Training Report

Prospective Study on Prevalence and Antimicrobial Susceptibility Pattern of Pathogens causing Urinary Tract Infection

Internship Training Report

Submitted to

Lovely Professional University, Punjab

in partial fulfillment of the requirements

For the degree of

Master of Science in Clinical Microbiology

Submitted by:
Haleema Zaid
(Reg No 11404371)

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

DECLARATION

I hereby declare that the work embodied in this internship report was carried by me under the supervision of **Ms Rupinder Kaur (lecturer)** (Internal supervisor), Lovely Professional University and This work has not been submitted in part or in full in any other university for any degree or diploma.

Name Haleema Zaid

Date:

Place:

CERTIFICATE

This is to certify that **Mr./*Ms.* Haleema Zaid** bearing **Registration Number 11404371** 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.

Internal Supervisor ***External Supervisor***

Date: Date:

LPU Punjab ……

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 tract infection vary from place to place and they also vary in their susceptibility and resistance patterns. 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 the isolates. Samples were processed on CLED medium and their antibiotic sensitivity on MH agar. This study is done in Tagore hospital and Heart Care Center. A total of 150 urine samples were taken from the suspected UTI patients. They were tested microbiologically and antimicrobial susceptibility test for the isolated pathogens.

The rate of culture positivity in females was 76.95% and in males was 23%. *E-coli* was the most frequently isolated urinary pathogen (53.84%), followed by *Klebsiella pneumoniae* (30.76%), *Pseudomonas aeruginosa* (5.76%). *E. coli* was 100% resistant to third generation cephalosporins and fluoroquinolones like ofloxacin .High level of resistance to the tune of 97% was also noted for Amoxycillin-clav, Cefixime, Cefuroxime and Cefipime. The drugs which showed higher susceptibility were Imipenem (67%), Amikacin(78%), Tigecycline (85%) and colistin (100%).

In Klebsiella pneumoniae higher resistance to Amoxy/clav and Cefotaxime (100%), Cefixime (93%), Levofloxacin(87%).The drug which showed higher suscepbility were Tigecycline(81%), colistin(81%), Nitrofuration (87.5%). Higher prevalence of UTI was seen in females. Gram negative organisms were the most commonly isolated organisms in UTI. Urinary pathogens showed resistance to commonly used antibiotics like Oflaxacin and Cefipime, Ceftrioxone**.** The susceptibility and resistance patterns of urinary pathogens should be considered before starting empirical treatment for UTI.

Key Words: *CLED, UTI, E.coli, Antimicrobial susceptibility, antibiotics, Gram negative, Gram positive bacteria.*

ACKNOWLEDGEMENT

It gives us an immense pleasure to express my profound gratitude to all those respectable personalities who helped me to complete my dissertation work. My first and for most expression of deepest and sincere gratitude goes to my supervisor **Dr. Raman preet kaur (MD Microbiologist Tagore Diagnostic center)** for her incisive observation, constant encouragement, tremendous support, patient guidance and suggestion, valuable supervision meticulous care throughout the research period. I would also like to thank my honorable Internal Supervisor **Lecturer Miss Rupinder kaur (LPU).**

I express my sincere thanks all the staff of Microbiology department of Tagore for their kind cooperation and support.

Finally, we would like to express special thanks to my family members for their constant encouragement and endless support throughout my student life and during the preparation of this thesis work.

…….

Haleema Zaid

TABLE OF CONTENTS

LIST OF TABLE

LIST OF FIGURES

Prevalence and antimicrobial susceptibility pattern of pathogens causing urinary tract infection

Introduction

Urinary tract infection is defined as a disease caused by microbial invasion of the genitourinary tract that extends from the renal cortex of the kidney to the urethra (1)Urinary tract infections are the second most common infection in community (2). Urinary tract infection is one of the important causes of morbidity and mortality. An estimated of about 150 million people were infected with UTI per annum in Worldwide (3). In the United States with approximately 13,000 deaths mortality rate 2.3% attributed to UTIs. Estimated cost per infection ranges from 750-1,000, total cost in the United State ranges from 340 million to 450 million annually (4). In India that UTI account for nearly 7 million office visits and 1 million emergency department visits, resulting in about 100,000 hospitalizations (5). Incidence of UTI is higher in women than men due to short urethera, absence of prostatic secretion, pregnancy and easy contamination of urinary tract with fecal flora (6).

Urinary tract infections are classified into two types uncomplicated and complicated urinary tract infection.Complicated urinary tract infections are deal with functional or structural abnormalities (7). Uncomplicated urinary tract infections are due to bacterial infection and the prevalence of UTI is higher in women than men [8]. The infection of cystitis or bladder infection occurs in the lower urinary tract includes the bladder and urethra. Infection that spreads to the upper tract (the ureters and kidneys) is called as polynephritis.

Micro-organisms can spread within the urinary tract by haematagenous and ascending route (9). Infection of the renal parenchyma by blood-borne organisms (*staphylococcus aureus*) occurs in human. Gram negative bacilli rarely occur by the hematogenous route. Ascending route of urinary tract infections in women develop when uropathogens from the fecal flora colonize the vaginal introitus and displace the normal flora (diphtheroids, lactobacilli, staphylococci and streptococcal species). Colonization of the vaginal introitus with *E. coli* seems to be one of the critical initial steps in the pathogenesis of both acute and recurrent UTIs. Most uropathogens originate in the rectal flora and enter the bladder via the urethera.

Causative organisms those are responsible for UTI *Escheria coli, Proteus mirabilis, Klebsiella, Pseudomonas aeruginosa, Enterobacter, Staphylococcus, Enterococcus, Salmonella typhii Staphylococcus* (10). Most common causes of UTI**s** in outpatients were *E. coli* 40%**,** *Klebsiella, Enterobacter, Seratia, and P.aeruginosa* 7%**,** *P.mirabilis* 6% Other Gram positive organisms*: staph epidermis, Staph aureus, and Enterococcus faecalis* 3%. Inpatients (Nosocomial infection**)** *E.coli* 40%and other gram negative organisms 25%**,** other gram positive organism 16%**,** *and P. mirabilis* 1%.

Therapeutic should be based on accurate and up-to-date antimicrobial susceptibility. The antibiotic drugs which have been used for the treatment of the UTI include Colistin, Tigecycline, Nitrofuration and imipenum demonstrated excellent effectiveness against the organisms. However, there are some drugs that show high resistivity towards an organism. So, these drugs will not use as a therapeutic agents.

LITERATURE REVIEW

Hajarnis S. (1995) at Seychelles' Victoria Hospital, E. coli isolates from urine samples of UTI patients showed a 78.6% resistance against Ampicillin and Amoxicillin and a 54.8% resistance against Cotrimoxazole. The same *E. coli* isolates showed a 75% susceptibility to Gentamicin, Nalidixic acid and Nitrofurantoin.

M.Vornea. et al (1999) The antibiotic resistance patterns of Gram-negative bacteria isolated from nursing home patients. *Escherichia coli* the most prevalent isolate (48%) followed by *Proteus spp*. (26%) and other *Enterobacteriaceae* (20%). In this study the susceptibility of *E. coli* decreased for co-trimoxazole (79% to 62%), increased for nitrofurantoin (79% to 91%) and remained unchanged for amoxycillin (41%).

