ISOLATION AND DETECTION *OF* EXTENDED SPECTRUM β -LACTAMASE PRODUCING *E.coli FORM* URINE SAMPLE AND ITS DRUG SENSITIVITY

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DISSERTATION

SUBMITTED FOR THE DEGREE OF MASTER OF SCIENCES, M.Sc (CLINICAL MICROBIOLOGY) BY

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CERTIFICATE

This is to certify that Karamjit kaur bearing Registration no. 11301053 has completed the Dissertation titled, ***"ISOLATION AND DETECTION OF* EXTENDED SPECTRUM β -LACTAMASE PRODUCING *E.coli FORM URINE SAMPLE AND ITS DRUG SENSITIVITY"***
under my guidance and supervision. The dissertation is fit for submission and the partial fulfilment of the conditions for the award of degree for Masters of Sciences in Clinical Microbiology.

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DECLAREATION

I am Karamjit kaur, student of M.Sc clinical microbiology 2nd year (4th semester) under Department of Paramedical Sciences of Lovely Professional University, Punjab, hereby declare that all the information furnished in this dissertation / capstone project report is based on my own intensive research and is genuine.

This dissertation / report do not, to the best of my knowledge, contain part of my work which has been submitted for the award of my degree either of this university or any other university without proper citation.

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ABBREVATION

1.XDR- Extremely drug resistances

2.MDR- Multi drug resistances

3.DOD- Disk on disk

4.MHA- Muller Hinton agar

5.ESBL- Extended-spectrum beta-lactamases

6.MBL - Metallo-beta-lactamases

7.DDST-Double disk synergy test

8.CDC – Centers for Disease Control and Prevention

9.AIDS- Acquired Immune Deficiency Syndrome

10.ICU- Intensive Care Units

11.UTI- Urinary tract infection

12.NCCLS – National Committee for Clinical Laboratory Standards

13.H_2O_2 - Hydrogen peroxide

14.MR – Methyl Red

15.VP - Voges Proskauer

16.KOH - Potassium hydroxide

17.CO_2 - Carbon dioxide

18.MIC - Minimal Inhibitory Concentration

ABSTRACT

The production of extended spectrum beta lactamases (ESBL) is an important mechanism for the resistance to the third generation cephalosporins. ESBL producing organisms is extremely found in Enterobacteriaceae family .The extended spectrum beta lactamases represent a major group of lactamases enzymes that mostly produced by Gram-negative bacteria.

The present study was done to detect the extended spectrum beta lactamases producing *E.coli* among urinary tract infections.Urine samples were cultured for aerobic bacteria and antimicrobial susceptibility testing was carried out by using Kirby-Baur agar diffusion method. The detection of ESBLin 17 clinical isolates of *Escherichia coli* was done by screening test ,one is Disk on Disk snd other is Double Disk synergy test.

In addition, ESBL producing bacteria maximum resistant to many antibiotics, it is mostly difficult to treat the infections. *Escherichia coli* and *Klebsiella pneumonia* species isolated from urine samples in AMAR HOSPITAL,PATIALA were investigated on the basis of biochemical test and their antimicrobial susceptibility pattern. Isolates varied in their susceptibility to various e.g. 80% Escherichia coli isolates were susceptible toIimipenem100% but in case, of extended spectrum beta lactamases (ESBL) maximum resistant to these antibiotics e.g.polymyxin-B, Meropenem , Faropenem and Piperacillin Tazobactam etc.The prevalence of *Escherichia coli* and *Klebsiella pneumonia* gained interest during last few decades due to its resistance to all antimicrobial agent.

CHAPTER 1

INTRODUCTION

1. INTRODUCTION

Urine analysis is a simple and oldest method in the clinical laboratory. We also do the urine analysis in clinical microbiology.(Praful B. Godkar){1}.Urine cytology is a test to look for abnormal cells in yours urine. Urine cytology is used along with other tests and procedures to diagnose urinary tract cancers . In today scenario , the most frequently test are urine strip method and urine microscopy. Urine analysis can detect a wide variety of disorder of urinary system. It is most valueable and highly important means diagnosis in clinical medicine. Although routine urine analysis is common , the results are important for diagnosis of the disease. For example, the exact cause of turbid or cloudy urine can be revealed by microscopic examination and quick chemical screening of urine for glucose .(Praful B.Godkar) The objective of basic urine analysis is to provide information for diagnosis, surveillance and the prevention.

1.Disease of urinary tract

2. Renal disease

The routine urine analysis is mainly performed for two purposes.

The purposes of urine analysis is to detect intrinsic conditions that may adversely affect the urinary tract or kidneys .Diseased kidneys cannot function normally in regulating the volume and composition of body fluids and also maintaining acid-base balance and homeostasis. Consequently structural elements , such as leukocytes ,red blood cells, cast or urinary tract .cells from the lower urinary tract may appear in urine. The second purposes of urine analysis to find out the metabolic or endocrine disturbances of the body.

For example,a normal urine does not contain a detectable amounts of bilirubin and glucose. Presence of bilirubin in urine gives indication of metabolic disturbances of bilirubin and thus indication of hepatic and post-hepatic condition. Similarly, the presence of glucose in urine may indicate the condition diabetes mellitus. i.e deficiency of insulin ,an endocrine disturbance.

1.1Composition of urine

Urine is a liquid substances which is produced by the kidney and to remove the waste products from bloodstream. Human urine is a yellowish color and is variable in physical and chemical composition.(Godkar) Normal urine contains 90-95% water and 60g per day of solid constituents which may be organic or inorganic. The human urine mainly consists of water, with organic solutes including urea, uric

acid, creatinine, hormones and carbohydrates. The inorganic ions such as sodium, potassium, chloride, magnesium and calcium

- Physical examination of urine
- Chemical examination of urine

1. 2.PREPARATION OF MEDIA:

1.C.L.E.D media:

Composition:

Peptic digest of animal tissue	4g/L
Casein enzyme hydrolysate	4g/L
Beef extract	3g/L
Lactose	10g.L
L –Cystine	0.128g/L
Bromothymol blue	0.02g/L
Andrade indicater	0.10g/L
Agar	15.00g/L
PH	7.5+0.2

Mix all the ingredients in distilled water and heat to dissolve properly .Autoclave at 121°C at 15lb pressure for 20min.

2.Mueller Hinton Agar

Composition

Beef infusion from	300g/L
Casein acid hydrolysate	17.5g/L
Starch	1.7g/L
Agar	17.00g/L
Final PH at 25C	7.3+0.1

Mix all the ingredients in distilled water and heat to dissolve properly .Autoclave at 121°C at 15lb pressure for 20 minutes.

3.Nutrient Agar

Composition:

Meat extract 10g/L

NaCl

8.5g/L

Peptone	10gL
Agar	20gL
Distilled Water	1000ml
pH	7.4-7.6

Mix all the ingredients in distilled water and heat to dissolve properly .Autoclave at 121°C at 15lb pressure for 20min.pour the media in Petri dishes when temperature reaches to 40-45°C.

4.Blood Agar

Blood agar is an **enriched**, bacterial growth **medium**. **Fastidious** organisms, such as streptococci, do not grow well on ordinary growth media.{2}. Blood agar is a type of growth medium that encourages the growth of bacteria, such as streptococci, that otherwise wouldn't grow well at all on other types of media.

