Training Report

Internship Training Report

Submitted to Mr. Harpreet Singh (Lecturer)

Lovely Professional University, Punjab

in partial fulfillment of the requirements

For the degree of

Master of Science in Clinical Microbiology

Submitted by:

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SCHOOL OF PHYSIOTHERAPY AND PARAMEDICAL SCIENCES

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May, 2016

PREVELANCE AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF ESCHERIA COLI ISOLATED FROM URINE SAMPLE INDOOR PATIENT IN A TERTIARY CARE HOSPITAL

CERTIFICATE

This is to certify that *Ms. Prabhjot kaur* bearing **Registration Number 11400007** has completed his/her Master of Science in Clinical Microbiology internship under our guidance and supervision. This report is record of the candidate own work carried out by him/her under my supervision. I certify that the matter embodied in this report is original and has been not submitted anywhere for the reward of any other degree.

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DECLARATION

I hereby declare that the work embodied in this internship report was carried by me under the supervision of Mr. Harpreet Singh (Internal supervisor), Lovely Professional University and Dr. Shashi Chopra (External Supervisor.), Professor Department of Microbiology. This work has not been submitted in part or in full in any other university for any degree or diploma.

Name: Prabhjot Kaur

Date:

Place: Lovely Professional University

ABSTRACT

Urinary tract infections (UTIs) are among the most common infectious diseases encountered in the community and in the hospital worldwide. *Escherichia coli (E. coli)* is one of the most important causes of community-acquired and nosocomial infections. Strains of *E.coli* that cause disease outside of the gastrointestinal tract are referred to as extraintestinal pathogenic *E.coli.* Early treatment of UTI is directly related to decrease in morbidity, which makes the selection of empirical therapy of great importance. Appropriate selection of antimicrobial therapy is an important strategy in the prevention of the spread of resistance, since use of overly broad therapy can lead to development of resistance. The aim of this study was to investigate microbial species isolated from indoor patients with UTI and evaluate *E. coli*prevalence and antibiotic susceptibility pattern in a tertiary care hospital. A prospective study was done on the 400 urine samples of indoor patients having symptoms of urinary tract infection, irrespective of their co-morbidities. Urine sample were processed within one hour of collection. Microscopic examination of urine sample was done. The samples were inoculated on Mac Conkey's agar by semi-quantitative method and incubated at 37˚ C for 18-24 hours. After 24 hours growth of bacteria was identified by colony characters and biochemical reactions. Antibiotic sensitivity of *Escherichia coli* isolate was done on Muller Hinton Agar and result recorded. Out of 400 urine samples 306 samples were of female patients and 94 urine samples were of male patients. The significant growth of bacteria was obtained in 76(19%) samples. The most common bacteria isolated among Gram negative bacteria were *Escherichia coli* (60.52%) followed by Klebsiella spp. and *Pseudomonas aeruginosa*(6.58%) each and among Gram positive bacteria *Staphylococcus aureus*(9.21%) and *Enterococci spp*. (5.27%). *Escherichia coli* (94%) were multidrug resistant. We conclude that acute uncomplicated UTI affects a large proportion of the population in the study area, and is more prevalent in females. To prevent UTI regular updated surveillance of local microbial prevalence and resistance patterns are needed to guide the empiric therapy for UTIs.

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I have no words to thanks my family, without the support and encouragement of which I was not able to reach this point where I am now.

I thanks God provide me all the sources to reach this point where I am now.

Prabhjot Kaur

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INDEX

LIST OF TABLES

LIST OF FIGURES:

LIST OF CHART:

CHAPTER-1: INTRODUCTION

Urinary tract infections (UTIs) are among the most common infectious diseases encountered in the community and in the hospital worldwide **(1).** They result in high rates of morbidity and high economic costs associated with Urinary tract infections (UTIs), including cystitis and pyelonephritis, are the most common infectious diseases **(2*).*** *Escherichia coli* is one of the most important causes of community-acquired and nosocomial infections. This organism is therefore of clinical importance and can be isolated from various clinical specimens **(3*).*** *E. coli* accounts for as much as 90 % of the community-acquired and 50% of the nosocomial UTIs **(4,5).** It is more common among women than men, although the prevalence in elderly men and women is similar. *Escherichia coli* are a bacterium commonly found in the large intestine of humans and other warm blooded animals. *Escherichia coli*, the most prevalent facultative Gram-negative bacillus in the human faecal flora, usually inhabit the colon as an innocuous commensal. Strains of *E.coli* that cause disease outside of the gastrointestinal tract are referred to as extraintestinal pathogenic *E.coli* (ExPEC and are divided into uropathogenic *E.coli* (UPEC) strains causing neonatal meningitis and septicemic *E.coli*. UPEC is the most common pathotype of ExPEC and is found in patients with urinary tract infections. Early treatment of UTI is directly related to decrease in morbidity, which makes the selection of empirical therapy of great importance **(6).** The correct choice of empirical antimicrobial requires a good understanding of the typical bacteriology involved in UTIs local resistance patterns **(7,8).** Appropriate selection of antimicrobial therapy is an important strategy in the prevention of the spread of resistance, since use of overly broad therapy can lead to development of resistance. In recent decades, the extended-spectrum β-lactamases (ESBLs) of the TEM, SHV, CTX-M, and OXA type, as well as the CTX-M have emerged as significant mechanisms of resistance in Gram-negative bacilli **(6).** In addition, carbapenem resistance, a worrisome public health threat, is being reported throughout the world. In addition, carbapenem resistance, a worrisome public health threat, is being reported throughout the world. The aim of this study was to investigate microbial species isolated from patients with UTI and evaluate *E. coli* prevalence and antibiotic susceptibility pattern in a tertiary care hospital.

CHAPTER-2: TERMINOLOGY:

UTI	Urinary tract infection
E.coli	*Escherichia coli*
ExPEC	Extra-intestinal pathotype *E. coli*
NMEC	Neonatal meningitis *E. coli*
UPEC	Uropathogenic *E. coli*
ABU	Asymptomatic bacteriuria
RTX	Repeat toxins
ESBLs	Extended-spectrum β-lactamases

CHAPTER-3: REVIEW OF LITERATURE

Urinary tract infections (UTIs) are one of the most common bacterial infections in humans both in the community and hospital setting **(9).** It is the commonest bacterial infectious disease in community practice with a high rate of morbidity and financial cost. It has been estimated that 150 million people were infected with UTI per annum worldwide which costing global economy more than 6 billion US dollars **(10).** It is defined as bacteriuria along with urinary symptoms **(11).** It may involve only the lower urinary tract or may involve both the upper and lower tract. The term cystitis has been used to describe lower UTI, which is characterized by a syndrome involving dysuria, frequency, urgency and occasionally supra pubic tenderness. However, the presence of symptoms of lower tract without upper tract symptoms does not exclude upper tract infection, which is also often present **(12).** Urinary tract infections (UTIs) occur more often in women than in men, at a ratio of 8:1. Approximately 50–60% of women report at least one UTI in their lifetime, and one in three will have at least one symptomatic UTI necessitating antibiotic treatment by age 24 **(13,14).** Normally, the urinary tract is sterile, but bacteria may rise from the perianal region, possibly leading to UTI. Pathogens in the bladder may stay silent or can cause irritative symptoms like urinary frequency and urgency, and 8% of women may have asymptomatic bacteriuria. If bacteria enter the blood stream, they could cause severe complications, including septicaemia, shock and, rarely, death **(15,16).** The definition of recurrent urinary tract infection (RUTI) is three UTIs with three positive urine cultures during a 12-month period, or two infections during the previous 6 months **(16, 17).**

