# PREVELANCE OF GRAM NEGATIVE BACTERIA IN URINARY TRACT INFECTION AND THEIR ANTIBIOTIC SENSITIVITY PATTERN TO NEWER ANTIBIOTICS

**Internship Training Report**

**Submitted to**

**Lovely Professional University, Punjab**

**in partial fulfilment of the requirements**

**For the degree of**

**Master of Science in Clinical Microbiology**

**Submitted by:**
**Jiban dip paul**
**(Reg. No. 11402712)**

**SCHOOL OF PHYSIOTHERAPY AND PARAMEDICAL SCIENCES**
**LOVELY PROFESSIONAL UNIVERSITY, PUNJAB, INDIA**
**May, 2016**

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**May, 2016**

# DECLARATION

I hereby declare that the work embodied in this internship report was carried by me under the supervision of Dr. Saurabh Saxena (Internal supervisor), Lovely Professional University and Dr. Renuka Bajaj (External supervisor), SRL Ltd. Fortis Escort Hospital, Amritsar. This work has not been submitted in part or in full in any other university for any degree or diploma.

Name: JIban dip paul
Date: 04- 05- 2016
Place: Lovely Professional University

## CERTIFICATE

This is to certify that Jiban dip paul (registration no.- 11402712) student of M.Sc. Clinical Microbiology (course code- 240A) in Lovely Professional University, Phagwara, Punjab has actively worked in a practical training from 1st January, 2016 to 30th April, 2016 in SRL Ltd. Fortis Escort Hospital, Amritsar, Punjab under supervision and proper guidance He has worked in microbiology department with full responsibility. We wish him good luck and success in future endeavor.

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# TO WHOM SO EVER IT MAY CONCERN

This is to certify that Mr. Jibandip Paul (Registration No- 11402712) S/O Sh. Sadhan Paul student of M.Sc Clinical Microbiology-2nd Year (Semester-4) from Lovely Professional University, Phagwara– 144402 has undergone 4 months of internship programme in SRL Limited., I/S Fortis Escorts Hospital, Amritsar from 01st January 2016 to 30th April 2016 as a part of his course curriculum.

He has worked as an observer in the field of Microbiology. I wish him success in all his future endeavors.

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# ABSTRACT

Urinary tract infection is one of the most common bacterial infections seen in clinical practice particularly in developing countries. The causative agents for urinary infection vary from place to place and they also vary in their susceptibility and resistance pattern. The objective was to assess the distribution of urinary tract bacterial pathogens with respect to age and gender and to determine the antibiotic susceptibility pattern of isolates. Single positive culture with a colony count of >105 CFU on CLED medium were identified and their antibiotic sensitivity and resistance pattern is represents in the form of antibiogram.This study is done in SRL limited in Amritsar. A total no. of 675 urine specimen suspected from patient and the specimen were tested microbiologically and antimicrobial susceptibility test were performed for isolation pathogen using of Vitec 2 compact.

More than half (53.79%) cases reported as *E.coli* followed by *Klebsiella* (8.23%), *Pseudomonas aeruginosa* (2.46%), *Proteus merabilis* (0.82%).

Higher prevalence of UTI was seen in males. A total no of 124(51.02%) positive cases in 388 specimens and 119 (48.97%) positive cases in 287 specimens was found.

Urinary pathogen showed more sensitivity in Colistin (100%), Imipenem (93.47%), and Meropenem (93.47%).

# ACKNOWLEDGEMENTS

I express my profound sense of gratitude to my project supervisor, **Dr. Saurabh Saxena** (Assistant professor, department of paramedical sciences), for his keen interest, much needed help and guidance throughout the course of this project work. His encouraging attitude reinforced my interest and inspired me throughout the project work. He was always with me when I needed a right decision. His words always help me to find myself when I was in doubt.

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The support of family and friends are worth mentioning. I am thankful to my father, mother and sisters; without whose living involvement, support and blessings, this project work would not have been possible.

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Most of all, I thank the Almighty for blessing me with the strength, light and wisdom to pursue my work.

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# CHAPTER- 1

# INTRODUCTION

## 1.1. URINARY TRACT INFECTION

Urinary tract infection including cystitis and pyelonephritis are the most common infection after kidney transplantation. Study by Fiorante and colleagues came with the findings that 50% patient of the kidney transplant had bacteriuria (1). Resistant to one of the most widely used antibacterial medicines for the treatment of urinary tract infections caused by E. coli–fluoroquinolones–is very widespread (2). In the 1980s, when these drugs were first introduced, resistance was virtually zero. But nowadays in half of the patient drugs are ineffective. *Escherichia coli* is one of the most common pathogen associated with urinary tract infection. In recent years, ESBL producing *E. coli* has been emerged as a potent pathogen and it is also associated with community-acquired infections. It is very essential to control the nosocomial spread of ESBL producing *E.coli*, and other pathogens to limit the rate of urinary tract infections. So newer antibiotics are now implemented in the treatment of UTI.

### 1.1.1. ANATOMY

The urinary tract consists of the kidneys, ureters, bladder and urethra. Often urinary tract infection is characterized as being either upper or lower based primarily on the anatomic location of the infection: the lower urinary tract encompasses the bladder and urethra, and the upper urinary tract encompasses the ureters and kidney.

The anatomy of the female urethra is of particular importance to the pathogenesis of UTIs. The4 female urethra is relatively short compared with the male urethra and also lies in close proximity to the warm, moist, which is teeming with microorganisms. Because of the shorter urethra, bacteria can reach the bladder more easily in the female host.

### 1.1.2. EPIDEMIOLOGY

UTIs are among the most common bacterial infections that lead patient to seek medical care. It has been estimated that more than 6 million outpatient visits and 300,000 hospitals stays every year due to UTIs (3, 4, 5). Approximately 10% human will have UTIs at some time during their lives .The exact prevalence of UTIs is the age and sex dependent. Among the males is low after age 1 and until approximately age of 60 and among the girl age 5 through 14 is 1% to 2%. This incidence increases gradually with time to as high to 5% in girls over age of 10.10-20% increase in elderly women. In women between ages of 20 to 40 who had UTIs, as many as 50% may become re- infected within 1 year.

