMLISDQGTLVRTRVDEVSLSGRNTQGVTLIKLASDEVL VGLERVQEPSGGDDEDLPEGEEAAESLGESAESESEPAAEAEGNEE	OXR86782.1
MGELAKEILPVNIEDELKQSYLDYAMSVIVGRALPDARDGLKPVHRRVLYAMSELGN DWNKPYKKSARVVGDVIGKYHPHGDTAVYDTIVRMAQPFSLRYMLVDGQGNFGSVD GDNAAAMRYTEVRMAKLAHELLADLEKETVDWVPNYDGTEQIPAVMPTKIPNLLVN GSSGIAVGMATNIPPHNLGEVIDGCLALMDNPDLTVDELMQYIPGPDFPTAGIINGRAGI IEAYRTGRGRIYIRARAVVEEMEKGGGREQIIITELPYQLNKARLIEKIAELVKEKKIEGI SELRDESDKDGMRVVIELRRGEVGEVVLNNLYAQTQLQSVFGINVVALVDGQPRTLNL KDMLEVFVRHRREVVTRRTVYELRKARERGHILEGQAVALSNIDPVIELIKSSPTPAEA KERLIATAWESSAVEAMVERAGADACRPEDLDPQYGLRDGKYYLSPEQAQAILELRLH RLTGLEHEKLLSEYQEILNLIGELIRILTNPARLMEVIREELEAVKAEFGDARRTEIVASQ VDLTIADLITEEDRVVTISHGGYAKSQPLAAYQAQRRGGKGKSATGMKDEDYIEHLLV ANSHATLLLFSSKGKVYWLRTFEIPEASRTARGRPLVNLLPLDEGERITAMLQIDLEAL QQNGGADDDLDEAEGAVLEGEVVEAAEVEEVEGETAELVAEPTGAYIFMATAFGTVK KTPLVQFSRPRSSGLIALKLEEGDTLIAAAITDGAKEVMLFSSAGKVIRFAESVVRIMGR	OXR83033.1

NARGVRGMRLGKGQQLISMLIPESGAQILTASERGFGKRTPLSKFPRRGRGGQGVIAM VTNERNGALIAAVQVQEGEEIMLISDQGTLVRTRTVDEVSLSGRNTQGVTLIKLASDEVL VGLERVQEPSGGDDEDLPEGEEAAESLGESAESESEPAAEAEGNEE	
MGELAKEILPVNIEDELKQSYLDYAMSVIVGRALPDARDGLKPVHRRVLYAMSELGN DWNKPYKKSARVVGDVIGKYHPHGDTAVYDTIVRMAQPFSLRYMLVDGQGNFGSVD GDNAAAMRYTEVRMAKLAHELLADLEKETVDWVPNYDGTEQIPAVMPTKIPNLLVN GSSGIAVGMATNIPPHNLGEVIDGCLALMDNPDLTVDELMQYIPGPDFPTAGIINGRAGI IEAYRTGRGRIYIRARAVVEEMEKGGGREQIIITELPYQLNKARLIEKIAELVKEKKIEGI SELRDESDKDGMRVVIELRSGEVGEVVLNNLYAQTQLQSVFGINVVALVDGQPRTLNL KDMLEVFVRHRREVVTRRTVYELRKARERGHILEGQAVALSNIDPVIELIKSSPTPAEA KERLIATAWESSAVEAMVERAGADACRPEDLDPQYGLRDGKYYLSPEQAQAILELRLH RLTGLEHEKLLSEYQEILNLIGELIRILTNPARLMEVIREELEAVKAEFGDARRTEIVASQ VDLTIADLITEEDRVVTISHGGYAKSQPLAAYQAQRRGGKGKSATGMKDEDYIEHLLV ANSHATLLLFSSKGKVYWLRTFEIPEASRTARGRPLVNLLPLDEGERITAMLQIDLEAL QQNGGADDDLDEAEGAVLEGEVVEAAEVEEVEGETAELVAEPTGAYIFMATAFGTVK KTPLVQFSRPRSSGLIALKLEEGDTLIAAAITDGAKEVMLFSSAGKVIRFAESVVRIMGR NARGVRGMRLGKGQQLISMLIPESGAQILTASERGFGKRTPLSKFPRRGRGGQGVIAM VTNERNGALIAAVQVQEGEEIMLISDQGTLVRTRTVDEVSLSGRNTQGVTLIKLASDEVL VGLERVQEPSGGDDEDLPEGEEAAESLGESAESESEPAAEAEGNEE	OXR82227.1
MGELAKEILPVNIEDELKQSYLDYAMSVIVGRALPDARDGLKPVHRRVLYAMSELGN DWNKPYKKSARVVGDVIGKYHPHGDTAVYDTIVRMAQPFSLRYMLVDGQGNFGSVD GDNAAAMRYTEVRMAKLAHELLADLEKETVDWVPNYDGTEQIPAVMPTKIPNLLVN GSSGIAVGMATNIPPHNLGEVIDGCLALMDNPDLTVDELMQYIPGPDFPTAGIINGRAGI IEAYRTGRGRIYIRARAVVEEMEKGGGREQIIITELPYQLNKARLIEKIAELVKEKKIEGI SELRDESDKDGMRVVIELRRGEVGEVVLNNLYAQTQLQSVFGINVVALVDGQPRTLNL KDMLEVFVRHRREVVTRRTVYELRKARERGHILEGQAVALSNIDPVIXLIKSSXTPAEA KERLIATAWESSAVEAMVERAGADACRPEDLDPQYGLRDGKYYLSPEQAQAILELRLH RLTGLEHEKLLSEYQEILNLIGELIRILTNPARLMEVIREELEAVKAEFGDARRTEIVASQ VDLTIADLITEEDRVVTISHGGYAKSQPLAAYQAQRRGGKGKSATGMKDEDYIEHLLV ANSHATLLLFSSKGKVYWLRTFEIPEASRTARGRPLVNLLPLDEGERITAMLQIDLEAL QQNGGADDDLDEAEGAVLEGEVVEAAEVEEVEGETAELVAEPTGAYIFMATAFGTVK KTPLVQFSRPRSSGLIALKLEEGDTLIAAAITDGAKEVMLFSSAGKVIRFAESVVRIMGR NARGVRGMRLGKGQQLISMLIPESGAQILTASERGFGKRTPLSKFPRRGRGGQGVIAM VTNERNGALIAAVQVQEGEEIMLISDQGTLVRTRTVDEVSLSGRNTQGVTLIKLASDEVL VGLERVQEPSGGDDEDLPEGEEAAESLGESAESESEPAAEAEGNEE	OXR65169.1
MGELAKEILPVNIEDELKQSYLDYAMSVIVGRALPDARDGLKPVHRRVLYAMSELGN DWNKPYKKSARVVGDVIGKYHPHGDTAVYDTIVRMAQPFSLRYMLVDGQGNFGSVD GDNAAAMRYTEVRMAKLAHELLADLEKETVDWVPNYDGTEQIPAVMPTKIPNLLVN GSSGIAVGMATNIPPHNLGEVIDGCLALMDNPDLTVDELMQYIPGPDFPTAGIINGRAGI IEAYRTGRGRIYIRARAVVEEMEKGGGREQIIITELPYQLNKARLIEKIAELVKEKKIEGI SELRDESDKDGMRVVIELRRGEVGEVVLNNLYAQTQLQSVFGINVVALVDGQPRTLNL KDMLEVCVRHRREVVTRRTVYELRKARERGHILEGQAVALSNIDPVIELIKSSPTPAEA KERLIATAWESSAVEAMVERAGADACRPEDLDPQYGLRDGKYYLSPEQAQAILELRLH RLTGLEHEKLLSEYQEILNLIGELIRILTNPARLMEVIREELEAVKAEFGDARRTEIVASQ VDLTIADLITEEDRVVTISHGGYAKSQPLAAYQAQRRGGKGKSATGMKDEDYIEHLLV ANSHATLLLFSSKGKVYWLRTFEIPEASRTARGRPLVNLLPLDEGERITAMLQIDLEAL QQNGGADDDLDEAEGAVLEGEVVEAAEVEEVEGETAELVAEPTGAYIFMATAFGTVK KTPLVQFSRPRSSGLIALKLEEGDTLIAAAITDGAKEVMLFSSAGKVIRFAESVVRIMGR NARGVRGMRLGKGQQLISMLIPESGAQILTASERGFGKRTPLSKFPRRGRGGQGVIAM VTNERNGALIAAVQVQEGEEIMLISDQGTLVRTRTVDEVSLSGRNTQGVTLIKLASDEVL VGLERVQEPSGGDDEDLPEGEEAAESLGESAESEPAAEAEGNEE	EKA34923.1

3.4.4 *Klebsiella pneumoniae*

Protein FASTA sequences for parental and mutated gyrA protein of *K. pneumoniae* were retrieved from Uniprot as mentioned in table 11.

