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**Original Research Article****DOI: 10.26479/2020.0603.08****ISOLATION AND MOLECULAR TYPING OF *ESCHERICHIA COLI* (*E. COLI*)  
SPECIES FROM VARIOUS SORTS OF WASTE WATER SOURCES****Ingilala Satheesh Kumar\*, Konatham Hanumohan Reddy, Chintagunta Anjani Devi,****Nune Satya Sampath Kumar, Dirisala Vijaya Ramu****Department of Biotechnology Vignan's University, Vadlamudi, Guntur,  
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**ABSTRACT:** In this present study RFLP (Restriction Fragment Length Polymorphism) as well as RAPD (Random Amplification of Polymorphic DNA) has been described as a powerful tools for molecular typing methods of microorganisms. Isolated bacterial species (*Escherichiasp*) were Biochemically characterized by Indole, Methyl red, and Citrate and Catalase tests. Genomic DNA was isolated and purified by enzymatic digestion methods for polymorphic studies by RAPD-PCR and isolation of plasmid DNA was done by using alkaline-Lysis method for RFLP examination. Both Genomic DNA and Plasmid DNA were separated by utilizing 1% Agarose gels and Measured by utilizing UV Visible Spectrophotometer at OD260 nm/OD280nm.

**Keywords:** Bacterial isolates, RAPD-PCR, RFLP, UV-Visible Spectrophotometer.

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**1. INTRODUCTION**

*Escherichia coli* (*E.coli*) is the most abundant gram-negative, facultative anaerobic, rod shaped bacterium that is ordinarily found in the lower digestive system of warm-blooded creatures. Majority of the *E. coli* strains are harmless, however a few, for example, serotype O157:H7, can cause serious food contamination in people, and are once in a while answerable for expensive item reviews [1]. *E. coli* are not constantly limited to the digestive tract, and their capacity to get by for brief periods outside the body makes them a perfect marker creature to test ecological examples for defilement.

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The microbes can likewise be developed effectively and its hereditary qualities are nearly straightforward and effortlessly controlled, making it extraordinary compared to other examined prokaryotic model living beings, and a significant species in biotechnology. *E. coli* was found by German bacteriologist Theodor Escherichia in 1885, and is presently delegated piece of the Enterobacteriaceae group of gamma-Proteobacteria [2]. *E. coli* is the most incessant urinary pathogen secluded from 50-90 % of every single uncomplicated disease. *E. coli* is the predominant pathogen. *E. coli*, the most widely recognized individual from the family Enterobacteriaceae represents 75-90 % of all diseases Distinguishing proof of *E. coli* strains necessitates that these life forms be separated from nonpathogenic individuals from the typical vegetation. The distinguishing proof of nonpathogenic individuals additionally needs to identify factors those decide harmfulness of this creature. Antimicrobial obstruction has become a significant issue around the world. Bacterial protection from antimicrobial operators has been developing and quickly dispersing among numerous nosocomial and network obtained pathogens. These living beings have wide assortment of anti-microbial affectability designs. The improvement of anti-infection obstruction in *E. coli* has significant clinical ramifications. The improvement of protection from more seasoned operators, for example, Ampicillin, gentamycin and ciprofloxacin opposition, may significantly constrain our anti-infection decisions. Anti-infection agents which might be utilized to treat *E. coli* disease incorporate amoxicillin just as other semi-engineered penicillin's, numerous cephalosporins, carbapenems, aztreonam, trimethoprim-sulfamethoxazole, ciprofloxacin, nitrofurantoin and the amino glycosides [3]. *E. coli* frequently conveys multidrug safe plasmids and under pressure promptly moves those plasmids to different species. For sure, *E. coli* is a regular individual from biofilms, where numerous types of microscopic organisms exist in closeness to one another. This blending of species permits *E. coli* strains that are pleased to acknowledge and move plasmids from and to other microscopic organisms. In this way *E. coli* and the other enterobacteria are significant repositories of transferable anti-infection obstruction. Protection from beta-lactum anti-infection agents has become a specific issue in late decades, as strains of microscopic organisms that produce expanded range beta-lactamase have gotten increasingly normal. These beta-lactamase chemicals make many, if not all, of the penicillin's and cephalosporins insufficient as treatment. Broadened range beta-lactamase creating *E. coli* are exceptionally impervious to a variety of anti-toxins and diseases by these strains [4]. As a wellspring of creature protein, goat meat has for since a long time ago involved an uncommon spot in the eating regimen for an assortment of reasons including taste inclination, eminence, religion, custom and accessibility, in practically all the networks of the nation with the healthful perspectives being incorporated all the more as of late. Meat was the principal significant nourishment that got together the craving of old individuals living in cavern [5]. It assumes an exceptionally fundamental job in keeping the human body solid so as to give vitality, wellbeing and power [6]. Be that as it may, microorganisms present in meat might be unsafe for human and may