Composition:-

Meat extract	10g/L
NaCl	8.5g/L
Peptone	10gL
Agar	20gL
Distilled Water	1000ml
Defibrinated blood	50-100ml

Procedure for the preparation of Blood Agar

1. Prepare the Blood Agar base as instructed by the manufacturer. Sterilize by autoclaving at 121ºC for 15 minutes.
2. Transfer thus prepared Blood Agar base to a 50ºC water bath.
3. When the agar base is cooled to 50ºC, add sterile blood agar aseptically and mix well gently. Avoid formation of air bubbles. You must have warmed the Blood to room temperature at the time of dispensing to molten agar base. (Note: If you are planning to prepare a batch of Blood Agar plates, prepare few blood agar plates first to ensure that Blood is sterile).
4. Dispense 15 ml amounts to sterile petri plates aseptically

5. Store the plates at 2-8 ºC, preferably in sealed plastic bags to prevent loss of moisture. The shelf life of thus prepared Blood Agar is up to four weeks[108]

5.MacConkey Agar

MacConkey agar was developed in 20th century by Alfred Theodore MacConkey. It was the first formulated solid differential media. MacConkey Agar is a selective and differential culture media commonly used for the isolation of enteric Gram-negative bacteria. It is based on the bile salt-neutral red-lactose agar of MacConkey.

Bile salts in incorporated in MacConkey Agar to prevent the growth of gram-positive bacteria and fastidious gram-negative bacteria, such as *Neisseria* and *Pasteurella*. Gram-negative enteric bacteria can tolerate to bile salt because of their bile-resistant outer membrane.

MacConkey Agar is selective for Gram negative organisms, and helps to differentiate lactose fermenting gram negative rods from Non lactose fermenting gram negative rods.{3}. It is primarily used for detection and isolation of members of family *Enterobacteriaceae* and *Escherichia coli and Klebsiella pneumonia.*

Composition:-

Peptone	20g/L
Agar	20g/L
Distilled Water	1000ml
Lactose(10%)	100ml
Sodium taurocholate	05g/L
Neutral red 2% in 50% ethanol	3.5ml
pH	7.5-7.7

at 25°C

Mix all the ingredients in distilled water and heat to dissolve properly .Autoclave at 121°C at 15lb pressure for 20min.pour the media in Petri dishes when temperatur

reaches to 40-45°C.

Figure 1: Those is picture shows the lactose and non-lactose fermenting colonies

6(a). MacConkey broth

composition:-

Peptic digest of animal tissue	40g/L
Lactose	20g/L
Bile salts	10g/L
NaCl	10g/L
Neutral red	0.15g/L
Distilled water	1000ml

Mix all the ingredients in distilled water adjust the pH to 7.4 +/- 0.2 at 25°C. Autoclave the media at 121°C for 20min at 15lb .When cooled store at 2-8°C in refrigerator.

7. XLD(Xylulose Lysine Deoxycholate Agar)

Composition:-

Yeast extract	03g/L
Lysine hydrochloride	05g/L
Xylose	3.7g/L
Lactose	7.5g/L
NaCl	5.0g/L
Sodium deoxycholate	2.5g/L
Sodium thiosulphate	6.0g/L
Ferric ammonium citrate	0.8g/L
Phenol red	0.08g/L
Agar	15g/L
Distilled Water	1000ml

Mix all the ingredients in distilled water adjust the pH to 7.4. Heat the media at 100°C for 10min.When cooled to 40-45°C pour the media in Petri dishes.

8.Peptone water

Peptone Water is used for the cultivation of non-fastidious microorganisms, indole testing, and as a basal medium for carbohydrate fermentation studies. it is enrichment medium.

Media:- Prepare water or tryptophan broth.

Composition:-

Peptone	05g/L
NaCl	5.0g/L
Distilled Water	1000ml
	pH

7.2-7.4

1.3Culture and antibiotic susceptibility:

First of all, urine sample were inoculated onto blood agar, C.L.E.D media and MacConkey agar media. After one hour, we take a inoculating loop and also take a flame. Then we do properly sterile the inoculating loop in the presence of heat. Afterthat we kept the petriplate into incubator at 37˚C for 24-48 hours.

After overnight incubation, we were checked the growth of bacteria. All organisms were identified by their colony morphology, staining characters, pigment production, motility and other relevant biochemical tests as per standard methods of identification. All Gram- negative bacteria isolates were tested for antimicrobial susceptibility by using commercially available antimicrobial disc on Mueller Hinton agar.

Escherichia coli and *Kllebsiella pneumonia* were isolated from clinical specimens were assessed using Kirby Bauer disk diffusion test.

Procedure : 3-5 isolated colonies were inoculated into 4-5 ml broth or and inoculated for 2-8 hour until the turbidity of the suspension reaches.

I. Muller-Hinton plates (150mm diameter, 3-5mm thick , and pH 7.2-7.4) were inoculated by dipping a sterile cotton swab into suspension , and evenly streaking the entire surfaces of the plate.
II. Commercially the entire surfaces of the plate.

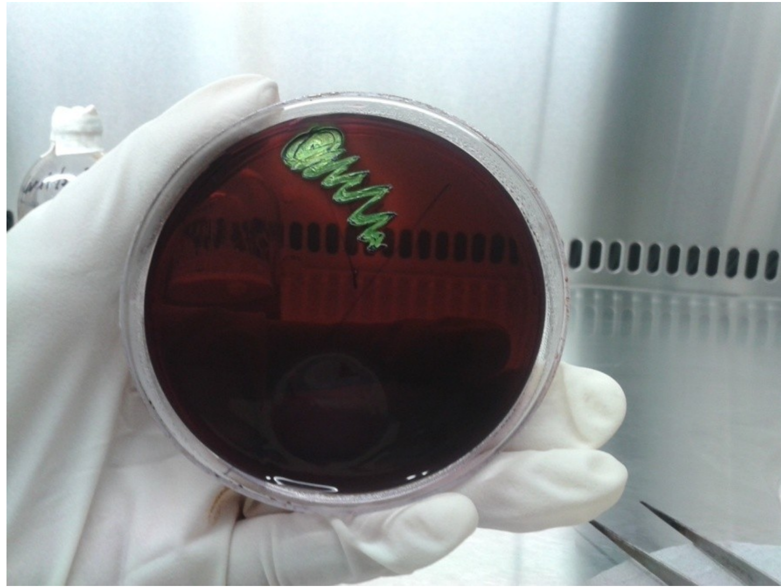


Figure 2- Growth of *E.coli* on XLD media

1.4.Antibiotics used :

Names	**Strength(mcg)**
Amikacin	30
Ciprofloxacin	5
Gentamicin	10
Cefotaxime	30
Imepenem	10
Meropenem	10
Cefoperazone	75
Azotreonam	75
Piperacillin-Tazobactam	100/10
Ceftazidime	30
Norfloxacin	10
Ampicillin	10
Cefopime	30
Ofloxacin	5
Amoxyclav	30
Polymyxin B	300
Levofloxacin	5
Faropenem	5
Cefozolin	30
Cefoxitin	10
Ceftriaxone	30
Nalidixic acid	30
Gentamicin	120

- The antimicrobial-containig discs were placed on the agar plates within min of inoculation by using a sterile forceps. The discs were pressed firmly against the agar surfaces and were inoculated for 16-18 hours at 35^{0}c.

- The diameter of zone of inhibition was measured.
- The zone size around each antimicrobial disc is interpreted as susceptible, intermediate or resistant based on the criteria indicated in tables provided by NCCLS.

