WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES

SJIF Impact Factor 7.421

Volume 7, Issue 12, 1077-1083 Research Article ISSN 2278 – 4357

IDENTIFICATION OF MULTI DRUG RESISTANT GENE IN *E.COLI*

P. Vigneshwaran*[1], R. Abishek[1], N. Saravanan[2] and N. G. Ramesh Babu[3]

[1,2,3]Department of Biotechnology, Adhiyamaan College of Engineering, Hosur-635130, India.

Article Received on
21 October 2018,

Revised on 10 Nov. 2018,
Accepted on 30 Nov. 2018

DOI: 10.20959/wjpps201812-12576

***Corresponding Author**

P. Vigneshwaran

Department of Biotechnology, Adhiyamaan College of Engineering, Hosur-635130, India.

ABSTRACT

This study based up on the isolation of multi drug resistant gene from *E.coli*. The sample is obtained from the urine of goat and that is cultured by standard quantitative method. From 24 hrs old culture the DNA were separated and PCR is done using that DNA sample. The PCR amplified product is subjected to the RFLP (Restriction Fragmentation Length Polymorphism) for the deduction of desired gene (β lactamase). To confirm that multi drug sensitivity test also has been performed.

KEYWORDS: Multi drug resistance, β-lactamase, PCR, RFLP.

INTRODUCTION

Among livestock population goats are known as poor person's bank or poor family's insurance policy and distributed mainly with landless and marginal farmers, but now-a-days the commercial goat farming is also gaining much importance. The major loss faced by the goat farmers is in terms of mortality and morbidity of young animals and is mainly due to diarrhoea which is a complex interaction of etiological, immunological, and manage mental factors. Escherichia coli play an important role in causing diarrhoea and other infectious diseases in goats. Pathogenicity of E. coli strains are due to the presence of one or more virulence factors including invasiveness factors like invasins, heat labile, heat stable enterotoxins, verotoxins and colonization factors or adhesins. In case of diarrhea in goat or other animals, the precise role of E. coli is not clear because this organism is part of normal intestinal flora in both healthy and diseased animals. It can be controlled following the maintenance of strict hygienic and sanitary measures in addition to antibiotic therapy.

Theodor Escherichia was first described as *E.coli* in 1885, which was isolated from the faces of new born, known as *Bacterium Coli Commune*. Later, it was renamed as *Escherichia Coli*.

Urinary Tract Infection (UTI) is one of the common and also life threatening infection in community practice. UTI may associate with both upper tract. Lower tract UTI describes as cystitis. Mostly UTI occurs as community acquired infection and is often treated with broad spectrum of antibiotics.[1] Microorganism can be considered as multi drug resistant (MDR) only when it is resistant to three anti-biotics for minimum.[2] In certain case *E.coli* is considered as a major cause of UTI, this study presents, a study to identify multi- drug resistant pattern of those *E.coli* strains.[1] For human and animals, usually *E.coli* is commensal bacterium. It is used an sentinel for drug resistance in fecal bacteria. For past few decades witnessed the increase in spread of multi drug resistant bacteria and increasing resistance to newer compounds.[3]

A study on multi drug resistant *E.coli* which is isolated from urine sample suggests that it isolates 271 originates from Bangladesh. The strain was reinforced that no other similar multi resistant *E.coli* strain had been isolated before.[4] A report states Nosocomial infections in dogs were isolated from genetic diversity in *E.coli*. Among 34 *E.coli* isolated from the type by PFGE, 9 distinct DNA patterns were observed while the 7 could not be typed by PFGE.[5] *E.coli* and Shigella are responsible for substantial proportion of acute diarrheal diseases worldwide which causes Bacillary dysentery. Diarrhea that are caused by multidrug resistant bacteria are the main problem among children and adult in the developing countries, and it represents a research priority of Diarrheal Disease Control Programme of the World Health Organization.[6]

Infections of the central nervous and circulatory system as well as urinary, respiratory and reproductive tracts are caused by the Extraintestinal Pathogenic Escherichia coli (ExPEC). ExPEC isolates virulence factor for invasion and colonization of extraintestinal site and typically belongs to *E.coli* pathogenic group.[7]

Work description

This study is based up on the identification of multidrug resistance gene from the *E.coli* by using PCR, RFLP.

MATERIALS AND METHODS

Source of inoculum

Samples of urine were routinely collected (clean-catch method) and cultured by standard quantitative methods at the beginning of the study (PPS). When symptoms of UTI in any

enrolled resident occurred throughout the study (continuing study), urine samples were also taken. Isolates were identified using polymerase chain reaction (PCR) with species-specific primers or phenotypic methods (API ID 32E, BioMerieux). Strains belonging to the Enterobacteriaceae family were further tested.