cause deterioration and might be utilized as marker living beings. Numerous analysts have disconnected and distinguished heterogeneous kinds of micro flora from new meat. The molecular characterization of microorganisms is frequently utilized by physicians, microbiologists, and disease transmission specialists to give proof of hereditary relatedness as a guide in the epidemiological examination of irresistible illnesses. Deciding the relatedness of creatures may emerge during an episode examination where a group of contaminations brought about by living beings of similar species indicating comparative antimicrobial opposition profiles and so as to decide clonal spread inside a microenvironment and to decide the wellspring of disease. The application of molecular examinations such as cell protein analysis and plasmid analysis to investigation of infectious disease outbreaks has resulted with the provider of many useful markers that recognize the epidemic clone of a pathogen and helped the distinguishing proof of specific vehicles illness.

## **2. MATERIALS AND METHODS**

### **Sugar plant water**

The short answer is truly, sugar helps plants develop. Be that as it may, unreasonable measures of sucrose can be hurtful to a plant. For plants developing hydroponically or in a specific medium, for example, in a Petri dish, sucrose is regularly utilized as a carbon hotspot for growing plants. Plants make sugars through photosynthesis by consolidating water and carbon dioxide. Plants use carbon dioxide as their principle carbon source, so they needn't bother with sugar in their substrate to develop. However, youthful plants and tissue plant clones that aren't yet proficiently creating sugars through photosynthesis can profit by the additional carbon put away in sucrose. Sugar water utilized in a plant's common habitat can likewise draw in different life forms and microbes.

### **Pond water**

Lake is an assortment of standing water, either regular or man-made, that is generally littler than a lake. A wide assortment of man-made waterways are named lakes, including water gardens intended for tasteful ornamentation, fish lakes intended for business fish rearing, and sun based lakes intended to store warm energy. Ponds and lakes are recognized from streams by means of ebb and flow speed. While ebbs and flows in streams are effectively watched, lakes and lakes have thermally driven micro currents and moderate breeze driven ebbs and flows. These highlights recognize a lake from numerous other sea-going territory highlights, for example, stream pools and tide pools.

### **Sewage water**

Recycled water, in some cases called reused water, is previous wastewater (sewage) that has been blessed to receive expel solids and certain polluting influences, and afterward permitted to revive the spring as opposed to being released to surface water. This energizing is frequently done by utilizing the treated wastewater for water system. In many areas, it is just expected to be utilized for no portable utilizations, for example, water system, dust control, and fire concealment, and there is debate about conceivable wellbeing and natural impacts for those employments. In certain areas

(not in the US), it is given further developed treatment and is utilized by implication for drinking. Testing for pathogens utilizing Polymer Chain Response (PCR) rather than more established refined methods and changing the undermined fecal coli form "marker living being" standard would be upgrades. In a huge report treatment plants indicated that they could essentially decrease the quantities of parasites in profluent, just by modifying the present utilized procedure. In any case, in any event, utilizing the best of flow innovation, danger of spreading drug obstruction in the earth through wastewater gushing, would remain. Seawater is denser than freshwater (which arrives at a most extreme thickness of 1.000 g/ml at a temperature of 4 °C (39 °F)) in view of the salts' additional mass.

### **Sample collection and transportation**

A complete number of water tests were gathered similarly from various beginning. Tests were gathered aseptically in sterile holders and brought to the research center inside 30-45 minutes utilizing fridge. After assortment, bacteriological examinations of the examples were performed to survey the chose microbial qualities.

