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Microbial and Molecular Characterization of Urinary Tract Infection Isolates of *Escherichia coli*

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ABSTRACT

A total of 50 urine samples are subjected to isolation of E. coli from 5 types of age groups of people. The sex wise distribution patterns showed a decided inclination towards one sex as; there were 25 from male and 25 from female patients. The isolates were obtained by selective media and standard biochemical tests. As for the age wise prevalence patterns of E. coli associated UTI, this study showed as interesting pattern, out of 50 samples 15 (30%) were obtained, the distribution of isolates from different age groups of male and female. The higher incidences were showed in female 32.0% followed by male 28%. Among the 15 isolates 12 biofilm producers were observed. Among the 5 types of age group 21-30 (100%) had highest occurrence followed by 11-20 and 41-60. In this study, biofilm producing isolates were not observed from 0-10 age group peoples. Among the 15 isolates 10 (66.6%) betalactamase producers were obtained. In this investigation highest betalactamase producers were observed from 11-20 and 41-60 age groups (75%) followed by 21-30 (60%) All fifteen isolates were subjected into PCR analysis for identification of ESBL producing isolates. Among the 3 antibiotics CTX-M were resistance to most of the isolates (60%) and second most TEM (40%) and followed by OXA (6.6%). In our current studies 31-60 age group peoples had multidrug resistance especially female peoples had highest resistance compared to male.

Key words:*E. coli,UTI,Biofilm,antibiotics CTX-M,ESBL.*

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INTRODUCTION

Urinary tract infection, commonly known as UTI, cystitis or bladder infection occurs when part of the urinary tract becomes infected. The urinary tract includes the following organs: kidneys, urethra, bladder. Both males and females are affected by UTI, it affects as many as 50% women at least once in their life time and 25% of those who acquire UTI will have recurrent infection within the following six months. Urine located within the urinary tract,excluding the distal region of the urethra is considered sterile in healthy individuals, as indicated by absence of cultivable bacterial cells [1].The urinary system consists of the two kidneys, two ureters and one urinary bladder, one urethra. It has three major functions: Excretion, Elimination, Homoeostatic regulation of the solute concentration of the blood plasma.

Each kidney is surrounded by three layer:*Renal capsule:* this is a layer of collagen fibers that covers the outer surface of the entire organ, *Fat:* this keeps the kidney in place and surrounds the renal capsule. *Renal fascia:* this is a dense fibrous outer layer that also secures the kidney to the posterior abdominal wall and to the surrounding structures. Nephron: The nephron is the functional unit of the kidney [2]. It is responsible for filtration of the blood and for the re-absorption of water and salts and the absorption of glucose. About 1.25 million nephrons can be found in the cortex. The nephron consists of a renal tubule and a renal corpuscle.

The bladder is a hollow, muscular organ that collects and stores urine. It is situated in the lower part of the abdomen and is lined with a membrane called the urothelium. The cells of this membrane are called transitional cells or urothelial cells. The bladder wall has three layers: mucosa, sub-mucosa and muscularis. The muscle of the bladder can then be contracted to force urine out of the body through a tube called the urethra [3].It is made up of stratified epithelium [2, 3].Kidney infection can mean serious health problems. There are two types of kidney infections. Pyelonephritisis caused by bacteria. Symptoms may include pain in the back, headache, fever, or chills [4]. Microorganisms causing urinary tract infection are bacteria, fungi, protozoa and viruses. The main bacteria causing urinary tract infection include Escherichia coli, Proteus species, *Pseudonronas aemginosa*, *Serratia marcescens* and Staphylococcus

saprophytic-us [5]. Among fungi feast like species called Candida Albican is commonly found in urinary infection, *Candida tropicalis* and *Candida parapsilosis* are less common [6, 7]. The urinary tract may be infected by a few parasites. The objective of this present study is to evaluate the presence of plasmid mediated multidrug resistance genes from *E. coli* causing UTI by the multiplex PCR.

MATERIALS AND METHODS

COLLECTION AND TRANSPORTATION OF SAMPLES:

A total of 50 urine samples were collected in each 10ml sterile plastic bottle from the different age group of urinary tract infected persons in around Namakkal hospitals. The samples were properly labeled indicating the source, date, time of collection, sex, age of patients. The urine samples were transported in cooler boxes to our Microbiology Laboratory, for bacteriological investigations within 4 – 6 h of collection.

BACTERIAL ISOLATION:

Culture plates of Eosin Methylene Blue Agar, MacConkey Agar (Hi media, Bombay, India) were used. The collected urine samples were streaked directly on the labelled agar plates and incubated at 37°C for 24 h. After incubation, cultures were examined for significant growth. Subcultures were then made into plates of nutrient agar and incubated for another 24 h. The primary identification of the bacterial isolates was made based on colonial appearance and pigmentation. Biochemical tests were performed to identify microbes. Biochemical tests applied were standard catalase test, citrate utilization, coagulase, oxidase, Methyl red, Voges-Proskauer, Indole production, motility, Glucose, sucrose, maltose, lactose, Characterization and identification of the isolates was done using the methods of Cowan [8], Fawole and Oso's [9]and Cheesbrough [10].