### 1.1.3 ETIOLOGICAL AGENT:

Escherichia coli are by far the most frequent cause of uncomplicated community-acquired UTIs. Other bacteria frequently isolated from patient with UTIs are Klebsiella spp., other Enterobacteriaceae and *Staphylococcus saprophyticus*. In more

complicated UTIs particularly in recurrent infections, the relative frequency of infection caused by Proteus, Pseudomonas, Klebsiella, and Enterobacter spp. Increase. Other less frequently isolated agent are other Gram negative bacilli, such as Acinetobacter and *Alcaligenes spp.Citrobacter spp.* and beta- haemolysis Streptococci.

## 1.1.4 MICROFLORA OF THE URETHERA:

1Lactobacilli.
2Coagulase negative Staphylococci.
3Diptheroids.
4Non-pathogenic Neisseria spp
5Anaerobic cocci
6Commensal Mycoplasma spp

The Infection involves interaction between the animal body (host) and the infection microorganism. A urinary tract infection is a condition in which one or more parts of the urinary system (kidney, bladder, ureters, urethra) become infected. Urinary tract infection is the most common of all bacterial infections and can occur at any time in the life of an individual. Nearly 95%of cases of urinary tract infection are caused by bacteria that typically multiply at the opening of the urethra and travel up to the bladder. Much less frequency, bacteria spread to the kidney from from the bloodstream . The male and female urinary tracts are similar expect for the length of urethra. The urinary system helps maintain proper water and salt balance throughout the body and also ejects urine from the body.

The ascending route is through faecal flora spreading to the premium and from there ascend in to the bladder. Urinary tract infection may be defined as the presence of bacteria undergoing multiplication in urine within the urinary drainage system. A count 10^5 organism/ml of urine denotes significant bacteriuria and indicate active urinary tract infection. Contamination account for less than 10^4 organism and usually less than 10/ml. Urinary tract infection is generally caused by one species, while contaminants are generally of mixed spices.

# CHAPTER- 2

# AIM AND OBJECTIVES

## 2.1 AIM

To determine the prevalence of Gram- negative bacteria in urinary tract infection and their sensitivity pattern to newer antibiotics.

## 2.2 OBJECTIVES

### 2.2.1 GENERAL OBJECTIVES

- To estimate the burden of urinary tract infection in patients admitted to hospital
- To evaluate the effect of antibiotics on different isolated organisms.

### 2.2.2 SPECIFIC OBJECTIVES

- To isolate and identify the uropathogens from urine specimen.
- To evaluate the effect of antibiotics on different organisms

# CHAPTER- 3
# LITERATURE REVIEW

Aghdam (2013**)** reported that urinary tract infections second to respiratory infections founds the highest statistics of infections among patients and has ranked first in terms of adult patients visits with doctor. In infectious diseases such as urinary tract infections, doctors often have to treat the infection before definitive identification of infectious agents and susceptibility testing to antibiotics; therefore, they have to have sufficient information in the context of infections and antibiotic susceptibility. The aim of this study was to determine the prevalence of bacterial agents of urinary tract infections and their antibiotic susceptibility pattern in Tabriz during the year 2011. In this study, 876 urine samples were cultured after being transferred in a completely sterile condition to the microbiology laboratory and finally the total of 135 samples were culture positive. Among the positive cases, the most frequency was related to *E. coli* with 63.7% of the whole positive samples and the highest sensitivity belonged to Ceftriaxone antibiotics with a sensitivity of 53.48% of all reported cases.(6)

Bano.K *etal* (2012) conduct a study of Urinary tract infections (UTI) are common and frequently encountered serious illness that affects its toll not only to all segments of human population but also results in increasing antibiotic resistance due to persistence and mis-management of the ailment. Pathogenic organisms' isolation and determination of antibiotic resistance by bacterial uropathogens in a cross section of patients was investigated at National Institute of Health, Islamabad. A total of 115 samples were collected from June to the August 2009. Identification was conducted by conventional biochemical tests and API 20E system. Percentage identification of API-20E was 100% for *Enterobacter cloacae and Klebsiella pneumoniae* while 98.9% for *E. coli.* Antibiotic sensitivity test was analyzed by disc diffusion method using different antibiotics and their zone of inhibition was measured. The bacterial isolates were identified as *Escherichia coli* (46.98 %) and *E. cloacae*, Methicillin resistant *Staphylococcus aureus* (MRSA), *Staphylococcus saprophyticus* (1.20 %). In this study it was found that *K. pneumoniae* showed highest sensitivity (80%) to cefepime and low susceptibility (13%) to ciprofloxacin, while the highest resistance (60%) to gentamicin and the lowest (6%) to meropenem, nitrofurantoin and ciprofloxacin was also observed. The susceptibility of *S. aureus* was highest (64%) to amikacin, augmenting and

oxacillin and lower sensitivity for ampicillin and moderate for erythromycin, methicillin, and cefotaxime with 45% outcome. The overall results obtained indicated varied patterns of antibiotic sensitivity and resistance, deserving therefore the judicious and rational use of antibiotic in the routine treatment of urinary tract infection to prevent the recurrence as well as resistant strains.(7)

Chaudhary and Murthy (2013) this study had undertaken to study the profile of uropathogenic bacterial flora in adult, their antibiotic resistant pattern, adherence factors of uropathogens and in vitro adherence capabilities of Uropathogens. The samples were tested microbiologically by standard procedure. Antibiotic susceptibility of the isolated pathogens was tested for commonly used antibiotics by Kirby-Bauer technique according to CLSI guidelines. Significant bacteriuria was present in 40% of samples. The most common pathogens isolated were *Escherichia coli* (52%), followed by *Klebsiella pneumoniae* (16%), *Acinetobacter anitratus* (11%), *Proteus mirabilis* (6%), *Enterobacter* species (5%), *Citrobacter* species (2%), *Pseudomonas aeruginosa* (2%), *Morganella morganii* (1%), *Enterococcus* species (3%) and *Staphylococcus aureus* (2%). The mean susceptibility of uropathogens was for amikacin (AK- 81%), nitrofurantoin (Nf-60%), cefotaxime (52%), ceftriaxone (Ci-47%), ciprofloxacin (Cf-45%), Norfloxacin (Nx-33%), Cotrimoxazole (Co-18%) and Nalidixic acid (Na-17%). *Escherichia coli* are the commonest cause of UTI. Adherence is one of the essential pre-requisites to establish UTI. Majority of UTI in men are mono-microbial. Most of uropathogens are susceptible to amikacin (81%).(8)

