ISOLATION AND CHARECTERIZATION OF UROPATHOGENS WITH CONVENTIONAL AND CHROMOGENIC MEDIA AND DETECTION OF EXTENDED SPECTRUM BETA LACTAMASES

Dissertation submitted in

Partial fulfillment of the Regulations required for the award of

M.D. DEGREE

In

MICROBIOLOGY– BRANCH IV

The Tamil Nadu

DR. M.G.R. MEDICAL UNIVERSITY

Chennai

APRIL 2015.

CERTIFICATE

This is to certify that the enclosed work "**ISOLATION AND CHARECTERIZATION OF UROPATHOGENS WITH CONVENTIONAL AND CHROMOGENIC MEDIA AND DETECTION OF EXTENDED SPECTRUM BETA LACTAMASES"** submitted by Dr.V.Vijayashree to The Tamilnadu Dr.M.G.R. Medical University is based on bonafide cases studied and analyzed by the candidate in the Department of Microbiology, Coimbatore Medical College Hospital, during the period from August 2013 to July 2014 under the guidance and supervision of **Dr.K.Rajendran B.Sc., M.D.,** Professor and Head of the Department of Microbiology and the conclusions reached in this study are her own.

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DECLARATION

I, Dr V.Vijayashree solemnly declare that the dissertation entitled **"ISOLATION AND CHARECTERIZATION OF UROPATHOGENS WITH CONVENTIONAL AND CHROMOGENIC MEDIA AND DETECTION OF EXTENDED SPECTRUM BETA LACTAMASES"** was done by me at Coimbatore Medical College Hospital, during the period from August 2013 to July 2014 under the guidance and supervision of **Dr. K. RAJENDRAN B.Sc., M.D.,** Professor and head of the department of Microbiology, Coimbatore Medical College, Coimbatore.

This dissertation is submitted to The Tamilnadu Dr.M.G.R. Medical University towards the partial fulfillment of the requirement for the award of M.D Degree (Branch - IV) in Microbiology.

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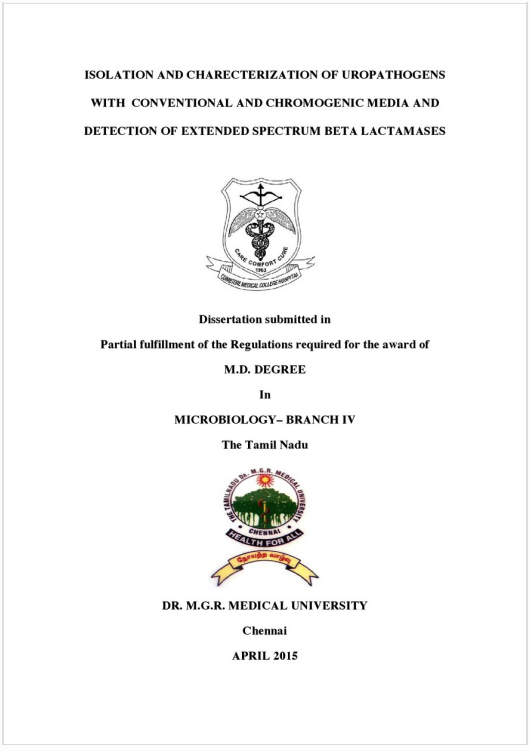

