Training Report

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:
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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.Naresh kumar** (Internal supervisor), Lovely Professional University and **Dr.Ramandeep kaur** (External supervisor) Tagore Hospital and Heart care Jalandhar (Punjab).This work has not been submitted in part or in full in any other university for any degree or diploma.

Name Raushan kumar

Date: 7/5/2016

Place: Lovely professional university

Phagwara

CERTIFICATE

This is to certify that **Mr. Raushan kumar** bearing **Registration Number** 11400012 has completed his/her Master of Science in Clinical Microbiology internship (programme code-240A), Lovely Professional University, Phagwara, Punjab has actively worked in a practical training from 1st January, 2016 to 30th April, 2016 in Tagore Hospital and Heart care Jalandhar(Punjab) under our supervision and proper guidance .He has worked in microbiology department with full responsibility. We wish her good luck and success in future endeavours.

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TOPIC

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LIST OF ABBREVIATION

CLED	Cysteine Lactose Electrolyte Deficient
E.coli	*Escherichia coli*
P.mirabilis	*Proteus mirabilis*
P.aeruginosa	*Pseudomonas aeruginosa*
S.aureus	*Staphylococcus aureus*
K.pneumoniae	*Klebsiella pneumoniae*
VUR	Vesico uretric reflux
UTI	Urinary tract infection
WHO	World Health organisation
Spp	Species
C.albicans	*Candida albicans*
ESBL	Extended spectrum beta latamase
MH	Muller Hinton agar
CFU	Colony forming unit

ANTIBIOTICS

Am	Ampicillin
Ak	Amikacin
Cfm	Cefuroxime
Cse	Ceftriaxone with sulbactum & EDTA
Imp	Imepenem
Mrp	Meropenem
Gen	Gentamicin
Nit	Nitrofurantoin
Nx	Norfloxacin
Of	Ofloxacin
Pit	Piperacillin/Tazobactom
Tgc	Tigecycline
Cl	Colistin
Cot	Co-trimoxazole
Ax/clav	Amoxy/clavulanic acid
Cft	Cefotaxime
Caz	Ceftazidime

ABSTRACT

Urinary tract infections (UTI) are common in humans and most often caused by uropathogenic *E. coli.* ESBL-producing *E. coli* and *K . pneumoniae* are increasing worldwide and they are frequently multidrug-resistant with limited treatment options so it reduces the effectiveness of treatment; thus patients remain infectious for a longer time,and increasing the risk of spreading resistant microorganisms to others. this is a complex problem driven by many inter connected factors. As such, single, isolated interventions have little impact .They are creating a major problem in hospitals , causing hospital and community acquired infections. Current study shows multidrug resistance of *E. coli* against the available anti biotics.

The present study in Tagore hospital and heart care jalandhar was conducted on 156 urine samples. Prevalence of *E. Coli was determined and* ESBL testing was done by modified double disc synergy test. (14.74%)samples were *E. coli* positive and ESBL was detected in (65.21%) of these *E. coli.* Maximum of the infections were found in the age group of 60 to 70 years. *E.coli* showed maximum resistance towards ampicillin, amoxycillin-clavulanic acid, cephalosporins and fluoroquinolones. Maximum susceptibility was observed for carbapenems, aminoglycosides, piperacillin- tazobactam, cotrimoxazole, nitrofurantoin, colistin and, tigecycline.

CHAPTER –1

INTRODUCTION

INTRODUCTION

A urinary tract infection is a condition in which one or more parts of the urinary system (kidneys,ureters,bladder,and urethra) become infected. They are common bacterial infections so thus every year affecting millions of people world wide [1-2]. According to a survey in india 2011 an estimate 93,000 UTI was detected in acute care hospital and annually global incidences of at least 250 million [3-4] .The incidences of UTI differs in males and females according to the age group. In infants it occurs more frequently in male than female due to improper cleaning of periurethral area and renal parencymal problem like VUR [2-3]. But as the age progresses this incidence increases in females than males due to shorter uretha than male so easily infected from bacterial infection . In old age male (50 +) they are more prevalent to exposure 10 to 30% chances in UTIs comparitively female due to hypertrophy of prostate in this age ratio between male and female 1 and 1.5[5-6]. In reproductive age of female UTI infections are more prevalent, due to taken Spermicides like nonoxynol-9 prolonged use of birth control device use (copper – T) they can upset the normal vagina flora . the ratio between male and female is 1 over 50 in age during 20 to 30 [7] . Acording to a data, *E .coli.* was responsible for 80% cases of UTI. *K.pneumoniae* caused 15% cases of UTI, *P.mirabillis , P.aeruginosa , Entero bacter , Serratia, Salmonella* less chances to causes UTI .Some fungus species such as *C.albicans* and *Cryptococcus neoformans* also causes UTI .Some viruses and parasite it causes UTI eg, *Herpes simplex* virus , *Adeno* virus , *Bk* virus and parasite such as Schistosoma *heamatobium* causes UTI [8-9]

1.1.1 Types of urinary tract infection

UTI are generally classified as :

- Uncomplicated or complicated ,depending on the factors that cause the infections
- Primary or reccurent ,depending on whether the infection is occuring for the first time or is a repeat occasion.

Uncomplicated Urinary Tract Infections (UTIs) : This is the most frequently encountered infections in the outpatient setting after respiratory tract infections. They effect women much more often than men.

Cystitis :- Cystitis ,or bladder infection,is the most common urinary tract infection .It occurs (the bladder and urethra) in women **.** In most cases ,the infection is breif and acute and only the surface of the bladder is infected .Deeper layers of the bladder may be injured if the infection becomes chronic.

Pyelonephritis (Kidney infection):-Sometimes the infection spreads to the upper urinary tract (the ureters and kidneys).This is called pyelonephritis or more commonly ,a kidney infection.

Complicated urinary tract infection

Complicated infections,which occur in men and women of any age ,are also caused by bacteria but they tend to be more severe ,more difficult to treat ,and repeated.

They are often result of :

- Some anatomical or structural abnormality that harms the ability of the urinary tract to clear out and therefore bacterial infection take place.
- Cathetar use in hospital setting or chronic indwelling cathetar in the outpateint setting ,also cause bacterial infection
- Bladder and kidney dysfunction,or kidney transplant (especially in the first 3 months after transplant).

Reappearance occur in up to 50-60% of pateints with complicated urinary tract infection if the basic structural or anatomical abnormalities are not corrected.[10-12]

1..2 Pathogenesis of UTI

Bacteria can invade and cause UTI through either two routes i.e. ascending route or descending route. The cells of bladder are coated with glycosaminoglycan mucus. Bacteria stick to this layer and if this layer is absent then , bacteria move and grows rapidly [13]

Ascending route:-

Urinary tract infection in female develops when pathogenic microbes colonize in the urinary tract and displace the normal floara *(Streptococcal* species ,diptheriods ,and *Lacto bacilli*) most uropathogen originate in the rectal floara and enter the bladder via the urethra. Bacterial infection develops depended upon the particular organisam ,the size of inoculum ,and host defence factor .Once the microbes ascend in to the bladder ,they may multiply and then pass up the ureters[14].