Nivas (2014) In UTI, most common organism is E.coli. Organism responsible for the hospital acquired infection may have tendency to develop multiple drug resistance. E.coli acquired from the Al- Quwayiyah General Hospital source may differ in their resistant against antibiotics. This was a surveying study conducted in an Al-Quwayiyah General hospital to know the prevalence and antibiogram of E.coli. Samples received include mid-stream clean catch urine, suprapubic aspirate and from Foley's catheter. All the plates were inspected for growth and the isolates were identified by observing colony morphology, Gram-stain characteristics and relevant biochemical tests. The isolates were tested for their antimicrobial susceptibility and the results were interpreted by Vitec 2 compact methods according to the

guidelines of Clinical and Laboratory Standards Institute. Out of 96 samples tested, 53 (55.2%) were positive for E.coli. E.coli was highly resistant to Ampicillin (85.1%) followed by Piperacillin (66.03%) and least resistance was seen with Nalidixic acid (5.6%).(9)

Rakesh *et al* (2013**)** *Klebsiella pneumoniae (K. pneumoniae)* accounts for 2nd highest organism isolated from urine samples of UTI patients after *Escherichia coli.* The present study is undertaken to determine the antimicrobial susceptibility pattern of *K.pneumoniae* isolated from urine samples of UTI patients of Peoples College of Medical Science & Research Centre and hospital. Between January 2010 and December 2011, a total of 1450 urine specimens processed in the laboratory, of which 65 (15.4%) yielded *K. pneumoniae.* Organisms were identified by conventional methods.(10)

Patel *et al* (2012) reported that symptomatic urinary tract infections (UTI) occurs in as many as 7 million visits to emergency units and 100,000 hospitalizations annually. UTI has become the most common hospital-acquired infection, accounting for as many as 35% of nosocomial infections, and it is the second most common cause of bacteremia in hospitalized patients. The prevalence of Urinary tract infections (UTI) was evaluated in 3046 patients attending G.G. Hospital & Medical College, Jamnagar. Results showed 1416 (46.48%) patients were positive. The most common organisms were Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Proteus mirabilis. In-vitro antibiotic susceptibility tests revealed that the gram negatives bacteria were sensitive to quinolones (Gatifloxacin, levofloxacin) and meropenem, while the gram positive isolates were sensitive to erythromycin, levofloxacin.(11)

# CHAPTER- 4

# MATERIAL & METHOD

## 4.1METERIALS

A list of materials used during the study is given appendix -I

## 4.2 METHODS

This study is carried out from 1st January to30th April, 2016 in the microbiology laboratory of SRL diagnostic centre in order to isolate and identify organism from urine sample along with their antibiotic susceptibility pattern there by covering a total period of 4 months. A total of 1356 urine sample were collected from the patient ranging in age from 3 month to 85 years.

## 4.3COLLECTION OF SAMPLE

For this study, early morning mid-stream urine samples were collected using Sterile, wide mouthed container with screw cap tops. On the urine sample container were indicated name, age, sex, and time of collection along with requisition forms.

### 4.4 PROCESSING OF SAMPLE

### 4.4.1 Macroscopic Examination / Physical Examination

The physical characteristics of urine sample were noted.

### 4.4.2 Microscopic Examination

The urine samples were mixed and centrifuged at 5000 rpm for 5 min. Discard the supernatant and place a drop of deposits on a glass slide and put a cover slip over it and were examined by using both 10X and 40X objectives. Samples with >10 white blood cells/mm3 were considered as pyuric. A volume of the urine samples were applied to a glass microscope slide, allowed to air dry, stained with gram stain, and examined microscopically. Bacterial isolates were identified generally using biochemical reaction.

The composition and preparation of staining reagent are given I appendix-II

### 4.5 CULTURE OF SAMPLE

The samples were culture for aerobic bacteria only and the study did not include anaerobic bacterial culture. The samples were inoculated in the CLED.

The inoculated plates were incubated at 37°C for 24 hours in incubator. The composition and preparation of culture media are given in appendix-III.

## 4.6 ISOLATION AND IDENTIFICATION OF ORGANISM

After overnight incubation, the culture plate incubated aerobically were examined for bacterial growth and identified by using standard microbial technique which involves colony characteristics, staining reaction and biochemical properties and also the antibiotic susceptibility pattern were noted.

## 4.7Identification of organism and antimicrobial susceptibility using VITEK 2 compact instrument:

- **PURPOSE:**
  To establish the standard operating procedure to determine automatically identification and sensitivity of the organism isolated from clinical specimen to various drug (Antibiotics) by using VITEC 2 compact system dedicated to the identification of bacteria and yeasts and susceptibility testing clinical significant bacteria.

- **PRINCIPLE**:
  VITEK2 compact system identified an organism by using methodology based on the characteristics of the data and knowledge about the organism and reaction being analysed. The printed lab report contains suggestion for any supplemental test necessary to complete the identification. If the tests are not sufficient to complete the identification, then standard microbiology references and literature should be consulted.
  The AST card for VITEK2 compact system is an automated test methodology based on the MIC technique reported by MacLowry and Marsh. The AST card is essential a miniaturized and abbreviated version of the doubling dilution technique MICs determined by the microdilution method. Each AST card contains 64 micro wells. A control well containing only microbiology culture medium is resident on all cards, with the remaining wells containing preleases amount of specific antimicrobials combined with culture medium. The organism suspension to be tasted must be diluted to a standardized concentration in 0.45% saline before being used to rehydrate the antimicrobial medium within the card . The card is filled, sealed and placed in to the

instrument. 24 hours for bacteria and 36 hours for yeast for complete incubation cycle.