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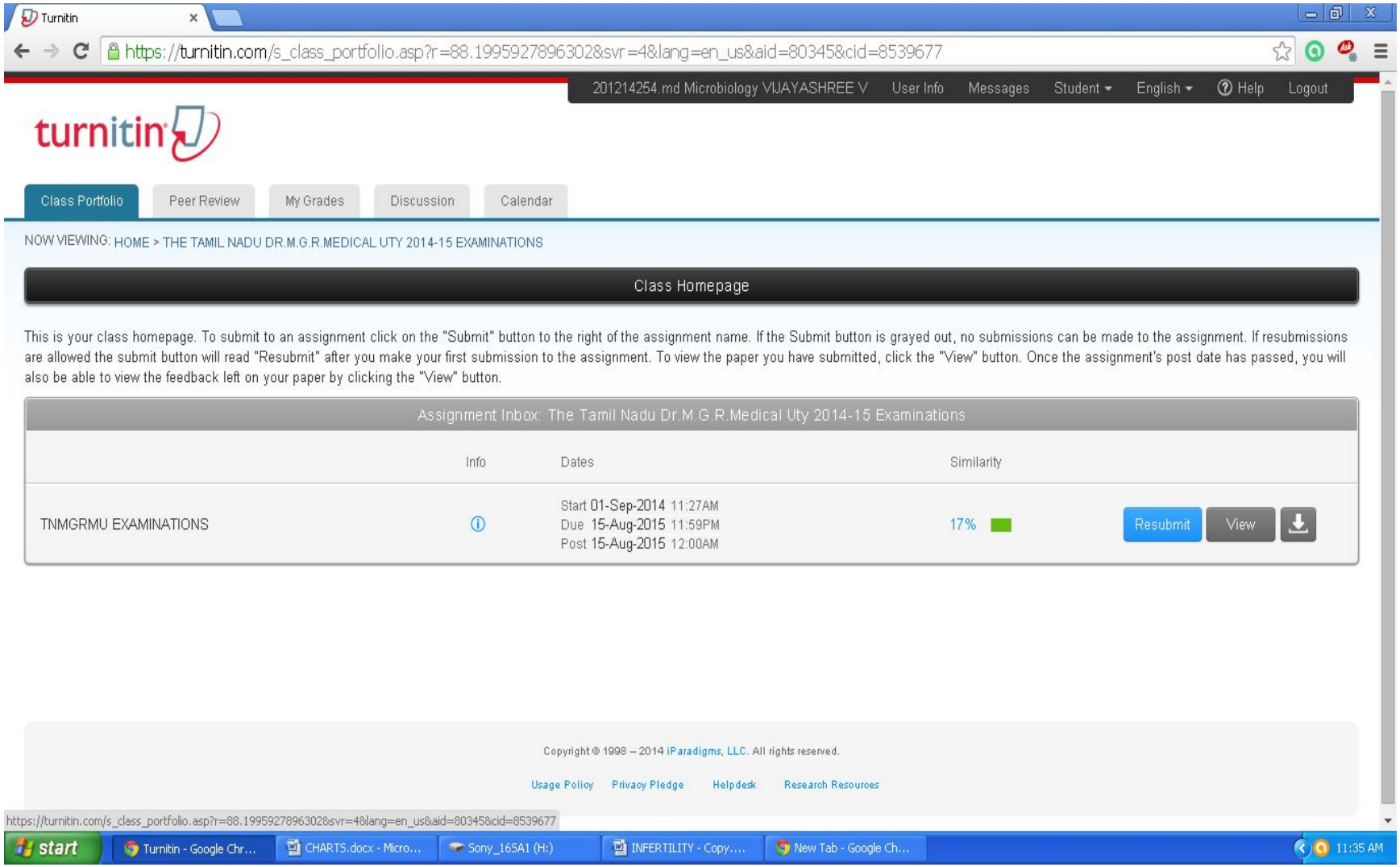
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CONTENTS

ISOLATION AND CHARECTERIZATION OF UROPATHOGENS WITH CONVENTIONAL AND CHROMOGENIC MEDIA AND DETECTION OF EXTENDED SPECTRUM BETA LACTAMASES

ABSTRACT

Background : Urinary tract infections are one of the most common bacterial infections in humans. It is also one of the common specimens sent to the laboratory. Worldwide, the data show that there is an increasing resistance among the organisms which cause UTI. A study on the changing antibiotic resistance pattern is pertinent for an appropriate treatment and for the prevention and control of the different mechanisms of resistance.

Aim : To compare the efficacy of chromogenic media with conventional media for the isolation and identification of uropathogens and also to detect their antibiotic susceptibility pattern. To detect the extended spectrum betalactamases production among Escherichia coli, Klebsiella pneumoniae and Proteus by double disc synergy test and Etest.

Methods : A total of 422 samples collected from various departments during the period of August 2013- September 2014 were processed in blood agar, MacConkey agar and chromogenic agar. Isolation rate was compared and antibiotic susceptibility tests were done. ESBL isolates were detected using DDST and E test.

Results : Escherichia coli was the common isolate. Chromagar detected all the uropathogens . Escherichia coli was the most common ESBL producer (67.5%). E test was more sensitive than DDST in detecting ESBL.

Conclusion : ESBL isolates are more susceptible to Meropenem, Nitrofurantoin, and Amikacin. DDST can also be adopted as a method of ESBL detection.

Key words : UTI, CHROM AGAR, ESBL, AST

INTRODUCTION

Urinary tract infections(UTIs) are one of the most prevalent bacterial infections in the developing countries. Among the hospital acquired infections , UTI s contribute to 35-40% of them. They also cause morbidity and increased mortality in hospitalized patients.[1] It has a spectrum of clinical entities with severity ranging from asymptomatic infection to acute pyelonephritis with sepsis.

The prevalence of UTI is age and sex dependent; with more of sex predilection towards females . The anatomy of female urethra is of particular importance to the pathogenesis of UTIs. Because of the short urethra and its close proximity to the perianal region, bacteria can reach the bladder more readily and cause infection in females .Also the anatomic and hormonal changes during pregnancy increase the incidence of bacteriuria. Other predisposing factors include diabetes, urinary calculi, structural and functional abnormalities of the urinary tract, renal disease and indwelling urinary catheters. Bacteria can invade and cause infection by two major routes. The ascending route is the most common route of spread of infections whereas hematogenous spread occurs as a result of bacteremia.

Urine samples are amongst the most common specimens sent to the microbiology laboratory. Only a small percentage of urine samples

give significant growth. Considerable amount of time is spent in evaluating samples that do not have clinical utility.[2]. Rapid identification of the causative organisms is necessary especially in hospitalized patients as increase in resistant organisms cause difficulty in treatment.[3] It provides useful information for the clinician to select appropriate antibiotics prior to the results of susceptibility tests . Therefore any medium with a ability to show results within a short time (i.e) reduced turnaround time and laboratory cost is preferred.

The main causative agent of urinary tract infection is Escherichia coli. Other Gram negative organisms like Klebsiella, Proteus, Pseudomonas, Enterobacter and Gram positive organisms such as Enterococcus faecalis and Staphylococcus saprophyticus are also responsible for the remainder of the UTIs .

