BIOFILM FORMATION IN UROPATHOGENIC *Escherichia coli* STRAINS; RELATIONSHIP WITH VIRULENCE FACTORS AND ANTIMICROBIAL RESISTANCE IN TERTIARY CARE HOSPITAL IN NORTH KARNATAKA REGION

A Thesis submitted to the Faculty of Medicine of

BLDE (Deemed to be University)

Vijayapura, India.

For the degree of

Doctor of Philosophy

In
Medical Microbiology

By

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SEPTEMBER 2019

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Certificate

This is to certify that the **Mr. Sudheendra Kulkarni** carried out the work on **"Biofilm formation in Uropathogenic *Escherichia coli* strains; Relationship with virulence factors and antimicrobial resistance in tertiary care hospital in North Karnataka region''** for the award of **Doctor of Philosophy in Medical Microbiology,** submitted to the **BLDE (Deemed to be University),** Vijayapura, India, for the requisite period, under the regulations in force and this thesis is the bonafide record of the work done by him under my supervision and guidance. This work is original and has not formed before the basis for the award to the candidate of any degree, diploma, associateship, fellowship or any other title.

I further certify that the entire thesis represents the independent work of **Mr. Sudheendra Kulkarni,** and all the experimental techniques employed in this work are actually undertaken by the candidate himself under my supervision and is worth submitting for the award of the said degree.

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This study represents the independent work conducted by him and has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or any other similar title.

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I hereby declare that the thesis entitled **''Biofilm formation in Uropathogenic *Escherichia coli* strains; Relationship with virulence factors and antimicrobial resistance in tertiary care hospital in North Karnataka region''** submitted to the **BLDE (Deemed to be University),** Vijayapura, India, for the fulfillment of the requirement for the degree of Doctor of Philosophy in Medical Microbiology, is a bonafide record of the original research work done by me under the supervision and guidance of **Dr. B.V. Peerapur**, Former Professor & HOD, Department of Microbiology, Shri B.M. Patil Medical college & Research Centre, Vijayapura.

This study represents the independent work conducted by me and has not previously formed the basis for award of any degree, diploma, associateship, fellowship or other similar title.

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September 2019.

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Sudheendra Kulkarni

My Parents……

Due to their dedication and support I have been able to accomplish all the things in my life, and thanks to them I have become the person that stands today.

CONTENTS

ABBREVIATIONS USED IN THE TEXT

Abbreviation	Explanation
%	Percentage
α	Alpha
β	Beta
µg	Micro gram
µl	Micro liter
µM	Micro molar
°C	Degree centigrade
≥	More than or equal to.
ABU	Asymptomatic bacteriuria
AMR	Antimicrobial resistance
ATCC	American Type Culture Collection
BHI	Brain heart infusion
bp	base pair
CDC	Centre for disease control
CFU	Colony forming unit
CLSI	Clinical and Laboratory Standards Institute
CNF	Cytotoxic necrotizing factor
DNA	Deoxyribose nucleic acid
DNTP	Deoxynucleotide triphosphates
ESBL	Extended spectrum β-lactamase
ELISA	The enzyme linked immunosorbent assay.
FQ	Fluoroquinolone.
g/L	Gram per litre.
h	Hour/hours
ICU	Intensive Care Unit
kDa	Kilodaltons
LPS	Lipopolysaccharides.

MDR	Multidrug Resistance.
mg	Milligram
MHA	Mueller Hinton agar.
MIC	Minimum inhibitory concentration
ml	Millilitre
MRHA	Mannose-resistant hemagglutination
MSU	Mid-stream urine sample
nm	Nanometer
O.D.	Optical Density
OMP	Outer membrane proteins
ORF	Open reading frame.
PAI	Pathogenicity islands.
Pap	Pyelonephritis associated pili
PBS	Phosphate buffer saline
PCR	Polymerase chain reaction
pH	pH value
pmol	picomole
rpm	Revolutions per Minute
RUTI	Recurrent Urinary tract infection
Taq	Thermus aquaticus
traT	Serum resistance.
TSB	Trypticase soy broth
UPEC	Uropathogenic *Escherichia coli*
UTI	Urinary tract infection
VFs	Virulence factors.

TABLES

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FIGURES

DETAILS OF MEDIA, REAGENTS, QUALITY CONTROLS AND EQUIPMENT'S USED FOR THE STUDY

1. Details of Media and Reagents

Product	Source
Agar agar	Hi-Media Laboratories, India.
Agarose gel	Sigma, USA.
Biochemical reactions media	Hi-Media Laboratories, India.
Brain Heart Infusion agar	Hi-Media Laboratories, India.
Congo red agar	In-house.
Congo red indicator	NICE Chemicals, India.
Crystal violet dye	NICE Chemicals, India.
ESBL Screen agar	Hi-Media Laboratories, India.
Ethidium bromide	Hi-Media Laboratories, India.
Glucose	NICE Chemicals, India.
MacConkey agar	Hi-Media Laboratories, India.
Milli Q water	Hi-Media Laboratories, India.
Molecular weight markers	ThermoFisher Scientific, USA.
Muller Hinton agar	Hi-Media Laboratories, India.
Nutrient agar	Hi-Media Laboratories, India.
Sheep Blood agar	In- house.
Peptone water	Hi-Media Laboratories, India.
PCR Master mix & buffers	QIAgen, Germany.
Phosphate buffered saline	Hi-Media Laboratories, India.
Primers	IDT DNA Technologies, USA.
Sucrose	NICE Chemicals, India.
Trypticase soy broth	Hi-Media Laboratories, India.

Maintenance of Isolates:

Isolates were maintained in Nutrient agar butts and stored in Refrigerator at 2-8˚C. Reconstituted in peptone water and sub cultured whenever required.

2. Quality control strains

Quality control strains	Tests
Escherichia coli ATCC 25922	Lactose fermentation on MacConkey agar, Antibiotic susceptibility testing. Biochemical reactions. Beta hemolysis on Sheep blood agar.
Klebsiella pneumoniae subsp. pneumoniae ATCC 700603	ESBL detection.
Staphylococcus aureus ATCC 25923	Beta Hemolysis on Sheep blood agar.
Staphylococcus epidermidis ATCC 35984	Positive control for Biofilm formation.
Staphylococcus epidermidis ATCC 12228	Negative control for Biofilm formation.

Source of control strains: Department of Microbiology, JIPMER Pondicherry.

3. Equipments

Name	Company
Centrifuge	REMI, India.
ELISA auto reader Model 680	BioRad, UK.
Gel-documentation system	BioRad, USA
Gel Electrophoresis Unit	Genei, India.
PCR Thermocycler	AB VERITI PCR, USA.
Sanger Sequencer	Applied biosystems, USA.

ABSTRACT

Background:

Urinary tract infection (UTI) is a clinical condition, with characteristic symptoms, colonization and multiplication of bacteria in significant numbers *i.e.*, 10^5 cfu/ml within the urinary tract. At this juncture, antimicrobial resistance (AMR) in uropathogens has become one of the major concerns globally.

Aim & Objectives:

The present study aimed to demonstrate the biofilm formation in Uropathogenic *Escherichia coli* strains; characterize the phenotypic & genotypic virulence factors and their relationship with antimicrobial resistance.

Methods:

In the present study, 1000 suspected UTI cases were included. Urine samples were processed for culture and antimicrobial drug susceptibility testing. Only *Escherichia coli* (*E. coli*) isolates were further studied for production of biofilm, ESBL and haemolysin enzymes by phenotypic methods. Virulence genes, *papEF*, *traT* & PAI were detected by multiplex PCR. Molecular confirmation of virulence genes was done by Sanger sequencing. Sequences were studied for characterization and mutations.

Results:

From the 1000 urine samples, 395 *E. coli* were isolated. Higher resistance was observed with antibiotics - ampicillin (82.53%), cefuroxime (72.41%), amoxicillin-clavulanic acid (71.90%), ceftriaxone (66.58%), ciprofloxacin (65.82%) and cefepime (57.47%). Further, in-vitro biofilm assay confirmed formation of biofilms by 71.39% isolates. Biofilm-forming *E. coli* strains developed higher degree of resistance towards antibiotics ampicillin (87.36%) followed by cefuroxime (81.58%), amoxicillin-clavulanic acid (77.61%), ciprofloxacin (71.48%), cefepime (64.98%) and ceftriaxone (54.6%). Phenotypic methods detected 62.3% isolates as ESBL producers and 40.2% were β-haemolytic. Virulence gene characterization revealed presence of gene *traT* in 73.2% isolates, PAI in 62.9% isolates and *papEF* among 33.5% isolates. ESBL & Hemolysin producing UPEC exhibited significantly higher resistant to ampicillin, amoxicillin-clavulanic acid, aztreonam, cefriaxone, cefuroxime, cefepime, ciprofloxacin, chloramphenicol, gentamicin and norfloxacin. Association between the expression of virulence genes and antimicrobial resistance was observed. Statistically proved association was seen with expression of genes RPAI and *traT* with antibiotics

nitrofurantoin and amikacin respectively. All the isolates were sensitive to antibiotics nitrofurantoin, piperacillin-tazobactam, and imipenem. Strains showed 99% sequence identity in Sanger sequencing and no mutations were detected among the study strains.

Conclusion:

Increase in trend of resistance was observed with antibiotics which were routinely used to treat UTI. Predominance of the *traT* gene and PAI markers was seen among UPEC strains. Results indicated significant correlation between phenotypic and genotypic virulence factors and antibiotic resistance. Antibiotics nitrofurantoin, piperacillin - tazobactam, and imipenem can be effective for severe UTIs. This study concludes that expression of virulence factors by UPEC strains is responsible for increased antibiotic resistance. Hence, characterizing the UPEC strains help clinicians and microbiologists to reach a better therapeutic outcomes and treatment regimens in this region.

CHAPTER 1
INTRODUCTION

CHAPTER 1

INTRODUCTION

The infection that affects any part of the urinary tract system i.e., urethra, bladder, ureters, and kidneys are termed as Urinary tract infections (UTIs). It is the second commonest bacterial infection causing serious health problems and morbidity accounting for more than 7 million hospital visits per year.[1-3] A person of any age or gender, including children, women and elderly, can develop UTI, but it is most predominant in women.[4] Young and healthy women are generally predisposed to UTI due to the host, genetic, biological and behavioural factors. Approximately 40% of women have had a UTI in their life time and over 20% of young sexually active women who had previous UTIs have recurrent UTIs.[5]

Most common pathogens causing urinary tract infections are Gram negative bacilli like *Escherichia coli, Klebsiella pneumoniae, Enterobacter species* and *Proteus species.* On the other hand, few gram-positive cocci such as *Staphylococcus aureus, Staphylococcus saprophyticus, Enterococcus faecalis* are also entitled to be uropathogens. *E. coli* is the predominant pathogen in UTIs and is found in 75% - 95% of both outpatient and inpatient UTIs as well as both community and hospital acquired UTIs leading to serious secondary health issues worldwide.[6]

UTI is more common in females than males because of their shorter urethra compared to male which allow bacteria quick access to the bladder and also regular sexual intercourse which increases a woman's risk of developing UTI. Fecal-perineal-urethral contamination in women also increases the risk of UTI in women.[7]

Currently, recurrent urinary tract infections (RUTIs) and antimicrobial resistance is a serious health concern for women despite the broad array of successful antimicrobial agents. Uropathogenic *Escherichia coli* (UPEC) cause 78% of the recurrent episodes.[8] RUTIs are commonly seen among young, healthy women with normal urinary tracts (both anatomically and physiologically) and represent a primary cause of morbidity and economic burden.[9] Recurrent and relapse UTIs may be due to bacterial virulence factors (VFs) exhibited by UPEC strains which enable colonization of the bacteria, evade host defences and invade the urinary tract.[10]

Biofilm formation is one of the most important virulence factors exhibited by *E. coli* among other VFs. Microbial biofilms are community of bacteria and other microorganisms that are irreversibly attached to self-produced extracellular polymeric

substances and adhere to a surface or each other. They play an imperative role in medicine and have proven to cause a wide range of microbial infections in the human body like UTIs, catheter associated infections or dental plaques.[11]

Biofilm decrease the susceptibility of organism to antimicrobial agents by enclosing them in an extracellular matrix.[12] High content of polysaccharides in biofilm prevents the access of antimicrobial agents. Limited penetration of antimicrobial agents into the biofilm may help in the development of persistent, hard to treat chronic illness. Biofilm forming bacteria exhibits higher resistance to common drugs used for UTI treatment and which also contribute to recurrent infections.[13]

UPEC display a high degree of genetic diversity and is due to pathogenicity islands (mobile genetic elements consisting of virulent genes). The distinct virulence markers present in the most UPEC isolates consists of adhesins (e.g. *P* and type I pili, fimbriae), host immune system evading factors (e.g., lipopolysaccharide, capsule), bacterial resistance to killing by serum through serum resistance, nutrient acquisition mechanisms (e.g. siderophores) and toxins[14] (e.g. hemolysin, cytotoxic necrotizing factor1).

Detection of these virulence factors of UPEC and its resistance to the routinely used antimicrobial agents to treat UTI is vital in improving the efficacy of empirical treatment. The management of multi-drug resistant pathogens causing UTI clinically is a challenge due to the rise in virulence and antimicrobial drug resistance of these uropathogens.

However, in spite of high incidence multidrug resistant (MDR) UPEC isolates in India, most of the studies did not characterize these isolates with respect to their VFs. Few Indian studies by Sharma *et al.*[15] and Naveen *et al.*[16] have phenotypically characterized the UPEC isolates but lack molecular characterization of the virulence traits of UPEC.

The knowledge of virulent traits and antibiotic susceptibility profile of UPEC strains in a particular geographical location would provide clinicians a clear picture in the management of UTI by formulating customized antibiotic policy for the hospital. There is insufficient data regarding the relationship between the biofilm formation and distribution of phenotypic & genotypic virulence factors (VFs) and their relationship with antimicrobial resistance among UPEC strains isolated from India. However until date, the incidence of UTI due to UPEC and their characteristics have not been investigated from a North-Eastern region of Karnataka.

So, this research was proposed to study the detection of biofilm formation, to correlate with carriage of specific phenotypic and genotypic virulence markers with patterns of antimicrobial resistance of UPEC strains isolated from patients attending a tertiary care hospital at Bidar, Karnataka.

CHAPTER 2
AIM & OBJECTIVES

CHAPTER 2
AIM AND OBJECTIVES

Aim:

To study the Biofilm formation in Uropathogenic *Escherichia coli* strains and their relationship with virulence factors and antimicrobial resistance.

Objectives:

1) Isolation and antibiotic susceptibility testing of *Escherichia coli* (*E. coli*) from suspected cases of Urinary tract infections (UTI) and analysis of incidence of multidrug resistance among isolates.
2) To detect in vitro bio film formation of *E. coli* isolates and analysis of their association with antimicrobial susceptibility patterns.
3) To detect the prevalence of phenotypic virulence factors and virulence genes of Uropathogenic *E. coli* (UPEC) by polymerase chain reaction (PCR) and their correlation with antibiotic resistance.
4) Molecular confirmation and characterization of virulent genes amplified from UPEC strains by Sanger Sequencing & Detection of mutations.

CHAPTER 3
REVIEW OF LITERATURE

CHAPTER 3

REVIEW OF LITERATURE

3.1 Urinary tract system

The kidneys and the urinary systems are responsible for removal of waste from the body. The urinary system comprises of kidneys, ureters, urinary bladder, two sphincter muscles, nerves in the bladder and the urethra which perform different functions. Waste in these systems are eliminated as liquid in the form of urea. Through the blood entering the kidneys, the urea is eliminated in a liquid form with water and other wastes. The kidney releases the waste from the body as urine.

3.2. Urinary tract infections

Urinary tract infections (UTI) are most common and are highly infectious. It has been estimated that this disease occupies second place in prominence as a bacterial infection in the community. In fact, it is the reason for health problems that may become serious enough to result in morbidity. UTI is responsible for infecting more than 7 million people a year. The disease is the cause for most visits to a physician each year, [1-3] and affects people belonging to all age groups; its distribution among both the sexes is also similar. Reports state that more than 10.8 million people were treated in the Emergency department (ED) for UTIs, in the United States alone, between the years 2006 and 2009. In fact, in this same period, about 1.8 million of people (16.7%) were treated in acute care hospitals.[17] It has been estimated that the treatment of UTIs may cost up to $2 billion every year. Moreover, UTIs is one of the infections that is always treated with an antibiotic on visiting a physician.[18]

3.3. Definition of UTI

UTI is detected by means of an amalgamation of symptoms related to urinary tract diseases and a positive result by a urine culture that proves the existence of a known uropathogen in large numbers, usually above the standard threshold (usually > 10^3 cfu/ml of urine).[19] However, for better identification standard thresholds range from 10^2 cfu/ml to 10^5 cfu/ml in extreme situations.[20] Nonetheless, signs signifying urinary tract diseases and bacteriuria repeatedly ensue individualistically. Approximately 20% of older females displaying “classic” UTI indications have negative urine cultures.[21] Similarly, large numbers of bacteria are frequently established in the urine of healthy, asymptomatic persons.[22]

3.4. Classification of UTI

UTIs need to be identified as per type and classified accordingly as it is important to arrive at appropriate clinical decisions. UTIs have been classified broadly into symptomatic and asymptomatic UTIs. These two types have been further classified on the basis of the place where the infection was acquired (community-acquired or hospital-acquired UTI) and the localization of infection within the urinary tract. Based on the localization within the urinary tract, UTI categories have been further subdivided into lower tract infections (urethritis and cystitis) and upper tract infections (pyelonephritis). Complications too have been known to arise because of UTIs, and on the basis of its chances for complication, UTIs are classified into complicated and uncomplicated UTIs. An uncomplicated UTI is when the host is normal and has no structural or functional abnormalities, is not pregnant, or does not have any internal instruments (for example, inserted with a catheter). All UTIs that do not fall under any of the aforementioned categories are considered complicated.[23]
Symptomatic bladder infections are distinguished on the basis of frequency of urination, urgency during urination, dysuria, or suprapubic pain in women. Complicated UTI covers a whole range of heterogeneous entities and includes factors such as anatomical variation, physiological changes in the urinary tract, diminished renal functioning as a result of parenchymal diseases, pre-intra or post-renal nephropathies, and any other associated diseases that may weaken the immune system of a patient.

Asymptomatic bacteriuria (ABU) is detected variedly across the genders. In women, if a patient exhibits no clinical signs of UTI and the urine's upper limit of $\geq 10^5$cfu /mL is exceeded twice in consecutive sample collection of midstream urine, ensuring the collection has been conducted properly, it is considered as a positive sign for ABU.

3.5. Recurrent or Relapse UTI

Recurrent urinary tract infections (RUTIs) are commonplace among young, healthy women; this is in spite of them generally having normal anatomy and physiology in the urinary tracts.[9] Globally, 25%–30% of all adult women having a first episode of UTI report a recurrence. Mabeck reported correlated data that approximately one-half of the women with uncomplicated UTIs that resolved spontaneously progressed to have RUTI within the first year.[24]

Women with RUTI have been revealed to have an augmented vulnerability to vaginal colonization with uropathogens and gram-negative bacilli. It has been postulated that most recurrences of cystitis are just reinfections by the strains existing in the fecal flora. These strains on eradication from the urinary tract, re-habitate it thereby causing recurrent infections.[25]

3.6. Epidemiology

UTIs have been reported to be the second most common bacterial contagions infecting children, as well as women and the elderly. However, the disease has been predominantly observed in women because of their structural anatomy (short urethra in comparison to men, with its adjacent association to the anus, provides easy access to the bacteria which results in colonization becoming easier, as the periurethral zone is at first infected by the intestinal bacteria).

Approximately 60% of women report at least once with symptomatic UTI through their lifespan. In the USA, about 10% of women report one or more incidents of symptomatic UTIs every year. Young, sexually-active women, in the age group of 18–24 years, have the maximum frequency of UTIs. Approximately 25% of these women show resolution of the symptoms by its own, but an identical number develop infection.[26] Studies estimate that every 1 in 3 women would have been infected with UTI by the time they reach 26 years of age. Approximately 25% to 50% of all young women may have had at least a single episode of UTI in their lives. Of these, at least 27% have a relapse in the six months of the first infection, whereas about 3% may have up to two relapses in the same time frame.[27]

The yearly frequency of UTI in the USA was assessed by means of self-reported UTI accounts for the preceding year by the National Health and Nutrition Examination Survey (NHANES). They noted that women with age ≥ 18 years had a projected prevalence of 12.6%; for men, however, their projected prevalence was only 3%.[28] Substantial bacteriuria has been reported in patients, with Nirmi, L., reporting an incidence level of up to 67.2%.[29] Among sexually active women, the frequency of cystitis is assessed at 0.5 to 0.7 occurrences per person per year.[30] Premenopausal married women reported an incidence of 4.6%, with nuns of similar age reporting an incidence level of only 0.7%. However, both pregnant and non-pregnant women reported comparable incidence levels of bacteriuria at 2%–7%. Bacteriuria is known to occur more frequently in women with diabetes, reporting a prevalence rate of 8%–

14%.[31] The positivity rate in urine cultures was 87.8% and 27.9% in females and males, respectively, in the study conducted by Razak, S.K, and Gurushantappa, V.[32] The occurrence of UTIs in men is considerably lesser than in women. In men it occurs predominantly in those with urologic anomalies in the structure of the system and in older adults. UTI, if left untreated, may result in kidney failure, septicemia, bacterial endocarditis, prostatitis, and infertility.[33]

Around 6%–15% of men (in the age group of 75 years and above) have been reported to display symptoms of bacteriuria. Documentation exists showing that the occurrence of UTI among the men in a community surges markedly on reaching 60 years. Such a finding may be associated with the impediments in the urethra that arise with age and the voiding dysfunction that is related to prostatic hypertrophy.[34] Adabara *et al.*[35] reported 75% of the urine samples collected from the population to display affirmative reports for bacterial development. Moreover, UTI incidence rates were noted to be 100% in the 30–39 age group, 94.4% in the 20–29 age group, and 64% in the 40–49 age group. Likewise, Mohammed Akram and Mohammed Shahid[36] described a 10.86% of bacterial incidence among the population members. The patients involved in the study were from a wide range of age groups. UTI cases were documented in greater frequency among the young and middle-aged patients (20 to 49 years; 51.04%). Pediatric patients (newborn to 19 years) comprised 36.45% of the incident cases and the elderly (50 to 80 years) constituted 16.66 %. Moreover, from a gender perspective, women were more prone to the disease, with a larger number of organisms being isolated from women (66.66%) rather than from men (33.34%). Patel *et al.*[37] noted a 65% incidence of UTI in their study. The study showed the highest frequency of UTI among the members of the age group of 61–70 years in males and 41–50 years in females. Mittal[38] reported that UTI occurrence was more common among women (in 72 of the studied; 53.3%) than men (in 63 of the studied; 46.7%). Moreover, they too attested to the fact that the highest incidence was noted among the young sexually active females, who were in the age range of 21 to 30 years (in 37 of the studied; 27%).

Increases in nosocomial UTI have been witnessed; such episodes mark the appearance of unfamiliar antibiotic resistant profiles. Most of these episodes are a result of nosocomial spread of outbreak strains via the hands of hospital employees. A projected 1 million cases of nosocomial UTIs transpire in the USA per annum, of

which 80% are ascribed to the usage of catheters. In fact, catheter-associated UTIs are the reason for 40% of all nosocomial infections.[39]

3.7. UTI during Pregnancy

UTIs are the primary bacterial infections detected in pregnant women. Examining and understanding the part played by these infections is of great urgency as UTI has been described to bring about complications such as pyelonephritis, chronic renal failure, premature delivery, and fetal mortality. The central cause for pregnant women being more disposed to both symptomatic and asymptomatic UTI is because of the physiological alterations that arise as a result of pregnancy. During pregnancy, woman's urethra tends to become shorter, with an expanded belly. Moreover, the compression created by the gravid uterus results in stasis of urine movement. These alterations in morphology obstruct appropriate upkeep of hygiene and normal urine flow, thereby making this population more prone to UTIs.[40]

Hence, it is mandatory that women during pregnancy undertake routine urine tests along with the usual customary tests and scans. Moreover, they are suggested to repeat the urine culturing (usually done in the third trimester of pregnancy) during all trimesters to circumvent complications.

UTI transpires roughly in 5%–10% of all pregnancies.[41-42] Pregnancy accompanied with ABU has been reported in 4%–7% of the cases, with pyelonephritis in 0.5%–2% of the cases.[43] Women with a history of RUTI, the presence of diabetes, and anatomical anomalies of the urinary tract are at an increased risk for UTI development during pregnancy.[44] Symptomatic infection in these women subjects them to a greater risk for developing ABU, which is the main cause for the progression of acute pyelonephritis.[45]

Rajaratnam *et al.*[46] reported the prevalence rate of UTI to be 13.2% among pregnant women. Mukherjee, Mandira *et al.*[47] collected 500 urine samples from asymptomatic pregnant women and screened for any significant display of bacteriuria. Among the urine samples tested, 22.6% displayed significant growth of the pathogenic bacteria. The average age for the distribution of ABU among the population was noted to be 25.14 ± 4.63 years, with the maximum belonging to the 22–29 years age group (68.2%).

Kasinathan and Thirumal[48] screened 174 women attending a antenatal care in a tertiary care hospital. They noted the occurrence of ABU among these women and

detected significant bacteriuria in 12.6% of the samples. Significant growth was noted in 59.1% of the samples that were from the age group of 26 to 30 years.

3.8. Pathophysiology of UTI

UTI has been considered to be linked with sexual activity by research conducted over several decades. Therefore, uncomplicated UTI has been nicknamed "honeymoon cystitis," as it more likely to appear in young sexually active women aged 18–29 years. Recent, regular vaginal intercourse carries the highest risk in developing UTI in this age group.[49]

Lower UTIs (also termed "cystitis") are considerably predominant in women due to their anatomic variances. UTIs characteristically begin with periurethral infection by an uropathogen living in the gut. This is followed by colonisation in the urethra and, finally ascent into the bladder or kidney through flagellar or pili-mediated locomotion. Bacterial attachment to the uroepithelium is crucial for the development of pathogenesis in patients with UTI. Host factors too play a role. These include genetic, biological, and behavioral dynamics. All of these factors incline young, healthy women to uncomplicated UTI. Infections occur when bacterial virulence mechanisms weaken the competent host immune machineries. Upper UTIs (commonly termed as "pyelonephritis"), progress when uropathogens mount to the kidneys through the ureters. Infections can transpire when bacteria bind to a kidney, or a bladder stone, or any medical device (urinary catheter for example), or when bodily impediment leads to their retaining in the urinary tract.

3.9. Predisposing Factors for development of UTI.

The non-pregnant adult women with no abnormalities in the urinary tract can rarely develop symptomatic cystitis or pyelonephritis even when they have bacteriuria. The collective influencing aspects for developing UTIs are enumerated in **Table 1**. The urethra is frequently populated with bacteria, and sexual contact can displace the bacteria into the female bladder. Besides, spermicides intensify the colonization potential of the vagina with uropathogens. Patients with physical anomalies develop UTIs mainly from impediments in the urine flow.[26]

Table 1: Predisposing risk factors for UTI.

RISK FACTORS FOR URINARY TRACT INFECTION		
Young adults	**Women**	Past history of UTI; Sexual intercourse; Diaphragm usage; Condom usage; Diabetes; Spermicide usage; Pregnancy; Parity; Sickle-cell anemia;
	Men	Instrumentation; No Circumcision; Homosexual activity.
Elderly people	**Women**	Estrogen deficiency; Incomplete emptying of bladder; Abnormalities of the urinary tract; Urinary catheterization; Functional or mental impairment;
	Men	Instrumentation; Prostatic disease; Benign enlargement; Calculi; Loss of bactericidal secretions.
Men and women with structural abnormalities		Extra-renal obstruction due to congenital abnormalities of the ureter or urethra, calculi, extrinsic ureteral compression, or benign prostate hypertrophy. Intra-renal obstacle due to nephrocalcinosis, uric acid nephropathy, polycystic kidney disease, hypokalemic or analgesic nephropathy, and renal injuries from sickle-cell disease.
Bacterial factors		Adherence factors; Siderophores; Bacteriocins; Toxins; Biofilm formation

Adapted from: Grabe, M *et al*.[50]

3.10. Host Defense

UTIs as a result of bacteria may arise by the role of various mechanisms. They are capable of binding, reproducing, inhabiting, colonizing, and invading the urinary tract. These processes occur in successive order. At each stage, the host would establish adaptive machineries to combat the bacteria. Additionally, at every single step there is a multifaceted interface between the bacteria and the host. This interaction either leads to disease progression or results in the infection process being

aborted. The halting of the infection takes place at any point from inception. These complicated state of affairs are best studied based on the unlikely positions that must be reached for the spread of infection.

Table 2: Host defense mechanism against UTI

Antibacterial Properties of Urine	Anti-adherence Mechanisms	Miscellaneous
Osmolality (extremes of high or low osmolalities inhibit bacterial growth).	Bacterial interference by normal flora present in the urethra, vagina, and periurethral region.	Mucopolysaccharide lining Of the bladder & urinary immunoglobulins.
High urea & organic acid concentration.	Urinary oligosaccharides (have the potential to detach epithelial bound *E. coli*).	Spontaneous exfoliation of uroepithelial cells with bacterial detachment
pH.	Tamm Horsfall protein: coating of *E. coli* by this protein might prevent attachment.	Mechanical flushing of micturition.

3.11. Etiology

Most infections in all inhabitants are instigated by uropathogenic *Escherichia coli* (UPEC). This organism has been detected in 75%–95% of both outpatient and inpatient UTIs, including community- and hospital-acquired UTIs.[6] The other organisms commonly associated are *Klebsiella pneumoniae*, *Enterobacter* species, and *Proteus* species. *Staphylococcus aureus*, *Staphylococcus saprophyticus*, and *Enterococcus faecalis* are the Gram-positive cocci that result in infection. Dispersal of uropathogens may vary according to the type of infection or the patient population type. Uropathogens causing different type of UTI are listed in the **Table 3**.

Table 3: Uropathogens classification as per the type of UTI.

Type	Common Uropathogens
Uncomplicated UTI	*E. coli* *S. saprophyticus* *Enterococcus spp*. *K. pneumoniae* *P. mirabilis*
Complicated UTI	*E. coli* *S. saprophyticus* *Enterococcus spp.* *K. pneumoniae* *P. mirabilis* MDR *E. coli* *P. aeruginosa* *Acinetobacter baumannii* *Enterococcus spp*. *Staphylococcus spp.*
CA-UTI	*P. mirabilis* *Morganella morganii* *Providencia stuartii* *C. urealyticum* *Candida spp.*

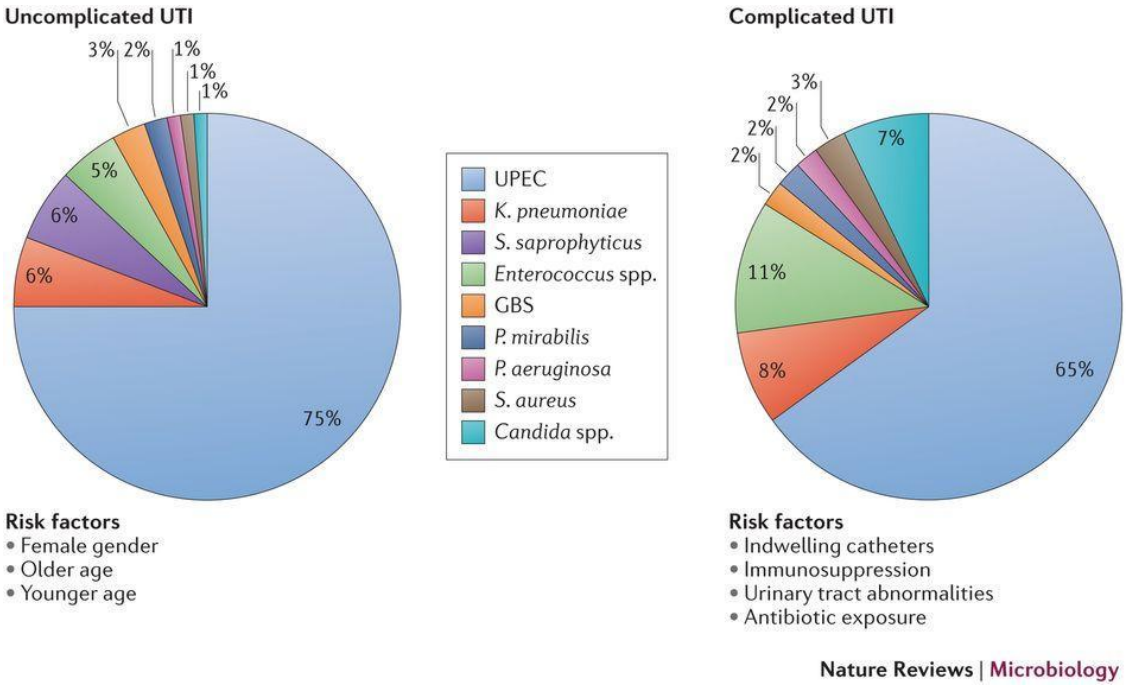


Fig 1: UTI Etiology.

Adapted from: Flores-Mireles, Ana L. *et al*.[51]

3.12. Complications of UTI

The complications in UTI usually result because of ascending infection. Because the infection spreads to other upper parts, it can lead to pyelonephritis, perinephric kidneys, intrarenal abscess, pyonephrosis, renal failure, and septicemia. Moreover, men who are affected with UTI report developing prostate-related infection.

3.13. Clinical Manifestations

Urinary-related manifestations include; Burning micturition, A recurrent or strong impulse to urinate, Leaking of urine, Sensation of partial bladder draining, Extreme urination in the night, Discomfort or pressure in the back or the lower part of the abdomen, Cloudy, dark, bloody, or abnormal smell in the urine, Tiredness or unsteady feeling, Tenderness of the infected parts, vaginal irritation, Fever or chills (a signal that the septicity may have extended to the kidneys), and Fatigue and Malaise.

3.14. Diagnosis of UTI

Symptoms registered in UTI are regulated by host factors that include time of life, sex, severity of the infection, and infected site. Moreover, patient's past history also has a bearing on the development of UTI. Therefore, data is collected pertaining to earlier UTI infections, sexual history, the usage of antibiotics, the presence of

deformity in the renal tract, the presence of diabetes, the usage of immunosuppressant's that include steroids, and the presence of a family history of UTI. All of the aforementioned data are imperative for the identification of UTI.

Characteristically, clean catch urine obtained midstream (MSU) is the ideal sample for testing of urine. Suprapubic aspiration is considered to be the best means to accumulate urine for testing in occasional settings. Nonetheless, urine collection by catheterization is considered as the finest practice as the collected urine samples have minimal contamination; customarily, however, preference for the method is minimal as it involves an invasive route

Urine analysis helps to provisionally diagnose UTI.[52] Pyuria may be identified and enumerated microscopically. This is done by estimating the leukocyte count in the urine sample. The occurrence of WBCs greater than 10 HPF-1 is an indication for infection.[53] Substantial pyuria is distinct by a leukocyte count of >5 HPF-1 in a fresh sample prior to centrifugation or a leukocyte count of >10 HPF-1 in a clean catch urine sample obtained midstream and prior to centrifugation.[54]

Bacteriuria may be identified by microscopy by employing Gram stain technique on urine samples that are not centrifuged and centrifuged.[55] Bacteriuria may be identified chemically using the nitrate test. The test necessitates a sample collected from the first urine passed in the morning. The test requires at least 4h, this is the time required for the bacteria to convert the present nitrate into nitrite. The reaction must produce levels of nitrite that are consistently detectable.

Culturing the MSU is universally acknowledged as the gold standard to diagnose UTI.[56] However, culturing should be done as soon as the samples are obtained usually within the first 2hr subsequent to collection. Even a slight delay, such as only of about 4 h, at room temperature can lead to 100-fold upsurge in the viable bacterial count. However, the specimens may be stored uncontaminated for longer duration by refrigeration.

Urine culturing with testing for antibiotic susceptibility should be done among pregnant women, empirical failure in treatment, or positive result or suspicion for upper tract septicity, complicated UTI, or RUTI. A sample must be attained for culturing before antimicrobial treatment.[52]

The investigative benchmarks for asymptomatic bacteriuria, uncomplicated cystitis, and catheter-associated UTI are comprehensively presented/ in **Table 4.**

Table 4: Diagnostic conditions for asymptomatic bacteriuria, uncomplicated cystitis, and catheter-associated UTI on the basis of the culturing of urine.

Asymptomatic Bacteriuria[34]	**Uncomplicated Cystitis**[57]	**Catheter-associated UTI**[58]
Two successive voided specimens of urine from women with $\geq 10^5$ CFU/mL with the identical bacterial strain	$\geq 10^3$ CFU/mL in a sample of urine from a patient displaying signs or indications of UTI	$\geq 10^3$ CFU/mL in a sample of urine from a patient displaying signs or indications of UTI
One voided urine sample in men along with $\geq 10^5$ CFU/mL of any one bacterial kind	…..	…..
One catheterized urine sample in either gender with $\geq 10^2$ CFU/mL of any one bacterial kind	…..	……

Abbreviation: Colony-forming unit (CFU)

3.15. Prophylaxis and Treatment

Numerous features require thought when choosing a suitable empirical antimicrobial element. These features include knowledge regarding the common causal pathogens, native resistance outlines, illness symptoms during severity. The outlines of resistance differ with the kind of patient population and the geographic location. Therefore, it is practical for physicians to be acquainted with their corresponding community-based or institution-based antibiogram.

Resistance to amoxicillin is highly prevalent among the populations; therefore, it is inapt to use this drug for empirical therapy.[59] Consequently, for empirical treatment, trimethoprim is as the foremost choicest drug for uncomplicated UTI; this is in spite of the fact that 10% to 20% of infections caused by *E. coli* are resistant to this drug.[18] As an alternative, Nitrofurantoin as well as cephalexin may be considered for the first-line treatment. For mild cases with uncomplicated pyelonephritis, the first-line treatment is done using Fluoroquinolones (FQs). Topical estrogen usage is restricted for short-duration treatments, especially in post-menopausal women.

Prophylactic treatment is recommended in the case of women having post-intercourse prophylaxis. Problematic UTIs that result because of extended-spectrum b-lactamase (ESBL) producing pathogens must be treated using carbapenems, an example of which is meropenem.[60] At present, researchers are trying to appraise the probable part of probiotics as well as vaccines for the inhibition of recurrent infections.[61]

3.16. Urinary Tract Infections due to *Escherichia coli*

Most infections in whole populations are instigated by Uropathogenic *E. coli*. As the most common pathogen in the urinary tract, it is detected in 74% of all outpatients with UTIs. It is the chief clinically applicable organism, responsible for 75% to 90% of all cases that have uncomplicated UTI isolates.[6] Amid otherwise healthy females aged 18–39 years, 80% of UTI are instigated by *E. coli*. UPEC are dissimilar from the *E. coli* strains that typically dwell in the gastrointestinal area by being better adjusted to live inside the urinary tract and evade the host's immune reaction.

3.16.1. Escherichia coli: General Properties & Characteristics

It was in the year 1885, *Theodor Escherich,* a German-Austrian Pediatrician, initially learned of this organism. He noted this organism's presence in feces obtained from healthy individuals. This genus fits in the bacterial group offhandedly identified as "coliforms," along with member of the Enterobacteriaceae family of the Gammaproteobacteria.[62]

3.16.1.1. Morphology

E. coli is an undersized, straight, bacillus that stains negatively with Gram's stain. It measures 1–3 µm x 0.4–0.7 µm, and is non-sporing. Almost all strains show motility, having peritrichous flagella. Frequently, the organism is fimbriated that arises singly, sometimes in pairs.