### **Isolation of bacteria from the water samples by serial dilution technique**

This is the common technique for collecting the pure culture of Microorganism and these microorganisms are successfully cultivated only in liquid media. Now we are going to isolate the pure form of microorganisms by using series of dilutions. At first, the inoculum is subjected for dilution in a sterile liquid medium as well as a large number of tubes of sterile liquid medium are inoculated with aliquots of each successive dilution. The goal of this dilution is series of inoculated tubes with microbial suspension, so dilute that some tubes showing growth of individual microbes only. Take out 1 ml of medium to this add 9 ml of fresh sterile liquid medium, at that point 100 microorganisms in 10 ml (or) 10 microorganisms /ml, add 1 ml of this suspension to another 9 ml of fresh liquid medium. Now each ml contains single microbes. If this tube shows any microbial growth, there is a very high probability this growth has resulted from the introduction of the single microorganism in the medium and it represents the pure culture of microorganism.

### **Laboratory diagnosis**

Under the microscopic observation all water samples contains the rod shapes (gram negative) with no particular cell arrangement. These microorganisms are grown on EMB agar followed by the formation of colonies. *E.coli* as its indole positive (red ring) and methyl red positive (splendid red), and citrate negative (no change-green shading).by using Gram's Method we detected this microorganism belongs to gram –negative.

### **Confirmatory test for *E.coli* species**

To confirm *Escherichia coli* sp., morphological characterization using Gram's staining and biochemical tests like Indole, methyl red, Catalase and citrate test were conducted according to the standard microbiological techniques.

### DNA isolation and purification

DNA is a nucleic corrosive that contains the hereditary guidelines utilized in the advancement and working of all known living beings. The fundamental job of RNA particles is the long haul stockpiling of data. DNA is frequently contrasted with a lot of plans, since it contains the guidelines expected to build different parts of cells, for example, proteins and RNA particles. The DNA was disconnected furthermore, subjective and quantitative investigation was finished by Spectrophotometer. The accompanying outcomes were delineated in the accompanying table acquired.

### 3. RESULTS AND DISCUSSION

In the present study, growth of *Escherichia coli* was observed in all the collected water samples of Andhra Pradesh, to identify the microbe loopful of the broth culture was streaked on to the EMB agar medium and incubated 37c for 24 h and observed the growth of single colonies. As shown in the Fig. No 1 and Fig. No 2. to identify the morphological characterization using Gram's staining and biochemical tests were carried out following standard microbiological technique. Gram's staining indicated that microbe is gram negative and it is present in rod like structures (Fig.No. 2). For further identification biochemical tests like indole, methyl red, citrate and Catalase tests were conducted. The results are represented in Table No.1 and Fig No. 3, 4, 5, 6 and 7. It was observed that indole, methyl red and Catalase tests are positive but citrate test is negative for all samples. The extraction of plasmid DNA as well as genomic DNA (Fig. No 8,9,10 and 11) was carried out on 1% Agarose gel followed by purity checked through qualitative and quantitative analysis at OD 260/280nm (Table 2 and 3). Genomic DNA was isolated and purified by enzymatic digestion methods for polymorphic studies by RAPD-PCR and isolation of plasmid DNA was done by using alkaline-Lysis method for RFLP examination.

**Table No.1: Biochemical characterization of bacterial isolates**

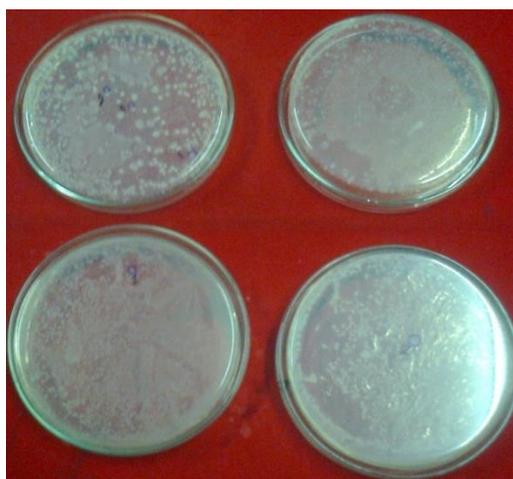
Gram staining	Indole test	Methyl red test	Catalase test	Citrate test	Water sample
-ve rod	+ve	+ve	+ve	-ve	Sewage water
-ve rod	+ve	+ve	+ve	-ve	Sugar plant water
-ve rod	+ve	+ve	+ve	-ve	Pond water