Most of the laboratories use blood agar, MacConkey agar or Cystine Lactose Electrolyte Deficient Agar (CLED) for primary inoculation of urine samples . Though blood agar is used as an enriched medium it lacks the ability to primarily differentiate between Gram positive or Gram negative organisms, which needs further identification tests and delay in result. Swarming of Proteus species is also not prevented in blood agar. Lactose fermenting and non lactose

fermenting organisms are differentiated by MacConkey agar using neutral red indicator[4]. Hence Blood agar and MacConkey agar medium are used together conventionally for the isolation of urinary pathogens. CLED (Cystine Lysine Electrolyte Deficient) medium supports the growth of Staphylococci, Streptococci and Candida. MacConkey agar and CLED agar media distinguishes Lactose fermenting and non lactose fermenting colonies and also prevent swarming of Proteus spp.. But further differentiation of organisms are not possible because these media lack the genus or species specific indicator properties .Also Gram positive organisms like Enterococci and Streptococci may be obscured by the heavy growth of Enterobacteriaceae.Hence it may be difficult to identify the predominant organism in mixed cultures[5]

Of late, Chromogenic media, as an alternative are available to overcome these limitations. These media help in identification of pathogens on the primary plate itself , by the typical colour and morphology produced by the organisms there by reducing the number of confirmatory tests. They also have the advantage that they support the growth of all uropathogens[6] and prevent swarming of Proteus.

Bacteria have many enzymes like β glucuronidase,and β galactosidase for their physiologic functions. Chromogenic substrates

incorporated in the Chromogenic media are specifically broken down by the enzymes present in the particular bacteria, imparting a distinct color to the growing bacterial colony, which can be observed visually. Chromogenic media help in rapid identification of the organisms . This in turn may help in initiating appropriate antibiotics earlier. These media also provide increased sensitivity in identification of some Gram positive cocci (e.g.,Enterococci) in mixed cultures.

Antimicrobial resistance is a growing threat worldwide. Resistance mechanisms are found for almost every class of antibiotic agents. The production of beta lactamases are the important mechanism of resistance in Gram negative bacteria. The production of extended-spectrum β-lactamases (ESBL) is an important mechanism of resistance to third-generation cephalosporins which are widely used in urinary tract infections because of lesser nephrotoxicity effects. During last few decades ESBL producing Gram-negative organisms have been a major problem in many settings.

Extended spectrum beta lactamases (ESBL) are enzymes capable of conferring bacterial resistance to the penicillins ,first, second and third generation cephalosporins and aztreonam(but not the cephamycins or carbapenems) by hydrolysis of these antibiotics. They are inhibited

by beta lactamase inhibitors such as clavulanic acid, sulbactum and tazobactum They are thought to have evolved by mutation of the TEM and SHV genes .Organisms responsible for UTIs such as E.coli and Klebsiella produce these enzymes. These enzymes are plasmid borne and confer multi drug resistance, difficulty in treatment and increased morbidity and mortality .Detection of ESBLs in urine samples are important because they represent epidemiological marker of colonization [7]. Disc diffusion methods have been proposed by CLSI for the detection of ESBLs in organisms such as Klebsiella, Escherichia coli and Proteus. The reduction in the zone diameters of antibiotics such as cefpodoxime, ceftriaxone, ceftazidime, cefotaxime or aztreonam indicate ESBL production. Testing with more than one drug increases the sensitivity of detection. Phenotypic confirmatory methods, such as double disc synergy test, combined disk method and E test (epsilometry methods) are available which are based on in vitro inhibition of ESBLs by betalactamase inhibitors, Genotypic detection methods are useful in distinguishing between specific enzymes (TEM, SHV, CTX-M) responsible for ESBL production and for epidemiological purposes.

As the specimen load (urine specimen) received in the laboratory have been increasing and the multidrug resistant organisms causing serious infections are on the rise, this study has been taken up. The spectrum of organisms causing UTI in our hospital with their antibiotic susceptibility pattern has been evaluated. In this study phenotypic methods such as double disc synergy tests and Etests are used in the detection of ESBLs among Gram negative isolates.

AIMS & OBJECTIVES

AIMS AND OBJECTIVES

AIM :

To isolate the uropathogens in conventional media such as blood agar, MacConkey agar and chromogenic agar, and to compare the rate of isolation. To study the antibiotic susceptibility pattern of the organisms and detection of extended spectrum beta lactamases in the Gram negative isolates.

SPECIFIC OBJECTIVES :

1. To isolate and characterize the uropathogenic organisms in blood agar , MacConkey agar and chromogenic agar.
2. To compare the isolation rate in chromogenic agar and conventional agar.
3. To study the antibiotic susceptibility pattern of the isolates.
4. To detect the extended spectrum beta lactamases among the isolates.
5. To confirm the extended spectrum beta lactamases producing strains with double disc approximation test and Etest or epsilometer test
6. To study the resistance pattern of the extended spectrum beta lactamases.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Urinary tract infection can be asymptomatic (subclinical infection) or symptomatic (disease). The term Urinary tract infection denotes a variety of clinical entities, including asymptomatic bacteriuria, cystitis, prostatitis and pyelonephritis.