IDENTIFICATION OF MICROORGANISMS:

PRELIMINERY TEST:

Gram Staining

Bacterial smears of 16-18 hrs old cultures were made on clean grease free slides, heat fixed and stained as follows. The slide was flooded with crystal violet solution for a minute, drained and rinsed with water; followed by Gram's iodine solution for one minute, drained and rinsed with water. Decolourised with ethyl alcohol for 30 Sec and later counterstained with safranin for one minute and observed under an oil immersion microscope [11].

Catalase Test

A small amount of culture was placed over a clean slide. A drop of 3% hydrogen peroxide was placed over the culture and observed for effervescence. The production of effervescence showed the ability to produce the enzyme catalase.

Oxidase Test:

The organism spotted on oxidase disc (HI Media) the blue or purple colour change was observed within 10 seconds.

ISOLATION AND IDENTIFICATION OF *ESCHERICHIA COLI.*

Urinary Tract Infections (UTIs) are one of the most common bacterial infections in humans, both in the community and the hospital settings. UTIs are amongst the most prevalent infectious diseases affecting approximately 150 million people worldwide annually which results in more than 6 billion US dollars loss to the global. The lifetime risk for UTI in females is greater than 50%. In the United States, about 8 million physician visits and more than 100,000 hospital admissions per year are due to UTIs. Urinary tract infections (UTI) are mostly caused by *Escherichia coli.*

In the present investigation to evaluate the *E. coli* from UTI infected persons in different age groups peoples. Out of 50 samples 15 (30%) isolates was obtained. Among the 5 types of age groups the highest prevalence occurs in 21-30 age groups. The second most incidences in 41-60 years age category, both males and females showed an unusually high incidence of UTI, 28.0% in male and 32.0% in female. In our overall observation the highest is in female patients. A total of 50 urine samples are subjected to isolation of *E. coli* from 5 types of age groups of people. The sex wise distribution patterns showed a decided inclination towards one sex as; there were 25 from male and 25 from female patients.

ASSAY FOR BETA LACTAMASE PRODUCTION:

Beta lactamase production was assayed using the method of Lateef(2004). Broth culture of the test organism was spot inoculated on to Mueller-Hinton agar and 1% starch and then incubated overnight at 37^0C. The plates were then flooded with freshly prepared phosphate buffered saline containing potassium iodide, iodine and penicillin. The presence of clear colourless zones around the bacterial growth is an indication of Beta lactamase production. Beta lactamase converts penicillin to penicilloic acid, which

reduces iodine to iodide monitored via decolourisation of the starch iodine complex. All the bacterial isolates were tested for the production of beta lactamase.

CULTURE MEDIA AND REAGENTS BETA LACTAMASE ASSAY :

Mueller-Hinton agar composition:

Starch **:**1gm

REAGENTS:

PBS (1X),

Sodium Chloride - 8grams
Potassium Chloride -0.2grams
Disodium Hydrogen Phosphate -1.5grams
Potassium Dihydrogen Phosphate -0.2grams

Dissolve in 800ml of triple or double distilled water. Adjust the pH 7.2, and then make up to one litre.

Penicillin solution preparation: Take 10000 U of Penicillin and dissolve in 100 ml of 1% PBS buffer and store in 4^0C.

RESULT AND DISCUSSION

ISOLATION AND IDENTIFICATION OF *Escherichia coli.*

Urinary Tract Infections (UTIs) are one of the most common bacterial infections in humans, both in the community and the hospital settings. UTIs are amongst the most prevalent infectious diseases affecting approximately 150 million people worldwide annually which results in more than 6 billion US dollars loss to the global. The lifetime risk for UTI in females is greater than 50%. In the United States, about 8 million physician visits and more than 100,000 hospital admissions per year are due to UTIs. Urinary tract infections (UTI) are mostly caused by *Escherichia coli.*

The isolates were obtained by selective media and standard biochemical tests. All urine samples were inoculated onto Eosin methylene blue (EMB) agar for primary screening of *Escherichia coli* and incubated aerobically at 37°C for 24 hours. Simultaneously inoculated into MacConkey agar and incubated at 37°C for 24 hours. After incubation time, observed morphological characters of colonies. *Escherichia coli* can be identified with Eosin methylene blue (EMB) agar based on the occurrence of green-metallic sheen and pink-red colonies in MacConkey agar. Suspected colonies of *E. coli* were sub-cultured into nutrient agar slant for further purpose. The standard biochemical tests summarized as (Table 1, and Fig. 1**)**