3.1 Classification of Urinary Tract Infections

UTIs are classified into 6 categories. The first category is an uncomplicated infection; this is when the urinary tract is normal, both structurally and physiologically, and there is no associated disorder that impairs the host defense mechanisms. The second category is an complicated infection; this is when infection occurs within an abnormal urinary tract, such as when there is ureteric obstruction, renal calculi, or vesicoureteric reflux. The third category, an isolated infection, is when it is the first episode of UTI, or the episodes are 6 months apart. Isolated infections affect 25–40% of young females. The fourth category, an *unresolved infection*, is when therapy fails because of bacterial resistance or due to infection by two different bacteria with equally limited susceptibilities. The fifth category, *reinfection*, occurs where there has been no growth after a treated infection, but then the same organism regrows two weeks after therapy,

or when a different microorganism grows during any period of time **(18,19).** This accounts for 95% of RUTIs in women. Bacterial persistence happens when therapy is impaired by the accumulation of bacteria in a location that cannot be reached by antibiotics, such as infected stones, urethral diverticula and infected paraurethral glands. The sixth category, *relapse,* is when the same microorganism causes a UTI within two weeks of therapy; however, it is usually difficult to distinguish a reinfection from a relapse **(20).** Reinfections and relapses are common in women who develop uncomplicated UTI. Understanding the pathogenesis of UTI may lead to better methods of prevention and treatment. There are 2 theories as to cause of recurrence, whether reinfection or relapse. The classic model of pathogenesis is that *E coli* emerge from an intestinal reservoir, colonize the vagina and periurethra, and ascend through the urethra to the bladder. To help shed more light on UTI pathogenesis, Thomas M. Hooton, MD **(21).** (USA) performed a study to identify temporal associations and dynamics between periurethral colonization with *E. coli*, bacteriuria, and recurrent UTI in 100 premenopausal adult women with acute cystitis. These women were followed for 3 months with daily urine and periurethral cultures; daily diary for symptoms, sex, and antibiotic use; and monthly fecal cultures. The *E. coli* strains causing recurrent UTI were identified in the periurethra of at least 75% of the women and in the urine of at least 35% 1 week prior to the onset of a new UTI. Furthermore, the recurrent UTI-causing strain was found in the rectum in 75% of women prior to the new UTI. These patterns overwhelmingly support the classic model of pathogenesis of UTI. A second hypothesis holds that some same strain episodes of recurrent UTI may originate from uropathogens lying dormant in the bladder following a previous UTI. Anthony J. Schaeffer, MD **(22).** (USA) presented new data that suggested some recurrences may be due to relapse from within the urinary tract and they identified bacteriuric pods that sequester bacteria in the deep mucosal layers of the bladder even though the urine shows no growth. In Hooton's study, described above, there were some patterns in which the recurrent UTI-causing strain was found in the urine without being detected in the periurethra just before onset of the new UTI. This is compatible with a bladder source for the recurrent UTI-causing strain. The prevalence of this phenomenon is unknown, but novel therapies should be considered for individuals with this predisposition to recurrent UTI.

3.2 Causes of UTI

Enterobacteriaceae are the most common cause of urinary tract infections (UTIs) in both community and healthcare settings. *Escherichia coli (E.coli)* is the most common uropathogen in both uncomplicated and complicated UTIs worldwide **(23).** Most episodes of UTI are caused by *Escherichia coli* (up to 85%) and Staphylococcus saprophyticus (up to 10%), while Klebsiella pneumoniae and Proteus species account for most of the remaining infections.

3.3 Escherichia Coli

E.coli typically colonizes the gastrointestinal tract of human infants with in few hours after birth. Usually, *E.coli* and its human host coexist in good health and with mutual benefit for decades. These commensal *E. coli* strains rarely cause disease except in immunocompromised hosts or where the normal gastrointestinal barriers are breached-as in peritonitis, for example **(24).**

3.4 Morphology

E.coli is a gram-Negative, straight rod measuring 1-3 ×0.4 -0.7μm arranged singly or in pairs. It is motile by peritrichate flagella, though some strains may be non-motile. Capsules and fimbriae are found in some strains. Spores are not formed **(25).**

PATHOGENESIS

In the debilitated or immunosuppressed host, or when the gastrointestinal barriers are violated, even non-pathogenic commensal strains of *E. coli* can cause infection **(26).** Diabetes in particular is associated with several syndroms of complicated UTI. Including intrarenal and perirenal abscess, emphysematous pyelonephritis and cystitis, papillary necrosis and xanthan granulomatous pyelonephritis **(27).** Some strains of *E. coli* can diverge from their commensal cohorts, taking on a more pathogenic nature. These strains acquire specific virulence factors (via DNA horizontal transfer of transposons, plasmids, bacteriophages, and pathogenicity islands), which confer an increased ability to adapt to new niches and allow the bacteria to increase the ability to cause a broad spectrum of diseases. The Extra-intestinal pathotype *E. coli* (ExPEC) strains have the capacity to exist in the gut normally, however, they can disseminate and colonize the blood, the central nervous system, and the urinary tract, thereby producing disease. ExPEC

strains includes two pathotypes namely, neonatal meningitis *E. coli* (NMEC) and uropathogenic *E. coli* (UPEC) **(28).** Haemolysin production, which is one of the virulence factors, is associated with pathogenic strains of *E. coli* in humans, especially those causing more clinically severe forms of UTI **(28,24).** Most haemolytic *E. coli* strains secrete a cytolytic protein toxin called alpha haemolysin. Approximately a half of UPEC strains causing upper UTIs, and a third causing lower UTIs, produce a haemolysin (HlyA) belonging to the repeat toxins (RTX) family **(24,29,30,31).** More than 80% of all UTIs, including both asymptomatic bacteriuria (ABU) and symptomatic UTIs are caused by UPEC. Pathogenesis of UTI in the women may be linked to close association of urethra with anus. Most of the uropathogens originate in the rectal flora and enter the bladder via the urethra with an interim phase of periurethral and distal urethra colonization. Vaginal acquisition of uropathogens from a women male sexual partner has been reported bur is probably only rarely the underlying cause of UTI. Vaginal colonization is a prerequisite to bladder infection. Factors that increase the risk of UTI generally do so at least in part by facilitating vaginal colonization. Whether subsequent UTI occurs is the results of a dynamic interaction between the host and uropathogens. Symptomatic UTIs develop when uropathogen in the bladder or kidney stimulate cytokine release, resulting in an inflammatory response and symptoms. The large difference in UTI prevalence between men and women is thoughts to results from a variety of factors of factors including the greater distance between the anus (the usual source of uropathogens) and the urethral meatus; the drier environment surrounding the male urethra; the greater length of the male urethra; and the antibacterial activity of prostatic fluid **(31).** Risk factors associated with UTI in healthy men include intercourse with an infected female partner, homosexuality and lack of circumcision, although often none of these factors is present in men with UTI **(32).** Haematogenous seeding of the urinary tract by potential uropathogens such as Staphylococcus aureus is the source of some UTIs. But this is more likely to occur in the setting of persistent bloodstream infection or urinary tract obstruction. The importance of lymphatic spread of uropathogens to the urinary tract in the pathogenesis of UTI is not known. Uropathogen virulence determinants appear to be of much less important in the pathogenesis of complicated UTIs than in uncomplicated UTIs. Infection with multiple drug resistant uropathogens is more likely than with uncomplicated UTI, especially in those infections. Which develop in institutional setting and in patients who require frequent course of antimicrobial agents Certain virulence determine of uropathogen

provide a selective advantage to those strains possessing them with regard to colonization and infection **(33,34).** Paradoxically bacteria causing complicated UTI often lack these virulence factors. While the majority of uropathogens causing uncomplicated UTI, especially pyelonephritis, express such virulence determinants.