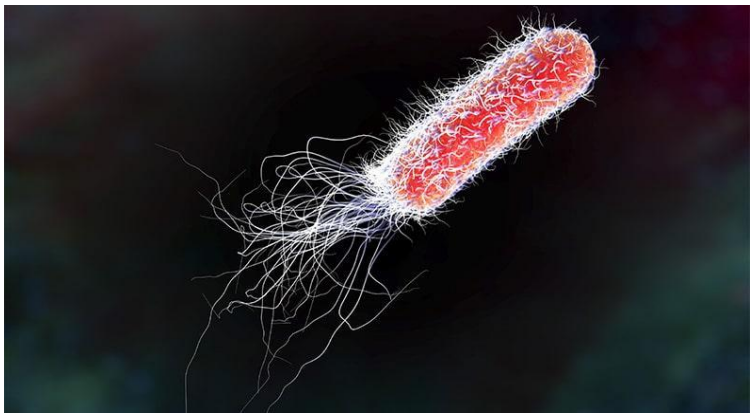


Fig 2: Electron microscope image of *Escherichia coli.*

3.16.1.2. Culture Characteristics

E. coli is an aerobic and facultative anaerobic organism. It cultivates on normal culture medium at optimal temperature of 37°C for 18–24 hours (10–40°C). It has been noted to grow rapidly in liquid cultures. Solubility of α-haemolysin is proven on blood agar. A few strains retain a cell-associated β-haemolysin activity, which is released on cell lysing. When cultured in MacConkey medium, its colonies appear pink because of lactose fermentation.[63]

3.16.1.3. Biochemical characteristics

Some significant biochemical features of *E. coli* have been concised in the following table.[64]

Table 5: Biochemical characteristics of *E. coli.*

Optimum growth temperature	37°C
IMViC Reactions:	+ + − −
Sugar fermentation tests:	
Glucose (acid+ gas); Lactose; Sucrose; mannitol; sorbitol;	90-100% Positive.
Lysine decarboxylase; motility;	76-89% positive.
Ornithine decarboxylase; dulcitol; salicin.	26-75% positive
Arginine dihydrolase.	11-25% positive
Other biochemical reactions:	
H2S; urease; phenylalanine deaminase; gelatin liquefaction; lipase; DNase.	0-11% positive

3.16.1.4. Antigenic characteristics

E. coli can be classified serologically based on numerous antigens - somatic (O) antigen, capsular (K) antigens, flagellar (H) antigens and fimbrial (F) antigen. O, K and H antigens are based on the typing scheme introduced by Kauffmann.[65] Currently; there are 173 types of O antigens, 80 types of K antigens, and 56 types of H antigens, which can all be further classified into partial antigens. The O, K, and H antigens can be found in nature in feasible amalgamations. Thus, the number of serotypes in *E. coli* is extremely high (≥50,000-100,000).

Somatic (O) antigens: O antigen is lipopolysaccharide (LPS) in nature. It is heat stable and key virulence factor accountable for endotoxic activity. It shields the

bacteria from phagocytosis and bactericidal effect of compliment. UPEC Serotypes O1, O2, O4, O6, O7, O8, O15, O16, O18, O21, O22, O25, O75 and O83 are responsible for majority of the UTIs.

Capsular (K) antigens:

K antigen is polysaccharide capsular antigen present on the envelope of a few strains of *E. coli*. It inhibits phagocytosis. It is expressed by only few strains of *E. coli*. Ex: those causing pyelonephritis.

Flagellar (H) antigens: It is flagellar antigen, which makes the bacteria motile.

3.16.2 Virulence factors (VFs):

Acknowledged VFs of UPEC include multiple surface virulence factors like, adhesions (P fimbriae, type 1 fimbriae, S and F1C fimbriae, and Dr-antigen specific adhesions), toxins (cytotoxic necrotizing factor and hemolysin), siderophores (enterobactin and aerobactin), lipopolysaccharide (LPS), polysaccharide coating (group II and group III) capsules and pathogenicity islands, serum resistance and biofilm.[66]

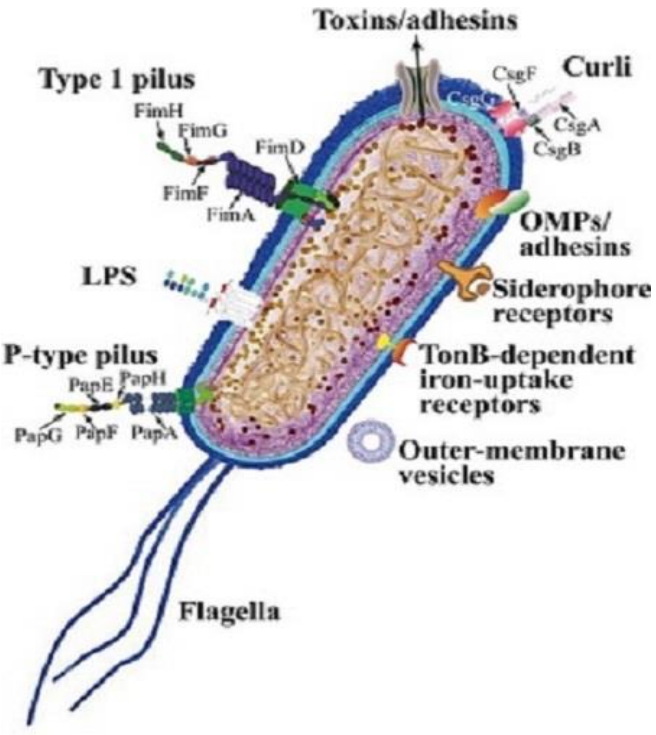


Fig 3: UPEC adhesins and harboring/motile structures concerned in transport, serum resistance, etc., OMP, Outer membrane proteins; LPS, lipopolysaccharide.

Adapted from: Terlizzi ME, Gribaudo G, Maffei ME.[67]

3.16.2.1. Adhesions:

Most of the pathogenic microbes like bacteria, yeasts, viruses have an adhesive property to fix them onto the host cell surface.[68] The adherence of *E. coli* to uroepithelial cells help in preventing the flush out of bacteria during the normal flow of body fluids in the renal system.[69] This attachment and fixation of a microbe to the host cell remains the first step in colonization and subsequent invasion in most of the pathogens.

Hemagglutination and Uroepithelial-Cell Adherence:

In 1970s, it was documented that UTI causing *E. coli* strains agglutinate erythrocytes even when mannose is present (Mannose resistant hemagglutination [MRHA]) and show adhesion to uroepithelial cell.[70-71] It was found that in individual strains of urinary isolates, there was a close association between MRHA and epithelial-cell adherence and both the properties are fimbriae mediated.

Role of fimbriae in Uroepithelial-Cell Adherence and MRHA:

The MRHA and epithelial cell adherence are mediated by fimbriae based on many studies.[72] Brinton coined the term "pili" (Latin: hairs) and used it for describing these fimbriae. The fimbriae are different from flagella and sex pili both in their morphology and function. While the flagella are longer, thicker and more flexible, they help only in motility and not in cell adhesion. Similarly, the sex pili are thicker and have an important role in conjugation. Brinton *et al*. studied the detailed structure of the type 1 fimbriae and found them to be 7nm diameter, 0.5 to 2 µm length and 0.2- to 0.25nm diameter across the central axis hole. They have repeated subunits with one subunit involved in eight turns. The structures of other types of fimbriae are almost similar.[73]

P fimbriae:

Initial investigations into the receptors of uroepithelial cells to which bacterial attachment happens led to other discoveries. In the presence of mannose, most of the strains with cell-adherence property also agglutinated RBC of the P blood group. Subsequently, the cell surface antigens of P-group RBC were implicated in these interactions. The purified fimbriae also have similar specificity to human erythrocytes, and hence these were labeled P-fimbriae.[74] The surface antigens of P-blood group RBC are members of oligosaccharides with Gal (αl-4) Gal-β moiety (Gal-Gal) either terminally or internally. They are also present in certain mammalian cells and have roles as glycosphingolipids. The P1 antigen is also detected among

human glycoproteins and certain bacterial cell surfaces. Much evidence points to the role of glycolipids with Gal-Gal moiety in *E. coli* cell adherence and are key determinants in binding.[75]

Structure and Genetics:

The P-fimbriae are composed of 103 subunits in a helical polymerization, with *papA* as the major subunit. The fimbrial tip consists of *papE, papF* and *papG* that are related to adherence.[76] The *pap* gene cluster located on the chromosome is multicistronic and encodes the fimbrial proteins and other accessory proteins.

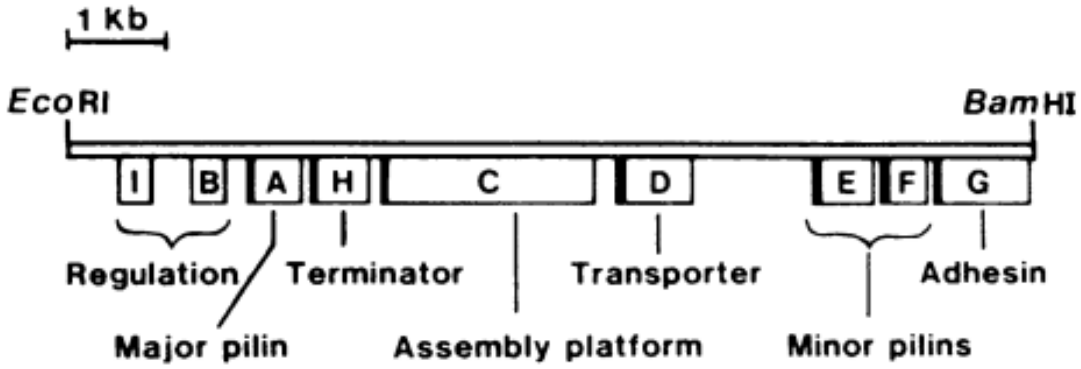


Figure 4: Overview of the pap gene cluster.

The P-fimbriae continue to be the key pathogenic component in developing UTI due to its activity in Gal-Gal specific adhesion between the host cells and bacteria. This facilitated colonization and initiation of the inflammatory responses which lead to establishment of infection and subsequent pathogenesis. Conversely, among the immune compromised host, the role of P-fimbriae is minimal.

X adhesins:

Dr Family of Adhesins:

Those urinary isolates which do not possess P-fimbriae would have MRHA (X adherence) in most of the cases. These MRHA can hybridize with non-fimbrial adhesin specific DNA probes. This can bind to various segments of Dr Blood group antigens.[77] All Dr Family of adhesins were initially detected among the UTI strains, and includes 075X adhesin, the afimbrial adhesin types I and III (AFA-I, AFA-III respectively) adhesins. The fimbrial and Dr Family of adhesins are structurally different: X-adhesins have a mesh-like coiled filamentous appearance.[78]

Receptors:

The Dr Antigen found on the decay-accelerating factor is involved in regulation of complement cascade preventing complement-mediated erythrocyte. They can bind various sites in urinary tract like Bowman's capsule, renal interstitium, tubular basement membrane, ureteral transitional epithelial cells and exfoliated epithelial cells in urine.[79]

Genetics:

The organization of adhesin gene clusters is similar among the Dr Family of proteins. They include five closely located genes one of which is structural gene that codes hemagglutinin. This 16-kDa protein and is variable among the different members of Dr Family and is different from *E. coli* fimbrial subunits. The number of copies of Dr gene cluster can vary, but many isolates have a single copy.[80]

S Fimbriae and FlC Fimbriae:

Some of the urinary strains can have two closely related adhesins- S fimbriae and F1C fimbriae. While the former binds to terminal sialyl-galactoside residues (hence the name), the F1C fimbriae are involved in binding with some renal or buccal epithelial cells only. The S fimbriae are known VFs in many animal experiments and UTI, and are commonly associated with meningitis and bacteremia.[81] The F1C fimbriae can be detected among 20% of urinary isolates upon subculture, and have an unclear in vivo pathogenicity role. One study couldn't identify F1C fimbriae among the twenty urine specimens.[82]

Mannose sensitive adhesins:

The type 1 fimbriae have roles in mannose-sensitive adherence in *E. coli* strains. The existence of these fimbriae are thus detected by using mannose-sensitive hemagglutination of guinea pigs erythrocytes in many clinical studies.[83]

Type 1 fimbriae of *Escherichia coli*:

E. coli has hair-like structures called fimbriae (belonging to type 1) that can bind with D-mannose present on various cell surfaces. These fimbriae can mediate binding of *E. coli* to mucosal inflammatory cells in both humans and other animals, and this property remains essential for bacterial infection.[84]

These fimbriae are arranged in peritrichous fashion, and each fimbria can be up to 1 mm long and 7nm wide. They are made of ~1000 molecules of FimA (a major subunit protein) that are arranged in a right-handed helix. In addition to FimA, the other minor components like FimF, FimG and FimH can also be detected. These

proteins are encoded by six genes in the 9.5 Kb fim locus present on the bacterial chromosome. This locus also encodes genes for biosynthesis and regulation of fimbriae.[85]

Role in virulence:

Almost all isolates of *E. coli* from UTI are characterized with the presence of type 1 fimbriae, and their attachment with host cells in the urinary tract may lead to cystitis. Also, their adherence to human polymorphonuclear leukocytes (hPMNLs) can promote renal scarring along with bacterial lysis. The fimbriae can further bind to Tamm-Horsfall protein (THP) allowing the bacterial clearances by the host even before colonization or infection are initiated.[86]

3.16.2.2. Iron and the virulence of *Escherichia coli*:

Aerobactin:

Bacterial Siderophores and the Superiority of Aerobactin:

Iron is one of the essential nutrients in all kinds of living cells, and has multiple roles like oxygen storage and transport, nucleic acid synthesis, electron transport and peroxide metabolism. *E. coli* uses hydroxamate siderophore aerobactin-mediated system (an iron chelation system) for iron absorption.[87] This system is advantageous over many other siderophores. This small protein has a molecular weight of 616Da and is synthesized by condensation of three molecules – two molecules of lysine and one molecule of citrate. The protein is encoded by an operon with five genes of which four genes are responsible for synthesis of aerobactin, and the fifth gene codes for outer membrane receptor protein (74 kDa).[88] The synthesis of aerobactin is regulated by fur (ferric uptake regulation) gene based on the intracellular iron concentration. After the aerobactin protein is secreted by *E. coli*, Fe3+ is extracted by this protein from the host iron-binding proteins which is then transported inside through the outer membrane receptor protein. This iron uptake by aerobactin is advantageous to the bacteria during growth in low-iron conditions like in serum and urine. Ferrichrome is not synthesized by *E. coli* and hence sufficient quantities of citrate cannot be transported in such low iron conditions.[89]

3.16.2.3. TOXINS:

Hemolysin: (Alpha Hemolysin)

The alpha-hemolysin (HlyA) is the cytolytic protein produced in most of the hemolytic *E. coli* strains. This toxin has non-apeptide repeat in its carboxy-terminal common to the RTX family of toxins.[90] It acts on many host cell types including

erythrocytes in warm-blooded animals and fish. The serum resistance in a few hemolytic *E. coli* is attributed to the increased production of this toxin.[91] Such persistence in host maybe one of the reasons for the development and recurrence of intestinal and extra-intestinal infections.

Cytotoxicity:

The human immune cells like polymorphonuclear granulocytes (PMNs), monocytes, mast cells, basophils and platelets involve in immune reaction towards *E. coli* α-hemolysin.[92] These cells, upon interacting with hemolysin, release mediators subsequent to degranulation of serotonin, histamine and enzymes or after the inception of 12-hydroxyicosatetraenoic acid (12-HETE) and leukotrienes. The release of common cytokines like interleukin-α, IL-6, and tumor necrosis factor (TNF) from human lymphocyte/monocyte/basophil cells (LMB) is also inhibited by the hemolysin.[93]

Genetics:

The production of hemolysin (110kDa) is mediated by four genes belonging to the hly operon. This operon is chromosomally-located among *E. coli* from human sources while among the zoonotic isolates, the operon is located on the plasmids. The secretion of this toxin does not involve cleavage of any signal peptide or bacterial cell lysis, and is hence unique among other *E. coli* toxins. Activation of this protein is mediated by a 20kDa intracellular HlyC85 protein before it is secreted, and requires several repeat regions in the carboxy end of HlyA.[94]

Role in virulence:

The α-Haemolysin is secreted outside to the membrane bi-layers and can't be found in the periplasmic space. It is produced in vivo by the pathogenic *E. coli* causing severe forms of UTI in humans. The toxin's provirulence activity can be multifactorial leading to release of iron from RBC, disruption of phagocytosis and direct cell toxicity.

Cytotoxic Necrotizing Factor (CNF):

The cytotoxic necrotizing factor (CNF) was described in 1983 by Caprioli *et al*.[95] and causes multinucleation ("cytotoxic") in vitro, and necrosis ("necrotizing") in rabbit skin. The 1014 residue long protein has multiple domains where N-terminal involves in receptor binding, and its carboxy terminal in catalysis. The C-terminal domain is specific and catalyses modification of cellular target within the host cytoplasm. The N- and C- terminal domains are separated by two short

transmembrane helices H1 (350-372) and H2 (387-412) which help in membrane translocation.[96]

The virulence factor CNF1 is involved in invasion into kidneys, and is produced in ~1/3rd of strains associated with pyelonephritis.[66] This protein is produced even in vitro, and is involved in stimulating actin stress fiber formation and membrane ruffle formation in Rho GTPase-dependent manner. This results in *E. coli* entry into the host cells, although the exact mechanism is unknown and is highly debated. Based on in vitro studies, CNF1 is found to interfere with PMN-phagocytosis and apoptosis of bladder epithelial cells, while in vivo, CNF1 can cause bladder cell exfoliation and subsequent bacterial access to deeper tissues.[97]

3.16.2.4. Capsular polysaccharide (K antigen)

E. coli has >80 capsular types which are linear polymers made of repeated carbohydrate subunits and can rarely has an amino acid or lipid components. Coating the cell, they interfere with the detection of O-antigen thereby providing immunity against host defense mechanisms.[98] Most of the extra intestinal pathogenic *E. coli* have thin, patchy capsules that are acidic, thermostable and highly anionic (common characteristics of group II polysaccharides). These group II polysaccharides can aggregate spontaneously due to the presence of phosphatidic acid group at their reducing end, and are allelic (Kl, K2, K5, K6, K12, K13, K14, K15, K20, K23, K51, K52, and K54). Among these, K1 is a polymer of repeating NeuNAc (sialic acid) linked 2-8 with random acetylation at C7 and C9, and is similar to that of *Neisseria meningitidis* group B in its structure, and to human trisialogangliosides having a NeuNAc(2-8)-NeuNAc moiety.[99]

Virulence:

The acidic capsular polysaccharides, in particular the K1 capsule, contribute to bacterial virulence by protecting them from phagocytosis and possibly from serum killing. The amount of polysaccharide is directly related to the degree of impairment of phagocytosis.[100] Among the human isolates, *E. coli* from urinary infections are encapsulated and can be typed using anti-K sera when compared with the fecal strains. The K types Kl, K2, K3, K5, K12, K13, K20, and K51 are more frequent among patients with cystitis and pyelonephritis than from fecal sources. Especially K1 and KS capsular types are found in 63% of women with pyelonephritis, while Kl, K2, K3, K12, and K13 account for 70% of isolates from girls with pyelonephritis.[101] While 018:K1 strains are rare in UTI, O1:K1 strains are common, and the association

between capsular types with UTI is influenced by the O group. Among the capsular types, K1 is frequently detected among both urinary strains.[99]

3.16.2.5. Serum resistance:

The complement-mediated lytic activity leads to bacterial cell death by normal human serum. The alternate pathway activated by the bacteria plays a significant role in serum killing when compared with the classic pathway. This is because, although the lipid A has the potential to activate classic pathway, its location in the cell (outer membrane) prevents access to complement components in intact bacteria (except in rough strains). The two pathways eventually merge in the formation of C5-9 membrane attack complex (MAC). MAC is a short hollow cylinder having 10nm and 22nm inner and outer diameters and has a molecular weight of $2x10^6$. Subsequent to its formation, MAC forms pores by inserting into the outer membrane through which lysozyme can target peptidoglycan of the cell wall. Later on MAC gets inserted into the inner membrane causing pores in the membrane and cell lysis.[102]

Bacterial resistance against serum killing can be multifactorial involving capsular polysaccharide, side chains of O-polysaccharide and cell-surface proteins, either alone or in synergistic mechanisms.[103]

3.16.2.6. Pathogenicity islands (PAIs):

The pathogenicity islands (PAIs) are the locations of the genes of UPEC and are first studied in these strains. Their sizes can range from 70-190kb (PAI-I and PAI-II) in 536 (06: K15) and J96 (04: K6), and are flanked by short direct repeats of 16-18bp length. These short repeats are implicated in their deletion during recombination at a frequency 10-3.[104] While PAI-I encodes the hly hemolysin, PAI-II codes for another hly operon along with prf (P-related fimbriae) pilus operon. The uropathogenic J96 strain has two PAI that are inserted in two different tRNA genes (pheV at 64 min for PAI-IV and pheR at 94 min for PAI-V).[105]

The *E. coli* J96 has several well-characterized factors that are involved in extra intestinal disease, and are chromosomally encoded. Some of these factors are the two types of P fimbriae (*Pap* and *Prs*), hemolysin, and the cytotoxic necrotizing factor 1 (CNF-1) toxin. The CNF-1 is produced in ~1/3rd of UPEC and in some gastrointestinal *E. coli*, and can induce major changes in the epithelial cell cytoskeleton including actin reorganization and membrane ruffling.[106] The strain *E. coli* CFT073 has the smallest (50kb) of the known five PAIs encoding a hly-operon, and is inserted near the metV gene.

3.16.2.7. Biofilm:

Biofilms are heterogenous, structured, matrix-encased bacterial communities formed due to novel behaviours of planktonic cells during bacterial interactions with a surface. These biofilm infections are important as they help in overcoming stress, antibiotics and host defense machinery.[107-108] The term was first coined in 1978, and bacteria were found to develop biofilms on virtually all the surfaces like soil, living tissues, medical devices and plumbing.[109]

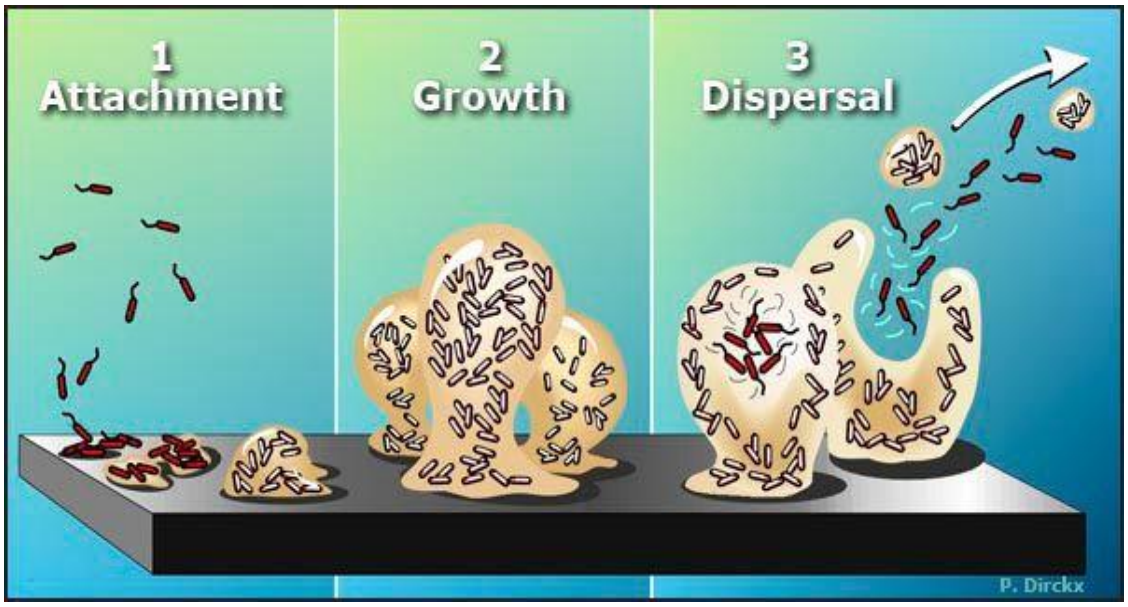


Fig.5. Life cycle of biofilm communities

1. Free-floating, bacteria encounter a submerged surface and attach within minutes to produce slimy extracellular polymeric substances (EPS) and colonize the surface.
2. A complex, three-dimensional structure develop within hours resulting in Biofilm communities.
3. Biofilms propagate through detachment of small or large clumps of cells, allowing them to attach to a new surface.

The composition of biofilm is complex, including water along with many biological components like proteins, lipids/phospholipids, nucleic acids, absorbed nutrients, metabolites, and exopolysaccaride polymers (like cellulose and colanic acid) along with bacteria.[110] These biofilms form when bacteria excrete a slimy, glue-like substance after their adherence, and can help in anchoring them to variety of substances including metals, medical implant materials and living tissues.

The bacterial cells initially adhere to the surfaces through van der Waals forces that are weak and reversible. The permanent adhesion is usually mediated by the cell adhesion molecules like proteins on their surfaces in a process called cell adhesion. They can then help in colonization of other pathogens by providing more diverse adhesion sites. They can also increase the matrix production which can subsequently help those pathogens that are unable to adhere to the cells directly.

The stage of biofilm development succeeds the biofilm formation and involves cell division. Various cells in the biofilm can have different patterns of gene expression, and lead to slow growth of biofilms in diverse locations. Hence most of the biofilm infections produce symptoms very late. Once established, the planktonic bacteria within the biofilm can leave or multiply rapidly and disperse, and these patterns are naturally formed. Due to the presence of the matrix, the host immune system cannot be able to initiate a defense mechanism.[111]

Biofilm can cause or exacerbate a variety of infections like periodontitis, cystic fibrosis pneumonia, recurrent tonsillitis, device related infections and chronic otitis media.[107] It also has a role in pathogenesis of UTI and RUTI, and is believed to play a role in persistent colonization as in ABU. The extracellular matrix prevents the activity of most of the antibiotics by causing limited penetration and due to high concentration of the polysaccharides. These can lead to development of chronic infections and bacteria that can produce biofilms can have increased resistance to antibiotics which further lead to recurrent infections.[13]

The important factors involved in biofilm formation are discovered by various molecular and genetic studies especially in *E. coli* K12 strains. Flagella are required in movement of bacteria to the surfaces, but its presence is not an absolute requirement. Many types of fimbriae (mainly type1 fimbriae, curli fimbriae) and conjugative pili help in attachment to cell surface and biofilm maturation. The outer membrane proteins like antigen 43 (A43) are involved in bacterial adhesion with each other and formation of biofilm architecture.[112]

Table 6: Different features of UPEC virulence characteristics.[113]

	Type of Virulence Factors	Virulence factors	Gene	Role	Association in UTIs
Superficial virulence factors	**Afimbrial Adhesins**	AFA-I, AFA-II, AFA-III, AFA-IV, AFA-V, AFA-VII, AFA-VIII	*afa*	Adhesion, Colonization, High tropism to kidney	Chronic cystitis/pyelonephritis, Recurrent cystitis/pyelonephritis, rarely in ABU
		Curli	*csg*	Adhesion, Colonization, Biofilm formation	All kinds of UTIs
	Fimbrial Adhesins	P fimbriae	*pap*	Adhesion, Colonization, Cytokine production, Invasion, Inflammation, Pain, Renal tropism, Pathogenesis	Most acknowledged in upper UTIs, Acute UTIs, Acute Pyelonephritis, renal failures, Acute Cystitis, Rarely in ABU
		Type 1 fimbriae	chaperone-	Adhesion, Biofilm	All types of UTIs

	Type of Virulence Factors	**Virulence factors**	**Gene**	**Role**	**Association in UTIs**
			usher class fimbrial genes: *fim*	formation, Colonization, Growth, Invasion, Rapid replication, Inflammation, Intracellular survival	
		Type 3 fimbriae	chaperone-usher class fimbrial genes: *mrk*	Biofilm formation	Generally in catheter associated UTIs
		Dr	*dra*	Adhesion, High tropism to kidney	Persistent cystitis/pyelonephritis, Repeated cystitis/pyelonephritis, rarely in ABU
		F1C	*foc*	Adhesion, Biofilm formation, Colonization	All classes of UTIs, renal failure

	Type of Virulence Factors	**Virulence factors**	**Gene**	**Role**	**Association in UTIs**
		S fimbriae	*sfa*	Adhesion, Colonization, Dissemination, Bacterial ascending factor	Meningitis, Septicemia, Mostly severe upper UTIs
		F9 fimbriae	chaperone-usher class fimbrial genes: *c*	Adhesion Biofilm formation	UTIs, commonly pyelonephritis
		chaperone-usher class fimbrial genes: *auf*	Adhesion Biofilm formation	All classes of UTIs	
	Capsule	K polysaccharides including: K1, K2, K3, K5, K12, K13, K20, K51/KspMT	*kps*	Adhesion, Biofilm formation, Antimicrobial non-susceptibility, Anti-phagocytosis, Anti-serum and anti-bactericidal	All categories of UTIs

	Type of Virulence Factors	**Virulence factors**	**Gene**	**Role**	**Association in UTIs**
				complement activity	
	Lipopolysaccharide	O serogroups UPEC including: O1, O2, O4, O6-O8, O15, O16, O18, O21, O22, O25, O75, O83	*rf*	Adjuvant, Anti-phagocytosis, Anti bactericidal complement activity, Induction of human cytokine production, Endotoxin activity, Acute inflammation pain	All categories of UTIs
	Motility	Flagella protein H antigen	*flic*	Biofilm development, Colonization, Facilitated ascending (dissemination), Invasion, Chemotaxis	Primarily cystitis and pyelonephritis

	Type of Virulence Factors	**Virulence factors**	**Gene**	**Role**	**Association in UTIs**
	Outer membrane proteins	OmpA, OmpC, OmpF, OmpT, OmpX	*ompA, ompC, ompF, ompT ompX*	Porin, transportation, Facilitating factor for UPEC intracellular virulence	Primarily persistent UTIs
	Serum Resistance	Serum resistant proteins	*iss, traT, cvaC*	Neutralization of anti-bactericidal effect of serum	Commonly cystitis and pyelonephritis, bacteremia
	Siderophores	Aerobactin/Enterobactin/ Salmochelin/Yersiniabactin	*aer,iutA/entS/ iroN/ fyuA, ybtP, ybtQ*	Growth, Iron uptake	Severe UTIs
		Hemin uptake system	*chuA, hma, ireA, iha,iutA*	Biofilm formation, Growth, Iron uptake	All categories of UTIs
	Autotransporter adhesins (Type V secretion system proteins)	Secreted Auto transporter Toxin (SAT)	*sat*	Colonization, Cytotoxic effect on bladder and kidney, Pathogenesis	Commonly pyelonephritis, UTIs

	Type of Virulence Factors	**Virulence factors**	**Gene**	**Role**	**Association in UTIs**
		Ag43 (outer membrane protein antigen), Upab, UpaC, Upag and UpaH proteins	*ompA*, *upab*, *upaC*, *upaG*, *upaH*	Adhesion, Biofilm development, Intracellular survival, Long term infection	Persistent UTIs
	Toxins	Cytolethal Distending Toxin	*cdt*	Cytolethal factor.	UTIs
		Cytotoxic Necrotizing Factor 1 (CNF1)	*cnf1*	Invasion, Apoptosis in cell bladder, Host cell malfunction	Grievous UTIs
		α-Haemolysin	*hlyA*	Host cell lysis, Hemolysis, Growth, Adhesion, Inflammation	Mostly in serious and symptomatic UTIs
		Serine protease autotransporter toxin (Sat)	*sat*	Cytotoxic effect on bladder and kidney	Mostly pyelonephritis
		Vacuolating autotransporter toxin (Vat)	*vat*	Cytotoxic effect on bladder and kidney endothelial tissue	Mostly pyelonephritis

	Type of Virulence Factors	**Virulence factors**	**Gene**	**Role**	**Association in UTIs**
		TosA	*tosA*	Adhesion, Colonization	UTIs
		Shigella enterotoxin-1	*set-1*	Invasion, Inflammation	Severe UTIs
		Arginine succinyltransferase	*astA*	Invasion, Cytotoxin, Inflammation	Critical UTIs
		Toll/interleukin receptor domain containing protein (Tcp)	*tcpC*	Bacterial existence, Human avoidance system, Cytopathic effect on kidney	Largely pyelonephritis
	Multi-functional factors	Usp	*usp*	Invasive, Inflammation	Severe UTIs

3.16.3. Epidemiology of *E. coli* UTI:

UPEC remains a primary pathogen causing community-acquired UTI, with 20% of women above 18 years of age being affected at least once in their lifetime. Overall, it can cause community-acquired UTI in 70-95% of the patients and 50% of all the nosocomial UTIs. It thus accounts for morbidity and mortality in substantive numbers apart from the economic implications. The infections can also be recurring with multiple episodes which can further increase these problems. The human intestinal tract remains the main reservoir for this pathogen, and multiple virulence factors are usually involved in proceeding to invasion.[114]

In Bangalore, Eshwarappa *et al.*[115] reported *E. coli* to be the predominant pathogen (341/510; 67%) among the patients and is more frequent in the elderly age group above 50 years of age (57.4%). In this study, only 9.8% of the children had *E. coli* infections, and the mean age was 55.47±21.51 years among complicated UTI with slight male predominance (1.63:1).

The expression of P-fimbriae also depends on the disease present and was investigated by Brauner *et al*.[116] The expression of P-fimbriae was higher in 70% of the patients with pyelonephritis, 71% (177/248) of the patients with bacteremia due to urosepsis and 36% of the cystitis patients. Similarly, 24% of ABU patients and 19% of the fecal strains only exhibited elevated P-fimbriae expression levels. Among isolates causing bacteremia from other foci of infection, only 28% (28/99) had elevated expression.

Raksha *et al.*[117] studied the MRHA and serum resistance in *E. coli* causing UTI among 220 patients from Bangalore. They found 41.4% (91/220) of the strains to be hemolytic, 30.9% (68/220) having MRHA, 26.4% (58/220) had hydrophobic cell surfaces, and 32.7% were serum-resistant. Thus a significantly higher number of isolates from cases were positive for hemolysis and MRHA. In this study, 14 atypical *E. coli* were from the urine samples and were positive for one or the other virulence markers.

Jadhav *et al.*[118] analyzed 150 UPEC isolates from Pune, among which 60% were α-hemolytic and 55% were serum-resistant. About 45% of the isolates had type 1 fimbriae. Among these 150 isolates, 21.3% were ESBL producers which predominantly belonged to serotype O25 (31.3%). Also, the ESBL-positive strains were associated with hemolysis than the ESBL-negative strains (65.6% vs. 58.5% respectively).

In a study from Vellore, the phenotypic characteristics of 163 UPEC were studied and 19% were found to be hemolytic and 20% were atypical.[16]

Soto *et al*.[119] studied *E. coli* from different diseases (75 from pyelonephritis, 44 from cystitis and 32 from prostatitis) in relation to their in vitro biofilm production, their phylogenetic groups, positivity for several uro-virulence factors and nalidixic acid resistance. The biofilm production was significantly higher in strains from prostatitis which also exhibited hemolysin production. The biofilm producing strains also had elevated expression of type 1 fimbriae.

A majority of the UPEC isolates belonged to group B2 (90.8%; 59/65) while only 34.3% (23/67) of the isolates from rectum were from the same group. In this study, the virulence factors were common among the isolates from urine than rectum. Johnson *et al*.[120]

Similar findings were reported by Bonacorsi *et al*.[121] where 76% of the 83 urine isolates from France belonged to phylogroup B2. The commonest virulence factor was *papC* (87%) followed by *iutA* (84%), *cnf1* (25%), K1 (40%) and *hly* (42%). Among the toxin-encoding genes, 34% of the isolates possessed *usp* while 26% and 12% harbored *hlyA* and cnf1 respectively. The presence of *hlyA* was correlated with hemolysis as 26% of the strains had the gene and 27% were hemolytic. Also 56% of the strains were biofilm producers, but none of the virulence factors either alone or in combination were associated with biofilm formation. Thus the biofilm production and multivirulent B2 group were not associated significantly, but it had association with other phylogroups like B1 and D. The presence of virulence factors was also assessed among the immunocompromised patients, and *usp, papC* and the adhesin-encoding *sfa/foc* only were associated statistically.

Kudinha *et al*.[122] detected 180 UPEC isolates identified in Australia. Among them, 173 (96%) were positive for the *fimH* gene. The next gene that was most frequent was *papC* 144 (80%), followed by *hlyA* 122 (68%), *iutA* 121 (67%), and *cnf1* 68 (38%)

Farshad *et al*.[123] considered 96 UPEC types of strains from Iran. On PCR analysis, *cnf1* was identified to be more prevalent (22.9%) in comparison to *hly* (13.5%). *Pap* was established in 27.1% of the isolates and *sfa* in 14.6% of the isolates. Among all isolates, about 32 (33%) showed the presence of at least a single gene and 14 (6.3%) exposed the presence of all four gene types.

3.17. Antimicrobial Activity

Antimicrobials are classes of compounds that can either kill (microbicidal) or halt the growth (biostatic) of microbes. They are usually classified according to their spectrum of activity against each microbial class (e.g. antibacterial and antifungal drugs). The use of antimicrobials to treat microbial infections is called antimicrobial chemotherapy, while their use to prevent such infections is termed antimicrobial prophylaxis.

3.17.1. Classification of Antimicrobial Agents

Antibacterial agents are classified based on their structure, mode of action and target specificity as narrow or broad-spectrum antibiotics. These agents may have either a bactericidal activity by targeting the cell wall (penicillins and cephalosporins); cell membranes (polymixins); bacterial enzymes (quinolones and sulfonamides); or bacteriostatic activity by targeting protein synthesis (aminoglycosides, macrolides, and tetracyclines). Owing to the rise in antimicrobial resistance, newer class of antibiotics have been developed which include, cyclic lipopeptides (e.g. daptomycin), oxazolidinones (e.g. linezolid) and glycylcyclines (e.g. tigecycline).[124] On the basis of pharmacological properties, the antibiotics are convened into a total of 11 classes.

Table 7: Categorization of Antibiotics established on Pharmacological Characteristics.

Antibiotics class	Action	Examples
Penicillin	Bactericidal	amoxicillin, ampicillin, augmentin
Cephalosporin	Bactericidal	cefixime, cefotaxime, ceftriaxone, ceftazidime.
Macrolide	Bacteriostatic	azithromycin, erythromycin
Tetracycline	Bacteriostatic	tetracycline, doxycycline, unidox
Aminoglycosides	High toxicity	amikacin, gentamicin, tobramycin
Glycopeptides	Bactericidal & bacteriostatic	vancomycin, teicoplanin
Lincosamides	Bactericidal & bacteriostatic	lincomycin, clindamycin
Fluoroquinolones	Bactericidal	ciprofloxacin, ofloxacin, norfloxacin
Nitrofurans	Bactericidal	nitrofurantoin
Monobactams	Bactericidal & bacteriostatic	aztreonam
Carbapenems	Bactericidal & bacteriostatic	imipenem, meropenem

3.17.2. Mode of Action of Antimicrobial Agents

Antibiotics function by numerous diverse mechanisms; however, all launch an attack on the critical parts or pathways of a bacterial cell. They act by exploiting differences between pathogens and human cells.[125] The methods of action of the antibacterial agents have been listed under Table.

Table 8: Mechanism of action of antibacterial agents.

Target site	Antibiotic group	Example
Obtrusion in cell Wall synthesis.	β – Lactams	Penicillins, cephalosporins, carbapenems, monobactams
	Glycopeptides.	vancomycin, teicoplanin.
Retardation of Protein synthesis.	50s ribosomal binding subunit.	Macrolides, chloramphenicol, clindamycin, quinupristin dalfopristin, linezolid.
	30s ribosomal binding subunit.	Aminoglycosides, tetracyclines
	Bacterial isoleucyl-tRNA synthesis binding.	Mupirocin
Obtrusion in nucleic acid synthesis.	DNA synthesis inhibition.	Fluoroquinolones
	RNA synthesis inhibition.	Rifampicin
Obstruction in metabolic pathway.	Metabolism interference.	Sulfonamides, folic acid analogues
Disturbance of bacterial membrane structure.	Dissolution of the membrane.	Polymyxins, daptomycin

3.17.3. Antibiotic Resistance.

Antibiotic resistance refers to a kind of drug resistance wherein bacteria have the potential of surviving contact with an antibiotic. Though an unprompted genetic alteration in bacteria might result in resistance toward antimicrobial preparations, genes that result in resistance are able to be transmitted between bacteria. This is done horizontally during conjugation, transduction, or transformation. Consequently, a genetic factor aimed at antibiotic resistance evolving through natural selection might be mutual. Numerous antibiotic resistant genes exist in plasmids, enabling their

transmission. If any bacterium transfers numerous drug resistance genes, it is termed as MDR or, more commonly, a superbug or super-bacterium.[126]

Genetic factors leading to resistance against antibiotics are primordial.[127] Nevertheless, the occurrence of antibiotic-resistant bacteria causing clinical infections is due to antibiotic usage for both human and animals. Any usage of antibiotics can lead to selective burden in a populace of bacteria by making the non-susceptible bacteria to flourish and susceptible bacteria to perish. This increase in non-susceptible bacteria led to exploring for unconventional therapy. Nevertheless, despite a impetus for new antibiotic treatments, a sustained weakening in the quantity has been noted among newly permitted drugs.[128]

3.17.4. Mechanism of antimicrobial resistance:

The resistance that arises for antibiotics may be naturally present or acquired. This resistance may be transmitted horizontally or vertically. The genes that encode such resistance (called resistance genes) have been known to be usually located in the transposons. As a result, resistance genes are usually transmitted via plasmids. A few transposons may contain complex DNA sections called "integron," which is a site proficient in incorporating diverse AMR genes. Consequently, it confers manifold AMR to the bacteria. As soon as a mutation transpires and effects a modification in bacterial genome, the genetic substance may be transported across bacterial cells using techniques such as conjugation, transformation, and transduction.[129]

Factors supporting antimicrobial resistance have been noted to be as follows: adjacent infected individuals; shifting into or from acute care hospitals, poor hand hygiene, incorrect usage of antibiotics, diminished immune status, operational disability, the usage of any invasive device; and chronic degenerative illness. In a few developing countries, treatment prior to laboratory investigations increases the possibilities of acquiring resistance for that particular antibiotic, particularly when administered during long periods of time.[130-131] Inadequate dose and/or only monotherapy favors the arise of resistance in definite infections.