Cystitis refers to symptomatic infection of the bladder and pyelonephritis denotes symptomatic infection of the kidneys.

Uncomplicated urinary tract infection refers to infection in a structurally and neurologically normal urinary tract.

Complicated urinary tract infection refers to infection in a urinary tract with functional or structural abnormalities, indwelling catheters and calculi.

Recurrent urinary tract infections may be caused by relapses or reinfections .

Relapse is caused by the persistence of microorganism in the urinary tract that was present before therapy was started**.**

Reinfection is a recurrence of bacteriuria with a different microorganism or with a same microorganism, which may have persisted in vagina or faeces.

Urosepsis is the sepsis syndrome caused by urinary tract infection.

Acute lobar nephronia, also called focal bacterial nephritis, is inflammation and edema of one kidney lobe in a patient with acute pyelonephritis.[8]

EPIDEMIOLOGY

Urinary tract infections are the most common bacterial infections. Approximately 10% of people experience urinary tract infection during their life time.[9] During the first year of life the incidence of urinary tract infection in males and females are about 2%. The incidence in males become relatively low after one year of age until about sixty years, when hypertrophy of prostate interferes with bladder emptying. The incidence in females increases with age to as high as 10%.Short urethra, close proximity to perirectal region, sexual activity, hormonal changes during pregnancy all contribute to the higher prevalence of urinary tract infections among females.

Age related risk of acquiring UTI[10]

Age	Prevalence	Predisposing factors
Neonate	1% males, females	Congenital urethral valve or stenosis
School children	1-2% in girls, 0.03% in boys	30-50% have vesico uretrric reflex
Young adults	1-3% in women, 0.1% in men	Sexual activity, altered vaginal flora
Older adults	20% in women, 10% in men	Bladder prolapsed, calculi, prostatic hypertrophy

Risk factors for urinary tract infections

1. Lack of circumcision.
2. Previous urinary tract infection
3. Urologic instrumentation or surgery
4. Urethral catheterization
5. Neurogenic bladder
6. Renal transplantation
7. Diabetes
8. Pregnancy

9. Bladder prolapse

10. Prostatic enlargement.

Etiology

In acute uncomplicated cystitis, Escherichia coli accounts for 75-90% isolates, Staphlococcus saprophyticus for 5-15%(in younger women),Klebsiella ,Proteus ,Enterococcus ,Citrobacter and other organisms for 5-10%.

In uncomplicated pyelonephritis E.coli is the predominant isolate.

In complicated UTI(catheter associated) E.coli is the predominant organism, but organisms such as Klebsiella, Proteus, Citrobacter, Acinetobacter, Morganella and Pseudomonas are also isolated. Gram positive organisms such as Enterococci, Staphlococcus aureus and yeasts also play an important role in complicated UTI.[11]

Adeno virus type 11 causes epidemic haemorrhagic cystitis in children , particularly in boys.[64]

Pathogenesis

Urinary tract infections are caused by the result of interaction of bacterial virulenc , as opposed by host defence mechanisms. The three routes by which bacteria can enter and spread within the

urinary tract are the hematogenous, ascending and lymphatic pathways.

Ascending route: Colonization of the vagina and periurethral area with the organisms from the intestinal flora is the initial step in the pathogenesis of UTI. Sexual intercourse, nonoxynol-9 in contraceptives, which is toxic to normal vaginal flora ,estrogen deficiency in post menopausal women and short urethra in females contribute to the increased vaginal colonization of E.coli and bacteriuria.

Anatomical and functional abnormalities: Any condition that produces urinary stasis or obstruction predisposes to UTI.Foreign bodies such as stones, urinary catheters, vesicoureteral reflux, ureteral obstruction secondary to prostatic hypertrophy, neurogenic bladder, urinary diversion and surgery create an environment favourable to UTI. In these conditions ,E.coli strains lacking typical urinary virulence factors cause infection. Decreased ureteral tone and inhibition of ureteral peristalsis are important pathogenesis of pyelonephritis in pregnant women.

Hematogenous route: In patients with Staphylococcus aureus bacteremia or endocarditic, kidney is frequently the site of abscess formation. Infection of the kidney with gram negative bacilli such

as Salmonella results from bacteremia .Less than 2% of urinary tract infections occur by hematogenous route. The infections with Candida occur mostly by hematogenous route. The presence of Candida in a noninstumented immunocompetent patient indicates either contamination or widespread dissemination.

Lymphatic route: The increased pressure in the bladder causes lymphatic flow to be directed towards the kidney .Thus the ascending pathway is the most important.

UROVIRULENCE IN BACTERIA

The commonest organism causing urinary tract infections are E.coli. Only a few serogroups of E.coli -01, 02, 06, 07, 08, 075, 0150, 018 ab cause infection. Serotypes such as O,K and H causes severe pyelonephritis. These isolates express chromosomally encoded virulence markers.[12] The virulence genes commonly associated with uropathogenic E.coli include **P fimbriae (pap), aerobactin (aer), afimbrial adhesion 1 (afal), aerobactin (aer), type 1 fimbriae, hemolysin (hly), Sfimbriae (sfa), cytotoxic necrotizing factor 1(cnf1), adhesins and fimbriae (1,5**).[13] These virulence genes help in bacterial colonization in urinary epithelium causing severe disease. **The p fimbriae** attach to the globoseries receptors present in the P blood group antigens of erythrocytes and uroepithelial cells. They

augument the infection by remaining longer in the intestinal tract and spreading efficiently to the urinary tract for colonizing and producing ascending infection. They are mannose resistant.