Kebira, A.N., Ochola, P. and Khamadi, S.A. **(**1997) carried out a prospective study at Thika District hospital, Kenya for the twelve months on 3,341 patients who presented symptoms of urinary tract infections. Among the cultures screened, bacteriuria of 10^5 per millilitre of urine was found in 831 (24%) of the samples having Escherichia coli, and of this (831) 30% occurred with other uropathogens **(35).**

Ronald et al. in 2001 reported that Urinary tract infections (UTIs) are one of the commonly encountered diseases in developing Countries with an estimated annual global incidence of at least 250 million and Ronald *et al* .in 2002 reported at UTI is extremely common infection in both female and males. Escherichi*a* coli *(E. coli*) is the major aetiological agent in causing UTI, which accounts for up to 90% of cases with other pathogens including *Enterococci, Staphylococcus saprophyticus,* Klebsiella spp., Proteus mirabilis and Pseudomonas **(36,37).**

In 2005 Shirishkumar Patel1, et al at G.G Hospital & Medical College, Jamnagar included in their study 3046 specimens of urine collected from indoor patients. More than one fourth (27.92%) samples were of male and near about two third (64.75%) of the female patients had positive test result. Overall positivity of growth in UTI infection was 46.48% and among the organisms they reported maximum percentage of Escherichia coli (53.38%) responsible for causing UTI **(38).**

In 2008 De Backer D, Christian's T, Heytens S, de Sutter A, Stobberingh EE, Verschraegen G from China reported that although UTIs occur in both men and women, clinical studies suggest that the overall prevalence of UTI is higher in women. Uncomplicated UTIs in healthy women have an incidence of 50/1000/year **(39).**

Jayanta Debnath, PradipKr. Das, Munmun Debnath and K.K. Haldar conducted a study for a period of two years, from June 2011 to May 2013, at Dr. B.R. Ambedkar Memorial Teaching Hospital, a tertiary health care centre in the state of Tripura and they processed, 2396 urine samples out of which1084 (45.2%) yielded significant bacterial isolates. The predominant isolate was *Escherichia coli* (43.9%) followed by Enterococcus faecalis (21.4%), *Klebsiella*

pneumoniae (11.8%), Staphylococcus aureus (10.3%) and others. Females were more affected than males (3.1:1), with majority of infections in the age group of 31-45 years (36%) followed by 16-30 years (29.3%) **(40).**

Das Niladri Sekharand and Pal Kuhu carried out one year study in the department of Microbiology, College of Medicine and JNM Hospital kalyani, west-Bengal during March 2011 to February 2012. This was an analysis of data generated from the records of consecutive urine samples received in the laboratory from hospital's indoor and outdoor during the study period and reported data. A total of 1092 consecutive urine samples were included in the study. Out of these, 731 (67%) were sterile, 181 (16.6%) showed significant growth, 22 (2%) showed insignificant growth and 158 (14.5%) were found to be contaminated. Out of the 181 culture positives, we isolated 65.75% gram negative (n=119) and 34.25% gram positive (n=62) bacteria. *Escherichia coli* was the most predominant (54%) isolate followed by Enterococcus species (15%), Staphylococcus aureus (10%), *Klebsiella* species (10%), *CONS* (8%), pseudomonas species (1%), Proteus species and Acinetobacter species (1%) **(41).**

Niranjan V. and Malini A. (2012) conducted the hospital record based cross-sectional study at a 750 bedded tertiary care centre located in Pudu cherry, south India. The analysis was done on all *E. coli* isolates obtained from urine samples among hospital inpatients, admitted during the period of August 2011 to July 2012. All the inpatients with UTI at the time of admission or acquired during their stay in the hospital were included in the study. A total of 2941 urine samples were received for culture and sensitivity during the study period. Among these, 547 samples (18.5%) yielded significant bacteriuria; 2323 samples (79.1%) showed no growth and 74 samples (2.4%) showed mixed growth. The various organisms isolated from urine culture are shown in Table I. *E. coli* was the commonest accounting for 56.8 per cent of the uropathogens of the 311 *E. coli* isolates, 119 (38.2%) were isolated from 119 inpatients (age range on 6 months-80 year) **(42).**

R Mahajan, S Gupta and Bella Mahajan (2014) carried out the study in the Department of Microbiology, Govt. Medical College, Jammu for a period of one year to know the etiological agents of UTIs and their antibio grams. *Esherichia coli* is the commonest isolate. The urine samples of 1644 patients were received during the above mentioned period. The age of patients included in the study ranged from 1 to 60 years. The total number of urine samples that showed significant growth was 300 (18.2%). Of these,182 (83.3%) were from outpatients and 118

(16.6%) from inpatient. These results indicated that the prevalence of UTI was higher in female patients than in males as 180 (60%) samples from females and 120 (40%) from males showed significant bacteriuria. The highest susceptible age group of patients to UTI was 40 years (32.33%) followed by 20-30 years (24%),30-40 years (22.3%),50-60years(10.66%) and 10- 20 years (7.3%), and Comparatively, however, more cases of UTI were observed in females than in males in all age groups.

The highest prevalence of UTI in females was found in the age group of 20-30 years (90.69%); however in males the highest susceptible age group to UTI was 40 years (71.15%). *Escherichia coli* was found the dominant bacteria among all isolated uropathogens with the prevalence rate of 60%. The second most prevalent isolate was Staphylococcus aureus (14%) followed by budding yeast (7.3%), *Klebsiella pneumoniae* (6.6%) *Enterococcus* (4.6%), *Citrobacter* (2.6%), *Proteus spp*. (2.6%) and *Pseudomonas aeruginosa* (2%) **(43).**

A Laghawe1, Apoorva Tripathi and S B Saxena (2015) reported that they had conducted a retrospective study at Chirayu Medical College and Hospital, Bhopal, MP state in Central India and analyzed data for years 2012 and 2013. Total 456 organisms were isolated during this period. Out of these isolates, 278 were from different wards, 103 were from ICU and 75 were from OPD patients. Out of these isolates, 214 (47%) were *E. coli*, followed by *Kleb. pneumoniae* 70 (15%) and *Enterobacter spp*. 46 (10%) **(44).**

CHAPTER-4: OBJECTIVE

The aim of this study was to investigate microbial species isolated from indoor patients with UTI and evaluate *E. coli* prevalence and antibiotic susceptibility pattern in a tertiary care hospital

CHAPTER-5: MATERIALS AND METHODS

Study Design and Criteria

A prospective study of four months (1st January 2016 to 31st April 2016) was done on the urine samples received in the department of microbiology at Punjab Institute of Medical Sciences (PIMS), Jalandhar from indoor patients having symptoms of urinary tract infection, irrespective of their co-morbidities. Samples were collected from In Patient Departments (IPD) which included gynaecology, surgery, medicine, emergency, paediatrics, ICU wards. Total 400 samples were processed during this period.

4.1 Sample Collection and Analysis

Urine was collected by midstream "clean catch" method and from catheters of the catheterized patients. Urine sample were processed within one hour of collection. Microscopic examination of urine sample was done. The samples were inoculated on Mac Conkey's agar by semi-quantitative method with calibrated loop (internal diameter 4 mm) and incubated at 37˚ C for 18-24 hours and significant bacteriuria was looked for. Growth of Gram-negative bacteria > 10^5 cfu/ml, while for gram-positive bacteria, growth of 10^3 – 10^5 cfu/ml was considered significant. The antibiotic sensitivity testing of *E. coli* isolates were done on Muller Hinton Agar media and results were recorded.

