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"Effect of antibiotic resistance on the genomic DNA of *E.coli* O157: H7 at sublethal concentrations"

Project Report

Submitted in partial fulfilment of the requirements for the degree of

Masters of science(Zoology Hons)

Submitted By

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Under the guidance of

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CERTIFICATE

This is to certify that TSIIKHII A (11510951) have completed the project, entitled '**EFECTS OF ANTIBIOTIC RESISTANCE ON THE GENOMIC DNA OF *E.coli* O157;H7 AT SUBLETHAL CONCENTRATIONS'** under my guidance and supervision. To the best of my knowledge, the present work is the result of her original investigation and study. No part of the report has ever been submitted for any other degree at any University. The report is fit for submission and the partial fulfilment of the conditions for the award of M.sc Zoology degree.

(Supervisor's Signature)

DECLARATION

I hereby declare that the project entitled **"EFECTS OF ANTIBIOTIC RESISTANCE ON THE GENOMIC DNA   OF** *E.coli* **O157;H7 AT SUBLETHAL CONCENTRATIONS**" is an authentic record of my own work carried out at School of Biotech and Biosciences, Lovely Professional University, Phagwara, for the partial fulfilment of the award of Masters Degree of Zoology in department of Biosciences, under the guidance of 'Dr. Mahiti Gupta'. This work is my original work and has not been submitted for any degree/diploma in this or any other University. The information furnished in this report is genuine to the best of my knowledge and belief.

ACKNOWLEDGEMENT

I take this opportunity to present my vote of thanks to all those who have guided me and acted as lightening pillars to enlighten my way throughout this project that has led to proper and effective completion of this study.

I am grateful to the university for providing with opportunity to work on this project and providing me with the necessary facilities. I am thankful to my supervisor Dr.Mahiti Gupta for her active support, valuable time and advice, whole-hearted guidance, sincere cooperation and pain-taking involvement during the study and completing the project within time stipulated.

Lastly, I would like to thank my friends and family and all those who had helped me, for their effort and time in completing this project within the stipulated time.

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ABBREVIATIONS

- o µl- microlitre
- o mm- millimetre
- o bp- basepairs
- o DAEC- diffusely adherent *Escherichia coli*
- o *E.coli- Escherichia coli*
- o EAEC- Enteroaggregative *Escherichia coli*
- o EHEC- enterohaemorhhagic colitis
- o HC- Haemorrhagic colitis
- o LEE- Locus for enterocyte effacement
- o SMAC- sorbitol mac conkey agar
- o MAC- mac conkey agar
- o STX- shiga toxin
- o VT- verocytotoxin
- o VTEC- verocytotoxigenic*E.coli*
- o EPEC- Enteropathogenic*Escherichia coli*
- o STEC- shiga toxigenic *Escherichia coli*
- o Hly- enterohaemolysin
- o AAE- attaching and effacing gene
- o CT-SMAC-cefiximetellutite sorbitol mac conkey agar
- o Cfu- colony forming unit
- o MIC- Minimal Inhibitory Concentration
- o RFLP- Restriction Fragment Length Polymorphism
- o ET BR- Ethidium Bromide
- o ETDA- Ethylene diaminatetra acitic acid
- o SDS- Sodium Dodecyl Sulphate

LIST OF SYMBOLS

- %   Percentage
- °C   Degree centigrade
- mg   Milligram
- g   Gram
- l   Liter
- ml   Microliter
- cm   Centimeter
- m   Meter
- h   Hour
- min   Minute
- µg/ml   Microgram per millilitre
- g/l   Gram per liter
- mM   Millimolar
- M   Molar
- sec   Second
- KPa   Kilopascal

ABSTRACT

E.coli O157:H7 is a causative agent of diarrhoea. The main source of *E.coli* infections are through the consumption of uncooked ground beef, unpasteurized milk and the contamination of surface and ground water. The antibiotics are used frequently for the treatment of this disease. But it is seen that this aggravated the infection rate where the bacteria become resistant to the antibiotics. The Ampicillin and Tetracycline are commonly available. The *E.coli* isolates from the sewerage water were used to check the resistance against the two antibiotics, Ampicillin and Tetracycline in the broth cultures at the concentration of 25 µg/ml, 50 µg/ml, 75 µg/ml and 100 µg/ml. The growth was seen in broths having concentrations 25 µg/ml and 50 µg/ml. So the genomic DNA was extracted from the bacteria growing under stress of 50 µg/ml antibiotics. The DNA from respective isolates was subjected to restriction digestion using EcoRI enzyme to check the change in restriction pattern. It was found that greater number of mutations in Ampicillin treated DNA then tetracycline led to resistance in microorganisms.

1. INTRODUCTION

Antibiotics are the substances which are used to kill microorganisms and cure bacterial infections. The antibiotic resistance occurs when the antibiotic loses its ability to inhibit or kill bacteria. This resistance maybe due to excessive or frequent use of antibiotics. The resistance is critical health hazard to the public in developing nations (World Health Organization, 1996; Karlowsky and Jones, 2003).

1.1 HOW BACTERIA ACQUIRE RESISTANT GENE

1. Vertical gene transfer

   Where the resistant gene is passed through bacterial replication. Maybe during the evolution. For example, spontaneous mutation for a resistant gene.

2. Horizontal gene transfer

   Resistant gene is transferred to bacteria through three ways

   i) Conjugation

       The bacteria has a pilus from where the gene is passed to other bacteria.

   ii) Transduction

       Transduction transfers through a virus called bactereriophage. The bacteriophage inject its DNA called phage DNA into the bacteria. The phage DNA leaves the bacterial DNA and starts replicating, destroying bacterial DNA in the process. The resistant gene which is part of bacterial DNA starts floating inside the damaged bacteria so that the viral DNA is being replicated and new bacteriophages are formed within the bacteria. This causes the bacteria to lyse releasing the phage DNA out.

   iii) Transformation

       Bacteria containing the resistant gene lyse/dies releasing the resistant gene and other bacteria will incorporate this into genome.

1.2 Modes of Resistance

i)   Genetic Mutation

ii) Acquiring Resistance genes from other bacteria

Gene mutation is alteration in the DNA sequence whereby the sequence differs. The bacteria change the permeability of their r the cell. The routes of entry of antibiotics into the cells are closed by pumping mechanisms like the efflux pumps whereby the bacteria export back the antibiotic back outside without allowing it to reach the target site. Some bacteria produce certain enzymes. For example enzyme beta-lactamases which destroy penicillin.

1.3 Mobile Genetic Elements

Mobile genetic elements are those which can move around within genome. Plasmids and transposons are mobile genetic elements in terms of antibiotic resistance. Plasmids are small circular extra chromosomal DNA within a cell and can replicate independently. The plasmid carries a gene which is beneficial for the survival of the organism, like resistance to antibiotics. Transposons are jumping genes, small DNA pieces, which can jump from one DNA molecule to another.

1.4   Gene cassettes and integrons

Gene cassettes are small mobile elements which carry a gene and have an attc recombination site which vary in length and sequence. They are usually inserted into integrons. The integrons consists of an Int1 gene and an att1 recombination site and a pc promoter.Int1 integrase is encoded by gene Int1. Integrons have ten to hundreds of cassettes depending on Int1 sequences and chromosomal integrons.