Multi drug resistant analysis

The mueller hinton agar was prepared and fresh *E.coli* culture was swag in that medium. Then the antimicrobial disk was placed .The culture was incubated for 24 hrs in the incubator. Then the antimicrobial sensitivity was measured.

DNA isolation

The 2ml of fresh culture of *E.coli* was collected. Centrifuge at 5 min for 5000 rpm and the pellet were collected and 20µl of lysozyme and 500µl of 1X TE buffer were added. Incubate at 37ºc and 150µl of 10% SDS is added. Incubate at 65ºc for 15 to 30 min. Phenol, chloroform, iso amyl alcohol in the ratio (25:24:1) were added and Centrifuge at 10 min for 10000 rpm. The supernatant were collected and 0.2 volume of 3 molarities of sodium acetate and 5 volume of isopropanol is added. Centrifuge at 1 min for 10000 rpm. Pellet were collected and add 500µl of 100% ethanol. Centrifuge at 5 min for 5000 rpm. Pellet were collected.500µl of 70% ethanol were added and Centrifuge at 5 min for 5000 rpm. Pellet were collected and air derided, after air dry, 30µl of 1xTE buffer were added.

PCR analysis

After the collection of DNA from the required *E.coli* sample then add PCR reaction mixture were added. That PCR mixture contain DNA 2µl and PCR master mix 8µl, the primer R and primer F each of 1µl respectively and water 6µl were added. Then the reaction is completed by using PCR (polymerised chain reaction) machine. After some time the agrarose gel was prepared and started the electrophoresis by using the PCR amplified product.

RFLP analysis

The RFLP analysis is used to identify the desired gene sequence in the DNA sequence. initially 2µl of DNA sample and restriction enzyme mixture of 1µl, this restriction enzyme mixture contain (HIND Ш, SAMU 3A, E.CORI R1), 10XTE buffer 2µl, blue dye of 5µl were added. Incubate that mixture at 37ºc for 3-4hr. then the agarose gel electrophoresis was performed.

Remedial measured

Nutrient agar for 30 ml were prepared and poured on the petriplate and allow it for certain time to solidification. then the *E.coli* culture was stroke and then make three wheel in each plate were makes for the addition of antimicrobial drug. 10µl of curkumin, ampicylin, gold nanoparticle, silver nanoparticles, ginger extract, neem extract were added in each well respectively. then incubate for 24 hr in the incubator.

RESULT AND DISCUSSION

Multi drug sensitivity test

From this test we are conclude that this *E.coli* was resistant to all the antibacterial drugs.

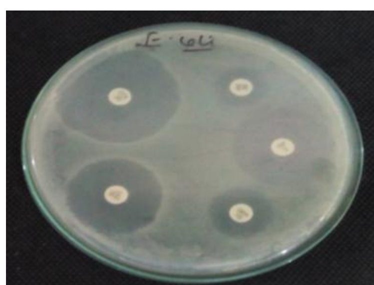


Figure1(A).

Figure1(B).

Table 1: The resistivity of the bacterial cell was shown in the table below.

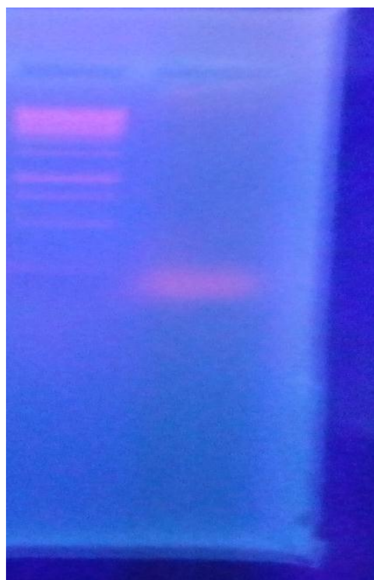
ANTIBACTERIA	ZONE FORMED(mm)	SENCITIVITY
Ceftazidime	15	Resistant
Amikacin	5	Resistant
Ciprofloxacin	13	Resistant
Ceftazidime	11	Resistant
Gentamicin	6	Resistant
Levofloxacin	10	Resistant
Erythromycin	9	Resistant
Chloramphenicol	5	Resistant
Ampicillin	2	Resistant
Cefixime	0	Resistant

- **DNA isolation**

Lane1=marker: lane2=sample.

This indicate that the isolation part is a DNA.

- **PCR analysis**

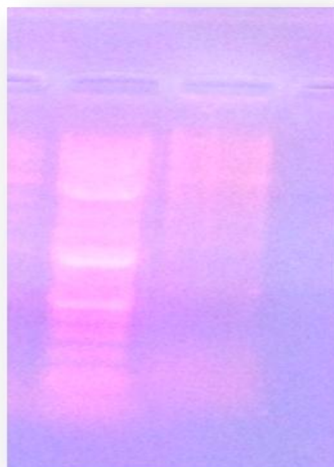


lane1 –matker lane 2- PCR amplified product:

From the above analysis it is confirmed that the required product is get amplified.