The limited number of mechanisms that are recognized are presented in Table.

Table 9: Mechanism of antimicrobial resistance:

Antibiotics	Resistance mechanism
Chloramphenicol	Diminished intake into cell
Tetracycline	Active effluence from the cell
β-lactams, erythromycin, lincomycin.	Abolishes the binding of antibiotic to cell target.
β-lactams, aminoglycosides, chloramphenicol	Enzymatic cleavage or alterations to deactivate antibiotic agent.
Sulfonamides, trimethoprim	Metabolic diversion of impeded reaction.

3.17.5. Mechanism of Antibiotic resistance in *Escherichia coli:*

E. coli presents antimicrobial resistance through either of the mechanisms – degrading or structurally modifying of antibiotic, decreasing cell permeability to the antibiotics, employing efflux pumps, modifying cell targets of the drug and enzymatically modifying the antibiotics.[132]

The antimicrobial resistance in *E. coli* among diverse sources (human, zoonotic or environmental) is one of the major public health concerns in the 21st century. The ESBL producing *E. coli* are now pathogens associated with majority of nosocomial infections and can also be detected from community settings.[133] This is because genes that encode ESBL production are present on large plasmids which may harbor genes conferring resistance to other antibiotics as well.[134] There has been a documented rise in FQ resistance among *E. coli* worldwide since 1990s, and use of FQ is now considered as a risk factor for acquiring such resistant strains especially in long-term hospitalized patients, which may eventually lead to treatment failure. Also, the multi-resistance (norfloxacin non-susceptibility along with two or more other antibiotics) has increased recently. It is also found that quinolone resistance has fitness costs for VFs and can reduce their expression or decrease their prevalence.[135-136] As the quinolone resistance involves mutation in gyrA (at codon 83), this may affect DNA supercoiling and concomitant changes in gene expression. The deletion or increased transposition of PAIs was also implicated in the above observation. Soto *et al.*[137] found loss of virulence genes (either partial or total) within PAIs when sub inhibitory concentrations of ciprofloxacin were used.

Among the complicated cases, ESBL-producing *E. coli* was commonest in a study by Eshwarappa *et al.*[115] while 50% of the uncomplicated cases had ESBL-negative strains. The carbapenem-resistance was least common (3.9%) while amikacin and nitrofurantoin resistance was detected in 28% and 28.6% respectively. Quinolone resistance was present in 74.1% of the isolates. Similar trends in antimicrobial resistance was seen among the isolates from other infections (4.1%, 29% and 31.2% of the isolates were resistant to carbapenems, amikacin and nitrofurantoin respectively). The nitrofurantoin resistance was least among the isolates from uncomplicated UTI (5.8%).

Higher resistance rates to ampicillin/sulbactam, aminopenicillins, tetracycline and amoxicillin/clavulanate were observed among *E. coli* in a study by Mladin *et al.*[138] and resistance to quinolones, carboxipenicillins, aminoglycosides and cotrimoxazole was frequently detected.

Poovendran *et al.*[139] found that ESBL-producing *E. coli* are responsible for community and hospital-acquired UTI, and resistance rates were higher among ESBL-producing *E. coli* in yet another study by Naik and Desai.[140]

Among the UTI cases studied by Mahesh *et al.*[141] 65.7% had *E. coli* as a pathogen, and 66.8% were ESBL-positive and 99% of these ESBL-positive isolates were also resistant to first generation FQs. On the other hand, carbapenem-resistance was least common. Similarly, high resistance to beta-lactams, FQs and aminoglycosides were detected among Gram-negative bacilli and Gram-positive cocci by Rizvi *et al.*[142]

Among the 150 UPEC isolates studied by Jadhav *et al.*[118] 32 were ESBL-positive, while 57.3% were sensitive to nitrofurantoin, 52% and 49% were sensitive for ciprofloxacin and nalidixic acid respectively. Resistance to amoxicillin predominated (67.3%) followed by non-susceptibility to tetracycline (61.3%) and cefotaxime (45.3%). Resistance to ciprofloxacin was present in 53.1% of the ESBL-producers, and nitrofurantoin and ciprofloxacin were superior to amoxicillin and cotrimoxazole in efficacy. All the uropathogens were resistant to any of the antibiotics tested.

From Vellore, Rebecca *et al.*[16] found 73% if the 163 UPEC isolates to be resistant to nitrofurantoin, followed by amikacin (70%), gentamicin (60%), ciprofloxacin (38%) and cotrimoxazole (37%).

3.17.6. Association of virulence factors and antimicrobial resistance:

A high proportion of ESBL-producers (31.3%) in Pune belonged to serotype O25 while the total number of ESBL-producers was 32 of the 150 UPEC strains. These ESBL-producers were also associated with hemolysis more frequently than the non-producers (65.6% vs. 58.5%).[118]

Among the UPEC strains, *traT* was most frequently expressed in a study by Neamati *et al*.[143] they found 74% of the UPEC expressing this gene, while 61.3% had PAI markers and 16.6% harbored the *pap* gene. The resistance to antibiotics was also higher among the study isolates, however, authors did not find out the association between virulence genes and antibiotic resistance.

Reports by Ghosh A and Mukherjee M.[144] also indicate a high prevalence of PAI markers among the 12 virulence genes in both symptomatic and asymptomatic *E. coli*.

CHAPTER 4

MATERIALS & METHODS

CHAPTER 4

MATERIALS & METHODS

4.1. Study design and Study duration:

This work is a cross sectional study and conducted from year 2012 to 2015.

4.2. Patients and participants:

The study population consists of patients with suspected UTI symptoms from urban and rural area of Bidar attending Outpatient departments of Medicine, OBG, & Surgery of Bidar Institute of Medical sciences (BRIMS) Teaching hospital Bidar. This study protocol was approved by Institute's Ethics Committee, and samples were collected after obtaining informed consent from the patients.

4.3. Sample size calculation:

The sample size (n=1000) was estimated with an expected prevalence of *E. coli* as 15% with 4% absolute precision and 95% confidence interval. An interim analysis was carried out and the estimate from the interim analysis was used to modify the sample size.

4.4. Sampling methods:

Convenience sampling method was adapted.

4.5. Patients Inclusion criteria:

Patients of all age groups and both sexes complaining of burning micturition and other associated illness were included in the study.

4.6. Patients Exclusion criteria:

Patients suspected of having a UTI, who had not received antimicrobials within the previous 48hours and patients with clinical symptoms of UTI but cultures negative were excluded from the study.

4.7. Isolation and Identification:

4.7.1. Sample collection:

Approximately 50 ml of a clean-catch, midstream urine sample (MSU) was collected in a sterile, wide-mouth, leak-proof plastic container after recording information about patient's age, sex, brief clinical history and demographic details.

4.7.2. Processing of samples:

- Samples were cultured on Blood & MacConkey agar to isolate *E. coli*, as per the recommendations of Kass.[145] At 37°C the culture plates were incubated for

24 hours and in case of culture negative, further plates incubated for 48h before considering it as no growth.

- Colony morphology and standard biochemical tests were used to identify the isolates.
- Diagnosis of UTI was made when *E. coli* was grown at the concentration of $>10^5$ colony forming unit (CFU)/ml of urine.

4.8. Antimicrobial susceptibility testing:

Antibiotics (obtained from HiMedia Laboratories, Mumbai, India) - ampicillin (AMP 10mcg), amikacin (AK 30 mcg), amoxicillin-clavulanic acid (AMC 30 mcg), aztreonam (AT 30 mcg), cefriaxone (CTR 30 mcg) cefuroxime (CXM 30mcg), cefepime (CPM 30mcg) ciprofloxacin (CIP 5mcg), chloramphenicol (C 30mcg) gentamicin (GEN 10mcg), imipenem (IPM 10mcg), nitrofurantoin (NIT 300mcg), norfloxacin (NX 10mcg), and piperacillin-tazobactam (PIT 100/10 mcg) tested according to Kirby Bauer's disc diffusion method as per CLSI guidelines.[146]

Quality control:

For antimicrobial susceptibility testing, the CLSI control strain of *Escherichia coli* ATCC 25922 was employed as control.

4.9. In-vitro Biofilm Detection:

Three different methods were employed for *In vitro* detection of biofilm i.e., Tube adherence method, Congo red agar method (CRA) and Tissue culture plate method (TCP).

4.9.1. Tube adherence method:

This is a qualitative method for biofilm detection developed by Christensen *et al*.[147] A loop full of test organisms was inoculated in 10 ml of Trypticase soy broth + 1% glucose in test tubes which are then incubated at 37ºC for 24 h. After this, tubes were decanted, washed with phosphate buffer saline (pH 7.3) and dried. Crystal violet (0.1%) was then used for staining, and excess stain was removed using deionized water. After drying the tubes in inverted position, the scoring for tube method was done by comparing with the results of the control strains. An isolate was considered biofilm-producer if a visible film lined the wall and the bottom of the tube. The amount of biofilm formed was scored as: 1-weak/none, 2-moderate and 3-high/strong. The experiment was performed in triplicate and repeated three times.

4.9.2. Congo Red Agar method:

Freeman *et al.*[148] have described a simple qualitative method to detect biofilm production by using Congo Red Agar (CRA) medium. CRA medium was prepared with brain heart infusion broth with 37 g/L, sucrose 50 g/L, agar No. 1 10 g/L (Hi Media Laboratories, Mumbai, India) and Congo red indicator (Nice chemicals Cochin) 8 g/L. Initially, an aqueous solution of Congo red stain was prepared and autoclaved (121ºC for 15 minutes) separately from the other constituents. The sterile concentrated stain was then added to the autoclaved brain heart infusion agar with sucrose at 55ºC. Five CRA plates were then inoculated with test organisms and incubated at 37ºC for 24 h aerobically. Biofilm production is indicated by formation of black colonies with a dry crystalline consistency. The experiment was performed in triplicate and repeated three times.

4.9.3. Tissue culture plate method:

This quantitative test described by Christensen *et al.*[149] is considered the gold-standard method for biofilm detection. Organisms isolated from fresh agar plates were inoculated in 10 ml of Trypticase soy broth containing 1% glucose. Broths were incubated at 37ºC for 24 h, and the cultures were diluted 1:100 with fresh medium. Individual wells of sterile 96 well-flat bottom polystyrene tissue culture plates were filled with 200 µL of the diluted cultures. The control organisms were also included after dilution. Negative control wells contained sterile broth. The plates were incubated at 37ºC for 24 h. After incubation, contents of each well were removed by gentle tapping. The wells were washed with 0.2 mL of phosphate buffer saline (pH 7.2) four times to remove non adherent cells free floating bacteria. Biofilm formed by bacteria adherent to the wells were fixed by 2% sodium acetate and stained using crystal violet (0.1%). Excess stain was removed using deionized water and the plates were dried. Optical density (OD) of stained adherent biofilm was obtained using micro ELISA auto reader (model 680, Biorad, UK) at wavelength 570 nm. The experiment was repeated in triplicate. The interpretation of biofilm production was done according to the criteria of Stepanovic *et al.*[150]

Quality control:

The biofilm producers *Staphylococcus epidermidis* ATCC 35984 (positive control) and the non-biofilm producers *Staphylococcus epidermidis* ATCC 12228 (negative control) were used as standard control strains.

4.10. Phenotypic characterization of *E. coli.*

4.10.1. ESBL detection:

ESBL was determined by inoculating the isolates on HI chrome ESBL screen agar (Hi Media Laboratories, Mumbai, India). At 37°C plates were incubated for overnight. The *E. coli* colonies capable of producing ESBL grew as either pink or purple colonies.

4.10.2. Haemolysin production:

Most of the haemolytic *E. coli* isolates secrete hemolysin - a cytolytic protein toxin. The synthesis of haemolysin was determined by the formation of a clear zone of lysis of erythrocytes on blood agar around the bacterial colony. For this purpose, 5% sheep blood agar is used to demonstrate clear zone of hemolysis by *E. coli* isolates upon overnight incubation at 37°C.

4.11. Genotypic characterization of *E. coli*:

4.11.1. Preparation of Template DNA:

A colony of *E. coli* strain was inoculated in a micro centrifuge tube containing 500µl of peptone water and incubated at 37˚C overnight. It was then centrifuged at 13,000 rpm for 10 minutes. Supernatant was discarded and 100-200 µl of Milli-Q water was added. Then the tube was vertexed vigorously for 1min. tube was heated at 95˚C degrees for 20 minutes in a dry bath and centrifuged again at13000rpm for 10min. The bacterial DNA was collected from the supernatant and stored at -20˚C until further use.

4.11.2. DNA Amplification:

PCR Reaction mixture: From bacterial DNA, virulence genes were targeted using specific primers. The 25µl of PCR reaction mixture including 2X Amplicon III Red Taq master mix (12.5 µL) (Master mix composition: (NH4)2 SO4, 4 mM MgCl2, Tris HCL pH 8.5, 0.2% Tween 20, Taq DNA polymerase, 0.4 mM dNTPs, and 0.2 units/µl amplicon). To the master mix 4 µM concentrations of both forward and reverse primers were added. Finally, 5µL of template DNA was added.

4.11.3. Multiplex PCR assay for the Detection of virulence factor (VF) genes:

The following 3 virulence genes of *E. coli* were detected.

4.11.3.1.papEF- gene coding for pilus associated pyelonephritis.

4.11.3.2.*traT*- gene coding and associated with serum resistance.

4.11.3.3.RPAI- marker for pathogenicity island of Uropathogenic *E. coli*.

Table 10: Primer sequence for virulence genes of UPEC used in this study

Sl. No	Virulence gene	Length	Primer sequence (5'-3')	Amplicon size, base pairs (bp)	Primer reference
1	*papEF*	20	F: TAGCCTGGGAACCATGAAAG R: GTTGAACGTGCTGTGTCCAG	336	Newly Designed in this study
2	*traT*	20	F: TTGATGATGGTTGCACTGGT R: GCAACATTGTCCGTTGTCAC	290	Newly Designed in this study
3	PAI	22	F: GGACATCCTGTTACAGCGCGCA R: TCGCCACCAATCACAGCCGAAC	930	Johnson JR *et al.*[151]

Table 11: Multiplex PCR Conditions for detection of virulence genes of *E. coli*

Gene	Initial Denaturation	Denaturation	Annealing	Extension	Final extension	No of cycles
PapEF	94°C for 2mins	94°C for 60secs	63°C for 30secs	72°C for 90secs	72°C for 5mins.	30
traT	94°C for 2mins	94°C for 60secs	63°C for 30secs	72°C for 90secs	72°C for 5mins.	30
PAI	94°C for 2mins	94°C for 60secs	63°C for 30secs	72°C for 90secs	72°C for 5mins.	30

Instrument:

Thermal Cycler apparatus (AB VERITI PCR MACHINE, USA.)

The PCR products were electrophoresed on 1.5% agarose gels containing Ethidium bromide and visualized in gel document system (Biorad, UK). The sizes of the DNA bands were determined by comparing them with a 100-bp DNA ladder as the molecular size marker (100 bp DNA ladder, MBI Fermentas).

4.12. DNA Sequencing:

Sanger Sequencing was carried out for molecular confirmation and characterization of *papEF*, *traT* and PAI genes. Samples those yielded good quantity of DNA were subjected to endpoint polymerase chain reaction followed by Sanger Sequencing. Sanger sequencing, also termed as chain termination method or dideoxy nucleotide sequencing is a first-generation method of determining the nucleotide sequence of DNA. This most widely used DNA sequencing method was developed by Frederick Sanger in the year 1977. In Sanger sequencing, polymerase chain reaction is performed where, first denaturation of the double-stranded DNA occurs and then the resulting single stranded DNA gets annealed to oligonucleotide primer. PCR mixture for sequencing contains buffer, DNA polymerase enzyme, Mg++ divalent ions and normal deoxynucleotide triphosphates (dNTPs) of all four nucleotides of Adenine (A), cytosine (C), Thymine (T), and guanine (G). In addition to these usual ingredients, Sequencing PCR mixture also contains small quantities of four chain-terminating dideoxynucleotides (ddATP, ddGTP, ddCTP, ddTTP) that are labelled with particular fluorescent marker and terminate the reaction. During chain elongation step in the PCR, when a particular ddNTP is attached to the daughter sequence, polymerase enzyme stops the further addition of dNTPs resulting in each sequence gets terminated at varying lengths. Once the sequencing PCR is completed then the resulting products are resolved on a gel in an automated machine that is embedded with laser to read the nucleotide sequence based on the detection of particular color emitted by particular ddNTP. By convention, Adenine is indicated by green fluorescence, Thymine by red fluorescence, Guanine by black fluorescence, and Cytosine by blue fluorescence and the intensities of fluorescence is electronically translated into a "peak."

Before proceeding for Sanger sequencing all PCR products were first column purified to remove impurities such as unbound primers and excess of dNTPs from amplicons. Sanger sequencing was done using the Big Dye Terminator v3.1 Kit

(Applied Biosystems) according to the kit manufacturer's protocol. 50ng of PCR product was used as initial template in the sequencing PCR. 8µL of sequencing master mix from the kit, 3.2 pmol of primer were used in a 20 µL reaction volume. The thermal condition for sequencing PCR was initial denaturation at 96^0C for 1 min, 25 cycles of 96^0C for 10 sec, 50^0C for 5 sec and 96^0C for 4 minutes. Sequencing PCR products were cleaned up using Big Dye X Terminator™ purification Kit (Applied Biosystems) and dissolved in 10 µL of Hi-di Formamide before subjecting them into an automated DNA analyzer machine 3500. The 3500 automated DNA analyzer machine performs Capillary electrophoresis to generate the raw DNA sequence data in terms peaks or plots for each nucleotide.

The sequencing raw data and sequencing plots were analyzed using the DNASTAR SeqMan software (DNASTAR, Inc.). Consensus sequences were generated for each sample and were checked for molecular confirmation in the PubMed using NCBI-BLAST tool (https://blast.ncbi.nlm.nih.gov). Nucleotide changes in the study genes were carefully checked and sequencing errors were omitted.

4.13. Detection of Mutations:

The strains subjected for sequencing were checked for any nucleotide changes they possess in the study genes.

4.14. Statistical Analysis:

Statistical software package SPSS version 22 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp) was used to analyse the data. Age, gender, organisms causing UTI, and its antibiotic sensitivity and resistance were included as variables in the model. Chi-square test was applied wherever necessary and *P*-value of <0.05 was considered statistically significant.

CHAPTER 5

RESULTS

CHAPTER 5

RESULTS

5.1. Isolation rate:

A total of 395 *Escherichia coli* (39.5%) were isolated from 1000 urine samples after identification by biochemical tests. From the **table-12** (provided below) it is evident that the highest isolation of *E. coli* were in the year 2013.

Table 12: Year wise isolation data of *Escherichia coli* from patients.

Year	No. of Samples Tested	Patients	*E. coli* Isolates	%
2012	250	Suspected UTI	72	28.8
2013	250	Suspected UTI	133	53.2
2014	250	Suspected UTI	75	30
2015	100	Suspected UTI	54	54
	150	Antenatal care patients	61	40.66
Total No Of Samples Tested	**1000**		**395**	**39.5**

5.2. Demographic details of UTI patients:

5.2.1. Age wise distribution:

Infection was predominant among age group of 20-29 with 154 *E. coli* isolates at the rate of 39%.

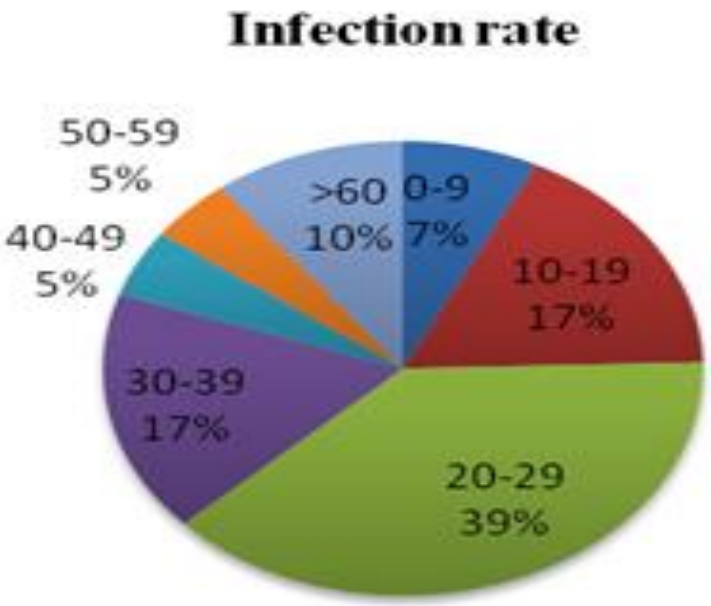


Figure 6: Age wise distribution of UTI.

5.2.2. Gender wise distribution:

UTI was seen in 316 (81.4%) female patients and in 72 (18.6%) male patients. Infection was highest among female patients.

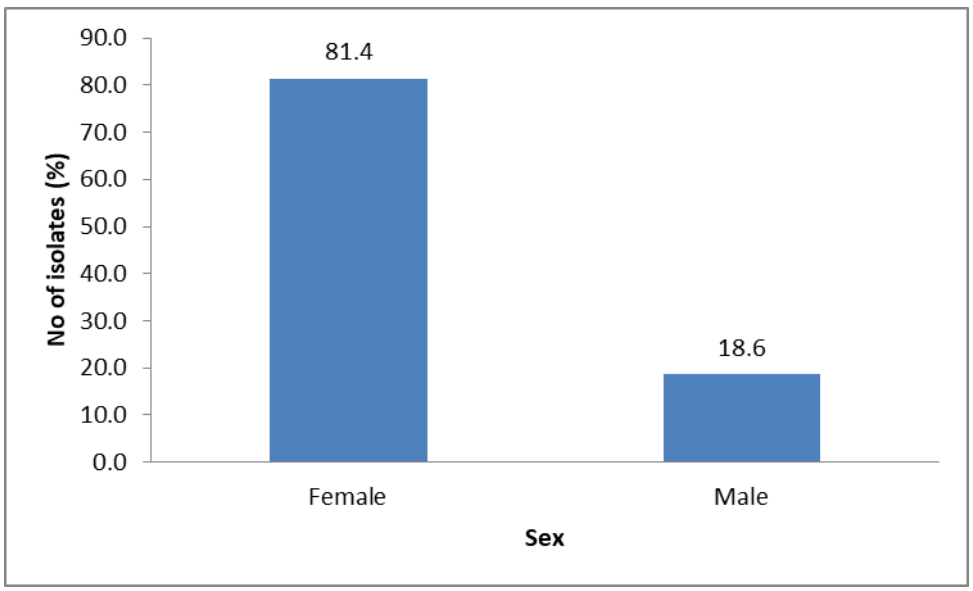


Figure 7: Distribution of UTI among Female & Male patients.

5.2.3. Prevalence of UTI demographic region wise:

UTI was seen high among rural patients 75% (291 of 388) as compared to patients from urban area. Only 25% (97 of 388) of infection was seen among patients from urban area.

5.3. Antibiotic susceptibility test (AST) pattern of *E. coli* isolates:

Antibiotic susceptibility pattern of *E. coli* isolates was analyzed year wise as below **(Table 13)**. Of the total 395 *E. coli* isolates, 170 (43%) were multi drug resistant (MDR). The isolates showed high level of resistance to ampicillin (82.53%), cefuroxime (72.41%), amoxicillin-clavulanic acid (71.90%), ceftriaxone (66.58%), ciprofloxacin (65.82%) and cefepime (57.47%). The isolates were sensitive to imipenem (96.71%), nitrofurantoin (92.41%), amikacin (90.89%), chloramphenicol (85.82%), piperacillin-tazobactam (80.76%), gentamicin (59.24%), azetreonam (54.43%) and norfloxacin (53.67%).

As the Minimum inhibitory concentration (MIC) of *E. coli* to AMP and CXM increased year wise, resistance to other antibiotics also increased. Based on the age of the patients, resistance to antibiotics AMP and CXM was more common among age group of 20 –29 years. In the year 2012, the isolates were sensitive to majority of the antibiotics compared to 2015. Only Carbapenems showed susceptibility to all the isolates from year 2012 to 2015.

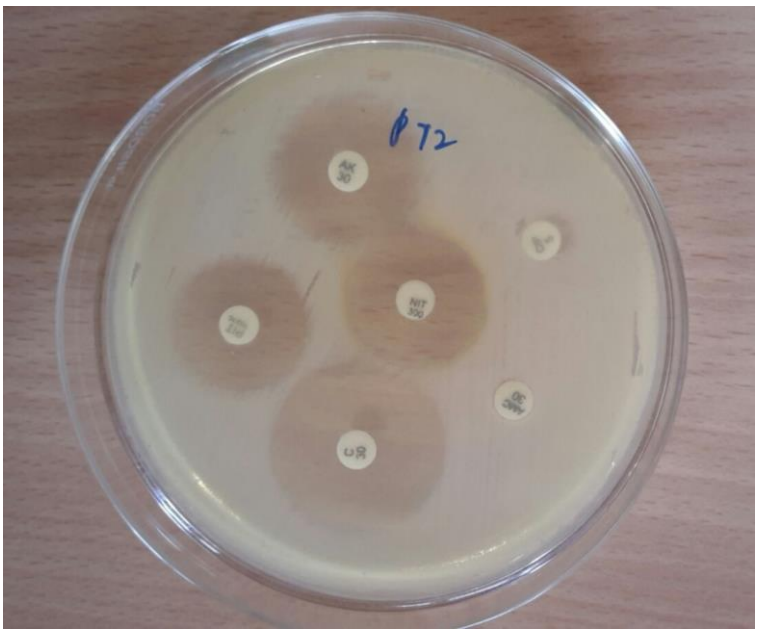


Fig 8: Antibiotic susceptibility pattern of *E. coli* on MHA.

Table 13: Analysis of AST Pattern of *E. coli* Year Wise:

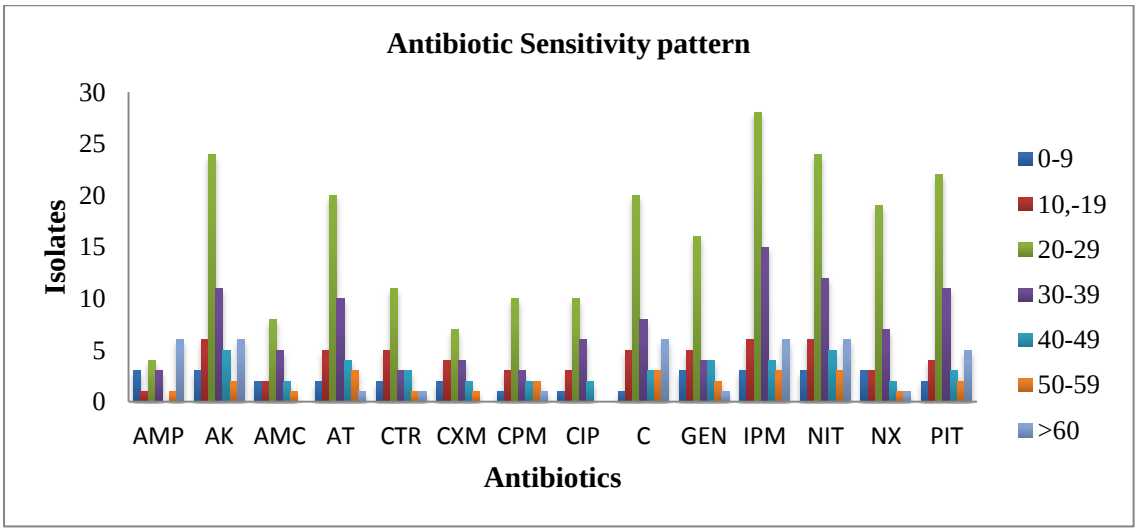
Year and Total Isolates	**Resistance Pattern**			**Sensitivity Pattern**	
2012 **72 *E. coli* of 250 samples tested**	***Age group***	***Isolates***	***Highest Resistance To Antibiotics***	***Isolates***	***Highest Sensitive To Antibiotics***
	0-9	02	CPM, CIP, C	03	AMP, AK,GEN, IPM, NIT, NX
	10-19	05	AMP	06	AK, IPM, NIT
	20-29	**28**	**AMP**	**24**	**IPM**
	30-39	13	AMP, CTR, CPM	15	IPM
	40-49	06	AMP	05	AK, NIT
	50-59	03	CIP	03	AT, C, IPM, NIT
	>60	06	AMC, CXM, CIP	06	AMP, AK, C, IPM, NIT
2013 **133 *E. coli* of 250 Samples tested**	0-9	01	AMP, AMC, CXM	06	AK, CTR, CPM, CIP, C, GEN, IPM, NIT, PIT, NX.
	10-19	23	AMP	27	AK, PIT, NIT
	20-29	**38**	**AMP**	**40**	**IPM, NIT**
	30-39	21	CXM	26	NIT
	40-49	06	AT, CXM, CIP	07	AK, C, IPM, PIT, NIT
	50-59	08	AMC, CXM	08	AK
	>60	16	AMP	19	NIT

2014 **75 *E. coli* of 250 Samples tested**	0-9	01	AT, CIP, GEN	09	AMP, AK,AMC, CTR, CXM, CPM, C, IPM, NIT, PIT, NX
	10-19	15	AMP	17	C, IPM
	20-29	**19**	**AMP**	**21**	**NIT**
	30-39	11	CXM	13	AK, C, IPM
	40-49	04	AMP	04	AK, C, IPM
	50-59	05	AMP, AT, CTR, CXM	05	AK, C, NIT
	>60	05	AMP, AMC	06	AK,IPM
2015 **54 *E. coli* of 100 Samples tested**	0-9	07	C	09	AK, IPM, PIT
	10-19	07	AMP	08	IPM
	20-29	**20**	**AMP, CXM**	**23**	**IPM**
	30-39	05	AMP, CXM	23	IPM
	40-49	01	AMP, AMC, AT, CTR, CXM, CPM, CIP, NIT, NX, PIT	01	AK, C, GEN, IPM
	50-59	01	AMP, AMC, AT, CTR, CXM, CPM, CIP, NIT, NX, PIT	01	AK, C, IPM, NIT
	>60	07	AMP, CTR, CXM, CPM, CIP	07	C, NIT
	10-19	10	AMP	10	IPM, NIT, PIT

C. *coli* from ANC samples	**20-29**	**34**	**AMP**	**35**	**IPM**
	30-39	09	AMP	09	IPM
	40-49	01	AMP, AMC, CPM	01	IPM, AK, NIT, C, CIP, NX, AT
61 of 150 samples tested					

Abbreviations:

AMP= Ampicillin, AK= Amikacin, AMC= Amoxicillin-clavulanic acid, AT= Aztreonam, CTR= Ceftriaxone, CXM= Cefuroxime CPM= Cefepime, CIP= Ciprofloxacin, C= Chloramphenicol, GEN= Gentamicin, IPM= Imipenem, NIT= Nitrofurantoin, NX= Norfloxacin, PIT= Piperacillin-tazobactam.



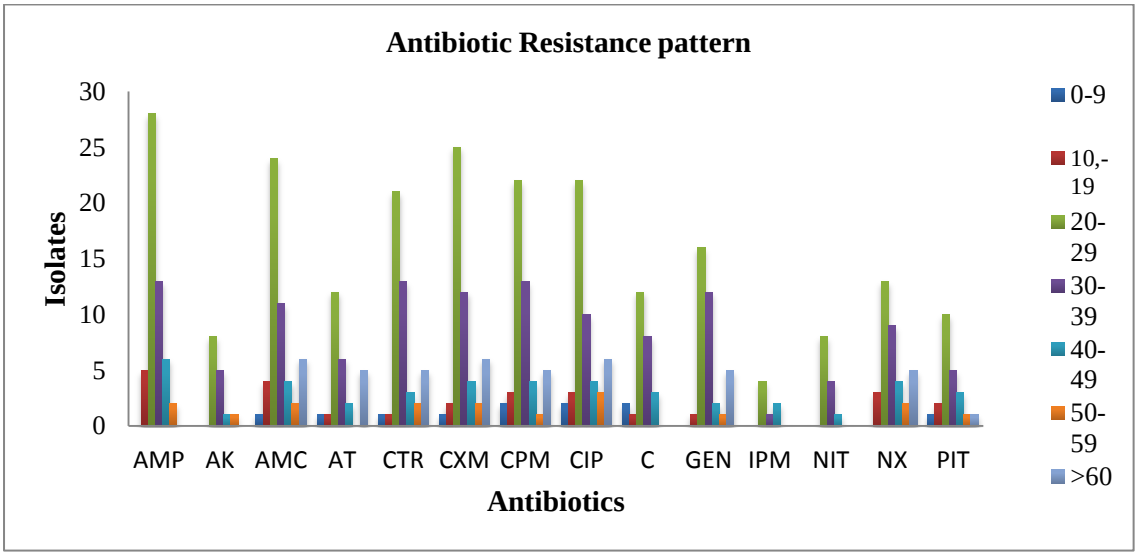
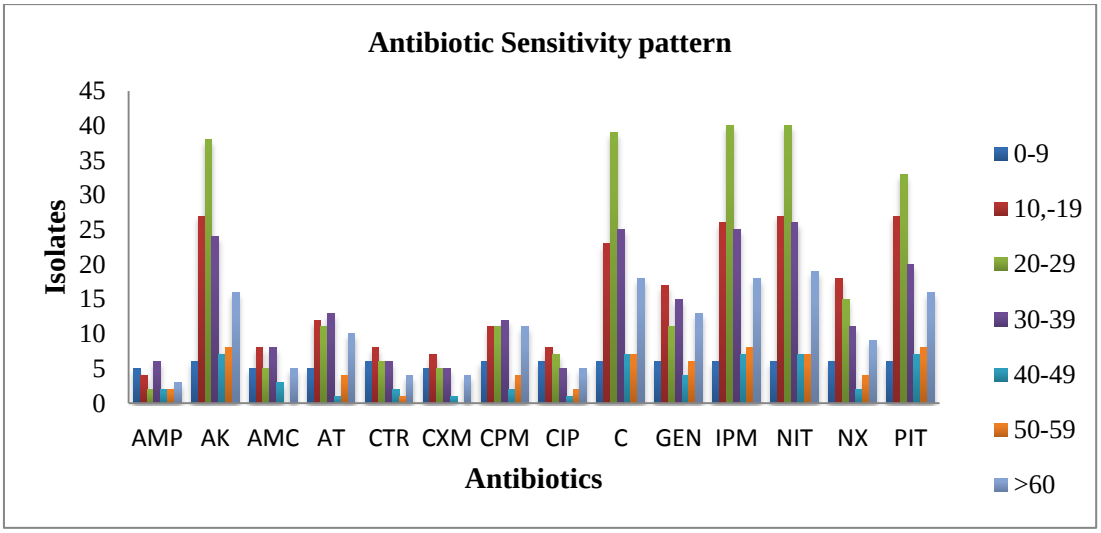


Figure 9: Antibiotic sensitivity and resistance pattern of *E. coli* isolated in the year 2012.



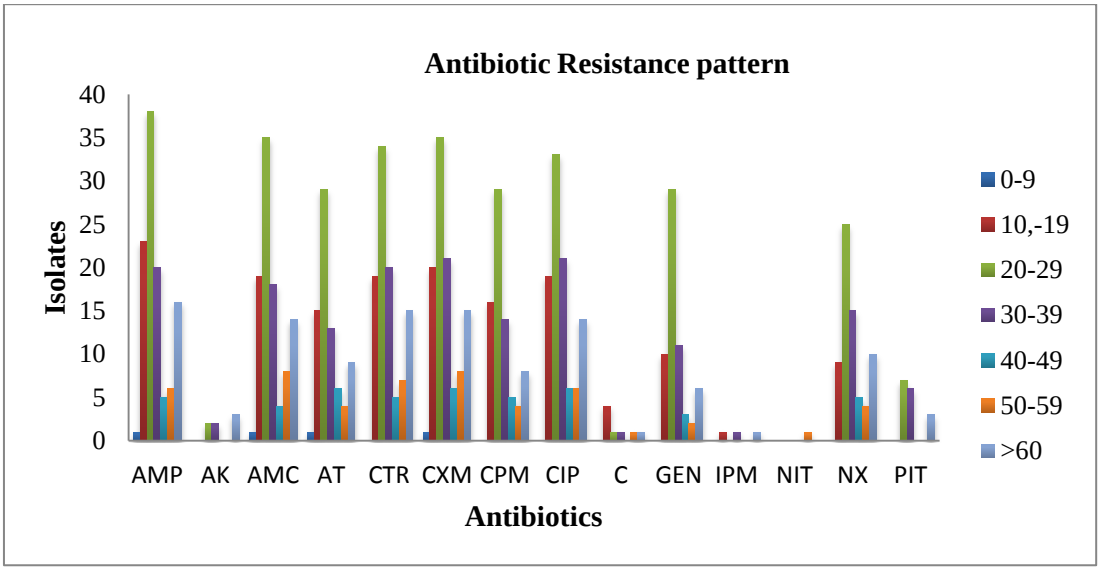
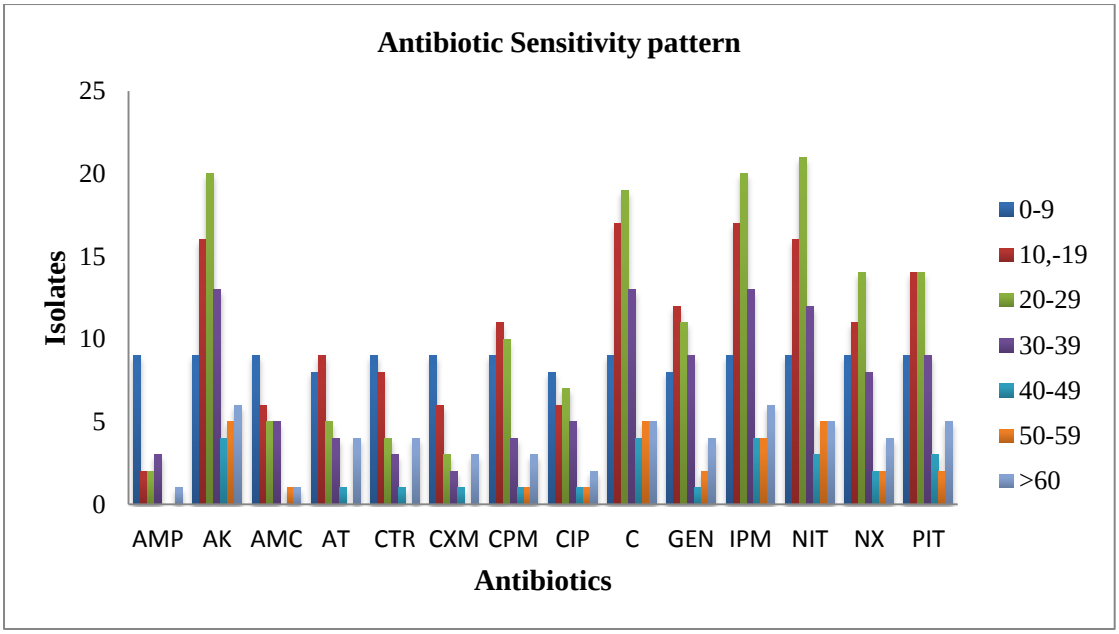


Figure 10: Antibiotic sensitivity and resistant pattern of *E. coli* isolated in the year 2013.