Menzue Kenechukwu, et al (2004) collected 1,814 urine samples at University of Nigeria Teaching Hospital. The commonest isolates were *Escherichia coli*, *Staphylococcus aureus, Streptococcus faecalis* and *Proteus spp*. (These represented 46.3%, 30.7%, 9.1% and 5.4% of isolates respectively). The Gram-positive organisms were very sensitive to Augmentin and the Fluoroquinolones. *E. coli* showed the highest sensitivity to Nitrofurantoin (76%) while it was also very susceptible to the fluoroquinolones (74%).The study clearly shows that Nitrofurantoin is a very effective first line drug for UTIs.

Hasan (2007) conduct a study to analyze the pathogenic organisms recovered from patients with urinary tract infection in a tertiary Indian hospital.
E coli were seen in 50.7% samples followed by Klebsiella spp (27.6%). Staphylococcus aureus was the commonest Gram- positive isolate (1.5%). Urinary tract infection (UTI) was seen in 70.5% females as compared to 29.5% males. A high recovery of isolates was noted from July to September. Multi drug resistance was commonest with Enterococcus (78.8%) followed by Pseudomonas (65.1%). Drugs, which retained usefulness for Gram-negative isolates, were amikacin, norfloxacin and cefotaxime. For Gram-positive isolates, vancomycin, teicoplanin, lincomycin and Norfloxacin were very effective.

EL. Mahmood Muhammad Abubakar (2009) investigated the prevalence of bacteria causing UTIs as well as their susceptibility to commonly used antibiotics at the Specialist Hospital, Yola. *E. coli* (24.5%), *K. pneumoniae* (17.3%), *P. mirabilis* (14.6%), *S. faecalis* (13.4%), *S. aureus* (5.3 %), *P. vulgaris* (4.7%), *P.stuartii* (4.1%), *S. epidermidis* (3.8%), *A. faecalis* (3.4%), *S. saprophyticus* (2.8%), *P. aeruginosa* (2.5%), *S. marsescens* (2.0%) and *C. fruendii* (1.7%). Sensitivity of the antibiotics were ofloxacin (63.8%), gentamycin (13.26%), streptomycin (37.0%), colistin (49.2%), ampicillin (25.4%), nalidixic acid (45.5%), nitrofurantoin 55.4%), augmentin (64.9%), tetracycline (27.0%), cotrimoxazole (41.8%), pefloxacin (34.9%), chloramphenicol (50.9%), and erythromycin (51.6%).

Manikandan.S *et al.* (2011) studied that the current situation of antimicrobial resistance of Urinary Tract Infections (UTIs) caused by human pathogens. About 10 midstream urine samples were collected from adult patients were analyzed for Multidrug Resistant (MDR) strain isolation and identified that *E. coli* was the predominant pathogen (31.5%) causing UTI, followed by *Staphylococcus aureus* (20.5%), *Klebsiella pneumonia* (15.8%), *Proteus mirabilis* (7.4%) and *Pseudomonas aeruginosa* (7.5%). Among this *E. coli*, *K. pneumoniae* and *P. aeruginosa* were highly resistance to most of the antibiotics, whereas *Staphylococcus* spp and *Serratia marcescens* exhibited sensitive to Cephalexin, Ciprofloxacin and Gentamicin.

Razak (2012) reported that urinary tract infection is one of the most common bacterial infections seen in clinical practice particularly in developing countries.. The rate of culture positivity in females was 87.82% and in males was 27.92%. E-coli were the most frequently isolated urinary pathogen (37.95%), followed by Klebsiella (21.41%) and Acinetobacter (10.94%). E-coli were highly sensitive to Nitrofurantoin (81.92%) and Amikacin (69.88%) and it was highly resistant to Ampicillin (1.0%). Klebsiella was highly sensitive to imipenem and it was highly resistant to Ampicillin

Bano.K *et.al* (2012) Acc. to this study 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 cefapime 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.

Patel *et al* (2012) reported that UTI has become the most common hospital- acquired infection, accounting for as many as 35% of nosocomial infections. 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 meropenum, while the gram positive isolates were sensitive to linazolid, erythromycin & quinolones (Gatifloxacin, levofloxacin).

Aghdam et.al (2013) reported that urinary tract infections second to respiratory infections found the highest statistics of infections among patients. In this study, 876 urine samples were cultured, 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.

Rakesh *et al* (2013) Urinary tract Infection (UTI) is among the most common infections described in outpatient department and hospitals inpatients. *Klebsiella pneumoniae* (*K.*

pneumoniae) accounts for 2nd highest organism isolated from urine samples of UTI patients after *Escherichia coli*. In this 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*.

Dash *et al* (2013): determined the antimicrobial resistance of urinary tract pathogens has increased worldwide. The prevalence of UTI was significantly higher in females compared with males (females 45.2%, males18.4%, Young females within the age group of 18to37 years and elderly males (≥68 years) showed high prevalence of UTI. *Escherichia coli* (68.8%) were the most prevalent isolate followed by *Enterococcus spp*. (9.7%).

Khatri.B *et al* (2014) reported that Urinary tract infection (UTI) is the most common infection in both community and hospital patients. This study was designed to find out the etiological agents of UTI and their prevalence, and to determine the antimicrobial susceptibility Gram negative bacteria, showed high resistance to cephalothin (88.16%) and high susceptibility to nitrofurantoin (90.78%). Also *Staphylococcus saprophyticus* isolates, as the most frequent Gram positive bacteria, exhibited high resistance to ampicillin, tetracycline, and erythromycin (92.31%) and high susceptibility to nitrofurantoin and vancomycin (92.3%).

Ashley Bryce, et al (2016) investigated *E coli* isolates in urine at OECD (Organization for Economic Co-operation and Development) countries, the pooled prevalence of resistance was 53.4% (95% confidence interval 46.0% to 60.8%) for ampicillin, 23.6% (13.9% to 32.3%) for trimethoprim, 8.2% (7.9% to 9.6%) for co-amoxiclav, and 2.1% (0.8 to 4.4%) for ciprofloxacin; nitrofurantoin was the lowest at 1.3% (0.8% to 1.7%).

AIM AND OBJECTIVES

AIM

To determine the prevalence of UTI isolated from urine sample and their antibiotic susceptibility pattern.

Specific Objectives

- To isolate and identify the pathogenic bacteria in urine sample collected from patient visiting Tagore Hospital and Heart Care Centre
- To study the bacteriological episodes in different age group with relation to gender.
- To perform antibiotic susceptibility test of bacterial isolates and to estimate the prevalence of Multi Drug Resistant microorganism from the isolates

MATERIALS & METHODS

Materials

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

This study is carried out from 2nd January to 2nd may, 2016 in the microbiology laboratory of Tagore Hospital and Heart Care 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 100 urine sample were collected from the patient. For this study, urine samples were collected using Sterile, wide mouthed container with screw cap tops.

COLLECTION OF SAMPLE

For this study mid-stream urine samples were collected using sterile wide mouthed container with screw cap tops. On the urine sample bottles were indicated name, age, sex, and time of collection.