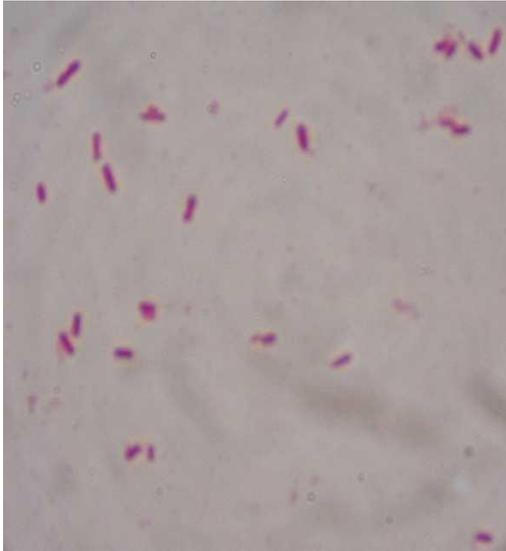
**Table No.2: Qualitative and Quantitative estimation of genomic DNA**

Water Samples	OD at 260nm	OD at 280nm	Concentration (ng/ml)	Purity
Sewage Water	0.073	0.051	730	1.4
Sugar Plant Water	0.080	0.050	800	1.6
Pond Water	0.074	0.049	740	1.1.5

**Table No.3: Qualitative and quantitative estimation of plasmid DNA**

Water Samples	OD at 260nm	OD at 280nm	Concentration (ng/ml)	Purity
Sewage Water	0.070	0.050	700	1.5
Sugar Plant Water	0.068	0.055	680	1.2
Pond Water	0.075	0.062	750	1.2

**Figure 1: *E.coli* culture *sp*****Figure 2: *E.coli* sub culture *sp***



**Figure 3: Gram staining of *E.coli sp***



**Figure 4: *E.coli sp* Indole positive**



**Figure 5: *E.coli sp* Methyl red positive**



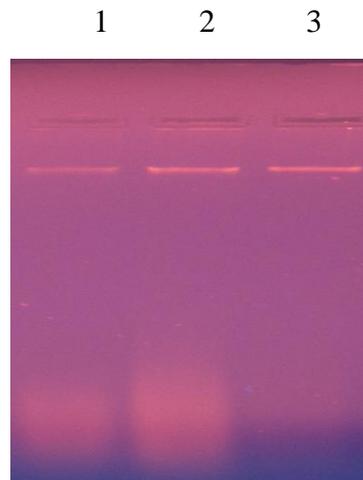
**Figure 6: *E.coli sp* Citrate negative**



**Figure 7: *E.coli sp* Catalase positive**

**DNA extraction**

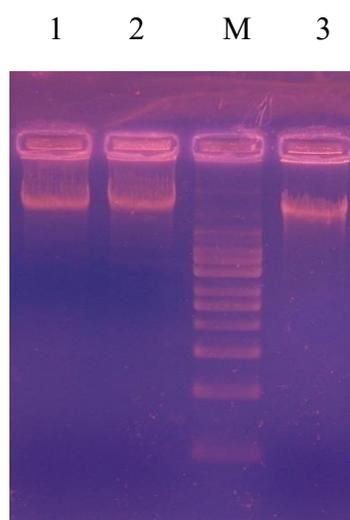
Water tests were gathered from various natural sub zones. Disconnect a high sub-atomic weight DNA from the indigenous bacterial networks present in the water. Indigenous bacterial populaces are harder to lyse than the seeded microorganisms in the water test.



**Figure 8: DNA extraction**

**Lane: 1:** Extracted DNA from Sewage Water Sample. **Lane: 2:** Extracted DNA from pond water Sample. **Lane: 3:** Extracted DNA from Sugar plant water Sample.