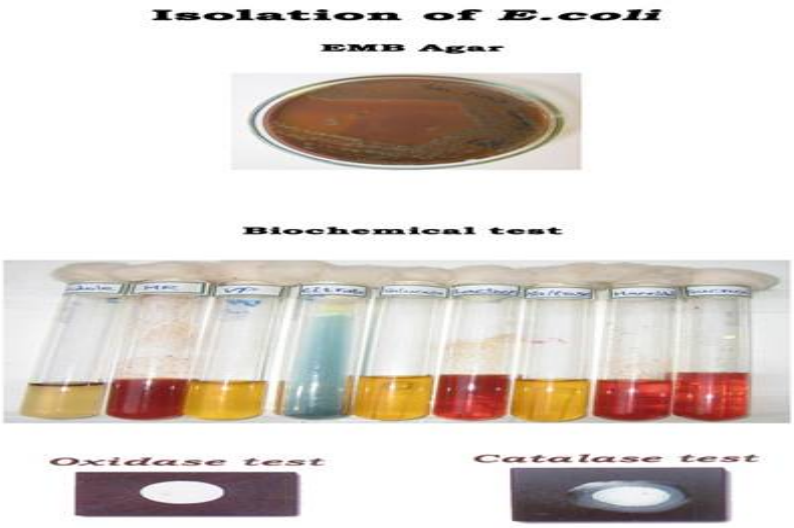

Fig. 1 The standard biochemical tests

Table1. Biochemical characteristics of the *Escherichia coli* isolates

S. no	Biochemical test	Clinical isolates of *E. coli*
1.	Gram's staining	Gram negative rods
2.	Catalase	Positive test
3.	Citrate utilization	Negative test
4.	Coagulase	Negative test
5.	Oxidase	Negative test
6.	Methyl red	Positive test
7.	Voges-proskauer	Negative test
8.	Indole	Positive test
9.	Motility	Positive test
10.	Glucose	Positive test
11.	Sucrose	Positive test
12.	Maltose	Positive test
13.	Lactose	Positive test

As for the age wise prevalence patterns of *E. coli* associated UTI, this study showed as interesting pattern, out of 50 samples 15 (30%) were obtained, the distribution of isolates from different age groups of male and female. The higher incidences were showed in female 32.0% followed by male 28%. In female out of 5 types of age groups 4 groups showed 40% occurrence except 0-10 years. In male the highest prevalence was in 21-30 (60%) and lowest in 11-20- and 41-60-years age groups. In both sex groups 0-10 did not show any prevalence (Table 2, Figure 2**).**In this result each age group persons exhibited different result. The reason for these differences may be a culmination of reasons stemming from poor hygienic conditions within our community, our house hold, a lack of education and proper personal hygiene practices.

BIOFILM PRODUCTION

All isolates of *E.coli* was subjected to biofilm production with Congo red method. Among the 15 isolates 12 biofilm producers were observed. Among the 5 types of age group 21-30 (100%) had highest occurrence followed by 11-20 and 41-60. In this study, biofilm producing isolates were not observed from 0-10 age group peoples **(**Table 3, Table 4**)).**

Biofilm formation allows the strains to persist for a long time in the genitourinary tract and interfere with bacterial eradication and initiate colonization and dispersion of pathogenic bacteria inside the host leading to bloodstream infection to UTI [12]. It was found that 60% of *Escherichia coli* isolates in our study were found to be slime producers. This record was slightly high compared to the previous records .

The very difficulty in treatment of UTI associated with biofilm formation because antimicrobial agents are ineffective to penetrating the biofilm decreasing the concentration acting on the bacterial cells. In addition biofilm formation suppresses the phagocytic activity [12]. Biofilm assays may be helpful in selecting patients who require a therapeutic approach to eradicate persistent biofilm-forming *E. coli* strains.

Table2.Prevalence of *Escherichia coli* from different age groups of people of UTI patients

S.No	Sex	Age group					% of occurrence	Total % of occurrence
		0-10	11-20	21-30	31-40	41-60		
1.	Male	0/5 (0%)	2/5 (40%)	3/5 (60%)	0/5 (40%)	2/5 (40%)	28.0% 32.0%	30%
2.	Female	0/5 (0%)	2/5 (40%)	2/6 (40%)	2/5 (40%)	2/5 (40%)		