gyrA parental sequence of *Klebsiella pneumoniae*

>BAH64436.1 DNA gyrase subunit A [*Klebsiella pneumoniae* subsp. pneumoniae NTUH-K2044]MSDLAREITPVNIEEELKNSYLDYAMSVIVGRALPDVRDGLKPVHRRVLYAMNVLGNDWNKAYKKSA RVVGDVIGKYHPHGDSAVYDTIVRMAQPFSLRYMLVDGQGNFGSIDGDSAAAMRYTEIRLAKIAHELMADLE KETVDFVDNYDGTERIPDVMPTKIPNLLVNGASGIAVGMATNIPPHNLTEVINGCLAYVDDEDISIEGLMAHIPG PDFPTAAIINGRRGIEEAYRTGRGKVYIRARAEVEVDAKSGRETIIVHEIPYQVNKARLIEKIAELVKEKRVEGIS ALRDESDKDGMRIVIEVKRDAVGEVVLNNLYSQTQLQVSFGINMVALHHGQPKIMNLKDIIAAFVRHRREVVT RRTIFELRKARDRAHILEALAVALANIDPIIELIRRAPTPAEAKTALVAQAWDLGNVAAMLERAGDDAARPEW LEPEFGVRDGKYYLTEQQAQAILDLRLQKLTGLEHEKLLDEYKELLEQIAELLHILGSADRLMEVIREELELIRD QFGDERRTEITANSADINIEDLINQEDVVVTLSHQGYVKYQPLTDYEAQRRGGKGKSAARIKEEDFIDRLLVAN THDTILCFSSRGRLYWMKVYQLPEASRGARGRPIVNLLPLEADERITAILPVREYEEGVNVFMATASGTVKKTA LTEFSRPRSAGIIAVNLNEGDELIGVDLTSGQDEVMLFSAAGKVVRFKEDAVRAMGRTATGVRGIKLAENDSV

24

VSLIIPRGEGAILTVTQNGYGKRTAAAEYPTKSRATQGVISIKVTERNGSVVGAVQVDDCDQIMMITDAGTLVR
TRVSEVSIVGRNTQGVILIRTAEDENVVGLQRVAEPVDDEELDAIDGSAAEGDDDIAPEADTDDDIAEDEE

Table 11-Mutations in gyrA sequence of *Klebsiella pneumoniae* are listed in the table

Protein FASTA Sequence	Accession Ids
MSDLAREITPVNIEEEELKNSYLDYAMSVIVGRALPDVRDGLKPVHRRVLYAMNVLGND WNKAYKKSARVVGDVIGKYHPHGDIAVYNTIVRMAQPFSLRYMLVDGQGNFGSIDGDS AAAMRYTEIRLAKIAHELMADLEKETVDFVDNYDGTERIPDVMPTKIPNLLVNGASGIAV GMATNIPPHNLTEVINGCLAYVDDEDISIEGLMAHIPGPDFPTAAIINGRRGIEEAYRTGRG KVYIRARAEVEVDAKSGRETIIVHEIPYQVNKARLIEKIAELVKEKRVEGISALRDESDKD GMRIVIEVKRDAVGEVVLNNLYSQTQLQVSFGINMVALHHGQPKIMNLKDIIAAFVRHRR EVVTRRTIFELRKARDRAHILEALAVALANIDPIIELIRRAPTPAEAKTALVAQAWDLGNV AAMLERAGDDAARPEWLEPEFGVRDGKYYLTEQQAQAILDLRLQKLTGLEHEKLLDEY KELLEQIAELLHILGSADRLMEVIREELELIRDQFGDERRTEITANSADINIEDLINQEDVVV TLSHQGYVKYQPLTDYEAQRRGGKGKSAARIKEEDFIDRLLVANTHDTILCFSSRGRLYW MKVYQLPEASRGARGRPIVNLLPLEADERITAILPVREYEEGVNVFMATASGTVKKTALT EFSRPRSAGIIAVNLNEGDELIGVDLTSGQDEVMLFSAAGKVVRFKEDAVRAMGRTATG VRGIKLAENDSVVSLIIPRGEGAILTVTQNGYGKRTAAAEYPTKSRATQGVISIKVTERNG SVVGAVQVDDCDQIMMITDAGTLVRTRVSEVSIVGRNTQGVILIRTAEDENVVGLQRVA EPVDDEELDAIDGSAAEGDDDIAPEADTDDDIAEDEE	KLA39053.1
MSDLAREITPVNIEEEELKNSYLDYAMSVIVGRALPDVRDGLKPVHRRVLYAMNVLGND WNKAYKKSARVVGDVIGKYHPHGDSAVYDTIVRMAQPFSLRYMLVDGQGNFGSIDGDS AAAMRYTEIRLAKIAHELMADLEKETVDFVDNYDGTERIPDVMPTKIPNLLVNGASGIAV GMATNIPPHNLTEVINGCLAYVDDEDISIEGLMAHIPGPDFPTAAIINGRRGIEEAYRTGRG KVYIRARAEVEVDAKSGRETIIVHEIPYQVNKARLIEKIAELVKEKRVEGISALRDESDKD GMRIVIEVKRDAVGEVVLNNLYSQTQLQVSFGINMVALHHGQPKIMNLKDIIAAFVRHRR EVVTRRTIFELRKARDRAHILEALAVALANIDPIIELIRRAPTPAEAKTALVAQAWDLGNV AAMLERAGDDAARPEWLEPEFGVRDGKYYLTEQQAQAILDLRLQKLTGLEHEKLLDEY KELLEQIAELLHILGSADRLMEVIREELELIRDQFGDERRTEITANSADINIEDLINQEDVVV TLSHQGYVKYQPLTDYEAQRRGGKGKSAARIKEEDFIDRLLVANTHDTILCFSSRGRLYW MKVYQLPEASRGARGRPIVNLLPLEADERITAILPVREYEEGVNVFMATASGTVKKTALT EFSRPRSAGIIAVNLNEGDELIGVDLTSGQDEVMLFSAAGKVVRFKEDAVRAMGRTATG VRGIKLAENDSVVSLIIPRGEGAILTVTQNGYGKRTAAAEYPTKSRATQGVISIKVTERNG SVVGAVQVDDCDQIMMITDAGTLVRTRVSEVSIVGRNTQGVILIRTAEDENVVGLQRVA EPVDDEELDAIDGSAAEGDDDIAPEADTDDDVAEDEE	PLJ21830.1
MSDLAREITPVNIEEEELKNSYLDYAMSVIVGRALPDVRDGLKPVHRRVLYAMNVLGND WNKAYKKSARVVGDVIGKYHPHGDIAVYDTIVRMAQPFSLRYMLVDGQGNFGSIDGDS AAAMRYTEIRLAKIAHELMADLEKETVDFVDNYDGTERIPDVMPTKIPNLLVNGASGIAV GMATNIPPHNLTEVINGCLAYVDDEDISIEGLMAHIPGPDFPTAAIINGRRGIEEAYRTGRG KVYIRARARKVEVDAKSGRETIIVHEIPYQVNKARLIEKIAELVKEKRVEGISALRDESDK DGMRIVIEVKRDAVGEVVLNNLYSQTQLQVSFGINMVALHHGQPKIMNLKDIIAAFVRH RREVVTRRTIFELRKARDRAHILEALAVALANIDPIIELIRRAPTPAEAKTALVAQAWDLG NVAAMLERAGDDAARPEWLEPEFGVRDGKYYLTEQQAQAILDLRLQKLTGLEHEKLLD EYKELLEQIAELLHILGSADRLMEVIREELELIRDQFGDERRTEITANSADINIEDLINQEDV VVTLSHQGYVKYQPLTDYEAQRRGGKGKSAARIKEEDFIDRLLVANTHDTILCFSSRGRL YWMKVYQLPEASRGARGRPIVNLLPLEADERITAILPVREYEEGVNVFMATASGTVKKT ALTEFSRPRSAGIIAVNLNEGDELIGVDLTSGQDEVMLFSAAGKVVRFKEDAVRAMGRTA TGVRGIKLAENDSVVSLIIPRGEGAILTVTQNGYGKRTAAAEYPTKSRATQGVISIKVTER NGSVVGAVQVDDCDQIMMITDAGTLVRTRVSEVSIVGRNTQGVILIRTAEDENVVGLQR VAEPVDDEELDAIDGSAAEGDDDIAPEADTDDDIAEDEE	PHQ12249.1