- **Reagent cards**

The reagent cards consist barcodes that have information about product type, lot number, date of expiry and a unique identifier. These cards have 64 wells; each contains an individual test substrate which measures metabolic activities such as acidification, alkalinization, enzyme hydrolysis and growth in the presence of inhibitory substances. On the both sides, an optically clear film is present that allows oxygen transmission and maintain a sealed vessel that prevents contact with the organism- substrate admixtures. A pre- inserted transfer tube is fixed to each card to inoculate the card with microbial suspension.

Recently five types of identification card are available for different organism classes-

1. GN - Gram-negative fermenting and nonfermenting bacilli
2. GP - Gram-positive cocci and non-spore-forming bacilli
3. YST - Yeasts and yeast-like organisms
4. BCL - Gram-positive spore-forming bacilli
5. NH – *Neisseria*, *Haemophilus*, and other fastidious species

- **Preparation of suspension**

Through a sterile applicator stick required amount to pure growth was transferred to 3 ml sterile saline (0.45%- 0.50% aqueous NaCL, pH- 4.5-7.0) in a sterile test tube made of polystyrene (marked with ID). Colonies were emulsified well and mixed by vortex mixture. The turbidity was adjusted to 0.5- 0.63 McFarland turbidity range and was measured by using a turbidity meter called DensiChek™. 145 µl of this suspension was transferred to another tube containing 3 ml of sterile saline (marked with AST).

- **Inoculation**

The suspension tubes were placed in cassettes and GN card was inserted into the first tube that has marked with ID and the AST-281 card was inserted into the second tube that has marked with AST. These filled cassettes then load manually into the vacuum chamber station of the instrument. After the vacuum is applied and theair is re-introduced into the station, the organism suspension is forced through the transfer tube into micro-channels that fill all the test wells.

- **Incubation**

After loading of suspension into the wells, the transfer tubes were cut and the cards were sealed by the machine itself. The cards then loaded into the carousel incubator where online incubation was given (35.5 +/- 1.0˚C). Each card was removed from the incubator once every 15 mins and transported to an optical system for reaction readings and then returned to the incubator until the next read time.

When the identification and sensitivity test has completed a print out of the result was taken and examined.

# CHAPTER- 5

# RESULT

The study was carried out in Microbiology Laboratory of SRL from 1st January to 30th April 2016 thereby covering a total period of 4 months. Urine samples collected were processed and analyzed by standard microbial techniques.

In this study a total of 675 urine sample were collected and processed for culture and sensitivity testing. Urine samples of patients of all age groups (l month-85years) and both sexes were processed. A total 166 positive cases were seen from 675 urine samples thus culture positive was 24.59 % (i.e. 166) and negative is 75.40 % (i.e.509) as shown in Table.

## Table 1: Organisms wise distribution of Positive cases (n=166)

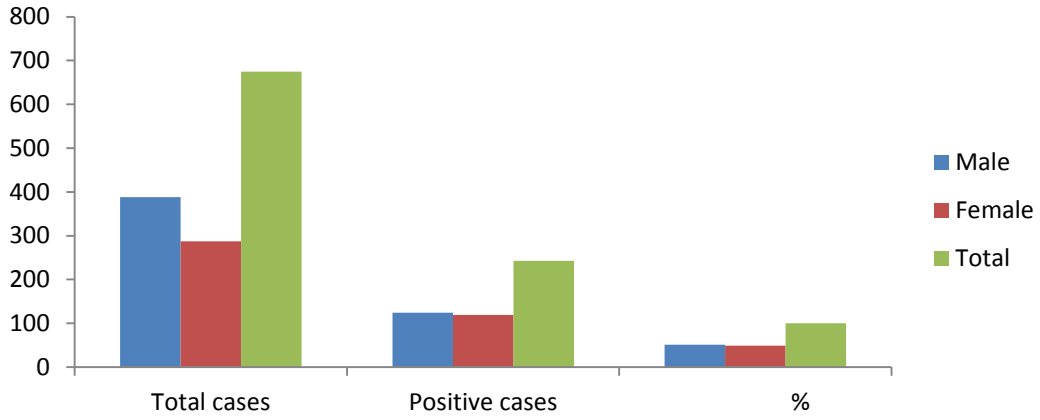
| NAME OF ORGANISMS | POSITIVE CASES | |
|---|---|---|
| | NUMBER | % |
| *E.coli* | 138 | 83.13 |
| *Proteus mirabilis* | 2 | 1.20 |
| *Pseudomonas aeruginosa* | 6 | 3.61 |
| *Klebsiella pneumoniae* | 20 | 12.04 |

More than half (83.13%) cases reported as *E.coli* followed by *Klebsiella* (12.04%), *Pseudomonas aeruginosa* (3.61%), *Proteus mirabilis* (1.20%),

## Table 2: Sex wise Distribution of the Cases according to their test results (n=675)

| Gender | Total cases | Positive cases | % |
|---|---|---|---|
| Male | 388 | 124 | 51.02 |
| Female | 287 | 119 | 48.97 |
| Total | 675 | 243 | 100 |

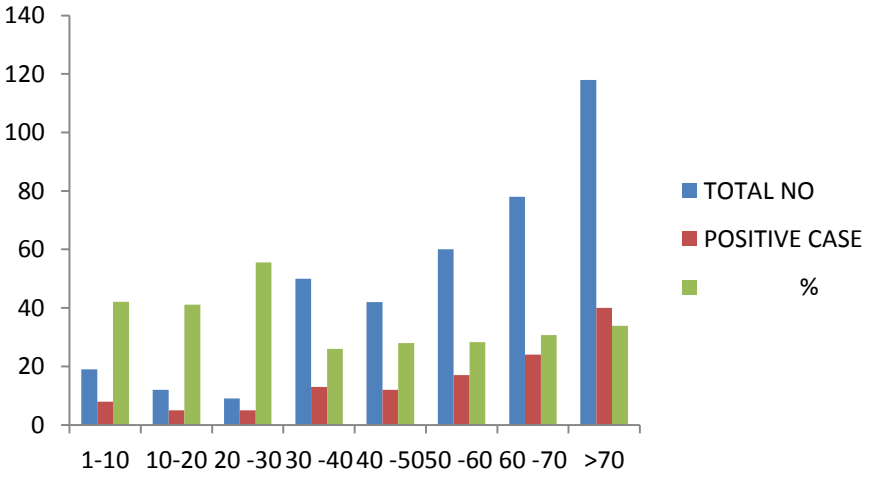
## Chart -1: Sex wise Distribution of the Cases according to their test results