Heamatogenous route :-

Infection of renal parenchyma by blood born micro organisam in human , heamatogenous route infection is less commonly than ,Ascending route infection .Most prevalent of causative microbes Gram positive organisam (*S.aureus*) is mainly responsible for bacteriaemia , Gram negative bacilli raerly cause infection in heamatogenous route[14].

1.3 Hospital acquired UTI

It can spread from the hospital environment, nursing home environment, rehabilitation facility, clinic,or other clinical settings. Infection can spread to the susceptible patient in the clinical setting by a number of means. Health care staff can spread infection, due to contaminated equipment like bed lining and indwelling urethral cathetar.Ipd patients with a long term indwelling catheter are most prevalent to bacteriuric,this infection can originate from the outside of environment,through another infected patient, or microbes coming from the patient's own skin commensal flora .Urinary catheter provides a focus for bacterial biofilm formation in some cases the sources of infection cannot be determined. Estimated roughly 1.7 million hospital-associated infection world wide occurs every year, either Gram positive or Gram negative .The infection caused by two or more organisms in which most prevalent is *E .coli* and *Klebseilla . spp* some time acid fast bacilli also causes UTI. One estimate mortality rate in Hospital aquired UTI is aproximately 99,000 deaths in every year .

Direct contact transmission :-

This involves a direct body surface to body surface contact and physical transfer of microorganism between a susceptible host and infected colonized person takes place. Direct contact transmission also can occur between two pateints ,with one serving as the source of infection and other as a susceptible host.[15-16]

1.4 Community acquired UTI

They are usually spread from external vaginal introitus to the bladder or external urethral meatus. Female are more prone to infection in age between 16 to 35 years due to shorter urethra than man.The chances of infection is 50 times more in female than male due to increases sexual activity leads to more frequent urinary tract infections especially when a spermicide is used and there have more than one sexual partner. Causative agent ,some time virus and fungal also causing uti community acquired urinary tract infections mostly related to urinary catheterization involve a much broader range of pathogens including *E.coli* (27%), *K.pneumoniae*(11%), *P.aeruginosa* (11%), *Enterococcus* (7%) and the fungal pathogen *C.albicans* (9%), among others. Urinary tract infections due to *S. aureus* typically occur secondary to blood-borne infections. *Chlamydia .trachomatis* and *Mycoplasma*

genitalium can infect the urethra but not the bladder.These infections are usually classified as a urethritis rather than urinary tract infection.[15-17]

1.4 Role of antibiotic resistance in UTI

Antibiotic resistance is a major clinical problem in world wide .The epidemiological research estimates an incidence of 8 million cases of UTI every year in which 5 million women and 3 million man. The emergence of microbial resistance problem is increasing day by day due to the inappropriate uses of antibiotic and the inadequate dosage of antibiotics. If Proper uses antibiotics helps reducing the frequency and clinical expression of UTI .Antimicrobial resistances have been reported as the most predominant pathogenic microorganisms like *S. aureus, E. coli, P. aeruginosa,*and *K. pneumoniae* [18-19]

1.5 Risk factor of urinary tract infection

The over use and inadequate doses of antibiotics can damage periurethral flora, allowing uropathogens to colonize and make biofilm formation so antibiotic not easily reach the target and subsequently to infect the urinary tract

1.5.1 GENDER :-

- Females are more prone to urinary tract infections than males.This is because the urethra is shorter which cuts down the distance that bacteria have to travel to reach the bladder. The urethral opening is also much closer to the anus and come into contact with bacteria more reaidly.
- As males age,they often have enlargement of the prostate gland .This causes an obstruction to the flow of urine.When the bladder does not completely empty ,bacteria are not fully flushed out and can multiply and cause an infection.
- In males who are not circumsised ,there are more bacteria livinig closer to the opening of the urethra which increases their risk for developing an infection.
- After menopause,female are also more prone to infections due to lack of estrogen[18-19].

1.6 Resistance mechanisam of microorganisam

Micro organisam have several ways to resistance the antibiotic via de novo mutation or the aquisition of resistance genes from other gram negative organisams through plasmid .Some other resistance mechanisam are shown below.

Decreased cell permeability
Lack of entry
Greater exit
Active eflux
Altered target modification of drug receptor site
Synthesis of metabolic pathway [20]

1.6.1 Mode of diffrent groups of drug resistance

Aminoglycosides resistance	mutation of ribosomal binding site
Flouroquionolone resistance	alteration of alpha subunit of dna
Beta – lactam resistance	fails to cross membrane
Vancomycin resistance	too large fails to gram negative outer membrane[21-23]

1.7 ESBL

Critically ill pateint are especially prone to the infection and the nature and epidemiology of causative agent can verry tremendously especially a multi drug resistant organisam are a major concern to mortality and morbidity . This is higher and difficult to identify by routine laboratory assays this can lead to delay an anti bacterial therapy,for infected pateint. ESBL is enzyme produced by gram negative lactose fermenter organisam such as *K..pneumonia* and *E. coli* they are hydrolyze the beta–lactam ring of antibiotic. ESBL enzyme transfer one generation to second generation through plasmid and gain resistance. Some non lactose fermenter organisam also produce ESBL such as *A.baumannii* and *P. aeruginosa* . ESBLs are usually inhibited by β-

lactamase inhibitors, such as clavulanic acid, sulbactam or tazobactam.For furthre uses of combination in this drugs in infected pateint against to ESBL producing organisam[24] .

1.7.1 ESBL enzyme devided in three major groupes

Cephalosporinase : this group of enzyme not inhibited by clavulanic acid
Broad spectrum enzyme : they are inhibited by clavulanic acid
Metallo beta – lactamases : hydrolyse the beta lactam antibiotic such as pencillin , Cephalosporins and monobactam, but inhibited by clavulanic acid. [25-26]

1.7.2 Types of ESBL

TEM - beta – lactamases	there are two types of TEM -1 and TEM -2 they are found in *E . coli* and *K..pneumoniae*
SHV - beta – lactamases	produce in *Enterobacteriaceae P.aeruginosa* and *Acinetobacter spp*
CTX - M beta – lactamases	produce in *Enterobacteriaceae P.aeruginosa* and *Acinetobacter spp*
Oxa type ,per type ,ges type 1	Produce many gram negative organisam

1.8 Various method for detection of ESBL

Types of test :

1.8.1 Epsilometer test

this is ready to use strip test determination the precise MIC value of a wide range of anti microbial agent against different micro organisam [28] .

Broth micro dilution test : This is phenotypic confirmatory test in which using ceftazidime and ceftazidime plus clavulanic acid. make >3 fold serial dilution decrease in MIC of either cephalosoprin in the presence of clavulanic acid [29].

Modified double disk synergy test : This is phenotypic method to detect the ESBL production in gram negative bacilli. among them, the double disc synergy test (DDST) which uses the third generation cephalosporins (3GC), is a simple and a reliable method. But the coexistence of ampC may give false negative results [30].

Double disk synergy test : This test is done by using a disc of clavulanic acid,cefotaxime ceftazidime and cefpodoxime placed around the clavulanic acid disc keep in the distance between 15 to 20 mm [30].