1.5Antibiotics and their zone size in millimeters :

Antibiotics	**Resistant**	**Intermediate**	**Sensitive**
Amikacin	14	15-16	17
Ciprofloxacin	15	16-20	21
Gentamycin	12	13-14	15
Cefotaxime	14	15-17	23
Imipenem	13	14-15	16
Meropenem	13	14-15	16
Cefopime	14	15-17	18
Cefotaxime	22	23-25	26
Piperacillin-Tazobactam	17	18-20	21
Ofloxacin	12	13-15	16
Ceftazidime	14	15-17	18
Norfloxacin	14	15-16	17
Azotreonam	17	18-20	21

According to these zones size , reporting was done and on the basis of these organisms is considered resistant, sensitive and intermediate.

1.6ANTIBIOTIC SUSCEPTIBILITY TESTING

Bacteria demonstrate two kinds of resistance to antibiotics, namely intrinsic resistance and acquired resistance. Intrinsic resistance means that the species was resistant to an antibiotic even before its introduction.

Acquired resistance means that the species was originally susceptible to an antibiotic, but later became resistant. Bacteria can acquire antibiotic resistance either by mutation or through exchange of genetic material among same or closely related species.

The sudden acquisition of resistance to antibiotics poses difficulties in treating infections. Resistance to several different antibiotics at the same time is even more significant problem. It is because of the acquired resistance that bacterial isolates must be subjected to antibiotic susceptibility testing. Bacteria showing reduced susceptibility or resistance to an antibiotic implies that it should not be used on the patient.

Muller-Hinton agar is widely used for antibiotic susceptibility testing.

Ingredients :

Beef infusion	: 300 ml
Casein hydrolysate	: 17.5 ml
Starch	: 1.5 g
Agar	: 10 g
Distilled water	: 1000 ml
Final pH	: 7.4

Preparation:Emulsified the starch in a small amount of cold water, poor into the beef infusion then add the casein hydrolysate and the agar. Make up the volume with distilled water. Dissolve the constituents by heating gently at 100^0c with agitation Dispense in screw capped bottle and sterilize by autoclaving 121^0 c for 20 minutes and pour on the plates.The methods most commonly used in clinical laboratories are disc diffusion, agar diffusion, macrobroth dilution. Additionally, automated methods are becoming widely recognized.

<u>Disk diffusion test:</u>

In this method the standardized bacterial isolate is spread on an agar plate and then paper disc containing specific concentration of antibiotics are placed and incubated at 37^oC overnight.

If the isolate is susceptible to the antibiotic, it does not grow around the disk thus forming a zone of inhibition. Strains resistant to an antibiotic grow up to the margin of disk.The diameter of zone of inhibition must be measured and result read from the Kirby Bauer chart

as sensitive, intermediate or resistant._

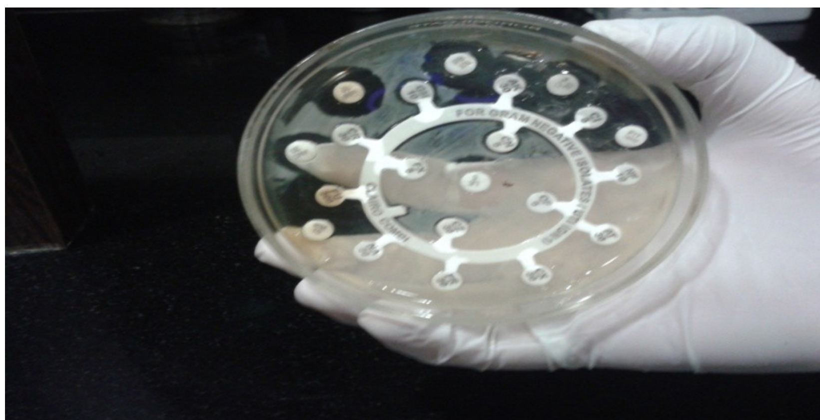
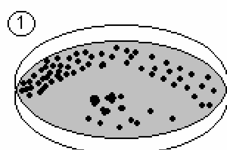
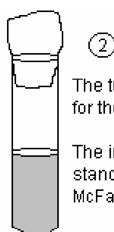
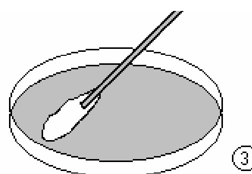


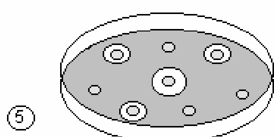
Figure 3- Antibiotic susceptibility test on Mueller Hinton Agar

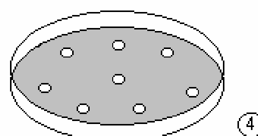
Procedure of disk diffusion method











1.7DATA INTERPRETATION OF URINE PATIENTS WITH ESCHERICHIA COLI AND KLEBSIELLA PNEUMONIA

ESCHERICHIA COLI ISOLATED WITH DIFFERENT ANTIBIOTIC SUSCEPTIBILITY :

AGE GROUP	*ANTIBIOTIC*	*RESISTANAT %*	*SENSITIVITY %*
20-65Y	*CPM*	*5%*	*95%*
	IPM	*0%*	*100%*
	AMC	*3.5%*	*97.5%*
	LE	*1%*	*99%*
	AK	*11%*	*85%*
	MRP	*0.5%*	*99.5%*
	PIT	*1.5%*	*98.5%*
	PB	*0.5%*	*99.5%*
	FAR	*7.5%*	*92.5%*
	NF	*90.5%*	*9.5%*
	AT	*21.5%*	*78.5%*
	CX	*99.5%*	*0.5%*

	FR	***92.5%***	***7.5%***
	CZ	***95.5%***	***4.5%***
	NA	***98.5%***	***1.5%***
	FU	***94.5%***	***5.5%***
	CR	***99.5%***	***0.5%***
	FX	***99.5%***	***0.5%***
	CL	***98.5%***	***1.5%***

Figure 4-Percentage of antibiotics against *E.coli*.

2.ESCHERICHIA COLI (E.coli)

E.coli was first recognized as a cause of human diarrheal illness in the 1960s. It have since emerged as a major bacterial cause of diarrhea among travelers and children in the developing world. Escherichia coli (E. coli) are a group of bacteria that can cause a variety of illnesses in humans, including diarrhea, urinary tract infections, respiratory illnesses pneumonia and other problems.(Kristina Duda.R.N)[4]. *E.coli* is increasingly recognized as an important cause of foodborne illness in developed nations, such as the United States. *E. coli* consists of a diverse group of bacteria. Pathogenic *E. coli* strains are categorized into pathotypes. Six pathotypes are associated with diarrhea and collectively are referred to as diarrheagenic *E. coli*.

What is the morphology of *E.coli* ?

E.coli is a Gram-negative bacteria. It is a straight, rod measuring 1-3*0.4-0.7um arranged singly or in pairs. It is motile by peritrichate flagella, though some strains may be nonmotile. Capsules and fimbriae are found in some strains. Spores are not formed.

What is the Cultural characteristics of *E.coli* ?

It is an aerobe and a facultative anaerobe. The temperature range in 10-40˚ C. Good growth occurs in ordinary media. Colonies are thick, large greyish white, moist, smooth opaque or partially translucent discs. On MacConkey's medium, colonies are bright pink due to lactose fermentation. After the identifies the colony characterstics, we will do the Gram staining for the confirmation of the bacteria.

What are the symptoms of *E.coli ?*

a. Painful burning sensation when urinating
b. Abdominal cramping
c. Discomfort , pressure
d. Cloudy and bloody urine
e. Loss of appetite/nausea
f. Vomiting (rare)
g. Fatigue

These symptoms usually resolve on their own within 5 to 7 days. E. coli infections are mild in most people, but they can be serious or even life threatening in others.(Johns Hopkins)[5]. Symptoms of an infection typically start 3 to 5 days after exposure to the bacteria, but they can occur as early as one day or as late as 10 days after exposure. (Ann Pietrangelo)[5].