MSDLAREITPVNIEEEELKNSYLDYAMSVIVGRALPDVRDGLKPVHRRVLYAMNVLGND WNKAYKKSARVVGDVIGKYHPHGDFAVYDTIVRMAQPFSLRYMLVDGQGNFGSIDGDS AAAMRYTEIRLAKIAHELMADLEKETVDFVDNYDGTERIPDVMPTKIPNLLVNGASGIAV GMATNIPPHNLTEVINGCLAYVDDEDISIEGLMAHIPGPDFPTAAIINGRRGIEEAYRTGRG KVYIRARAEVEVDAKSGRETIIVHEIPYQVNKARLIEKIAELVKEKRVEGISALRDESDKD GMRIVIEVKRDAVGEVVLNNLYSQTQLQVSFGINMVALHHGQPKIMNLKDIIAAFVRHRR EVVTRRTIFELRKARDRAHILEALAVALANIDPIIELIRRAPTPAEAKTALVAQAWDLGNV AAMLERAGDDAARPEWLEPEFGVRDGKYYLTEQQAQAILDLRLQKLTGLEHEKLLDEY KELLEQIAELLHILGSADRLMEVIREELELIRDQFGDERRTEITANSADINIEDLINQEDVVV TLSHQGYVKYQPLTDYEAQRRGGKGKSAARIKEEDFIDRLLVANTHDTILCFSSRGRLYW MKVYQLPEASRGARGRPIVNLLPLEADERITAILPVREYEEGVNVFMATASGTVKKTALT EFSRPRSAGIIAVNLNEGDELIGVDLTSGQDEVMLFSAAGKVVRFKEDAVRAMGRTATG VRGIKLAENDSVVSLIIPRGEGAILTVTQNGYGKRTAAAEYPTKSRATQGVISIKVTERNG SVVGAVQVDDCDQIMMITDAGTLVRTRVSEVSIVGRNTQGVILIRTAEDENVVGLQRVA EPVDDEELDAIDGSAAEGDDDIAPEADTDDDIAEDEE	APB52988.1
MSDLAREITPVNIEEEELKNSYLDYAMSVIVGRALPDVRDGLKPVHRRVLYAMNVLGND WNKAYKKSARVVGDVIGKYHPHGDIAVYDTIVRMAQPFSLRYMLVDGQGNFGSIDGDS AAAMRYTEIRLAKIAHELMADLEKETVDFVDNYDGTERIPDVMPTKIPNLLVNGASGIAV GMATNIPPHNLTEVINGCLAYVDDEDISIEGLMAHIPGPDFPTAAIINGRRGIEEAYRTGRG KVYIRARAEVEVDAKSGRETIIVHEIPYQVNKARLIEKIAELVKEKRVEGISALRDESDKD	KTG72022.1

GMRIVIEVKRDAVGEVVLNNLYSQTQLQVSFGINMVALHHGQPKIMNLKDIIAAFVRHRR EVVTRRTIFELRKARDRAHILEALAVALANIDPIIELIRRAPTPAEAKTALVAQAWDLGNV AAMLERAGDDAARPEWLEPEFGVRDGKYYLTEQQAQAILDLRLQKLTGLEHEKLLDEY KELLEQIAELLHILGSADRLMEVIREELELIRDQFGDERRTEITANSADINIEDLINQEDVVV TLSHQGYVKYQPLTDYEAQRRGGKGKSAARIKEEDFIDRLLVANTHDTILCFSSRGRLYW MKVYQLPEASRGARGRPIVNLLPLEADERITAILPVREYEEGVNVFMATASGTVKKTALT EFSRPRSAGIIAVNLNEGDELIGVDLTSGQDEVMLFSAAGKVVRFKEDAVRAMGRTATG VRGIKLAENDSVVSLIIPRGEGAILTVTQNGYGKRTAAAEYPTKSRATQGVISIKVTERNG SVVGAVQVDDCDQIMMITDAGTLVRTRVSEVSIVGRNTQGVILIRTAEDENVVGLQRVA EPVDDEELDAIDGSAAEGDDDIAPEADTDDDIAEDEE	
MSDLAREITPVNIEEEELKNSYLDYAMSVIVGRALPDVRDGLKPVHRRVLYAMNVLGND WNKAYKKSARVVGDVIGKYHPHGDIAVYDTIVRMAQPFSLRYMLVDGQGNFGSIDGDS AAAMRYTEIRLAKIAHELMADLEKETVDFVDNYDGTERIPDVMPTKIPNLLVNGASGIAV GMATNIPPHNLTEVINGCLAYVDDEDISIEGLMAHIPGPDFPTAAIINGRRGIEEAYRTGRG KVYIRARARKVEVDAKSGRETIIVHEIPYQVNKARLIEKIAELVKEKRVEGISALRDESDK DGMRIVIEVKRDAVGEVVLNNLYSQTQLQVSFGINMVALHHGQPKIMNLKDIIAAFVRH RREVVTRRTIFELRKARDRAHILEALAVALANIDPIIELIRRAPTPAEAKTALVAQAWDLG NVAAMLERAGDDAARPEWLEPEFGVRDGKYYLTEQQAQAILDLRLQKLTGLEHEKLLD EYKELLEQIAELLHILGSADRLMEVIREELELIRDQFGDERRTEITANSADINIEDLINQEDV VVTLSHQGYVKYQPLTDYEAQRRGGKGKSAARIKEEDFIDRLLVANTHDTILCFSSRGRL YWMKVYQLPEASRGARGRPIVNLLPLEADERITAILPVREYEEGVNVFMATASGTVKKT ALTEFSRPRSAGIIAVNLNEGDELIGVDLTSGQDEVMLFSAAGKVVRFKEDAVRAMGRTA TGVRGIKLAENDSVVSLIIPRGEGAILTVTQNGYGKRTAAAEYPTKSRATQGVISIKVTER NGSVVGAVQVDDCDQIMMITDAGTLVRTRVSEVSIVGRNTQGVILIRTAEDENVVGLQR VAEPVDDEELDAIDGSAAEGDDDIAPEADTDDDIAEDEE	OIV73604.1
MSDLAREITPVNIEEEELKNSYLDYAMSVIVGRALPDVRDGLKPVHRRVLYAMNVLGND WNKAYKKSARVVGDVIGKYHPHGDSAVYDTIVRMAQPFSLRYMLVDGQGNFGSIDGDS AAAMRYTEIRLAKIAHELMADLEKETVDFVDNYDGTERIPDVMPTKIPNLLVNGASGIAV GMATNIPPHNLTEVINGCLAYVDDEDISIEGLMAHIPGPDFPTAAIINGRRGIEEAYRTGRG KVYIRARAEVEVDAKSGRETIIVHEIPYQVNKARLIEKIAELVKEKRVEGISALRDESDKD GMRIVIEVKRDAVGEVVLNNLYSQTQLQVSFGINMVALHHGQPKIMNLKDIIAAFVRHRR EVVTRRTIFELRKARDRAHILEALAVALANIDPIIELIRRAPTPAEAKTALVAQAWDLGNV AAMLERAGDDAARPEWLEPEFGVRDGKYYLTEQQAQAILDLRLQKLTGLEHEKLLDEY KELLEQIAELLHILGSADRLMEVIREELELIRDQFGDERRTEITANSADINIEDLINQEDVVV TLSHQGYVKYQPLTDYEAQRRGGKGKSAARIKEEDFIDRLLVANTHDTILCFSSRGRLYW MKVYQLPEASRGARGRPIVNLLPLEADERITAILPVREYEEGVNVFMATASGTVKKTALT EFSRPRSAGIIAVNLNEGDELIGVDLTSGQDEVMLFSAAGKVVRFKEDAVRAMGRTATG VRGIKLAENDSVVSLIIPRGEGAILTVTQNGYGKRTAAAEYPTKSRATQGVISIKVTERNG SVVGAVQVDDCDQIMMITDAGTLVRTRVSEVSIVGRNTQGVILIRTAEDENVVGLQRVA EPVDDEELDAIDGSAAEGDDDIAPEADTDDDVADDADE	RIU59463.1
MSDLAREITPVNIEEEELKNSYLDYAMSVIVGRALPDVRDGLKPVHRRVLYAMNVLGND WNKAYKKSARVVGDVIGKYHPHGDIAVYDTIVRMAQPFSLRYMLVDGQGNFGSIDGDS AAAMRYTEIRLAKIAHELMADLEKETVDFVDNYDGTERIPDVMPTKIPNLLVNGASGIAV GMATNIPPHNLTEVINGCLAYVDDEDISIEGLMAHIPGPDFPTAAIINGRRGIEEAYRTGRG KVYIRARAEVEVDAKSGRETIIVHEIPYQVNKARLIEKIAELVKEKRVEGISALRDESDKD GMRIVIEVKRDAVGEVVLNNLYSQTQLQVSFGINMVALHHGQPKIMNLKDIIAAFVRHRR EVVTRRTIFELRKARDRAHILEALAVALANIDPIIELIRRAPTPAEAKTALVAQAWDLGNV AAMLERAGDDAARPEWLEPEFGVRDGKYYLTEQQAQAILDLRLQKLTGLEHEKLLDEY KELLEQIAELLHILGSADRLMEVIREELELIRDQFGDERRTEITANSADINIEDLINQEDVVV TLSHQGYVKYQPLTDYEAQRRGGKGKSAARIKEEDFIDRLLVANTHDTILCFSSRGRLYW MKVYQLPEASRGARGRPIVNLLPLEADERITAILPVREYEEGVNVFMATASGTVKKTALT EFSRPRSAGIIAVNLNEGDELIGVDLTSGQDEVMLFSAAGKVVRFKEDAVRAMGRTATG VRGIKLAENDSVVSLIIPRGEGAILTVTQNGYGKRTAAAEYPTKSRATQGVISIKVTERNG SVVGAVQVDDCDQIMMITDAGTLVRTRVSEVSIVGRNTQGVILIRTAEDENVVGLQRVA EPVDDEELDAIDGSAAEGDDDIAPEADTDDDIAEDEE	KTG68338.1