Molecular techniques : Plasmid profile ,pulse feild gel electro phoresis , ribotyping ,pcr , random amplifeid poly morphic DNA

1.9 Frequency of occurance

ESBLs are the main cause of resistance to beta-lactam antibiotics in members of the *Enterobacteriaceae.* . Due to the clinical importance of the detection of ESBLs, screening and confirmatory methods have been routinely used to investigate the production of these enzymes in *E. coli* and *Klebsiella spp*. As their occurrence in other *Enterobacterias* has been increasing, it becomes essential to evaluate their occurrence

- The occurrence of ESBL acording to a data collection in india *E.coli* occurance ,(7.4%) *p. mirabillis* ,(7.5%) and *k.pneumoniae* (21.5%) it shows the frequency of ESBL is higher in (india)[31 -32]

CHAPTER – 2

AIM&OBJECTIVES

AIM AND OBJECTIVES

OBJECTIVES

2.1 General objectives

To determine the prevalence of ESBL producing *E.coli* from urine sample. Double disc synergy test method used for detection of ESBL and antibiotic susceptibility pattern of isolates in pateint visiting Tagore Hospital and Heart care Jalandhar.

2.2 Specific objectives

- To isolate and identify the pathogenic bacteria in urine sample collected from pateint visiting Tagore Hospital and Heart care Jalandhar
- To study the ESBL positive organisam in different age group with relation to gender.
- To perform antibiotic susceptibility test of bacterial isolates.

CHAPTER-3

REVEIW OF LITRATURE

3.1 REVIEW OF LITRATURE

Knothe et al., 1983 .conducted a study in which plasmid transferable β-lactamase was first time showed in the 1960s and named TEM-1 after the name of a Greek girl Temoniera, who carried *E. coli* from which the TEM-1 enzyme was discovered. A large number of plasmid-transferable enzymes have been discovered since the 1980s. The ESBLs related problem was also begun in Western Europe in early 1980s . In *K. pneumoniae*, ESBL was first described in 1983 in Germany (Knothe et al., 1983). [33]

Sirrot et al., 1992 conducted a study the frequency of nosocomial ESBLproducing *K. pneumoniae* was 13% in a survey carried out between 1988-1990 in france [34].

Syeda Jabun Nahar et al conducted a study in dhaka (sdc). A total 2000 urine samples were analyzed during the winter (November 1999 - January 2000) and summer (April -June 2000) which, showed 47.6% E. coli infection. In summer, the prevalence of the UTI were 60.7% in females and 39.2% in males, similarly in winter, the prevalence was 61% in females and 39% males [35] .

Winokur et al., 2001 conducted a study for ESBL strains showed high levels of co-resistance to aminoglycosides, tetracycline, trimethoprim-sulfamethoxazole, and ciprofloxacin. Imipenem remains highly effective against ESBL strains. Organisms expressing an ESBL are widely distributed worldwide, although prevalence rates are significantly higher in certain geographic regions. it was demonstrated that about 5.3% of *E. coli* in the United States harbored ESBLs [36]

Meera sharma et al 2002 in pgimer chandigarh 4800 sample taken in between three months jan to march 2000 . 110 sample positive in which most prevalent was *P. aeruginosa* (45.4%) *Acinetobacter spp.* (39.0%), maximum resistance was observed for strains of *Acinetobacter baumanii*. however, all the strains of nonfermenting bacilli except *PP fluorescence* showed very high level of resistance as depicted in . Piperacillin (41.8% resistance) and amikacin (47.2% resistance) were the most effective in vitro antibiotics [37]

C. R. V. Kiffer,et al Iran (2003) 3363 urine sample was collected in which 404 sample positive (12.5%) , in which *e .coli* 290 (72%) positive, *enterobacter* 23 (5 .7%) positive *p.mirabilis, k.pneumoniae* and *p. aeruginosa* 94 (15.8%) positive.[38-39]

Ghedria et al., 2004 In a tunisian study conducted in the hospital of Fattouma Bourguiba, 96% of uropathogens strains were resistant to ampicillin, 26 amoxicillin and cefalotin, 67% of strains were resistant to amoxicillin with clavulanic acid and only 34% of them were resistant to cotrimoxazole(Pape et al., 2004; [40] .

Yuksel et al., 2006 conducted In a turkish study to assess the resistance patterns of urinary isolates to commonly used antimicrobials and to evaluate the options for empirical treatment of UTI ampicillin has the highest resistance rate against E. coli (74.2%) followed by co-trimoxazole (61.3%) whereas nitrofurantoin has the lowest resistance rate against E. coli (2.2%), followed by amikacin (4.9%), ceftriaxone (7.5%) and ciprofloxacin (12%) [41] .

Kiffer et al ,2007 conducted a study , in terms of patient's population (both males and females of any age), number of isolates (35 782), and selected age groups. They also found (1) a lower percentage of *E. coli* isolation in patients younger than 13 years or older than 60 years (69.0% and 68.8%, resp.) as compared to the age group 13–60 years (79.7%); (2) a higher difference in *E. coli* rates of isolation, between males and females, in the youngest (27.2%) and the oldest (25.8%) age groups with respect to the 13–60 years age group (8.9%); (3) a higher prevalence of *E. faecalis* (16.4%) and *P. aeruginosa* (14.7%) in males older than 60 years, approximately three and six times higher, respectively, as compared to females of the same age group; (4*) P. mirabilis* to be the second leading cause of UTI in pediatric population [42]

Mshana et al 2009 done a study in a total of 377 Gram-negative bacteria (GNB) recovered from 377 clinical specimens they were analyzed and out of which 76.9% were *Enterobacteriaceae*. Among all GNB, 110/377 (29.2%) were found to be ESBL producers. Species specific ESBLs rate among *Klebsiella pneumoniae*, *Escherichia coli*, *Acinetobacter spp*, *Proteus spp* and other *enterobacteria* were 63.7%, 24.4%, 17.7%, 6.4% and 27.9% respectively . [43] .

L. S. Briongos et .al conducted a study in 2009, 23,839 urine samples were processed in the Microbiology Service of rio hortega university hospital (Valladolid, Spain) of which 4522 were positive. *e. coli* was isolated in 60% ($N = 2725$), of which 6% ($N = 162$) were ESBL-producing

strains(14] - In 2010, 30,438 cultures were processed of which 5062 were positive. *e. coli* was isolated in 59.4% (*N* = 3007), of which 7% (*N* = 210) were ESBL-producing strains.)[44]

A1 Benwan et al.2010. ESBL-producing isolates was significantly larger among in patients (15.4%) than in out patients (4.5%). Moreover, data collected over three years in kuwait showed that the levels of ESBLs were lower in community isolates of *k. pneumoniae* (17%) and *e. coli* (12%) than in the corresponding hospital isolates (28% and 26%, respectively)[45] .

Rupinder et al., 2013 ESBL positive isolates were obtained from 63 male and 105 female patients with a male to female ratio of 1:1.6 . Ninety two percent of ESBL producers were inpatients. Out of 357 isolates, there were 246(69%) E. coli, 91(25.5%) K. pneumoniae and 20(5.6%) P. aeruginosa isolates in a tertiary hospital in Patiala, Punjab. Maximum number of isolates were obtained from urine sample[46]..