How someone become infected with *E.coli* ?

Infection occurs when a person eats food, or drinks water or ice contaminated with ETEC bacteria. Human or animal wastes (e.g.,feces) are the ultimate source of ETEC contamination.

What can be done to prevent infection with *E.coli*?

For travelers in developing countries, ETEC infection can be prevented by avoiding foods and beverages that could be contaminated with bacteria. Although both cooked and uncooked foods have been associated with ETEC infections, high-risk foods are raw fruits and vegetables (e.g., salads), raw seafood or undercooked meat or poultry, unpasteurized dairy products, food from street vendors, and untreated water (including ice) in areas lacking adequate chlorination.

There have been rare cases of ETEC infection reported in the United States associated with eating salads, raw fruits, and vegetables. However, these foods are generally safe if handled and prepared appropriately.

In developing countries, food can be made safe to eat by thoroughly cooking it and by keeping it hot. Fruits and vegetables should be peeled by the person eating them. Water used for drinking (including brushing teeth) or for washing food in these countries should be bottled, boiled, or chemically treated with iodine, chlorine or

another disinfectant. Handwashing with soap and water also can prevent contamination of food and beverages with ETEC and prevent transmission from person to person as well.

What are the risk factor of *E.coli* ?

E. coli can affect anyone who is exposed to the bacteria. But some people are more likely to develop problems than are others. Risk factors include:

I. **Age.** Young children and older adults are at higher risk of experiencing illness caused by *E. coli* and more-serious complications from the infection.

II. **Weakened immune systems.** People who have weakened immune systems — from AIDS or drugs to treat cancer or prevent the rejection of organ transplants — are more likely to become ill from ingesting E. coli.

III. **Eating certain types of food.** Riskier foods include undercooked hamburger; unpasteurized milk, apple juice or cider; and soft cheeses made from raw milk.

IV. **Time of year.** Though it's not clear why, the majority of E. coli infections in the U.S. occur from June through September.

V. **Decreased stomach acid levels.** Stomach acid offers some protection against *E. coli.* If you take medications to reduce your levels of stomach acid, such as esomeprazole (Nexium), pantoprazole (Protonix), lansoprazole (Prevacid) and omeprazole (Prilosec), you may increase your risk of an *E. coli* infection.

What are the lifestyle and home remedies ?

Follow these tips to prevent dehydration and reduce symptoms while you recover:

I. **Clear liquids.** Drink plenty of clear liquids, including water, clear sodas and broths, gelatin, and juices. Avoid apple and pear juices, caffeine, and alcohol.

II. **Add foods gradually.** When you start feeling better, stick to low-fiber foods at first. Try soda crackers, toast, eggs or rice.

III. **Avoid certain foods.** Dairy products, fatty foods, high-fiber foods or highly seasoned foods can make symptoms worse.

IV. Drink pasteurized milk, juice and cider. Any boxed or bottled juice kept at room temperature is likely to be pasteurized, even if the label doesn't say so.

V. Wash raw produce thoroughly. Washing produce won't necessarily get rid of all *E. coli* — especially in leafy greens, which provide many spots for the bacteria to attach themselves to. Careful rinsing can remove dirt and reduce the amount of bacteria that may be clinging to the produce.

1.8 .GRAM STAINING:

Introduction :

Gram staining is one of the most important and widely used differential staining techniques in diagnostic microbiology.

The gram staining procedure was develop by Christian Gram in 1883 , a Danish physician who was working as a pathologist in Municipal Hospital IN Berlin.

Principle : Gram-positive cells have a thick peptidoglycan cell wall that is able to retain the crystal violet-iodine complex that occurs during staining, while Gram-negative cells have only a thin layer of peptidoglycan. Thus Gram-positive cells do not decolorize with ethanol, and Gram-negative cells do decolorize. This allows the Gram-negative cells to accept the counter stain safranin. Gram-positive cells will appear blue to purple, while Gram-negative cells will appear **pink to red**[6]

Reagents:

1.1%CRYSTAL VIOLET SOLUTION

Crystal violet - 1.0 g

Distilled water - 100 mL

2.GRAM'S IODINE SOLUTION

Iodine - 1.0 g

Potassium iodide - 2.0 g

Distilled water - 300 mL

3.ACETONE

4.0.5% Safranin Solution

Procedure:

1.First of all, we take a clean and grease free glass slide and add a drop of normal saline and take a colony and prepare a glass slide. Before staining the glass slide should be dry.

2. Place slides on a staining rack and add crystal violet for solution for 1 minute.

3. Rinse in tap water.

4. Flood slides with Gram's iodine solution for 1 minute.

5. Rinse in water

6. Differentiate briefly with acetone

7. Counter Stain with 0.5% safranin solution.

8. Rinse the slide in water.

9. Dry the smear in air, observe under oil immersion lens.

Observation :

Violet color : Gram positive bacteria

Red/Pink color : Gram negative bacteria

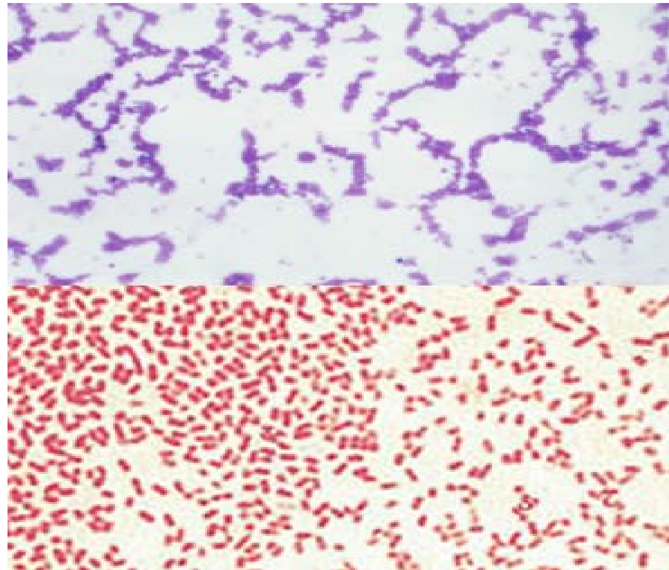


Figure 4- Gram staining shows Gram positive and Negative bacilli

CHAPTER 2

REVIEW OF LITERATURE

2. INTRODUCTION

Extended spectrum beta lactamase (ESBL) enzymes were first reported in Germany in1983 from *E.coli* and *Kelbsiella pneumonia* .They are group of enzymes capable of hydrolysing the third generation of oxyminocephalosporins such as cefotaxime, ceftriaxone and ceftazidime ,the monobactam (azotreonam) but not cephamycin (cefoxitin,cefotetam) or the carbapenems (imipenem meropenem)(Kliebe C,Nies BA 1995)[7].These enzymes are produced mainly *Klebsiella pneumonia, E.coli* and *proteus* species although they have detected in *Pseudomonas* species and other Enterobacteriaceae .(Yates RR,1999)[8]..ESBL strains can survive in the hospital environment.

ESBL produced mostly by members of Enterobacteriaceae have revealed as serious nosocomial pathogen globally.(Hobson RP 1996)[9]. ESBL are plasmid mediated enzyme capable of hydrolyzing and inactivating a wide variety of B lactams ,including the third generation of cephalosporins penicillins and against monobactams e.g. azotreonam .(Philip J.Turner 2005)[10].There are various reports of ESBL producing organisms worldwide from hospital environments , patients and others.