Type 1 fimbriae are mannose sensitive. They bind to mannose epitopes on secretory IgA , urinary mucus, Tamm-Horsfall protein, bladder uroplakin protein and fibronectin. Adherent bacteria multiply into biofilm like inclusions or enter into a quiescent phase for reemergence. This may contribute to clinical relapse. Bacterial adherence to urinary catheters is also type 1 fimbriae dependent.[14] Type 3 fimbriae contribute to biofilm formation and urethral catheter related infections. Other adhesins like t**ype1c,G,Dr fimbriae and M and X adhesins .**Dr fimbriae positive E.coli persist in renal infections and play a role in chronic pyelonephritis and interstitial nephritis.All these adhesins bind to uroepithelial cells and cause recurrent urinary tract infections.

Organization of virulence factors: In uropathogenic Escherichia coli virulence factors like fimbriae and hemolysin are clustered in segments of DNA ,termed pathogenicity islands. An organism may contain more than one of these islands . A cross talk between the genes encoding the pathogenicity islands and chromosomal loci has been described.

Other uropathogens like Proteus mirabilis and Klebsiella species also have showed the adherence in the pathogenesis of urinary tract infections.

Other virulence factors: Motile bacteria can ascend against the flow of urine. Endotoxins of Gram-negative bacilli decrease uretral peristalsis and contribute to renal parenchymal inflammatory response.

In Proteus species, and in Staphylococcus saprophticus the production of urease increases the urinary pH and the increased concentration of ammonia lead to precipitation of struvite or carbonate-apatite. Also urease may increase the adhesion of bacteria to the urothelial cells.

The K capsular antigen protects bacteria from leukocyte phagocytosis. The hemolysin produced facilitates tissue invasion and causes renal tubular and parenchymal damage, making iron available to invading E.coli. Aerobactin, an iron scavenging protein or siderophore is present in the uropathogenic strains of E.coli. Uropathogenic strains have a lot of iron acquisition systems than fecal or commensal strains, which shows the adaptation to the iron limiting urinary environment.[15]

In Staphlococcus saprophyticus ,a hemagglutinin that recognizes a protein receptor on the erythrocyte is present. This adhesin is responsible for the attachment to uroepithelial cells.In Enterococci an aggregation substance that contains an RGDS and RGDV motif is expressed which binds to cells expressing integrins which are the receptors.

The susceptibility to infection is different for various parts of the urinary tract. The medulla is easily susceptible to infection because of the high concentration of ammonia,high osmolality, low pH, and low blood flow which may inactivate the complement.

Anti bacterial host defences in the urinary tract

The bacterial adherence to uro epithelial cells are inhibited by Tamm-Horsfall protein, bladder mucopolysaccharide, secretory immunoglobulin A, lactoferrin, polymorphonuclear neutrophils and cytokines.

Host signalling in response to uropathogens

Toll-like receptor 4 (TLR4) is essential for signalling innate immunity in response to UTI. The enhanced expression of TLR 4 causes the secretion of interleukin-6, and interleukin-8 which prevents the invasion of the bacteria and helps in expulsion of uropathogenic organisms.[16]TLR1,TLR2 are also secreted in response

to urinary tract infections. Polymorphism in the interleukin 8 receptor gene CXCR 1 impairs neutrophil dependent host defence against bacterial invasion and increases the chance of infection.[17]

Immunity

Systemic antibody response is produced in acute pyelonephritis. Antibodies against type 1 fimbriae, P fimbriae ,Kantigen and O antigen are produced .Ig M antibodies are found in response to upper tract infections. High level of Ig G antibodies correlate with the progression of renal parenchymal destruction. Lower UTI is associated with a reduced serologic response, indicating the superficial nature of the infection .Secretory IgA is also secreted in response to the microorganisms. In spite of the urinary antibody response, the protective role is not clear. Antibodies may be useful in limiting the damage produced in the kidney and in preventing the colonization which precedes recurrence.

Structural abnormalities which produce obstruction to urinary flow, result in stasis which in turn increase the susceptibility to infection.

Extra renal obstructions like congenital anomalies of the urethra or ureter, such as stenosis, valves or calculi, uterine prolapse , benign prostatic hypertrophy and intrarenal obstructions such as

nephrocalcinosis, analgesic nephropathy, uric acid nephropathy, , poly cystic kidney disease, hypokalemic nephropathy, inhibit the normal urinary flow and increase the susceptibility to infection. Vesicoureteral reflux , especially in children contribute to infection of ureter and kidneys which result in subsequent scarring.

Patients with incomplete emptying of the bladder or neurologic abnormalities like diabetic neuropathy and spinal cord injuries are prone to frequent urinary tract infections.[18]

Genetic factors like decreased expression of CXCR 1 and Toll like receptor 4 result in decreased neutrophil migration and increased susceptibility to asymptomatic bacteriuria.