Ghaley et al. 2009 conduct a another study showed that the antibiotic ofloxacin (58% sensitivity) is more effective against isolated UTI isolates followed by amikacin (54% sensitivity), chloramphenicol (52% sensitivity), norfloxacin (51% sensitivity), azithromycin (47% sensitivity), ampicillin (11% sensitivity), cefotaxime (2% sensitivity), and penicillin-G (0% sensitivity) (Mangiarotti, 2000).

Adeymo et al. 1994 reported that all the urinary isolates were poorly susceptible to trimethoprim-sulfamethaxazole and ampicillin but good susceptible to nalidixic acid, nitrofurantoin, norfloxacin, and gentamycin.

CHAPTER –4

MATERIAL AND METHOD

MATERIAL & METHOD

4.1 MATERIALS

A list of materials used during the study is given appendix -1

4.2 METHODS

This study is carried out from 1st January to 30th april,2016 in the microbiology laboratory of Tagore Hospital Jalandhar in order to isolate and identify organisam from urine sample along with their antibiotic susceptibility pattern there by covering a total period of 4 months. A total of one hundred fifty six urine samples were collected from the ranging in age from 21 to 91 years.

4.3 COLLECTION 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 bottles 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 charecteristics of urine sample were noted.

4.4.2 Microscopic Examination

The urine sample 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 10X and 40X objectives.Sample with >10 white blood cells/mm3 were considered as pyuric .

4.5 Culture of sample

The urine culture is used to diagnose a urinary tract infection (UTI) and to identify the bacteria or yeast causing the infection. It may be done in conjunction with susceptibility testing to determine which antibiotics will inhibit the growth of the microbe causing the infection.

4.5.1Culture procedure in CLED agar

1. Inoculated the urine sample through sterile inoculating loop the tip of loop insert in to the sample and inoculated in culture plate make inoculum in the periphery of plate .
2. Same loop without heat sterile start with edge of the inoculum and done primary streaking .
3. After heated the inoculating loop and done secondary streaking .Again heated the loop and done tertiary streaking and finally make zig zag motion.
4. Incubated for 24 hr at 37^0c aerobically for asceptic condition in incubator .

4.5.2 Results:

The diffrent types of colony after 24 hr incubation were observed on the basis of colony charecteristic either lactose fermenter or non lactose fermenter i.e. yellow colony with deeper yellow (LF) and center mucoid with whitish blue colonies (NLF) and observed the colony size ,shape,edges ,concave or convex

4.5.3 Motility test

Hanging drop method :

A clean glass in which make a ring of vaseline around the center of slide and taken a cover slip in which placed over one drop of specimen, after slide is inverted over the cover slip due to hang the drops and obsereved under microscope (40 X) and focus in the edge of cover slip .

4.6 Gram stain

This is most valuable stain in microbiology, it is used to distinguish between gram negative and gram positve organism .

Reagent

- Crystal voilet
- Gram iodine
- Acetone

- Safranin

Procedure :

1. A clean dry slide was taken , in which placed over a drop of distilled water and mix with single isolated colony , mix it many time in circular motion
2. Heat fixed slide was taken and placed over staining rack
3. Poured primary stain crystal voilet for 1 minute after washed through d/w
4. Gram iodine poured was 30 second after washed through d/w
5. Decolourise used acetone or alcohal poured for 1 minute after washed through d/w
6. Flood slide with safranin solution and allow to counterstain for 30 seconds.after washed through d/w
7. Drain and blot dry with tissue paper and observed under oil immerson (100 x)\
   Result: Gram positive-blue colour Gram negative –red colour

FIG: GRAM POSITIVE COCCI

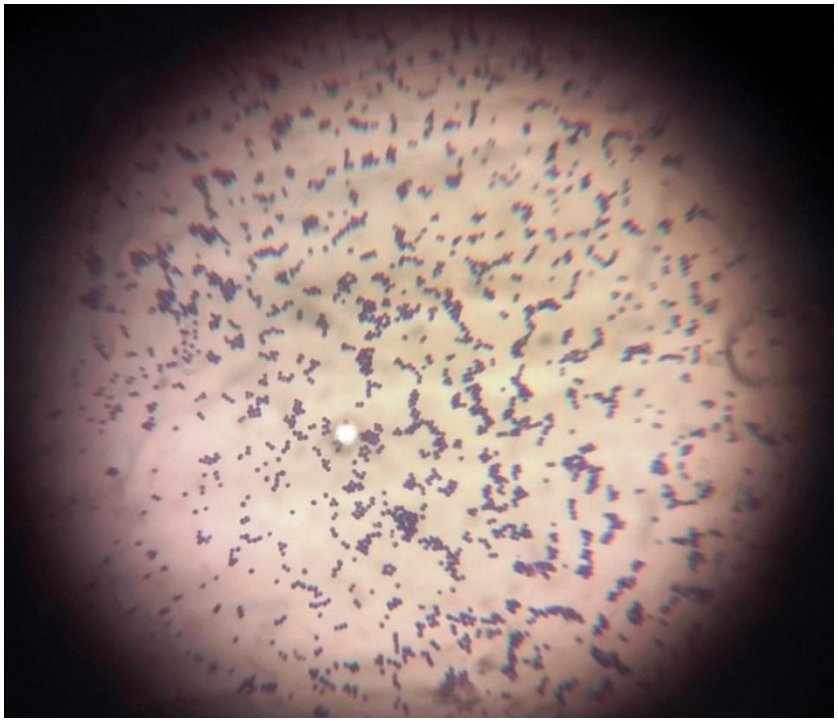
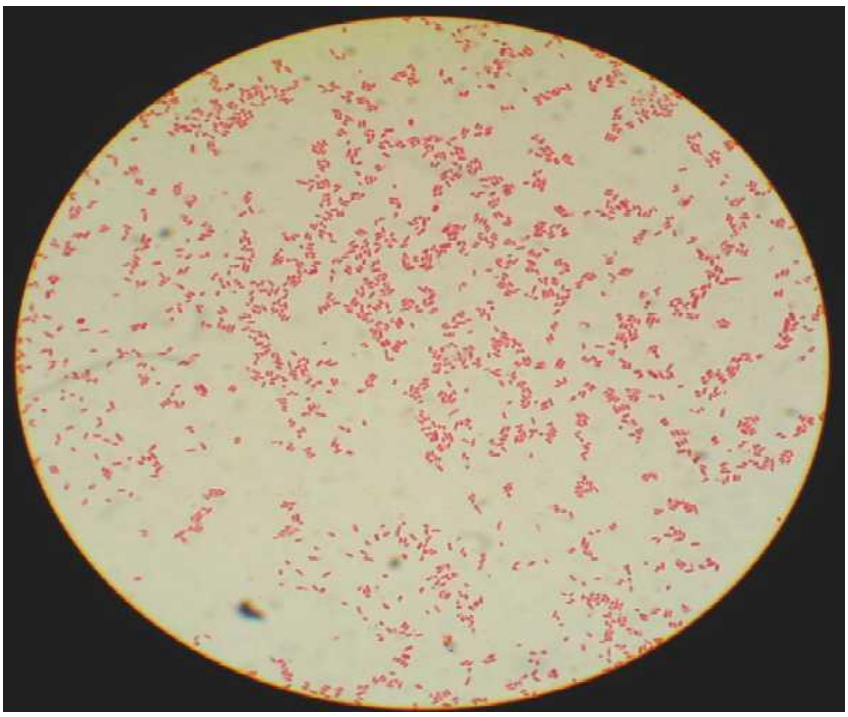


FIG:2 GRAM NEGATIVE BACILLI



Biochemical test for identification of *E. coli* :

Triple sugar iron test

This agar is used for identification of gram negative bacilli on the basis of glucose ,sucrose and lactose fermentation and hydrogen sulphide production.