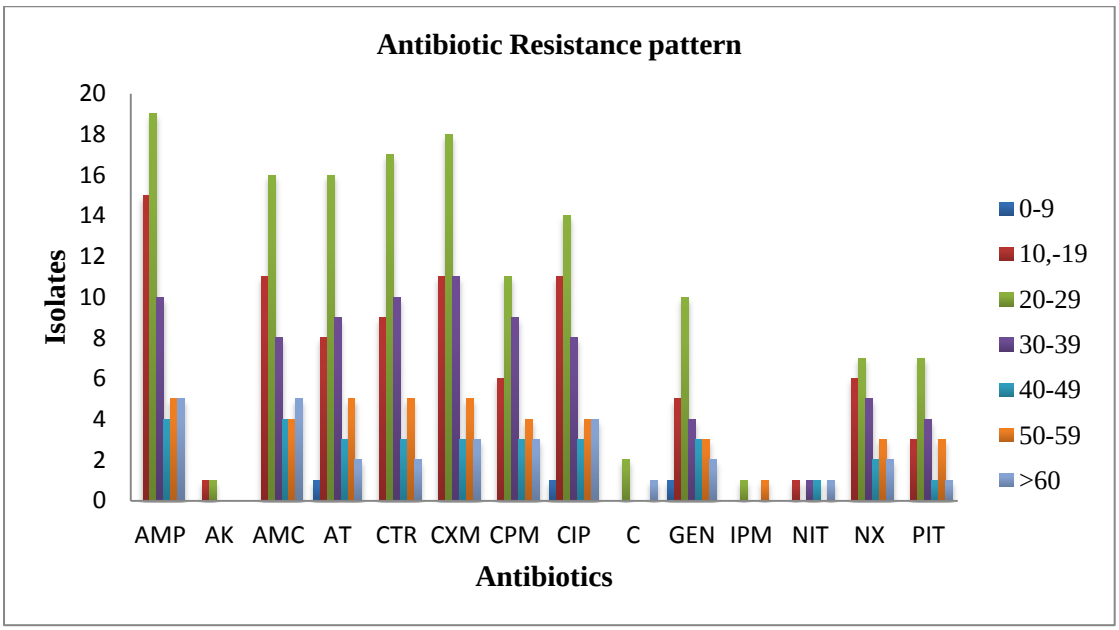
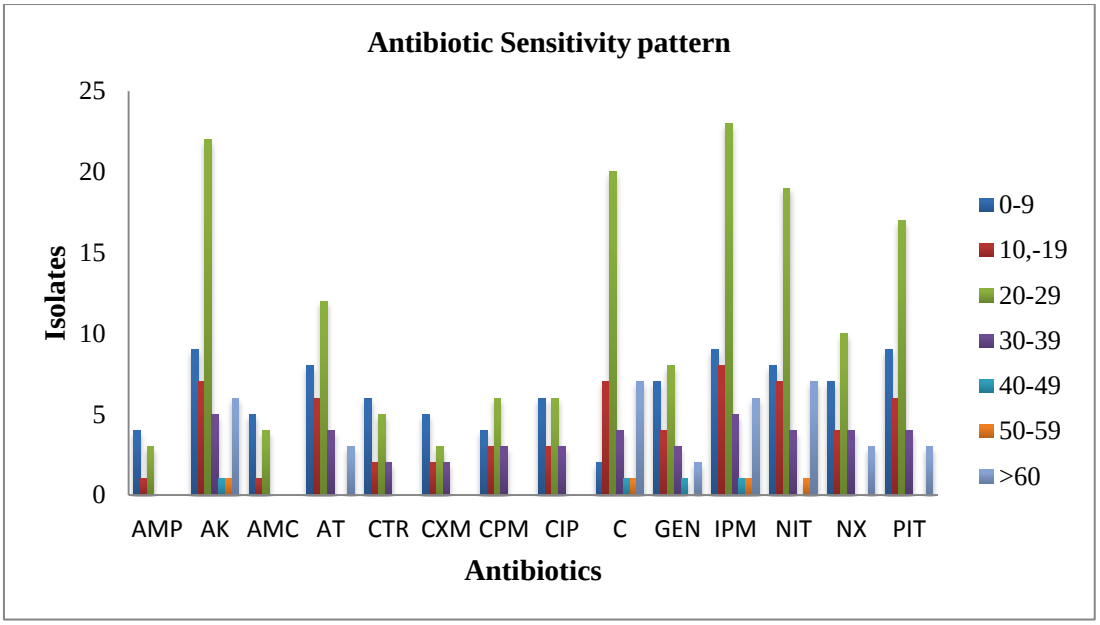


Figure 11: Antibiotic sensitivity and resistant pattern of *E. coli* isolated in the year 2014.



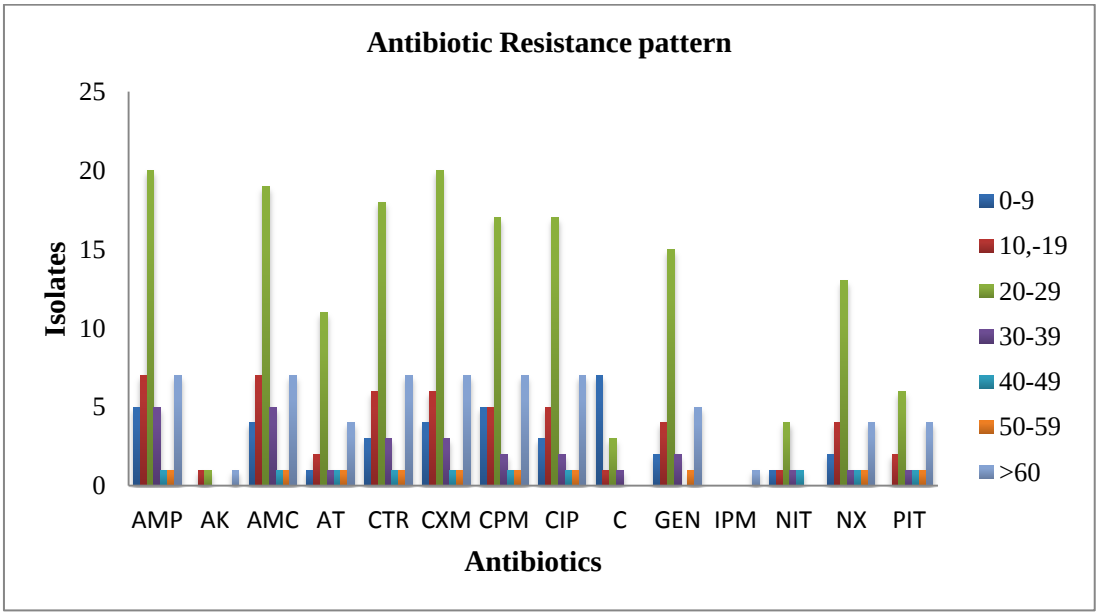
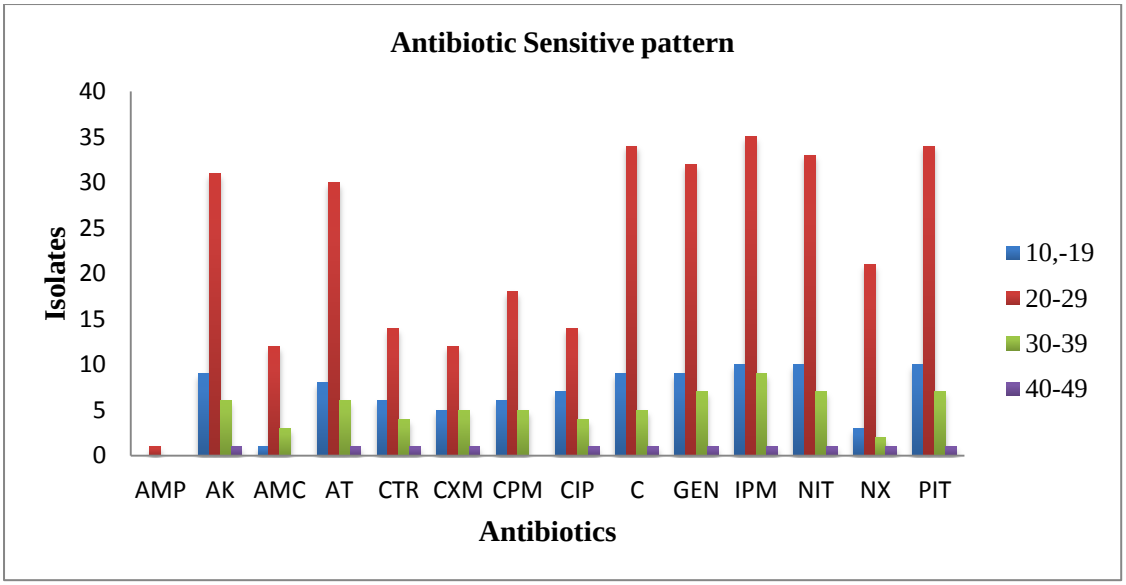


Figure 12: Antibiotic sensitivity and resistant pattern of *E. coli* isolated in the year 2015



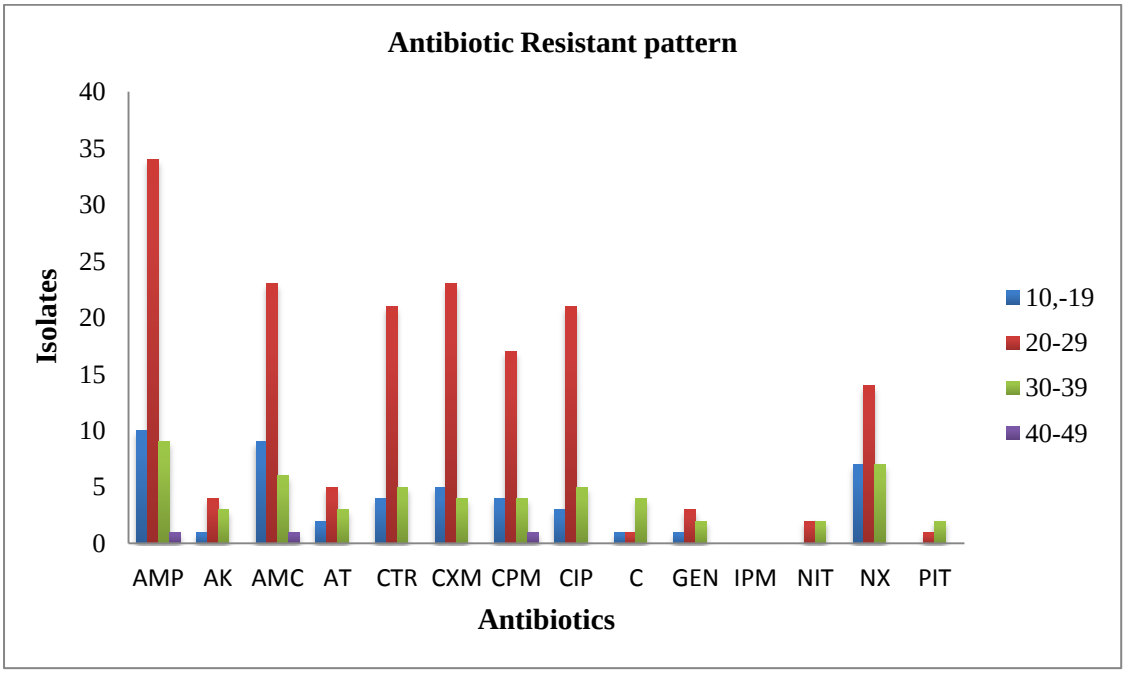


Figure 13: Antibiotic sensitivity and resistant pattern of *E. coli* isolated from pregnant women in the year 2015.

5.4. Multidrug resistance (MDR) among *E. coli* isolates.

Out of the total 395 *E. coli* isolates, 170 (43%) *E. coli* were MDR. High level of resistance to antibiotics - ampicillin (82.53%), cefuroxime (72.41%), amoxicillin-clavulanic acid (71.90%), ceftriaxone (66.58%), ciprofloxacin (65.82%) and cefepime (57.47%) were seen respectively.

Table 14: Incidence of Multidrug resistance among *E. coli* isolates.

MDR	Incidence rate (N, %)
Resistant to 2 antibiotics	20 (5.2)
Resistant to 3 antibiotics	8 (2.1)
Resistant to 4 antibiotics	24 (6.2)
Resistant to 5 antibiotics	14 (3.6)
Resistant to 6 antibiotics	24 (6.2)
Resistant to 7 antibiotics	59 (15.2)
Resistant to 8 antibiotics	76 (19.6)
More than 8 antibiotics	163 (23.8)

5.5. In-vitro biofilm detection:

Among 388 *E. coli* isolates subjected for in vitro biofilm production, 277 isolates (71.39%) produced biofilm by all the three methods.

In vitro biofilm formation by different methods was as follows: (Table 15).

5.5.1. By tube adherence method:

Among the biofilm producers, 40 (10.3%) strains were high biofilm producers, 35 strains (9%) produced biofilms moderately and 91 strains (23.5%) produced weak biofilm formation.

5.5.2. By Congo red Agar method (CRA):

254 strains (65.5%) produced biofilm by this method.

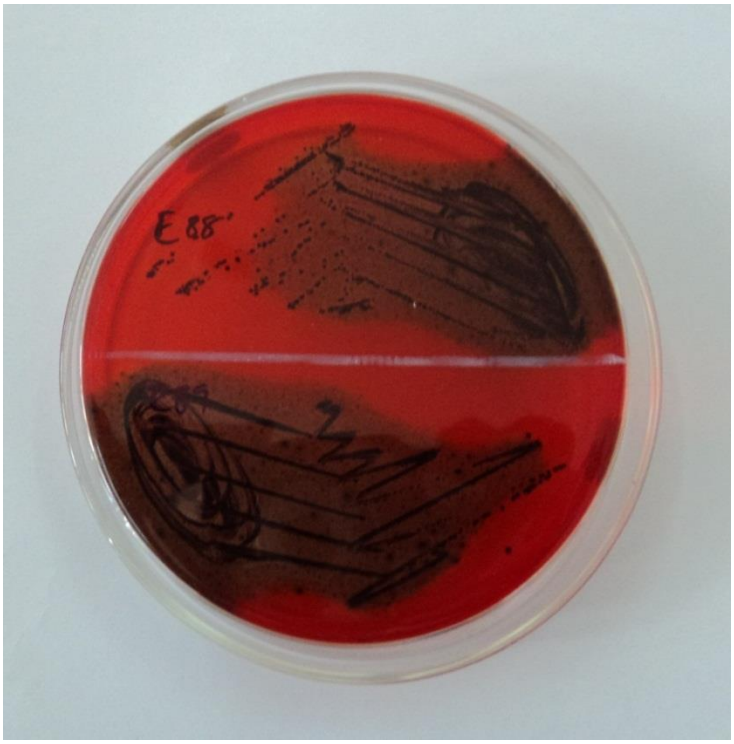
5.5.3. By Tissue Culture Plate Method (TCP):

By this method, 284 (73.2%) strains produced abundant biofilms, 23 strains (5.9%) produced biofilms moderately and 81 strains (20.9%) showed weak biofilm formation.

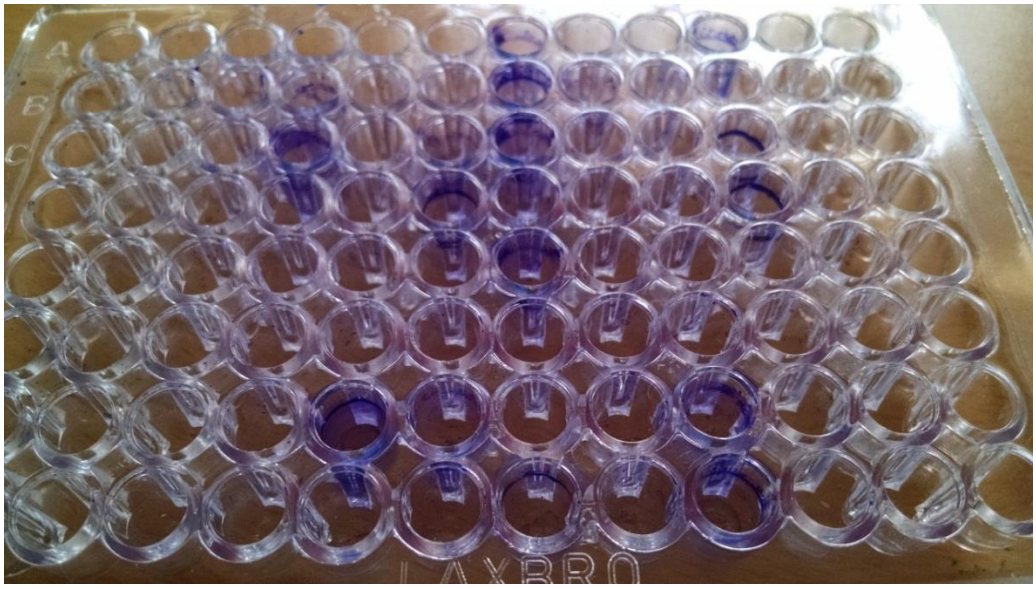
Table 15: Screening of the *E. coli* isolates for biofilm formation by Tube Adherence method, Congo Red Agar (CRA) method and Tissue Culture Plate method (TCP).

	Tube adherence Method.	CRA method.	TCP method.
Strong	40 (10.3%)	254 (65.5%)	284 (73.2%)
Moderate	35 (9%)	……	23 (5.9%)
Weak	91 (21.5%)	……	81 (20.9%)
Negative	222 (57.2%)	134 (34.5%)	……
Total	**388**	**388**	**388**

Highly positive biofilm formation by Tube Adherence method



Positive biofilm formation on Congo Red Agar.



Positive biofilm formation by Tissue Culture Plate method

Fig 14: Biofilm formation of *E. coli* by three different methods

5.6. Antimicrobial susceptibility profile of biofilm and non-biofilm producing isolates:

Biofilm producing *E. coli* isolates demonstrated high resistance to antibiotics ampicillin (87.36%) followed by cefuroxime (81.58%), amoxicillin-clavulanic acid (77.61%), ciprofloxacin (71.48%), ceftriaxone (54.6%) and cefepime (64.98%) than non-biofilm producers. **(Table.16)**

Table 16: Antibiotic susceptibility profile of biofilm forming and non-biofilm forming *E. coli* strains.

Antibiotic	Biofilm producers n=277		Non-Biofilm producers n=111	
	No. of Resistant Isolates N (%)	No. of Sensitive Isolates N (%)	No. of Resistant Isolates N (%)	No. of Sensitive Isolates N (%)
ampicillin	242 (87.36)	35 (12.2)	88 (79.3)	23 (20.7)
amikacin	32 (11.55)	245 (88.4)	10 (9.1)	101 (90.9)
amoxicillin-clavulanic acid	215 (77.61)	62 (22.3)	62 (55.9)	49 (44.1)
aztreonam	139 (50.18)	138 (49.8)	37 (44.4)	74 (66.6)
ceftriaxone	198 (71.48)	79 (28.5)	57 (51.4)	54 (48.6)
cefuroxime	226 (81.58)	51 (18.4)	61 (55)	50 (55)
cefepime	180 (64.98)	97 (35)	47 (42.4)	64 (57.6)
ciprofloxacin	198 (71.48)	79 (28.5)	56 (50.5)	55 (49.5)
chlorampheni	33	244	19	92

col	(11.91)	(88)	(13.2)	(82.8)
gentamicin	128 (46.2)	149 (53.7)	34 (30.7)	77 (69.3)
imipenem	08 (2.88)	269 (97.1)	05 (4.6)	106 (95.4)
nitrofurantoin	21 (7.58)	256 (96)	06 (5.4)	105 (94.5)
norfloxacin	134 (48.37)	143 (51.6)	42 (37.9)	69 (62.1)
piperacillin tazobactam	06 (2.16)	271 (97.8)	18 (12.3)	93 (83.7)

5.7. Association between biofilm formation and antimicrobial resistance among UPEC isolates.

Statistically Significant ($p<0.05$) correlation was observed between biofilm forming *E. coli* isolates and Multi drug resistance with antibiotics amikacin, amoxicillin-clavulanic acid, aztreonam, ceftriaxone, cefuroxime, cefepime, ciprofloxacin, and chloramphenicol. **Table.17**.

Table 17: Association between biofilm formation of UPEC by TCP method & antibiotic resistance.

Biofilm formation		**Antimicrobial drugs**									
		AMP %	AK %	AMC %	AT %	CTR %	CXM %	CPM %	CIP %	C %	GEN %
TCP Method	Strong	6.1	11.9	6.6	8.7	06	6.6	7.1	5.6	12.5	7
	Moderate	22.7	38.1	25.3	25.4	26.3	24.5	26.2	25	35.7	32.4
	Weak	71.2	50	68.1	65.9	67.7	69	66.7	69.4	51.8	68.8
	p value	NS	0.002 *	0.002 *	0.009 *	0.002 *	0.010 *	0.003 *	0.020 *	0.001 *	NS

Abbreviations- AMP = ampicillin, AK = amikacin, AMC = amoxicillin-clavulanic acid, AT = aztreonam, CTR = ceftriaxone, CXM = cefuroxime, CPM = cefepime, CIP = ciprofloxacin, C = chloramphenicol, GEN = gentamicin.

* Significant at $p < 0.05$ TCP= Tissue culture plate.

5.8. Correlation between overall biofilm formation and antimicrobial resistance.

Biofilm forming strains showed the highest resistance to the antibiotics compared to non-biofilm forming isolates **(Table 18)**. Biofilm producers demonstrated resistance to antibiotics ampicillin (87.36%) followed by cefuroxime (81.58%), amoxicillin-clavulanic acid (77.61%), ciprofloxacin (71.48%), ceftriaxone (54.6%) and cefepime (64.98%).

Table 18: Correlation between overall biofilm formation and antibiotic resistance.

		Antimicrobial Agents (N, %R)													
S. No	**Biofilm Formation (by three methods)**	**AMP**	**AK**	**AMC**	**AT**	**CTR**	**CXM**	**CPM**	**CIP**	**C**	**GEN**	**IMP**	**NIT**	**NX**	**PIT**
01	Positive	266 (95)	40 (14.3)	262 (93.6)	156 (55.7)	218 (77.9)	238 (75)	208 (74.3)	215 (76.8)	60 (21.4)	155 (55.4)	23 (8.2)	41 (14.6)	148 (52.9)	67 (23.9)
02	Negative	98 (90.7)	16 (14.8)	80 (84.2)	50 (46.3)	76 (70.4)	84 (77.8)	66 (61.1)	79 (73.1)	20 (18.5)	41 (38)	2 (1.9)	19 (17.6)	42 (38.9)	20 (18.5)
03	***P* value**	0.156	0.873	***0.01****	0.112	0.146	0.098	***0.013****	0.509	0.577	***0.001****	***0.021****	0.531	***0.017****	0.279

* Significant at $p < 0.05$

AMP= ampicillin, AK= amikacin, AMC= amoxicillin-clavulanic acid, AT= aztreonam, CTR= ceftriaxone, CXM= cefuroxime CPM= cefepime, CIP= ciprofloxacin, C= chloramphenicol, GEN= gentamicin, IPM= imipenem, NIT= nitrofurantoin, NX= norfloxacin, PIT= piperacillin-tazobactam.

5.9. Antibiotic sensitivity pattern of biofilm and non -biofilm forming isolates:

Both biofilm forming and non-biofilm forming isolates were highly sensitive to antibiotics piperacillin-tazobactam, (97.83%), imipenem (97.14%), and nitrofurantoin (92.41%).

5.10. Phenotypic characterization of UPEC:

5.10.1. ESBL Detection:

Of the 388 *E. coli* isolates, 242 were confirmed as ESBL producers indicating a prevalence of 62.37% (242/388).

5.10.2. Haemolysin formation:

On sheep blood agar plate's, 156 (40.20%) strains produced β-haemolysis (156/388).

Fig 15: ESBL production by *E. coli* on ESBL screen agar.

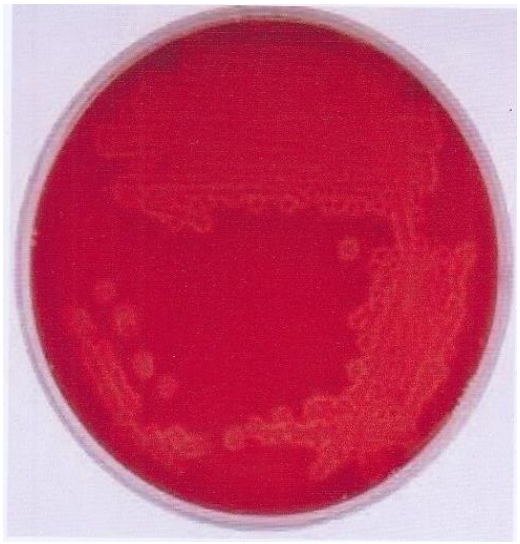


Fig 16: Blood agar showing β- hemolysis of *E. coli.*

5.11. Overall number of *E. coli* strains expressing phenotypic VFs.

388 *E. coli* strains exhibited the following percentage of phenotypic characters. Biofilm formation: 72.1% strains; ESBL production: 62.3% strains; & Hemolysin production: 40.2% strains.

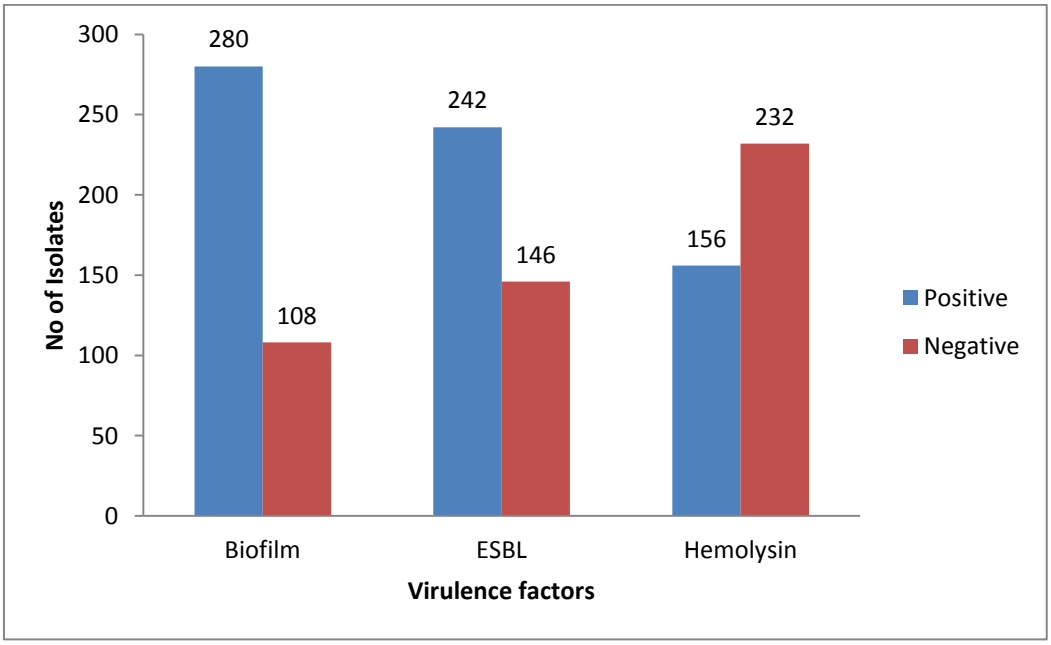


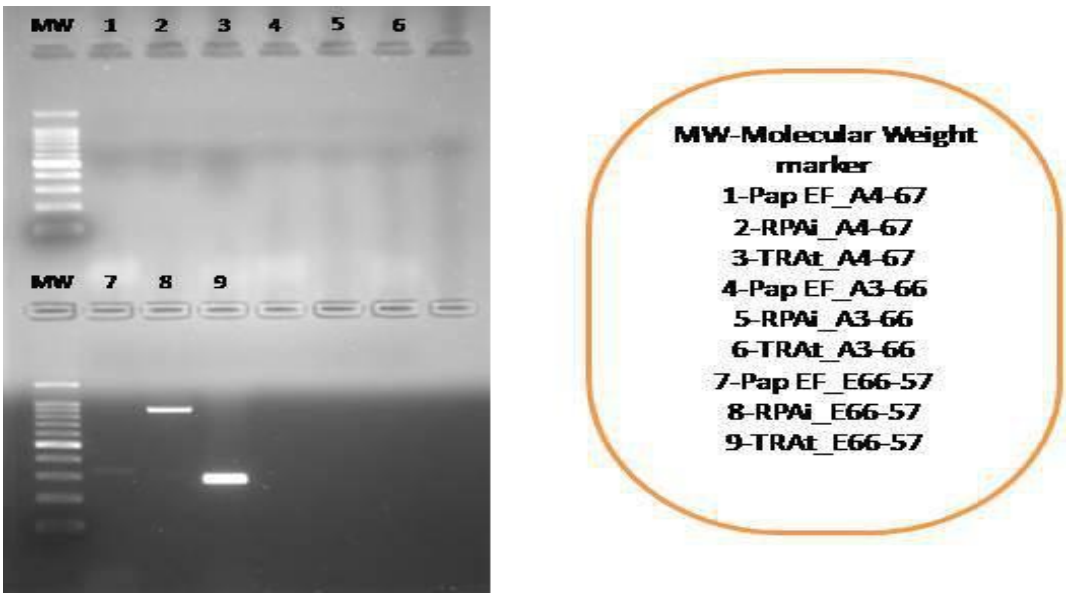
Fig 17: Graph showing overall number of *E. coli* exhibiting phenotypic virulence factors.

5.12. Detection of Virulence factor genes:

PCR's were developed, standardized and used for detection of virulence genes among *E. coli* strains. A total of 318 (81.95%) UPEC strains harbored the virulence genes. 70 strains (18%) lacked these virulence genes. Among the virulence genes detected, 76 strains (19%) possessed only one gene, 123 strains (31.70%) two genes; and combinations of three virulence genes were detected in 49 (12.62%) strains. The frequency of the detected virulence genes is shown in below **Table 19.**

Table 19: Prevalence of three different virulence genes among UPEC strains.

S.No	Virulence factors	Genes	N=388 (%)
01	Pilus associated pili	*papEF*	130 (33.5)
02	Serum resistance	*traT*	284 (73.2
03	Pathogenicity island	PAI	244 (62.9)



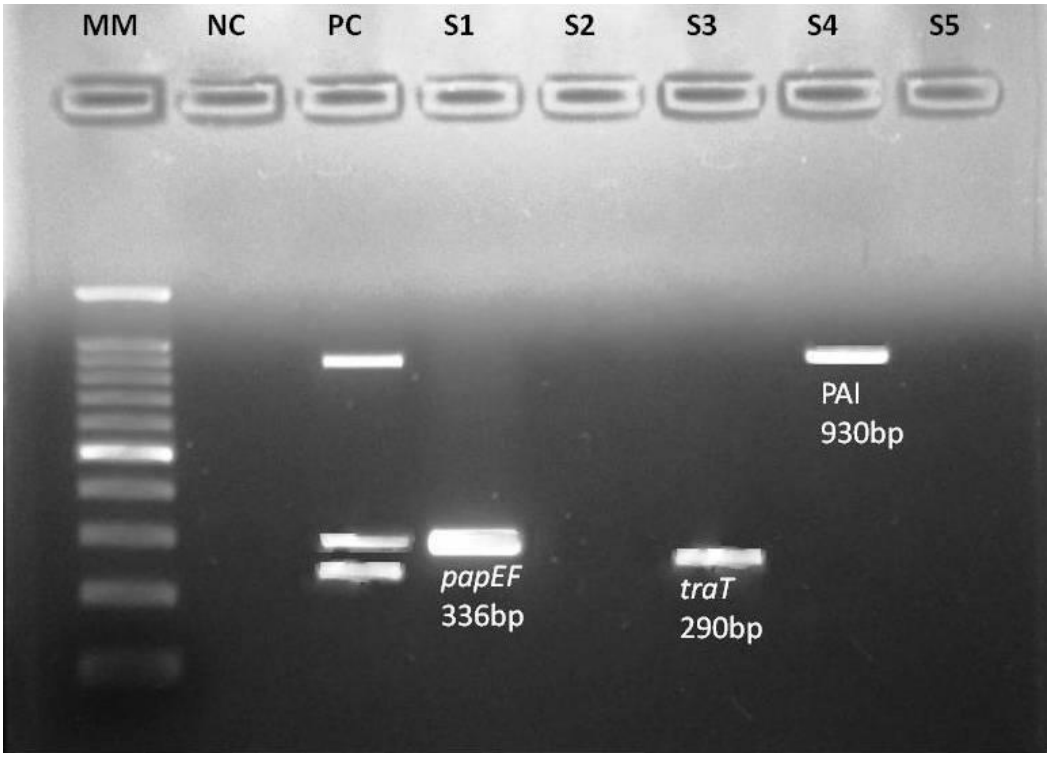


Fig 18: Agarose gel electrophoresis of PCR products showing bands of amplified genes. MM; DNA marker; NC; negative control, PC; positive control, S1; positive strain for *papEF*, S2; negative isolate; S3; positive strain for *traT*, S4; **positive strain for PAI; S5; negative isolate.**

5.13. Distribution of phenotypic virulence factors and virulence genes of UPEC sex wise:

Distribution of Virulence factors & virulence genes was found high among female patients than males **(Table 20).**

Table 20: Phenotypic virulence factors and virulence genes of UPEC among male & female patients.

S. No	Virulence factors	No of isolates positive among females	N=316 %	No of isolates positive among males	N=72 %
01	ESBL	191	60.44	51	70.83
02	Hemolysin	126	39.87	30	41.66
03	*papEF*	111	35.12	53	73.61
04	*traT*	234	74.05	50	69.44
05	*PAI*	202	63.92	42	58.33

5.14. Association between Phenotypic virulence factors & virulence genes of UPEC with Biofilm formation.

5.14.1. Association of phenotypic virulence factors with biofilm formation.

Significant relationship between ESBL & hemolysin with biofilm formation was seen. ESBL & Hemolysin producing UPEC strains had a higher ability of biofilm formation indicating statistically significant values **(Table 21)**.

Table 21: Association of ESBL & Hemolysin formation of UPEC with biofilm formation.

* Significant at $p < 0.05$.

S. No.	Phenotypic Virulence Factors	Biofilm Formation		*P* Value
		Negative (N, %)	Positive (N, %)	
01	**ESBL**			***<0.001****
	Negative	60 (55.6)	86 (30.7)	
	Positive	48 (44.4)	194 (69.3)	
02	**Hemolysin**			***0.001****
	Negative	79 (73.1)	153 (54.6)	
	Positive	26 (26.9)	127 (45.4)	

5.14.2. Association between Virulence genes of UPEC and biofilm formation.

Association was established between virulence genes and biofilm formation. But not proved statistically significant. Biofilm forming strains made no impact on gene carriage by strains. This may imply there is no significant correlation between presence of virulence genes & biofilm formation **(Table 22)**.

Table 22: Association between Virulence genes of UPEC and biofilm formation.
* Significant at $p < 0.05$.

Sl. No	Genotypic Virulence Factors	Biofilm Formation		*P* Value
		Negative (N, %)	Positive (N, %)	
1	***papEF***			0.473
	Negative	75 (69.4)	183 (65.4)	
	Positive	33 (30.6)	97 (34.6)	
2	***traT***			0.075
	Negative	36 (33.3)	68 (24.3)	
	Positive	72 (66.7)	212 (75.7)	
3	**RPAI**			0.907
	Negative	41 (38)	103 (36.8)	
	Positive	67 (62)	177 (63.2)	

5.15. Association between phenotypic & genotypic virulence factors of UPEC strains and antimicrobial resistance.

Expression of Phenotypic virulence factors & antibiotic resistance:

An analysis of the phenotypic characteristics expressed by the UPEC strains with their possession of virulence/drug resistance characters yielded the following information:

Statistically significant association was proved between ESBL & Hemolysin producing UPEC strains and multidrug resistance. ESBL & Hemolysin producing UPEC exhibited resistant to antibiotics ampicillin, amoxicillin-clavulanic acid, aztreonam, ceftriaxone, cefuroxime, cefepime, ciprofloxacin, chloramphenicol, gentamicin and norfloxacin **(Table 23).**

Expression of Virulence genes & antibiotic resistance:

Upon analysis of the correlation between the expression of virulence genes by UPEC strains and antibiotic resistance, it was found virulence genes expression made an impact on the development of antibiotic resistance. Strains expressing different virulence genes exhibited increase in resistance to antibiotics ampicillin, amoxicillin-clavulanic acid, aztreonam, cefepime, ciprofloxacin, chloramphenicol, gentamicin. But it was not proved to be significant statistically. Only the association between gene RPAi with antibiotic nitrofurantoin and *traT* with amikacin was proved significant statistically **(Table 24).**

Table 23: Association between phenotypic virulence factors of UPEC and antimicrobial resistance.

S. No.	Virulence marker	Antimicrobial agent													
		AMP (N, %R)	**AK (N, %R)**	**AMC (N, %R)**	**AT (N, %R)**	**CTR (N, %R)**	**CXM (N, %R)**	**CPM (N, %R)**	**CIP (N, %R)**	**C (N, %R)**	**GEN (N, %R)**	**IMP (N, %R)**	**NIT (N, %R)**	**NX (N, %R)**	**PIT (N, %R)**
1	**ESBL**														
	Positive	236 (97.5)	37 (15.3)	227 (98.7)	139 (57.4)	202 (83.5)	222 (91.7)	191 (78.9)	198 (81.8)	67 (27.7)	138 (57)	14 (5.8)	38 (15.7)	132 (54.5)	60 (24.8)
	Negative	128 (87.7)	19 (13)	115 (79.3)	67 (45.9)	92 (63)	100 (68.5)	83 (56.8)	96 (65.8)	13 (8.9)	58 (39.7)	11 (7.5)	22 (15.1)	58 (39.7)	27 (18.5)
	P value	***<0.001****	0.655	***<0.001****	***0.028****	***<0.001****	***<0.001****	***<0.001****	***0.001****	***<0.001****	***0.002****	0.426	1.00	***0.006****	0.168
2	**Hemolysin**														
	Positive	152 (97.4)	27 (17.3)	146 (98.6)	94 (60.3)	130 (83.3)	141 (90.4)	120 (76.9)	125 (80.1)	43 (27.6)	89 (57.1)	13 (8.3)	25 (16)	83 (53.2)	41 (26.3)
	Negative	212 (91.4)	29 (12.5)	196 (86.3)	112 (48.3)	164 (70.7)	181 (78)	154 (66.4)	169 (72.8)	37 (15.9)	107 (46.1)	12 (5.2)	35 (15.1)	107 (46.1)	46 (19.8)
	P value	***0.017****	0.189	***<0.001****	***<0.023****	***0.005****	***0.001****	***0.031****	0.116	***0.007****	***0.038****	0.291	0.886	0.180	0.139

* Significant at $p < 0.05$.

AMP= Ampicillin, AK= Amikacin, AMC= Amoxicillin-clavulanic acid, AT= Aztreonam, CTR= Ceftriaxone, CXM= Cefuroxime CPM= Cefepime, CIP= Ciprofloxacin, C= Chloramphenicol, GEN= Gentamicin, IPM= Imipenem, NIT= Nitrofurantoin, NX= Norfloxacin, PIT= Piperacillin-tazobactam

Table 24: Association between Virulence genes of UPEC and antimicrobial resistance.

S. No	Virulence marker	Antimicrobial agent													
		AMP (N, %R)	AK (N, %R)	AMC (N, %R)	AT (N, %R)	CTR (N, %R)	CXM (N, %R)	CPM (N, %R)	CIP (N, %R)	C (N, %R)	GEN (N, %R)	IMP (N, %R)	NIT (N, %R)	NX (N, %R)	PIT (N, %R)
1	***papEF***														
	Positive	119 (91.5)	19 (14.6)	116 (89.2)	65 (50)	99 (76.2)	107 (82.3)	93 (71.5)	94 (72.3)	28 (21.5)	71 (54.6)	10 (7.7)	18 (13.8)	63 (48.5)	35 (26.9)
	Negative	245 (95)	37 (14.3)	239 (92.6)	141 (54.7)	195 (75.6)	215 (83.3)	181 (70.2)	200 (77.5)	52 (20.2)	125 (48.4)	15 (5.8)	42 (16.3)	127 (49.2)	52 (20.2)
	P value	0.189	1.00	0.255	0.391	1.00	0.886	0.814	0.261	0.791	0.282	0.514	0.556	0.915	0.156
2	***traT***														
	Positive	268 (94.4)	47 (16.5)	262 (92.3)	152 (53.5)	211 (74.3)	236 (83.1)	205 (72.2)	219 (77.1)	64 (22.6)	149 (52.5)	20 (7.8)	45 (15.8)	142 (50)	70 (24.6)
	Negative	96 (92.3)	9 (8.7)	93 (89.4)	54 (51.9)	83 (79.8)	86 (82.7)	69 (66.3)	75 (72.1)	16 (15.4)	47 (45.2)	5 (4.8)	15 (14.4)	48 (46.2)	17 (16.3)
	P value	0.478	***0.05****	0.412	0.189	0.287	1.00	0.314	0.349	0.156	0.210	0.494	0.874	0.567	0.099
3	***PAI***														
	Positive	229 (93.9)	33 (13.5)	222 (91.0)	123 (50.4)	183 (75)	201 (82.4)	173 (70.9)	184 (75.4)	53 (21.7)	122 (50)	16 (6.6)	29 (11.9)	116 (47.5)	54 (22.1)
	Negative	135 (93.8)	23 (16)	93 (92.4)	83 (57.6)	111 (77.1)	121 (84)	101 (70.1)	110 (76.4)	27 (18.8)	74 (50.4)	9 (6.3)	31 (21.5)	74 (51.4)	33 (22.9)
	P value	1.00	0.551	0.709	0.173	0.713	0.780	0.908	0.903	0.518	0.834	1.00	***0.013****	0.528	0.900

* Significant at $p < 0.05$.