1.5   *Escherichia coli (E.coli)*

Escherichia coli was first discovered by Theoder Escherichia in 1885. This bacterium is found as a commensal organism in the large intestine. The first outbreak of *E.coli* was reported in 1935 among infants. *E.coli* is often referred

to as Enteric bacteria because they inhabit the intestinal tracts of humans and other animals. *E.coli* belongs to the family of "Enterobacteriaceae" which include gram negative, non-spore forming, rod shaped, motile due to flagella. Majority of the strains can grow in laboratory media both in presence or absence of oxygen and metabolism by transpiration and fermentation. *E.coli* act as a useful organism by inhibiting the growth of other harmful bacteria which produce the shiga toxin. *E.coli* is member of normal flora of the large intestine. They remain commensal unless they acquire the virulence factors. Resistant *E.coli* strains have the ability to acquire resistance from other organisms (Osterbald et al 2000).

1.6   Structure Classification and Antigenic Types

E.coli are grouped according to the presence or absence of specific heat-stable somatic antigens (O antigens) made up of polysaccharides linked to core lipopolysaccharide (LPS) complex. More than 170 different O-specific antigens are found since 1943 when Kauffmann began this method of typing in *E.coli*. The H antigens are flagellar antigens and are of 56 types. O;H antigenic makeup helps in the identification strains which cause outbreaks. The *E.coli* isolates proteins which are unrelated to O and H. These antigens are thin and rigid filamentous structures called pile (fimbriae).

1.7 Classes of *E.coli*

i) Enterotoxigenic *E.coli* (ETEC)

They are the main causative agents of diarrhea in infants, dogs, sheep and cattles. This strain of *E.coli* produces enterotoxins. Ingestion of contaminated food and water leads to ETEC. Enterotoxins produced by ETEC are LT (heat-labile) toxin. Both the genes may occur in the same or separate plasmids. The molecular weight of ST is about 2000 Daltons. As a result of this small size they are not inactivated by heat. The ST leads to increase GMP in host cell cytoplasm leading to secretion of fluid and electrolytes resulting to diarrhea.They bind to the enterocyte cells with fimbriae adhesion

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ii) Enteroinvasive *E.coli* (EIEC)

EIEC are invasive organism. Seen only in humans. Their pathogenic mechanisms resemble that of Shigella. They can penetrate the epithelial cells of colon and multiply rapidly causing wide damage. They do not have fimbriae, LT and ST toxins, nor Shiga toxins. They cause dysentery like diarrhae with fever.

iii) Enteropathogenic *E.coli* (EPEC)

EPEC causes watery diarrhea.They possesses an outer membrane protein called intimin which can bind to the host intestinal cells. EPEC have a virulence plasmid that encodes the bundle forming pilus(BFP) (Stone et al.,1996).Two protein namely espA and espB are secreted by the EPEC.

iv) Enterohaemorrhagic *E.coli* (EHEC)

These are generally found in goats, cattles, and humans. EHEC is the causative agents of HUS and kidney problem. They attach to the host by fimbriae and possess shiga toxins.The shiga toxins are also called as verotoxin. This EHEC also have 90 kbp plasmid po157, which helps the fimbriae in the attachment to the intestinal epithelial cells.

1.8 *Escherichia coli* O157; H7

Escherichia coli O157; H7 is a causative agent of diarrhea (Chalmers et al., 2000).It is known that the transmission of *E.coli* O157;H7 is due to contaminated food and water but person to person contact might also be the cause of transmission. It is a gram negative bacterium and produces shiga toxins (Wong et al., 2000) *E. coli* O157; H7 has somatic (O) antigen 157 and (H) antigen flagella 7. E. Coli O157; H7 has a chromosomal size of 5.5M6. It is known that some of the DNA sequences are foreign DNA which are transferred horizontally like prophage and some are loss. This might have led to evolution of *E.coli* O157; H7. *E.coli* O157; H7 has acid tolerance due to the presence of gene "RPOS" which is associated with oxidative arginine dependent and glutamate dependent.

E.coli O157:H7 can survive in a very low pH (<pH 3.0) and this have led them to their survival and ability to colonize intestine and cause the infection. This *E.coli*

O157:H7 has some mechanism to this acid resistance like RpOS, glucose repression and the glutamate which gives protection. Several proteins like DNA binding protein DpS, RNA polymerase associated protein SspA and chaperone HdeA are involved in the acid resistance of *E.coli* O157:H7.

1.9   Virulance factor

Ability of organism to cause disease in a host. Virulence in latin meaning poisonous. Virulence factors helps in distinguishing from non pathogenic bacteria.

i)   Shiga toxins (stxs)

It is a major virulence factor which can lead to death. The Stxs is grouped into stx1 and stx2 (Nataro and kapper, 1998). Among the 2, stx2 is more toxic and is associated with HC or HUS. Shiga toxin is made up pf active and receptor binding subunits forming a pentameric ring. The active subunit is 32 KDa and binding site is 7.7 KDa monomers. The Shiga toxin can cause damage to intestinal epithelial cells and vascular endothelial cells.

Different subtypes of Stx1

   a) Stx 1
   b) Stx 1c
   c) Stx 1d

Different subtypes of Stx2

   a) Stx 2 (associated with HUS)
   b) Stx 2c
   c) Stx 2c2
   d) Stx 2d
   e) Stx 2c
   f) Stx 2f

ii) Plasmid O157;H7 (pO157)

Plasmid usually carries the resistant gene. For plasmid to make the resistant gene, it has to incorporate into the circular DNA. But the plasmid is synthesizing RNA i.e. MRNA. MRNA is read by ribosome to make polypeptide to make proteins. This proteins will become enzymes or structure that help bacteria become resistant to antibiotic.

pO157 is a plasmid found in *E.coli* O157;H7. It is 92 to 104kb in size, non-conjugative F-like plasmid. pO157 include the insertion sequence, transposons and prophages. The insertion sequence are associated with the virulence segments similar to those of shigella species. pO157 might have being formed from the integrated fragments originally from different species. The pO157 consists of 100 open reading frames of which 35 proteins are associated with the pathogenecity. The biological significance of pO157 in pathogenecity is not well known.

iii) Locus of enterocyte effacement.

EHEC causes are the formation of attaching and effacing lesions (A/E). During the A/E lesions there is lose of adherence of bacteria to the epithelial cells and microvilli. The genes responsible for this are called the LEE. The LEE has 41 different genes found in three regions. The intimin ,Esp and type III secretion system.

iv) Stx converting bacteriophage

It was first described by Bradley in 1967. It is consists of nucleic acid molecule which maybe DNA or RNA. It is surrounded by a capsid and has a tail and spikes (Grabow et al., 2002). Toxin synthesis is carried out by integrated toxins converting phage from host organisms. The phage release is done by UV exposure. This UV mediated DNA damage causes the release of the previously silent bacteriophage gene like the stx gene.

v) Pathogenicity Island (PI)

The pathogenicity island was first coined by Jorg Hacker and colleagues in 1980's. This pathogenicity island is obtained through the horizontal transfer. This pathogenicity island is associated with the virulence factor

in UPEC. This pathogenicity island has a region called P-fimbriae and production site for haemolysin (hly). Different mobile genetic elements like transposons, integrons and insertion element are present. During the deletion, certain parts of virulence factors are loss, not by mutations but due to removal of the PI. Protien secretion is required for pathogenicity.The protein are required for the cell wall formation, for defense against external agents and metabolism. There are five protein secretion system namely Type I secretion system, Type II, Type III, Type IV and Type V.