The spread of ESBLs in Gram –negative bacteria represents a major challenge to the antimicrobial therapy of infections caused by these organisms either in hospital or in a community.(Paterson DL 2005)[11]. AmpC enzymes are chromosomally encoded B-lactamases which confer resistance to the oxyimino group containing cephalosporins (cefotriaxone,cefotaxime andceftazidime) and not infected by the inhibitors (clavulanic acid and tazobactam). They are commonly found in *Enterobacter, Serratia, Citrobacter freundii* and *Pseudomonas aeruoginosa* (George A.Jacoby 2009)[12].

Since the parental enzymes are sensitive to beta lactamase inhibitors, like clavulanic acid, the ESBLs are also sensitive to these inhibitors (Bush *et al*., 1995)[13]. ESBL producingorganism, are very difficult to eliminate because of their multi-drug resistance to various classes of antibiotics such asCephalosporins, monobactam, carbapenems, Ciprofloxacin, erythromycin and β-lactam, β-lactamase inhibitor combination (Iroha, 2009)[14].

A major advantage of the double disk diffusion test is technically simple (Peterson and Bonomo, 2005)[15].

ESBL gene are usually found in large plasmids . ESBL have serine at their active sites which attact the amide bond in the lactam ring of antibiotics causing their hydrolysis. These enzymes which are number more than 150 were initially limited to *Escherichia coli* and *Klebsiella* species. AmpC production enzyme hyper production and porin loss (Sturenburg E,Lang M,Horstkotte 2004)[16].ESBLs producing organism are resistant to aminoglycosides, fluororquinolones, tetracyclines, cephalosporins and sulphonamides(Mario Tumbarello 2006)[17].

The Epsilometer test (E-test) method is used for the confirmation of ESBL production (Stobberingh EE 1999)[18].The E-test is also used for determing the MIC values of the antimicrobial agents .Several different method like disk approximation or double disk synergy test or modified double disc test (MDDT) NCCLs phenotypic confirmatory method , E- test ESBL strips have been suggested for the detection of ESBL in clinical isolates. While definitive guidelines for the management of patients infected with ESBL-producing bacteria are still awaited, there is strong evidence that failure to detect ESBL-mediated resistance can lead to treatment failure. During the late 1990s and early 2000s CTX-M producing enterobacteriacea has emerged as the most common ESBL type in many parts of the world including Africa, South America,Asia and some parts of Europe(Livermore DM)[19].

Detection of ESBL producing organisms is a challenge for laboratories since routine methods of antibiotic susceptibility testing are not sensitive enough to detect them. ESBLs can be detected by disc diffusion methods, three dimensional agar tests, rapid automated systems, E-test and Polymerase chain reaction (AA Cagatay ,T Kocagoz 2003)[20].

This study was undertaken to determine the prevalence of ESBL production .Among urinay isolates of *E.coli* and *Klebsiella pneumonia* and their impact on their clinical outcome(Ebbing Lautenbach 2001)[21].The main objective of this study was to identify the ESBL producing organisms of Enterobacteriaceae family from urinary specimen of the patients suffering from urinary tract Infection (UTI) and comparison with double disk diffusion.

CHAPTER 3

MATERIAL AND METHODS

3. <u>MATERIAL AND METHODS</u>

This study was carried in the department of Microbiology in Amar hospital ,Patiala during the period of training. Five hundred (500) adult male and female patient who had a history of urinary tract infection .We have included total isolates of *Escherichia coli* and *Klebsiella pneumonia* in this study.

3.1SAMPLE COLLECTION:

Urine sample was collected into the wide mouth disposable container. A catch midstream urine specimen voided under the instruction given to them was collected. The sample were collected from Urology, Nephrology and Gynaecology. One sample to 30-50ml midstream urine is taken after of at least stay in bladder and then processed the urine sample within one hour. Specimens from infants and young children can be collected in a disposable collection apparatus(Ezaz Ahmed)[22]It consists of a plastic bag with an adhesive backing about the opening to fasten it to the child so that the voids directly into the bag. Care must be taken to avoid fecal contamination.(Praful.B Godkar)[23]

Figure 5- Urine specimen

Signs of inclusion criteria:

1. Those patients are considered which are signs and symptoms relating to urinary tract infection.

.**Exclusion criteria:**

1. Patient having known congenital abnormalities.
2. Some patient already on antibiotic or who had taken antibiotic two weeks ago.
3. Some patients have metabolic disorder.
4. Patient with chronic indwelling

3.2 URINE MICROSCOPY

The microscopic examination is a valuable diagnostic tool for the detection and evaluation of renal and urinary tract disorders and other systemic diseases.

PRINCIPLE:

The microscopic elements present in urine are collected in the form of deposit by centrifugation. A small drop of the sediment is examined by making a coverslip preparation under microscope.

REQUIREMENTS:

1. Cover slips
2. Glass slides
3. Centrifuge tube or test tube
4. Microscope
5. Pasteur pipettes

SPECIMEN:

Urine sample is taken freshly voided, midstream and morning urine.

PROCEDURE

i. First of all, we mix the urine and pour into a centrifuge tube until it is 3\4 full (about 5ml) Centrifuge with another balanced test tube for 5 minutes at 25,000 RPM.
ii. Pour off the supernatant quickly and completely into another test tube for protein determination
iii. Resuspend the deposit by shaking the tube.
iv. Place one drop of the deposit on a glass slide and cover it with a cover slip and mark it with the identification number.

v. Observe it first under power objective in subdued light The various finding observed in sediment may as follows.

1.Leukocytes or pus cell: The pus cell can enter in urine anywhere from the glomerulus to the urethra.Normal urine can contain 2-3 pus cell/per h.p.f..These are mostly neutrophils. The approximate diameter is 10-12. The leukocytes also known as pus cell.

Figure 6-This picture shows the pus cell in urine microscopy

2.Epithelial cell : These cells may originate from any site in the genitourinary tract from the proximal convoluted tubule to the urethra or from vagina. Normally few cells 3-5 per h.p.f from these sites can be found in the urine due to sloughing off of old cells.

Three main types of epithelial cells may be recognized:

I. Tubular epithelial cell
II. Transitional epithelium cell
III. Squamous epithelium cell

I. **Tubular epithelium cell:** These cell may be cuboidal, flat or columnar. These are slightly larger than leukocytes and contain large round nucleus.

II. **Transitional epithelial cell**: They may be pear shaped or round. These are two to four times as large as white cells. Occasionally these may contain two nuclei.

III. **Squamous epithelial cell: Squamous** epithelial cell are large, flat, and irregularly shaped. They contain abundant cytoplasm and small central nuclei.

3.Erythrocytes:

The erythrocytes are appear smooth, biconcave disks about 7umdiameter and 2um thick. In fresh urine these cells have a normal, pale or yellowish appearance. hey contain nuclei. In dilute or hypertonic urine red cells swell up and lyse. Then the lysed cell appears as colorless circles.