### 5.1.3 Age wise distribution of male positive patient:

| AGE | TOTAL NO | POSITIVE CASE | % |
|---|---|---|---|
| 1 - 10 | 19 | 8 | 42.10 |
| 10- 20 | 12 | 5 | 41.16 |
| 20 -30 | 9 | 5 | 55.55 |
| 30 -40 | 50 | 13 | 26.00 |
| 40 -50 | 42 | 12 | 28.00 |
| 50 -60 | 60 | 17 | 28.33 |
| 60 -70 | 78 | 24 | 30.76 |
| >70 | 118 | 40 | 33.89 |

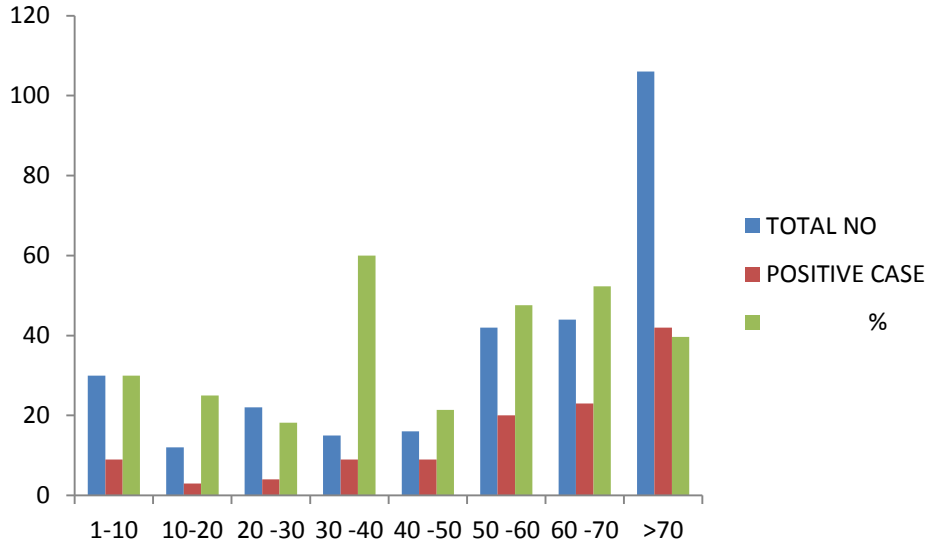
## Chart -2: Age wise distribution of male positive patient



### 5.1.4 Age wise distribution off female positive patient:

| AGE | TOTAL NO | POSITIVE CASE | % |
|---|---|---|---|
| 1 -10 | 30 | 9 | 30.00 |
| 10 -20 | 12 | 3 | 25.00 |
| 20 -30 | 22 | 4 | 18.18 |
| 30 -40 | 15 | 9 | 60.00 |
| 40 -50 | 16 | 9 | 21.42 |
| 50 -60 | 42 | 20 | 47.61 |
| 60 -70 | 44 | 23 | 52.27 |
| >70 | 106 | 42 | 39.62 |

### Chart – 3: Age wise distribution off female positive patient




## 5.2 ANTIBIOTIC SUSCEPTIBILITY PATTERN OF THE BACTERIAL ISOLATES

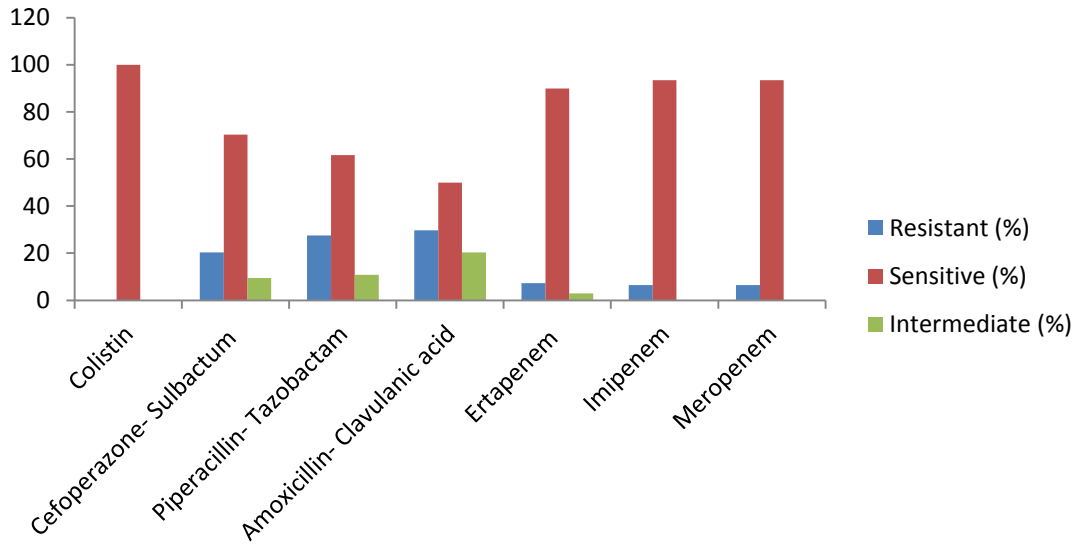
**5.2.1** Antibiotic susceptibility pattern of *E.coli* amongst the bacterial isolates