PROCESSING OF SAMPLE

Microscopic Examination

Wet Mount: one drop of urine was placed on the slide and cover with coverslip, taking care to avoid air bubbles. Then, observed under light microscope for the presence of pus cells, epithelial cells, red blood cells and bacteria.

Culture of sample

The samples were inoculated on the CLED agar **(**Cysteine-Lactose-Electrolyte-Deficient Agar).The inoculated plates were incubated at 37°C for 24 hours in an incubator.

ISOLATION AND IDENTIFICATION OF ORGANISM

After overnight incubation, the culture plates were examined for bacterial growth and identified by using standard microbial technique which involves colony characteristics are showing in table 1, staining reaction, motility test, biochemical properties and also the antibiotic susceptibility pattern.

Table 1. Lactose fermenting and non – lactose fermenting colony morphology on CLED

Organisms	Colony Morphology	Images

Escherichia coli	mucoid colonies, irregular shapes	
Klebsiella spp	Translucent blue colonies	
Proteus spp	rough periphery greenish colony appear, earthy smell	
Pseudomonas aeruginosa		
Enterococci	small yellow colony	
Staphylococcus aureus	deep yellow colony	

Gram Staining- Gram staining was done to differentiate between Gram negative and Gram positive bacteria. For Gram negative organisms: TSI test (Trilple sugar iron), Simmon citrate test, urease test, PPA test (Phenyl pyruvic acid) and Indole test.
For Gram positive organisms: Coagulase test, Catalase test and Oxidase test

Procedure

- Heat fixed smear of specimen is stained with crystals violet for one minute.
- Pour Gram's iodine over the slide for1 minute.
- Decolourise with acetone for 10-30 seconds.
- Wash the smear with water.
- Counterstain with safranin for 1 minute.

Gram positive bacteria

Resist decolourisation and retain the color of primary stain (violet color)

Gram negative bacteria

Stain red or pink due to retaining the counter staining (Safaranin) shown as figure 1a and 1b

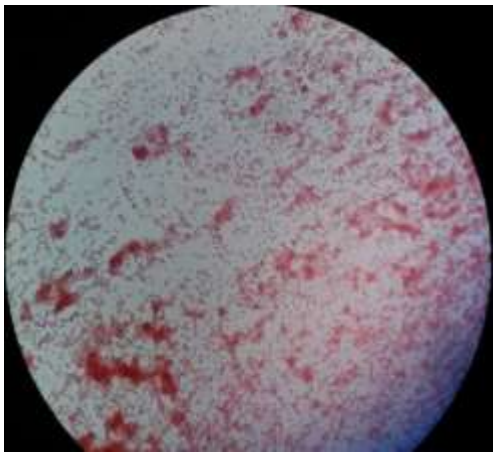


Figure 1a showing Gram negative bacilli

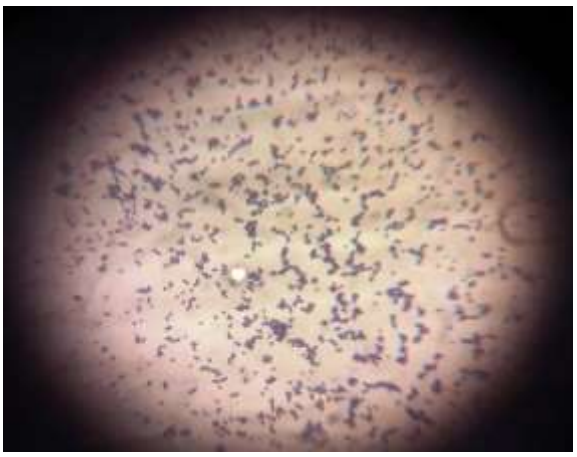


Figure 1b showing Gram positive cocci

MOTILITY TEST:HANGING DROP METHOD

It helps to distinguish between motile bacteria from non-motile ones.

- Take a clean and grease free slide a ring of vaseline make around the center of the slide and one drop of bacterial growth is placed over the cover slip after immediately cover slip placed over there slide and see under microscope.

BIOCHEMICALTEST

TSI (triple sugar iron test) This is a deferential medium that contain lactose , sucrose and

dextrose , ferrous sulphate, phenol red is ph indicator. Medium is inoculated with bacterial culture by a straight wire deep in the butt, incubated at 37°c for overnight.If organism ferment any of the three sugar, the medium will turn yellow as shown in figure 2a:

SLANT/BUTT	COLOR	INTERPRETATION
K/A	Red/yellow	Glucose ferment only
A/A	Yellow/yellow	Glucose, lactose and sucrose fermentation
K/K	Red/red	Neither glucose, lactose, nor sucrose fermented
K/A, H2S	Red/yellow with black Precipitate	Glucose fermentation only ,H2S produced

Figure 2a showing TSI

Phenylalanine Deaminase Test ability of an organisam to produce the enzyme deaminase. This enzyme is removed to the phenylalanine and release phenyl pyruvic acid. Medium is inoculated with bacterial growth an incubated at 37°c for overnight, after incubation 10% ferric chloride is added to the medium, due to the andprovidencia,PPApositive)
Interpretation: positive-green color, negative-no color change as shown in figure 2b.

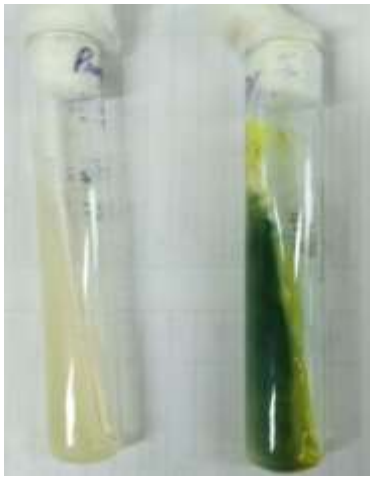


Figure 2b showing Phenylalanine Deaminase Test

Citrate Utilisation Test ability of an organisms to utilize citrate as a carbon source**.** Inoculated the media with bacterial colony by a straight wire and incubated at 37°c for overnight.
(*Klebsiella sp, Salmonella spp except S.*typhii citrate positive)
Interpretation: Positive-growth with an intense blue color, Negative-no growth with no change in color as shown in figure 2c.

Figure 2c showing Citrate test

Indole Test the ability of an organisam to split amino acid tryptophan to form the compound indole, tryptophan is hydrolysed by tryptophanase to produce the end product one is indole. Indole production is detected by inoculating the bacterium into peptone water and incubated at 37°c for overnight. 0.5ml kovac's reagent (which contain 4(p) dimethyl amino benzaldehyde) is added and gently shaken, (E.coli, proteus sp, indole positive

Interpretation: Indole positive-red colored ring near the surface of the medium,
Indole negative-yellow colored ring near the surface of the medium as shown in figure 2d.

Figure 2d showing Indole test

Urease test the ability of an organism to produce an enzyme urease which hydrolyze urea to ammonia and carbon dioxide. Organism is inoculated on the medium and incubated at 37°c for overnight. *Klebsiella spp, proteus spp*. Urease positive.
Interpretation: positive-pink color, negative-pale yellow color as shown in figure 2e.