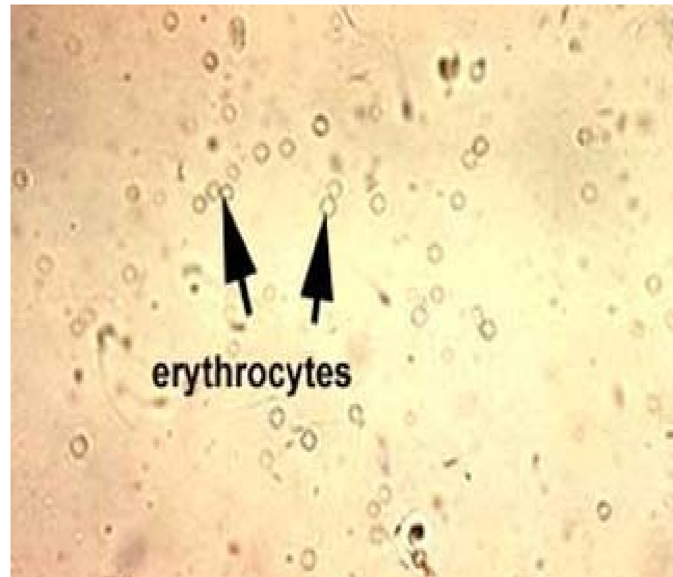


Figure 7-This picture shows red blood cell in urine microscopy

4.Casts: Urinary casts are formed in the lumen of the tubules of the kidney. The renal tubules secrete a mucoprotein called Tamm-horsfall protein which is believed to form the basic matrix of all casts. Cast formation takes place in the distal and collecting tubule. Cast dissolve in alkaline urine. The cast have nearly parallel sides and rounded or blunt ends. They may be convoluted, straight or curved. The can a form as the result of:

- Precipitation of gelatine of Tamm-horsfall mucoprotein
- Clumping of cells on other material within protein matrix.
- Coagulation of material within the lumen.

4(a).Granular casts: These casts always indicate renal disease. The casts are present due to the degeneration of cellular casts due to direct aggregation of serum proteins in a Tamm- horsfall protein.

4(b)Hyaline cast: They are colourless, homogeneous, transparent and with rounded end. These casts can be seen in increased number even in the mildest kind of renal disease.

5.Mucus threads: These are long, thin waxy threads of ribbon like structures. They may be present in normal urine but found in high proportion in the presence of inflammation or irritation of urinary tract. .

6.Yeast cell: These are smooth, colourless and usually ovoid cells. These can vary in size and have doubly refractile walls. They often show budding. Unlike red cells, they are insoluble in acid and alkali. Yeast cell may be found in urinary tract infection. They may be present in urine as a result of skin or vaginal contamination.

7.Crystals: Many of the crystals that are present in the urine have a little significance although they may be found in calculus formation, metabolic disorders, metabolic disorders and regulation of medication. Crystals present in urine are uric acid crystals calcium oxalate crystals.

8.Bacteria: Freshly voided normal urine is generally free of bacteria .Contamination may occur from bacteria present in urethra, vagina or other external source. Presence of large number of bacteria with many pus cells indicates urinary tract infections.

3.3 METHODS

A total 75 isolates *E.coli* from various clinical specimens were obtained and identified by standard microbiological techniques. Antibiotic susceptibility test (AST) was performed on Mueller Hinton Agar by Kirby Bauer Method. After performing the susceptibility test according to National Committee for Clinical Laboratory standard (NCCLs) standard methodology using Mueller Hinton agar There are two method for the confirmation of ESBL production those are Disc on Disc (DOD) and Double Disc Synergy Test (DDDT) was carried out on a Mueller Hinton Agar.

The equivalent single cephalosporins disc on a plate .i.e ESBL detection was carried out by double disc diffusion method(A susceptibility disc containing augmentin was placed as the inhibitor of beta lactamase in the centre of the plate. Then the ceftazidime(30ug) , cefotaxime(30ug), ceftriaxone and azotreonam were placed 15mm apart centre to centre on the plates with the sterile forceps. Ceftazidime (30 μg) disc and a ceftriaxone (20 μg) disc were placed apart on MHA surface in such a manner that the distance between the two discs was approximately twice the zone of inhibition (ZOI) produced around the ceftazidime disc in screening method . Ceftazidime discs were used as a source of beta lactamase inhibitor clavulanic acid.

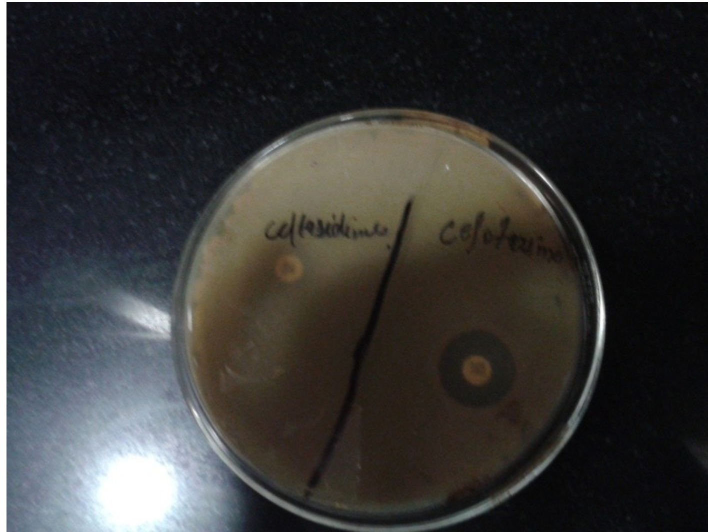


Figure 8- Disk on Disk Method on Mueller Hinton agar

The agar plates were incubated at 37°C for 18 hours. The organism was considered as ESBL producer if the zone of inhibition around the Ceftriaxone disc (RS Miles,SGB Amyes 1996)[24].

After the overnight incubation, a clear and enhanced zone of inhibition from 5mm above in the presence of Augmentin means clavulanic acid is regarded as positive for phenotyping production of ESBL enzyme. An inhibition zone of <22mm ceftriaxone was considered as indication of ESBL production. In disc on disc (DOD) method, a lawn culture approximately 5+1CFU/ml was prepared on Mueller Hinton Agar. Ceftriaxone disc with clavulanic acid overlaid car carefully with ceftriaxone was placed 25mm away from the another disc. Then agar plates were incubated at 37C for 18 hour. A test was considered positive if the difference in diameter Zone of Inhibition of ceftazidime with clavulanic acid more than 5mm and combination of other antibiotics. . A test was considered positive if the difference in diamete Zone of Inhibition of ceftriaxone with clavulanic acid more than 5mm and combination of other antibiotics

3.4.BIOCHEMICAL REACTION FOR THE IDENTIFICATION OF ISOLATES

1.OXIDASE TEST:

PURPOSE :

To determine the presences of an enzymes . cytochrome oxidase which catalyse oxidation of reduced cytochrome by molecular oxygen.

Principle :

This test depends on the presence of cytochrome oxidase in bacteria that will catalyze the transport of electrons between electron donors and redox dye. Tetramethyl-p-phenylene diamine dihydrochloride in the reagent is reduced to deep purple color. This test is used for the screening of Pseudomonas, Vibrio, Neisseria, Brucella and Pasteurella, which give positive test. Enterobacteriaceae are oxidase negative[25].

Reagents :

Oxidase reagent is specially prepared as 10g/l or 1% solution of tetramethyl-p-phenylene diamine dihydrochloride.

Procedure :

Filter Paper Method

Place a piece of filter paper in petri dish and add 3 drops of freshly prepared oxidase reagent. Using a sterile glass rod, remove a colony of test organisms from a culture plate and smear it on the filter paper.

Interpretation :

Oxidase positive organisms give blue color within 5-10 seconds, and in oxidase negative organisms, color does not change[25].

2.CATALASE TEST

Principle :

Catalase is an enzyme that breaks down hydrogen peroxide (H_2O_2) into H_2O and O_2. A catalase is a way to get rid of the peroxide in a cell. The test is performed by placing a culture on a microscope slide and adding a few drops of H_2O_2 onto the smear. A positive result is bubbling due to a rapid evolution of O_2. A negative result is no bubbles or scattered bubbles[26].