1.10 Haemorrhagic colitis (HC)

E. coli O157;H7 is the causative agent of haemorrhagic colitis(HC).It is characterized by abdominal cramps, watery diarrhaea, right lower quadrant pain nausea, vomiting with little or no fever and chills. In elderly patients this HC could lead to several complications like HUS. Haemorrhagic colitis outbreak was reported in 1982 in Oregon and Michigan with bloody diarrhea and abdominal pain. (Morbid, Mortal Weekly Rep 31:580, 1982).

1.11 Hemolytic Uremic Syndrome (HUS)

HUS caused *E. coli* O157:H7. This HUS leads to kidney failure and a low platelet count. This HUS carries about 5-10% mortality rate. In 1955 HUS was first seen as a syndrome. This HUS is also caused by some shiga toxin-producing strains of shigella dysenteriae.The shiga like toxin I and shiga like toxin II usually associated with HUS are due to *E.coli* O157;H7.

1.12 Urinary Tract Infection (UTI)

The most common agent for UTI are the Uropathogenic *E.coli* (UPEC). The UTI is characterized by inflammation in bladder, fever and chills. The UPEC colonize the epithelium cells. The UTI are commonly seen in women compared to men. The UPEC colonize the cells and starts multiplying. There are greater chances of UTI in women than men (Johnson and Stamm, 1989; Lipsky, 1989). The most commonly used drugs for UTI are trimethoprim.

1.13 Antibiotic resistance

E.coli has evolved the mechanism to resist some antibiotics. But the antibiotic sensitivity varies among different strains. The antibiotics which are used to treat *E.coli* infection are amoxicillin, cephalosporins, trimethoprim-sulfamethoxazole, penicillins, ciprofloxacin, aminoglycosides, tetracycline, aztreonam, streptomycin, chloramphenicol, gentamycin. There is a high chance that this resistance maybe due to overuse of antibiotics. *E.coli* has the ability to transfer the resistant gene to another by conjucation or horizontal gene transfer. *E.coli* can exist in close proximity with others. Thus the mixing of different species allows the transfer of plasmids to one another.It is known that under stress condition *E.coli* can transfer the resistant plasmid to other causing the other bacteria to be resistant to certain antibiotics.

1.14 Antibiotic resistant gene

They form antibiotic degrading enzyme like Betalactamases which essentially breaks betalactam rings. Betalactam rings are found in penicillin. The penicillin is active because the betalactam ring is closed. However when penicillin encounters a betalactamase in the bacteria, the betalactamases will break the betalactam ring causing the penicillin to become inactive.

1.15 Methods of Resistance and the antibiotics involved

i) Efflux mechanism

The antibiotics involved are macrolides, tetracyclines, quinolones. This level of resistance is low.

iii)   Penetration

Chloramphenicol,trimethoprim,tetracycline and β-lactams show this mechanism.

The level of resistance is low.

iii) Alteration of the target

The antibiotics involved are chloramphenicol, tetracycline, β-lactams, quinolones, Rifampicin, glycopeptides and macrolides. The resistance level is variable.

iv) Detoxification of enzyme

The antibiotics involved are β-lactams, macrolides, chloramphenicol and Aminiglycosides. The resistance level is high.

v) Bypass mechanism

The antibiotics involved are sulphonamides and trimethoprim. The reistance level is high.

1.16 Efflux pump

Efflux pump are found on the membrane. Some antibiotics like tetracycline interfere in the protein syntheses. The bacteria used to pump out the antibiotic.

1.17 Resistant Gene to modify antibiotic binding target

Penicillin (betalactam) targets penicillin binding protein found in peptidoglycan layer of bacteria. But the bacteria has gene to modify penicillin binding protein which change the structure of protein. The penicillin is unable to bind into the protein anymore.

1.18 Chloramphenicol

Chloramphenicol inhibits the enzyme peptidyl transferase by binding the 50S ribosomal subunit in protein synthesis. Cat genes activate antibiotic. Resistance of gram negative bacteria to chloramphenicol is due to plasmid. The enzyme acetyl transferase inactivates the drugs.

1.19 β – lactams

β- lactams are widely used class of drugs. The enzyme Beta lactamases helps in the resistance against β-lactams antibiotics. These antibiotics bind to the PBP which are involved in cell wall synthesis in bacteria. The enzyme inactivates the

antibiotics by hydrolyzing the beta lactam amide bond. In the early 1960s the first plasmid mediated β- lactamases (TEM-1) was described in gram negative bacteria (Datta and Kontomichalou 1995).Genes like bla_{Tem-1},bla_{tem-52},bla_{CMY-2} are known to cause resistance in *E.coli* O157;H7.

1.20 Ampicillin

Ampicillin inhibits the growth of bacteria by interfering in the peptidoglycan layer and kills the dividing cells in the cell wall. The ampicillin resistance genes present on the pGLO plasmid produce β-lactamases, which dissociate the ampicillin molecule. β- lactamases cleaves the β-lactam ring essential for the antibiotic action. β- lactamases also passes through the cellular envelope and disables the ampicillin into the surrounding medium (Idika et al., 2010).

1.21 Trimethoprim sulphamethoxazole

This antibiotic inhibits the enzyme dihydrofolate reductase (DHFR).Various factors like mutation, excessive production of DHFR, presence of gene dfr leads to theresistance.It is also knwn that the resistance of trimethoprim is linked with some other drugs like *Ampicillin* .

1.22 Tetracycline

Tetracycline acts on 30S ribosomal subunit. The bacteria shows resistance to tetracycline by pumping the tetracycline out of bacterial cell and EF-G like protein which protects the ribosome. Some genes like Tet(A), tet(B), tet(C), tet(D), tet(E), tet(Y) and tet(I) helps in antibiotic resistance against tetracycline.There is inhibition of the 30S rRNA of bacteria and blockage of amincyl- tRNA's which subsequently leads to the prevention of protein production.

1.23 Fluoroquinolone

They are used for the treatment of infectious disease which are usually caused by enteric bacteria.The mechanism of bacterial resistance to fluoroquinolone involves the changes in drug target enzymes. There is

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alteration in domains near enzyme active sites .There is inhibition of bacterial enzymes. Point mutations in the fluoroquinoles resistance genes have been detected by using various sequencing of the target genes, sequence specific oligonucleotide probe hybridization, restriction fragment length polymorphism, amplification mutation assay, radio isotopic or non radio isotopic SSCP analysis, mismatch PCR and allele specific PCR (Fluit et al., 2001).

1.24 Efflux protein

They are found in the lipid bilayer with hydrophobic amino acid loops. The efflux genes have 2 domains, α and β which are functional. The efflux protein requires intact membranes to function. The Tet efflux genes code for membrane associated proteins which export tetracycline from the cell. Most of the efflux proteins shows resistance to tetracycline. But mutations in tet (A) or tet (B) led to glycylcycline resistance, which shows that bacterial resistance to this group of drugs may have develop over time and with increased clinical use.