Figure 2e showing urease test

Catalase test certain bacteria have an enzyme catalase which acts on hydrogen peroxide to release oxygen. Pick up a few colonies of bacterial growth and mix it in a drop of hydrogen peroxide on a clean glass slide.

Interpretation: positive result-immediate bubbling, (O_2 formed), Negative-no bubbling (no O_2 formed) as shown in figure 2f.

Figure 2f showing catalase test

Oxidase test to determine the presence of an enzyme cytochrome oxidase which catalyses the oxidation of reduced cytochrome by molecular oxygen.(pseudomonas aeruginosa oxidase positive) **.**

DRY FILTER PAPER METHOD: 1 filter paper soaked in 1% solution of tetramethyl-p-phenylene-diamine dihydrochloride, drainned for about 30secs and freeze dried and stored in a dark tightly sealed bottle , a strip was removed. The colony to be tested was picked up with a platinum loop and smeared over the moist area.
Interpretation: positive-deep purple within 10 seconds, Negative-no color change shown as figure 2g.

Figure 2g showing oxidase test

Coagulase Test The coagulase slide test is used to identify the presence of bound coagulase which is attached to the cell walls of bacteria. Bound coagulase react with the fibrinogenin the plasma, causing the fibrinogen to precipitate. This causes the cells to clump togather, on a clean glass slide in a drop of normal saline few colonies of bacteria were emulsified and then mixed with a drop of human plasma. Prompt clumping of the suspension occurs with coagulase positive stains while clumping was absent in negative stains as shown in figure.

Bile Esculin Test this is a selective and deferential medium to identify enterococci and group d streptococci based on the ability of an organisam to hydrolyze esculin (bile salts) to inhibit the

gram positive organisam other than enterococci and group d strepto cocci. If an organisam can hydrolyze esculin, the media will turn brown or black as shown in figure 2h.

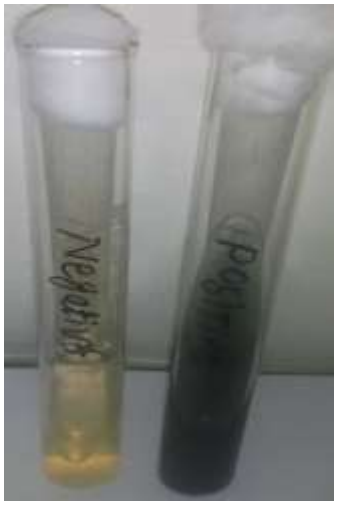


Figure 2h showing Bile Esculin test

ANTIBIOTIC SENSITIVITY TEST

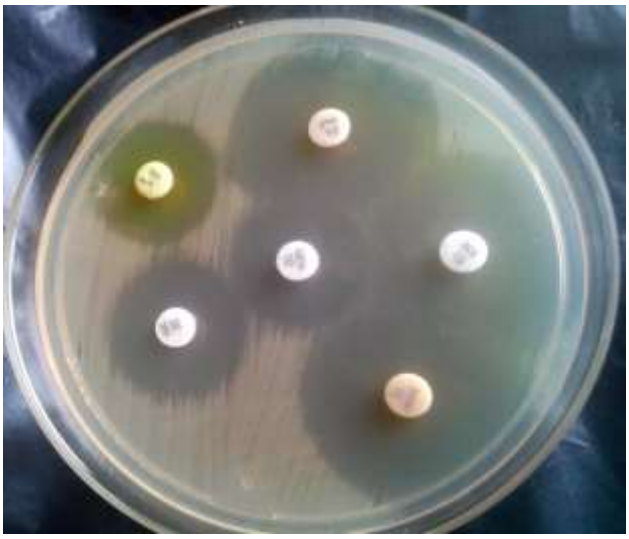
Mueller -Hinton Agar This medium is used for determination of susceptibility of microorganism to antimicrobial agents.

KIRBY-BAUER METHOD this method allows the rapid determination of the efficacy of a drug by measuring the diameter of the zone of inhibition that results from the diffusion of the agent into the medium surrounding the disc.

PROCEDURE

- The colonies of organism from culture plates were passed into peptone water and incubated at 37^0c for 1hrs.
- After 1hrs a cotton swab was dipped into inoculum and streaking with swab on muller Hinton agar plate.
- Using a sterile foreceps antibiotic discs were applied to the MH plate. On a plate of 100mm diameter, seven discs may be applied, one in the centre and six in the periphery.
- The plates were then incubated at 37^0c for 16-24hrs.
- After incubation, the zone of complete growth inhibition around each discs were measured and the diameter of the disc was included in these measurements.
- The interpretation of zone site into sensitive, intermediate and resistant were based on interpretation

The below figure is showing the sensitivity of antibiotic discs towards *E.coli*



RESULTS

The study was carried out in Microbiology Laboratory from 2nd January to 2nd may. Urine samples were collected and processed and analyzed by standard microbial techniques. In this study a total of 150 urine sample were collected and processed for culture and sensitivity testing. Urine samples of patients of all age groups and both sexes were processed. Different organisms were isolated from 150 urine samples, thus culture positive as shown in Table1and a graph 1a shows the distribution of organisms.

Table 1: Organisms wise distribution of Positive cases (No-52)

Types of organisms	**Positive Cases**	
	No.	**%**
Esxhericae.coli	28	53.84%
Klebsiella pneumoniae	16	30.76%
Pseudomonas aeruginosa	3	5.76%
Staphylococcus aureus	3	5.76%
Proteus mirabilis	1	1.92%
Enterococcus	1	1.92%
TOTAL	52	