The spectrum of presentation

- Asymptomatic bacteriuria
- Symptomatic acute cystitis and urethritis
- Acute pyelonephritis
- Acute prostatitis
- Acute urethral syndrome
- Septicemia

Sometimes urinary tract infections are classified as complicated and uncomplicated. Uncomplicated infections occur in

healthy females, occasionally in male infants, and adolescents and adult males.

Complicated urinary tract infections occur with the following risk factors:

- Underlying diseases that predispose kidney to infection (diabetes, sickle cell anemia)
- Kidney stones
- Structural or functional abnormalities of the urinary tract
- Indwelling urinary catheters.

Uncomplicated infections respond readily to antibiotics and complicated infections are difficult to treat and have higher morbidity and mortality rates.

Asymptomatic bacteriuria[19]

It is defined as $> 10^5$ organisms/ml in the urine of apparently healthy patients. Its prevalence varies with age, gender, and the presence of genitourinary abnormalities. The prevalence is 1% among children under the age of 1 and school girls. It is of 0.03% for school boys and men,3% in non pregnant adult women and 5% for pregnant women. Screening and treatment for asymptomatic bacteriuria is recommended only for pregnant women ,patients

undergoing transurethral resection of prostate or other urological surgeries, and renal transplant recepients. Screening and treatment is not recommended for premenopausal, diabetic women, non pregnant women, persons with spinal cord injury, and catheterized patients. E.coli is the commonest organism causing asymptomatic bacteriuria. Pregnant women with asymptomatic bacteriuria are more prone for pyelonephritis and hypertension. Preterm deliveries and low birth weight infants are also common in pregnant women with asymptomatic bacteriuria . These organisms possess lesser virulence characteristics than those causing symptomatic infections.

Acute cystitis [20]

The features of cystitis are

- abrupt onset of micturition and urgency
- dysuria(i.e) scalding pain in the urethra during micturition,suprapubic pain during and after voiding
- desire to pass urine after micturition due to spasm of inflamed bladder wall
- Cloudy urine with an odour
- Presence of haematuria.

Fever and other signs of systemic illness are usually absent because cystitis is a local infection.

Urethritis

Urethritis is a common infection with symptoms of dysuria and frequency. The common causes of urethritis is Escherichia coli. The non specific urethritis is due to organisms such as Chlamydia trachomatis, Neisseria gonorrhea, Trichomonas vaginalis , herpes simplex and cytomegaloviruses.

Pyelonephritis[21,22]

Pyelonephritis is a bacterial infection of the kidneys that results from ascent of the pathogens from bladder to the kidneys through the ureters.The incidence is higher in healthy women between 15-29 years of age, followed by infants and older persons. E.coli is responsible for 80% of acute pyelonephritis cases. Other causative organisms include Enterobacteriaceae, Pseudomonas aeruginosa, group B streptococci and enterococci. Risk factors include structural abnormalities of the urinary tract, previous urinary tract infections, and diabetes. The clinical features are lower urinary tract symptoms (urinaryfrequency, urgency, dysuria), along with fever, rigors, nausea, vomiting, and flank pain. The fever is high, spiking “picket-fence” pattern and resolves after 72 hours of

therapy. In 20-30% of patients pyelonephritis leads to bacteremia. Papillary necrosis may occur in patients with diabetes, sickle cell disease and analgesic nephropathy. It produces obstruction and rise in serum creatinine level.

Emphsematous pyelonephritis, a necrotizing form of pyelonephritis with gas formation and is seen in patients with diabetes mellitus.

Xanthogranulomatous pyelonephritis, is a chronic infection, that resembles a tumour, and is usually associated with obstruction and generally requires nephrectomy.

Acute urethral syndrome

Patients with this syndrome are young, sexually active women, who have cardinal symptoms of dysuria, frequency and urgency. They yield less than 10^5 cfu/ml (colony forming units per ml).90% of patients have pyuria.(presence of ≥8 puscells per cubic millimeter on microscopic examination of uncentrifuged urine). Urethritis, vaginitis and prostatitis are the causes of acute urethral syndrome. Non specific urethritis anaerobic infection and genital herpes account for some of the cases.[23]

Catheter associated urinary tract infections(CAUTI)

Catheter associated urinary tract infection is defined as the presence of symptoms and signs compatible with urinary tract infections in a patient who has been catheterized within the previous 48 hours., with no other source of infection, along with $\geq 10^3$ cfu/ml of urine.[24]CAUTI accounts for 30% of nosocomial infections and is associated with prolonged hospital stay. Long term catheterization, old age, female sex, and diabetes are risk factors associated with CAUTI. The order of frequency of pathogens were E.coli, Klebsiella, Pseudomonas, Candida, Enterococcus, Coagulase negative staphylococcus, Citrobacter, and Proteus species .This can be avoided by using catheters in situations, except where there is an absolute indication and observing strict asepsis during the procedure.[25]

Diagnosis

Detailed history and physical examination is necessary. In case of pyelonephritis imaging studies may be necessary. Urine can be sent to the laboratory for analysis . Proper collection of urine is very important since urine specimen is much more prone for contamination.