The efflux gene can code for approx 46 kDa membrane-bound efflux proteins.

Group a	Tet (A),(B),(C), (D),(E),(G),(H), (Z).
Group b	Tet (K), Tet (L).
Group c	Otr (B), Tcr (3)
Group d	Tet A (p)
Group e	Tet (V)
Group f	Unnamed determinants.

1.25 Ribosomal protection proteins

They are cytoplasmic proteins which protects ribosome from the action of tetracycline. They have two elongation factors EF-G and EF-TU. This RPP are

present on mobile genetic elements. In the presence of Tet(M) protein aminoacyl tRNA binds to the ribosome which in turn inhibits the translation. The tet(O) and tet (M) can remove tetracycline from ribosome thus removing the inhibitory effect of the drugs.

1.26 Transmission

The transmission of *E.coli* O157; H7 is mainly by water and food. Transmission can also be person to person, from recreational water, animal exposure or laboratory. Cattle were identified as the primary reservoir of *E.coli* strain O157;H7 (Montenegro et al.., 1990).*E.coli* O157;H7 was first reported in contaminated ground beef in 1982. The outbreak due to groundbeef is high during the summer months. This is linked to consumption of undercooked hamburgers. Several outbreaks have been reported due to some dairy products, raw milk and cheese. The outbreak resulting from contaminated water are larger compared to any other outbreaks. The largest *E.coli* O157 outbreak in 1999, US was due to contaminated drinking water.

1.27 Infected hosts.

1. Humans
2. Cattles.

1.28 Pathogenesis

The pathogenicity of *E.coli* O157:H7 involve different complex mechanism. In order to colonize the intestine, it has to overcome the acidic condition. The colonization of the colon and small intestine leads to some attachment-effacement lesions accompanied by bloody diarrhea. Steps involved are colonization of gut, effect of the virulence factors on the host and the disease caused by

- Colonization of gut- Attachment or adherence to the wall of the gut mainly distal small intestine, colon and rectum.
- The peristaltic mechanism does not remove the bacteria and so they colonize and multiply in the gut forming attaching and effacing adherence.

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- The *E. coli* O157:H7 derives its nutrients from the mucosa. The attachment prevents the loss of organisms into the environment.
- The *E. coli* O157:H7 have a 35kb Locus of enterocyte attachment. The Locus of enterocyte effacement has a cluster of genes which encode intimin an outer membrane protein such EspA, EspB,EspD and type 3 secretion pathway. The gene eaeA helps in the attachment and effacement in intestine.

1.29 DNA isolation.

DNA is the extraction of the cells of interest, purification of the cells extract and other insoluble particles. Disruptions of the cell wall are done by some salt or detergents and are centrifuged.Isopropanol or etanol can be used for precipitaton of DNA. The following steps take place in the DNA separation like disruption, lysis, removalof proteins and other contaminants and DNA recovery.

1.30 Agarose gel electrophoresis

Electrophoresis is the process of separating molecules based on the charge and size by applying an electric field. Agarose is extracted from seaweed. It is a polysaccharide. Agarose is used for separating of large molecules especially DNA. Agarose is used to form the electrophoresis gel which act as separating medium. The agarose gel has larger pore size and lower resolving power. Some factors also affect the rate of migration of nucleic acid such as the voltage used, size of DNA, the ionic strerngth of buffer, the dimension of the gel pores and the concentration of dye such as Ethidium Bromide. DNA is negatively charge and so it migrates to the positive (i.e anode). Small molecules migrate faster than the bigger ones as small molecules can move easily through the pores. DNA fragments of length from 50bp to several millions. Bp can be separated by using agarose gel electrophoresis. The DNA exist in 3 forms i.e linear form, open circular and super coiled form. There is a break in one strand of DNA in the open circular form.. There is less friction in the super coiled form and it runs faster in the gel.

1.31 Electrophoresis buffer

Mostly TAE (Tris Acetate EDTA) is used as the buffer in gel electrophoresis. The buffer provides the ionic charge and also maintains the pH.

1.32 Restriction fragment length polymorphism to check the effects of antibiotics (*Ampicillin* and tetracycline) on the genomic DNA of *E.coli* O157; H7

It is a technique that exploits variations in homologous DNA sequence. The DNA are broken into fragments and digested by the restriction enzymes which can recognize the DNA and cut into shorter sequence and then the resulting fragments are analysed using gel electrophoresis. Different fragments are obtained according to recognition sequence. RELP can be used in genome mapping, paternity test, determining disease status of individual, and detection of mutation. The restriction endonucleases type 1 is not specific.Type 2 cuts specific recognition sites. The restriction enzymes used are Ecor RI, HIND III, BamHI and Sma I.

2. SCOPE AND OBJECTIVE

Due to excessive use of antibiotics, the microorganisms are becoming resistant to them due to certain changes in their metabolic process. The greatest known example is resistance of bacteria to 'First generation penicillins'. Just in two years bacteria managed to escape the action of these penicillins. After that came into play 'Second generation penicillins' with modified mode of action. Again bacteria became resistant to these antibiotics. Now we have 'Superbugs'. It is usually believed that resistance to antibiotics is due to plasmids which are responsible for multiple drug resistance. The present study explores the chances of resistance to most common two antibiotics due to mutations in genomic DNA. Till date no work has been done on mutation in genomic DNA of *E. coli* O157:H due to Tetracycline and Ampicillin.

The objectives of the present study are:

1. Isolation of *E. coli* O157:H7 from sewerage water.

2. Isolation of DNA from *E. coli* O157:H7 and *E. coli* O157:H7 grown in antibiotics at sublethal concentrations.

3. Restriction digestion of the DNA to determine the difference in the pattern.

3. REVIEW OF LITERATURE

Preez M. et al 2007 reported that the rural household depend mainly on rivers, spring, boreholes for household water requirement. Their study have revealed that water stored in containers are prone to contamination. This study was done to check the origin of *E.coli*, by assessing the genetic relativeness of *E.coli* which are stored in water and those present in some external environment. MFC agar was used.

Kuzewewaski.A et al(2002) studied the antimicrobial resistance strain in 200 verocytotoxic and 216 non-verocytotoxic *Escherichia coli*. The strains were isolated from different domestic food source. It was found that non-VTEC shows higher resistance level than the VTEC irrespective of the source. The VTEC strains were isolated from sick animals. It also revealed that 6.8% VTEC strains isolated from human origin shows the antibiotic resistance.

J G wells et al a total of 1266 fecal specimens from healthy cattle to check the HUS. This Hemolytic uremic syndrome resulted from consumption of raw milk. The specimens were examined using sorbitol mac conkey agar. The study revealed that 19% of calves and 8% of adult cows shows positive to shiga toxin producing. It shows that diary cattles are reservoir of *E.coli* O157; H7

Wong,c.sJelacis et al (2000) studied the risk of haemolytic uremic syndrome after the treatment of antibiotic of *E.coli* o157;h7 in human. A total of361 *E.coli* isolates were collected and examined and based on the broth dilution results it was found that 61% were susceptible to all the 13 antimicrobials tested. The *E.coli* O157;H7 shows 27% resistance to tetracycline,17% resistance to cephalothin, 26% resistance to sulfamethoxazole and 13 % resistance to ampicillin.