5.16. Association between ESBL producing UPEC and Ciprofloxacin resistance.

Statistically significant association was seen between ESBL & ciprofloxacin resistance. 67.3% (198/294) of UPEC were ESBL positive, signifying their resistance to the available fluoroquinolones – which is the drug of choice for the management of UTI by ESBL-producing strains. **Table 25**.

Table 25: Association between ESBL producing UPEC & Ciprofloxacin resistance.

* Significant at $p < 0.05$.

S. No	Antibiotic	ESBL		P value
		Negative (N, %)	Positive (N, %)	
1	**Ciprofloxacin**			***0.001****
	Resistant	96 (65.8)	198 (81.8)	
	Sensitive	50 (34.2)	44 (18.2)	

5.17. Molecular confirmation and characterization of virulent genes amplified from UPEC isolates by Sanger Sequencing.

Sanger sequencing was done for four samples of '*Pap*' gene and eleven samples of '*traT*' gene. All these samples were selected and subjected for DNA sequencing based on their satisfactory PCR amplifications and convenient band density of PCR product on TAE-Agarose gel electrophoresis.

***PapEF*:** n=3 & ***traT*:** n=11

Three PCR product samples of '*Pap*' gene and eleven samples of '*traT*' gene were successfully sequenced. Pictures of Sequence chromatograms/plots are shown below.

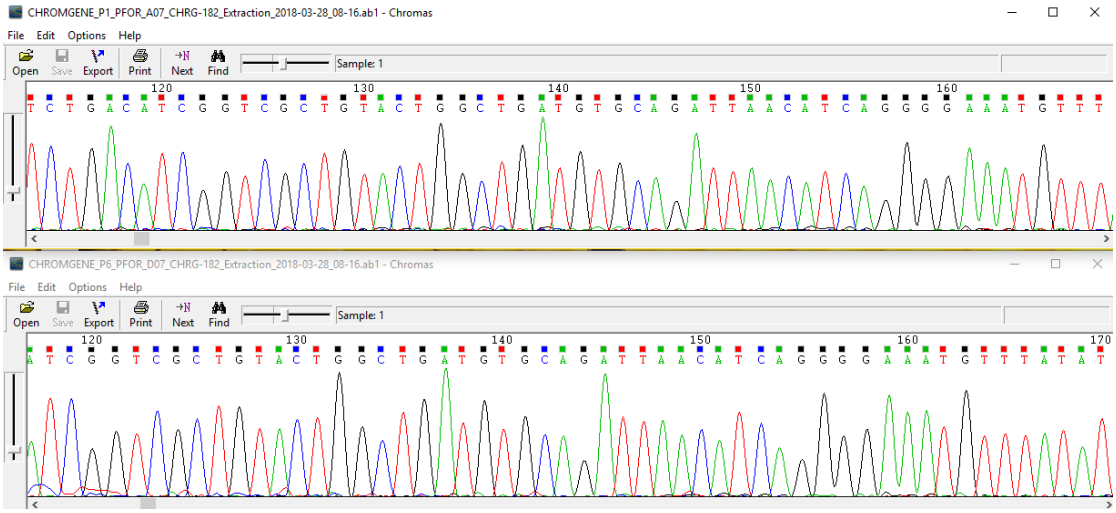


Figure 19: Sequencing plots of '*Pap*' gene (P-1 and P-6 bacterial isolate).

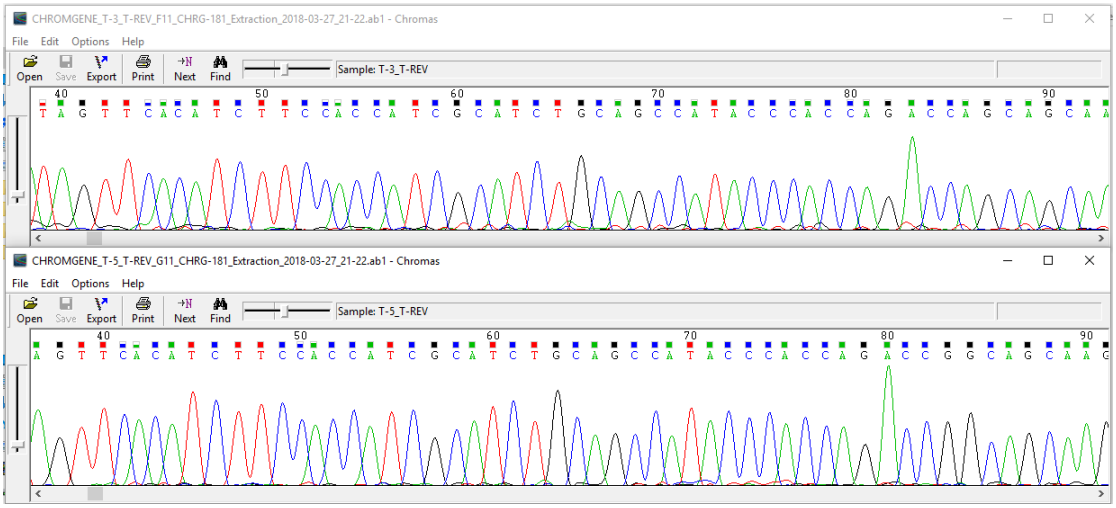


Figure 20: Sequencing plots of '*traT*' (T-3 and T-5 bacterial isolates).

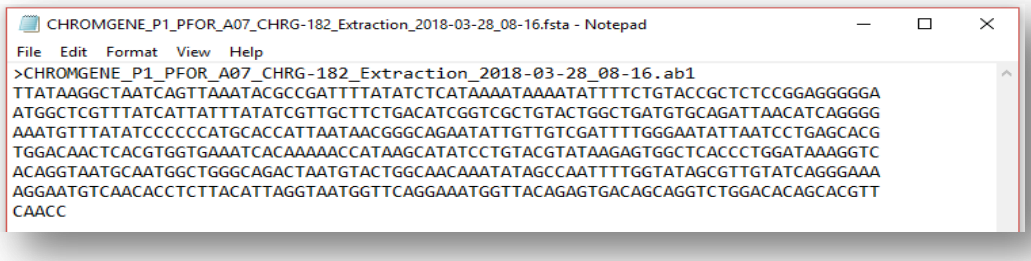


Figure 21: Raw sequence data of '*Pap*' gene (P-1 bacterial isolate).

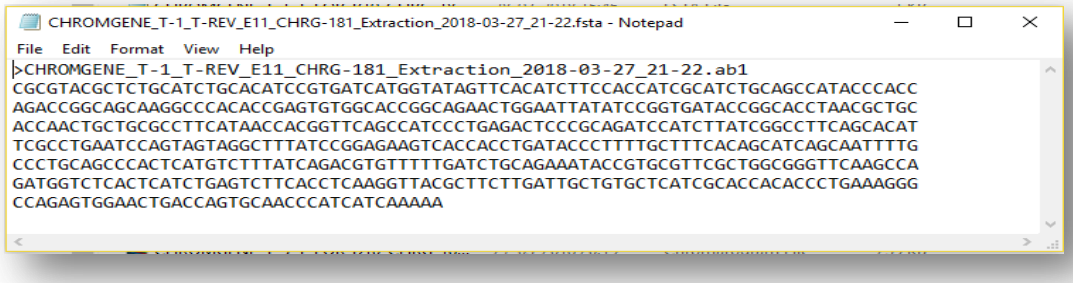


Figure 22: Raw sequence data of '*traT*' gene (T-1 bacterial isolate).

5.18. Molecular confirmation results:

Results for the molecular confirmation of both the study genes are tabulated below **(Table 26)**. Pictures showing matching of Query sequences aligned with PubMed sequence deposit are shown below after the results table **(Figures 23-26)**.

Table 26: Results of Molecular confirmation of *papEF & traT* genes of the study UPEC strains.

Isolate ID	Forward Sequencing	Reverse Sequencing	Molecular confirmation/Sequence alignment results
P1	Worked	Worked	Both Forward and reverse sequencing confirmed *E. coli papEF* DNA
P5	Worked	Worked	Both Forward and reverse sequencing confirmed *E. coli papEF* DNA
P6	Worked	Worked	Both Forward and reverse sequencing confirmed *E. coli papEF* DNA

Results of Molecular confirmation of *Trat* gene of study UPEC strains.

Isolate ID	Forward Sequencing	Reverse Sequencing	Molecular confirmation/Sequence alignment results
T1	Worked	Worked	Both forward and reverse sequencing confirmed *E. coli traT* DNA.
T3	Worked	Worked	Both forward and reverse sequencing confirmed *E. coli traT* DNA.
T5	Worked	Worked	Both forward and reverse sequencing confirmed *E. coli traT* DNA.
T6	Worked	Worked	Both forward and reverse sequencing confirmed *E. coli traT* DNA.
T11	Worked	Worked	Both forward and reverse sequencing confirmed *E. coli traT* DNA.
T15	Worked	Worked	Both forward and reverse sequencing confirmed *E. coli traT* DNA.
T16	Worked	Worked	Both forward and reverse sequencing confirmed *E. coli traT* DNA.
T17	Worked	Worked	Both forward and reverse sequencing confirmed *E. coli traT* DNA.
T24	Worked	Worked	Both forward and reverse sequencing confirmed *E. coli traT* DNA.
T26	Worked	Worked	Both forward and reverse sequencing confirmed *E. coli traT* DNA.
T28	Worked	Worked	Both forward and reverse sequencing confirmed *E. coli traT* DNA.

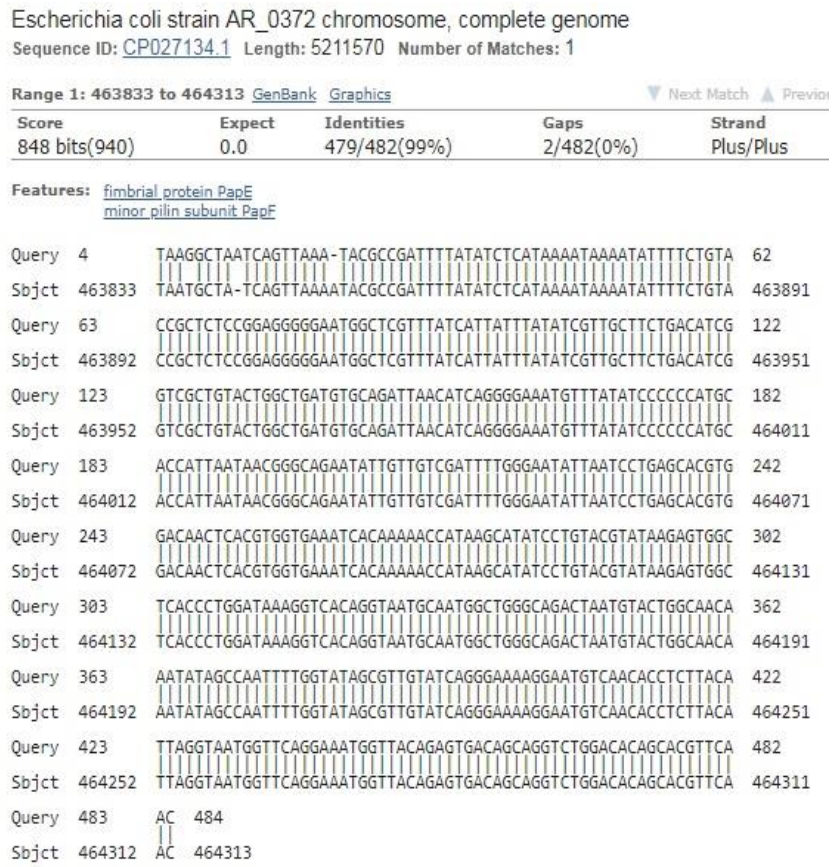


Fig 23: NCBI-BLAST alignment of UPEC *'pap'* gene (P1 Forward sequence).

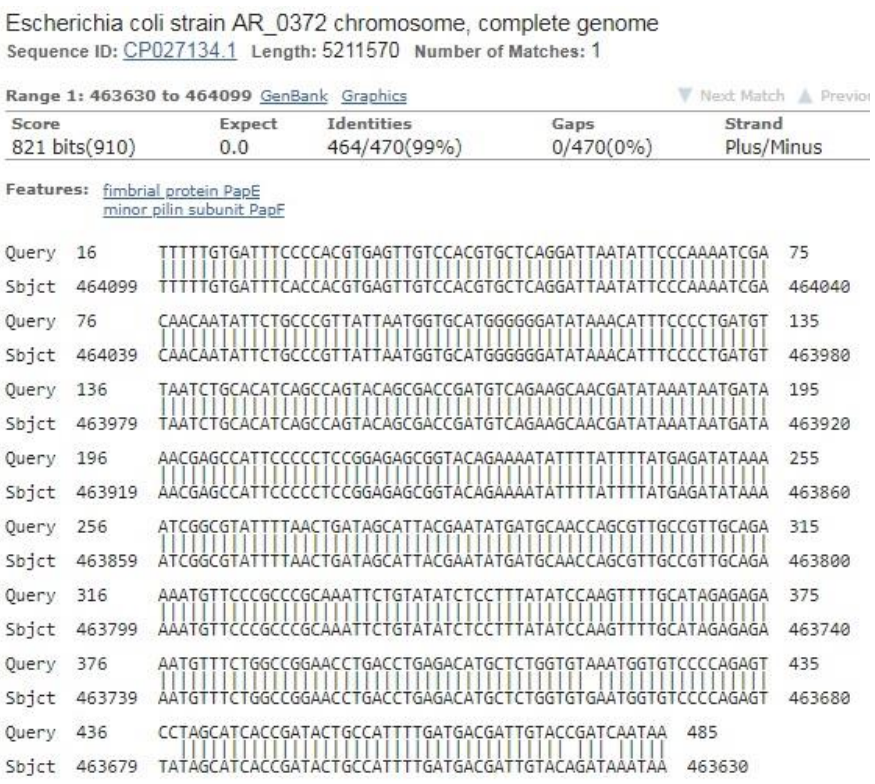


Fig 24: NCBI-BLAST alignment of UPEC *Pap* gene (P1 Reverse sequence)

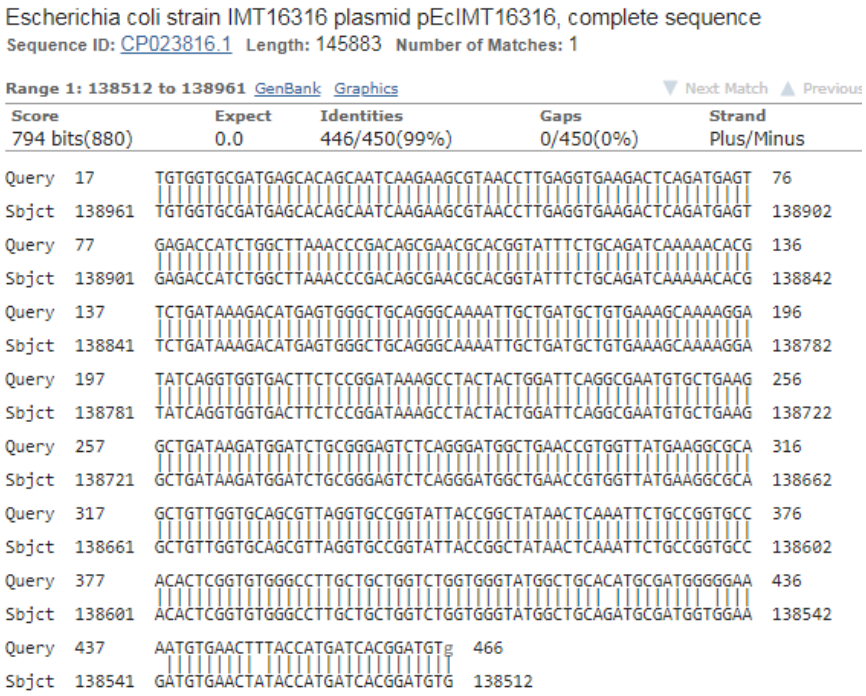


Fig 25: NCBI-BLAST alignment of UPEC '*traT*' gene (T17 Forward sequence).

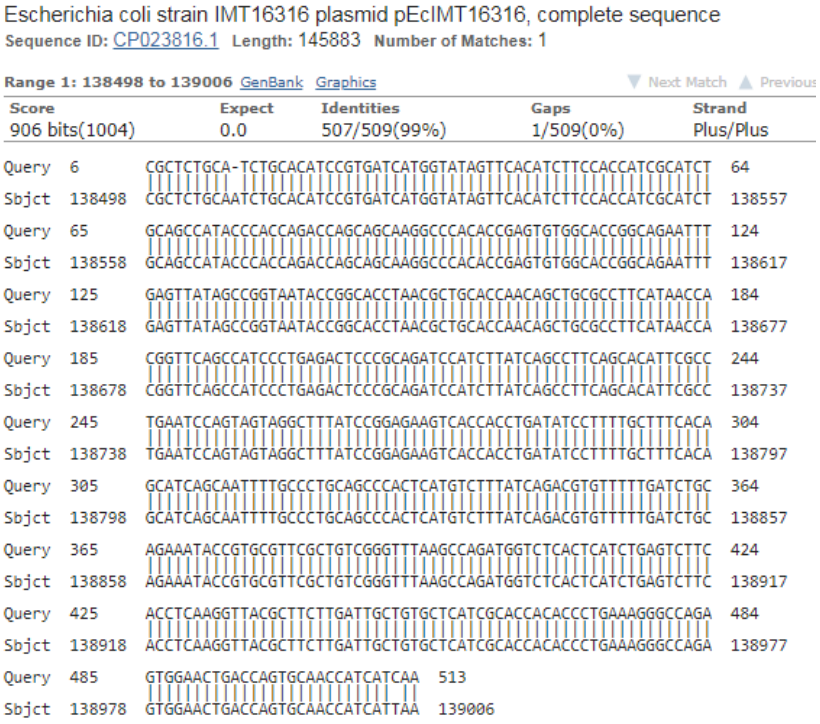


Fig 26: NCBI-BLAST alignment results of UPEC '*traT*' gene (T17 Reverse sequence).

5.19. Accession numbers for sequences from Genbank:

We have deposited *papEF* sequences to NCBI-Gen Bank DNA database and have got accession numbers shown below.

SOURCE *Escherichia coli* ORGANISM *Escherichia coli*

Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacterales; Enterobacteriaceae; Escherichia.

REFERENCE 1 (bases 1 to 460)

AUTHORS Kulkarni,S. and Peerapur,B.

TITLE Molecular Characterization of Escherichia coli isolates from patients with Urinary Tract infections in North Karnataka

JOURNAL Unpublished REFERENCE 2 (bases 1 to 460)

AUTHORS Kulkarni,S. and Peerapur,B.

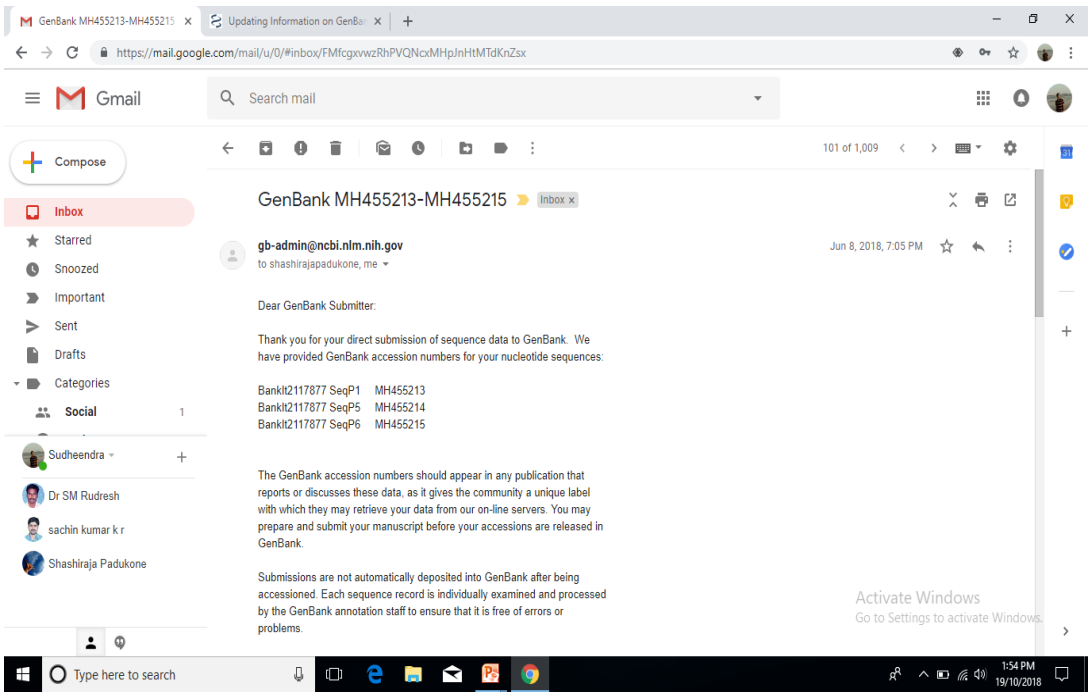
TITLE Direct Submission

Accession numbers:

BankIt2117877 SeqP1 MH455213

BankIt2117877 SeqP5 MH455214

BankIt2117877 SeqP6 MH455215



5.20. Detection of mutations in sequences of genes of UPEC strains:

All the isolates subjected for Sanger sequencing were checked for any nucleotide changes they possess in the study genes. *Pap* gene from all the three isolates showed 99% sequence identity and less than 2 nucleotide gap in overall product length with the aligned sequences in PubMed. This may indicate *Pap* gene in all the three isolates did not possess much molecular differences indicating homogenous genetic composition. **However, Bacterial isolate, P1 showed "T to G" nucleotide change and another isolate, P5 showed "A to G", "G to A" and "G to C" nucleotide changes.** These changes were confirmed in both forward and reverse sequencing but functional relevance of these single nucleotide changes on the activity of protein are yet to be deduced. Similarly *Trat* gene from all the eleven isolates possessed 98-99% sequence identity with very less nucleotide gaps (only one sequence possessed 98% identity but rest all were 99% identical).

Changes were seen at the nucleotide positions 799, 800 (A to G), 924 (G to C) and 966 (G to A)

Complete gene sequence

>M94076.1 Escherichia coli papE gene, papF gene, papG gene, complete cds

TTTTTGTACAGGATATTTCAGATGAAAAAGATAAGAGGTTTGTGTCTTCCGGTAATGCTGGGGGCAGTGT
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TGTAACAAAGGCAGAGGTTGACTGGGGAAATGTAGAGATTCAGACATTGAGCCCAGATGGAAGCAGACAT
CAAAAAGACTTTTCTGTCGGTATGAACTGCCCCTATAGCCTGGGAACCATGAAAGTCACAATAACATCAA
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TCTTTACAACAGTAATAACAGTAGTATTGGTAACGCAGTCACTTTAGGAAGTCAGTTTACGCCCGGAAAA
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CAGACTCTTTCTGTGTCTTGCGATGTGCCTGCAAATATTCGTTTTATGCTGTTAAGAAATACAACTCCGA
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>ORF number 1 in reading frame 1 on the direct strand extends from base 1 to base 546.

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GATATGAGAAAGCTGCAGGCTAAAGCATTTTCTGCAACGGCAACGCTGGTTGCATCATAT
TCGTAA

Translated protein sequence

FLYRIFQMKKIRGLCLPVMLGAVLMSQHVHAADNLTFKGKLIIPACTVTKAEVDWGNVEI
QTLSPDGSRHQKDFSVGMNCPYSLGTMKVTITSNGQTGNSILVPDTSSVSGDGLLIYLYN
SNNSSIGNAVTLGSQFTPGKITGVGQSKNITLYAKLGYKGDMRKLQAKAFSATATLVASY
S*

Complete gene sequence after incorporating nucleotide changes (highlightened in yellow color) that were shown in sequencing results

Escherichia coli papE gene, papF gene, papG gene, complete cds

TTTTTGTACAGGATATTTCAGATGAAAAAGATAAGAGGTTTGTGTCTTCCGGTAATGCTGGGGGCAGTGT
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>ORF number 1 in reading frame 1 for the above sequence

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S*

No changes were seen in the open reading frame.

CHAPTER 6

DISCUSSION

CHAPTER 6

DISCUSSION

Globally, UTI is the most common bacterial infection and is caused by *E. coli* which accounts to 90% of the UTIs, despite the availability of diverse antimicrobial agents. The emergence of antimicrobial resistance has caused a serious impact on the healthcare system globally. The rise in antimicrobial resistance among uropathogens is mainly attributed to the overuse, abuse and at times misuse of antibiotics along with empirical prescription without urine culture and sensitivity testing. The key method to tackle this issue is through appropriate use of antibiotics, knowledge of *E. coli* virulence factors, development of new agents and improved infection control policies. Susceptibility pattern of *E. coli* to the various antibiotics differ among geographical regions, thus leading to the development of an empirical therapy. .

This is the first study of its kind from North Karnataka to work on patients with UTI and the phenotypic & molecular characterization of the virulence factors of UPEC strains.

In this study, among 1000 urine samples tested, 39.5% (395) samples grown *E. coli* with colony count of 10^5 CFU/ml of urine **(Table 12**). We included only *E. coli* isolates in our study as *E. coli* is the predominant pathogen causing UTI. Studies from different parts of India have reported *E. coli* as one of the most common etiology causing UTI. The studies by Barate *et al.*[152] Amin *et al.*[153] and Shwetha *et al.*[154] have reported *E. coli* as the most common uropathogen in their studies.

In our study population, we observed that age was an important risk factor for UTI infection with *E. coli.* A large prospective study on sexually active young women revealed that recent sexual intercourse, use of diaphragm with spermicide and alteration of vaginal flora by OCP usage were the predisposing factors for UTI. Most women of child bearing age fall within this group, thus, amounting to disabilities during their reproductive life. Increasing incidence of UTI in elderly males is attributed to the upsurge in prostate disease and diabetes mellitus.[155]

`In this study, the prevalence of UTI was observed in the age group 20–29 years **(Fig 6)**. This is similar to the findings of the studies done by Janifer *et al.*[156] and Kamat *et al.*[157] We observed a higher prevalence of UTI in females (81.26%) than in males (19.07%). This is due to the anatomy of the female genitourinary tract making

them more susceptible to ascending infection by fecal flora, especially *E. coli* and is a consistent trend worldwide.

E. coli UTI caused by the MDR *E. coli* has seen a rise in the current decade probably due to the increasing and irrational use of antibiotics. The etiological profile and their antimicrobial susceptibility pattern differs geographically, thus routine testing of antibiotic agents is really imperative.[158] *E. coli* is the predominant pathogen isolated in our study population. The isolates were tested for fourteen different antibiotics and showed resistance to fluoroquinolones (FQs) and other commonly used antibiotics to treat UTI.

Out of 395 *E. coli* isolates, 170 (43%) were MDR **(Table 14)**. The isolates were highly resistant to ampicillin (82.53%), cefuroxime (72.41%), amoxicillin-clavulanic acid (71.90%), ceftriaxone (66.58%), ciprofloxacin (65.82%) and cefepime (57.47%) which is in agreement with the previous studies conducted by Akram *et al.*[36] and Aypak *et al.*[159] in other parts of India. In the current investigation, *E. coli* showed 71.90% resistance for amoxicillin-clavulanic acid; whereas, Sire *et al.*[160] reported 67.0% resistance to amoxicillin-clavulanic acid, which is lower than the present study finding. We monitored the year wise antibiotic sensitivity pattern of *E. coli*. 72 *E. coli* were isolated in the year 2012 and were analysed for antibiotic sensitivity pattern among patients of different age group as shown in the **Table 13**. Maximum isolates shown sensitivity and resistance to antibiotics in the age group 20-29 with highest resistance of 28 isolates for antibiotic AMP and sensitivity of 24 isolates for IPM. This trend continued till year 2015. Highest resistance of 38 isolates for AMP and sensitivity of 40 isolates for antibiotics IPM & NIT in the year 2013, highest resistance of 19 isolates for AMP and sensitivity of 21 isolates for NIT in the year 2014, highest resistance of 20 isolates for AMP, CXM and sensitivity of 23 isolates for IPM in the year 2015. And in the same year 2015, 34 *E. coli* isolates showed highest resistance to antibiotic AMP and sensitivity of 35 isolates to IPM among the *E. coli* isolated from urine samples of pregnant women. In the year 2012, the isolates were sensitive to the most of the antibiotics compared to 2015 **(Fig 9-13)**. Thus, these findings clearly indicate that MIC of *E. coli* to AMP and CXM has increased along with the resistance to other antibiotics. Our study findings are in concordance with the study results conducted by Biswas *et al.*[161]

The rapid upsurge in the rate of antibiotic resistance of UPEC isolates is a major cause of concern. In our study isolates, we observed a high degree of resistance

pattern to commonly used antibiotics such as ampicillin, amoxicillin-clavulanic acid, ciprofloxacin and cefuroxime. Higher sensitivity was observed in nitrofurantion (92.41%), amikacin (90.89%), chloramphenicol (85.82%) and piperacillin-tazobactum (80.76%). Several studies have also reported high level of resistance to these antibiotics used for the treatment of UTI.[118, 162] A study by Banu *et al.*[163] in patients with *E. coli* UTIs found 96% ampicillin resistance, 74% co-trimoxazole, 44% ciprofloxacin, 56% gentamicin and 35% amikacin resistance respectively. Another study by Zhanel *et al.*[162] found that about 38% *E. coli* isolates were ampicillin resistant and around 21% were co-trimoxazole resistant. Eshwarappa *et al.*[115] studied the clinico‑microbiological profile of UTI in South India. Their study also revealed that *E. coli* was the most common organism causing UTIs which recorded a high resistance to commonly used antibiotics in the UTI treatment and least resistance against carbapenems (3.9%). A study by Sharma *et al.*[15] in Mangalore also reported high prevalence of antibiotic resistance among the *E. coli* isolates. This finding is helpful in guiding early appropriate empirical therapy for UTI infections. Among all the antibiotics tested, highest degree of sensitivity was seen with the antibiotic imipenem (96.71%).

Pregnant women are more susceptible to asymptomatic and symptomatic UTI because of increased urinary content of amino acids, vitamins, and other nutrients, which encourage the persistence of infection. Manjula *et al.*[164] studied the incidence and prevalence of UTI among pregnant women in Karnataka and found that 49.4% of pregnant women have had UTI, and *E. coli* was the predominant pathogen isolated. The present study revealed 40.66% of the asymptomatic UTI rate due to *E. coli* in pregnant women of Bidar district in North Karnataka region and their resistant pattern to different antibiotics.

Current investigation reveals that the significant numbers of the UTI are caused by MDR *E. coli*, which is evident in our study population. Our study findings also might help the physicians in choosing the appropriate treatment for UTI patients. There is a need for continued surveillance both locally and nationally to administer safe and effective empirical therapy for UTIs in order to combat both infection and drug resistance.

Fluoroquinolones (FQs) are the paramount drugs in the treatment of UTIs. Renal excretion of these molecules and the availability of oral and parenteral formulations have allowed them to compete with aminoglycosides and beta-lactams

group of antibiotics which are commonly used in the therapy of UTIs, especially in hospital settings. FQs such as ciprofloxacin (CIP) have been suggested as an effective empirical treatment for uncomplicated urinary tract infections with high levels (~10%) of resistance among uropathogens to trimethoprim sulfamethoxazole or trimethoprim, in both community and hospital settings in north America.[165] However, in recent years, emergence of resistance to CIP was reported amongst these uropathogens.[166] One of the reasons for appearance of higher resistance to ciprofloxacin might be due to the fact that these particular groups of antibiotics are among the first choice of drug for treating the UTI by many physicians in Indian subcontinent. Lee, Seung Ju, *et al.*[167] from South Korea and Manjunath GN *et al.*[168] from South Karnataka have revealed high incidence of resistance to CIP (73%-92.7%) amongst the UPEC isolates. In the present study, 65.82 % and 46.33% of *E. coli* isolates were resistant to ciprofloxacin and norfloxacin respectively. The rate of resistance to ciprofloxacin is alarming as it is far above the 20.0% rate recommended for empirical use of antibiotics for treatment of UTI. This may be due to the FQs cross-resistance phenomenon of sharing same enzyme target, and also frequent prescription of FQs to FQs resistant *E. coli*. Furthermore, ciprofloxacin resistance was seen commonly among women with prior UTI history. Thus concluding that resistance to FQs may be a significant issue only in higher risk group of patients with prior UTI and antibiotic use. A study by Aypak *et al.*[159] reported that, the treatment duration for UTI with FQs were significantly longer than the recommended regime and suggested to discourage the empirical use of FQs in UTI treatment. In addition, socio demographic differences like lifestyle, occupation, culture, and literacy between the populations might have also contributed for antibiotic resistance.

UTI continues to persist regardless of positive antimicrobial therapy. It is also known to recur despite treatment with a broad array of antibiotics prescriptions. UPEC is receiving increased attention, due to the fact of its high degree of morbidity and mortality rates seen. Recurrent and relapse of UTIs may be attributed to the presence of bacterial virulence factors. Some of these virulence factors are cytotoxic and strain specific to *E. coli*. UPEC virulence factors have been demonstrated to play an important role in pathogenesis and cause significant antimicrobial resistance.[169] The importance of the UPEC VFs is accredited to the fact that they facilitate the colonization of the bacteria by assisting the organism overcome host defences, thereby infesting the urinary tract. Hence it is essential to differentiate UPEC from

non UPEC isolates in the cultures. VFs of UPEC develop multi-drug resistance. Apart from these, antimicrobial resistance may develop on account of abuse of broad-spectrum antibiotics. Among the various identified VFs, the most significant are expression of adhesins or fimbriae which allow UPEC to bind and invade host cells and tissues within the urinary tract. Other important virulence factors are biofilm formation, haemolysin, (which produces toxin), serum resistance & Pathogenicity islands.

Further, in our study, we studied the multiple phenotypic and genotypic virulence factors of UPEC like biofilm formation, ESBL production and Hemolysin formation. Results of our study showed the presence of more than one virulence markers in a majority of *E. coli* isolates. Thus, in this study we conclude that UPEC strains are associated with the aetio-pathogenesis of UTI, and the presence of multiple VFs in these strains further strengthens the concept of association of UPEC with urinary pathogenicity.

In the urinary tract, biofilm has been noted to develop on catheters and epithelial cells. This is a matter of concern because, in a biofilm, bacteria are more resilient to antimicrobial agents in contrast to planktonic bacteria.[170] Several studies have reported that UPEC strains were frequent biofilm producers than other strains.[107-108] In this study, the incidence of in vitro biofilm formation by UPEC was 71.39% which was similar to the study findings of Sharma *et al.* [171] and Subramanian Pramodhini *et al.*[172] who reported biofilm formation at the rate of 63%, and 67.5% respectively. In our study, we analysed the in vitro biofilm formation of the strains by three different methods. 42.78% isolates were positive by tube adherence method. We classified them as highly positive (10.3%), moderate positive (9%), and weakly positive (23.5%). By Congo red agar (CRA) method, 65.5% isolates were positive. In tissue culture plate (TCP) method, again it was classified as strong positive (73.2%), moderate positive (5.9%) and weakly positive (20.9%) **(Table 15).** These findings were much closer to the study results reported by Tabasi, Mohsen, *et al.*[173] In our study, we detected biofilm formation in all 388 *E. coli* isolates (100%) by TCP method which was similar to the other study findings reported by Fattahi *et al.*[174] who reported biofilm formation in 100% isolates by TCP method.

The rapid increase of antibiotic resistance in biofilm forming UPEC strains is a major cause of concern. So, analysis of the association of biofilm forming *E. coli* strains and their antimicrobial susceptibility pattern was taken up in this study.

Biofilm‐producing strains showed the highest resistance to the antibiotics compared to non-biofilm‐producing isolates. Biofilm producers demonstrated resistance to AMP (87.36%) followed by CXM (81.58%), AMC (77.61%), CIP (71.48%), CTR (54.6%), and CPM (64.98%). Our study results revealed significant correlation between biofilm formation and MDR. There was an increase in resistance pattern of the drugs amoxicillin-clavulanic acid, aztreonam, cefriaxone, cefuroxime, cefepime, ciprofloxacin, and chloramphenicol which were routinely used to treat UTI from long time **(Table 16, 17 & 18)**. This pattern of resistance coincides with the study findings reported by Mittal *et al.*[38] and Poovendran Ponnusamy *et al.*[175] strains exhibited sensitivity to drugs amikacin (88%) and nitrofurantoin (92.41%). Among all the antibiotics tested, highest degree of sensitivity was seen with imipenem (97.14%) and piperacillin‐tazobactam (97.83%).

In the present study, the drugs AK, NIT, PIT, and IPM, were effective against biofilm‐producing UPEC strains and these drugs can serve as useful reserved drugs for the treatment of UTI but it should be considered that overuse of these antibiotics can gradually lead to increasing antibiotic resistance. Understanding biofilms in UTIs will help clinicians in decision‐making towards effective treatment guidelines for recurrent UTI in this geographical region.

We carried out phenotypic detection of virulence factors of *E. coli* strains. 62.37% of the strains were ESBL producers. (242 of 388 *E. coli* strains) Increase in the ESBL-producers is evident globally, indicating the need in continuous monitoring systems and effective infection control measures. Current studies on ESBL production among *E. coli* isolated from urine specimens shows an increase in the incidence of ESBL producers. Studies conducted in different parts of India have reported 34% and 88% prevalence of ESBL production by UPEC strains.[122,38] In the studies done outside India by Sanjeev, *et al.*[176] and Abu Jaffal A *et al.*[177] in Kathmandu and Saudi Arabia, reported 33.2 % and 33% of ESBL production by UPEC strains respectively, which is lesser than our study results. We found high prevalence of ESBL production by UPEC strains. Previous studies have shown that ESBL producing *E. coli* strains were frequently resistant to non- β-lactam antibiotics such FQs and aminoglycosides.[15, 178] In our study we found a high degree of resistance to multiple classes of antibiotics among ESBL producing strains. This finding is supported by the study reports of Poovendran *et al.* and Naik and Desai.[139-140] Further, in this study,

more than half of the ESBL producing strains (81.81%) were resistant to ciprofloxacin. This finding established a statistically significant association between ESBL formation & ciprofloxacin resistance suggesting that they might be resistant to all available FQs - the drug of choice for treatment of UTI caused by ESBL-producing strains **(Table 25)**. Similar trend has been reported by Jadhav *et al.*[118] in their study conducted in semi urban locality of India.

Most of the UPEC strains secrete a cytolytic protein toxin called as haemolysin. In our study, 40.20% *E. coli* strains were observed to be β haemolytic on sheep blood agar plates. In the previous studies conducted by Siegfried *et al.*[179] reported 68.45% of *E. coli* strains producing hemolysin, Raksha *et al.*[113] reported 41.36%, and Mittal *et al.*[180] reported 47.4% of hemolysin production respectively, which is higher than our study results. Hemolysin production is associated with human pathogenic strains of *E. coli*, especially those causing more clinically severe forms of UTI. It is toxic to a range of host cells in ways that probably contribute to inflammation, tissue injury and impaired host defenses.[181] Our study results also showed a relationship between multiple phenotypic virulence factors of UPEC strains. Our results demonstrated statistically significant relationship between UPEC strains exhibiting virulence factors, ESBL and hemolysin with biofilm formation **(Table 21)**. This shows that ESBL & Hemolysin producing UPEC strains had a higher ability of biofilm formation among UPEC strains, thereby increase in the antibiotic resistance. This finding is similar with the other study findings.[119, 139, 182] It has been suggested that a number of chromosomal gene re-arrangement occurs upon acquisition of the ESBL plasmid and its expression, leading to an increased mortality and severity of infection.[183]

Multiple drug resistance patterns of *E. coli* isolates and the correlation between ESBL and biofilm producing *E. coli* strains were also determined. Statistically significant association was seen between ESBL & Hemolysin producing UPEC and MDR. This study revealed more than 90% of ESBL & Hemolysin producing UPEC are resistant to more than 5 drugs, **(Table 23)**. These findings are in concordance with the results reported by Supriya S. *et al.*[184] The results of present study indicated a need for continued surveillance of antimicrobial resistance among ESBL and biofilm producing uropathogens causing UTI, so as to increase the positive outcomes of clinical interventions.