Procedure :-

- With a sterilized straight inoculating loop touch the top of a well-isolated colony
- Inoculate TSI Agar by first stabbing through the center of the medium to the bottom of the tube and then streaking on the surface of the agar slant.
- Leave the cap on loosely and incubate the tube at 35°C for 24 hours

Slant /butt	Colour	Interpretation
K/A (alkaline/acidic)	Red /yellow	Glucose fermented only peptone catabolized
A/A (acidic /acidic)	Yellow/yellow	All three sugars fermented
K/K (alkaline /alkaline)	Red/red	No fermentation peptone catabolized
NC/NC	Not colour change	No fermentation
Black percipitate	-	H2s production
K/A G,	-	Glucose fermentation only ,gas produced
A/A G,	Yellow /yellow with bubbles	Glucose and lactose or sugar fermentation ,gas produced

FIG:3 TSI SLANT

E.coli ***Salmonella Typhi*** ***Proteus Mirabillis*** **Control**

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 is indole .

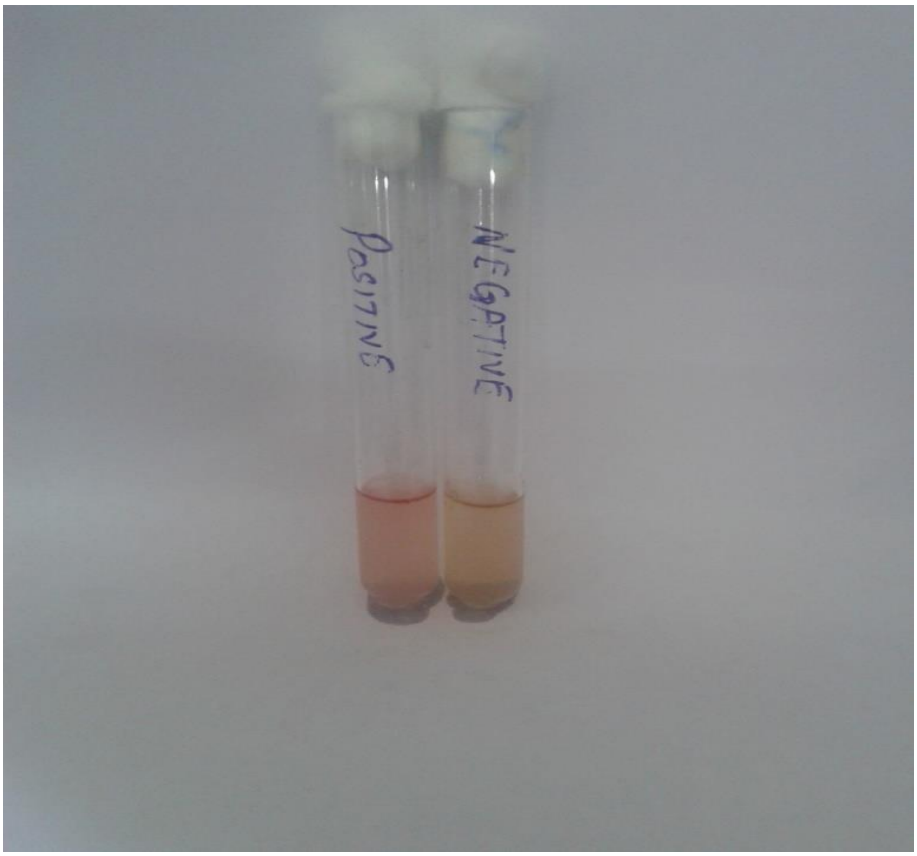
- Inoculate a single isolated colony in the peptone broth medium and incubate at 37^0c for 24 hr
- Aid with one drop of kovac reagent after over night incubation and shake gently.

Result

Red colour ring appear – positive

Greenish colour ring appear –negative

FIG:4 INDOLE TEST



ANTI MICROBIAL SENSITIVITY TEST

Antimicrobial susceptibility testing as a routine procedure in all microbiology laboratories. In laboratories it can be made available by using antibiotic disc which will diffuse slowly into the medium where the suspected organism is grown. Antimicrobial susceptibility tests (ASTs) basically measures the ability of an mueller hinton agar .

Mueller Hinton Agar (MHA):- The use of a suitable medium for testing the susceptibility of microorganisms It is a non-selective, non-differential medium It contains starch . Starch is known to absorb toxins released from bacteria, so that they cannot interfere with the antibiotics. It also mediates the rate of diffusion of the antibiotics through the agar.Antibiotic or other antimicrobial agent to inhibit the in vitro microbial growth. Sensitivity test was performed by kirby bauer method with available disc on.

Procedure :-

1. Single colony was passed in peptone broth for 3 to 4 hr at 37^0c
2. After 4hrs a cotton swab was dipped into inoculum and streaking with swab on muller hinton agar plate was done three to four times over the entire agar surface.
3. Wait few minute to dry the surface
4. Using a sterile forceps disc diffuse in the agar plate ,seven disc may be applied one disc in center and 6 in periphery whole plate daimmeter is 100 mm.
5. Plates were incubated in aseptic condition at 37^0c for 24 hr
6. After incubataion zone of complete growth inhibition measured with calibrated scale
7. Acording to zone, interpret the result sensitive ,intermediate,and resistant

Antimicrobial agents in gram negative organisam.

imepenam	Ofloxacin
amikacin	Cefuroxime (cfm)
Piperacillin tazobactom	Nitrofurontoin
meropenam	Colistin
norfloxacin	Gentamicin
cotrimoxazole	Tigecycline
ampicillin	Cefoperazone /tazobactom
amoxy /clav	Cefotaxime
cefixime	Ceftriaxone
ceftazidime	Levofloxacin
cefepime	

Antibiotic sensitivity was performed by the Kirby- Bauer, disc diffusion method.