Vincent N chigor et al have studied the multidrug resistance and plasmid pattern of *E.coli* O157 isolated from diarrhoeal stool and surface water. From the study it was found that about 2.2% of *E.coli* O157 was found in surface water and its prevalence rate in children with diarrhaea shows 5.4%. The drug resistance were more in aquatic isolates compared to the clinical isolates. The study shows that 35% of *E.coli* O157 contains plasmids. These *E.coli*

shows resistance to ampicillin, ciprofloxacin,cefuroxime, nalidixic acid, cotrimoxazoles, tetracycline and nitrofurantoin.The plasmid are prevalent in both clinical and the aquatic isolates showing that MDR is plasmid mediated.

Jiyounlim et al Characterization of *Escherichia coli* O157; H7 plasmid; The study shows presecnce of pO157 in *E.coli* O157;H7. It was found that pO157 have similarties with plasmids which are found in enterohemorrhagic *E.coli* serotypes. The growth rate ,surivival in the environment and the resistance pattern of po157 mutant from the wild type was compared.The survival rate of pO157 was higher in the bovine gastrointestinal tracts compared to the wild type.Some proteins such as glutamate decarboxylase isozymes and tryptophanase are involved which increase the survival rate of pO157 mutant.

Nancy d. Hanson et al studied the plasmid mediated AmpC β-lactamases genes using pcr multiplex.The multiplex PCR was used to identify the family specific AmpC β-lactamases in gram negative bacteria. AmpC specific primers was used with 90 bp to 520bp and are run in gel electrophoresis. The ampc differentiates the plasmid mediated ampc families such as klebsiella pneumonia, *E.coli*,and salmonella enteric.

Caprioli. A et al (2004);cattle are main source of human infections with *E,coli* o157;H7.The role of the organism leading to this infection have been studied.It also suggest that there are certain food source which act as a vehicle in transmitting this infections to the host organism.Certain routes of transmission involve are like exposure to the animal, and the environment infected also result in the transmission.

Rice H D (1995);different methods for the identification of *E.coli* from the water samples . In this study different broth, different incubation period, temperature and the immune magnetic separation were analyzed on the artificial and naturally contaminated water. It was seen that increase in the temperature(44.5 degree celcius), incubation period(24 hrs) and the use of tryptic soya broth and the IMS increases the sensitivity of *E.coli* o157 culture from the water samples.

Josefa M Rangel et al studied outbreaks of *E.coli* O157; H7 between 1982-2002 in the united states**;** The outbreaks of *Escherichia coli* is due to the consumption of uncooked beef. This leads to HUS and acute kidney failure. The outbreaks from different sources were identified, mode of transmission like how the patient acquired t he infection were analyzed.There were 17.4% hospitalizations, 4.1% HUS, 0.5% deaths. The outbreaks were reported in Minnesota, Washington, Newyork, California and Oregon. The transmission routes for 14% person to person,52% were food borne,6% recreational water, 3% animal contact and drinking water and 0.3% laboratories.

Journal of animal and veterinary advances 11(1); 52-55, 2012.Yahya kuyucuoglu et al; Studied the antibiotic resistance of *E.coli* O157;H7 *E.coli* commonly present in human and animal intestinal tracts due to faecal contamination (Schroeder et al). Fluoroquinolones resistance is mediated by chromosomes but recent studies shows that such resistance could be carried by resistance plasmid (smith et al,2003).In this study 75 faeces sample were collected. Isolation of *E.coli* was done on mac conkey agar and incubation at 37∘c. Lactose positive colonies were restreaked onto mac conkey agar and incubated. The resulting *E.coli* was identified as oxidase negative, in dole positive,urease negative and hydrogen sulphide negative. The susceptibility testing was done by disk diffusion method on Mueller Hinton agar (NCCLS, 2002). *E.coli* O157;H7 were resistant to the penicillin, ertiyromisin and neomycin (95.6%), tetracycline (73.3%) and ampicillin (60 %).There was more resistance to tetracycline. Tetracycline resistance was highest for *E.coli*i,e 73% and for *E.coli* O157;H7 is 78.5%.The resistance rate of cephalothin was (14.2%) for *E.coli O157;H7* isolated from cattle. *Escherichia.coli* and *Escherichia.coli O157; H7* shows susceptibility to ciprofloxacin and trimethoprim-sulfamethoxazole.

David G.White et al studied the Integron which confers the Antibiotic Resistance among shiga toxin producing *E.coli* isolates:

In this study 50 isolates of stx producing *Escherichia coli* was analyzed to identify Integron and their antibiotic susceptibility. It was found that 78% isolates shows resistance to two or

more antibiotic. The antibiotics include sulfamethoxazole, tetracyclin and streptomycin. Class integrons were also indentified and isolates contain aadA gene which is resistance to spectinomycin and spretomycin . DNA sequence shows that O103;H2 and *E.coli* O157:H7 possessed similar aadA gene. The integrons found in O157:H7 can be transferred through conjugation.

Chopra,I et al(2001);studied the bacterial resistance to tetracycline and the action of tetracycline; All the *E.coli* shows wide range of resistance to tetracycline. This resistance is due to the presence of tetracycline resistance gene and efflux mechanism where the bacterial export the tetracycline out of the cell wall and the presence of ribosomal protection protienthat protects bacterial ribosomes. These genes like tet(A),TET(M) are associated with plasmids and transposons.

R.C. Reuben et al; 420 fermented milk sample were used for the study. Several cultural techniques like Tryptonsoybroth and the selective plating and CT-sorbitol MacConkey agar were used for the study. It shows that 45% were contaminated with *E.coli* O157:H7. 94.7% were resistant to erythromycer, 84.2% to amoxicillin trimethoprim and oxacillin, 42.1% resistant to streptomycin, and 68.4% resistant to chloramophenicol while 78.9% and 89.5% were sensitive to gentamicin and ciprofloxacin.

Daniel A.Tadesse et al studied drug resistance in *E.coli* in humans and animal food in between 1950-2002 to assess the changes of bacterial resistance. 15 antimicrobial drugs were tested. They found a significant increase in the trend of resistance for ampicillin, tetracycline and sulfonamide. This study shows the increased in resistance from 7.2% in 1950s to 63.6% in 2000s. 29.7% resistance to tetracycline and streptomycin and 29% to sulfonamide.

Journal of applied microbiology: Virulence factors of *Escherichia coli* 0157 and other shiga toxin producing *E.coli*.

This review describes the virulence factors of *E.coli* 0157. In this review, the factors like enterohaemolysin, catalase and serine protease (Espp) are described. The enzyme catalase helps the bacteria to recover from stress and escape the host defence.

Daphney P.Shandukani et al studied the induction of Shiga toxins in the isolates collected from groundwater of *E.coli* O157:H7 using two antibiotics ampicillin and tetracycline**.** They found that the isolates treated with ampicillin have more proportion of *E.coli* O157:H7 at 72 hours treatment as compared to the ones treated with tetracycline. After 24 hours incubation, there was no detection of Shiga toxins using EIISA ESSAY. This might be the indication that, more exposure of antibiotics might lead to more resistance of *E.coli*.