Graph 1a showing the frequency distribution of microorganisms

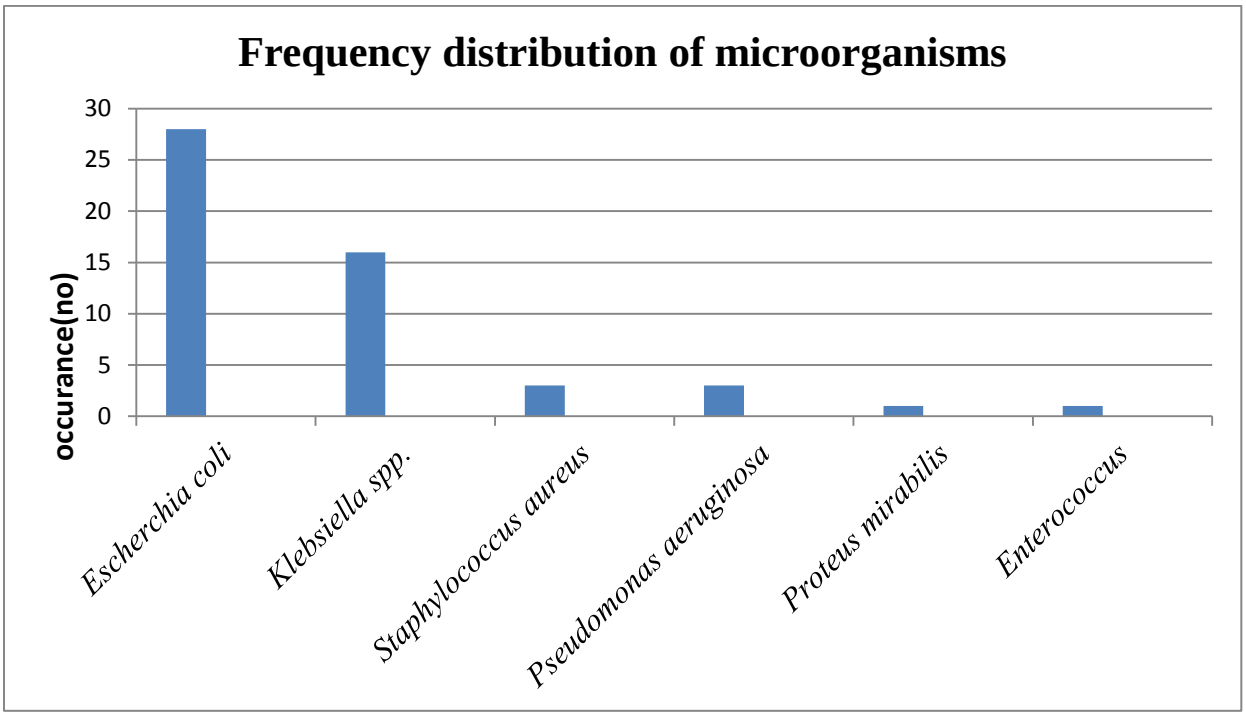


Table 2: Sex wise Distribution of the Cases according to their test results (No-52) and also shown in pie chart figure 2a

GENDER	**POSITIVE C ASES**		**TOTAL PERCENTAGE OF MALES AND FEMALES**
	IPD	**OPD**	**%**
Male	6	6	23.07
Female	16	24	76.90

Figure 2 a shows the pie chart of growth pattern of positive sample

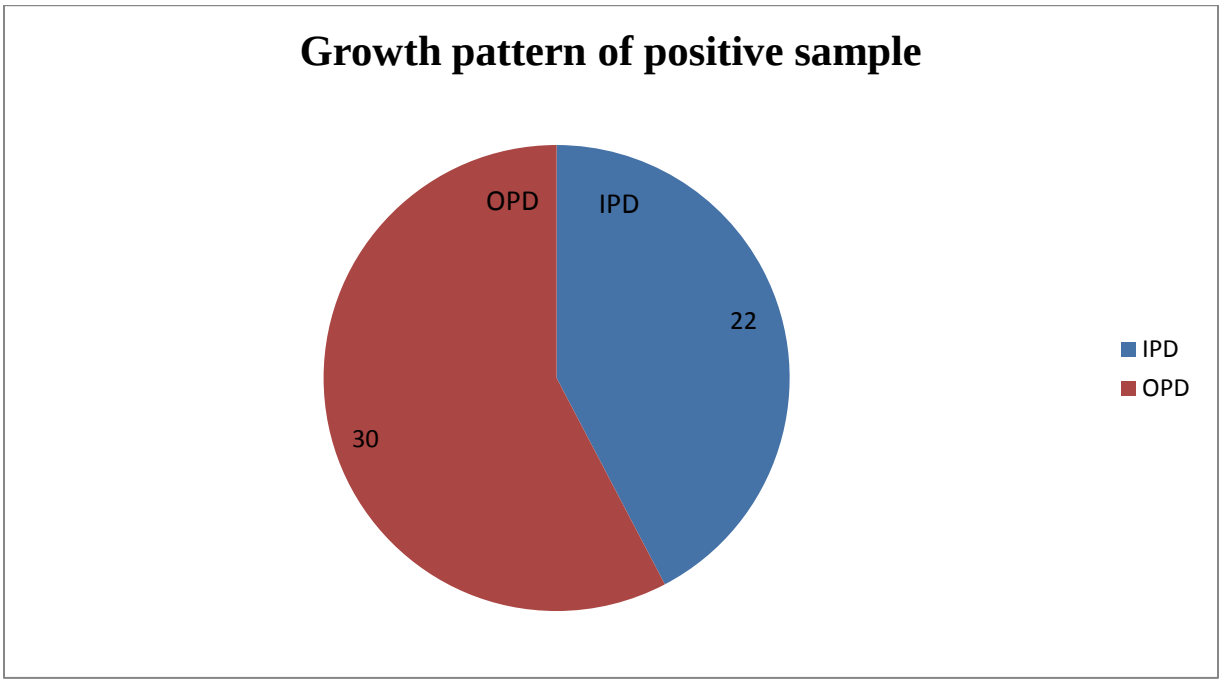
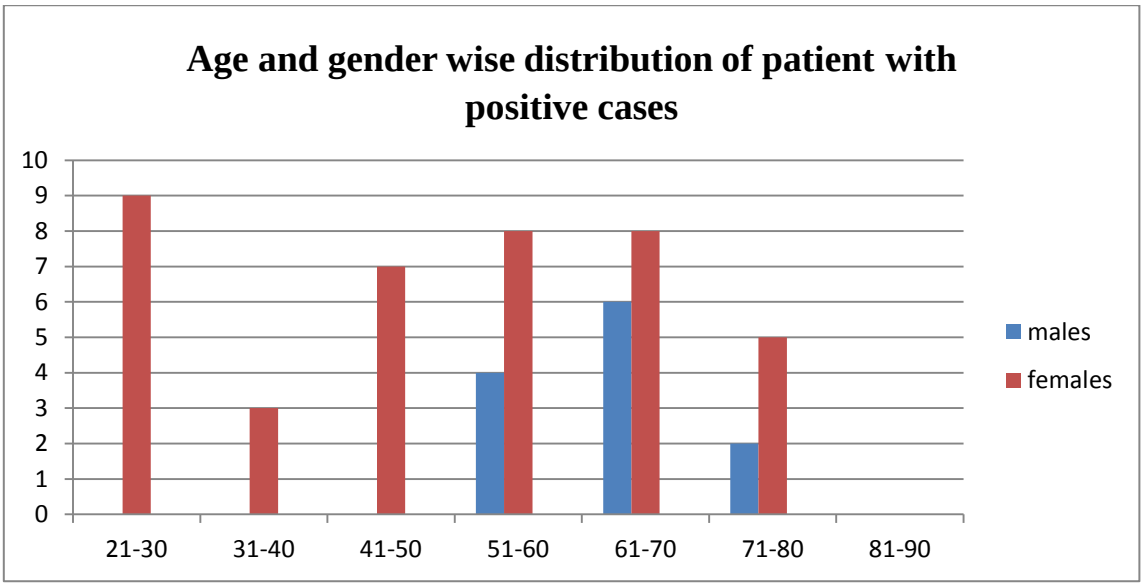


Table 3: Age and gender wise distribution of patient with positive Cases and figure 3a shows the bar graph distribution

AGE GROUP	POSITIVE CASES	
	GENDERE	
	MALE	FEMALE
10-20		
21-30		9
31-40		3
41-50		7
51-60	4	8
61-70	6	8
71-80	2	5
81-90		
TOTAL	12	40

Figure 3a shows the bar graph of age and gender wise distribution



ANTIBIOTIC SUSCEPTIBILITY PATTERN OF THE BACTERIAL ISOLATES

Table 4: Antibiotic susceptibility patterns of *E.coli* (N-28) among the bacterial isolates.