MSDLAREITPVNIEEEELKNSYLDYAMSVIVGRALPDVRDGLKPVHRRVLYAMNVLGND WNKAYKKSARVVGDVIGKYHPHGDYAVYNTIVRMAQPFSLRYMLVDGQGNFGSIDGDS AAAMRYTEIRLAKIAHELMADLEKETVDFVDNYDGTERIPDVMPTKIPNLLVNGASGIAV GMATNIPPHNLTEVINGCLAYVDDEDISIEGLMAHIPGPDFPTAAIINGRRGIEEAYRTGRG KVYIRARAEVEVDAKSGRETIIVHEIPYQVNKARLIEKIAELVKEKRVEGISALRDESDKD GMRIVIEVKRDAVGEVVLNNLYSQTQLQVSFGINMVALHHGQPKIMNLKDIIAAFVRHRR EVVTRRTIFELRKARDRAHILEALAVALANIDPIIELIRRAPTPAEAKTALVAQAWDLGNV AAMLERAGDDAARPEWLEPEFGVRDGKYYLTEQQAQAILDLRLQKLTGLEHEKLLDEY KELLEQIAELLHILGSADRLMEVIREELELIRDQFGDERRTEITANSADINIEDLINQEDVVV TLSHQGYVKYQPLTDYEAQRRGGKGKSAARIKEEDFIDRLLVANTHDTILCFSSRGRLYW MKVYQLPEASRGARGRPIVNLLPLEADERITAILPVREYEEGVNVFMATASGTVKKTALT EFSRPRSAGIIAVNLNEGDELIGVDLTSGQDEVMLFSAAGKVVRFKEDAVRAMGRTATG VRGIKLAENDSVVSLIIPRGEGAILTVTQNGYGKRTAAAEYPTKSRATQGVISIKVTERNG SVVGAVQVDDCDQIMMITDAGTLVRTRVSEVSIVGRNTQGVILIRTAEDENVVGLQRVA EPVDDEELDAIDGSAAEGDDDIAPEADTDDDIAEDEE	PTD91237.1
MSDLAREITPVNIEEEELKNSYLDYAMSVIVGRALPDVRDGLKPVHRRVLYAMNVLGND WNKAYKKSARVVGDVIGKYHPHGDSAVYDTIVRMAQPFSLRYMLVDGQGNFGSIDGDS AAAMRYTEIRLAKIAHELMADLEKETVDFVDNYDGTERIPDVMPTKIPNLLVNGASGIAV GMATNIPPHNLTEVINGCLAYVDDEDISIEGLMAHIPGPDFPTAAIINGRRGIEEAYRTGRG KVYIRARAEVEVDAKSGRETIIVHEIPYQVNKARLIEKIAELVKEKRVEGISALRDESDKD	PLI77147.1

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GMRIVIEVKRDAVGEVVLNNLYSQTQLQVSFGINMVALHHGQPKIMNLKDIIAAFVRHRR
EVVTRRTIFELRKARDRAHILEALAVALANIDPIIELIRRAPTPAEAKTALVAQAWDLGNV
AAMLERAGDDAARPEWLEPEFGVRDGKYYLTEQQAQAILDLRLQKLTGLEHEKLLDEY
KELLEQIAELLHILGSADRLMEVIREELELIRDQFGDERRTEITANSADINIEDLINQEDVVV
TLSHQGYVKYQPLTDYEAQRRGGKGKSAARIKEEDFIDRLLVANTHDTILCFSSRGRLYW
MKVYQLPEASRGARGRPIVNLLPLEADERITAILPVREYEEGVNVFMATASGTVKKTALT
EFSRPRSAGIIAVNLNEGDELIGVDLTSGQDEVMLFSAAGKVVRFKEDAVRAMGRTATG
VRGIKLAENDSVVSLIIPRGEGAILTVTQNGYGKRTAAAEYPTKSRATQGVISIKVTERNG
SVVGAVQVDDCDQIMMITDAGTLVRTRVSEVSIVGRNTQGVILIRTAEDENVVGLQRVA
EPVDDEELDAIDGSAAEGDDDIAPEADTDDDIAEDEE
```

3.5 Multiple sequence alignment

MSA (Multiple sequence alignment) was performed using the gyrA protein FASTA sequence in four different bacteria. Ten mutated sequences of each bacterium were compared with non mutated parental sequence in order to trace mutation in amino acid sequence. The tool used to perform MSA is Clustal Omega.

Steps to perform MSA

1) Clustal Omega program was selected

2) Query sequence or the file of the sequence was uploaded.

3) Algorithmic and parameter was selected

4) MSA program was run.

Fig 3- Clustal Omega tool used to perform MSA of different bacteria

CHAPTER 4
RESULTS AND
DISCUSSION

4.1 Media preparation and bacterial culturing

E. coli and *A. baumannii* isolates were inoculated in Luria broth and showed optimal growth at 37°C. In order to obtain isolated colonies, the cultures were streaked on MacConkey agar plate (Fig 4).

Fig 4- Bacterial isolated colonies on MacConkey agar plate

4.2 Antimicrobial susceptibility test (AST)

Antimicrobial susceptibly of *A. baumannii* and *E. coli* isolates was determined for different quinolones including ciprofloxacin, nalidixic acid and norfloxacin. Type strain ATCC 19606 and ATCC 25922 were used as a control for determination of antibiotic susceptibility. The zone of inhibitions listed in table no. 12 showed that there was a very small zone of inhibition in strain AB1, AB2, EC1 and EC2 in comparison to respective type strains.

Table 12- Zone of inhibition of bacterial isolates for different quinolones

	Zone of inhibition (mm)		
Bacterial isolates	**Ciprofloxacin**	**Nalidixic acid**	**Norfloxacin**
ATCC 19606	16	15	15
AB1	10	10	9
AB2	9	11	11
ATCC 25922	15	14	15
EC1	12	8	11
EC2	8	10	9

4.3 Multiple sequence alignment of *Acinetobacter baumannii* gyrA

The comparison *Acinetobacter baumannii* gyrA parental sequence with gyrA of ten antibiotic resistant strains showed conserved regions of the

protein except substitution mutations at certain positions. The substitution of Ser to Leu in 8 sequences of *A. baumannii* strains in comparison to parental sequence is highlighted in Fig 5. Similarly, the substitution of Ile with Phe and Ala with Thr was observed in 3 sequences. The substitution of Glu with Lys, Arg with His, Lys with Asn, and Gly with Cys was observed in single sequence of resistant strains out of ten in comparison to parental sequence.