Table 4. Antibiotic susceptibility pattern of *E.coli* amongst the bacterial isolates

| NAME OF ANTIBIOTIC | RESISTANT | | SENSITIVE | | INTERMEDIATE | |
|---|---|---|---|---|---|---|
| | Number | % | Number | % | Number | % |
| Colistin | 0 | 0 | 138 | 100 | 0 | 0 |
| Cefoperazone-Sulbactum | 28 | 20.28 | 97 | 70.28 | 13 | 9.42 |
| Piperacillin-Tazobactam | 38 | 27.53 | 85 | 61.59 | 15 | 10.86 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Amoxicillin-Clavulanic acid | 41 | 29.71 | 69 | 50 | 28 | 20.28 |
| Ertapenem | 10 | 7.24 | 124 | 89.85 | 4 | 2.89 |
| Imipenem | 9 | 6.52 | 129 | 93.47 | 0 | 0 |
| Meropenem | 9 | 6.52 | 129 | 93.47 | 0 | 0 |

**Chart- 4: Antibiotic susceptibility pattern of *E.coli* amongst the bacterial isolates:**



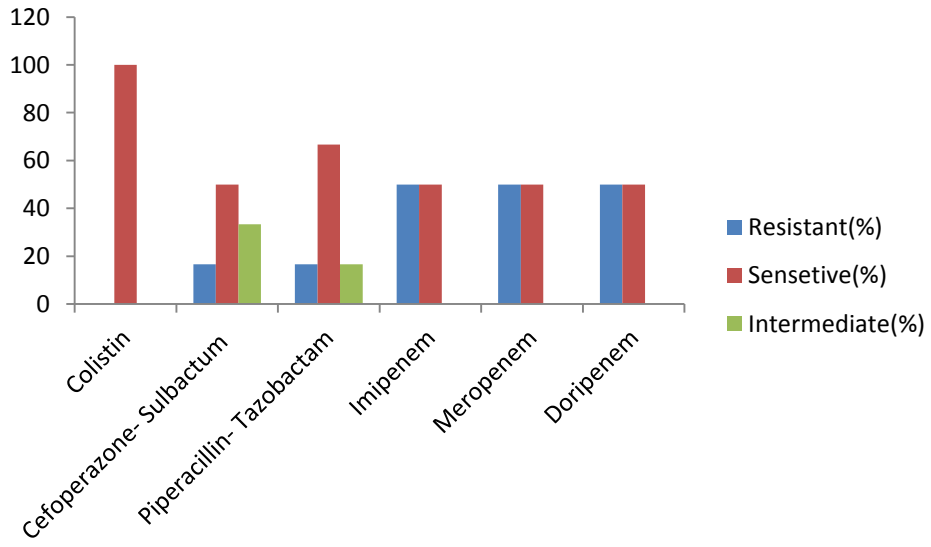

In vitro the antibiotic susceptibility if E.coli isolates Colistin(100%) was found most sensitive followed by Imipenem (93.47%) , Meropenem (93.47%), while Amoxicillin- Clavulanic acid (50%) respectively .

**5.2.3** Antibiotic susceptibility pattern of *Pseudomonas aeruginosa* amongst the bacterial isolates.

**Table 6: Antibiotic susceptibility pattern of *Pseudomonas aeruginosa* amongst the bacterial isolates**

| Name of antibiotic | Resistant | | Sensitive | | Intermediate | |
|---|---|---|---|---|---|---|
| | Number | % | Number | % | Number | % |
| Colistin | 0 | 0 | 6 | 100 | 0 | 0 |
| Cefoperazone-Sulbactum | 1 | 16.66 | 3 | 50 | 2 | 33.33 |
| Piperacillin-Tazobactam | 1 | 16.66 | 4 | 66.66 | 1 | 16.66 |
| Imipenem | 3 | 50 | 3 | 50 | 0 | 0 |
| Meropenem | 3 | 50 | 3 | 50 | 0 | 0 |
| Doripenem | 3 | 50 | 3 | 50 | 0 | 0 |

**Chart- 5: Antibiotic susceptibility pattern of *Pseudomonas aeruginosa* amongst the bacterial isolates**



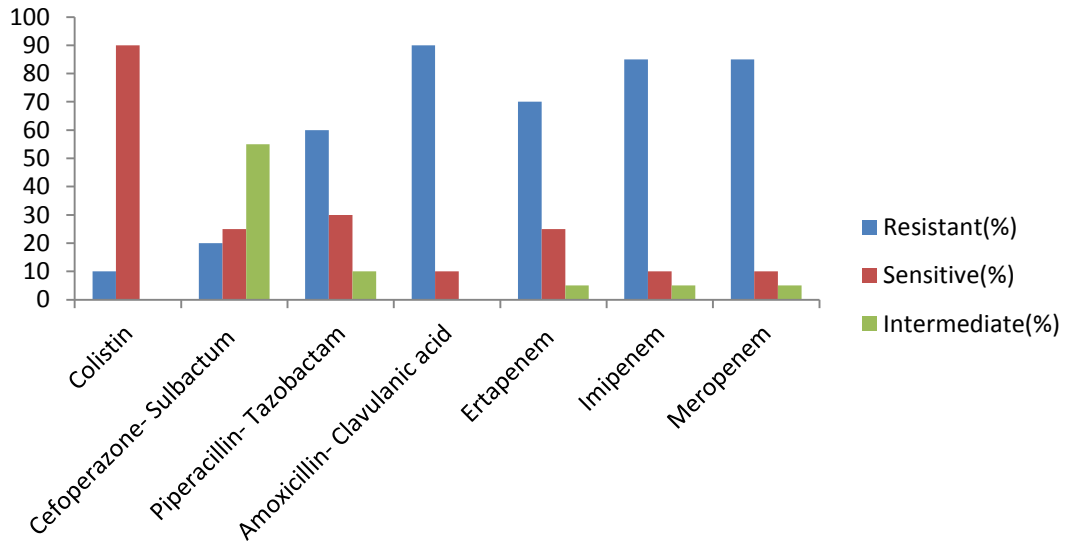

In vitro the antibiotic susceptibility of Pseudomonas aeruginosa isolates Colistin (100%)was most sensitive followed by Piperacillin- Tazobactam (66.66%), Imipenem (50%) respectively