Procedure :

1. Place a small amount of growth from your culture onto a clean microscope slide. If using colonies from a blood agar plate, be very careful not to scrape

up any of the blood agar— blood cells are catalase positive and any contaminating agar could give a false positive.

2. Add a few drops of H_2O_2 onto the smear. If needed, mix with a toothpick. DO NOT use a metal loop or needle with H_2O_2. it will give a false positive and degrade the metal.
3. A positive result is the rapid evolution of O_2 as evidenced by bubbling.
4. A negative result is no bubbles or only a few scattered bubbles.
5. Dispose of your slide in the biohazard glass disposal container.

IMViC REACTIONS

IMViC reactions are a set of four useful reactions that are commonly employed in the identification of members of family enterobacteriaceae. The four reactions are: Indole test, Methyl Red test, Voges Proskauer test and Citrate utilization test. The letter "i" is only for rhyming purpose[27].

(A).INDOLE TEST:

Principle: Some bacteria can produce indole from amino acid tryptophan using the enzyme typtophanase. Production of indole is detected using Ehrlich's reagent or Kovac's reagent. Indole reacts with the aldehyde in the reagent to give a red color. An alcoholic layer concentrates the red color as a ring at the top.

Media:-prepare water or tryptphan broth.

Composition:-

Peptone	05g/L
NaCl	5.0g/L
Distilled Water	1000ml
pH	7.2-7.4

Reagent:- Kovac's reagent

Composition:-

Amy or isoamyl alcohol	150ml
Paradiamethylaminobenzenaldehyde	10g

HCL(Conc)

50ml

Procedure

1. We will inoculate the tryptophan broth with broth culture.
2. After that we emulsify the bacterial isolated colony of the test organism in tryptophan broth.
3. Incubate at 37C for 24-28 hrs in ambient air.
4. Then add a Kovac reagent to the broth culture.

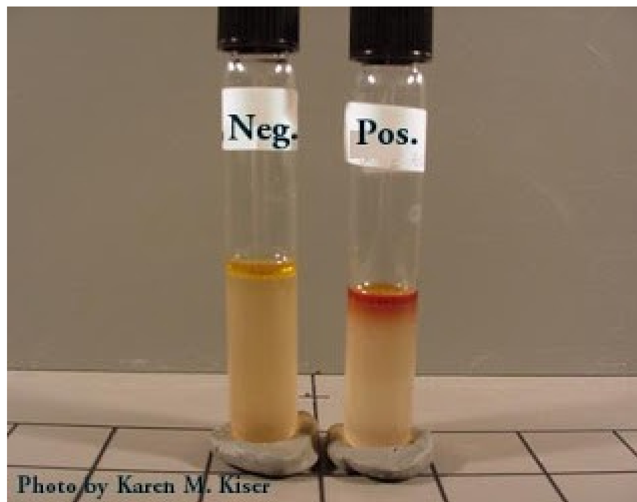


Figure 9- This picture shows indole test

Result: -

Negative: No colour change.

Positive: Dark pink coloured ring at the surface of medium.

(B).METHYL RED TEST

Principal: This is to detect the ability of an organism to produce and maintain stable acid end products from glucose fermentation. Some bacteria produce large amounts of acids from glucose fermentation that they over come the buffering action of the system. Methyl Red is a pH indicator, which remains red in colour at a pH of 4.4 or less.

.

Media:- Glucose phosphate broth as for methyl red test.

Composition:-

Peptone	05g/L
Glucose	5.0g
Distilled Water	1000ml
pH	7.6
K2HPO4	05g

Reagent:-

Methyl red	0.1g
95% Ethyl alcohol	300ml
Distilled Water	200ml

Procedure

1. The bacterium to be tested in inoculated into glucose phosphate broth, which contains glucose and phosphate buffer and incubated at 37oC for 48 hours.
2. Over the 48 hours the mixed-acid producing organism must produce sufficient acid to overcome the phosphate buffer and remain acid.
3. The pH of the medium is tested by the addition of 5 drops of MR reagent. Development of red colour is taken as positive.
4. MR negative organism produces yellow colour.

Example: *Eschericihia coli*: Positive; *Klebsiella pneumoniae*: Negative

Result:-

Negative: No colour change

Positive: Dark pink coloured ring at the surface of medium.

(C) Citrate utilization test:-

Citrate utilization test is used to detect the ability of bacteria to utilize sodium citrate. When as its only carbon source and inorganic sole is fixed nitrogen source. The organic acid is used as a carbon and alkaline carbonate and biocarbonate are produced ultimately. The ammonium hydroxide is produced when ammonium salts in the medium are used as the sole of the nitrogen source.

When the color change of the indicater due to alkali production by test organism as it grow in the medium .If the microorganism grows then presence of bromothymol blue color and turning green to blue.

Media:- Simmon's Citrate Agar.

Composition:-

NaCl	05g
$MgSO_4$	0.2g
Ammonium dihydrogen phosphate	01g
Sodium Citrate	05g
Agar	20g
Bromothymol blue (0.2%)	40ml

Procedure:

1. Bacterial colonies are picked up from a straight wire and inoculated into slope of Simmon's citrate agar and incubated overnight at 37ºC.
2. If the organism has the ability to utilize citrate, the medium changes its color from green to blue[27].

Examples: *Escherichia coli* Negative, *Klebsiella pneumonia* Positive .

Result: Negative: No colour change
Positive: Green to blue colour.

(D)VOGES PROSKAUER (VP) TEST:

Principle: While MR test is useful in detecting mixed acid producers, VP test detects butylene glycol producers.

Acetyl-ethyl carbinol (acetoin) is an intermediate in the production of butylene glycol. In this test two reagents, 40% KOH and alpha-naphthol are added to test broth after incubation and exposed to atmospheric oxygen. If acetoin is present, it is oxidized in the presence of air and KOH to diacetyl. Diacetyl then reacts with guanidine components of peptone, in the presence of alphanaphthol to produce red color. Role of alpha-naphthol is that of a catalyst and a color intensifier.

Procedure:

1. Bacterium to be tested is inoculated into glucose phosphate broth and incubated for at least 48 hours. 0.6 ml of alpha-naphthol is added to the test broth and shaken. 0.2 ml of 40% KOH is added to the broth and shaken.
2. The tube is allowed to stand for 15 minutes. Appearance of red color is taken as a positive test. The negative tubes must be held for one hour, since

maximum color development occurs within one hour after addition of reagents.

Examples: *Escherichia coli*: Negative; *Klebsiella pneumoniae*: Positive

(D) Urease test

Media:- Christensen's medium

Composition:-

Peptone	01g
NaCl	5.0g
Distilled Water	100ml
pH	6.8-6.9
Agar	20g
Potassium dihydrogen phosphate	02g
Phenol red(1:500 aq solution)	06ml

Result:- Positive: Pink colour

Negative: No colour change

(E) Catalase test:

Principal:Catalase test is a enzyme that is produced by the microorganisms. Catalase enzyme neutralize the bactericidal effect of hydrogen peroxide. It live in oxygenated environments to neutralize to toxic forms of oxygen metabolites. Catalase test is used to distinguish among Gram-positive cocci. The catalase test is also used for the detection of Enterobacteriaceae .

Reagent :- 3% Hydrogen peroxide

Procedure:

1. Take a clean and grease free glass slide
2. After that we take a small amount of bacterial colony on a glass slide using a sterile wooden stick.
3. Place a drop of 3% of hydrogen peroxide on to the slide and mix well.
4. If the test is positive then bubble formation on a glass slide.

5. If test is negative then there is on bubble formation.

Result:-

Positive: Bubble formation occurs.