FIG:5 Showing AST plate and zone of inhibition

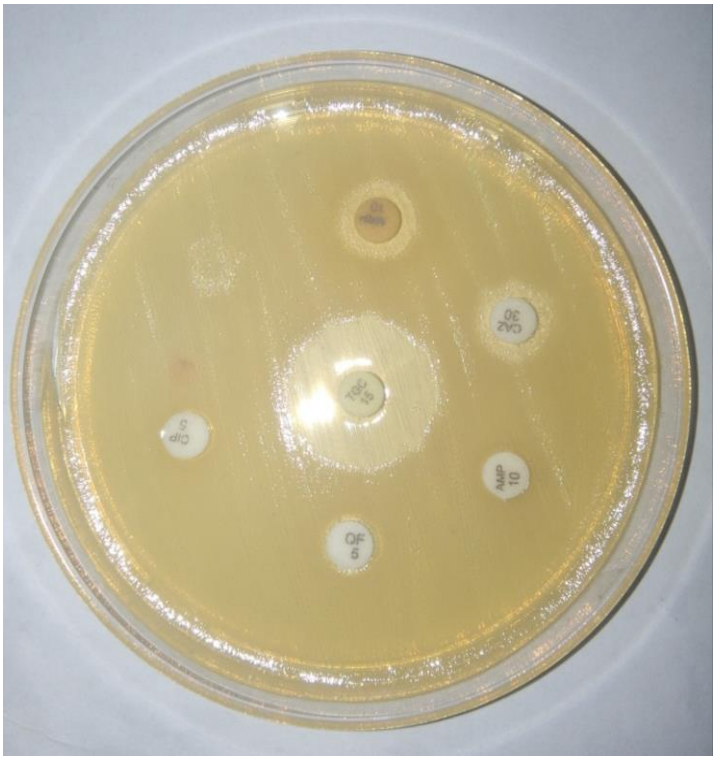
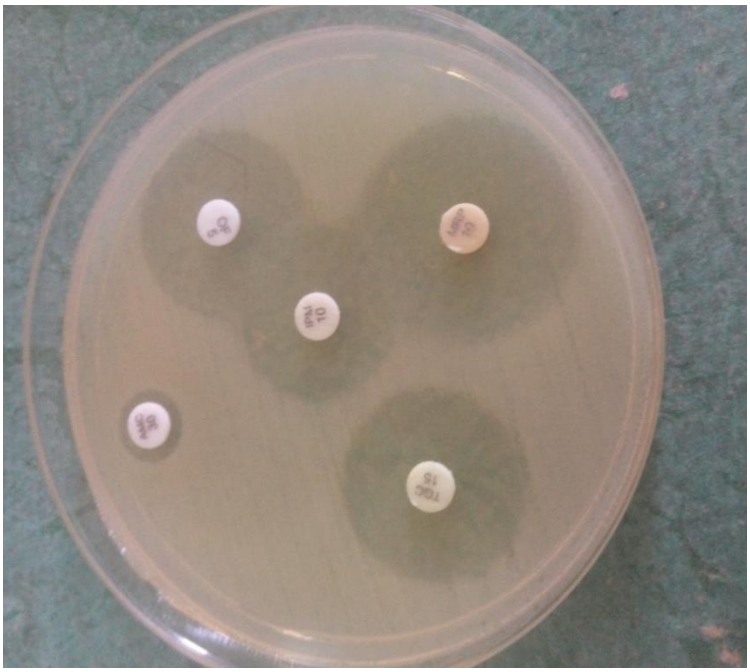


FIG :6 Showing ESBL positive.



CHAPTER –5

RESULT

RESULT

The study was carried out in Microbiology Laboratory of Tagore Hospital and Heart care Jalandhar from 1st january to 30th april 2016 there by covering a total period of 4 months .Urine sample collected were processed and analyzed by standard microbial techniques.

In this study a total of 156 urine sample were collected and processed for culture and sensitivity testing.Urine sample of pateint of all age groups (21 to 91years) and both sexes were processed.A total 45 diffrent organisams were isolated from 156 urine samples thus culture positive was 28.8%(i.e ,45)and negative is 71.1% (i.e.156) as show in table.

Table 1;Organisams wise distribution of positive cases (n=45)

Types of organisam	**Positive cases**	
	NO.	**%**
E.coli	23	51.08
Staphylococcus aureus	7	15.61
Klebsiella pneumoniae	5	11.11
Pseudomonas aeruginosa	3	6.66
Enterococcus faecalis	3	6.66
Citrobacter freundii	2	4.44
Enterobacter	1	2.22
Proteus mirabillis	1	2.22
TOTAL	45	100

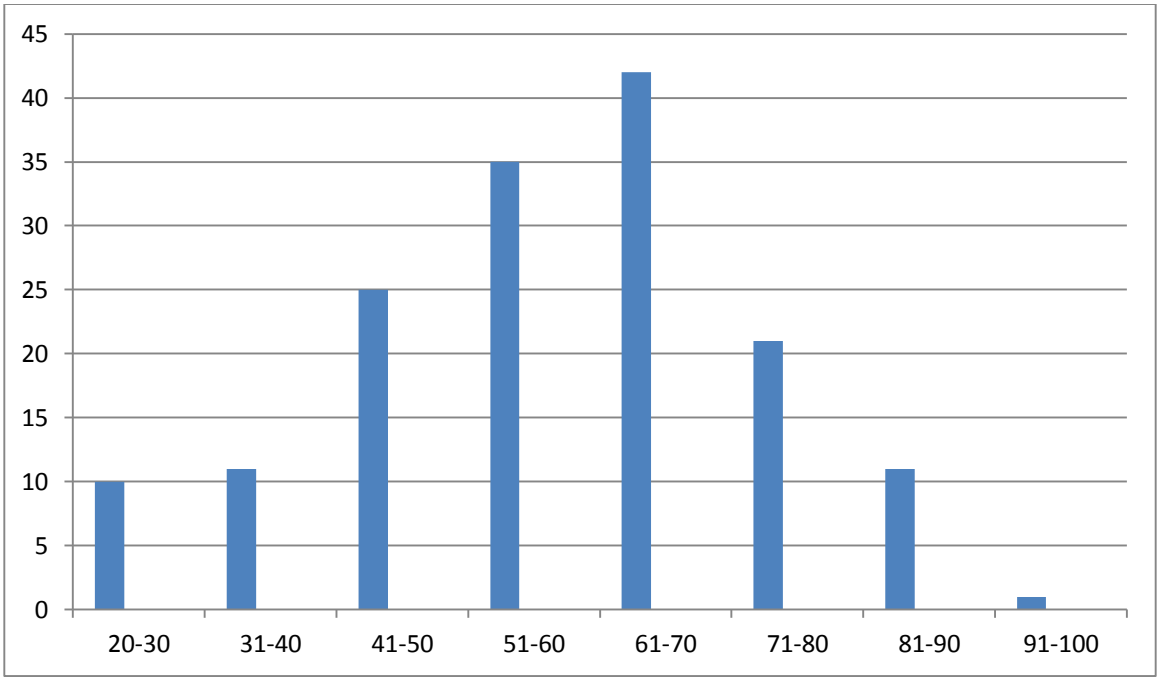
More than half (51.68%) cases reported as *E.coli* followed by (15.61%),Klebsiella (11.11%),Pseudomonas (6.66%), Enterococcus (6.66%) ,Citrobacter (4.44%),Enterobacter (2.22%) Proteus (2.22%)respectively.