ANTIBIOTICS	SENSITIVITY	
	NO	%
Ampicilin	2	7.14
Amikacin	22	78.57
Gentamicin	12	42.85
Amoxy/clav	1	3.57
Cefixime	1	3.57
Cefuroxime	1	3.57
Cefotaxime	00	00
Ceftrixone	00	00
Ceftazidime	00	00
Cefipime	1	3.57
Nitrofuration	26	90
Norflaxacin	2	7.14
Levoflaxacin	2	7.14
Oflaxacin	00	00
Piperacillin+Tazobactum	11	39.28
Cefoparazone+Sulbactum	20	71.42
Trimethoprim/sulfomethazole	3	10.71
Imipenem	19	67.85

Meropenem	1	3.57
Colistin	28	100
Tigecycline	24	85.71

Antibiotic susceptibility pattern of *E.coli* isolates Tigecycline (85.71%) was found to be most sensitive followed by Amikacin (78.57%) , Cefoparazone+sulbactum (71.52%), Colistin (67.85%), Imipenem (67.85%), Nitrofuration (60.71%), while Gentamicin (42.85%) and Piperacillin+Tazobactum (39.28%) being the least sensitive antibiotic respectively.

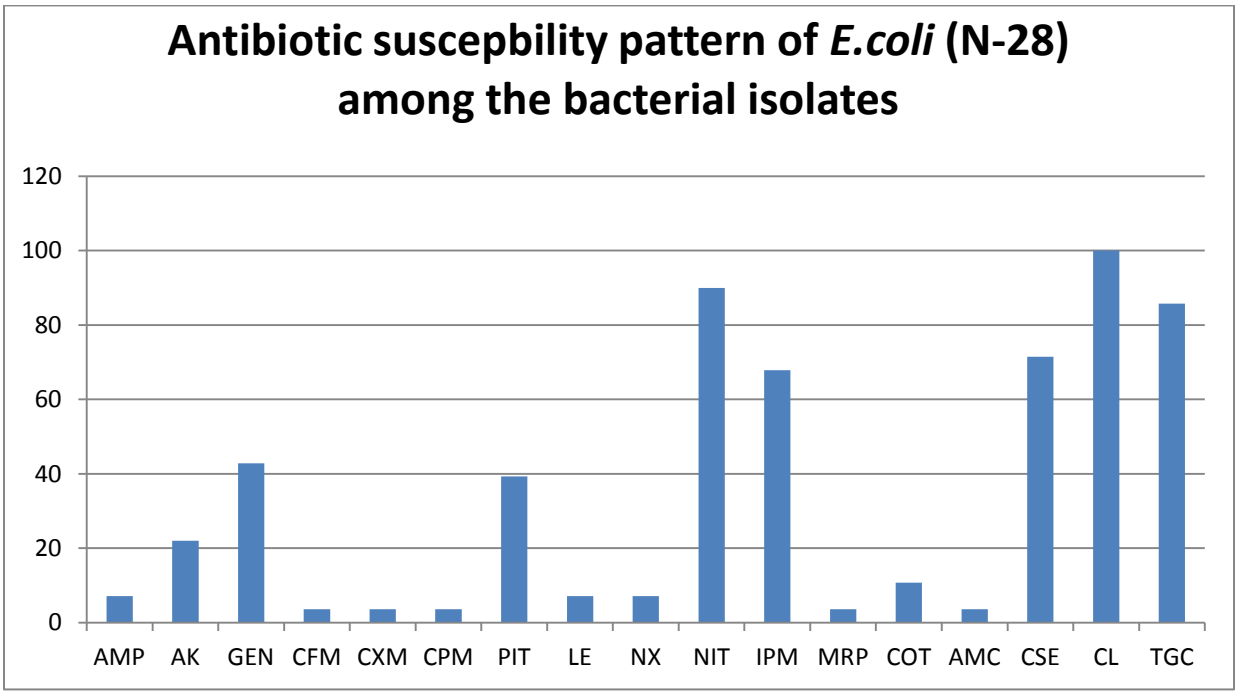
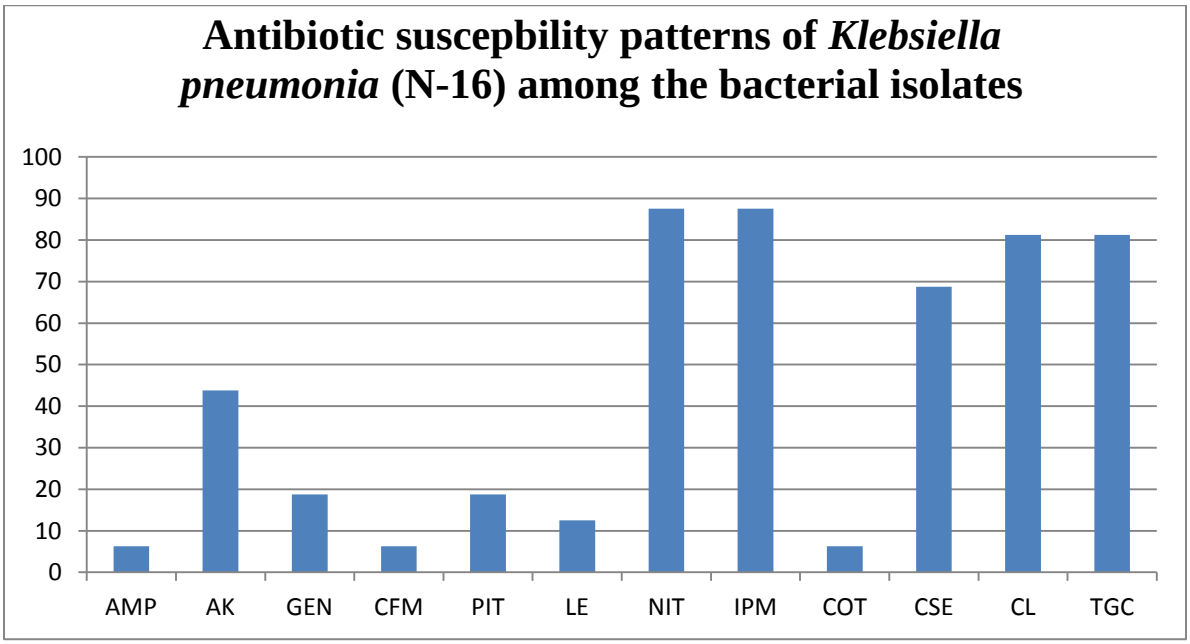


Table 5: Antibiotic susceptibility patterns of *Klebsiella pneumonia* (N-16) among the bacterial isolates.