4.2 Mac Conkey Agar

Composition:

Peptone	17 g
Lactose	10g
Bile Salt	1.5
Sodium chloride	5g
Neutral red	0.03g
Agar	13.5

Water –add to make 1 litre;

pH adjusting to 7.1 $\pm$0.2

Test Procedure

Sample was inoculated directly on the surface of a Mac Conkey agar plate by standardized loop with 4 mm diameter to pick up 0.004 ml of urine. The inoculated plate was placed aerobically at 35-37˚ C. Growth was examined after 18-24 hours of incubation. The observed growth was identified by colony characters, Gram's staining and biochemical reactions by standard methods (CLSI, 2008) **(45).**

Interpretation:

Pink colour Colonies of *Escherichia coli* bacilli seen.

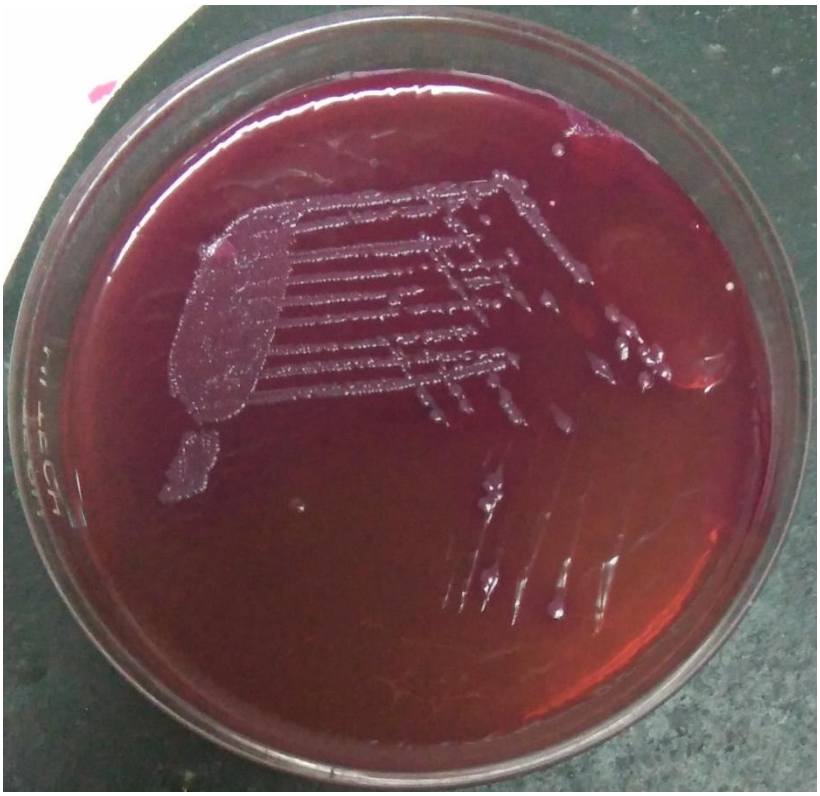


Figure 1: Showing growth of *E.coli* on Mac Conkey agar

MICROSCOPY

WET MOUNT: Urine wet mount was performed to look for bacteria along with pus cells, RBC`S and casts.

Identification by Gram staining

Preparation of smear-

Smear was prepared on a clean and dry glass slide. Drop of normal saline was taken on the slide, then colony was touched with the help of sterilized loop and mix with the normal saline on the Slide and the smear was prepared and fixed.

Gram Staining

PRINCIPLE

Gram staining is a differential staining and use to differentiate between two large groups of bacteria (Gram positive and gram negative). It is based on the chemical and physical properties of their cell wall.

Method of Gram staining

Procedure of Gram staining

1. Slide was placed on rack so that smear was facing upwards.
2. Crystal violet was poured over the smear for 1 min.
3. Gently washed with tap water.
4. Covered with Gram`s iodine and allowed to stand for 1Min.
5. Gently washed with tap water.
6. Decolorize the smear with acetone and smear was washed.
7. Then counter stain, diluted saffrainine was added for one min.

8. The smear was washed, dried and observed under oil immersion objective (100x) microscopically

Observation

Pink coloured *Escherichia coli* was seen.

4.3 Biochemical test for the identification of E. coli

Biochemical tests used to identify Escherichia coli are catalase test, oxidase test, urease test, citrate test, Indole test, TSI test, Motility test, MR Test, VP Test etc. Some of the widely used biochemical tests are described as follow.

SUGAR FERMENTATION

PRINCIPLE

To determine the ability of an organism to ferment a specific carbohydrate (sugar) incorporated in a medium producing acid or acid with gas.

PROCEDURE

Test organism is inoculated in a sugar medium containing 1% sugar and indicator (Andrade`s indicator) and incubated at 37˚ C for 18 to 24 hrs. Glucose, Sucrose, lactose and Mannitol are widely used sugar

INTERPRETATION OF *E.COLI*

All sugars were fermented with production of acid and gas. Acid production was seen by change in colour of sugar media from pink to yellow and gas production was seen as bubbles in Durham`s tube.

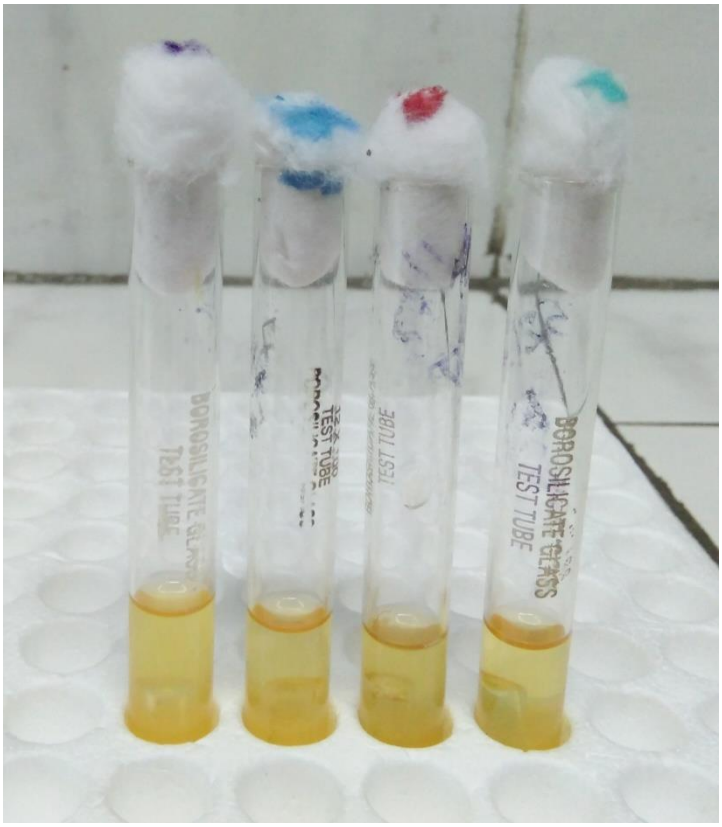


Figure2: Showing the sugar fermenters results of *E.coli*

2. INDOLE PRODUCTION

Principle:

To determine the ability of an organism to decompose amino acid tryptophan into Indole. Tryptophan is decomposed by an enzyme tryptophan produced by certain bacteria.

PROCEDURE

Indole production is detected by inoculating the test bacterium into peptone water (tryptophan rich) and incubating it at 37˚c for 18-24 hrs.0.5/ Kovac`s regents is added to the bacterial growth and gently shaken.

INTERPRETATION OF *E.COLI*

E. coli is Indole positive.

A red coloured ring near the surface of the medium (example Escherichia coli.)