Alternative choice of antimicrobial agent for the treatment of ESBL positive UPEC is limited. carbapenems are the most effective in this situation, which has been proved in the present study also; but the drawback is that it needs intravenous or intramuscular administration. Furthermore, the very high rate of ESBL-producing UPEC strains is a matter of concern. It also warrants for a change in the empirical therapy for UTI and calls for a continuous monitoring of the ESBL production and antimicrobial sensitivity testing, to prevent treatment failure. The early detection and reporting of suitable antibiotics would definitely minimize the treatment failure in ESBL UTI as the high prevalence of MDR and ESBL among UPEC strains is a major threat for healthcare system especially in a resource constrained developing country like India. On the other hand, due to the high cost of antibiotics, it may not be possible for the weaker socioeconomic sector to procure these antibiotics. Continued use of these drugs is likely to be associated with a high risk of treatment failure. This study paves a way for strict antibiotic policy implementation in hospitals, to estimate the impact of increased resistance in bacteria and also to take steps for reducing their resistance. There is need to formulate an appropriate hospital antibiotic policy for the treatment of UTI. In the interim, clinicians could take these research findings into consideration, whenever prescribing empirically for UTI. The present study recommends that amikacin and nitrofurantoin can be used for empirical treatment of UTI and carbapenems (imipenem and meropenem) are the most effective reserved drugs against ESBL producing UPEC strains.

The urine represents the most important barrier to microbial colonization of the urinary tract by UPEC strains. These strains carry multiple virulence genes such as adhesins, toxins and siderophores that contribute to the development of the infectious process. A previous study indicated that although virulence of an organism cannot be accurately predicted on the basis of its measurable virulence factor phenotype, the presence of multiple virulence factor genes does increase the pathogenicity of organisms. The VFs function additively or synergistically in overcoming normal host defenses and the strains with a more widespread complement of VFs are more effective pathogens.[66] The generally accepted hypothesis is that UPEC evolved from non-pathogenic strains by acquiring new VFs from accessory DNA by horizontal transfer located at the chromosomal or plasmid level and progress in the molecular technology has facilitated studies on UPEC.[185-186]

The present research aimed to evaluate the prevalence of different virulence genes among UPEC strains and also to correlate the genetic virulence traits and antibiotic resistance of the strains. We used a genotypic assay to detect the virulence factor genes in potential UPEC strains. PCR is highly specific, informative and a powerful genotypic assay, used for the detection of three most important virulence genes of UPEC namely, adhesin-encoding operon, i.e. Pilus associated pili (*papEF*), genes encoading Serum resistance (*traT*) and gene for Pathogenicity Island (PAI) which contributes to the virulence in UTI.

E. coli strains were examined for the above three virulence genes by using specific primers. Our study results revealed that, UPEC strains expressed multiple virulence factor genes. Overall, the virulence genes were detected in 318 (81.95%) of UPEC strains. 76 strains (19%) possessed only one gene, 123 strains (31.70%) had two genes; and a combination of three virulence genes were observed in 49 (12.62%) strains. In 70 strains (18%) none of these virulence genes were found. Several other investigators have also reported the presence of multiple virulence factor genes among the UPEC strains.[187-189] The frequencies of the studied virulence genes are depicted in **Table 19.**

P fimbriae, the principal mannose - resistant adherence organelles of extra intestinal pathogenic *E. coli*, is known to contribute to the pathogenesis by promoting bacterial colonization of host tissues and by stimulating an injurious host inflammatory response. With regard to adhesin virulence determinants, the *papEF* gene was detected in 33.5% (130/388) of the UPEC strains. Among the genes coding for serum resistance, *traT* was the most common virulence gene and was detected in 73.2%, (284/388) strains and PAI was present in 62.9% (244/388) UPEC strains. Expression of all these three genes was high in female patients compared to males **(Table 20**). *PapEF* is responsible for the assembly platform for the fimbrial growth and helps the isolates to adhere to eukaryotic cells. Johnson *et al.*[190] and Brauner A[116] have suggested that P fimbriae contribute to the ability of *E. coli* strains to cause UTI, especially the more clinically severe forms. We found the lower frequency of *pap* gene among UPEC strains in the present study, less than the study findings of Chakraborty, *et al.*[191] (45%) in South India & Fattahi *et al.*[174] (43%) in Iran and Abe CM *et al.*[192] (45.8%) from Brazil. But higher than the findings of study conducted in China by Qin, Xiaohua, *et al.*[193] who reported the gene frequency of 28% suggesting that it may not be important in UPEC pathogenesis.

Taylor PW[102] reviewed that bacteria are killed by normal human serum through lytic activity of alternative complement system. Bacterial resistance to killing by serum results from individual or combined effects of capsular polysaccharide, O polysaccharide and surface proteins.[194] Current study results showed that 73.2% of UPEC strains contained *traT* gene. Oliveira *et al.*[195] showed that 76% of the urine samples were contaminated with multidrug-resistant bacteria carrying *traT* gene and Kudinha *et al.*[122] reported a frequency of 77% for *traT* gene among *E. coli* strains isolated from patients with cystitis. These results are compatible with those of the current study, which suggest that the *traT*, as a common and important virulence factor, and could be considered as a target for therapeutic interventions.

Earlier reports demonstrated that UPEC strains might harbor various VFs, usually encoded on PAIs, providing a mechanism for coordinated horizontal transfer of virulence genes, known to contribute to the bacterial pathogenesis and survival in a specific environment.[196-197] The PAI marker showed a frequency of 62.9% in the current study. Johnson *et al.*[198] reported a frequency of 71% and Najafi *et al.*[199] reported PAI markers in substantial percentage of UPEC strains in their study (98.6 %). Ghosh, A & Mukherjee M.[200] found higher prevalence of the PAI markers in their study. It is PAIs are capable of horizontal virulence genes transfer between species; therefore, a frequency of 62.9% for PAI markers in UPEC strains isolated in our study is notable.

However, in this study, even though there was an association between the prevalence of *papEF*, *traT* & RPAI genes of UPEC strains and their biofilm formation potential, it was not proved statistically, which rather suggests a role in the in vivo colonization of uroepithelial cells. Our findings are in accordance with other study results.[20-21] Our study data reveals that among 280 biofilm positive UPEC strains, 183 strains did not express the gene *papEF* but only 97 strains expressed. Similarly with *traT* & RPAI genes also, where biofilm forming strains made no impact on the expression of virulence genes by UPEC strains. This revealed no significant correlation between presence of virulence genes & biofilm formation. But earlier study reported the association between higher biofilm formation potential and some virulence genes including P and type1 fimbriae.[22]

The interplay between resistance and virulence is poorly understood. In the clinical management of infectious diseases, with respect to multidrug-resistant pathogens, it has been frequently assumed that more antimicrobial drug resistance

equates with greater virulence. Hence, we tried to establish association between the expression of virulence genes with multidrug resistance. Earlier studies on UPEC by Kawamura-Sato *et al.*[201] & Basu *et al.*[202] reported a correlation between antibiotic resistance and reduction in the virulence factor genes. Authors suggested that quinolone resistance may be directly associated with the loss of virulence. In this study, MDR UPEC strains exhibited significantly higher prevalence of gene *traT* than other virulence genes. UPEC strains exhibited lower prevalence of *pap* and PAI genes. The prevalence of *papEF* gene was associated with resistance of antibiotics AMP (91.5%), AMC (89.2%), CTR (76.2%), CXM (82.3%), CPM (71.5%), and CIP (72.3%) but not proven to be statistically significant. Gene *traT* was associated with AMP (94.4%), AMC (92.3%), CTR (74.3%), CXM (83.1%), CPM (72.2%), and CIP (77.1%). RPAI was associated with AMP (93.9%), AMC (91%), CTR (75%), CXM (82.4%), CPM (70.9%), and CIP (75.4%) respectively. Only the gene RPAI was associated with antibiotic NIT and *traT* with AK and this association was proved to be statistically significant **(Table 24)**. Resistance to ceftriaxone, cefuroxime & cefepime may be related to the production of ESBL. These results reflect the heterogeneous distribution of virulence genes and antibiotic resistance among UPEC strains. Our findings conclude that VFs and antibiotic resistance of UPEC vary significantly. Similar findings were reported by Miranda-Estrada, Laura Iveth, *et al.*[202] in their study. A study by Basu *et al.*[203] and Ghosh A. & Mukherjee, M.[200] indicated a statistically significant reduction in the distribution of urovirulence genes amongst the NA and CIP resistant and susceptible UPEC strains circulated in Kolkata.

Earlier studies conducted in different parts of the world stated that pathogenic *E. coli* evolved from commensal *E. coli* through horizontal gene transfer.[204-205] Therefore, characterizing the UPEC virulence genes provides a detailed insight into the characteristics of UPECs isolated from North Karnataka region and helps the microbiologists and clinicians to understand the emerging pathogenic potential of UPECs that may colonize and persist in human urinary tract.

In our study, molecular confirmation & characterization of *pap* gene revealed that, among the *E. coli* strains employed for Sanger sequencing, all strains showed 99% sequence identity **(Table 26)**. This may indicate no variation in the *pap* gene. Isolate, P1 showed *"T to G"* nucleotide change and another isolate, P5 showed *"A to G", "G to A"* and *"G to C"* nucleotide changes. Functional relevance of these single nucleotide changes on the activity of protein are yet to be deduced. UPEC strains

sequenced for *traT* gene possessed 98-99% sequence identity with very less nucleotide gaps (only one sequence possessed 98% identity but rest all were 99% identical). Changes were seen at the nucleotide positions 799, 800 (*A to G*), 924 (*G to C*) and 966 (*G to A*).

Mutation analysis revealed no changes in the open reading frame and no mutations were detected among the sequenced genes.

CHAPTER 7

SUMMARY & CONCLUSION

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SUMMARY AND CONCLUSION

Summary:

- The research was performed on 1000 patients suffering with symptoms and complaints of UTI attending to a tertiary care hospital in North Karnataka. The aim of the study was to estimate the prevalence of UTI due to *E. coli* and its antibiotic susceptibility pattern year wise, determination of biofilm formation, and correlation with antibiotic resistance with special reference to expression of phenotypic and genotypic characters of uropathogenic *E. coli*.
- The clinical details such as evidence of fever, burning micturition, pain in lower abdomen and other symptoms associated with UTI were collected in a proforma, along with the details of antibiotics prescribed and the clinical outcome.
- The *E. coli* isolates were identified phenotypically based on their colony morphology on Blood & MacConkey agar, and by standard biochemical tests.
- 395 *E. coli* (39.5%) were isolated from 1000 urine samples. Infection was predominant in females (81.26%) between the age group of 20-29 with 154 isolates at the rate of 39% and highest among rural population (75%).
- Antimicrobial susceptibility testing was performed using Kirby-Bauer disk diffusion methods in accordance with CLSI guidelines. This study monitored the year wise antibiotic susceptibility pattern of *E. coli* isolates to different antibiotics. Isolates have shown a low but steady increase in resistance to antibiotics such as ampicillin (82.53%), cefuroxime (72.41%), amoxicillin-clavulanic (71.90%), ceftriaxone (66.58%), ciprofloxacin (65.82%) and cefepime (57.47%) which are commonly used to treat UTI.
- Of 395 *E. coli* isolates, 170 (43%) *E. coli* were MDR. High level of resistance to antibiotics ampicillin (82.53%), cefuroxime (72.41%), amoxicillin-clavulanic acid (71.90%), ceftriaxone (66.58%), ciprofloxacin (65.82%) and cefepime (57.47%) were seen respectively.
- Biofilm formation: In-vitro Biofilm formation of isolates was detected by three different methods. Tube adherence method, Congo red agar method and Tissue culture plate method. 71.39% isolates produced biofilm by all the three above

methods. Biofilm forming *E. coli* strains developed higher degree of resistance towards antibiotics ampicillin (87.36%) followed by cefuroxime (81.58%), amoxicillin-clavulanic acid (77.61%), ciprofloxacin (71.48%), cefepime (64.98%) and ceftriaxone (54.6%).

- Significant association was observed between biofilm formation and multi drug resistance which was found to be statistically significant ($p<0.05$) with regards to the following antibiotics - amikacin, amoxicillin-clavulanic acid, aztreonam, ceftriaxone, cefuroxime, cefepime, ciprofloxacin, and chloramphenicol.
- Phenotypic characterization of *E. coli* isolates was done as follows: Isolates were tested for ESBL via HI chrome ESBL screen agar media. And haemolysin production on 5% sheep blood agar. Phenotypically 62.3% *E. coli* isolates were ESBL producers. 40.2% isolates were found to be β- haemolytic.
- There was significant correlation between ESBL & hemolysis producing strains with biofilm formation. ESBL & Hemolysis producing UPEC had a higher ability of biofilm formation indicating that the haemolytic strains carry maximum number of virulence genes.
- Statistically significant association was seen between ESBL & Hemolysin producing UPEC and multidrug resistance. ESBL & Hemolysin producing UPEC exhibited resistant to antibiotics ampicillin, amoxicillin-clavulanic acid, aztreonam, ceftriaxone, cefuroxime, cefepime, ciprofloxacin, chloramphenicol, gentamicin and norfloxacin.
- In correlation with ESBL producing UPEC and ciprofloxacin resistance, significant association was seen between ESBL & ciprofloxacin resistance. 67.3% of UPEC were ESBL positive.
- On analysis of virulence genes among UPEC strains, genes *traT* and PAI were found to be the most prevalent (73.2% & 62.9%). *PapEF* was found in 33.5% strains.
- There was no statistically proven significant association seen in the distribution of virulence genes among the biofilm producing strains.
- In this study, we found that, there was correlation with the possession of virulence genes and drug resistance. The prevalence of *papEF* gene was associated with

resistance of antibiotics AMP (91.5%), AMC (89.2%), CTR (76.2%), CXM (82.3%), CPM (71.5%), and CIP (72.3%). Gene *traT* was associated with AMP (94.4%), AMC (92.3%), CTR (74.3%), CXM (83.1%), CPM (72.2%), and CIP (77.1%). RPAI was associated with AMP (93.9%), AMC (91%), CTR (75%), CXM (82.4%), CPM (70.9%), and CIP (75.4%) respectively

- Only gene RPAi was associated statistically significantly with antibiotic NIT and gene *traT* with AK which is statistically proven, indicating that, host factors and early use of appropriate antibiotics may influence the outcome.
- Molecular confirmation & characterization of study virulence genes was done by Sanger sequencing. *E. coli* strains employed for Sanger sequencing, showed 99% sequence identity.
- *papEF* sequences were deposited to NCBI-Gen Bank DNA database and got the accession numbers.
- Mutation analysis revealed no changes in the open reading frame and no mutations were detected among the sequenced genes.
- None of the UPEC strains from this study population were 100% susceptible to any of the antimicrobial drugs tested. Antibiotics nitrofurantoin, piperacillin - tazobactam, and imipenem can be effective for severe UTIs.

Conclusion:

Knowing the present trends of virulence patterns of the Uropathogenic *E. coli* from particular geographical region will help the clinicians to formulate guidelines for the empirical treatment of UTI while awaiting culture & sensitivity results.

This study gives clinicians an insight on the pattern of virulence traits of UPEC and its association with antibiotic resistance which will help in treating patients of North Karnataka region effectively.

In this study, we noticed high prevalence of UTI in this region along with increasing trends of *E. coli* isolates to antibiotics which are routinely used for the treatment of UTI. Further, we found the expression of phenotypic virulence factors, which are responsible for higher resistance to antibiotics and established a relationship

between the two. We also observed the correlation where UPEC strains carrying virulence genes did establish a statistical significant association with the drug resistance.

Hence we conclude in this study that, there is significant relationship between the phenotypic and genotypic virulence factors of UPEC strains and antibiotic resistance isolated from this region.

As per our knowledge, this is the first study of its kind, reported from North Karnataka on virulence factors of UPEC strains and molecular characterization of genotypic virulence of UPEC strains.

CHAPTER 8
STUDY LIMITATIONS & FUTURE DIRECTIONS

CHAPTER 8

STUDY LIMITATIONS & FUTURE DIRECTIONS

Study limitations:

1. Host factors like time of symptomatic presentation, prior antibiotic treatment history of the patient and also the phenotypic and genotypic characters of the infecting *E. coli* strains limit the results of the study.
2. However, this study has certain limitations, which could serve as a basis for advanced future studies. The presence of *pap, traT and* PAI genes were targeted and expression of other possible virulence genes were not included in this study.
3. Sequencing and characterization on a large number of isolates is essential to confirm the mutations.

Future prospective:

Increased resistance to almost all the antimicrobials tested in this research directs towards a further study with large number of isolates on the genetic propensity of the strains accumulating mutations, conferring Multi drug resistant phenotypes.

CHAPTER 9

BIBLIOGRAPHY

CHAPTER 9

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1. Hooton TM, Stamm WE. Diagnosis and treatment of uncomplicated urinary tract infection. Infect Dis Clin North Am. 1997 Sep;11(3):551-81.
2. Patton JP, Nash DB, Abrutyn E. Urinary Tract Infection: Economic Considerations. Med Clin North Am. 1991 Mar;75(2):495-513.
3. Schappert SM, Burt CW. Ambulatory care visits to physician offices, hospital outpatient departments, and emergency departments: United States, 2001-02. Vital Health Stat 13. 2006 Feb;(159):1-66.
4. Al-Jiffri O, El-Sayed ZMF, Al-Sharif FM. Urinary Tract Infection with *Esherichia coli* and Antibacterial Activity of Some Plants Extracts. Int. J. Microbiol. Res 2011 Jan;2(1):1-7.
5. Kunin CM. Urinary Tract Infections in Females. Clin Infect Dis. 1994 Jan;18(1):1-12.
6. Dromigny JA, Nabeth P, Juergens-Behr A, Perrier-Gros-Claude JD. Risk factors for antibiotic-resistant *Escherichia coli* isolated from community-acquired urinary tract infections in Dakar, Senegal. J Antimicrob Chemother. 2005 Jul;56(1):236-9.
7. Minardi D, d'Anzeo G, Cantoro D, Conti A, Muzzonigro G. Urinary tract infections in women: etiology and treatment options. Int J Gen Med. 2011;4:333-43.
8. Finer G, Landau D. Pathogenesis of urinary tract infections with normal female anatomy. Lancet Infect Dis. 2004 Oct;4(10):631-5.
9. Sobel JD. Pathogenesis of urinary tract infection. Role of host defenses. Infect Dis Clin North Am. 1997 Sep;11(3):531-49.
10. Wang X, Lunsdorf H, Ehren I, Brauner A, Romling U. Characteristics of biofilms from urinary tract catheters and presence of biofilm-related components in *Escherichia coli*. Curr Microbiol. 2010 June;60(6):446-53.
11. Tambyah PA. Catheter-associated urinary tract infections: diagnosis and prophylaxis. Int J Antimicrob Agents. 2004 Sep;24 Suppl 1:S44-8.
12. Tajbakhsh E, Ahmadi P, Abedpour-Dehkordi E, Arbab-Soleimani N, Khamesipour F. Biofilm formation, antimicrobial susceptibility, serogroups and virulence genes of uropathogenic *E. coli* isolated from clinical samples in Iran. Antimicrob Resist Infect Control. 2016 Apr;5:11.

13. Tayal R, Baveja S, De A. Analysis of biofilm formation and antibiotic susceptibility pattern of uropathogens in patients admitted in a tertiary care hospital in India. Int J Health Allied Sci. 2015 Nov;4(4):247.
14. Foxman B. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. Am J Med. 2002 Jul 8;113 Suppl 1A:5s-13s.
15. Sharma S, Bhat GK, Shenoy S. Virulence factors and drug resistance in *Escherichia coli* isolated from extraintestinal infections. Indian J Med Microbiol. 2007 Oct;25(4):369-73.
16. Naveen R, Mathai E. Some virulence characteristics of uropathogenic *Escherichia coli* in different patient groups. Indian J Med Res. 2005 Aug;122(2):143-7.
17. Sammon JD, Sharma P, Rahbar H, Roghmann F, Ghani KR, Sukumar S, et al. Predictors of admission in patients presenting to the emergency department with urinary tract infection. World J Urol. 2014 Jun;32(3):813-9.
18. Abbo LM, Hooton TM. Antimicrobial Stewardship and Urinary Tract Infections. Antibiotics (Basel). 2014 Jun;3(2):174-92.
19. Rubin RH, Shapiro ED, Andriole VT, Davis RJ, Stamm WE. Evaluation of new anti-infective drugs for the treatment of urinary tract infection. Infectious Diseases Society of America and the Food and Drug Administration. Clin Infect Dis. 1992 Nov;15 Suppl 1:S216-27.
20. Warren JW, Abrutyn E, Hebel JR, Johnson JR, Schaeffer AJ, Stamm WE. Guidelines for antimicrobial treatment of uncomplicated acute bacterial cystitis and acute pyelonephritis in women. Infectious Diseases Society of America (IDSA). Clin Infect Dis. 1999 Oct;29(4):745-58.
21. Ferry SA, Holm SE, Stenlund H, Lundholm R, Monsen TJ. Clinical and bacteriological outcome of different doses and duration of pivmecillinam compared with placebo therapy of uncomplicated lower urinary tract infection in women: the LUTIW project. Scand J Prim Health Care. 2007 Mar;25(1):49-57.
22. Nicolle LE. Asymptomatic bacteriuria--important or not? N Engl J Med. 2000 Oct;343(14):1037-9.
23. Mittal R, Aggarwal S, Sharma S, Chhibber S, Harjai K. Urinary tract infections caused by *Pseudomonas aeruginosa*: a minireview. J Infect Public Health. 2009;2(3):101-11.
24. Mabeck CE. Treatment of uncomplicated urinary tract infection in non-pregnant women. Postgraduate medical journal. 1972;48(556):69-75.

25. Mulvey MA, Lopez-Boado YS, Wilson CL, Roth R, Parks WC, Heuser J, et al. Induction and evasion of host defenses by type 1-piliated uropathogenic *Escherichia coli*. Science. 1998 Nov;282(5393):1494-7.
26. Sobel JD KD. Urinary tract infections. In: Mandell GL BJ, eds, editor. Principles and Practice of Infectious Diseases. 8 ed. Philadelphia: Elsevier Saunders; 2014. p. 886-913.
27. Foxman B. Recurring urinary tract infection: incidence and risk factors. Am J Public Health. 1990 Mar;80(3):331-3.
28. Johnson CC. Definitions, classification, and clinical presentation of urinary tract infections Med Clin North Am. 1991 Mar;75(2):241-52.
29. Nimri L. Community-Acquired Urinary Tract Infections in a Rural Area in Jordan: Predominant Uropathogens, And their Antimicrobial Resistance. Webmed Centeral. 2010 Jan;1(9):660.
30. Brazilian Federation of Gynecology and Obstetrics Associations BSoID, Brazilian Society of Family and Community Medicine, Brazilian Society of Nephrology, Brazilian College of Radiology. Uncomplicated urinary infection in women: diagnosis. Revista da Associacao Medica Brasileira (1992). 2011;57(3):255-8.
31. Nicolle LE, Bradley S, Colgan R, Rice JC, Schaeffer A, Hooton TM. Infectious Diseases Society of America guidelines for the diagnosis and treatment of asymptomatic bacteriuria in adults. Clin Infect Dis. 2005 Mar;40(5):643-54.
32. Razak S, Gurushantappa V. Bacteriology of urinary tract infection and antibiotic susceptibility pattern in a tertiary care hospital in South India. Int J Med Sci Public Health. 2012;1(2):109-12.
33. Prabhu V, Pandian SR. Study on the prevalence of urinary tract infection among the Paliyar Indian tribe. Int J Curr Res. 2012 Jan;4:44-8.
34. Nicolle LE. Asymptomatic bacteriuria: when to screen and when to treat. Infect Dis Clin. 2003 Jun 1;17(2):367-94.
35. Adabara NU, Momoh JA, Bala JD, Abdulrahaman AA, Abubakar MB. The prevalence of bacterial urinary tract infections (uti) among women attending antenatal clinic in the general hospital, minna in niger stateInt J Biomed Res. 2012 Apr;3(3):171-3.
36. Akram M, Shahid M, Khan AU. Etiology and antibiotic resistance patterns of community-acquired urinary tract infections in J N M C Hospital Aligarh, India. Ann Clin Microbiol Antimicrob. 2007 Mar;6:4.

37. Patel P, Bairy, KL,Amberkar, Mohan. Antibiotic Resistance Pattern Among in-patients of Urinary Tract Infection at Tertiary Care Hospital of Coastal Karnataka-A Retrospective Study. Int. J. Pharma Bio Sci. 2016 Apr;7(2):18-23.
38. Mittal S, Sharma M, Chaudhary U. Biofilm and multidrug resistance in uropathogenic *Escherichia coli*. Pathog Glob Healt. 2015 Feb;109(1):26-9.
39. Maki DG, Tambyah PA. Engineering out the risk for infection with urinary catheters. Emerg Infect Dis. 2001 Mar-Apr;7(2):342-7.\
40. Nowicki B. Urinary Tract Infection in Pregnant Women: Old Dogmas and Current Concepts Regarding Pathogenesis. Curr Infect Dis Rep. 2002 Dec;4(6):529-35.
41. Sengupta S. Cystone in Urinary Tract Complaints during Pregnancy. The Medicine and Surgery. 1987;27:7-11.
42. Todar K. Bacteriology: Science Magazine; 2008.
43. Patterson TF, Andriole VT. Detection, significance, and therapy of bacteriuria in pregnancy. Update in the managed health care era. Infect Dis Clin North Am. 1997 Sep;11(3):593-608.
44. Golan A, Wexler S, Amit A, Gordon D, David MP. Asymptomatic bacteriuria in normal and high-risk pregnancy. Eur J Obstet Gynecol Reprod Biol. 1989 Nov;33(2):101-8.
45. Whalley P. Bacteriuria of pregnancy. Am J Obstet Gynecol. 1967 Mar;97(5):723-38.
46. Rajaratnam A, Baby NM, Kuruvilla TS, Machado S. Diagnosis of asymptomatic bacteriuria and associated risk factors among pregnant women in Mangalore, karnataka, India. J Clin Diagn Res: JCDR. 2014 Sep;8(9):Oc23-5.
47. Mukherjee M, Koley S, Mukherjee S, Basu S, Ghosh B, Chakraborty S. Phylogenetic background of E. coli isolated from asymptomatic pregnant women from Kolkata, India. J Infect Dev Ctries. 2015 Jul;9(7):720-4.
48. Kasinathan A, Thirumal P. Prevalence of asymptomatic bacteriuria in antenatal women attending a tertiary care hospital. Int J Reprod Contracept Obstet Gynecol. 2014 Jun;3(2):437-41.
49. Foxman B, Gillespie B, Koopman J, Zhang L, Palin K, Tallman P, et al. Risk factors for second urinary tract infection among college women. Am J Epidemiol. 2000 Jun;151(12):1194-205.
50. Grabe M, Bjerklund-Johansen TE, Botto H, et al. European Association of Urology. Guidelines on urological infections 2013.

51. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol. 2015 May;13(5):269-84.
52. Srivastava RN, Bagga A. Urinary tract infection: Pediatric Nephrology. 5th ed. New Delhi: Jaypee Brothers; 2011.
53. Cohen KR, Frank J, Israel I. UTIs in the Geriatric Population: Challenges for Clinicians. US Pharm. 2011;36(6):46-54.
54. Vijayakumar M, Kanitkar M, Nammalwar BR, Bagga A. Revised statement on management of urinary tract infections. Indian Pediatr. 2011 Sep;48(9):709-17.
55. Wilson ML, Gaido L. Laboratory diagnosis of urinary tract infections in adult patients. Clin Infect Dis. 2004 Apr;38(8):1150-8.
56. Schmiemann G, Kniehl E, Gebhardt K, Matejczyk MM, Hummers-Pradier E. The diagnosis of urinary tract infection: a systematic review. Dtsch Arztebl Int. 2010 May;107(21):361-7.
57. Barber AE, Norton JP, Spivak AM, Mulvey MA. Urinary tract infections: current and emerging management strategies. Clin Infect Dis. 2013 Sep;57(5):719-24.
58. Hooton TM, Bradley SF, Cardenas DD, Colgan R, Geerlings SE, Rice JC, et al. Diagnosis, prevention, and treatment of catheter-associated urinary tract infection in adults: 2009 International Clinical Practice Guidelines from the Infectious Diseases Society of America. Clin Infect Dis. 2010 Mar;50(5):625-63.
59. Gupta K, Hooton TM, Naber KG, Wullt B, Colgan R, Miller LG, et al. International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: A 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. Clin Infect Dis. 2011 Mar;52(5):e103-20.
60. Gupta K, Bhadelia N. Management of urinary tract infections from multidrug-resistant organisms. Infect Dis Clin North Am. 2014 Mar;28(1):49-59.
61. Mazumdar K, Dutta NK, Dastidar SG, Motohashi N, Shirataki Y. Diclofenac in the management of *E. coli* urinary tract infections. In Vivo. 2006 Sep-Oct;20(5):613-9.
62. George MG. The Gammaproteobacteria. Bergey's Manual of Systematic Bacteriology 2B 2nd ed. New York: Springer; 1984.
63. Smith HW. The haemolysins of *Escherichia coli*. J Pathol Bacteriol. 1963 Jan;85:197-211.

64. Bergey DH, N.R. K, Holt JG. Family I. Enterobacteriaceae. In Bergey's Manual of Systematic Bacteriology: Baltimore, MD : Williams & Wilkins, ©1984-©1989.; 1984.
65. Kauffmann F. Zur Serologie Der Coli- Gruppe. Acta Pathologica Microbiologica Scandinavica. 1944 Mar;21:20-45.
66. Johnson JR. Virulence factors in *Escherichia coli* urinary tract infection. Clin Microbiol Rev. 1991 Jan;4(1):80-128.
67. Terlizzi ME, Gribaudo G, Maffei ME. UroPathogenic *Escherichia coli* (UPEC) Infections: Virulence Factors, Bladder Responses, Antibiotic, and Non-antibiotic Antimicrobial Strategies. Front Microbiol. 2017 Aug;8:1566.
68. Baddour LM, Christensen GD, Simpson WA, Beachey EH. Microbial adherence. In: G. L. Mandell RGD, Jr., and J. E. Bennett, editor. Principles and practice of infectious diseases. 3rd ed. Churchill Livingstone, Inc., New York.1990.
69. Reid G, Sobel JD. Bacterial adherence in the pathogenesis of urinary tract infection: a review. Rev Infect Dis. 1987 May-Jun;9(3):470-87.
70. Green CP, Thomas VL. Hemagglutination of human type O erythrocytes, hemolysin production, and serogrouping of *Escherichia coli* isolates from patients with acute pyelonephritis, cystitis, and asymptomatic bacteriuria. Infect Immun. 1981 Jan;31(1):309-15.
71. Eden CS, Eriksson B, Hanson LA. Adhesion of *Escherichia coli* to human uroepithelial cells in vitro. Infect Immun. 1977 Dec;18(3):767-74.
72. Duguid JP, Anderson ES. Terminology of bacterial fimbriae, or pili, and their types. Nature. 1967 Jun;215(5096):89-90.
73. Brinton CC, Jr. The structure, function, synthesis and genetic control of bacterial pili and a molecular model for DNA and RNA transport in gram negative bacteria. Trans N Y Acad Sci. 1965 Jun;27(8):1003-54.
74. Kallenius G, Mollby R, Svenson SB, Winberg J, Hultberg H. Identification of a carbohydrate receptor recognized by uropathogenic *Escherichia coli*. Infection. 1980;8 Suppl 3:288-93.
75. Svenson SB, Hultberg H, Kallenius G, Korhonen TK, Mollby R, Winberg J. P-fimbriae of pyelonephritogenic *Escherichia coli*: identification and chemical characterization of receptors. Infection. 1983 Jan-Feb;11(1):61-7.

76. Lindberg F, Lund B, Johansson L, Normark S. Localization of the receptor-binding protein adhesin at the tip of the bacterial pilus. Nature. 1987 Jul;328(6125):84-7.
77. Nowicki B, Labigne A, Moseley S, Hull R, Hull S, Moulds J. The Dr hemagglutinin, afimbrial adhesins AFA-I and AFA-III, and F1845 fimbriae of uropathogenic and diarrhea-associated *Escherichia coli* belong to a family of hemagglutinins with Dr receptor recognition. Infect Immun. 1990 Jan;58(1):279-81.
78. Orskov I, Birch-Andersen A, Duguid JP, Stenderup J, Orskov F. An adhesive protein capsule of *Escherichia coli*. Infect Immun. 1985 Jan;47(1):191-200.
79. Nowicki B, Truong L, Moulds J, Hull R. Presence of the Dr receptor in normal human tissues and its possible role in the pathogenesis of ascending urinary tract infection. Am J Pathol. 1988 Oct;133(1):1-4.
80. Arthur M, Johnson CE, Rubin RH, Arbeit RD, Campanelli C, Kim C, et al. Molecular epidemiology of adhesin and hemolysin virulence factors among uropathogenic *Escherichia coli*. Infect Immun. 1989 Feb;57(2):303-13.
81. Ott M, Hacker J, Schmoll T, Jarchau T, Korhonen TK, Goebel W. Analysis of the genetic determinants coding for the S-fimbrial adhesin (sfa) in different *Escherichia coli* strains causing meningitis or urinary tract infections. Infect Immun. 1986 Dec;54(3):646-53.
82. Pere A, Nowicki B, Saxen H, Siitonen A, Korhonen TK. Expression of P, type-1, and type-1C fimbriae of *Escherichia coli* in the urine of patients with acute urinary tract infection. J Infect Dis. 1987 Oct;156(4):567-74.
83. Duguid JP, Old DC. Bacterial Adherence. Adhesive Properties of Enterobacteriaceae: Springer; 1980. p. 185-217.
84. Brinton CC, Jr. Non-flagellar appendages of bacteria. Nature. 1959 Mar;183(4664):782-6.
85. Hull RA, Gill RE, Hsu P, Minshew BH, Falkow S. Construction and expression of recombinant plasmids encoding type 1 or D-mannose-resistant pili from a urinary tract infection *Escherichia coli* isolate. Infect Immun. 1981 Sep;33(3):933-8.
86. Enerback S, Larsson AC, Leffler H, Lundell A, de Man P, Nilsson B, et al. Binding to galactose alpha 1----4galactose beta-containing receptors as

potential diagnostic tool in urinary tract infection. J Clin Microbiol. 1987 Feb;25(2):407-11.

87. Warner PJ, Williams PH, Bindereif A, Neilands JB. ColV plasmid-specific aerobactin synthesis by invasive strains of *Escherichia coli*. Infect Immun. 1981 Aug;33(2):540-5.
88. Weinberg ED. Iron and infection. Microbiol Rev. 1978 Mar;42(1):45-66.
89. Gross R, Engelbrecht F, Braun V. Genetic and biochemical characterization of the aerobactin synthesis operon on pColV. Mol Gen Genet: MGG. 1984;196(1):74-80.
90. JG C. The RTX toxins of Gram-negative bacterial pathogens: modulators of the host immune system. Rev Med Microbiol. 1996 Jan;7(1):53.
91. Boehm DF, Welch RA, Snyder IS. Domains of *Escherichia coli* hemolysin (HlyA) involved in binding of calcium and erythrocyte membranes Infect Immun. 1990 Jun;58(6):1959-64.
92. Jorgensen SE, Mulcahy PF, Louis CF. Effect of *Escherichia coli* hemolysin on permeability of erythrocyte membranes to calcium. Toxicon. 1986;24(6):559-66.
93. Scheffer J, Vosbeck K, Konig W. Induction of inflammatory mediators from human polymorphonuclear granulocytes and rat mast cells by haemolysin-positive and -negative *E. coli* strains with different adhesins. Immunology. 1986 Dec;59(4):541-8.
94. Felmlee T, Welch RA. Alterations of amino acid repeats in the *Escherichia coli* hemolysin affect cytolytic activity and secretion. Proc Natl Acad Sci U S A. 1988 Jul;85(14):5269-73.
95. Caprioli A, Falbo V, Roda LG, Ruggeri FM, Zona C. Partial purification and characterization of an *Escherichia coli* toxic factor that induces morphological cell alterations. Infect Immun. 1983 Mar;39(3):1300-6.
96. Pei S, Doye A, Boquet P. Mutation of specific acidic residues of the CNF1 T domain into lysine alters cell membrane translocation of the toxin. Mol Microbiol. 2001 Sep;41(6):1237-47.
97. Mills M, Meysick KC, O'Brien AD. Cytotoxic necrotizing factor type 1 of uropathogenic *Escherichia coli* kills cultured human uroepithelial 5637 cells by an apoptotic mechanism. Infect Immun. 2000 Oct;68(10):5869-80.

98. Jann K, Jann B. The K antigens of *Escherichia coli*. Host Parasite Relationships in Gram-Negative 33: Karger Publishers; 1983. p. 53-79.
99. Orskov F. Virulence factors of the bacterial cell surface. J. Infect. 1978 Jan;137(5):630-3.
100. Howard CJ, Glynn AA. The virulence for mice of strains of *Escherichia coli* related to the effects of K antigens on their resistance to phagocytosis and killing by complement. Immunology. 1971 May;20(5):767-77.
101. Sandberg T, Kaijser B, Lidin-Janson G, Lincoln K, Orskov F, Orskov I, et al. Virulence of *Escherichia coli* in relation to host factors in women with symptomatic urinary tract infection. J Clin Microbiol. 1988 Aug;26(8):1471-6.
102. Taylor PW. Bactericidal and bacteriolytic activity of serum against gram-negative bacteria. Microbiol Rev. 1983 Mar;47(1):46-83.
103. Roberts I, Mountford R, High N, Bitter-Suermann D, Jann K, Timmis K, et al. Molecular cloning and analysis of genes for production of K5, K7, K12, and K92 capsular polysaccharides in *Escherichia coli*. J Bacteriol. 1986 Dec;168(3):1228-33.
104. Hacker J. Genetic determinants coding for fimbriae and adhesins of extraintestinal *Escherichia coli*. Curr Top Microbiol Immunol. 1990;151:1-27.
105. Swenson DL, Bukanov NO, Berg DE, Welch RA. Two pathogenicity islands in uropathogenic *Escherichia coli* J96: cosmid cloning and sample sequencing. Infect Immun. 1996 Sep;64(9):3736-43.
106. Falzano L, Fiorentini C, Donelli G, Michel E, Kocks C, Cossart P, et al. Induction of phagocytic behaviour in human epithelial cells by *Escherichia coli* cytotoxic necrotizing factor type 1. Mol Microbiol. 1993 Sep;9(6):1247-54.
107. Donlan RM, Costerton JW. Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev. 2002 Apr;15(2):167-93.
108. Hall-Stoodley L, Stoodley P. Evolving concepts in biofilm infections. Cell Microbiol. 2009 Jul;11(7):1034-43.
109. Costerton JW, Geesey GG, Cheng KJ. How bacteria stick. Sci Am. 1978 Jan;238(1):86-95.
110. Beloin C, Roux A, Ghigo JM. *Escherichia coli* biofilms. Curr Top Microbiol Immunol. 2008;322:249-89.
111. Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin-Scott HM. Microbial biofilms. Annu Rev Microbiol. 1995;49:711-45.

112. Schembri MA, Kjaergaard K, Klemm P. Global gene expression in *Escherichia coli* biofilms. Mol Microbiol. 2003 Apr;48(1):253-67.

113. Jahandeh N, Ranjbar R, Behzadi P, Behzadi E. Uropathogenic *Escherichia coli* virulence genes: invaluable approaches for designing DNA microarray probes. Cent European J Urol. 2015;68(4):452-8.

114. Foxman B. The epidemiology of urinary tract infection. Nat Rev Urol. 2010 Dec;7(12):653-60.

115. Eshwarappa M, Dosegowda R, Aprameya IV, Khan MW, Kumar PS, Kempegowda P. Clinico-microbiological profile of urinary tract infection in south India. Indian J Nephrol. 2011 Jan;21(1):30-6.