T.T.Callaway et al studied the toxin production of *E.coli* O157:H7 under the effects of Sodium chlorate**.** The *E.coli* O157:H7 culture where treated with MIC (Minimal Inhibitory Concentrations) of antibiotics like tylosin, penicillin, ampicillin, ciprofloxacin, tetracycline, ceftiofur, gentamicin novobiocin and monensin. This study shows that the chlorate does not stimulate the toxin production but it kills the *E.coli* O157:H7.

Mashkoor Mohsin et al (2010) studied the release of shiga toxins by the effects of Ampicillin, Cefotoxime and gentamacin from Shiga-toxin producing *Escherichia coli* from Faisalabad, Pakistan. The frequency of STEC was 21.7% (5) of 23 patients. Their study revealed that exposure of MIC of gentamicin, cefotaxime and ampicillin shows a reduced release of toxin as compared to the controlled i.e. without antibiotics. But exposure of sub MIC shows increased release of STX and cytoxicity as compared to gentamicin and cefotaxime.

Farah J.Nassar et al (2013), studied the effects of subinhibitory concentrations of antimicrobial agents on *E.coli* O157:H7**.** The different subinhibitory concentrations like gentamicin, rifampicin, azithromycin and imipenem were studied and compared with Norfloxacin (SOS inducer). This has been done to check the role of the SOS response in STX release. The sub MICs of RIF and IMC increase the STX production.

A.mahanti et al (2013) studied the isolation, antibiotic resistance and molecular characterization of shiga toxin-producing *Escherichia coli* from buffalo in India. This study shows that out of 363 *E.coli* isolates, 6.61% carried at least one gene of STEC. The virulence factor stx2 were found in 83.3% i.e. 23 of isolates and stx1 in 95.8% i.e. 20 of isolates.

Tareq M.Osaili et al studied the prevalence and antimicrobial susceptibility of *E.coli* O157; H7 on beef cattle slaughtered in Amman abattois. This study was done to check the virulence factor and the antimibrobial resistance level. Theier study showed that 50 *E.coli* isolates have eaeA and hlyA while 60%, 22% and 12% shows virulence factor Stx_1, Stx_2 and Stx_1 and stx_2 respectively. The isolates show high resistance to erythromycin, vancomycin and neomycin.

Steen Ethelberg et al studied the virulence factor of HUS. The study shows that out of 343 STEC patients, 6.1% shows HUS symptoms while 36.4% shows bloody diarrhaea. They found the Stx_2 gene for the HUS symptoms while eae gene for the diarrhaea.

Ichiro Tatsono et.,al studied the role of toxB gene on PO157 of *E.coli* O157:H7. The study shows that toxB gene help in the adherence of O157:H7 to the epithelialcells by production of type III proteins like Esp A, Esp B, Esp D, Tir and Intimin.

Tadashi Shimamoto et.,al analyzed the multi drug reisitance in Shiga-toxin producing *E.coli* O157:H7 from meat products. The study showed the genetic level of *E.coli* O157:H7 when exposed to multi drugs and PCR and DNA sequencing was done. Their study shows that 57.4% isolates from meat and dairy products shows resistance to at least 3 different drugs. The highest resistance shown by the *E.coli* O157:H7 is Kanamycin, next Spectinomycin, ampicillin, streptomycin and lastly tetracycline. DNA sequencing shows class 1 and class 2 integrons, β-lactams genes like bla_{Tem-1}, bla_{Tem-52}. Certain quinolones resistance genes like qnrB, qnrS and florfenicol gene floR were also identified

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Anju Thangam et al investigated the effects of ZnO Nanoparticles against the strains of *Escherichia coli*. In this study as a model of the gram-negative bacteria *E.coli* strains were investigated for the antimicrobial effect of zinc oxide nanoparticles. The zinc oxide nanoparticles that were synthesized were characterized by Scanning Electron Microscopy (SEM),Ultraviolet analysis(UV) and X-Ray Diffraction(XRD).These test confirmed the size and surface morphology of ZnO nanoparticles. In the study different concentration of ZnO nanoparticles were used and were performed by well diffusion assays and growth rate studies too understand the antimicrobial effect. Through the results it is understood that the ZnO nanoparticles acts as an effective bactericide agent. The Scanning Electron Microscopy result was used to understand the bactericidal action of ZnO nanoparticles, as the SEM technique provides the morphological features, the effects were easily studied. The *E.coli* that was treated with ZnO nanoparticles showed the formation of pits in the cell wall due to the damages done by nanoparticles. This results in death of the cell wall if such cell morphology is exhibited by the membrane which significantly decreases the permeability.

Maria Braoudaki et.,al compared the resistance level of *E.coli* K 12 and *E.coli* O55 with *E.coli* O157.This study shows that there is more resistance level of antibiotics by *E.coli* O157 as compared to the other two.*E.coli* O157 showed resistance to various antibiotics like chloramphenicol,tetracycline,trimethoprim and amoxicillin while *E.coli* K-12 and *E.coli* O55 showed resistance to chloramphenicol and trimethoprim respectively.The study shows that the level of resistance maybe dependent on the different strains of bacteria instead of the mechanism shown by them.

Dan I. Anderson et al reviewed the microbiological effects of sublethal levels of antibiotics. In this study it is reviewed the widespread use of antibiotics and the concentration gradients of the antibiotics in the humans, livestock and also the environment by their use. Recent studies suggest that in the evolution of the antibiotic resistance by the bacterial entities has been resulted from the frequent exposure of non lethal concentration such as drugs etc. Through this review the antibiotic ecology and their ability at the sub inhibitory concentrations to choose for bacterial resistance has been discussed. The exposure on bacterial physiology by low level drugs and its effects are also discussed in the review.

Roselli et al investigated that Zinc Oxide can protect cultured enterocytes from the damage induced by *Escherichia coli*. From some evidences it can be understood that the Zinc Oxide (ZnO) can be proctective against intestinal diseases. Although it has been reported that ZnO has antibacterial effect, the study and the mechanism of its protective nature is not much elucidated. Through this study the protection of intestinal cells from *E.coli* (ETEC,Strain K88) with the help of ZnO. It was understood that on treating with ZnO under controlled concentrations the cell permeability remain unaffected and also the transfer of 14(C)-insulin increased as compared to untreated cells. Treatment with ZnO also reduced the bacterial adhesion and helped in blocking bacterial invasion. ZnO showed protective effects due to the viability of ETEC grown under a medium which contained ZnO was unaffected and not due to any antibacterial activity. By preventing the adhesion and internalization of bacteria the ZnO can be used to protect intestinal cells from ETEC infection.

Katja Hille et.,al studied the cefotaxime-resistant *E.coli* in diary and beef cattle farms. This study was done to check the cefotaxime resistant in *E.coli*.70% of beef cattle and 85% of dairy cattle in each of which at least one sample of cefotaxime-resistant *E.coli* were isolated. Their result shows that 35% of beef cattle and 45% of dairy cattle shows resistant to cefotaxime. Hygeine condition can also cause a change in the resistance level.

Eduardo L.Lopez et.,al studied the infection caused by shiga like toxin in children associated with haemolytic uremic syndrome. 51 children with HUS symptoms were studied to check the rate of infection caused by shiga like toxins. 39% shows free toxin,antisera neutralization done and found 6 with shiga like toxin I and 10 with shiga like toxin II.