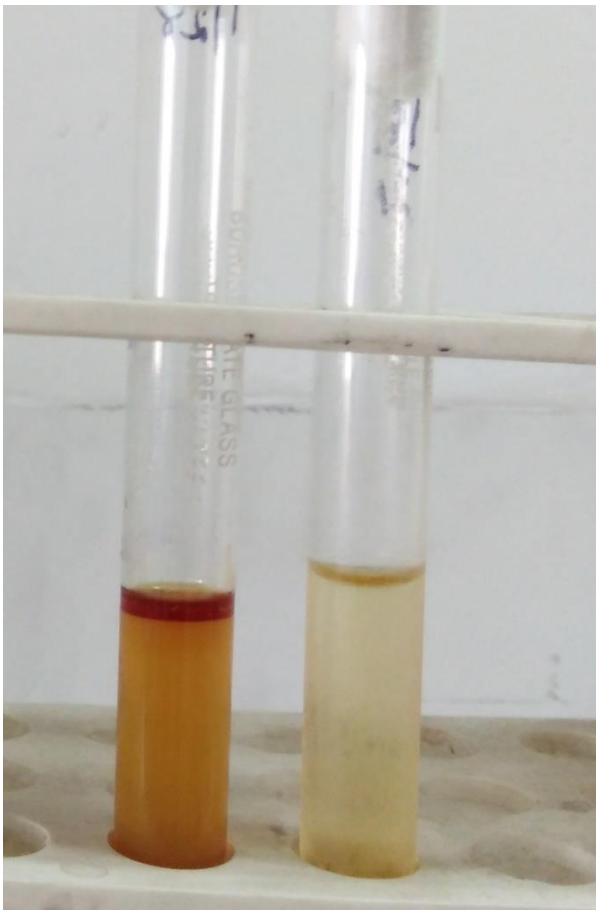


Figure3: Showing the Indole positive a red colour ring testing results of *E.coli*

1. UREASE PRODUCTION

Principle

TO determine the ability of an organism to produce an enzyme urease, this splits urea to ammonia. Ammonia makes the medium alkaline and thus phenol red indicator changes to pink/ red in colour.

Procedure:

The test is done in Christensen`s urease medium. The test organism is inoculated in the entire slope of the medium and incubated at 37˚c. It is examined after four hours and after overnight incubation **(45).**

INTERPRETATION OF *E.COLI*

Urease test is negative (pale yellow colour) in *E. coli*

Figure4: Showing the urease testing results of pale colour in *E.coli*

CITRATE UTILISATION TEST:

Principle:

Citrate agar is used to test an organism`s ability to utilize citrate as a source of energy. The medium contains citrate as the sole carbon source and inorganic ammonium salts as the sole source of nitrogen. Bacteria that can grow on this medium produce an enzyme, citrate- permease, capable of converting citrate to pyruvate. Pyruvate can then enter the organism`s metabolic cycle for the production of energy. Growth is indicative of utilization of citrate an intermediate metabolite in the Krebs cycle. When the bacteria metabolize citrate; the ammonium salts are broken down to ammonia, which increases alkalinity. The shift in pH turns the bromothymol blue indicator in the medium from green to blue above pH 7.6.

PROCEDURE:

1. Streak the slant back and forth with a light inoculum picked from the canter of a well isolated colony.

2. Incubate aerobically at 35 to 37c for up to 4-7 days.

3. Observe a colour change from green to blue along the slant **(46).**

INTERPRETATION OF *E.COLI*:

Negative test

No growth and no colour change occur in *E. coli*

Figure5: Showing the no growth citrate testing results of *E.coli*

METHYL RED (MR) TEST

Principle:

This test **detects** the production of sufficient acid during fermentation of glucose by bacteria and sustained maintenance of pH below 4.5.

PROCEDURE:

The test organism is inoculated in glucose phosphate broth and incubated at 37˚c for 2-5 days. Then add five drops of 0.004/ solution of methyl redmix well and read the results immediately.

INTERPRETATION OF *E.COLI*:

1. **Positive –** Red colour in *E.coli* immediately

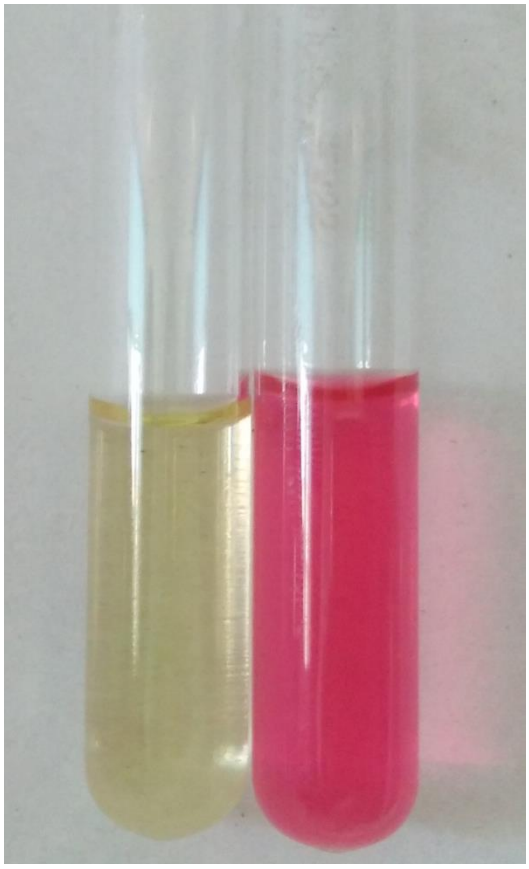


Figure6: Showing the MR testing results of *E.coli*

VOGES-PROSKAUER (VP) TEST OR ACETOIN PRODUCTION TEST:

Principle:

This test depends upon the production of carbinol (acetone) from pyruvic acid in the media. In the presence of alkali and atmospheric oxygen, acetone is oxidized to diacetyl. Which react with α- naphthol to give red colour.

PROCEDURE:

Test organism is inoculated in glucose phosphate broth and incubated at 37˚c for 18 t0 24 hours. Then add 1 ml of 40% KOH and 3 ml of a 50% solution of α- naphthol in absolute alcohol.

INTREPRETATION OF *E.COLI:*

1. **Positive – pink colour** with 2-5 minutes, deepening to crimson colour in 30 min.
2. **Negative- colourless for 30 mints**

TRIPLE SUGAR IRON (TSI):

PRINCIPLE:

To determine the ability of an organism to attack specific carbohydrate in a growth medium with or without the production of gas, alone with the determination of possible hydrogen sulphide production. Triple sugar iron medium is a differential medium that can distinguish between a number of gram negative enteric bacteria based on their physiological ability (or lack thereof) to:

1. Metabolise lactose and/ or sucrose.
2. Conduct fermentation to produce acid.
3. Produce gas during fermentation.
4. Generate H_2S.

The medium contains 1.0% each of sucrose and lactose and 0.1% glucose. If only glucose is fermented, acid produced in the butt will turn it yellow, but insufficient acid products. **(45)**

Figure7: Showing the TSI testing results of *E.coli*

Motility Test:

E. coli is a motile bacillus and it is visible in semisolid agar as shown above.

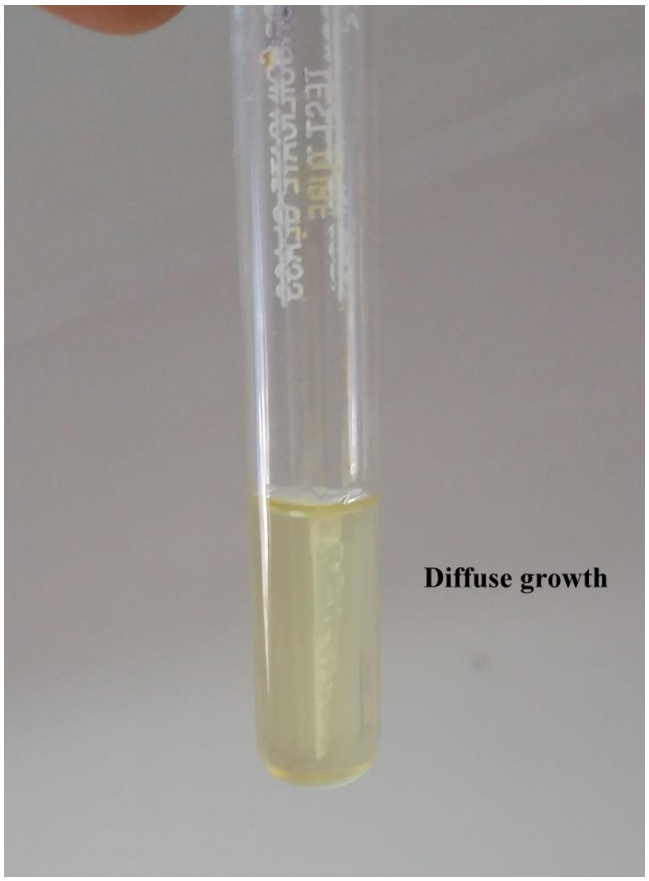


Figure No 8: Showing motility testing results of *E.coli*

2. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility of *E. coli* was tested by the disk diffusion method according to the CLSI recommendations, using the Mueller-Hinton agar. Antimicrobial agents tested were Nitrofurantion, Norfloxacin, Ciprofloxacin, Gentamicin, Netilimicin, cefuroxime, Cefpodoxime, ceftriaxone, ceftazidime, Cefipime, Polymyxin B, Piperacillin+tazobactam, Imipenem, Meropenem, Colistin, Cefoperozone, Ciprofloxacin, Amoxyclav, Amikacin, Cefoperazone + sulbactum, Ceftazidime + Clavulanic Acid,. The diameter of the zone of inhibition each antibiotic disk was measured using engineer calipers. The result was interpreted as susceptible or resistance to the antibiotic agent used, depending on the length of zone of inhibition produced compared to reported standard length [23].

Figure 9: Showing antimicrobial susceptibility testing results of *E.coli*

CHAPTER-6: RESULTS

Four hundred urine samples of patients having symptoms of UTI were screened. Out of these 306 samples were of female patients and 94 urine samples were of male patients. The age and sex wise distribution of patients having symptoms of UTI is shown in table and chart number I. The significant growth of bacteria was obtained in 76(19%) samples. Sex wise Distribution of the Cases according to their test results (n=76) is shown in table and chart number II

The details of organisms are shown in table and chart number III. Out 76 samples *Escherichia coli* was obtained in 46(60.52%) samples. The percentage of Escherichia coli infection in females patients was 83.33% (35) and in male's patients was 16.67% (7). The age and sex wise distribution of patients having *Escherichia coli* infection is shown in table and chart number IV.Susceptibility rate of *E. coli* to Nitrofurantion, Aminoglycosides, Fluroquinolones, Cephalosporin's, Carbapenem and β lactam inhibitors combination is shown in table number V and Pie diagram I.

Table Number 1

The age and sex wise distribution of patients having symptoms of UTI

AGE	FEMALE	PERCENTAGE	MALE	PERCENTAGE
1-10	4	1.30	0	0
11-20	20	6.53	1	1.06
21-30	96	31.38	4	4.25
31-40	104	34.00	10	10.64
41-50	38	12.41	12	12.77
51-60	26	8.50	28	29.79
>60	18	5.88	39	41.49
TOTAL	306	100	94	100

Chart number 1

The age and sex wise distribution of patients having symptoms of UTI

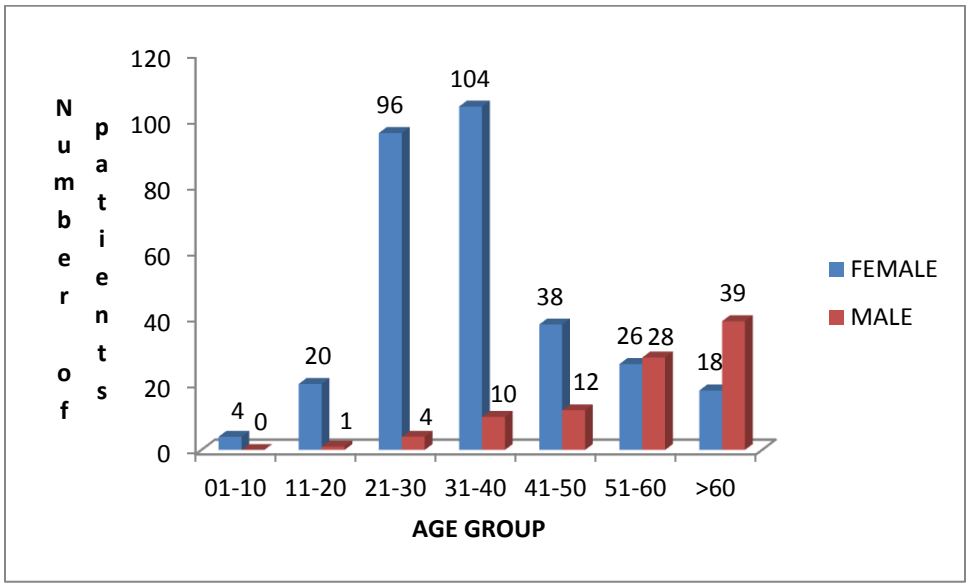


Table 2

Sex wise Distribution of the Cases according to their test results (n=76)

sex	Total cases	Positive cases
Male	94	8(8.51%)
Female	306	68(22.22%)
Total	400	76(19%)

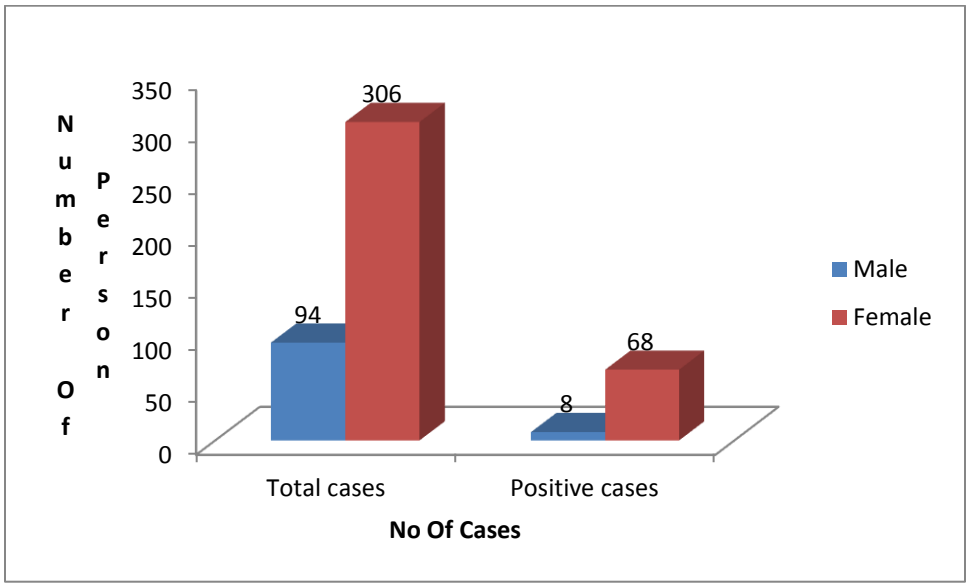


Table 3

The distribution and percentage of organisms isolated

ORGANISMS	NUMBER	PERCENTAGE
Escherichia coli	46	60.52
Klebsiella spp.	5	6.58
Proteus spp.	3	3.94
Pseudomonas aeruginosa	5	6.58
Acinetobacter spp.	4	5.27
Staphylococcus aureus	7	9.21
Enterococcus spp.	4	5.27
Candida spp.	2	2.63
Total	76	100