116. Brauner A, Leissner M, Wretlind B, Julander I, Svenson SB, Kallenius G. Occurrence of P-fimbriated *Escherichia coli* in patients with bacteremia. Eur J Clin Microbiol. 1985 Dec;4(6):566-9.

117. Raksha R, Srinivasa H, Macaden RS. Occurrence and characterisation of uropathogenic *Escherichia coli* in urinary tract infections. Indian J Med Microbiol. 2003 Apr-Jun;21(2):102-7.

118. Jadhav S, Hussain A, Devi S, Kumar A, Parveen S, Gandham N, et al. Virulence characteristics and genetic affinities of multiple drug resistant uropathogenic *Escherichia coli* from a semi urban locality in India. PLoS One. 2011 Mar;6(3):e18063.

119. Soto SM, Smithson A, Martinez JA, Horcajada JP, Mensa J, Vila J. Biofilm formation in uropathogenic *Escherichia coli* strains: relationship with prostatitis, urovirulence factors and antimicrobial resistance. J Urol. 2007 Jan;177(1):365-8.

120. Johnson JR, Scheutz F, Ulleryd P, Kuskowski MA, O'Bryan TT, Sandberg T. Phylogenetic and pathotypic comparison of concurrent urine and rectal *Escherichia coli* isolates from men with febrile urinary tract infection. J Clin Microbiol. 2005 Aug;43(8):3895-900.

121. Bonacorsi S, Houdouin V, Mariani-Kurkdjian P, Mahjoub-Messai F, Bingen E. Comparative prevalence of virulence factors in *Escherichia coli* causing urinary tract infection in male infants with and without bacteremia. J Clin Microbiol. 2006 Mar;44(3):1156-8.

122. Kudinha T, Kong F, Johnson JR, Andrew SD, Anderson P, Gilbert GL. Multiplex PCR-based reverse line blot assay for simultaneous detection of 22

virulence genes in uropathogenic *Escherichia coli*. Appl Environ Microbiol. 2012 Feb;78(4):1198-202.

123. Farshad S, Emamghorashi F. The prevalence of virulence genes of *E. coli* strains isolated from children with urinary tract infection. Saudi J Kidney Dis Transpl. 2009 Jul;20(4):613-7.

124. Finberg RW, Moellering RC, Tally FP, Craig WA, Pankey GA, Dellinger EP, et al. The importance of bactericidal drugs: future directions in infectious disease. Clin Infect Dis. 2004 Nov;39(9):1314-20.

125. Tenover FC. Mechanisms of antimicrobial resistance in bacteria. Am J Med. 2006 Jun;119(6 Suppl 1):S3-10; discussion S62-70.

126. Centre for disease control and prevention. Antibiotic Resistance Questions & Answers. 2013.

127. D'Costa VM, King CE, Kalan L, Morar M, Sung WW, Schwarz C, et al. Antibiotic resistance is ancient. Nature. 2011 Aug;477(7365):457-61.

128. Donadio S, Maffioli S, Monciardini P, Sosio M, Jabes D. Antibiotic discovery in the twenty-first century: current trends and future perspectives. J Antibiot (Tokyo). 2010 Aug;63(8):423-30.

129. Alanis AJ. Resistance to antibiotics: are we in the post-antibiotic era? Arch Med Res. 2005 Nov-Dec;36(6):697-705.

130. Ogbodo S, Okeke A, Ugwuoru C, Chukwurah E. Possible Alternatives to Reduce Antibiotic Resistance. Life Sc Med Res. 2011.

131. Hoffmann K, Wagner G, Apfalter P, Maier M. Antibiotic resistance in primary care in Austria - a systematic review of scientific and grey literature. BMC Infect Dis. 2011 Nov;11:330.

132. Drawz SM, Bonomo RA. Three decades of beta-lactamase inhibitors. Clin Microbiol Rev. 2010 Jan;23(1):160-201.

133. Oteo J, Lazaro E, de Abajo FJ, Baquero F, Campos J. Antimicrobial-resistant invasive *Escherichia coli*, Spain. Emerg Infect Dis. 2005 Apr;11(4):546-53.

134. Livermore DM, Woodford N. The beta-lactamase threat in Enterobacteriaceae, Pseudomonas and Acinetobacter. Trends Microbiol. 2006 Sep;14(9):413-20.

135. Goettsch W, van Pelt W, Nagelkerke N, Hendrix MG, Buiting AG, Petit PL, et al. Increasing resistance to fluoroquinolones in *Escherichia coli* from urinary tract infections in the netherlands. J Antimicrob Chemother. 2000 Aug;46(2):223-8.

136. Hopkins KL, Davies RH, Threlfall EJ. Mechanisms of quinolone resistance in *Escherichia coli* and *Salmonella*: recent developments. Int J Antimicrob Agents. 2005 May;25(5):358-73.

137. Soto SM, Jimenez de Anta MT, Vila J. Quinolones induce partial or total loss of pathogenicity islands in uropathogenic *Escherichia coli* by SOS-dependent or -independent pathways, respectively. Antimicrob Agents Chemother. 2006 Feb;50(2):649-53.

138. Mladin C, Chifiriuc MC, Palade A, C.R. U, Slavu CL, Alina R, et al. Phenotypic and genetic analysis of the antibiotic resistance patterns in uropathogenic *Escherichia coli* strains. Rom Biotechnol. 2010 Mar;15(2):5078-86.

139. Poovendran P, Vidhya N, Murugan S. Antimicrobial Susceptibility Pattern of ESBL and Non-ESBL Producing Uropathogenic *Escherichia coli* (UPEC) and Their Correlation with Biofilm Formation. Int J Microbiol Res. 2013;4(1):56-63.

140. Naik J, Desai P. Antibiotic resistance pattern in urinary isolates of *Escherichia coli* with special reference to extended spectrum β-Lactamases. International journal of pharmacy & life sciences. 2012;3(3):1498-502.

141. Mahesh E, Ramesh D, Indumathi VA, Punith K, Kirthi R, Anupama HA. Complicated Urinary Tract Infection in a Tertiary Care Center in South India. Al Ameen J Med S ci. 2010;3(2):120-7.

142. Rizvi M, Khan F, Shukla I, Malik A. Rising prevalence of antimicrobial resistance in urinary tract infections during pregnancy: necessity for exploring newer treatment options. J Lab Physicians. 2011 Jul;3(2):98-103.

143. Neamati F, Firoozeh F, Saffari M, Zibaei M. Virulence genes and antimicrobial resistance pattern in uropathogenic *Escherichia coli* isolated from hospitalized patients in Kashan, Iran. Jundishapur J Microbiol. 2015 Feb;8(2): e17514.

144. Ghosh A, Mukherjee M. Incidence of multidrug resistance, pathogenicity island markers, and pathoadaptive FimH mutations in uropathogenic *Escherichia coli* isolated from asymptomatic hospitalized patients. Folia Microbiol (Praha). 2019 Jul;64(4):587-600.

145. Kass EH. Pyelonephritis and bacteriuria. A major problem in preventive medicine. Ann Intern Med. 1962 Jan;56:46-53.

146. Clinical and Laboratory Standards Institute. 2009. Performance standards for antimicrobial disk susceptibility tests, Approved standard M2-A10. Clinical and Laboratory Standards Institute; Wayne PA.

147. Christensen GD, Simpson WA, Bisno AL, Beachey EH. Adherence of slime-producing strains of *Staphylococcus epidermidis* to smooth surfaces. Infect Immun. 1982 Jul;37(1):318-26.

148. Freeman DJ, Falkiner FR, Keane CT. New method for detecting slime production by coagulase negative staphylococci. J Clin Pathol. 1989 Aug;42(8):872-4.

149. Christensen GD, Simpson WA, Younger JJ, Baddour LM, Barrett FF, Melton DM, et al. Adherence of coagulase-negative staphylococci to plastic tissue culture plates: a quantitative model for the adherence of staphylococci to medical devices. J Clin Microbiol. 1985 Dec;22(6):996-1006.

150. Stepanovic S, Vukovic D, Hola V, Di Bonaventura G, Djukic S, Cirkovic I, et al. Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. APMIS. 2007 Aug;115(8):891-9.

151. Johnson JR, Kuskowski MA, Gajewski A, Sahm DF, Karlowsky JA. Virulence characteristics and phylogenetic background of multidrug-resistant and antimicrobial-susceptible clinical isolates of *Escherichia coli* from across the United States, 2000-2001. J Infect Dis. 2004 Nov;190(10):1739-44.

152. Barate D, Ukesh C. The bacterial profile and the antibiotic resistance pattern of urinary tract infections. Dav International Journal Of Science. 2012;1:21-4.

153. Amin M, Mehdinejad M, Pourdangchi Z. Study of bacteria isolated from urinary tract infections and determination of their susceptibility to antibiotics. Jundishapur J Microbiol. 2009 Oct;2(3):118.

154. Vijayaswetha V, Sreenivasa RU, Hema Prakash P, Subbarayudu S. Aerobic bacteriological profile of urinary tract infections in a tertiary care hospital. Int.J.Curr.Microbiol.App.Sci. 2014;3(3):120-5.

155. Akoachere JF, Yvonne S, Akum NH, Seraphine EN. Etiologic profile and antimicrobial susceptibility of community-acquired urinary tract infection in two Cameroonian towns. BMC Res Notes. 2012 May;5:219.

156. Janifer J, Geethalakshmi S, Satyavani K, Viswanathan V. Prevalence of lower urinary tract infection in South Indian type 2 diabetic subjects. Indian J Nephrol. 2009 Jul;19(3):107-11.

157. Kamat US, Fereirra A, Amonkar D, Motghare DD, Kulkarni MS. Epidemiology of hospital acquired urinary tract infections in a medical college hospital in Goa. Indian J Urol. 2009 Jan;25(1):76-80.

158. Ullah F, Malik S, Ahmed J. Antibiotic susceptibility pattern and ESBL prevalence in nosocomial *Escherichia coli* from urinary tract infections in Pakistan. Afr. J. Biotechnol. 2009;8(16):3921-6.

159. Aypak C, Altunsoy A, Duzgun N. Empiric antibiotic therapy in acute uncomplicated urinary tract infections and fluoroquinolone resistance: a prospective observational study. Ann Clin Microbiol Antimicrob. 2009 Oct;8:27.

160. Sire JM, Nabeth P, Perrier-Gros-Claude JD, Bahsoun I, Siby T, Macondo EA, et al. Antimicrobial resistance in outpatient *Escherichia coli* urinary isolates in Dakar, Senegal. J Infect Dev Ctries. 2007 Dec;1(3):263-8.

161. Biswas D, Gupta P, Prasad R, Singh V, Arya M, Kumar A. Choice of antibiotic for empirical therapy of acute cystitis in a setting of high antimicrobial resistance. Indian J Med Sci. 2006 Feb;60(2):53-8.

162. Zhanel GG, Hisanaga TL, Laing NM, DeCorby MR, Nichol KA, Weshnoweski B, et al. Antibiotic resistance in *Escherichia coli* outpatient urinary isolates: final results from the North American Urinary Tract Infection Collaborative Alliance (NAUTICA). Int J Antimicrob Agents. 2006 Jun;27(6):468-75.

163. Banu A, Kabbin S J, Anand M. Extra intestinal Infections due to *Escherichia Coli*: An Emerging Issue. J Clin Diagn Res: 2011 June;5(3):486-490.

164. G. MN, Math. GC, Patil SA, Gaddad SM, Shivannavar CT. Incidence of Urinary Tract Infections and Its Aetiological Agents among Pregnant Women in Karnataka Region. Adv Microbiol. 2013;3(6):473-8.

165. Drews SJ, Poutanen SM, Mazzulli T, McGeer AJ, Sarabia A, Pong-Porter S, et al. Decreased prevalence of virulence factors among ciprofloxacin-resistant uropathogenic *Escherichia coli* isolates. J Clin Microbiol. 2005 Aug;43(8):4218-20.

166. Kahlmeter G, Menday P, Cars O. Non-hospital antimicrobial usage and resistance in community-acquired *Escherichia coli* urinary tract infection. J Antimicrob Chemother. 2003 Dec;52(6):1005-10.

167. Lee S, Cho Y, Kim B, Lee J, Jung S, Lee S, et al. A Multicenter Study of Antimicrobial Susceptibility of Uropathogens Causing Acute Uncomplicated Cystitis in Woman. Korean J Urol. 2003 Jul;44(7):697-701.

168. Manjunath G, Prakash R, Vamseedhar A, Shetty k. Changing trends in the spectrum of antimicrobial drug resistance pattern of uropathogens isolated from hospitals and community patients with urinary tract infections in tumkur and bangalore. International Journal Of Biological and Medical Research 2011;2(2):504-7.

169. Slavchev G, Pisareva E, Markova N. Virulence of uropathogenic *Escherichia coli.* J Cult Collect 2008-2009;62:3-9.

170. Hall-Stoodley L, Costerton JW, Stoodley P. Bacterial biofilms: from the natural environment to infectious diseases. Nat Rev Microbiol. 2004 Feb;2(2):95-108

171. Sharma M, Aparna, Yadav S, Chaudhary U. Biofilm production in uropathogenic *Escherichia coli*. Indian J Pathol Microbiol. 2009 Nov;52(2):294.

172. Subramanian P, Shanmugam N, Sivaraman U, Kumar S, Selvaraj S. Antiobiotic resistance pattern of biofilm-forming uropathogens isolated from catheterised patients in Pondicherry, India. Australas Med J. 2012;5(7):344-8.

173. Tabasi M, Asadi Karam MR, Habibi M, Yekaninejad MS, Bouzari S. Phenotypic Assays to Determine Virulence Factors of Uropathogenic *Escherichia coli* (UPEC) Isolates and their Correlation with Antibiotic Resistance Pattern. Osong Public Health Res Perspect. 2015 Aug;6(4):261-8.

174. Fattahi S, Kafil HS, Nahai MR, Asgharzadeh M, Nori R, Aghazadeh M. Relationship of biofilm formation and different virulence genes in uropathogenic *Escherichia coli* isolates from Northwest Iran. GMS Hyg Infect Control. 2015 Jul;10:Doc11.

175. Ponnusamy P, Natarajan V, Sevanan M. In vitro biofilm formation by uropathogenic *Escherichia coli* and their antimicrobial susceptibility pattern. Asian Pac J Trop Med. 2012 Mar;5(3):210-3.

176. Neupane S, Pant ND. Correlation between biofilm formation and resistance toward different commonly used antibiotics along with extended spectrum beta

lactamase production in uropathogenic *Escherichia coli* isolated from the patients suspected of urinary tract infections visiting Shree Birendra Hospital, Chhauni, Kathmandu, Nepal. Antimicrob Resist Infect Control. 2016 Feb 15;5:5.

177. Alqasim A, Abu Jaffal A, Alyousef AA. Prevalence of Multidrug Resistance and Extended-Spectrum beta-Lactamase Carriage of Clinical Uropathogenic *Escherichia coli* Isolates in Riyadh, Saudi Arabia. Int J Microbiol. 2018 Sep;2018:3026851.

178. Ghenghesh KS, Elkateb E, Berbash N, Abdel Nada R, Ahmed SF, Rahouma A, et al. Uropathogens from diabetic patients in Libya: virulence factors and phylogenetic groups of *Escherichia coli* isolates. J Med Microbiol. 2009 Aug;58(Pt 8):1006-14.

179. Siegfried L, Kmetova M, Puzova H, Molokacova M, Filka J. Virulence-associated factors in *Escherichia coli* strains isolated from children with urinary tract infections. J Med Microbiol. 1994 Aug;41(2):127-32.

180. Mittal S, Sharma M, Chaudhary U. Study of virulence factors of uropathogenic *Escherichia coli* and its antibiotic susceptibility pattern. Indian J Pathol Microbiol. 2014 Jan-Mar;57(1):61-4.

181. Stanley P, Koronakis V, Hughes C. Acylation of *Escherichia coli* hemolysin: a unique protein lipidation mechanism underlying toxin function. Microbiol Mol Biol Rev. 1998 Jun;62(2):309-33.

182. Marhova M, Kostadinova S, Stoitsova S. Biofilm-Forming Capabilities of Urinary Escherichia coli isolates. Biotechnol Biotec Eq. 2014 Apr;24(sup1):589-93.

183. Sahly H, Navon-Venezia S, Roesler L, Hay A, Carmeli Y, Podschun R, Hennequin C, Forestier C, Ofek I. Extended-spectrum β-lactamase production is associated with an increase in cell invasion and expression of fimbrial adhesins in *Klebsiella pneumoniae*. Antimicrob Agents Chemother. 2008 Sep 1;52(9):3029-34.

184. Tankhiwale SS, Jalgaonkar SV, Ahamad S, Hassani U. Evaluation of extended spectrum beta lactamase in urinary isolates. Indian J Med Res. 2004 Dec;120(6):553-6.

185. Johnson JR, Kuskowski MA, O'Bryan T T, Colodner R, Raz R. Virulence genotype and phylogenetic origin in relation to antibiotic resistance profile among

Escherichia coli urine sample isolates from Israeli women with acute uncomplicated cystitis. Antimicrob Agents Chemother. 2005 Jan;49(1):26-31.

186. Farmer JJ. Enterobacteriaceae: introduction and identification. In: P.R. Murray EJB, M.A. Phaller, F.C. Tenover, R.H. Yolken, editor. Manual of clinical microbiology. Washington D.C: American Society for microbiology; 1999. p. 452-5.

187. Mora A, Lopez C, Dabhi G, Blanco M, Blanco JE, Alonso MP, et al. Extraintestinal pathogenic *Escherichia* coli O1:K1:H7/NM from human and avian origin: detection of clonal groups B2 ST95 and D ST59 with different host distribution. BMC Microbiol. 2009 Jul;9:132.

188. Watt S, Lanotte P, Mereghetti L, Moulin-Schouleur M, Picard B, Quentin R. *Escherichia coli* strains from pregnant women and neonates: intraspecies genetic distribution and prevalence of virulence factors. J Clin Microbiol. 2003 May;41(5):1929-35.

189. Arisoy M, Aysev D, Ekim M, Ozel D, Kose SK, Ozsoy ED, et al. Detection of virulence factors of *Escherichia coli* from children by multiplex polymerase chain reaction. Int J Clin Pract. 2006 Feb;60(2):170-3.

190. Johnson JR, Kuskowski MA, Owens K, Gajewski A, Winokur PL. Phylogenetic origin and virulence genotype in relation to resistance to fluoroquinolones and/or extended-spectrum cephalosporins and cephamycins among *Escherichia coli* isolates from animals and humans. J Infect Dis. 2003 Sep;188(5):759-68.

191. Chakraborty A, Adhikari P, Shenoy S, Saralaya V. Molecular characterisation of uropathogenic *Escherichia coli* isolates at a tertiary care hospital in South India. Indian J Med Microbiol. 2017 Apr-Jun;35(2):305-10.

192. Abe CM, Salvador FA, Falsetti IN, Vieira MA, Blanco J, Blanco JE, et al. Uropathogenic *Escherichia coli* (UPEC) strains may carry virulence properties of diarrhoeagenic E. coli. FEMS Immunol Med Microbiol. 2008 Apr;52(3):397-406.

193. Qin X, Hu F, Wu S, Ye X, Zhu D, Zhang Y, et al. Comparison of adhesin genes and antimicrobial susceptibilities between uropathogenic and intestinal commensal *Escherichia coli* strains. PLoS One. 2013 Apr;8(4):e61169.

194. Montenegro MA, Bitter-Suermann D, Timmis JK, Aguero ME, Cabello FC, Sanyal SC, et al. traT gene sequences, serum resistance and pathogenicity-related

factors in clinical isolates of *Escherichia coli* and other gram-negative bacteria. J Gen Microbiol. 1985 Jun;131(6):1511-21.

195. Oliveira FA, Paludo KS, Arend LN, Farah SM, Pedrosa FO, Souza EM, et al. Virulence characteristics and antimicrobial susceptibility of uropathogenic *Escherichia coli* strains. Genetics and molecular research : Genet Mol Res. 2011 Oct;10(4):4114-25.

196. Kryger J, Burleigh A, Christensen M, Hopkins W. Genetic Evaluation of *E. coli* Strains Isolated from Asymptomatic Children with Neurogenic Bladders. Int J Chronic Dis. 2015 Nov;2015:206570.

197. Sabate M, Moreno E, Perez T, Andreu A, Prats G. Pathogenicity island markers in commensal and uropathogenic *Escherichia coli* isolates. Clin Microbiol Infect. 2006 Sep;12(9):880-6.

198. Johnson JR, Stell AL. Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. J Infect Dis. 2000 Jan;181(1):261-72.

199. Najafi A, Hasanpour M, Askary A, Aziemzadeh M, Hashemi N. Distribution of pathogenicity island markers and virulence factors in new phylogenetic groups of uropathogenic *Escherichia coli* isolates. Folia Microbiol (Praha).. 2018 May;63(3):335-43.

200. Ghosh A, Mukherjee M. Incidence of multidrug resistance, pathogenicity island markers, and pathoadaptive FimH mutations in uropathogenic *Escherichia coli* isolated from asymptomatic hospitalized patients. Folia Microbiol (Praha). 2019 Jul;3(4):587-00.

201. Kawamura-Sato K, Yoshida R, Shibayama K, Ohta M. Virulence genes, quinolone and fluoroquinolone resistance, and phylogenetic background of uropathogenic *Escherichia coli* strains isolated in Japan. Jpn J Infect Dis. 2010 Mar;63(2):113-5.

202. Miranda-Estrada LI, Ruíz-Rosas M, Molina-López J, Parra-Rojas I, González-Villalobos E, Castro-Alarcón N. Relationship between virulence factors, resistance to antibiotics and phylogenetic groups of uropathogenic *Escherichia coli i*n two locations in Mexico. Enferm Infecc Microbiol Clin.. 2017 Aug 1;35(7):426-33.

203. Basu S, Mukherjee SK, Hazra A, Mukherjee M. Molecular Characterization of Uropathogenic *Escherichia coli*: Nalidixic Acid and Ciprofloxacin Resistance,

Virulent Factors and Phylogenetic Background. J Clin Diagn Res. 2013 Dec;7(12):2727-31.

204. Bien J, Sokolova O, Bozko P. Role of Uropathogenic *Escherichia coli* Virulence Factors in Development of Urinary Tract Infection and Kidney Damage. Int J Nephrol. 2012 Mar;2012:681473.

205. Naderi G, Haghi F, Zeighami H, Hemati F, Masoumian N. Distribution of pathogenicity island (PAI) markers and phylogenetic groups in diarrheagenic and commensal *Escherichia coli* from young children. Gastroenterol Hepatol Bed Bench. 2016;9(4):316-24.

ANNEXURES

ಒಪ್ಪಿಗೆ ಪತ್ರ

ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಿದ ರೋಗಿ ಅಥವಾ ರೋಗಿಯ ತಂದೆ–ತಾಯಿ ಅಥವಾ ರೋಗಿಯ ಪೋಷಕರ ಒಪ್ಪಿಗೆ ಪತ್ರ.

ಭಾಗವಹಿಸಿದವರ ಹೆಸರು: **ವಿಳಾಸ:**

ಪ್ರಬಂಧದ ಶೀರ್ಷಿಕೆ:

ಬಯೋಫಿಲ್ಮ್ ಫಾರ್ಮೇಶನ್ ಇನ್ ಯುರೋಪ್ಯಾಥೊಜನಿಕ ಎಷಿರೆಶಿಯಾ ಕೊಲೈ ಸ್ಟ್ರೇನ್ಸ್; ರಿಲೇಶನ್‌ಶಿಪ್ ವಿಥ್ ವಿರುಲೆನ್ಸ್ ಫ್ಯಾಕ್ಟರ್ಸ್ & ಆಂಟಿಮೈಕ್ರೋಬಿಯಲ್ ರೆಸಿಸ್ಟಾನ್ಸ್ ಇನ್ ಟೆರಿಶರಿ ಕೇರ ಹಾಸ್ಪಿಟಲ್ಸ್ ಇನ್ ನಾರ್ಥ ಕರ್ನಾಟಕ ರೀಜನ್.

ಅಧ್ಯಯನದ ವಿವರಗಳು, ಬರವಣಿಗೆ ನನಗೆ ಒದಗಿಸಿದ್ದು ನನ್ನ ಭಾಷೆಯಲ್ಲಿಯೇ ಮತ್ತು ನನ್ನ ಭಾಷೆಯಲ್ಲಿಯೇ ನನಗೆ ವಿವರಿಸಿದರು. ನಾನು ನಿರ್ದಿಷ್ಟಪಡಿಸಿದ ಮೇಲಿನ ಅಧ್ಯಯನದ ವಿಷಯದ ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳಲು ಅವಕಾಶವಿತ್ತು ಮತ್ತು ಅವುಗಳನ್ನು ಅಥೈಯಿಸಿಕೊಂಡು ನಾನು ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಸ್ವಯಂಪ್ರೇರಿತನಾಗಿ ಭಾಗವಹಿಸಿದ್ದೇನೆ. ನಾನು ನೀಡಿದ ಮಾಹಿತಿಯನ್ನು ಯಾವುದೇ ಕಾರಣವನ್ನು ನೀಡದೆ ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಹಿಂದಕ್ಕೆ ಪಡೆಯಬಹುದು. ವೈದ್ಯಕೀಯ ಪರಿಣಾಮವನ್ನು ಹೊರತುಪಡಿಸಿ ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಸಾಮಾನ್ಯವಾಗಿ ಉದ್ಭವಿಸುವ ಯಾವುದೇ ಮಾಹಿತಿ ಅಥವಾ ಫಲಿತಾಂಶಗಳು ಅಧ್ಯಯನದ ವೈಜ್ಞಾನಿಕ ಉದ್ದೇಶಗಳಿಗೆ ನೀಡಿದೇವು ಆಗಿವೆ. ಈ ಅಧ್ಯಯನದ ವಿವರಗಳನ್ನು ಒಂದು ಮಾಹಿತಿ ಹಾಳೆಯಲ್ಲಿ ನೀಡಲಾಗಿದೆ. ನಾನು ಸಂಪೂರ್ಣವಾಗಿ ಮೇಲಿನ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಒಪ್ಪಿಗೆಯನ್ನು ನೀಡಿದ್ದೇನೆ.

ದಿನಾಂಕ_____ **ಭಾಗಹಿಸಿದವರ ಸಹಿ_____**

CONSENT FORM

(For children above 7 years and below 18 years of age)

Title of the project:

Biofilm formation in Uropathogenic *Escherichia coli* strains; Relationship with virulence factors and antimicrobial resistance in tertiary care hospital in North Karnataka region.

Child Participant's name: Date of birth/Age:

Parent/LAR's name: Address:

The details of the study have been provided to me in writing and explained to me in my own language. I confirm that I have understood the above study and had the opportunity to ask questions. I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without the medical care that will normally be provided by the hospital being affected. I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s). I understand that following completion of study as well as during publication of the results, confidentiality of my identity will be maintained. I fully assent to participate in the above study

Signature/ thumb impression of the Child participant: _____ Date: _____

Signature/ thumb impression of the parent/guardian: _____ Date: _____

Name and address of the witness: _____ Date: _____

Signature of the witness: _____ Date: _____

Signature of the investigator: _____ Date: _____

CONSENT FORM (Adult)

Title of the project:

Biofilm formation in Uropathogenic *Escherichia coli* strains; Relationship with virulence factors and antimicrobial resistance in tertiary care hospital in North Karnataka region.

Participant's name: Address:

The details of the study have been provided to me in writing and explained to me in my own language. I confirm that I have understood the above study and had the opportunity to ask questions. I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without the medical care that will normally be provided by the hospital being affected. I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s). I fully consent to participate in the above study.

Signature/ thumb impression of the participant: _____ Date: _____

Signature/ thumb impression of the witness: _____ Date: _____

Name and address of the witness: _____ Date: _____

Signature of the investigator: _____ Date: _____

PUBLICATIONS & PRESENTATIONS RELATED TO THESIS WORK

Research Publications

1. Kulkarni SR, Peerapur BV, Sailesh KS. Isolation and antibiotic susceptibility pattern of *Escherichia coli* from urinary tract infections in a tertiary care hospital of North Eastern Karnataka. Journal of natural science, biology, and medicine. 2017 Jul;8(2):176
2. Sudheendra KR, Basavaraj PV. Analysis of antibiotic sensitivity profile of biofilm-forming uropathogenic *Escherichia coli*. Journal of Natural Science, Biology and Medicine. 2018 Jul 1;9(2):175.

Conference presentations

Oral presentations

1. Kulkarni SR, Peerapur BV. Association of phenotypic virulence determinants of uropathogenic *Escherichia coli* with antibiotic resistance isolated from a tertiary Care hospital. **38th IABMS 2017, Saveetha University, Chennai.**
2. Kulkarni SR, Peerapur BV. Virulence Factors and Antibiogram of *Escherichia coli* – The Causative Agent of Urinary Tract Infection among Pregnant Women of Bidar District. **Conference of the Clinical Scientists on Research in Basic Medical Sciences August 2019, Hyderabad.**

Poster presentations

1. Kulkarni SR, Peerapur BV. Prevalence of Asymptomatic Urinary Tract infection due to *Escherichia coli* among pregnant women attending ANC Clinic at BRIMS Teaching Hospital. Annual conference of Indian Association of Medical Microbiologists, **39th MICROCON 2015, JIPMER Pondicherry.**
2. Kulkarni SR, Peerapur BV. Isolation and antibiotic susceptibility pattern of *Escherichia coli* from urinary tract infections in a tertiary care hospital of North Eastern Karnataka **GUT MICROBIOME-2016; An International Perspective. Manipal University, Manipal.**
3. Kulkarni SR, Peerapur BV. Antibiotic susceptibility pattern of Biofilm producing Uropathogenic *Escherichia coli* isolated from a Tertiary care hospital of North Karnataka. Annual conference of Indian Association of Medical Microbiologists **40th MICROCON 2016, PGIMER, Chandigarh.**

Original Article

Isolation and Antibiotic Susceptibility Pattern of *Escherichia coli* from Urinary Tract Infections in a Tertiary Care Hospital of North Eastern Karnataka

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Abstract

Introduction: Clinical management of the urinary tract infections (UTI) is influenced by the antimicrobial vulnerability patterns. **Objective:** The study aimed to analyse the resistance pattern of the *Escherichia coli* (*E. coli*) causing UTI in patients over a period of 4 years from 2012 to 2015. **Materials and Methods:** 1000 samples from patients suspected of having urinary tract infections were collected and processed for culture and antimicrobial drug susceptibility as per the routine microbiological techniques. **Results:** Of the total 1000 samples, 395 cases were culture-positive for E. coli. These isolates were tested for antibiotic susceptibility by disk diffusion method. Of the total 395 E. coli isolates, 170 (43%) were multi drug resistant (MDR). The isolates showed high level of resistance to Ampicillin (82.53%), Cefuroxime (72.41%), Amoxycillin-clavulinic acid (71.90%), Ceftriaxone (66.58%), Ciprofloxacin (65.82%) and Cefepime (57.47%). The isolates were sensitive to Imipenem (96.71%), Nitrfurantion (92.41%), Amikacin (90.89%), Chloramphenicol (85.82%), Piperacillin-tazobactum (80.76%), Gentamicin (59.24%), Azetreonam (54.43%) and Norfloxacin (53.67%). **Conclusion:** We conclude that a significant number of the urinary tract infections in our study subjects were caused by multiple drug resistant E. coli. The sensitivity pattern showed a continued decline from 2012 to 2015, with Imipenem being currently the most effective antibiotic.

Keywords: *Escherichia coli*, multidrug resistant, therapy, urinary tract infection

Introduction

Urinary tract infection (UTI) can be caused by Gram-negative bacteria such as *Escherichia coli*, *Klebsiella* species, *Enterobacter* species, and *Proteus* species. *E. coli* is the most common organism causing both community as well as hospital-acquired UTI,[1] often leading to serious secondary health issues.[2] Different factors such as age, gender, immunosuppression, and urological instrumentation can affect prevalence of UTI.[3] Detection of UTI causing pathogens and analyzing resistance pattern of these pathogens to commonly prescribed antibiotics in the clinical practice is essential and helpful in improving the efficacy of empirical treatment.[4] UTI caused by multidrug-resistant (MDR) *E. coli* increases the cost of treatment, morbidity, and mortality, especially in developing countries like India.[5,6] The resistance rate of uropathogenic *E. coli* to various antibiotics has been reported as beta-lactams (57.4%), co-trimoxazole (48.5%), quinolones (74.5%), gentamicin (58.2%), amikacin (33.4%), cefuroxime (56%), and nalidixic acid (77.7%).[2,7-10] However, these antibiotic sensitivity patterns may vary in different geographical locations. Hence, here, we aimed to isolate *E. coli* and study the antibiotic sensitivity profile from patients with UTIs from a tertiary care hospital of North Karnataka, India.

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Materials and Methods

Sample collection

The study was performed from the year 2012–2015 to investigate symptomatic and asymptomatic UTI among patients attending OPD of Bidar Institute of Medical Sciences

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(BRIMS) Teaching Hospital, Bidar, Karnataka, India. One thousand patients complaining of burning micturition and other associated illness were included in this study. A clean-catch midstream urine sample was collected in a sterile wide mouth container labeled with information on the patients age, sex, and brief clinical history. The samples were transported immediately to the laboratory, Department of Microbiology, BRIMS, Bidar and processed for culture and antimicrobial drug susceptibility as per the routine microbiological techniques. Semi-quantitative urine culture using a calibrated loop was used to isolate bacterial pathogens on blood and MacConkey agar as per the recommendations of Kass.[11] The plates were incubated at 37°C for 24 h and further incubated for 48 h in culture (growth) negative cases. Following this, the isolates were identified by standard biochemical tests, and diagnosis of UTI was made when pathogens were present at a concentration of at least 10^5 colony-forming unit (CFU)/ml of urine. Isolates other than *E. coli* were not considered for this study.

Antibiotic sensitivity testing

Antibiotic sensitivity testing was done on Mueller-Hinton agar by Kirby–Bauer disc diffusion method[12] using following antibiotic discs (HiMedia, Mumbai) ampicillin (AMP 10 mcg), amikacin (AK 30 mcg), amoxicillin-clavulanic acid (AMC 30 mcg), aztreonam (AT 30 mcg), ceftriaxone (CTR 30 mcg), cefuroxime (CXM 30 mcg), cefepime (CPM 30 mcg), ciprofloxacin (CIP 5 mcg), chloramphenicol (C 30 mcg), gentamicin (GEN 10 mcg), imipenem (IPM 10 mcg), nitrofurantoin (NIT 300 mcg), norfloxacin (NX 10 mcg), and piperacillin-tazobactam (PIT 100/10 mcg) as per CLSI guidelines.[13]

Statistical analysis

Statistical software package SPSS version 16 (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp) was used to analyze the data. Age, gender, organisms causing UTI, and its antibiotic sensitivity and resistance were included as variables in the model.

Results

Among 1000 samples tested, 39.5% (395) samples showed growth of *E. coli* with colony count of over 10^5 CFU/ml of urine. Of the total 395 *E. coli* isolates, 170 (43%) were MDR. The isolates showed high level of resistance to ampicillin (82.53%), cefuroxime (72.41%), amoxicillin-clavulanic acid (71.90%), ceftriaxone (66.58%), ciprofloxacin (65.82%), and cefepime (57.47%) and were sensitive to imipenem (96.71%), nitrofurantoin (92.41%), amikacin (90.89%), chloramphenicol (85.82%), piperacillin-tazobactam (80.76%), gentamicin (59.24%), aztreonam (54.43%), and norfloxacin (53.67%).

Seventy-two *E. coli* positive samples were isolated in the year 2012 and were analyzed for the antibiotics sensitivity and resistance pattern among the patients of different age group as shown in Figure 1. The age group of 0–9 showed the highest resistance to two isolates for CPM, CIP, and C and sensitivity to three isolates for AMP, AK, GEN, IPM, NIT, and NX. The age group of 10–19 showed the highest resistance to five isolates for AMP and sensitivity to six isolates for AK, IPM, and NIT. Age group of 20–29 showed the highest resistance to 28 isolates for AMP and sensitivity to 24 isolates for IPM. Age group of 30–39 showed the highest resistance to 13 isolates for AMP, CTR, and CPM and sensitivity to 15 isolates for IPM. The age group of 40–49 showed the highest resistance to six isolates for AMP and sensitivity to five isolates for AK and NIT. The age group of 50–59 showed the highest resistance to three isolates for CIP and sensitivity to three isolates for AT, C, IPM, and NIT. Age group >60 years showed the highest resistance to six isolates for AMC, CXM, and CIP and sensitivity to six isolates for AMP, AK, C, IPM, and NIT. The maximum isolates were both sensitive and resistant in the 20–29 age group.

In the year 2013, 133 isolates were analyzed for the antibiotic sensitivity pattern among the patients of different age group as shown in Figure 2. The age group of 0–9 showed the highest resistance to isolates for AMP, AMC, and CXM and sensitivity to six isolates for AK, CTR, CPM, CIP, C, GEN, IPM, NIT, PIT, and NX. Age group of 10–19 showed the highest resistance to 23 isolates for AMP and sensitivity to 27 isolates for AK, PIT, and NIT. Age group of 20–29 showed the highest resistance to 38 isolates for AMP and sensitivity to 40 isolates for IPM and NIT. Age group of 30–39 showed the highest resistance to 21 isolates for CXM and sensitivity to 26 isolates for NIT. Age group of 40–49 showed the highest resistance to six isolates for AT, CXM, and CIP and sensitivity to seven isolates for AK, C, IPM, PIT, and NIT. Age group of 50–59 showed the highest resistance to eight isolates for AMC and CXM and sensitivity to eight isolates for AK. Age >60 years showed the highest resistance to 16 isolates for AMP and sensitivity to 19 isolates for NIT. In this year also, maximum isolates were both sensitive and resistant in the 20–29 age group.

In the year 2014, 75 isolates were analyzed for the antibiotic sensitivity pattern among the patients of different age group as shown in Figure 3. The age group of 0–9 showed the highest resistance to one isolates for AT, CIP, and GEN and sensitivity to nine isolates for AMP, AK, AMC, CTR, CXM, CPM, C, IPM, NIT, PIT, and NX. Age group of 10–19 showed the highest resistance to 15 isolates for AMP and sensitivity to 17 isolates for C and IPM. Age group of 20–29 showed the highest resistance to 19 isolates for AMP and sensitivity to 21 isolates for NIT. Age group of 30–39 showed the highest resistance to 11 isolates for CXM and sensitivity to 13 isolates for AK, C, and IPM. Age group of 40–49 showed the highest resistance to four isolates for AMP and sensitivity to four isolates for AK, C, and IPM. Age group of 50–59 showed the highest resistance to five isolates for AMP, AT, CTR, and CXM and sensitivity to five isolates for AK, C, and NIT. Age group of >60 years showed the highest resistance to five isolates for AMP and AMC and sensitivity to six isolates for AK and IPM. Again in this year also, maximum isolates were both sensitive and resistant in the 20–29 age group.