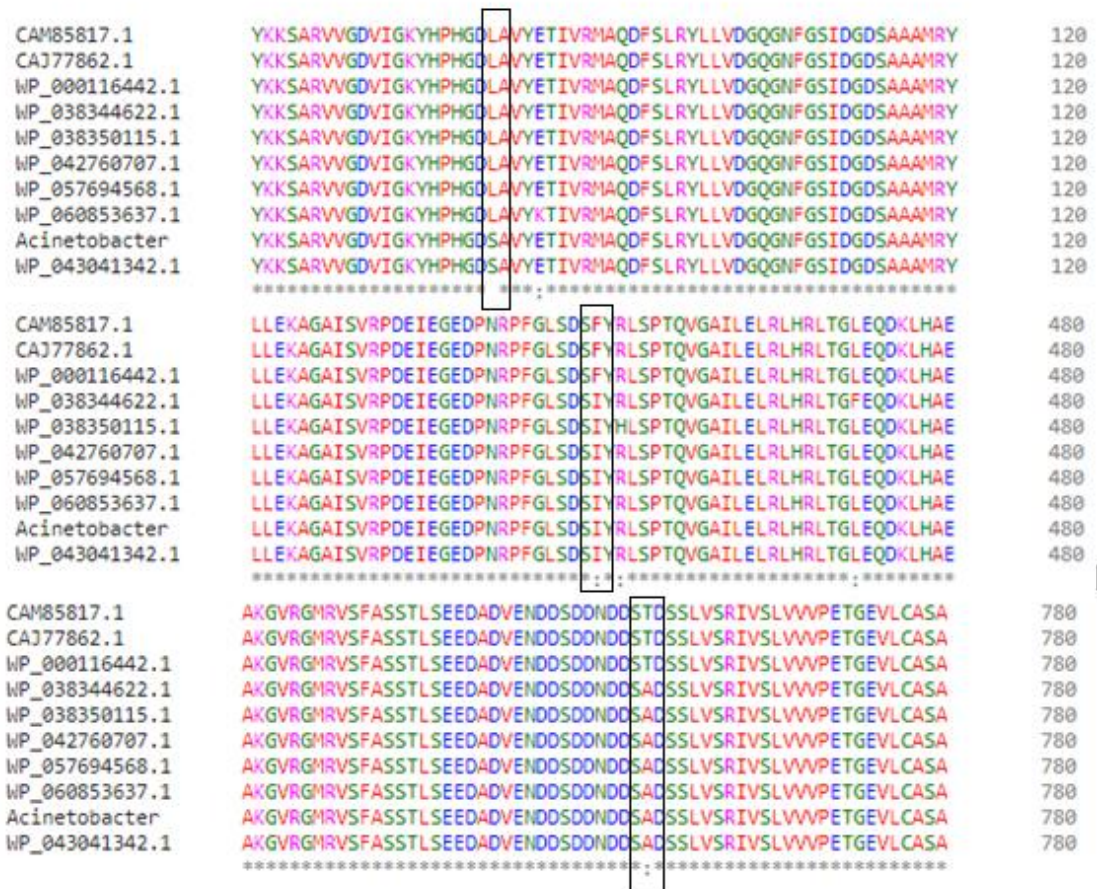

CAM85817.1	YKKSARVVGDVIGKYHPHGDLAVYETIVRMAQDFSLRYLLVDGQGNFGSIDGDSAAAMRY	120
CAJ77862.1	YKKSARVVGDVIGKYHPHGDLAVYETIVRMAQDFSLRYLLVDGQGNFGSIDGDSAAAMRY	120
WP_000116442.1	YKKSARVVGDVIGKYHPHGDLAVYETIVRMAQDFSLRYLLVDGQGNFGSIDGDSAAAMRY	120
WP_038344622.1	YKKSARVVGDVIGKYHPHGDLAVYETIVRMAQDFSLRYLLVDGQGNFGSIDGDSAAAMRY	120
WP_038350115.1	YKKSARVVGDVIGKYHPHGDLAVYETIVRMAQDFSLRYLLVDGQGNFGSIDGDSAAAMRY	120
WP_042760707.1	YKKSARVVGDVIGKYHPHGDLAVYETIVRMAQDFSLRYLLVDGQGNFGSIDGDSAAAMRY	120
WP_057694568.1	YKKSARVVGDVIGKYHPHGDLAVYETIVRMAQDFSLRYLLVDGQGNFGSIDGDSAAAMRY	120
WP_060853637.1	YKKSARVVGDVIGKYHPHGDLAVYKTIVRMAQDFSLRYLLVDGQGNFGSIDGDSAAAMRY	120
Acinetobacter	YKKSARVVGDVIGKYHPHGDSAVYETIVRMAQDFSLRYLLVDGQGNFGSIDGDSAAAMRY	120
WP_043041342.1	YKKSARVVGDVIGKYHPHGDSAVYETIVRMAQDFSLRYLLVDGQGNFGSIDGDSAAAMRY	120
	********************:* .***:***********************************	
CAM85817.1	LLEKAGAISVRPDEIEGEDPNRPFGLSDSFYRLSPTQVGAILELRLHRLTGLEQDKLHAE	480
CAJ77862.1	LLEKAGAISVRPDEIEGEDPNRPFGLSDSFYRLSPTQVGAILELRLHRLTGLEQDKLHAE	480
WP_000116442.1	LLEKAGAISVRPDEIEGEDPNRPFGLSDSFYRLSPTQVGAILELRLHRLTGLEQDKLHAE	480
WP_038344622.1	LLEKAGAISVRPDEIEGEDPNRPFGLSDSIYRLSPTQVGAILELRLHRLTGFEQDKLHAE	480
WP_038350115.1	LLEKAGAISVRPDEIEGEDPNRPFGLSDSIYHLSPTQVGAILELRLHRLTGLEQDKLHAE	480
WP_042760707.1	LLEKAGAISVRPDEIEGEDPNRPFGLSDSIYRLSPTQVGAILELRLHRLTGLEQDKLHAE	480
WP_057694568.1	LLEKAGAISVRPDEIEGEDPNRPFGLSDSIYRLSPTQVGAILELRLHRLTGLEQDKLHAE	480
WP_060853637.1	LLEKAGAISVRPDEIEGEDPNRPFGLSDSIYRLSPTQVGAILELRLHRLTGLEQDKLHAE	480
Acinetobacter	LLEKAGAISVRPDEIEGEDPNRPFGLSDSIYRLSPTQVGAILELRLHRLTGLEQDKLHAE	480
WP_043041342.1	LLEKAGAISVRPDEIEGEDPNRPFGLSDSIYRLSPTQVGAILELRLHRLTGLEQDKLHAE	480
	**********************************:.*:*********************:********	
CAM85817.1	AKGVRGMRVSFASSTLSEEDADVENDDSDONDDSTDSSLVSRIVSLVVVPETGEVLCASA	780
CAJ77862.1	AKGVRGMRVSFASSTLSEEDADVENDDSDONDDSTDSSLVSRIVSLVVVPETGEVLCASA	780
WP_000116442.1	AKGVRGMRVSFASSTLSEEDADVENDDSDONDDSTDSSLVSRIVSLVVVPETGEVLCASA	780
WP_038344622.1	AKGVRGMRVSFASSTLSEEDADVENDDSDONDDSADSSLVSRIVSLVVVPETGEVLCASA	780
WP_038350115.1	AKGVRGMRVSFASSTLSEEDADVENDDSDONDDSADSSLVSRIVSLVVVPETGEVLCASA	780
WP_042760707.1	AKGVRGMRVSFASSTLSEEDADVENDDSDONDDSADSSLVSRIVSLVVVPETGEVLCASA	780
WP_057694568.1	AKGVRGMRVSFASSTLSEEDADVENDDSDONDDSADSSLVSRIVSLVVVPETGEVLCASA	780
WP_060853637.1	AKGVRGMRVSFASSTLSEEDADVENDDSDONDDSADSSLVSRIVSLVVVPETGEVLCASA	780
Acinetobacter	AKGVRGMRVSFASSTLSEEDADVENDDSDONDDSADSSLVSRIVSLVVVPETGEVLCASA	780
WP_043041342.1	AKGVRGMRVSFASSTLSEEDADVENDDSDONDDSADSSLVSRIVSLVVVPETGEVLCASA	780
	***********************************:* *****************************	

Fig 5 – MSA result of *Acinetobacter baumannii* gyrA

Phylogenetic Tree

This is a Neighbour-joining tree without distance corrections.