- **RFLP analysis**

Lane :1=marker Lane :2=sample



From this RFLP we are confirm that the required gene (β latamace).

- **Remidial Measure**

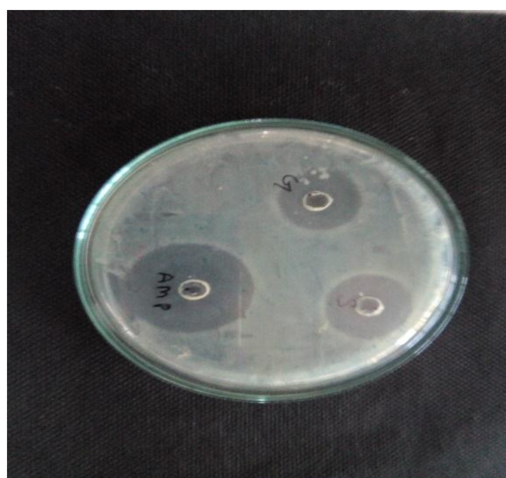


Figure 2 (A).

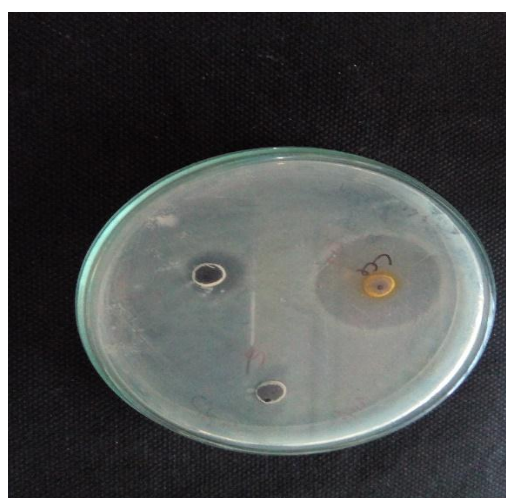


Figure2(B).

From the above experiment its seen that the the *E.coli* is highly killed for the copper nanoparticle.

DISCUSSION

From the above experiment we concluded that the multidrug resistant gene (MDR) was identified as the β lactamase. If the MDR is isolated and incorporated to the other useful organisms to make that organism Multi Drug resistant .This is done by using CRISPR.

REFERENCES

1. Sandeep Kumar Dash, Subhankari Prasad Chakraborty, Debasis Mandal and Somenath Roy. Isolation and Characterization of Multi Drug Resistant Uropathogenic Escherichia Coli from Urine Sample of Urinary Tract Infected Patients. Int. Life. Sci. Pharma. Res., 2012; 2(1): 29-39.
2. Santo E, Salvador MM and Marin JM. Multidrug-resistant Urinary Tract Isolates of Escherichia Coli from Ribeirao Preto, Sao Paulo, Brazil. The Brazilian Journal of Infectious Diseases., 2007; 11: 575-578.
3. Daniel A. Tadesse, Shaohua Zhao, Emily Tong, Sherry Ayers, Aparna Singh, Mary J. Bartholomew and Patrick F. McDermott. Antimicrobial Drug Resistance in Escherichia Coli from Human and Food Animals. www.cdc.gov/eid, 2012; 18(5): 741-749.
4. Laurent Poirel, Emilie Lagrutta, Peter Taylor, Jeanette Pham and Patrice Nordmann. Emergency of metallo-β- Lactamase NDM-1- Producing Multidrug Resistant Escherichia Coli in Australia. Antimicrobial Agents and Chemotherapy, 2010; 54(11): 4914-4916.
5. Susan Sanchez, M. A. McCrackin Stevenson, Charlene R. Hudson, Maria Maier, Tameka Buffington, Quyen Dam and John J. Maurer. Characterization of Multidrug-resistant Escherichia Coli Isolates Associated with Nosocomial Infections in Dogs. Journal of Clinical Microbiology, 2002; 40(10): 3586-3595.
6. Ashraf M. Ahmed, Shin-ichi Miyoshi, Sumio Shinoda and Tadashi Shimamoto. Molecular Characterization of Multidrug-resistant Strain of Enteroinvasive Escherichia Coli O164 isolated in Japan. J. Med. Microbio., 2005; 54: 273-278.
7. Justine S. Gibson, Rowland N. Cobbold and Darren J. Trott. Characteristics of Multidrug-resistant Escherichia Coli Isolated from Extraintestinal Clinical Infections in Animals. J. Med. Microbio., 2010; 59: 592-598.