Heramb M Kulkarni et al studied about the protective role of *E.coli* membrane vesicles against antibiotics. Since the outer membranes (OMVs) of bacteria are defensive as well as protective in nature and thus take part in community related functions. Through this study the producer bacterium are shown to be protected by the OMVs from growth inhibitory effects of some antibiotics. The isolated OMVs protected the bacteria from colistin,melittin which are

the membrane active antibiotics. The OMVs from the *E.coli* MG1665 were seen to be effective in protecting the bacteria from P.aeruginosa and A.radioresistens respectively.Thus OMVs were seen to protect the bacteria from membrane active antibiotics and not any other antibiotics. It was also found out that the protective property of OMVs from *E.coli* against antibiotics were also situation dependent since the mechanism is different for different situations. The study suggests that the bacterial community gets a common defense from the OMVs against specific antibiotics.

Yahya kuyucuoglu et al, 2012 Antibacterial resistance of commensal *E.coli* and *E.coli* O157; H7 strains isolated from cattle and calves faeces samples. Journal of animal and veterinary advances 11(1); 52-55. *E.coli* and *E.coli* O157; H7 were isolated to check the resistance level to antibiotics. The study shows that all *E.coli* isolates were resistant mostly to penicillin while *E.coli* O157; H7 shows resistant to neomycin, penicillin and ertiyromisin. The level of ampicillin resistant was high in both the isolates and the least resistance were shown by ciprofloxacin and cefoxitim at 7.2%.

4. MATERIALS AND METHODS

Materials and equipments

1. Sorbitol MacConkey agar
2. MacConkey agar, MacConkey broth
3. Antibiotics (tetracycline, ampicillin)
4. Sewage water
5. Agar
6. Autoclave
7. UV laminar airflow
8. Petriplates
9. Agarose
10. Ethidium bromide
11. 500bp DNA ladder
12. TAE buffer
13. SDS

4.1 Preparation of culture media (MacConkey agar)

Weigh 3.45gm of macConkey agar in 100ml of H_2O in 250ml conical flask. Then 1gm of agar was added. The solution was heated for few minutes to completely dissolve. Then it was autoclaved at 121lbs for 30mins. The solution was cooled and then poured in 5 autoclaved petriplates at 20mm thickness and let it solidify. Then spreading was done with the sewage water inside the UV laminar air flow. Then the petriplates were sealed using parafilm wax and then incubation was done at 37°C for 24hrs. The growth of *E.coli* was observed.

4.2. Preparation of sorbitol macConkey agar

5gm of sorbitol macConkey agar was taken in 200ml of H_2O. Then add 1gm agar was added. The solution was heated for few minutes to dissolve completely. It was then sterilized in autoclave at 121lbs for 30minutes. The solution was cooled and then it was poured in 8 sterilized petriplates inside the UV laminar air flow. Then after the

solidification, with the help of micropipette, sewage water was added and spreading was done. Seal it and incubate at 37°C for 24hrs.

4.3. Streaking

Streaking is for selective medium. With the help of sterile inoculating loop, a loopful of culture was taken and streaked on sorbitol macConkey agar plates. It was then sealed with parafilm wax and incubated at 37°C for 24hrs.

4.4. Preparation of macConkey broth

1.3gm of macConkey broth was taken in conical flask (50ml). Then 50ml of dH$_2$O was added in both the flask. The media was autoclaved at 121lbs for 30mins. It was then cooled. After cooling, 2 antibiotics (tetracycline and amphicilin were taken and filtered) and added in 15µl in each of the two conical flask and 100µl in the other two conical flask. Then with the inoculating loop inoculate the colonies from the cultured plates into the broth. Then the flask were plugged with cotton and allowed to incubate at 37°C.

4.5 Bacterial growth and DNA isolation

E.coli was cultivated in macconkey broth at 37°C. 2ml of culture was taken in the eppendorfs. The bacterial cells were pelleted by centrifugation at 7000rpm for 10 minutes and then 800µl of saline EDTA (0.15m NaCl, 0.5m EDTA pH 8.0) and 50µl of lysozyme (10mg/1ml) were added in each eppendorf and incubated again at 60°C for 15 minutes. Equal value of PCI was added and centrifuged at 8000rpm for 10 minutes. The supernatant was transferred to new eppendorfs and equal volume of isopropanol was added and mixed thoroughly and left at 4°C for 30 minutes to 40 minutes. It was then centrifuged at 12000rpm for 15 minutes. The supernatant was discarded and the extract was precipitated with 400µl of 70% ethanol and centrifuged at 12000rpm for 5 minutes. Then ethanol was discarded, air dried and the pellet was dissolved in 10µl of TE buffer (10mM Tris, 1mM EDTA).

4.6 Preparation of Agarose gel

0.4gm of agarose was dissolved in 50:1x of distilled water and TAE buffer. The solution was then heated to dissolve it completely. Then it was cooled and 0.3µl of Etidium bromide was added to the agarose solution before pouring the agarose into the gel tray which is sealed at both the ends. Now, wells were made with the help of the combs. After the gel solidifies, the gel comb is slowly removed and the seal of gel tray is removed. The gel is then placed in the TAE buffer.

4.7 Running a gel

5µl of distilled water,5µl of loading dye and 2µl of DNA samples were loaded in each wells and the power was turned on to run the gel with the voltage set at 50volt for 3 hours. The running of gel can be confirmed by checking the bubbles from the electrodes.

4.8 Visualization

Ethidium bromide is used as a dye that binds to DNA and fluoresces under UV light.

4.9 Restriction Digestion

For genomic DNA 16µl of distilled water, 2µl of buffer,2µl of DNA and 1µl of EcoR1 enzyme were taken in two eppendorfs and incubated at 37°C for 2hrs and 15 minutes respectively. For the isolates treated with ampicillin and tetracycline 16µl of distilled water, 2µl of buffer, 2µl of DNA and 0.5µl of EcoR1 enzyme were taken and incubated for 15 minutes and 30 minutes respectively.

4.10 Gel Electrophoresis

The samples were taken and loaded in each of the wells. First well loaded with 500bp of DNA ladder in 5µl and 2 µl dye and in subsequent wells 10 µl of sample containing Ampicillin and 3µl of loading dye followed by tetracycline and controlled which were incubated for 30 minutes and 2hrs of genomic DNA and in 3 wells, the samples kept for 15 minutes were loaded and perform agarose gel electrophoresis at 50 volts.

5. RESULT AND DISCUSSION

After treating with antibiotics tetracycline and ampicillin in amount of 50µl there was turbidness in the broths containing the culture but the broth culture with 100µl of antibiotics shows little or no turbidness. There might be a possibility that the resistance level is lower when there is increased in the concentration of antibiotics. This show that the *E.coli* is able to resist the antibiotics and grow well.