Chart 3

The distribution and percentage of organisms isolated

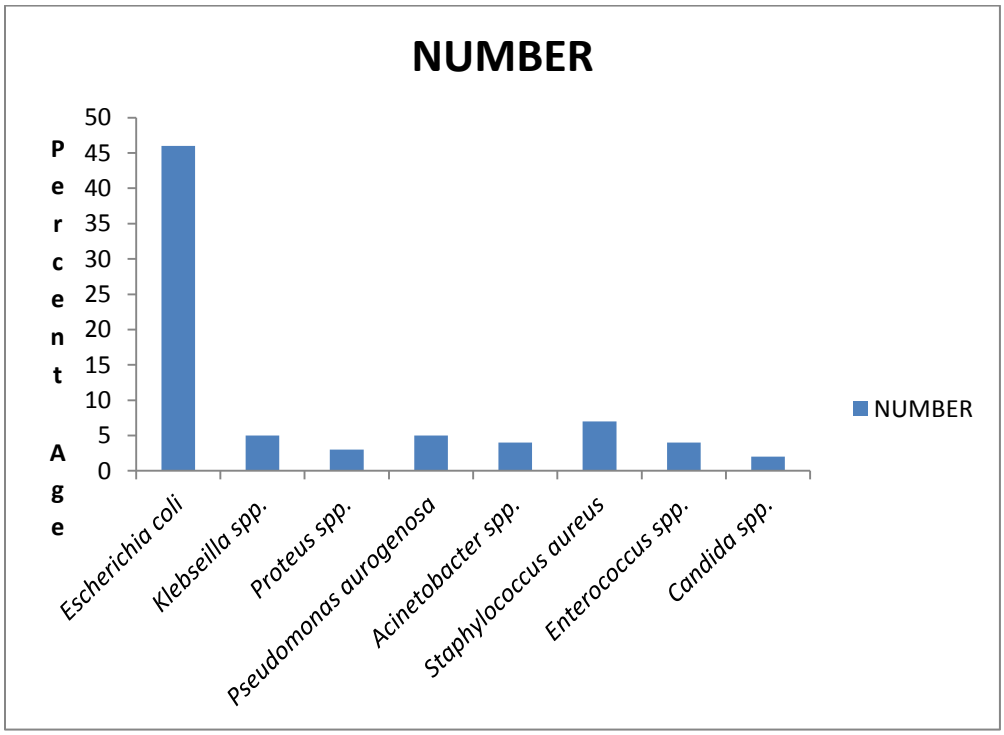


Table 4

The age and sex wise distribution of patients having Escherichia coli infection

AGE IN YEARS	FEMALES	PERCENTAGE	MALES	PERCENTAGE
1-10	0	Nil	Nil	Nil
11-20	1	2.56	Nil	Nil
21-30	4	10.25	Nil	Nil
31-40	5	17.95	1	14.28
41-50	9	28.21	1	14.28
51-60	9	23.08	2	28.58
>60	7	17.95	3	42.86
Total	35	100	07	100

Chart 4

The age and sex wise distribution of patients having *Escherichia coli* infection

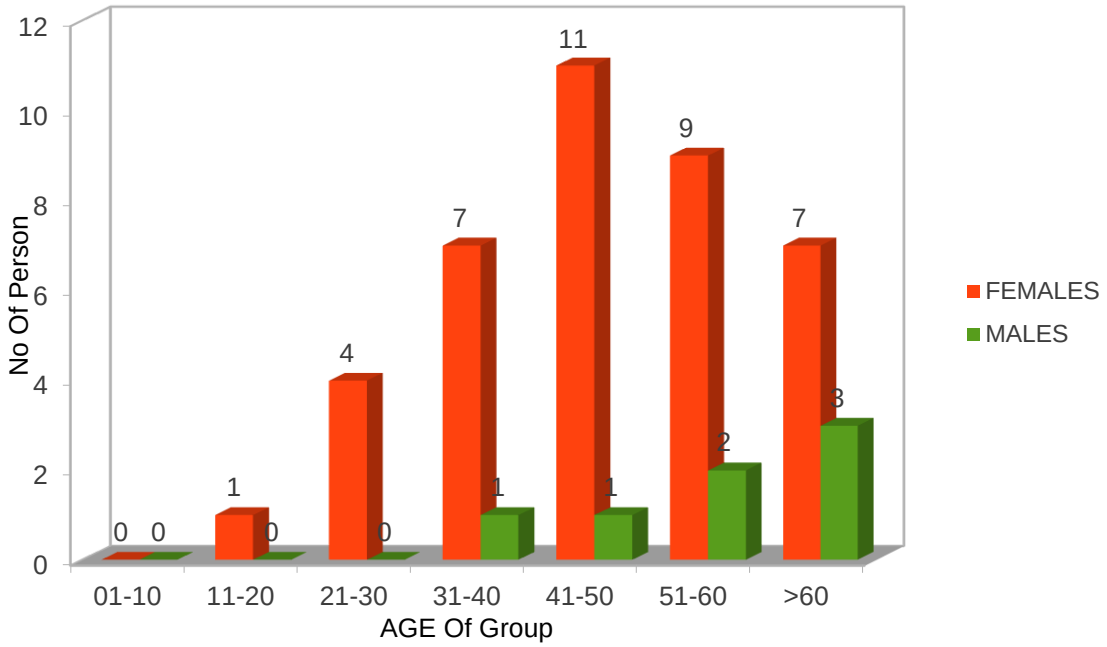


Table number 5

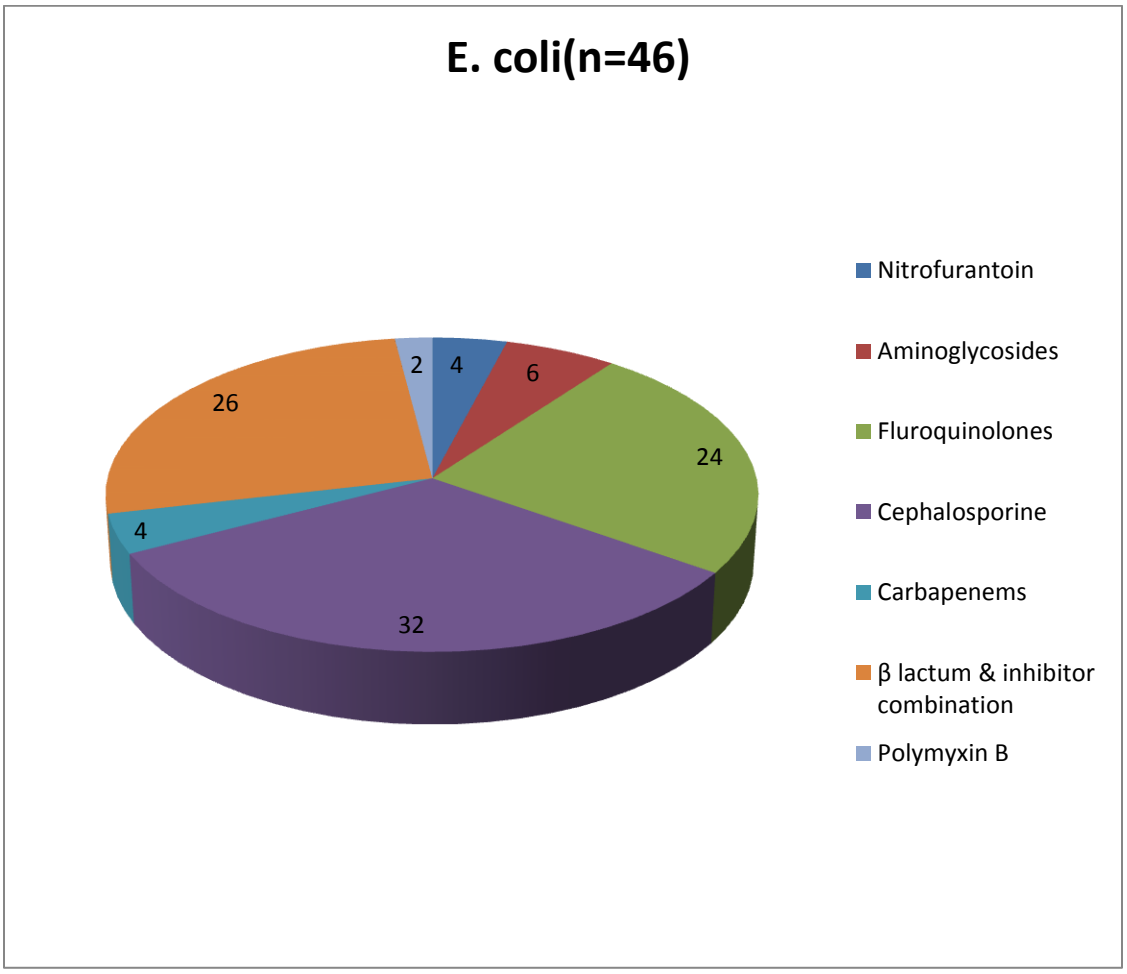
Antibiotic Sensitivity Pattern of *Escherichia coli* isolate (% resistance)