Collection of urine

Clean catch midstream specimen : It is the most commonly used method of urine specimen collection. The technique involved is based on collecting urine aseptically avoiding the first portion which is likely to be contaminated. Clean catch midstream urine should be collected in a sterile, wide mouthed, screw capped bottle, after thorough cleansing of external genitalia with soap and water. Antiseptics should not be used for this purpose.

Indwelling or Foley's catheter: Urine is collected by disinfecting a portion of the catheter tube with alcohol, puncturing the tube with a sterile needle and syringe and aspirating the urine .Urine should not be collected from the collection uro bag.

Suprapubic aspirate : Since urethral contamination is avoided suprapubic aspirate is preferable. It is usually performed in children from whom it is difficult to collect urine. In this method skin above the bladder is cleaned with a disinfectant ,a sterile needle and syringe is introduced into the bladder, urine is aspirated and placed in a sterile container.[26]

Specimen transport : Since urine acts as an excellent culture medium for bacteria , it must be sent to the laboratory as soon as it is collected and should be cultured immediately .If this is not

possible it may be refrigerated at 4-6◦C maximum for 2 hours before plating .When delay in delivery of more than 2 hours is anticipated, boric acid preservative(0.1gm/10 ml) should be added to urine. Specimen containing boric acid need not be refrigerated.[27] Other preservatives used are tartaric acid, boric acid ethyl paraben, chlorhexidine, thymol and sodium propionate. Using this preservatives urine can be stored for upto 24-72 hours without refrigeration.[28]

Screening tests for bacteriuria and pyuria

Microscopic examination:

Examination of an uncentrifuged urine :Detecting bacteria in uncentifufed urine indicates urinary tract infection .i.e bacteriuria in excess of 10^4 /ml. Pyuria can be quantified by counting White blood cells or estimating numbers by examining a drop of urine on a slide. Alternatively, an inverted microscope is used, in which 60µl of urine is examined in a flat bottom well of a microtitration plate and cell numbers calculated using a simple formula.

Preparation and examination of a wet preparation: Aseptically transfer about 10 ml of urine to a conical tube, centrifuge at 500-1000g for 5 minutes. Transfer one drop of well mixed sediment to a slide, cover with a cover glass and examine the preparation using

10x and 40x objective. Report for bacteria, pus cells, red cells, casts, epithelial cells, yeast cell and eggs of schistostoma hematobium.

Examination of the Gram stained preparation. In the examination of the Gram stained preparation of the uncentrifuged urine ,presence of at least one organism per oil immersion field has a sensitivity of 94% and specificity of 90% for detecting colony counts of atleast 10^5colony forming units per ml.[29]

Tests for bacterial products[30]: Griess nitrite test :Nitrite is absent in normal urine. The presence of nitrite detectable by a simple test, indicates the presence of nitrate reducing bacteria in urine. This was first applied to urine by J.Cruishank and J.Moyes in 1914.[31]

Catalase test : On addition of hydrogen peroxide, frothing indicates the presence of catalase .It is an indication of bacteriuria and hematuria.

Triphenyltetrazoliumchloride :It is based on the production of pink red precipitate in the reagent caused by the respiratory activity of growing bacteria.

Dip slide culture methods :Agar coated slides are immersed in urine or exposed to the stream of urine during voiding, incubated,

and growth estimated by colony counting or by color change of the indicators.

Tests for pyuria

Leucocyte esterase test strip: This is based on the esterase activity of granulocytes to hydrolyse an indoxyl ester to free indoxyl which reacts with a diazonium salt to form a violet dye. Automatic test strip reading is now available to improve the reliability of the results.

Leucocyte counting by Rant-Shepherd method: J D Rant and Dr W Shepherd devised a method of using an inverted binocular microscope with a mechanical stage and plastic flat bottomed microtitre wells. Fixed volume of urine is pipetted out into the tray and observed microscopically .From the knowledge of the area of the field and depth of the urine in the well ,the number of polymorphs in the urine can be estimated.[31,32]

Haemophilus, Gardnerella, Mycobacteria, Mycoplasma, and Chlamydia could be the possible agents, if there is presence of pus cells without culture growth.

Unacceptable specimens : Foley catheter tips, sediment from centrifuged urine which are inoculated into broth , urine stored for

more than 2 hours at room temperature or dried dip slides are specimens unacceptable for culture.[33]

Urine Culture

Urine culture is the Gold standard in the diagnosis of UTI.[11]

Surface viable count by Miles and Misra method: An inoculam is deposited as drops from a calibrated dropping pipette with different dilutions in separate plates..Counts are made in drop area showing the largest number of colonies without confluence. The total of the counts give the viable count per 0.1 ml of dilution. This method is too laborious for routine use.[34]

Semi-quantitative culture: The methods used for the semi quantitative culture are calibrated loop technique and the filter paper dip strip method.[35]