Branch length: ● Cladogram   ○ Real

CAM85817.1 0
CAJ77862.1 0
WP_000116442.1 0
Acinetobacter 0
WP_043041342.1 0.00111
WP_038344622.1 0.00111
WP_038350115.1 0.00111
WP_042760707.1 0.00111
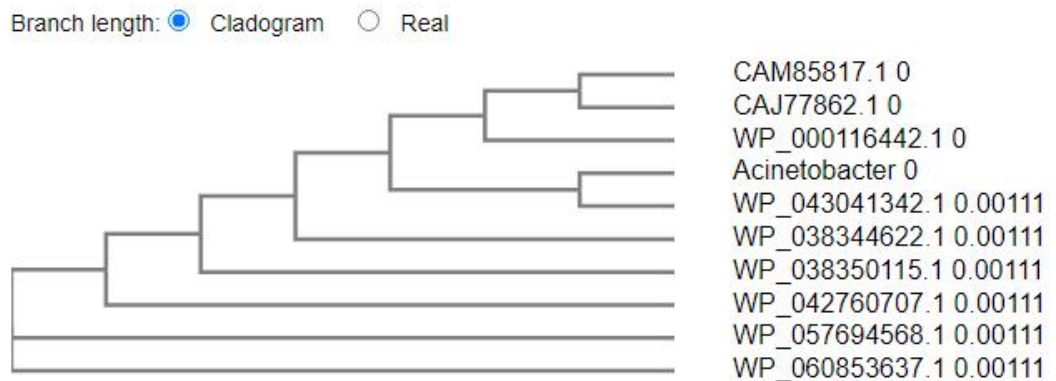WP_057694568.1 0.00111
WP_060853637.1 0.00111

Fig 6- Phylogenetic Tree of *Acinetobacter baumannii* gyrA

4.4 MSA result of *Escherichia coli.*

The comparison *Escherichia coli* gyrA parental sequence with gyrA of ten antibiotic resistant strains showed conserved regions of the protein except substitution mutations at certain positions. The substitution of Glu to Asp in 8 sequences of *E. coli* resistant strains out of 10 is highlighted in Fig 7. Similarly, the substitution of Phe with Ser and Ser with Leu was observed in 3 sequences. The substitution of Ala with Ser, Leu with Met, Gln with X (any amino acid) was observed in single sequence of resistant strains out of ten in comparison to parental sequence.
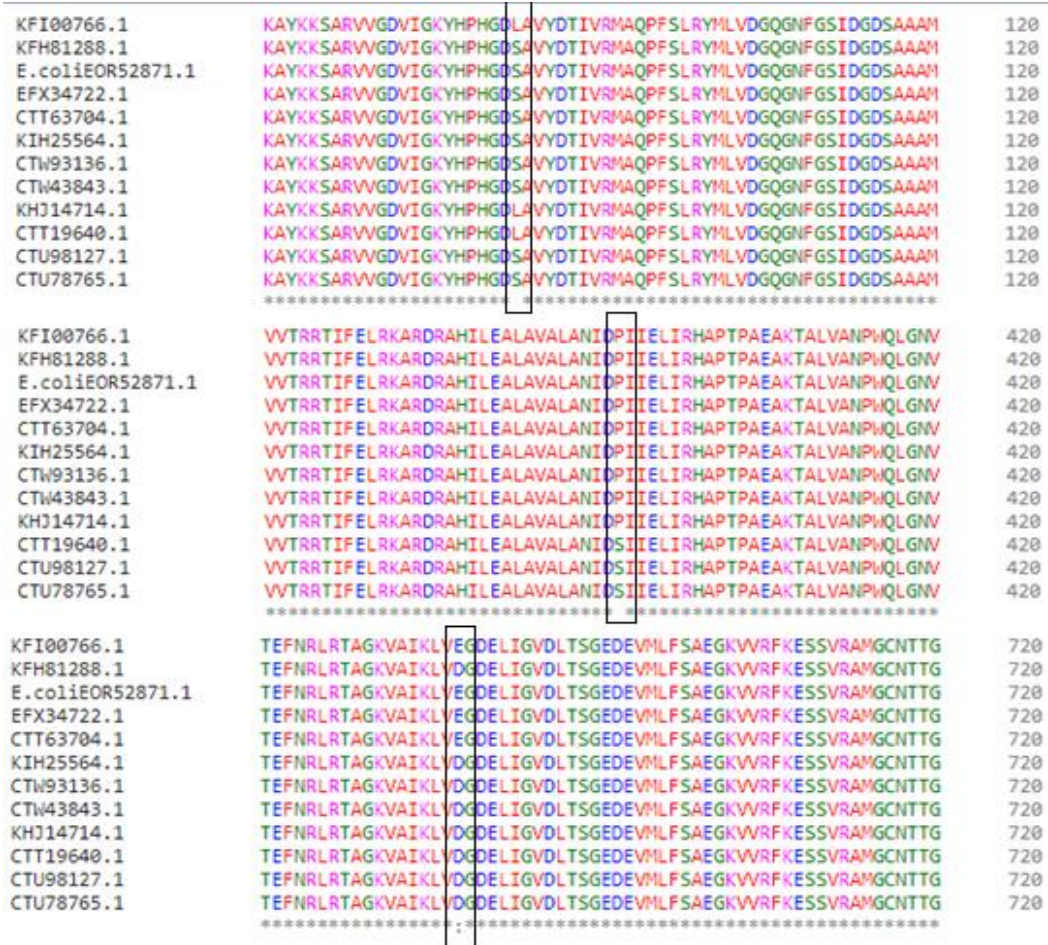
Fig 7- MSA result of *Escherichia coli*.

Phylogenetic Tree

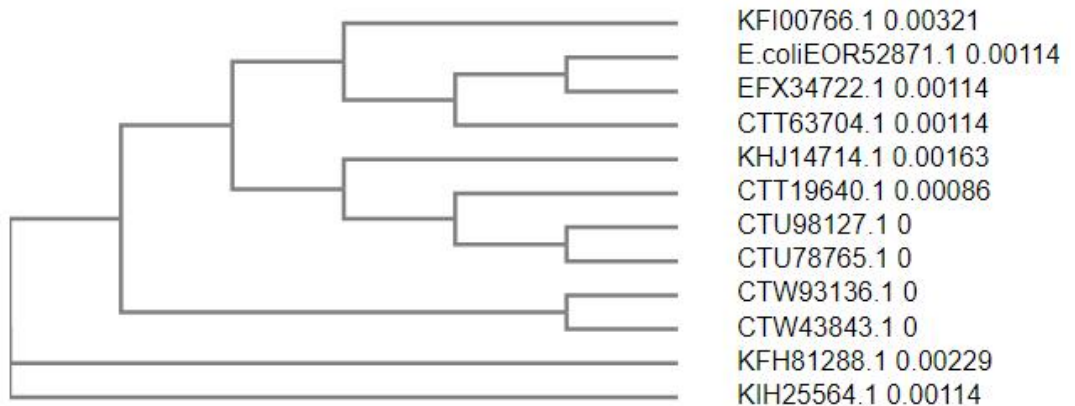*This is a Neighbour-joining tree without distance corrections.*

Branch length: ● Cladogram   ○ Real

KFI00766.1 0.00321
E.coliEOR52871.1 0.00114
EFX34722.1 0.00114
CTT63704.1 0.00114
KHJ14714.1 0.00163
CTT19640.1 0.00086
CTU98127.1 0
CTU78765.1 0
CTW93136.1 0
CTW43843.1 0
KFH81288.1 0.00229
KIH25564.1 0.00114

Fig 8 - Phylogenetic tree result of *Escherichia coli*

4.5 MSA result of *Pseudomonas aeruginosa*

The comparison *Pseudomonas aeruginosa* gyrA parental sequence with gyrA of ten antibiotic resistant strains showed conserved regions of the protein except substitution mutations at certain positions. The substitution of Asp to Asn in single sequence of *P. aeruginosa* resistant strains out of 10 is highlighted in Fig 9. Similarly, the substitution of Arg with Ser, Phe with Cys, Glu with X(any amino acid), and Pro with X(any amino acid) was observed in single sequence of resistant strains out of ten in comparison to parental sequence.