ANTIBIOTICS	SENSITIVITY	
	NO	%
Ampicilin	1	6.25
Amikacin	7	43.75
Gentamicin	3	18.75
Amoxy/clav	00	

Cefixime	1	6.25
Cefuroxime	00	00
Cefotaxime	00	00
Ceftrixone	00	00
Ceftazidime	00	00
Cefipime	00	00
Nitrofuration	14	87.5
Levoflaxacin	2	12.5
Oflaxacin	00	00
Piperacillin+Tazobactum	3	18.75
Cefoparazone+Sulbactum	11	68.75
Trimethoprim/sulfomethazole	1	6.25
Imipenem	14	87.5
Meropenem	00	00
Colistin	13	81.25
Tigecycline	13	81.25


Antibiotic suscepbility patterns of Klebsiella pneumonia (N-16) among the bacterial isolates
100
90
80
70
60
50
40
30
20
10
0
AMP
AK
GEN
CFM
PIT
LE
NIT
IPM
COT
CSE
CL
TGC

DISCUSSION

The urinary tract infections are the common infections worldwide. The study of Tagore hospital has shown that out of total 150 urine specimens, 34% showed positivity. The prevalence rate of UTI in our study has shown the similar correlation with other studies from India like B. Foxman et al, (35.5%)(11) and M. Dash et al (34.5%)(12), whereas, the prevalence rate of UTI in a study from South Trinidad was a little higher(49%)(13). In our study, females showed the highest rate of infection (76.92%) than males (23.07%) to the tune of about 3:1. This highest prevalence correlates with the fact that females are more prone to UTIs than males because of their shorter urethra and closer proximity to anus. This finding has been supported by various other studies including the study by Prakash et al, in which the prevalence of UTI was also higher in female patients in the ratio of 2:1 (14). It is also an established finding in literature that approximately 1 in 3 women will require antimicrobial treatment for a urinary tract infection before age 24, and 40% to 50% of women will suffer from urinary tract infection during their lifetime.

In this study maximum affected age group was 51-60 years(50%) followed by 61-70 years(42%). The similarity to these results has seen in other studies as well where higher incidence of UTI among 50-69yrs (41.25%) was mentioned in a study by R. Raval et.al (15). As mentioned in our study, increasing incidence of UTI with advancing age is occurring due to increased use of indwelling catheters in elder patients, low hygienic practices, weak immune system due to spinal cord injuries, diabetes or multiple sclerosis etc.

Moreover, from total positive cases, 57% OPD and 42% IPD Cases were reported in our study. The reason for higher cases of positive cases in OPD patients may be due to community acquired infection, low social economical status, malnutrition, poor hygiene.

The most commonly isolated bacteria were Gram-negative with high prevalence of *Escherichia coli* 28(53.84%) followed by *Klebsiella pneumoniae* 16(30.76%), *Pseudomonas aeruginosa* 3(5.76%), *Proteus mirabilis* 1(1.92%). while 3 isolates of *Staphylococcus aureus* (5.76%) and a single isolate of *Enterococcus* spp. (1.92%) was found among gram positive bacteria. Our study indicates that *E. coli (54%)* is the most common cause of urinary tract infection, it is observed in OPD UTI cases (30.78%) and IPD UTI cases (23%) our study correlates with other one (Raval et.al) having IPD cases(35%) and OPD cases (39%).

Incidence of *E.coli* in our study was higher when compared with the Nigerian studies reporting (42.10%) [16]

In our study *Klebsiella pneumoniae (*30.76%) was the second most common causative agent of UTI. This result correlates with other study which reports *Klebsiella pneumoniae (28.5%)* as the second frequently isolated organism in UTI [17]. The other microorganisms including gram-positive ones contributed very little to our UTI cases.

While performing antimicrobial susceptibility on these positive isolates we analyzed that *E. coli* was 100% resistant to third generation cephalosporins and fluoroquinolones like ofloxacin. High level of resistance to the tune of 97% was also noted for Amoxycillin-clavulinic acid, Cefixime,

Cefuroxime and Cefipime and levofloxacin 78%. The drugs which showed higher susceptibility were colistin (100%), Nitrofuration (90%), Tigecycline (85%), Amikacin (78%) and Imipenem (67%). In a study by Juliana Christyaningsih et al, levofloxacin(75%) resistance while amikacin was highly sensitive(92%)[18] which is similar to our study while according to mohan koshaiya et al., study Ofloxacin(84%) was resistance[19], similar to our study(100%) while Nitrofuration(40%) sensitive opposite to our study(90%).In talukder's study Nitrofuration was 90% sensitive which favour our study[20].

In Klebsiella pneumoniae higher resistance to Amoxy/clav and Cefotaxim (100%), Cefixime (93%), Levofloxacin(87%).The drug which showed higher suscepbility were Nitrofuration (87.5%), Tigecycline(81%) and colistin(81%), acc to Akter et al, imipenem was 88% sensitive followed by Nitrofuration(80%) similar to our study[21].

As this study have found majority of antibiotics such as cephalosporins, fluoroquinolones are resistant and colistin, Tigecycline, nitrofuration and imipenem still has a role to play in the treatment of UTI as its antibiotic sensitivity profile is very effective.

CONCLUSION

UTIs are one of the common infections in the Indian community. The high prevalence of urinary tract infection was seen in older age group between 50-80 years due to increased use of indwelling catheters in elder patients, low hygienic practices, weak immune system due to spinal cord injuries, diabetes or multiple sclerosis etc.

Gram negative organisms being the most commonly isolated organisms in urinary tract infection and out of them *E. coli* was the most frequent causative agent. Urinary pathogens showed resistance to commonly used antibiotics like Cefotaxime, Ceftrioxone, Ceftazidime and Oflaxacin. 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 can alert us against indiscriminate usages of antibiotics to prevent development of resistance against an antibiotic.

REFERENCES

1)**Rutuja Raval, et al (2015)** clino-pathological. Features of Urinary Tract Infection in Rural India Advances in Infectious Diseases, 5,132-139

2)**D. Leigh** "Urinary Tract Infection," In: G.R Smith and S.F. Easma Charles, Eds., Topley and Wilson's Principles of Bacteriology, Virology and Immunity, Butler and Tanler Ltd, Frome and London, 1990, pp. 197-214.

3)**Devanand parakash et al(2013)** Distribution and Antimicrobial suscepbility pattern of Bacterial pathogens Causing Urinary Tract Infection in Urban community of Meerut city, India

4)**Forman B.** Epidemiology of urinary tract infections: incidence, morbidity and economic costs. Am J Med 2002; 113

5)**Gulati, S., Kher, V., Gupta, A., Arora, P., Rai, P.K. and Sharma, R.K. (1995**) Spectrum of infection in India Children with Nephrotic Syndrome. Pediatric Nephrology, 9, 431-434.

6)**A.M Awaness, M.G. Al-Saadi and S.A Aadoas,** 'Antibiotics Resistance in Recurrent Urinary Tract Infection," Kufa Medical Journal, vol. 3, 2000.

7)**Gonzalez, C.M and Schaeffer, A.J. (1999)** Treatment of Urinary Tract Infection: What's Old, What's New, and What Works. World Journal of Urology, 17, 372-382.

8)**Poonam U. Sharma and Ulka Bidwai (2013)** Int. J.Curr. Microbial. App. Sci 2(4):97-103

9)**Roberts, J.A. (1991)** Etiology and pathophysiology of pyelonephritis. American Journal of Kidney Diseases, 17, 1-9.