Negative: No bubble formation.

(H) Coagulase test:

Principal: Coagulase test is used to differentiate Staphylococcus aureus. Coagulase is an enzyme which is produced from Staphylococcus aureus. Afterthat it converts (soluble) fibrinogen plasma to (insoluble) fibrin. Staphylococcus aureus is produced in two forms free and bound.

Composition:-

Citrated plasma	100µl
Normal saline	400µl
Bacterial suspension	500µl

Mix all the ingredients properly in a test tube and incubate the tube at 37°C for 4-6hrs.

Procedure: There are two methods which detect coagulase.

1. Slide coagulase test
2. Tube coagulase test

Slide coagulase test:

1. First of all, we will take a clean and grease free glass slide.
2. After that add a drop of plasma.
3. Then place a drop of saline or distilled water. then next add a drop of plasma as a negative control.
4. Emulsify a portion of the isolated colony being tested in a each drop. Make a smooth milky suspension.
5. Rotate the glass slide gently for 5-10 seconds and look for clumps.

Tube coagulation test :

1. Take a clean test tube.

2. Then emulsify a isolated colony of test organism and add 0.5ml rabbit plasma to give a milky suspension.
3. Incubate the tube at 35C in ambient air for 4 hour.

Result:

Positive: Clot formation

Negative: No clot formation

CHAPTER 4

RESULT AND DISCUSSION

4.RESULT

According to our study from 19rd January 2015 – 30rd april 2015. . During the training period we have collected 445 urine samples. A urine sample were examined and isolates the *E.coli* and *Klebsiella pneumonia.*

Number of the patients n = 445

Mean age of the patients=25-65 year

Total Bacteria isolates = 147

These patients were contacted who were complaining the symptoms of urinary tract infection. Out of these 211are male patients and 234 are female patients of the same symptoms and age group included in this study. After taking the all urine samples of the males and females were processed in the laboratory of bacteriology and also for the detection of extended spectrum of beta lactamases (ESBL) producing organisms of Enterobacteriaecae family *Escherichia coli* and *Klebsiella pneumonia*. The frequency of ESBL among *E.coli* that isolated from the urine shows in Table 1.

Table 1 Distribution of ESBL producing isolates

Total no. of specimen	Total no. of positive organism isolated	% of positive isolates	Total no. of +ve organism of Enterobacteriaceae family	Total no. of ESBL producing organisms	% of ESBL producing organism
445	155	35%	75	26	34.66%

Table 2 Distribution of urinary isolates producing ESBL in both sexes

Organisms	No. of ESBL producing isolates	% of ESBL producing isolates
Escherichia coli	12	46.1%
Klebsiella pneumonia	08	30.7%
Pseudomonas	06	23.07%

Figure10 – Distribution of ESBL producing isolates

Figure 11- urinary isolates producing ESBL in male and female

Table 3 Distribution of ESBL producing organisms from the urine sample of female patients

Organisms	No. of ESBL producing isolates	% of ESBL producing Isolates
E.coli	07	54.0%
Pseudonas	02	15.38%
Klebsiella pneumonia	04	30.7%

Table 4 Distribution of ESBL producing organisms from the urine sample of male patients

Organisms	No. of ESBL producing isolates	% of ESBL producing Isolates
E.coli	06	46.15%
Pseudomonas	02	15.38%
Klebsiella pneumonia	05	38.46%

According to the data given above it is clear that *Escherichia coli* and *Klebsiella pneumonia* showed highest resistances against Cefalexin(100%), Cefepime (96.5), Ampicillin (100%) , Azithromycin (96-98%) , Oflaxacin(70-85%) , Chlororamphenical (97%) and showed less resistances against Imepenem (35%),Levofloxacin(40%) and Pipracillin/tiazobactam (56%), Amikacin(60%) had showed great activity against *Escherichia coli* and *Klebsiella pneumonia* in great activity showed by Amoxyclave (45%), and Chloramphenical (56%).

4.1.DISCUSSION

The extended spectrum beta lactamases (ESBL) commonly producing by Enterobacteriacea confer resistance to beta lactam and monolactam antibiotics. In our study, 75 E. coli isolates were ESBL-producers. Greater and fewer percentages of ESBL production have been reported worldwide by other investigators(Kaye KS, Fraimow HS,2012)[28].

ESBL has emerged as a major contributor of cephalosporin resistant spread of ESBLs in Gram-negative bacteria represent a major challenge to the antimicrobial therapy of infection caused by these microorganisms either in hospital or in community(Paterson DL,Hujer KM,2003)[29] Maximum samples were of urine and from the ESBL production was detected by the disc on disc and double disc synergy test. The urine samples shows the highest rate of ESBL production by DOD and DDST. The pus sample also shows the extended spectrum of beta lactameses. The blood isolates did not show the ESBL production by Disc on Disc and Double Disc Synergy Test.

Antibiotic susceptibility studies of ESBL bacteria showed that *E.coli* isolated were totally resistant to ampicillin, amoxicillin, gentamicin, ciprofloxacin, ceftriaxone,aminoglycosides and cefoperazone. But E.coli was showed maximum susceptibility to imipenem and amikacin. The study revealed that there is high resistance pattern for these antibiotics that is shown under this Table 1.

Table 1 Resistance pattern of ESBL producing E.coli to individual drugs

S.No	ANTIBIOTICS	RESISTANT
1.	Ampicillin	98.7%
2.	Amoxicillin	97.6%
3.	Imipenem	0%
4.	Polymyxin-B	96%
5.	Faropenem	95.5%
6.	Ceftriaxone	97.5%
7.	Ciprofloxacin	92.5%

Figure12:- Resistance pattern of ESBL producing E.coli to individual drug______

Increasingly resistance to broad spectrum cephalosporins amongst *Klebsiella pneumonia* and *E.coli* species producing the ESBL enzyme were reported in different countries(Soek et.al.2014)[30]. Many reports from different regions and different countries have shown the prevalence rates ofESBL producing Enterobacteriaceae causing urinary tract infections. But *E.coli* and *Klebsiella pneumonia* are most common extended spectrum beta lactamases positive species. First , these strains were most likely to be more prevalent and these strains were most widely used and tested for b-lactams. In our study , antibiotics susceptibility shows that ESBL producing *E.coli* isolates were maximum susceptibility to imipenem. A study from North India on uropathogens such as *Klebsiella pneumonia*, *Escherichia coli, Enterobacter* have showed that these isolates were ESBL producers.

CHAPTER 5

CONCLUSION

5.CONCLUSION

Escherichia coli and *Klebsiella pneumonia* has revealed as a highly troublesome pathogen. Worldwide. Due to its ability antibiotic drug resistances, it has propelled to the forefront of scientific attention. Apart from its predilection for the seriously ill patients within intensive care units, *Escherichia coli* and *Klebsiella pneumonia* has more recently caused a wide range of urinary tract infection . This study details the significant advances , including current taxanomy , mechanism of antibiotic resistances ,epidemiology, clinicial manifestation of infection , and treatment regarding *Escherichia coli.*

At the end, we conclude based on our study that there is a high frequency of ESBL producers amongst *E.coli* in our hospital. In order to control the problem of multidrug resistant organisms, strict measures must be taken as to have continuous monitoring for the presence of these organism. It should be mandatory for all the laboratories to incorporate DDST detection of ESBL, especially for Gram-negative as it an convenient test to performs. The complex epidemiology of these resistant strains needs to be further studied in order to design measures to control their spread. New agents and treatment paradigms are required to provide clinicians with the means to treat these potentially dangerous pathogens.

CHAPTER 6

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