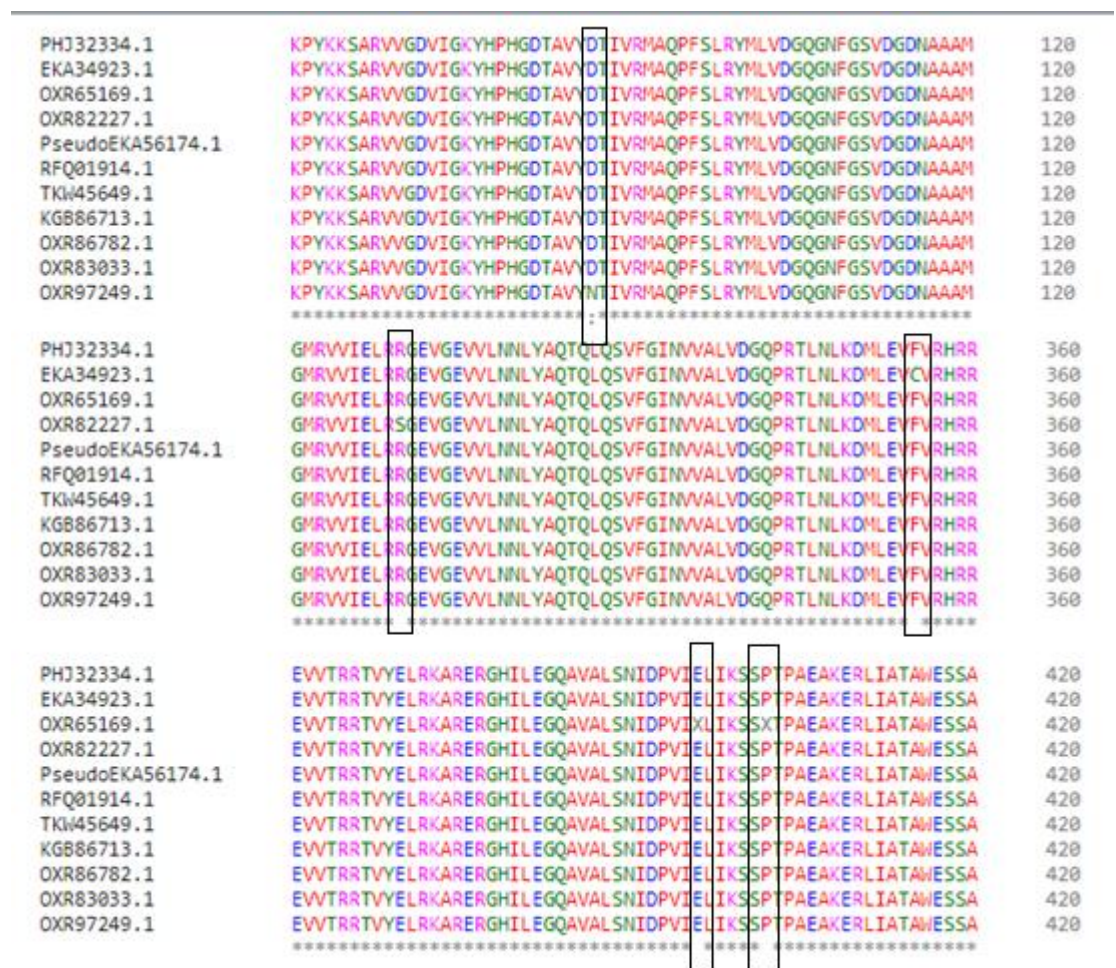

Fig 9- MSA result of *Pseudomonas aeruginosa*

Phylogenetic Tree

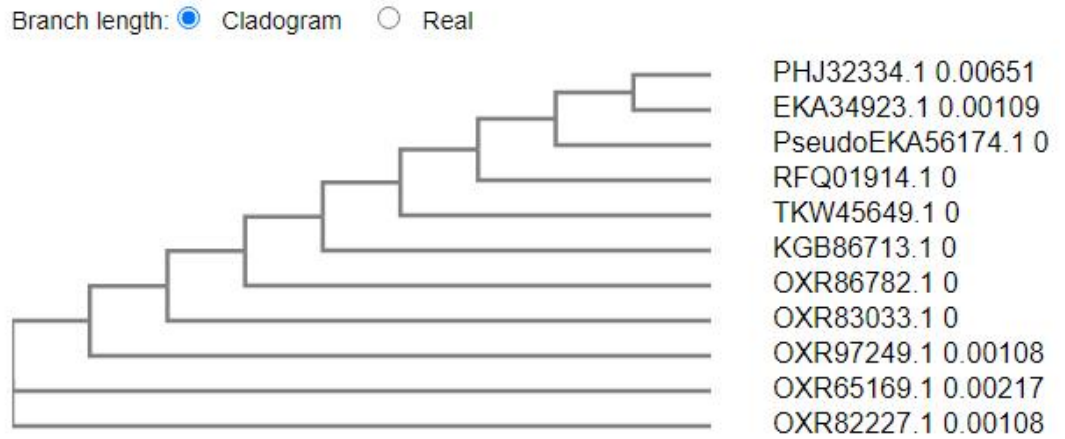*This is a Neighbour-joining tree without distance corrections.*

Branch length: ● Cladogram ○ Real

```
                                    PHJ32334.1 0.00651
                                    EKA34923.1 0.00109
                                    PseudoEKA56174.1 0
                                    RFQ01914.1 0
                                    TKW45649.1 0
                                    KGB86713.1 0
                                    OXR86782.1 0
                                    OXR83033.1 0
                                    OXR97249.1 0.00108
                                    OXR65169.1 0.00217
                                    OXR82227.1 0.00108
```

Fig 10- MSA result of *Pseudomonas aeruginosa*

4.6 MSA result of *Klebsiella pneumoniae*

The comparison *Klebsiella pneumoniae* gyrA parental sequence with gyrA of ten antibiotic resistant strains showed conserved regions of the protein except substitution mutations at certain positions. The substitution of Ser to Ile/Tyr/Phe respectively in 7 sequences of *K. pneumoniae* resistant strains out of 10 is highlighted in Fig 11. Similarly, the substitution of Ile with Val and Glu with Lys was observed in 2 sequences. The substitution of Glu with Asp, Glu with Lys was observed in single sequence of resistant strains out of ten in comparison to parental sequence.
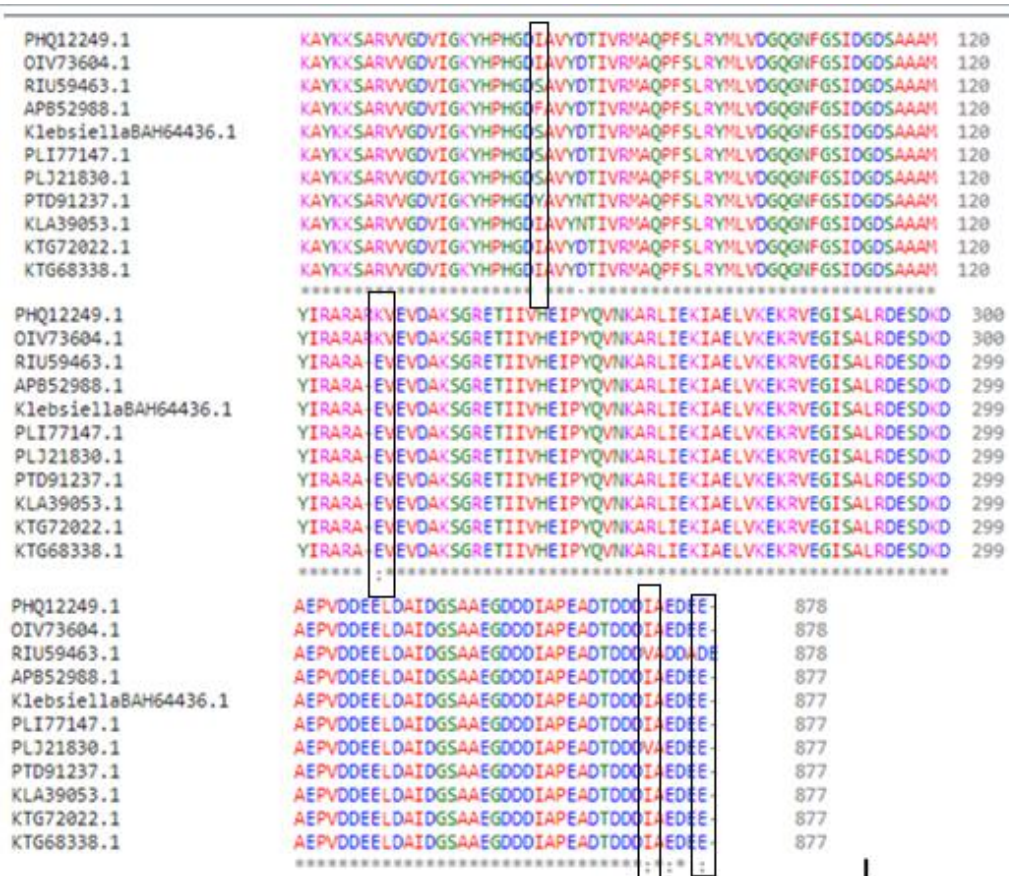
Fig 11- MSA result of *Klebsiella pneumoniae*

Phylogenetic Tree

This is a Neighbour-joining tree without distance corrections.