5.2.5 Antibiotic susceptibility pattern of *Klebsiella pneumoniae* amongst the bacterial isolates.

**Table 8: Antibiotic susceptibility pattern of *Klebsiella pneumoniae* among the bacterial isolates**

| Name of antibiotic | Resistant | | Sensitive | | Intermediate | |
|---|---|---|---|---|---|---|
| | Number | % | Number | % | Number | % |
| Colistin | 2 | 10 | 18 | 100 | 0 | 0 |
| Cefoperazone-Sulbactum | 4 | 20 | 5 | 25 | 11 | 55 |
| Piperacillin-Tazobactam | 12 | 60 | 6 | 30 | 2 | 10 |
| Amoxicillin-Clavulanic acid | 18 | 90 | 2 | 10 | 0 | 0 |
| Ertapenem | 14 | 70 | 5 | 25 | 1 | 5 |
| Imipenem | 17 | 85 | 2 | 10 | 1 | 5 |
| Meropenem | 17 | 85 | 2 | 10 | 1 | 5 |

## Chart- 6: Antibiotic susceptibility pattern of *Klebsiella pneumoniae* among the bacterial isolates:



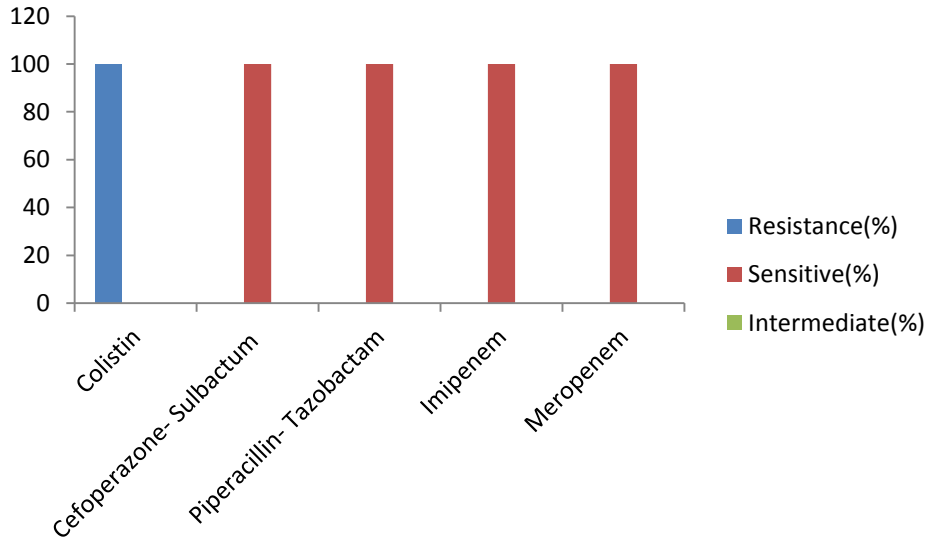

In vitro the antibiotic susceptibility of *Klebsiella pneumonie* Colistin (100%) was most sensitive while Imipenem, Meropenem were (10%) respectively.

Antibiotic susceptibility pattern of *Proteus.mirabilis* amongst the bacterial isolates

## Table – 9 Antibiotic susceptibility pattern of *Proteus.mirabilis* among the bacterial isolates

| Name of antibiotic | Resistant | | Sensitive | | Intermediate | |
|---|---|---|---|---|---|---|
| | Number | % | Number | % | Number | % |
| Colistin | 2 | 100 | 0 | 0 | 0 | 0 |
| Cefoperazone-Sulbactum | 0 | 0 | 2 | 100 | 0 | 0 |
| Piperacillin-Tazobactam | 0 | 0 | 2 | 100 | 0 | 0 |
| Imipenem | 0 | 0 | 2 | 100 | 0 | 0 |
| Meropenem | 30 | 0 | 2 | 100 | 0 | 0 |

**Chart- 7: Antibiotic susceptibility pattern of *Proteus.mirabilis* among the bacterial isolates**




In vitro the antibiotic susceptibility of *Proteus mirabilis*, Colistin, Meropenem, Imipenem was (100%) sensitive.

# CHAPTER 6

# DISCUSSION

Urinary tract infection is one of the commonest bacterial infections and the enterobacteriaceae are the most frequent pathogen detected causing 80% of urinary tract infection. The present study conduct to achieve resistance summary of clinical isolates from SRL diagnostic limited against commonly prescribed antibiotic. A total of 675 urine sample were received from SRL diagnostic center *from* 1st January to 30th April 2016 and these were processed in laboratory. Significant bacteria (culture with >105 cfu of bacteria/ml of urine) was found in 243 of 675(36%) of the urine sample. Gram-negative bacteria were (76.13%), and Yeast like spp (23.86%).

Aghdam, Bano K et al, Chaudhary and Murthy and Nivas reported in their study that *E.coli* and *Klebsiella pneumoniae* are mostly associated in the urinary tract infection and well compare to my study *E.coli* and *Klebsiella pneumoniae* mostly associated in urinary tract infections. In urinary tract infection, most common organism is E.coli, is responsible for the hospital acquired infection may have tendency to develop multiple drug resistance. *Klebsiella pneumoniae* is the second highest isolated organism from urine sample of UTIs patient.

In a total 675 clinical specimen, 243 isolates were obtained as positive and among them most common isolated bacteria found were *E.coli*(83.13%) followed by *Klebsiella pneumoniae* (12.04%), *Pseudomonas aeruginosa* (3.61%), *Proteus merabilis* (1.20%).
In this study, E.coli demonstrated a very high microbial resistance to antibiotics. The analyzed result of antibiotic susceptibility test showed that *E.coli* was highly resistance to Colistin(100%) was found most sensitive followed by Imipenem (93.47%) , Meropenem (93.47%), and Amoxicillin- Clavulanic acid (50%).

*Klebsiella pneumoniae* showed that Colistin(90%) was most sensitive while Imipenem, Meropenem were (10%) respectively.

*Pseudomonas aeruginosa* isolates Colistin (100%) was most sensitive followed by Piperacillin- Tazobactam (66.66%), Imipenem (50%) respectively.

*Proteus mirabilis*, Colistin, Meropenem, Imipenem was (100%) sensitive.