Table 2: Sex wise distribution of the cases acording to their test results (n=45)

Gender	**Total cases**	**Positive cases**	**%**
Male	**77**	**20**	**44.45**

Female	**79**	**25**	**55.55**
TOTAL	**156**	**45**	**100**

Chart 1 : Age wise distribution of E.coli positive cases



5.1 Table 3 : Age wise distribution of pateint with positive cases.

Age groups	No of pateints	*E. coli* positive
21 to 30	10	3
31 to 40	12	-
41 to 50	24	4
51 to 60	34	5
61 to 70	43	5
71 to 80	21	4
81 to 90	11	2
91 to 100	1	-
total	156	23

Table 4 :Gender wise distribution of pateint with positive cases male and female

AGE	GENDER			
	MALE NO :	POSITIVE CASES	FEMALE NO	POSITIVE CASES
21 -30	3	1	7	2
31-40	8	1	4	1
41-50	9	5	15	4
51-60	19	4	15	6
61 -70	21	7	22	5
71-80	9	1	12	4
81-90	7	3	4	1
Above 90	1	-	-	-
TOTAL	77	22	79	23

Out of 156 urine sample collected 77(49.35%) were from male pateint with(22, 14.11%) positive cases and 79 (50.64%) were from female pateints with (23 14.74%) positive cases .The samples were collected from age group ranging from 21 to 91years .Age group 61-70 year was found to be most affected age group(12, 24.66%) followed by 51 to 60 years age group 30 to 40 years being the least affected group.

Chart 2: Growth pattern of total sample

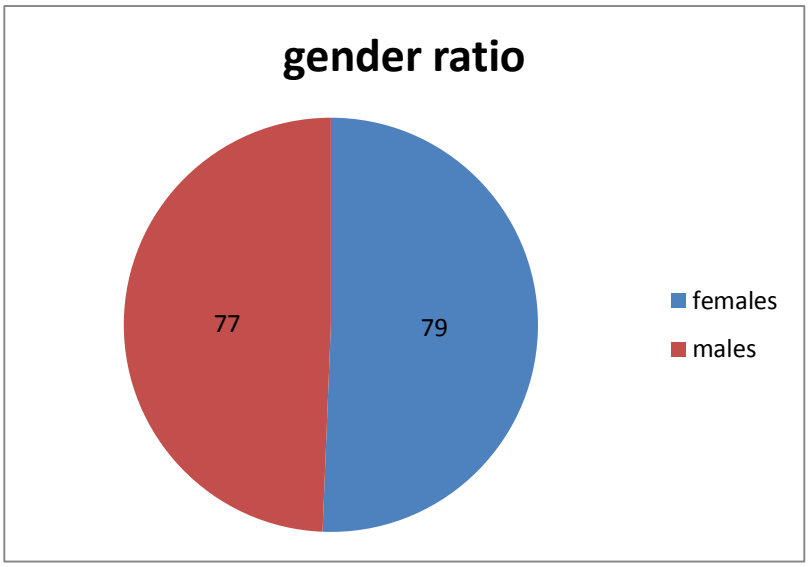
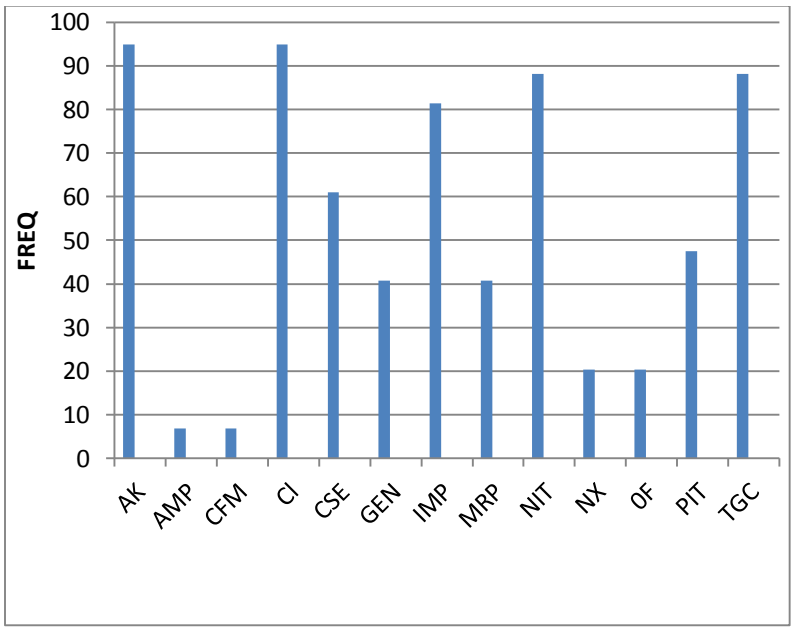


Chart 3 :Antibiotic susceptibility pattern of E.coli



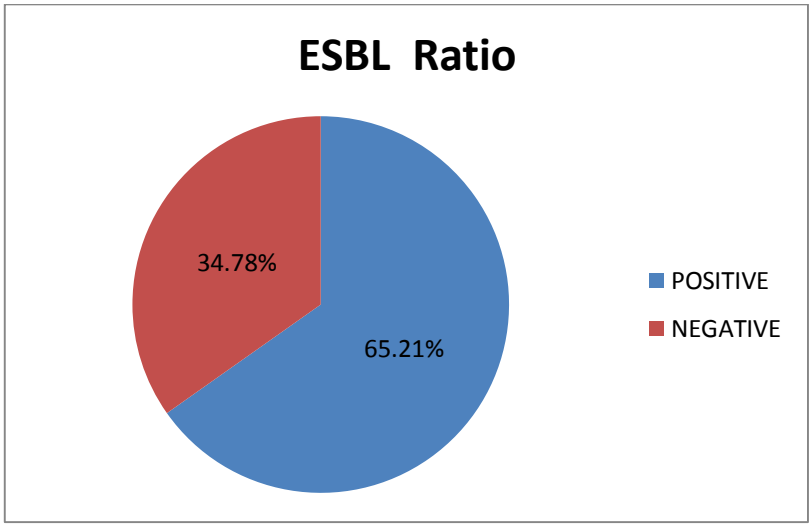
5.2 ANTIBIOTIC SUSCEPTIBILITY PATTERN OF THE BACTERIAL ISOLATES

5.2.1 Antibiotic susceptibility patterns of E.coli (N=45) amongs the bacterial isolates

Table 5: Antibiotic susceptibility patterns of E.coli (N-45) amongst the bacterial isolates

ANTIBIOTIC	% SENSITIVE
colistin	94.95
amikacin	94.95
imepenam	81.39
nitrofurontoin	88.17
tigecycline	88.17
Ceftriaxone /sulbactom	61.4
Piperacillin/tazobactom	47.47
meropenem	40.69
gentamicin	40.69
norfloxacin	20.34
cotrimoxazole	33.91
ofloxacin	20.34
ampicillin	6.78
cefuroxime	6.78

Chart 4: ESBL ratio positive /negative



Out of 156 urine sample collected 23 were E.coli positive in which 15 sample was ESBL positive (65.21%) and 8 sample were ESBL negative(34.78%)

CHAPTER -6

DISCUSSION

DISCUSSION

Urinary tract infection is one of the commonest bacterial infections. The present study provides epidemiological data on ESBL-carrying enterobacteriaceae in the clinical setting of a Tagore hospital Jalandhar. A total of 156 urine specimens were received from Tagore Hospital Jalandhar Jan 2016 to April 2016 and these were processed in the laboratory .Significant bacteria (cultures with $>10^5$ colony forming units (cfu) of bacteria /ml of urine)was found in 45/156 (28.84%)of the urine specimen.Gram negative bacteria were more prevalent (84.45%) and Gram positive bacteria (15.55)% . Identification was done on the basis of morphological ,biochemical,and phenotypic charecteristics. Acording to our current study showed 14.8% sample was *E.coli* positive this is lower compared to Mashana et ,el in which out of 377 sample procesed in which 24% found *E.coli* and 64% *K .pneumoniae* positive[43]. In current study show 65.21% is ESBL positive and 34.79% is ESBL negative . This is comparitively higher acording to Rupinder et al., Recently ESBL production was observed in 48% of *E. coli*, 44% of *K . pneumoniae* and 50% of *P. aeruginosa* isolates in a tertiary hospital in Patiala [46] . our study shows 10 .4% OPD pateints had ESBL infection as ci=ompared to 9.25% in ipd pateints . Males were 39.25% infected from ESBL infection and females (26 %) .Acording to A1 Benwan et. al ESBL-producing isolates was significantly larger among IPD pateint (15.4%) than in OPD pateint (4.5%)[45].