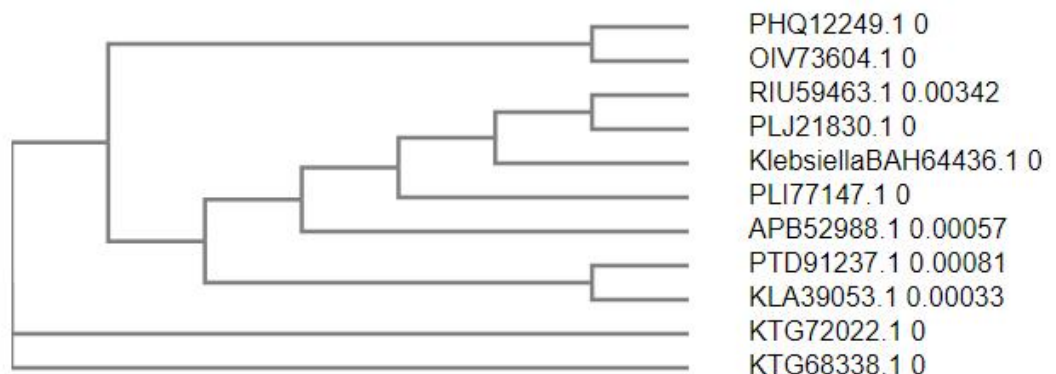
Branch length: ● Cladogram   ○ Real

PHQ12249.1 0
OIV73604.1 0
RIU59463.1 0.00342
PLJ21830.1 0
KlebsiellaBAH64436.1 0
PLI77147.1 0
APB52988.1 0.00057
PTD91237.1 0.00081
KLA39053.1 0.00033
KTG72022.1 0
KTG68338.1 0

Fig 12- Phylogenetic tree of *Klebsiella pneumoniae*

4.7 Parental sequence MSA

The bacterial species considered to carry out present study are gram negative bacteria which have developed antibiotic resistance to almost all available antibiotics. Hence, an attempt was carried to compare gyrA sequence of susceptible type *Acinetobacter baumannii*, *Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumonia.* The results of MSA after comparing parental sequences are mentioned in Fig. 13. The MSA indicate that the gyrA sequence of *A. baumannii* contains maximum substitutions, but still it has similarly with sequence *Pseudomonas aeruginosa*. Similarly, the gyrA sequence of *Escherichia coli* and *Klebsiella pneumoniae* are more related to each other.
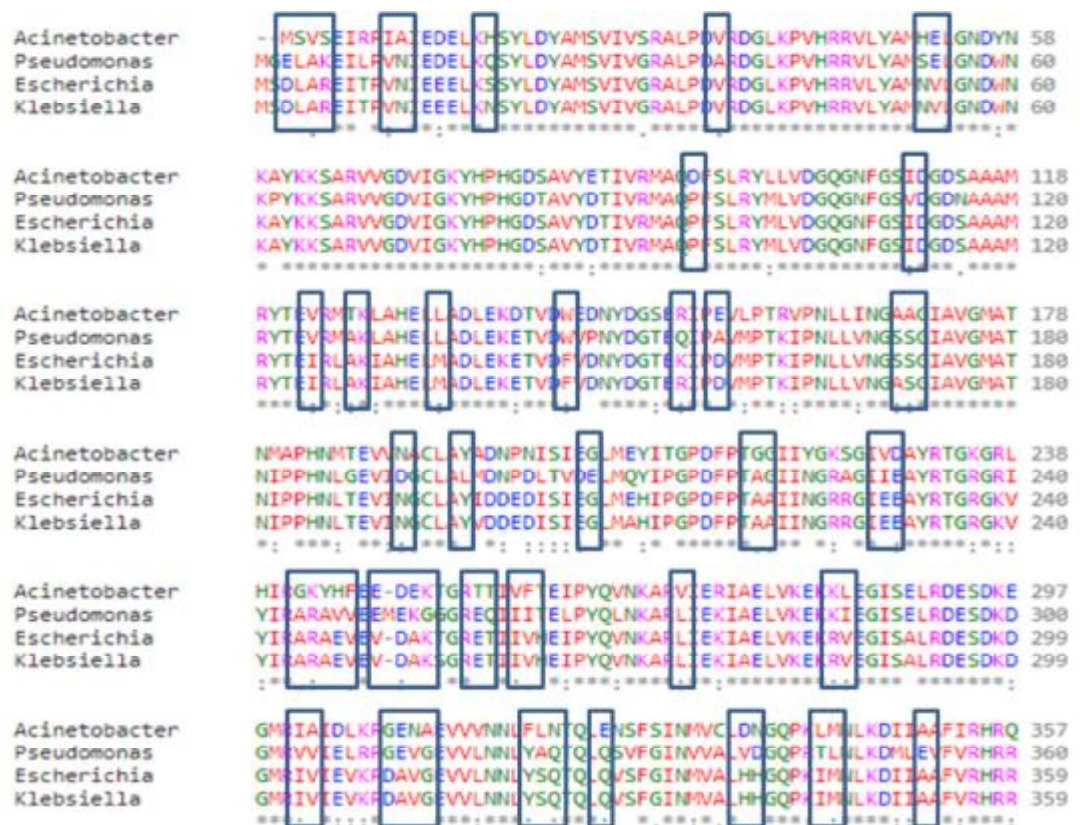

Fig 13- MSA of non mutated gyrA protein sequences of *Acinetobacter baumannii, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella Pneumoniae*

Phylogenetic Tree

This is a Neighbour-joining tree without distance corrections.

Branch length: ● Cladogram   ○ Real

Acinetobacter 0.21599
Pseudomonas 0.16376
Escherichia 0.03819
Klebsiella 0.03839

Fig 14 – Phylogenetic tree for gyrA protein sequences of *Acinetobacter baumannii, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella Pneumoniae*

CHAPTER 5

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CHAPTER 6
APPENDIX

5.1 Bacteriological media:

All the media mentioned below were dissolved in distilled water and prepared by autoclaving at 15 psi for 20 mins.

5.1.1 MacConkey agar (Himedia)

Table 14- MacConkey Agar ingredients and Quantity

Sr. No.Ingredients	Quantity /1000ml
1. Peptones (meat and casein)	3 gm
2. Pancreatic digest of gelatin	17gm
3. Lactose monohydrate	10gm
4. Bile salts	1.5gm
5. Sodium chloride	5gm
6. Crystal violet	0.001gm
7. Neutral red	0.030gm
8. Agar	13.500

5.1.2 Luria broth (Himedia)

Table 15 – Luria broth ingredient and quantity

Sr. No.	Ingredients	Quantity/1000ml
1.	Casein enzymic hydrolysate	10 gm
2.	Yeast extract	5gm
5	Sodium chloride	5gm

5.2 Preparation of Mc Farland

1% BaCl2 and 1% H2SO4

1gm in 100 ml of baCl2 and 1ml of H2SO4 in 99ml of water

0.05ml BaCl2 in 9.95ml of H2SO4.

i.e., 1×1 8 CFU/ml

Turbidity standard no. = 0.5