10) **Niranjan V. et al (2014)** Antimicrobial resistance pattern in Escherichia coli causing urinary tract infection among inpatients. Indian J Med Res 139, pp 945-948

11**) B. Foxman and P. Brown,(2013)** "Epidemiology of urinary tract infections: transmission and risk factors, incidence, and costs," Infectious Disease Clinics of North America, vol, no. 2, pp. 227-241,

12) **M. Dash, S. Padhi, I. Mohanty, p. pand, and B. parida(2013) "**Antimicrobial resistance in pathogens causing urinary tract infections in a rural community of Odisha, India," *Journal of Family and Community Medicine,* vol. 20, no.1, pp. 20-26.

13) **F.A. Orrett,(2001)** "Urinary tract infections in general practice in rural community in South Trinidad," Saudi Medical journal, vol.22, no. 6, pp.537-540

14) **K.Shigemura, K. Tanaka, H. Okada et al.,(2005)** "pathogen occurrence and antimicrobial susceptibility of urinary tract infection cases during a 20-year period (1983-2002)

at a single institution in japan," *Japanese Journal of Infectious Diseases,* vol. 58, no. 5, pp. 303-308 **I.O.**

15**)Okonko, L.A. Ijandipe, O.A. Ilusanya, et al.,(2009)** "Incidence of Urinary Tract Infection (UTI), South-Western Nigeria," African Journal of Biotechnology, Vol. 8, No. 23, pp. 6649-6657

16) **Rutuja Raval, et al (2015)** clino-pathological. Features of Urinary Tract Infection in Rural India Advances in Infectious Diseases, 5,132-139

17) **18) D, Sahm, C. Thomsbery, D. Mayfield, M. Jones and J. Karlowsky,(2001)** "Multidrug Resistant Urinary Tract Isolates of Escherichia coli: prevalence and patient demographic in the United States in 2000, and " *Antimicrobial Agents Chemotherapy*, Vol. 45, No. 5, pp. 1402-1406.

18**) J.C. Uwawzuoke and N. Ogbulie,(2006)** "Antibiotic sensitivity pattern of urinary tract pathogens in Port-Harcourt, Nigeria, *Journal of Applied Sciences and Environmental Management*, vol.10, no, 5, pp, 103-107

19) **Juliana Christyaningsih et al (2014)**The Pattern of Resistance of Antibiotics to *Escherichia Coli* Causes Urinary Tract Infection in East Java, Indonesia. Research Journal of Pharmaceutical, Biological and Chemical Sciences.

20) **Mahim Koshariya et al (2015)** Prevalence of pathogens and their antimicrobial susceptibility in catheter associated urinary tract infectionIAIM,2015, 2(4): 96-113

21) **Talukder, M.A.S., Bakheshwain, S.M. and Altasan, F. (1987)**.Urinary tract infection in children at the Armed Forces Hospital, Riyadh, Saudi Arabia-A pilot study.*Bangladesh J. Child. Health*. **11**, 55-58.

22) ***Akter et al (2013)***Antibiotic Sensitivity of Pathogens Causing Urinary Tract Infection. *Bangladesh Pharmaceutical Journal 16(1): 53-58,*

www.ncbi.nlm.nih.gov>NCBI>Litereture>pubMed Central(PMC)

Reference for an rivew literature:

1)**Hajarnis S.(1995)** Suspected urinary tract infection: identification of microorganism and sensitivity to antibiotics in seychellus. SMDJ P.3

2)**M.Vormena. A.J.A.M Vandervenb, A.Knolsc and M.E.E stabbering b, (1999).** Antimicrobial resistance patterns in urinary isolates from nursing home residents. Journal of Antimicrobial Chemotherapy. Volume 44, issue 1. Pp 133-166)

3)**Mezue Kenechukwu*,of ong chinekwu*, Nmezi Davidson*, Ugochukwu Obi Golibe* (2004)** study of Antibiotic Sensitivity Patterns in Urinary Tract Infections at a Tertiary hospital.JOURNAL OF THE UNIVERSITY OF NIGERIA MEDICAL STUDENTS.

4)**Hasan. A.S., Nair.D., Kaur.J., Baweja.G., Deb.M., Aggarwal., (2007)** resistance patterns of urinary isolates in a tertiary *Med. Coll. Abbottabad* 19 (1) Indian hospital, *J Ayub***)**

5)**EL. Mahmood Muhammad Abubakar(2009),** Antimicrobial susceptibility pattern of pathogenic bacteria causing urinary tract infections at the Specialist Hospital, Yola, Adamania State, Nigeria. Journal of clinical Medicine and Research vol.1 (1) pp.001-008

6)**Manikandan.S, Ganesapandian.S, Shigh.M, Kumaraguru.A.K (2011)** Antimicrobial susceptibility pattern of urinary tract infection causing human pathogenicbacteria).*Asian Journal of Medical Sciences* 3(2):56-60.

7)**Razak,S.K.,Gurushantappa.D.,(2012).**Bacteriology of urinary tract infection and antibiotic susceptibility pattern in a Tertiary Care Hospital in South India. *International Journal of Medical Science and Public Health*. Vol 1 109-122

8)**Bano.K et.al (2012).**Patterns of antibiotic sensitivity of bacterial pathogens among urinary tract infections (UTI) patients in a Pakistani population. *African Journal of Microbiology Research*. 6(2), 414-420.

9)**Patel et al (2012)** urinary tract infections (UTI) among patients at G. G. Hospital & Medical College, Jamnagar.*Nationa Journal of Community Medicine* Vol 3 (2012).

10)**Aghdam, V.B., Anzabi, Y., hassanzadeh, M., Anvarian, M. and Zangir, S.B.M. (2013).**Study of causative bacterial agents of urinary tract infections and determining their antibiotic susceptibility pattern in clients of Tabriz's Clinics. *Middle-East Journal of Scientific Research*. 13 (2): 183-186

11) **Rakesh et al (2013).** Prevalence and antimicrobial susceptibility pattern of *Klebsiella pneumonia* causing urinary tract infection and issues related to the rational selection of antimicrobials. *Sch. J. App. Med. Sci., 2013**;*** 1(5):395-399.

12) **Dash.M., Padhi.S., Mohanti.I., Panda. P., Parida.D (2013)**Antimicrobial resistance in pathogens causing urinary tract infections in a rural community of Odisha, India. *Journal of Family and Community Medicine* (20)1 20-60

13)**Khatri.B et al (2014).**Etiology and antimicrobial susceptibility pattern of bacterial pathogens from urinary tract infection**.** *Nepal Med. Coll. J 2012;* 14(2**)**: 129-132.

14) **Ashley Bryce, et al. (2016).** Global Prevalence of Antibiotic resistance in paediatric urinary tract infections caused by E. coli and association with routine use of antibiotics in primary care: systematic review and meta analysis. *BMJ* 2016;340:c2096. doi:10.1136/bmj.c2096.

Appendix I

A. LIST OF MATERIALS

1. Equipment

- Autoclave.
- Bunsen burner.
- Biosafety cabinet .
- 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.
- Gloves

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

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

3. Acetone-alcohol decolorize

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 DIFFERENT TYPES OF CULTURE MEDIA

1. CLED Agar (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.