In the year 2015, 54 isolates were analyzed for the antibiotics sensitivity pattern among the patients of different age group as shown in Figure 4. The age group of 0–9 showed the highest resistance to seven isolates for C and sensitivity to nine isolates for AK, IPM, and PIT. Age group of 10–19 showed the highest resistance to seven isolates for AMP and sensitivity to eight isolates for IPM. Age group of 20–29 showed the highest resistance of twenty isolates for AMP and CXM and sensitivity to 23 isolates for IPM. Age group of 30–39 showed the highest resistance to five isolates for AMP and CXM and sensitivity to five isolates for AK and IPM. Age group of 40–49 showed the highest resistance to single isolate except AK, C, GEN,

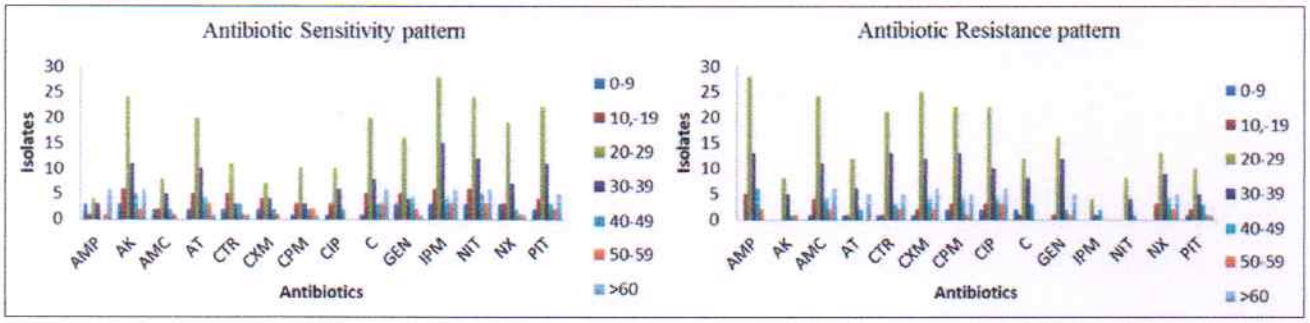

Figure 1: Antibiotic sensitivity and resistance pattern of *E. coli* isolates in the year 2012 in different age groups

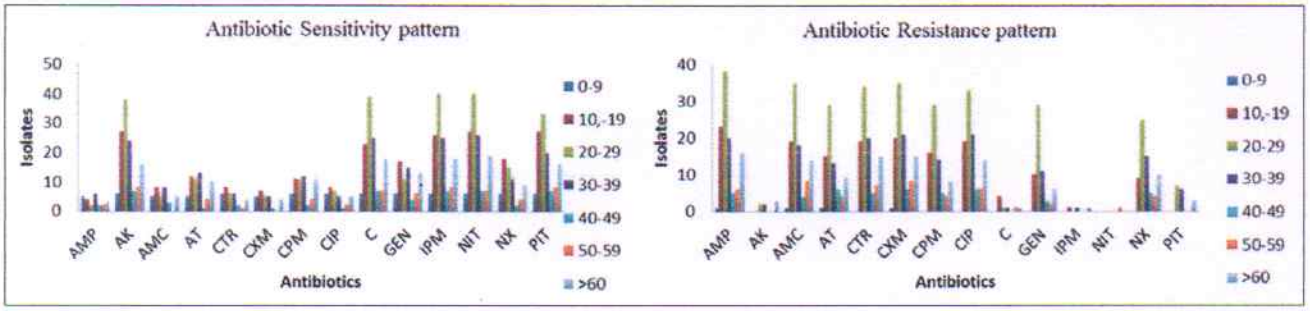

Figure 2: Antibiotic sensitivity and resistant pattern of *E. coli* isolates in the year 2013 in different age groups

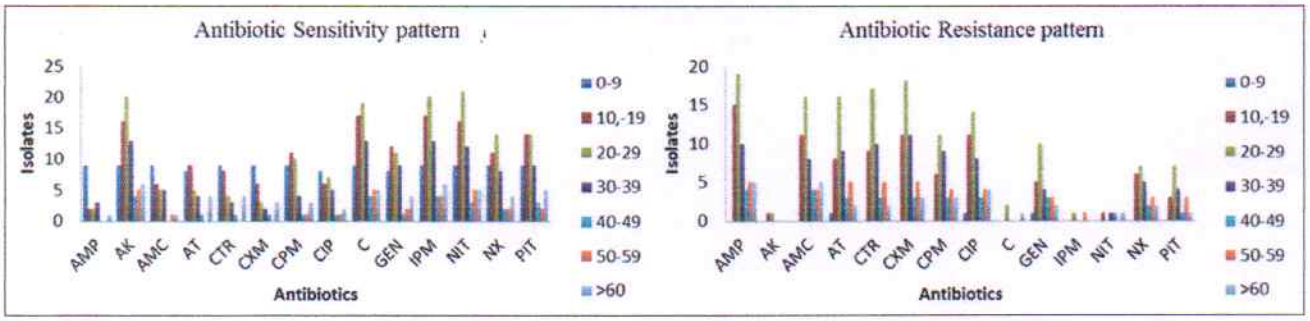

Figure 3: Antibiotic sensitivity and resistant pattern of *E. coli* isolates in the year 2014

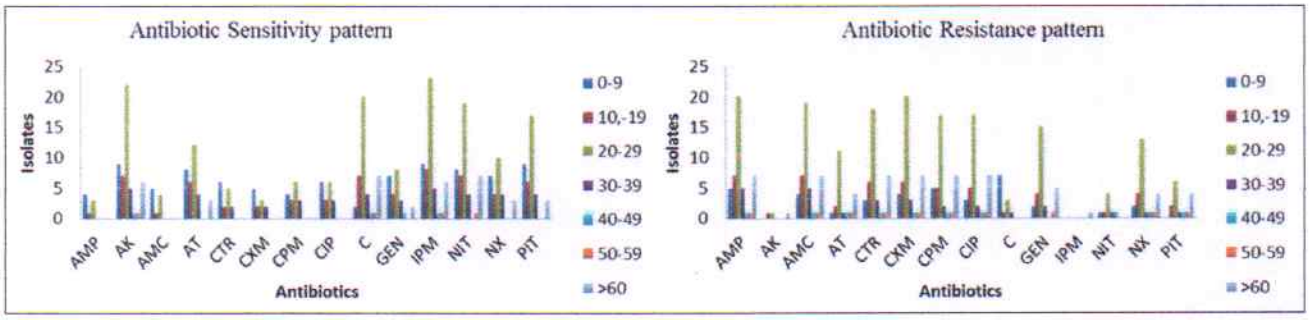

Figure 4: Antibiotic sensitivity and resistant pattern of *E. coli* isolates in the year 2015

and IPM and sensitivity to single isolate for AK, C, GEN, and IPM. Age group of 50–59 showed the highest resistance to single isolate except AK, C, IPM, and NIT and sensitivity to single isolate for AK, C, IPM, and NIT. Age group >60 years showed the highest resistance to seven isolates for AMP, CTR, CXM, CPM and CIP and sensitivity to seven isolates for C and NIT. The 20–29 age group showed the maximum isolates both sensitive and resistant to the antibiotics.

In the year 2015, 61 *E. coli* were isolated from pregnant women and were analyzed for the antibiotics sensitivity pattern among different age groups as shown in Figure 5. The age group of 10–19 showed the highest resistance to ten isolates for AMP and sensitivity to ten isolates for IPM, NIT, and PIT. Age group of 20–29 showed the highest resistance to 34 isolates for AMP and sensitivity to 35 isolates for IPM. Age group of 30–39 showed the highest resistance to nine isolates for AMP and sensitivity to nine isolates for IPM. Age group of 40–49 showed the highest resistance to single isolate for AMP, AMC, and CPM and sensitivity to single isolate except AMP, AMC, and CPM. The 20–29 age group showed both sensitive and resistant to the maximum isolates.

Our findings indicate that MIC of *E. coli* to AMP and CXM has increased and also the resistance to other antibiotics has increased with increase in MIC. Resistance to antibiotics AMP and CXM was more common among 20–29 years age group in all the years studied. The infection rate was higher in female patients (81.26%) and in the 20–29 age group [Figure 6].

Discussion

UTIs are the most frequent infection in women often caused by bacteria. *E. coli* UTI caused by the MDR *E. coli* has increased in the current years probably due to the increasing and irrational use of antibiotics. The distribution of species and their susceptibility to antibiotics vary with time and place.[14] *E. coli* was the most predominant species isolated in our study population. The isolates were tested for 14 different antibiotics and showed resistance to fluoroquinolones and other commonly used antibiotics to treat UTI which was similar to the studies conducted by Akram *et al.*[15] and Aypak *et al.*[16] in other parts of India. Our study also monitored the year-wise sensitivity pattern of *E. coli* isolates to the antibiotics. In the year 2012, the isolates were more sensitive to the most of the antibiotics compared to 2015. The carbapenems could be the promising antibiotics which showed only sensitive in the year 2015. Formation of films by bacteria inside the bladder leads to recurrent infections and also increases the possibility of MDR strain causing UTI.[17,18] Studies from India have reported *E. coli* as one of the most common organisms causing UTI.[15,19,20] In the year 2006, Biswas *et al.* studied the prevalence of antimicrobial resistance among urinary isolates in Uttaranchal, India, and in this study, *E. coli* was isolated from 67.5% samples and more than 35% isolates showed resistant to commonly used antibiotics to treat UTI.[21] Eshwarappa *et al.* studied the clinico-microbiological profile of UTI in South India. Their study also revealed that *E. coli* was the most common organism causing UTIs with extended spectrum beta-lactamase which recorded least resistance against carbapenems (3.9%) and a high resistance to commonly used antibiotics, which is of significant concern for future options in treating these infections.[22] Pregnant women are more susceptible to UTI because of increased urinary content of amino acids, vitamins, and other nutrients, which encourage the persistence of infection. Manjula *et al.* studied the incidence and prevalence of UTI among pregnant women in Karnataka and found that 49.4% had UTI, and *E. coli* was the predominant pathogen isolated.[23] The present study revealed the infection rate due to *E. coli* in pregnant women of North Karnataka region and their resistant pattern to different antibiotics.

Conclusion

We conclude that significant numbers of the UTI are caused by MDR *E. coli*. The antimicrobial resistance patterns of the causes of UTI are highly variable, and continuous surveillance of trends in resistance patterns of uropathogens is necessary. The treatment of UTI by antimicrobial agents needs to be strongly promoted by *in vitro* susceptibility testing to evade advance spread of antimicrobial resistance in patients and eventual development of MDR. This is the first study conducted in North Karnataka which helps further researchers to keep monitoring on the ever-changing trend in the antibiotic susceptibility pattern of *E. coli* isolates in this

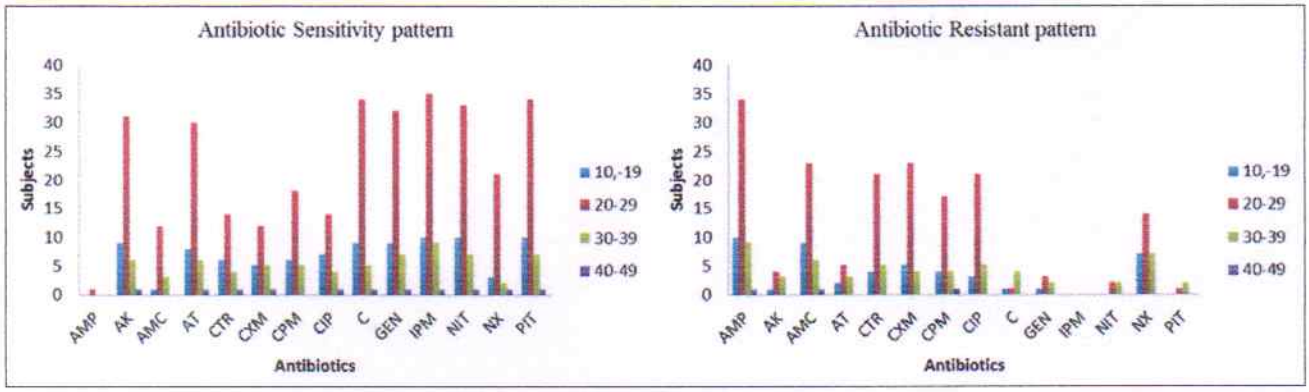

Figure 5: Antibiotic sensitivity and resistant pattern of *E. coli* isolated from pregnant women in the year 2015(P)

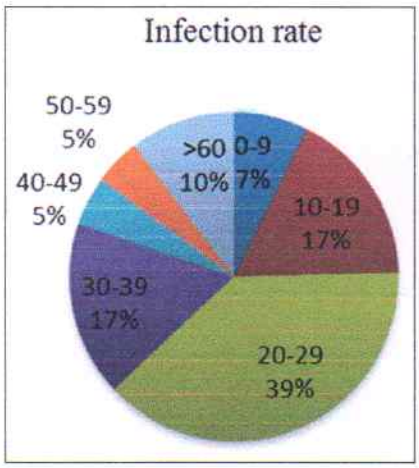

Figure 6: Percentage of infection rate in age group

region and facilitates evidence-based judicious antibiotic use policy to treat UTI.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Sobel JD, Kaye D. Urinary tract infections. In: Mandell GL, Bennett JE, Dolin R, editors. Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases. 7th ed., Vol. 1. Philadelphia, USA: Churchill Livingstone Elsevier Publication; 2010. p. 958-72.
2. Kalsoom BA, Jafar KH, Begum H, Munir S, ul AKBAR N, Ansari JA, Anees M. Patterns of antibiotic sensitivity of bacterial pathogens among urinary tract infections (UTI) patients in a Pakistani population. African Journal of Microbiology Research 2012;16;6:414-20.
3. Al-Jiffri O, El-Sayed Z, Al-Sharif F. Urinary tract infection with Escherichia coli and antibacterial activity of some plants extracts. Int J Microbiol Res 2011;2:1-7.
4. Gupta V, Yadav A, Joshi RM. Antibiotic resistance pattern in uropathogens. Indian J Med Microbiol 2002;20:96-8.
5. Iqbal T, Naqvi R, Akhter SF. Frequency of urinary tract infection in renal transplant recipients and effect on graft function. J Pak Med Assoc 2010;60:826-9.
6. Fink R, Gilmartin H, Richard A, Capezuti E, Boltz M, Wald H. Indwelling urinary catheter management and catheter-associated urinary tract infection prevention practices in nurses improving care for health system elders hospitals. Am J Infect Control 2012;40:715-20.
7. Manikandan S, Ganesapandian S, Singh M, Kumaraguru AK. Antimicrobial susceptibility pattern of urinary tract infection causing human pathogenic bacteria. Asian J Med Sci 2011;3:56-60.
8. Humayun T, Iqbal A. The culture and sensitivity pattern of urinary tract infections in females of reproductive age group. Ann Pak Inst Med Sci 2012;8:19-22.
9. Afzal S, Naemullah S. Spectrum of the microorganisms in children with urinary tract infection. J Rawalpindi Med Coll 2008;12:44-6.
10. Ramanath KV, Shafiya SB. Prescription pattern of antibiotic usage for urinary tract infection treated in a rural tertiary care hospital. Indian J Pharm Pract 2011;4:57-63.
11. Kass EH. Pyelonephritis and bacteriuria. A major problem in preventive medicine. Ann Intern Med 1962;56:46-53.
12. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 1966;45:493-6.
13. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Disk. Approved Standard M2-A10. Wayne, PA, USA: Clinical and Laboratory Standards Institute; 2007.
14. Ullah F, Malik SA, Ahmed J. Antibiotic susceptibility pattern and ESBL prevalence in nosocomial *Escherichia coli* from urinary tract infections in Pakistan. Afr J Biotechnol 2009;8:3921-6.
15. Akram M, Shahid M, Khan AU. Etiology and antibiotic resistance patterns of community-acquired urinary tract infections in J N M C Hospital Aligarh, India. Ann Clin Microbiol Antimicrob 2007;6:4.
16. Aypak C, Altunsoy A, Düzgün N. Empiric antibiotic therapy in acute uncomplicated urinary tract infections and fluoroquinolone resistance: A prospective observational study. Ann Clin Microbiol Antimicrob 2009;8:27.
17. Stamm WE. Urinary tract infections, pyelonephritis, prostatitis. In: Fauci AS, Braunwald E, Kasper DL, Hauser SL, Longo DL, Jameson JL, *et al.*, editors. Harrison's Principles of Internal Medicine. 17th ed., Vol. 2. New York, USA: Mc-Graw Hill; 2008. p. 1820-5.
18. Elder JS. Urinary tract infections. In: Kliegman RM, Behrman RE, Jenson HB, Stanton BE, editors. Nelson Textbook of Pediatrics. 18th ed., Vol. 2. Philadelphia, USA: Saunders Elsevier; 2007. p. 2224-5.
19. Hasan AS, Nair D, Kaur J, Baweja G, Deb M, Aggarwal P. Resistance patterns of urinary isolates in a tertiary Indian hospital. J Ayub Med Coll Abbottabad 2007;19:39-41.
20. Kothari A, Sagar V. Antibiotic resistance in pathogens causing community-acquired urinary tract infections in India: A multicenter study. J Infect Dev Ctries 2008;2:354-8.
21. Biswas D, Gupta P, Prasad R, Singh V, Arya M, Kumar A. Choice of antibiotic for empirical therapy of acute cystitis in a setting of high antimicrobial resistance. Indian J Med Sci 2006;60:53-8.
22. Eshwarappa M, Dosegowda R, Aprameya IV, Khan MW, Kumar PS, Kempegowda P. Clinico-microbiological profile of urinary tract infection in South India. Indian J Nephrol 2011;21:30-6.
23. Manjula NG, Math GC, Patil A, Gaddad SM, Shivannavar CT. Incidence of urinary tract infections and its aetiological agents among pregnant women in Karnataka region. Advances in Microbiology 2013;3:473-8.

Original Article

Analysis of Antibiotic Sensitivity Profile of Biofilm-Forming Uropathogenic *Escherichia coli*

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Abstract

Introduction: Biofilms are group of microorganisms which are embedded within a self-produced matrix of extracellular polymeric substance which adhere to each other. They are found to be involved in a wide range of infections in the body like urinary tract infections (UTIs). Biofilms are considered to be highly resistant to antimicrobial agents. *Escherichia coli* (*E. coli*) is the most common organism causing both community as well as hospital acquired UTI leading to serious health issues. **Objectives:** This study was conducted to analyse the antibiotic sensitivity profile of biofilm forming *Escherichia coli* (*E. coli*) isolated from patients with suspected UTI attending a Teaching hospital of North Karnataka. **Materials And Methods:** 388 *E. coli* isolates recovered from 1000 suspected cases of UTI were tested for susceptibility to fourteen different antibiotics. In vitro biofilm formation was detected by Tube adherence method, Congo red agar method and Tissue culture plate method. **Results:** 277 isolates (71.39%) produced biofilm in-vitro by all the three methods. Biofilm forming *E. coli* developed significantly higher degree of resistance towards antimicrobial drugs Ampicillin (87.36%), Cefuroxime (81.58%), Amoxicillin clavulanic acid (77.61%), Ciprofloxacin (71.48%) and Ceftriaxone (71.48%). They were sensitive to higher antibiotics like Imipenem, Piperacillin-tazobactam, Nitrofurantoin, and Amikacin. **Conclusion:** Detection of biofilm in *E. coli* and its resistance to commonly prescribed antibiotics in the clinical practice is essential in improving the efficacy of empirical treatment. This study revealed the prevalence and antimicrobial susceptibility pattern of biofilm forming *E. coli* which helps clinicians to treat UTI effectively.

Keywords: Antibiotic resistance, biofilm, *Escherichia coli*, urinary tract infection

Introduction

Urinary tract infections (UTIs) are the major and most important cause of serious health problems and morbidity. UTIs account for more than 7 million visits to physicians per year[1-3] affecting persons of all ages including children, women, and elderly but most predominant in women, especially in developing countries such as India.[4,5] Approximately 40% of women have had a UTI in their lifetime and over 20% of young sexually active women who had previous UTIs have recurrent UTIs.[6]

Escherichia coli is the most common organism causing both community and hospital-acquired UTIs, leading to serious secondary health issues worldwide.[7,8] Currently, recurrent UTI is a serious health problem for many women despite our broad array of very successful antimicrobial agents. Recurrent and relapse UTIs may be due to bacterial virulence factors exhibited by uropathogenic *E. coli* (UPEC) which enable colonization of the bacteria and help the organism overcome host defenses and invade the urinary tract.[9]

Biofilm formation is one of the most important virulence factors exhibited by *E. coli* among other virulence factors. Microbial biofilms are community of bacteria and other microorganisms that are irreversibly attached to self-produced extracellular polymeric substances and adhere to a surface or each other. Biofilms are ubiquitous and can be found in a variety of niches or sites or devices. They play an important role in medicine and have been proven to cause a wide range of microbial infections in the human body such as UTIs, catheter-associated infections, or dental plaques.[10]

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Biofilms decrease the susceptibility of organism to antimicrobial agents by enclosing them in an extracellular matrix.[11] A high content of polysaccharides in biofilm prevents the access of antimicrobial agents. Limited penetration of antimicrobial agents into the biofilm and slow rate of cell multiplication of organisms in the biofilm may contribute to the development of chronic infections. Biofilm-forming bacteria exhibit higher resistance to antimicrobial drugs used for the treatment of UTIs, which also lead to recurrent infections.[12]

Our study aimed to unveil the association of biofilm-forming *E. coli* and their antimicrobial susceptibility pattern. This study would help the clinicians in choosing suitable antibiotics for effective treatment of UTI.

Materials and Methods

Sample collection and processing

This study was conducted in the Department of Microbiology, Bidar Institute of Medical Sciences (BRIMS), Bidar, after getting approval from the Institutional Ethical Committee. One thousand patients of all age groups and both sexes complaining of burning micturition and other associated illness attending the outpatient department of BRIMS teaching hospital were included in this study. Informed consent was obtained from all the patients. Clean-catch midstream urine samples were collected in a sterile widemouthed container along with information about their age, sex, and brief clinical history. Samples were transported to the laboratory immediately and processed for culture and antimicrobial drug susceptibility testing as per the routine microbiological techniques and recommendations of Kass.[13] Further, the isolates were identified by standard biochemical tests, and a diagnosis of UTI was made when pathogens were grown at least 10^5 colony forming unit/ml of urine. Only *E. coli* isolates were included in this study.

Antibiotic sensitivity testing

Antibiotics (obtained from HiMedia Laboratories, Mumbai, Maharashtra, India) such as ampicillin (AMP 10 µg), amikacin (AK 30 µg), amoxicillin-clavulanic acid (AMC 30 µg), aztreonam (AT 30 µg), ceftriaxone (CTR 30 µg), cefuroxime (CXM 30 µg), cefepime (CPM 30 µg), ciprofloxacin (CIP 5 µg), chloramphenicol (C 30 µg), gentamicin (GEN 10 µg), imipenem (IPM 10 µg), nitrofurantoin (NIT 300 µg), norfloxacin (NX 10 µg), and piperacillin-tazobactam (PIT 100/10 µg) were tested according to Kirby–Bauer's disc diffusion method[14] as per the Clinical and Laboratory Standards Institute's (CLSI) guidelines.[15]

Quality control

The CLSI control strain of *E. coli* ATCC 25922 was used as a control for antimicrobial susceptibility testing.

In vitro Biofilm Detection

In vitro detection of biofilm was done by three different methods as follows: tube adherence method, Congo red agar method (CRA), and tissue culture plate method (TCP).

Tube adherence method

This test described by Christensen *et al.* is a qualitative method for biofilm detection.[16] A loopful of test organisms was inoculated in 10 ml of trypticase soy broth with 1% glucose in test tubes. The tubes were incubated at 37°C for 24 h. After incubation, tubes were decanted and washed with phosphate-buffered saline (pH 7.3) and dried. Tubes were then stained with crystal violet (0.1%). Excess stain was washed with deionized water and dried. The scoring for tube method was done according to the results of the control strains. Biofilm formation was considered positive when a visible film lined the wall and the bottom of the tube.

Congo red agar method

Freeman *et al.*[17] have described a simple qualitative method to detect biofilm production using CRA medium. CRA medium was prepared with brain–heart infusion broth 37 g/L, sucrose 50 g/L, agar no. 1 10 g/L (HiMedia Laboratories, Mumbai, Maharashtra, India), and Congo red indicator 8 g/L (Nice chemicals, Cochin). Initially, Congo red stain was prepared as a concentrated aqueous solution and autoclaved (121°C for 15 min) separately from the other medium constituents. It was then added to the autoclaved brain–heart infusion agar with sucrose at 55°C. CRA plates were inoculated with test organisms and incubated at 37°C for 24 h aerobically. Black colonies with a dry crystalline consistency indicated biofilm production.

Tissue culture plate method

This quantitative test described by Christensen *et al.* is considered the gold standard method for biofilm detection.[18] Isolates were inoculated in 10 ml of trypticase soy broth with 1% glucose and incubated at 37°C for 24 h. The cultures were then diluted 1:100 with fresh medium. Individual wells of sterile TCPs were filled with 200 µL of the diluted cultures including control strains. Plates were incubated at 37°C for 24 h. After incubation, contents of each well were removed by gentle tapping. The wells were washed with 0.2 mL of phosphate-buffered saline (pH 7.2) four times. Biofilms formed by bacteria adherent to the wells were fixed by 2% sodium acetate and stained by crystal violet (0.1%). Excess stain was removed by using deionized water and plates were dried. Optical density of stained adherent biofilms was obtained using micro ELISA autoreader (model 680, Biorad, UK) at a wavelength of 570 nm. The interpretation of biofilm production was done according to the criteria of Stepanovic *et al.*[19]

Quality control

The biofilm producers such as *Staphylococcus epidermidis* ATCC 35984 (positive control) and the nonbiofilm producers such as *S. epidermidis* ATCC 12228 (negative control) were used as standard control strains.

Statistical analysis

Statistical software package SPSS version 22 (IBM SPSS Statistics for Windows, IBM Corp., Released 2013, Armonk, NY, USA) was used to analyze the data. Chi-square test was applied. $P < 0.05$ was considered statistically significant.

Results

Of 1000 urine specimens processed from patients of suspected UTI, 388 *E. coli* were isolated (38.8%). Infection was predominant in females with a rate of 80.92% between the age group of 20 and 29 years (39%). Among males, the infection rate was 19.07%.

Among 388 *E. coli* strains subjected to *in vitro* biofilm production, 277 isolates (71.39%) produced biofilm by all the three methods. *In vitro* biofilm formation by different methods was as follows: 40 (10.3%) strains showed highly positive, 35 strains (9%) showed moderately positive, and 91 strains (23.5%) showed weakly positive by tube method [Figure 1]. Similarly, in CRA method, 254 strains (65.5%) showed highly positive [Figure 2], whereas in TCP method, 284 (73.2%) strains showed strongly positive, 23 strains (5.9%) showed moderately positive, and 81 strains (20.9%) showed weakly positive [Figure 3 and Table 1].

Biofilm-producing isolates showed the highest resistance to the antibiotics compared to nonbiofilm-producing isolates. Biofilm producers demonstrated resistance to AMP (87.36%) followed by CXM (81.58%), AMC (77.61%), CIP (71.48%), CTR (54.6%), and CPM (64.98%) [Table 2]. Significant association was observed between biofilm formation and multidrug resistance which was proved to be statistically significant regarding antibiotics such as AK, AMC, AT, CTR, CXM, CPM, CIP, and C [Table 3]. Isolates were sensitive to antibiotics such as PIT (97.83%), IPM (97.14%), and NIT (92.41%).

Discussion

E. coli is the most prominent causative agent of both symptomatic and asymptomatic UTIs, which accounts for more than 80% of the infections.[20,21] In our study, we found that the frequency of UTI was higher in females compared to males, which concord with other studies conducted.[22,23] This difference in frequency may be due to several clinical factors, including anatomic differences and hormonal effects. UTI is associated with an expression of different virulence factors including biofilm formation. Biofilm formation is closely related to the susceptibility pattern of *E. coli* toward the antimicrobial drugs which are commonly used to treat UTIs. The resistance pattern in UTI patients of this region is not known. Understanding the resistance pattern will be helpful for treatment. Resistance to antibiotics by biofilm-producing *E. coli* increases the chronicity and recurrence of UTI as bacteria are enclosed within the biofilm and do not allow the antibiotic access to the bacteria.

In this study, the incidence of *in vitro* biofilm formation by UPEC was 71.39%, which was similar to the studies conducted by Subramanian *et al.*,[24] Sharma *et al.*,[25] and Suman *et al.*[26] who reported biofilm formation at the rates of 63%, 67.5%, and 92.0%, respectively. In our study, we analyzed the *in vitro* biofilm formation by three different methods. Nearly 42.78% of isolates were found positive by tube adherence method,

Figure 1: Strong biofilm formation of *Escherichia coli* by tube adherence method

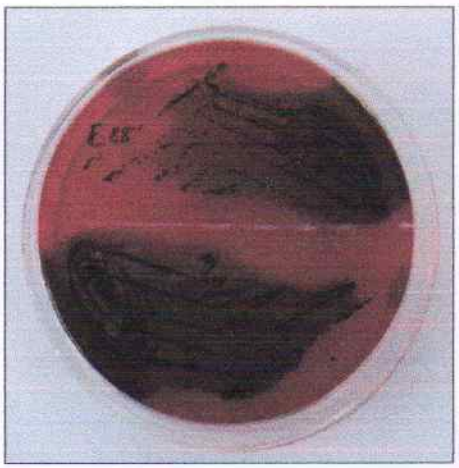

Figure 2: Biofilm formation of *Escherichia coli* on Congo red agar

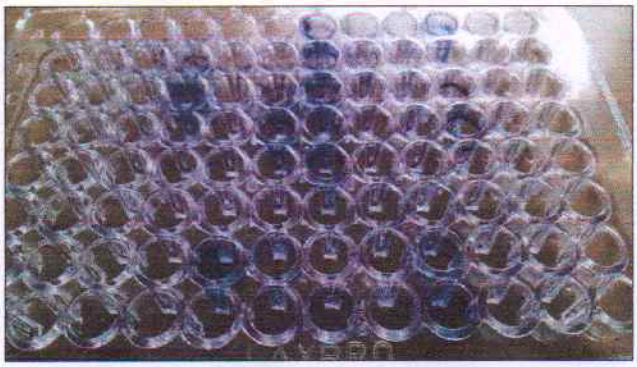

Figure 3: Positive biofilm formation of *Escherichia coli* by tissue culture plate method

and we classified them as highly positive (10.3%), moderately positive (9%), and weakly positive (23.5%). In CRA method, 65.5% isolates were found positive. In TCP method, again it was classified as strongly positive (73.2%), moderately positive (5.9%), and weakly positive (20.9%). These findings were much closer to the study results reported by Tabasi *et al.*[27]

In our study, we detected biofilm formation in all the 388 *E. coli* isolates (100%) by TCP method which was similar to the findings reported by Fattahi *et al.*[28] who outlined biofilm formation in 100% of isolates by TCP method.

We studied antibiotic susceptibility pattern for all UPEC isolates. We analyzed the antibiotic resistance pattern of biofilm- and nonbiofilm-forming *E. coli* isolates. Biofilm-forming isolates demonstrated increased resistance to the commonly used antibiotics to treat UTI compared to nonbiofilm producers. Our study results revealed significant correlation between biofilm formation and multidrug resistance. There was an increase in resistance pattern of the drugs such as AK, AMC, AT, CTR, CXM, CPM, CIP, and C which were routinely used to treat UTIs from a long time. This pattern of resistance coincides with the study findings reported by Mittal *et al.* and Ponnusamy *et al.*[29,30]

Bacterial biofilms are associated with long-term persistence of the organisms in various environments. Biofilms make the organisms impermeable to antibiotics and bind the agents at the outer surface of the matrix layer which protects the bacteria from penetration of the antibiotics. This causes recurrent infection and results in the organism developing multidrug resistance. These strains respond poorly or not respond at all to conventional and routine antimicrobial therapies.

In the present study, the drugs PIT, IPM, and NIT were effective against biofilm-producing UPEC isolates and these drugs can serve as useful reserved drugs for the treatment of UTI. Understanding biofilms in UTIs will help clinicians in decision-making toward effective treatment guidelines for recurrent UTI in this geographical region.

Table 1: Screening of the *Escherichia coli* isolates for biofilm formation by tube adherence method, Congo Red Agar method, and tissue culture plate method

	Tube adherence method (%)	CRA method (%)	TCP method (%)
Strong	40 (10.3)	254 (65.5)	284 (73.2)
Moderate	35 (9)		23 (5.9)
Weak	91 (21.5)		81 (20.9)
Negative	222 (57.2)	134 (34.5)	
Total	388	388	388

CRA: Congo Red Agar, TCP: Tissue culture plate

Table 2: Antibiotic sensitivity profile of biofilm-forming and nonbiofilm-producing *Escherichia coli* isolates

Antibiotic	Biofilm producers (n=277)		Nonbiofilm producers (n=111)	
	Number of isolates showing resistance (%)	Number of isolates showing sensitive (%)	Number of isolates showing resistance (%)	Number of isolates showing sensitive (%)
AMP	242 (87.36)	35 (12.2)	88 (79.3)	23 (20.7)
AK	32 (11.55)	245 (88.4)	10 (9.1)	101 (90.9)
AMC	215 (77.61)	62 (22.3)	62 (55.9)	49 (44.1)
AT	139 (50.18)	138 (49.8)	37 (44.4)	74 (66.6)
CTR	198 (71.48)	79 (28.5)	57 (51.4)	54 (48.6)
CXM	226 (81.58)	51 (18.4)	61 (55)	50 (55)
CPM	180 (64.98)	97 (35)	47 (42.4)	64 (57.6)
CIP	198 (71.48)	79 (28.5)	56 (50.5)	55 (49.5)
C	33 (11.91)	244 (88)	19 (13.2)	92 (82.8)
GEN	128 (46.2)	149 (53.7)	34 (30.7)	77 (69.3)
IPM	8 (2.88)	269 (97.1)	5 (4.6)	106 (95.4)
NIT	21 (7.58)	256 (96)	6 (5.4)	105 (94.5)
NX	134 (48.37)	143 (51.6)	42 (37.9)	69 (62.1)
PIT	6 (2.16)	271 (97.8)	18 (12.3)	93 (83.7)

AMP: Ampicillin, AK: Amikacin, AMC: Amoxicillin-clavulanic acid, AT: Aztreonam, CTR: Ceftriaxone, CXM: Cefuroxime, CPM: Cefepime, CIP: Ciprofloxacin, C: Chloramphenicol, GEN: Gentamicin, IPM: Imipenem, NIT: Nitrofurantoin, NX: Norfloxacin, PIT: Piperacillin-tazobactam

Table 3: Association between antimicrobial resistance and biofilm-forming uropathogenic *Escherichia coli* isolates

Biofilm formation	Antimicrobial drugs									
	AMP (%)	AK (%)	AMC (%)	AT (%)	CTR (%)	CXM (%)	CPM (%)	CIP (%)	C (%)	GEN (%)
TCP method										
Strong	6.1	11.9	6.6	8.7	6	6.6	7.1	5.6	12.5	7
Moderate	22.7	38.1	25.3	25.4	26.3	24.5	26.2	25	35.7	32.4
Weak	71.2	50	68.1	65.9	67.7	69	66.7	69.4	51.8	68.8
P	NS	0.002*	0.002*	0.009*	0.002*	0.010*	0.003*	0.020*	0.001*	NS

*Significant at P<0.05. TCP: Tissue culture plate, AMP: Ampicillin, AK: Amikacin, AMC: Amoxicillin-clavulanic acid, AT: Aztreonam, CTR: Ceftriaxone, CXM: Cefuroxime, CPM: Cefepime, CIP: Ciprofloxacin, C: Chloramphenicol, GEN: Gentamicin, NS: Not significant

Conclusion

Conclusively, we have noticed significant association between biofilm production and multidrug resistance. We believe that the detection of biofilm formation might be worth in the management of UTI therapy. Therefore, the knowledge of biofilm formation by *E. coli* and their antibiotic susceptibility pattern will help in deciding on an appropriate antibiotic treatment for UTI. It also helps restrain the emergence of drug-resistant strains.

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Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Patton JP, Nash DB, Abrutyn E. Urinary tract infection: Economic considerations. Med Clin North Am 1991;75:495-513.
2. Hooton TM, Stamm WE. Diagnosis and treatment of uncomplicated urinary tract infection. Infect Dis Clin North Am 1997;11:551-81.
3. Schappert SM, Burt CW. Ambulatory care visits to physician offices, hospital outpatient departments, and emergency departments: United states, 2001-02. Vital Health Stat 13 2006;(159):1-66.
4. Al-Jiffri O, El-Sayed ZM, Al-Sharif FM. Urinary tract infection with *Escherichia coli* and antibacterial activity of some plants extracts. Int J Microbiol Res 2011;2:01-7.
5. Williams NS, Bulstrode CJ, O'Connell PR, editors. Bailey & Love's Short Practice of Surgery. 25th ed. London, United Kingdom: Edward Arnold Publishers; 2008. p. 1329-30.
6. Kunin CM. Urinary tract infections in females. Clin Infect Dis 1994;18:1-10.
7. Schito GC. Why fosfomycin trometamol as first line therapy for uncomplicated UTI? Int J Antimicrob Agents 2003;22 Suppl 2:79-83.
8. Rodríguez-Baño J, Navarro MD, Romero L, Muniain MA, Perea EJ, Pérez-Cano R, *et al.* Clinical and molecular epidemiology of extended-spectrum beta-lactamase-producing *Escherichia coli* as a cause of nosocomial infection or colonization: Implications for control. Clin Infect Dis 2006;42:37-45.
9. Wang X, Lünsdorf H, Ehrén I, Brauner A, Römling U. Characteristics of biofilms from urinary tract catheters and presence of biofilm-related components in *Escherichia coli*. Curr Microbiol 2010;60:446-53.
10. Tambyah PA. Catheter-associated urinary tract infections: Diagnosis and prophylaxis. Int J Antimicrob Agents 2004;24 Suppl 1:S44-8.
11. Tajbakhsh E, Ahmadi P, Abedpour-Dehkordi E, Arbab-Soleimani N, Khamesipour F. Biofilm formation, antimicrobial susceptibility, serogroups and virulence genes of uropathogenic *E. coli* isolated from clinical samples in Iran. Antimicrob Resist Infect Control 2016;5:11.
12. Tayal RA, Baveja SM, De Anuradha S. Analysis of biofilm formation and antibiotic susceptibility pattern of uropathogens in patients admitted in a tertiary care hospital in India. Int J Health Allied Sci 2015;4:247.
13. Kass EH. Pyelonephritis and bacteriuria. A major problem in preventive medicine. Ann Intern Med 1962;56:46-53.
14. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 1966;45:493-6.
15. Clinical and Laboratory Standards Institute (CLSI) Approved standard M2-A10. Performance Standards for Antimicrobial Disk. Wayne, PA, USA: Clinical and Laboratory Standards Institute; 2007.
16. Christensen GD, Simpson WA, Bisno AL, Beachey EH. Adherence of slime-producing strains of *Staphylococcus epidermidis* to smooth surfaces. Infect Immun 1982;37:318-26.
17. Freeman DJ, Falkiner FR, Keane CT. New method for detecting slime production by coagulase negative staphylococci. J Clin Pathol 1989;42:872-4.
18. Christensen GD, Simpson WA, Younger JJ, Baddour LM, Barrett FF, Melton DM, *et al.* Adherence of coagulase-negative Staphylococci to plastic tissue culture plates: A quantitative model for the adherence of Staphylococci to medical devices. J Clin Microbiol 1985;22:996-1006.
19. Stepanović S, Vuković D, Hola V, Di Bonaventura G, Djukić S, Cirković I, *et al.* Quantification of biofilm in microtiter plates: Overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. APMIS 2007;115:891-9.
20. Hedlund M, Duan RD, Nilsson A, Svensson M, Karpman D, Svanborg C, *et al.* Fimbriae, transmembrane signaling, and cell activation. J Infect Dis 2001;183 Suppl 1:S47-50.
21. Svanborg C, Godaly G. Bacterial virulence in urinary tract infection. Infect Dis Clin North Am 1997;11:513-29.
22. Miller O, Hemphill RR. Urinary tract infection and pyelonephritis. Emerg Med Clin North Am 2001;19:655-74.
23. Foxman B. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. Am J Med 2002;113:5-13.
24. Subramanian P, Shanmugam N, Sivaraman U, Kumar S, Selvaraj S. Antibiotic resistance pattern of biofilm-forming uropathogens isolated from catheterised patients in Pondicherry, India. Australas Med J 2012;5:344-8.
25. Sharma M, Yadav S, Chaudhary U. Biofilm production in uropathogenic *Escherichia coli*. Indian J Pathol Microbiol 2009;52:294.
26. Suman E, Jose J, Varghese S, Kotian MS. Study of biofilm production in *Escherichia coli* causing urinary tract infection. Indian J Med Microbiol 2007;25:305-6.
27. Tabasi M, Asadi Karam MR, Habibi M, Yekaninejad MS, Bouzari S. Phenotypic assays to determine virulence factors of uropathogenic *Escherichia coli* (UPEC) isolates and their correlation with antibiotic resistance pattern. Osong Public Health Res Perspect 2015;6:261-8.
28. Fattahi S, Kafil HS, Nahai MR, Asgharzadeh M, Nori R, Aghazadeh M, *et al.* Relationship of biofilm formation and different virulence genes in uropathogenic *Escherichia coli* isolates from Northwest Iran. GMS Hyg Infect Control 2015;10:Doc11.
29. Mittal S, Sharma M, Chaudhary U. Biofilm and multidrug resistance in uropathogenic *Escherichia coli*. Pathog Glob Health 2015;109:26-9.
30. Ponnusamy P, Natarajan V, Sevanan M. *In vitro* biofilm formation by uropathogenic *Escherichia coli* and their antimicrobial susceptibility pattern. Asian Pac J Trop Med 2012;5:210-3.