The pinkish colour shows the presence of lactose fermenting bacteria. The whitish or colourless colonies are those which does not ferment lactose. The gram negative bacteria which are unable to ferment lactose usually grow in colourless colonies.
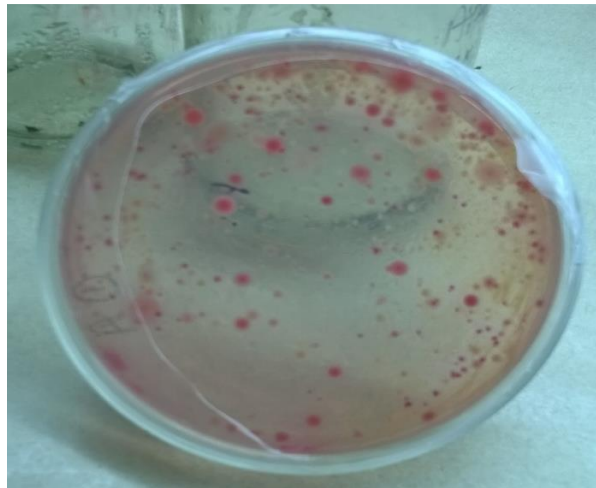

Fig1: Growth of *E.coli* on mac conkey agar

Fig2: Selective growth of *E.coli* on sorbitol mac conkey agar.

Fig3: Streaking on sorbitol mac conkey agar.

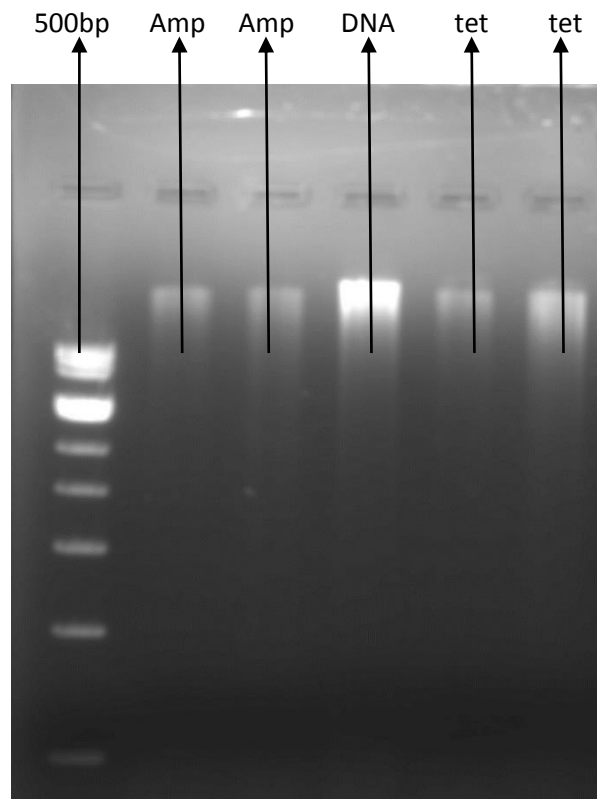*Fig4: E.coli* isolates treated with antibiotics in MacConkey broth

Fig5: Restriction digestion with EcoRI enzyme

Lane I- 500bp DNA ladder

Lane II – Genomic DNA treated with Ampicillin at 50µg/ml

Lane III- Genomic DNA treated with Ampicillin at 50µg/ml

Lane IV- Genomic DNA controlled

Lane V- Genomic DNA treated with Tetracycline at 50µg/ml

Lane VI- Genomic DNA treated with Tetracycline at 50µg/ml

Restriction digestion of *E.coli* O157:H7 genome was done by using EcoRI enzyme. In this we found high intensity of shearing in lane IV i,e genomic DNA contolled.(without antibiotics).This might be possibly due to mutation. And compared to the DNA treated with Ampicillin and tetracycline we could see that after the restriction digestion more of ampicllin treated DNA is digested. The mutations might have arised due to the stress inorder to resist the antibiotics.

E.coli is a causative agent of diarrhoea. The *Escherichia coli* outbreak is mostly due to the consumption of uncooked beef, unpasteurized milk and recreational water etc. The STEC cause a life threatening Haemolytic Uremic Syndrome (HUS) in both elderly and children.

In this study sewerage water was collected and this was analysed for the presence of *E.coli* O157:H7 strains. The result gives the resistance level shown by *E.coli* O157; H7 when treated with ampicillin and tetracycline. The level of exposure and variation between regions may also lead to different resistant profiles. The isolates may also find their way through the water bodies and grazing animal (Kidd et al 2002; Dancer 2009). The widespread and frequent use of antibiotics may also lead to the increased resistance. There is an increase in inducing the shiga toxins in the broth culture when treated with ampicillin and tetracycline. This shows that ampicillin is a potential inducer of shiga toxins in broth cultures of *E.coli* O157:H7 (Daphney P et al). The stress response gene RpOS also might have a link with the bacterial evolution and its pathogenecity in the environment. The antimicrobial agent like ampicillin might also have contributed to the resistance of trimethoprim – sulfamethoxazole. Trimethoprim treatment shows longer duration of diarrhea and there are high chances of haemolytic uremic syndrome development. Some major causes of antibiotic resistance are due to excessive use of antibiotics and the transfer of the resistance gene from the resistant donor to susceptible host. Some antimicrobial agents are also administered in the water to prevent disease. The unabsorbed antibiotics can enter the environment via sewage. The bacteria in the environment are subjected to various effects from fungi, plants and even some bacteria which produce antimicrobial substance in order to survive. Integrons are also responsible for the transfer of resistance genes in gram negative bacteria.(Rowe-magnus and mazel,2002).The gene responsible for the resistance to florfenicol is floR resistance gene.(Bischoff et al.,2002). (McKeon et al., 1995) observed an 87% resistance of at least one

antibiotic isolated from groundwater while 80% of resistance strains were seen from river, estuarine and municipal waste. Ground water contamination is also due to run off water from the cattle farms.

The main reservoirs of STEC are the sheep and the cattle..Barlow and Mellor reported the presence of STEC in fecal samples of cattle from Australia. To increase the food productivity there is also increased use of antimicrobial agents like tetracycline which is commonly used as the first line of defence. The genes like tetA are responsible for the tetracycline resistance and Str A genes for the streptomycin. Benson C. Iweriebor et.,al studied the tet determinants. The study shows similarities with the coliforms of humans and animals having the tet (A) efflux gene. Integrons are also responsible for the induction of resistance genes in gram negative bacteria. (Rowe-magnus and Mazel, 2002).

In the study conducted by Yahya Kuyu Cuoglu et al 2012 from cattle and calves faeces samples, the resistant level of *E.coli* and *E.coli* O157:H7 were same. But the resistant level of ampicillin was high in *E.coli* only. The study by Ajayi et al 2011 shows that the *E.coli* from cattle has high resistances to antibiotics. Tetracycline resistance shown by the isolates are high as these tetracyclines are used as a common feed supplement for young animals. Our finding shows more resistance to ampicillin.

6. CONCLUSION

The current study reports that antibiotic resistance is not only due to plasmids but also due to mutations in genomic DNA of *E. coli* O157:H7. Bacteria undergo certain mutations in their genomic DNA also that make them resistant to a particular antibiotic. This mutated DNA is passed to future generations also hence making them resistant too. So, the current study concludes the fact that not only plasmids but also genomic DNA is responsible for resistance. If the particular probes are designed and further sequencing of these mutated DNA can tell us where the exact mutations are present. Hence, giving us the knowledge of changes that occur at certain specific sites or they are random.

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