Antimicrobial agents	*E. coli*(n=46)	Percentage
Nitrofurantion	4	8.69
Aminoglycosides	6	13.04
Fluroquinolones	24	52.17
Cephalosporin	32	69.56
Carbapenem	4	8.69
B lactam & inhibitor combination	26	56.52
Polymyxin B	2	4.34

* Aminoglycosides (Gen,Ak,Net), Fluroquinolones (Nx,CIP), Cephalosporin (CXM,CFM,CAZ,CPD,CFR,CPZ,CPM,CTX,Cx,CTR), Carbapenems(IPM,MRP),β lactum & inhibitor combination (AMC,CFS,CAC,PIT)

Pie diagram number I

Antibiotic Sensitivity Pattern of Escherichia coli (46)



CHAPTER-7: DISCUSSION

Urinary tract infections (UTI) are one of the most common bacterial infections **(24).** The spectrum of UTI varies from asymptomatic bacteriuria to symptomatic cystitis, pyelonephritis and blood stream infections. In India prevalence of uropathogens ranges from 10.86% to 45.32% **(47,48).** The most frequent pathogen causing UTI among Gram-negative bacteria is *E. coli*. The present study included 400 specimens of urine collected from indoor patients. Out of these 96 urine samples were of male patients and 306 were of female patients. The prevalence of UTI was found to be 19%(76) in this study which correlates with the studies done in, Jammu18.2% and Aligarh 17.19 % **(48,49).**This figure is lower than prevalence rate of 31.35% and 66.78% recorded by Savitha, T et al and Mahesh E et al **(50,51).** The high prevalence may be due to genuine population susceptibility because factors like sexual intercourse, peer group influence, pregnancy, low socio-economic status.

In our study group, 8.51% male and 22.22% female urine samples had positive results. Similar study was done by other workers and they reported more than one fourth (27.92%) male and near about two third (64.75%) female who had positive test result **(38).** Our study showed a high prevalence of UTI in females than in males which correlates with other studies which revealed that the frequency of UTI is greater in females as compared to males (**41).**

The reason behind this high prevalence of UTI in females is due to close proximity of the urethral meatus to the anus, shorter urethra, sexual intercourse, incontinence and bad toilet **(52).** The most common organism isolated in our study population was *E. coli* (60.52%), Klebsiella (6.58%) *P. aeruginosa* (6.58%), *Proteus spp*. (3.94%), and *S. aureus* (9.21%). This findings pattern were similar with study of Shirishkumar Patel et al. like *E. coli* (53.38%), *Klebsiella* (18.92%) *P. aeruginosa* (10.74%), *P. mirabilis* (5.38%), and *S. aureus* (7.2%) **(38).** In study of Mahesh El et al. organisms isolated was *E. coli* (65.7%),%), Klebsiella (15.9%), P. aeruginosa (11.14%) **(51).**

In our study amongst the 76 isolates, *E.coli* was most common isolates (n=46) (60.52%).

Other workers also reported E. coli the most common isolates in their studies (65.7%, 54%, 53.38%) respectively (**51,41,38).** In our study range of patients age was between 1 to =/>60 years. Among them significant numbers of *E.coli* were isolated from age group of 21 - 50 years and above. Similar study was done by JignaNaik and Pretibial Desai. Numbers of *E.coli* were

isolated from age group of 15 - 50 years and above **(53).** In the our study the most common organism causing UTI in women between 21 – =/>60 year of age was *E coli,* which is in accordance with study carried out by the Annabelle *et al;*1999 **(54).** The more isolates were recovered from females (n=39) as compared to males (n=7). It is stated that UTI is predominantly a disease of the females due to a short urethra and proximity to anal opening. In our study too there was a female preponderance for this infection. Present findings were also in agreement with the findings of the Olaf son M *et al,* 2000 **a**nd Gupta *et al,* 1999; they found *E. coli* as the most common isolates in females in their study on uropathogens **(55, 56).** In our study *E. coli isolates,* 43(94%) were multi drug resistant whereas **Niranjan V. et al.** reported it to be 76.51% in their study **(46).** The isolates in our study showed high levels of resistance to sFluroquinolones (52.17%), Cephalosporin (69.56%), β lactam and inhibitor combination (56.62%) where as other workers reported high levels of resistance to ampicillin (88.4%), amoxicillin-Clavulanic acid (74.4%), Norfloxacin (74.2%), cefuroxime (72.2%), ceftriaxone (71.4%) and co-trimoxazole (64.2%) **(46).**The isolates were sensitive to Polymyxin B (95.66%), Carbapenem (91.31%), Nitrofurantion (91.31%) and 86.46% to Aminoglycosides where as other workers reported susceptibility to Amikacin (82.6%), piperacillin-tazobactum (78.2%), Nitrofurantion (82.1%) and Imipenem (98.9%) **(46).** Similar study was done by and they also reported resistant *E. coli* isolates, Ciprofloxacin (64%), Aminoglycosides (46.75%), Cephalosporin (58.9%), Nitrofurantion (17.5%) and Imipenem (9.3%) in their study.

Limitations of the study include that some of the isolates included in the analysis may have been associated with asymptomatic bacteriuria rather than true UTI. While we used standard surveillance criteria to define UTI. The results of this study and those of others may not be representative of the general population; urinary tract infections are often treated empirically and culture and susceptibility tests are often carried out only when the patient has failed one or more courses of antibiotics.

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CHAPTER-8: CONCLUSION

For optimal interpretation of cumulative susceptibility data in the primary healthcare setting, it is necessary to take into account the type of UTI (uncomplicated vs. complicated), as well as the sex and age of each patient. Based on the findings of this study, it is concluded that acute uncomplicated UTI affects a large proportion of the population in the study area, and is more prevalent in females. As reported previously by other workers **(55,56)** also, our study confirmed *Escherichia coli* to be a major uropathogen. Regularly updated surveillance of local microbial prevalence and resistance patterns are needed to guide the empiric therapy for UTIs.

CHAPTER-9: LIST OF APPENDICES: 1

A. LIST OF MATERIALS

1. Equipment

Autoclave

Bunsen burner

Incubator

Microscope

Refrigerator

Weighing machine.

2. Glass wares:

Petri plates

Test tube

Slides

Conical flask

Cover slips

3. OTHERS

Cottons

Forceps

CHAPTER-10: Bibligraphy

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