Moreover, considering susceptibility pattern of antibiotic agents for urinary tract infection therapy, particularly caused by *E.coli* as the most significant uropathogens, it seems that the drugs like Colistin, Imipenem should be prescribed cautiously especially against to those, show suitable respond to other antibiotics such as Meropenem.

In this study, 388 specimen was male and 287 specimen was female was conducted in SRL diagnostics and 124(51.02%) male specimen was positive and 119 (48.97%) female specimen was positive.

The age group of 20-30 year male was most effected (55.55%) by urinary tract infection followed by 1-10 year (42.10%), 10-20 year (41.16%), > 70 year (33.89%) 60-70 (30.76%). Female of age group 30-40 year (60.00%) was most affected followed by 60-70 year (52.27%), 50-60 year (47.61%), > 70 year (39.62).

This study describes the relation between sex and isolated bacterial agents of urinary tract infection. Microbial infection of the UTI is one of the most common infectious disease worldwide. The reason of prevalence of male with UTI is may be because of medical interventions applied during hospital stay. Proximity between vagina and anus, and small length of urethra are the two major reasons for female with UTI. Approximately 1 in 3 women will require antimicrobial treatment for UTI before age of 24. Moreover, management of micturition in women is important. Management mistakes made by women include cleaning premium forward from the anus to the vulva that can cause UTI. Sexually activity has been reported to influence higher prevalence of UTI. Considering the fact that most infecting organism are commensals of perianal and vaginal region, emphasis on personal hygiene especially in females.

# CHAPTER- 7

# CONCLUSION

It studied that higher prevalence of urinary tract infection was seen in male. In females urinary tract infection is seen in patients among 41-60 and above 60 years age group and in males it was seen in older age group between 60-80 years. Gram negative organisms were the most commonly isolated organisms in urinary tract infection among which E. coli was the most frequent causative agent. Urinary pathogens showed resistance to commonly used antibiotics like Colistin, Imipenem, Ertapenem etc. The susceptibility and resistance patterns of urinary pathogens should be considered before starting empirical treatment for urinary tract infection. Development of resistance to commonly used antibiotics for treating urinary tract infection alert us against indiscriminate usages of antibiotics to prevent development of resistance against an antibiotic.

# CHAPTER- 8

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# APPENDIX- I

## A. LIST OF MATERIALS

### 1. Equipment

- Autoclave
- VITEK2 compact
- Bunsen burner
- Biosafety cabinet II
- Incubator
- Microscope
- Refrigerator
- Weighing machine

### 2. Glass wares

- Petri plates
- Tubes
- Slides
- Glass rods
- Conical flask

### 3. Others

- Cottons
- Scissors
- Forceps
- Inoculating loops.
- Racks
- Face mask

# APPENDIX-II

## B. COMPOSITION AND PREPARATION OF GRAM'S STAIN REAGENT

### 1. Crystal violet reagent

| Composition | gram/lit |
|---|---|
| **Solution A** | |
| Crystal violet | 2.00 gm |
| Ethanol | 20.00 ml |
| **Solution B** | |
| Ammonium oxalate | 0.80 gm. |
| Distilled water | 8.00 ml |

**Preparation**

a) Mix solution A to solution B and store 24 hours to dissolve the stain completely.
b) Filter the solution through filter paper after 24 hours and then use.
c) Level the reagent bottle with preparation data and reagent name.

### 2. Gram's Iodine Solution

| Composition | gram/lit |
|---|---|
| Potassium iodide | 2.00 gm |
| Iodine | 1.00 gm |
| Distilled water | 100.00 ml |

**Preparation**

1. Dissolve the potassium iodide in distilled water and then add iodine.
2. Store in a tightly stopper bottle with preparation date and reagent name.

### 3. Acetone-alcohol decolourizer

| Composition | Volume (ml) |
|---|---|
| Acetone | 250 ml |
| Ethanol (absolute) | 250 ml |

**Preparation**

a) Mix the 250 ml of acetone into a 250 ethanol.
b) Store in a tightly stopper bottle with preparation date and reagent name.

### 4. Counterstain solution

| Composition | gram/lit |
|---|---|
| Safranine | 0.34 gm |
| Absolute alcohol | 10.0 ml |
| Distilled water | 90.0 ml |

**Preparation**

**a)** Dissolve the safranine in 0.34 gm. into 10 ml absolute alcohol and then add 90 ml distilled water.
**b)** Filter the solution through filter paper and then use.
**c)** Label the reagent bottle with preparation date and reagent name.

# APPENDIX- III

## C. COMPOSITION AND PREPARATION OF MEDIUM

### A.CLED (cysteine Lactose Electrolyte-Deficient Agar) (Hi-Media)

| **Composition** | **gram/lit** |
|---|---|
| Pancreatic digest of Gelatin | 4.0 gm. |
| Pancreatic digest of casein | 4.0 gm. |
| Beef extract | 3.0 gm. |
| Lactose | 10.0 gm |
| L-cystine | 0.128 gm |
| Bromthymol blue | 0.02 gm. |
| Agar | 15.0 gm |

**Preparation**

As directed by manufacturing company 40 gram of medium is dissolved in 1000 ml of distilled water and autoclave it at 15 lbs. pressure (121˚C) for 15 minutes.

# APPENDIX- IV

| | |
|---|---|
| % | Percentage |
| AST | Antimicrobial Susceptibility Testing |
| BCL | Gram- Positive spore forming bacilli |
| CFU | Colony Forming Units |
| CLED | Cysteine Lactose Electrolyte Deficient |
| E.coli | Escherichia coli |
| GNB | Gram Negative Bacilli |
| GP | Gram-Positive |
| *K.pneumoniae* | *Klebsiella pneumoniae* |
| MRSA | Methicillin Resistance Staphylococcus aureus |
| MIC | Minimum Inhibitory Concentration |
| NH | Neisseria, Haemophilus and other fastidious species |
| P.mirabilis | Proteus mirabilis |
| Spp. | Species |
| UTI | Urinary Tract Infection |
| YST | Yeast and Yeast like organism |