The mode of resistance to antibiotics used against the infectious disease has been introduced since 1940 . Irregular intake of antibiotic give rise to multi –drug resistant against the micro organisam. Antibiotic susceptibility testing in vitro determine sensitivity ,resistance and intermediate response against the bacterial infections .ESBL are commonly due to nosocomial infection . Bacteria show their resistance against the changing of genomes and have some resistance mechanisam. According to Yuksel et al,resistance rate of ampicillin is high in canada ,europe and africa 45%, 50%,and 100% in *E.coli.* respectively amikacin 4.9% nitrofurantoin 2.2% gentamicin 13.9% co-trimoxazole 63.3%[41] . In our study *E. coli* demonstrated a verry high microbial resistance to antibiotics.The analysed results of antibiotic susceptibility test showed that *E.coli* was highly resistant to Ampicillin (93.22%) cefuroxime (93%) ,Norfloxacin (79.12%),Ofloxacin (79.66%) Gentamicin (59.31%) Co-trimoxazole (66.9%) .On the other hand

,verry low resistance was detected to antibiotics such as Amikacin (5.5%), Tigecycline (11.83%) imepenem (18.61%),and Nitrofurantoin (11.83%) .

Result showed that the best activity against these bacteria was acheived by Amikacin ,Tigecycline,Imepenem,colistin,and Nitrofurontion . Ghaley et al. Conducted a study in which ofloxacin 58% sensitive, followed by amikacin (54%) ,chloromphenicol (52%) and norfloxacin was (51%) 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 ampicillin should be perscribed cautiously especially against to those isolates which show suitable response to other antibiotics such as Amikacin and Nitrofurantoin.

This over all data shows that how much ESBL were detected from gram negative organisam and how much resistance and sensitivity of different antibiotics against the bacteria were found in study .

CHAPTER -7

CONCLUSION

CONCLUSION

To conclude, our study showed higher prevalence of urinary tract infection in females in the age group of 51-60 years. *E .coli* was the most common pathogenic organism isolated in this prospective study followed by *P.aeruginosa, K .pneumoniae* ,and *S.aureus.* The rate of detection of ESBLs were more in hospitalised patients compared to outpatient settings. The rate of ESBL production in *E.* coli was found to be (65.23%) .(15/23). Most of our *E. coli* isolates showed resistance to ampicillin, norfloxacin, levofloxacin, ofloxacin, and there was good susceptibility against imepenem, amikacin, meropenam, piperacillin/tazobactom, cotrimoxazole and colistin. Hence, it is essential to routinely screen the presence of ESBLs in urinary isoaltes so as to properly manage these resistant cases and avoid further transfer of resistance. This can also help any laboratory to apply some prophylactic measures to tackle the problem of resistance once they know the baseline prevalence of ESBLs in their hospital. Hence it should be a routine in all the laboratories.

CHAPTER -8

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APPENDIX

APPENDIX I

MATERIAL :-

1 . EQUIPMENT

: Weighing machine

: Microscope

: Cold refrigerator

: Bunsen burner

: Laminar chamber

: Incubator

: Hot air oven

: Autoclave

2 . GLASSWARE

: Conical flask

: Measuring cylinder

: Glass slides

: Petri plates

: Tubes

: Glass rods

3 . OTHERS

: Inoculating loop

: Antiseptics

: Cotton

: Facemask

: Forceps

: Gloves

APPENDIX II

Reagents :-

1 . CRYSTAL VIOLET STAIN :-

Composition	**gm/litre**
Solution A	
Crystal	2 gm
Ethanol	20 ml
Solution B	
Amonium oxalate	0.8 gm
Distilled water	80 ml

PREPARATION :-

. Mixed solution A to solution B and store 24 hr to dissolve the stain completely.

. Filter the solution

. Label the reagents bottle

2 . GRAM'S IODINE

COMPOSITION	**gram/litre**
Potassium iodide	2gm
Iodine	1gm
Distilled water	100 ml

PREPARATION:-

.Dissolve the potassium iodide in distilled water and then add iodine & and store it, and label date and name.

3.ACETONE

COMPOSITION	**VOLUME**
Acetone	250 ml
Ethanol	250 ml

PREPARATION

Mix them properly labeled it and stored

4 .SAFRANIN

Composition	**gram/litre**
Safranin	0.34 gm
Absolute alcohol	10 ml
Distilled water	90 ml

PREPARATION :-

Dissolve the safranin in 10 ml absolute alcohal and then add 90 ml distilled water filter the solution and labelled them .

PROCEDURE :-

1 . Flood air-dried, heat-fixed smear for 1 minute with **crystal violet**

2. Wash slide in a gentle and indirect stream of tap water for 2 seconds.

3. Flood slide with the mordant: **Gram's iodine.** Wait 1 minute.

4. Wash slide in a gentle and indirect stream of tap water for 2 seconds.

5. Flood slide with **decolorizing agent for** 15 seconds drop by drop .

6. Flood slide with counterstain, **safranin**. Wait 30 seconds to 1 minute.

7. Wash slide in a gentile and observe under microscope with oil immerson.

Gram positive blue/purple and **Gram negative** pink/red

APPENDIX III

PREPARATION OF CULTURE MEDIA :-

CLED AGAR :-

COMPOSITION	gram/litre
Lactose	10
L-cystine	0.128
Pancreatic digest of gelatin	4.0
Pancreatic digest of casein	4.0
Beef extract	3.0
Bromothymol blue	0.02
Agar	15.0
Final ph at 25 c	7.3+_0.2

PREPARATION :-

1. Suspend 36.0 g of the medium in one liter of distilled or deionized water.
2. Heat slowly while stirring frequently. Boil for a minute and sterilize at 121ºC (15 lbs. of psi.) for 15 minutes.
3. Pour into Petri dishes. When the medium is solidified, invert the plates to avoid excess moisture

2. MUELLER HINTON AGAR

COMPOSITION	grm/litre
Beef extract	2.0
Acid hydrolysate of casein	17.50
Starch	1.50
Agar	17.0
Distilled water	1 litre
Final ph at 25 degree celcius	7.3+/-0.1

PREPARATION:-

1 . Suspend 38 grams in 1000 ml distilled water.

2 . Heat to boiling to dissolve the medium completely.

3 . Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

4 . Mix well before pouring.