**Gene amplification:** The explanation behind this methodology is to isolate the DNA from its related proteins with the goal that further controls should be possible to it. Catalysts added to cleanse DNA in vitro can have unhindered access to it. An ineffectively refined DNA planning might be incompletely open to the compounds. Hence, unique consideration must be taken to guarantee an unadulterated DNA readiness



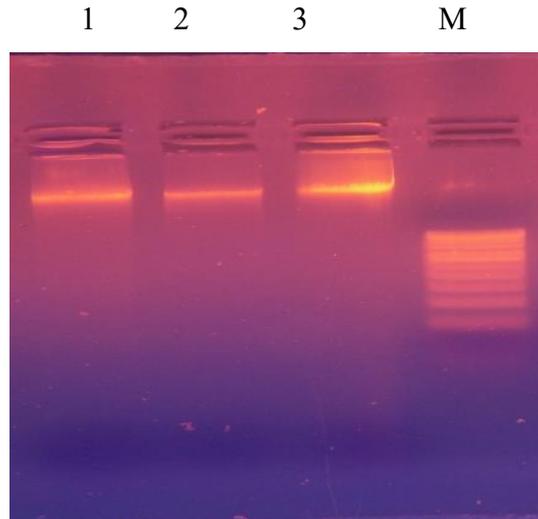
**Figure 9: Amplified DNA sample**

**Lane: 1:** Amplified DNA From Sewage Water. **Lane: 2:** Amplified DNA from sugar plant water  
**M:** 1Kb DNA Marker **Lane: 3:** Amplified DNA from pond water Sample

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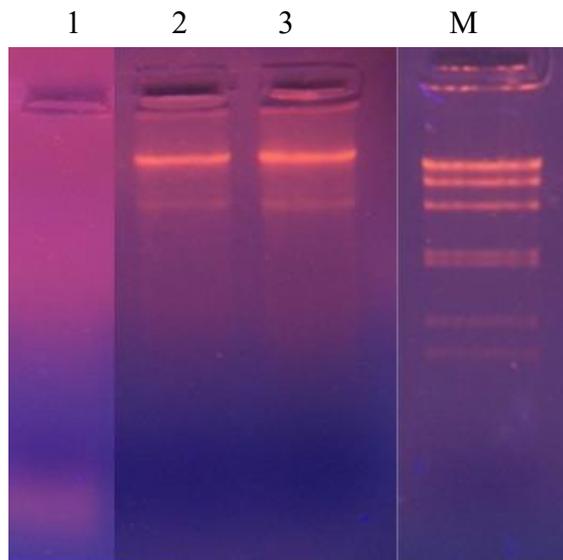
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**Genomic DNA and plasmid DNA extraction:****Figure 10: Genomic DNA separated on 1% Agarose gel**

**Lane: 1:** Extracted genomic DNA from Sewage Water Sample. **Lane: 2:** Extracted genomic DNA from sugar plant water Sample. **Lane: 3:** Extracted genomic DNA from pond water Sample.

**Lane: 4:** 1kb DNA

**Figure 11: Plasmid DNA separated on 1% Agarose gel**

**Lane: 1:** Extracted plasmid DNA from Sewage Water Sample **Lane: 2:** Extracted plasmid DNA from sugar plant water Sample. **Lane: 3:** Extracted plasmid DNA from pond water Sample.

**Lane: 4:** 1kb DNA Marker

**4. CONCLUSION**

The current investigation was attempted to decide *Escherichia coli sp.*, in sea wage water, sugar plant water and pond water tests are gathered from Andhra Pradesh. The isolation, identification and characterization of bacterium were completed by observing the standard microbiological tests like

streak plate technique, Gram's staining and biochemical tests. Genomic DNA was isolated and purified by enzymatic processing techniques for polymorphic investigations by RAPD-PCR and isolation of plasmid DNA was finished by utilizing basic Lysis strategy for RFLP assessment. Both Genomic DNA and Plasmid DNA were isolated by using 1% Agarose gels and Estimated by using UV visible Spectrophotometer at OD260 nm/OD280nm. The result showed that indole, methyl red test, and Catalase test are positive incase of citrate negative. From all of these discoveries it was directed that the tested organism may be *Escherichia coli*. Effectively disconnected genomic DNA just as plasmid DNA for the assessment of RFLP just as RAPD-PCR contemplates. These examinations will help for the total investigation of segregated *E.coli* from different sorts of water samples.

### **ETHICS APPROVAL AND CONSENT TO PARTICIPATE**

Not applicable.

### **HUMAN AND ANIMAL RIGHTS**

No Animals/Humans were used for studies that are base of this research.

### **CONSENT FOR PUBLICATION**

Not applicable.

### **AVAILABILITY OF DATA AND MATERIALS**

The authors confirm that the data supporting the findings of this research are available within the article.

### **FUNDING**

None

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### **CONFLICT OF INTEREST**

Authors have no conflict of interest.

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