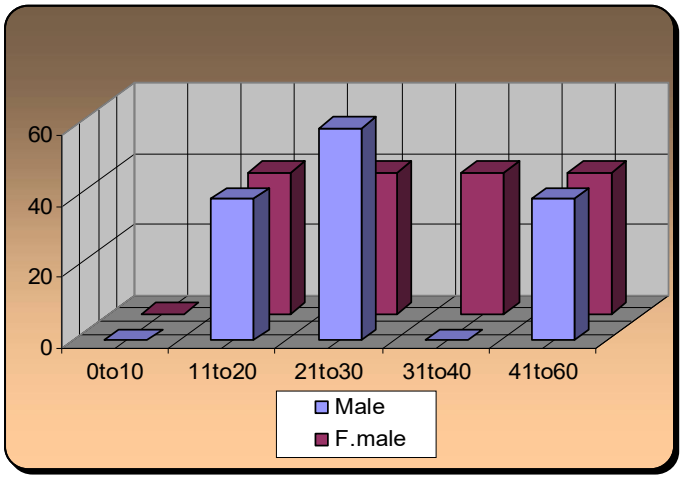

Fig.2 Prevalence of *E.coli* isolates from different age groups of UTI patients

In this investigation, most of drug resistance isolates produce betalactamase enzymes. Number of authors suggested that biofilm is responsible for antibiotic resistance [8, 10].At the same time, our reports evaluate argument with other reports for growing resistance of UPEC.

BETA LACTAMASE ASSAY

Betalactamase analysis was subjected to all isolates of *E.coli* with betalactam antibiotic. Among the 15 isolates 10 (66.6%) betalactamase producers were obtained. In this investigation highest betalactamase producers were observed from 11-20 and 41-60 age groups (75%) followed by 21-30 (60%) **(**Table 3**).**Many studies worldwide have also reported a sharp increase in ciprofloxacin resistant *E. coli* isolates from UTIs. For example, in China, from 1998 to 2002, he prevalence of ciprofloxacin resistance has increased steadily from 46.6% to 59.4%, and in Bangladesh the prevalence was 26%. ESBLs are known as extended-spectrum because they are able to hydrolyze a broader spectrum of B-lactam antibiotics than the simple parent B-lactamase from which they are derived. They are acquired plasmid-mediated B-lactamases.

Table3 Prevalence of biofilm and betalactamase producing E.coli

Test	**Age group**					**% of occurrence**
	0-10	**11-20**	**21-30**	**31-40**	**41-60**	
Biofilm	0%	75%	100%	50%	75%	60%
β lctamase	0%	75%	60%	50%	75%	52%

Table 4. Prevalence of Biofilm producing *E.coli*

S.No	**Isolates**	**Result**
1.	E1	Negative
2.	E2	Strong
3.	E3	Moderate
4.	E4	Week
5.	E5	Week
6.	E6	Strong
7.	E7	Week
8.	E8	Moderate
9.	E9	Strong
10.	E10.	Strong
11.	E11	Moderate
12.	E12	Strong
13.	E13	Negative
14.	E14	Negative
15.	E15	Strong

High level resistance to broad beta lactam antibiotics in grams negative bacteria usually results from beta lactamase activity. Plasmid mediated extended spectrum beta lactamase (ESBLs) is the predominant

causes of transferable resistance to third generation cephalosprins in gram negative bacteria. In the past few years CTX-M types ESBLs have become more prevalent worldwide.Production of ESBL is frequently plasmid encoded and bears clinical significance. Plasmids responsible for ESBL production frequently carry genes encoding resistance to other drug classes also. Therefore, antibiotic options in the treatment of ESBL producing organisms are extremely limited. Detection of ESBL production is important. One major concern is the spread of ESBL positive bacteria within hospitals, which may lead to outbreaks or to endemic occurrence.

Among the beta-lactamases tested, the carbapenems have the widest spectrum of activity. Imipenem was the most active antimicrobial agent having 98.3% activity. However, Akram *et al.* [2] from India have reported 100% activity for imipenem against *E .coli.* Imipenem was followed by meropenem with 97.4% activity.

CONCLUSION

In this study, the plasmid mediated multidrug resistant uropathogenic*Escherichia coli* were identified by multiplex polymerase chain reaction. A total of 50 urine samples were collected from 5 different age group of urinary tract infected persons in and around the Namakkal hospitals. Among the 50 urine samples, 25 samples were collected from male and 25 samples were collected from female patients.The *Escherichia coli* colonies were identified by using Gram's staining and biochemical test such as standard catalase test, oxidase test. After identification of *Escherichia coli* 30% (15/50) of positive isolates were obtained. Among the 50 samples 28.0% of isolates were obtained from males and 32.0% of isolates were obtained from females. Mostly 21-30 and 41-60 age groups of peoples were affected by urinary tract infection. All isolates were subjected to biofilm and betalactamase. Totally 60% and 52% of were produced respectively. Hence, there exists a great need for antimicrobial resistance surveillance at local, national and international level. And additional necessary to evaluate, despite significant value of antibiotics, the increase of bacterial resistance had restricted their clinical application.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

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