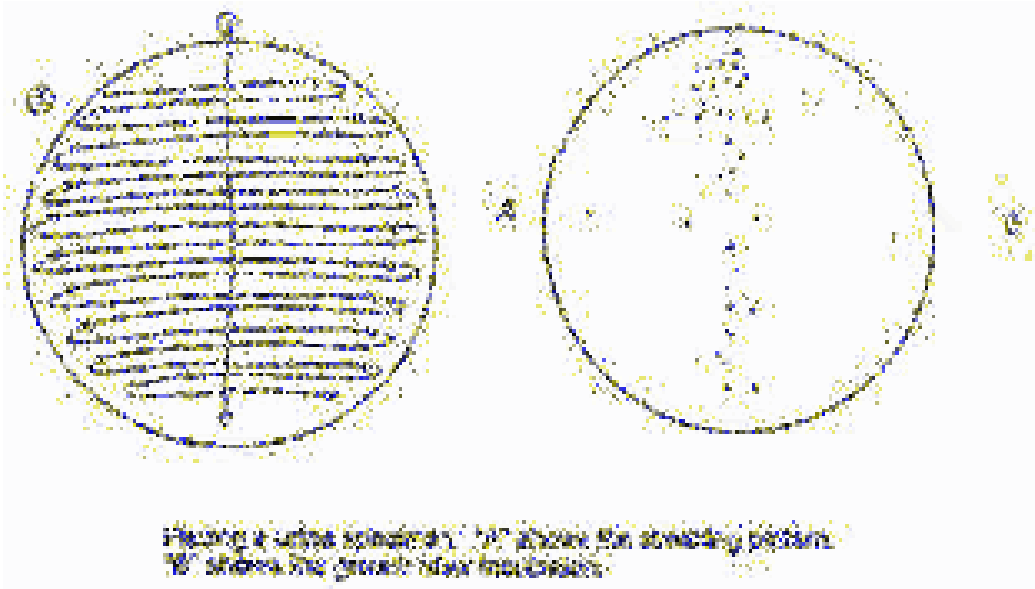
Calibrated loop technique:

To evaluate the clinical significance of a positive urine culture estimation of the number of organisms present is essential. A measured amount of urine is inoculated to each of the appropriate media. Urine should be thoroughly mixed before plating. Serial dilutions of urine is made by mixing 0.1 ml of urine with sterile normal saline. A calibrated loop with a diameter of 2mm or 4 mm can be used. The loop should be made up of metal or

plastic .A 4mmloop delivers 0.01 ml of urine and 2mm loop delivers 0.001ml of urine.

Method:

1. Insert the loop vertically into the urine to allow urine to adhere to the loop
2. Spread the loopful of urine over the surface of the agar plates
3. Loop should touch to the centre of the plate, from which the inoculam is spread in a line across the diameter of the plate.
4. Without flaming or reentering urine, loop should be drawn across the entire plate, crossing the first inoculam streak numerous times to produce isolated colonies.



Incubate the plates overnight at35◦C .

Filter paper method: The filter paper dip strip method of Leigh and Williams is based on the absorption and transfer of a fixed amount of urine to a suitable plating agar medium. But growths are often confluent and mixed .To obtain pure cultures for sensitivity testing, it may require further plating. Estimate the number of viable bacteria per ml of urine from the count of colonies on the impression area or the pattern of semi-confluent or confluent growth.

Choice of media

The choice of media to inoculate varies with the different Microbiology laboratories .Use of 5% sheep blood agar plate and a MacConkey agar plate allows detection of most Gram negative bacilli, Staphylococci, Streptococci and Enterococci. In some circumstances enterococci and streptococci may be obscured by the heavy growth of Enterobacteriaceae. Hence some laboratories add a selective media like Columbia colistin nalidixic acid agar or phenylethylalcohol agar for Gram positive organisms. Cystine lactose electrolyte deficient (CLED) agar is also used which differentiates lactose fermenting and non lactose fermenting colonies.

MacConkey agar This medium was developed by **Alfred Theodore MacConkey** while working as a bacteriologist for the Royal Commission on sewage disposal. This was the first solid differential media. It is primarily used in the isolation of Gram negative bacteria from faeces, urine, water and food. It is a selective and differential media, used for the isolation of enteric Gram negative bacteria. Gram negative bacteria that grow on MacConkey are differentiated by their ability to ferment lactose. If the organisms ferment lactose the production of acid drops the pH of the media, indicated by the change of neutral red indicator to pink. Gram negative bacteria that grow on MacConkey agar do not ferment lactose and appear colourless on the medium.

Composition of MacConkey agar

Enzymatic digest of gelatin, casein, and animal tissue provides vitamins ,minerals, nitrogen and aminoacids essential for growth. Lactose is a carbohydrate which provides carbon and energy. Sodium taurocholate and crystal violet are the selective agents inhibiting Gram positive organisms. Neutral red is a pH indicator which is red in colour at pH 6.8.

Modifications of MacConkey agar

MacConkey agar without crystal violet :Lack of crystal violet permits the growth of Staphlococcus and Enterococcus.

MacConkey agar without sodium chloride is used to prevent swarming of Proteus.

Blood agar :

In 1919, **James Brown**[36] used a blood agar medium to show the haemolytic properties of Streptococcus. It is an enriched and an indicator medium showing the type of haemolysis such as alpha,beta, and gamma haemolysis. The medium is prepared by adding sterile blood to sterile nutrient agar that has been melted and cooled to 50◦C. Blood may be used at a concentration varying from 5-10%.Either human or animal blood can be used. It is a medium used for the culture of fastidious organisms .Sheep blood has proved to be the more versatile additive. Incorporation of blood provides enrichment for growth and also haemolytic activity. Staphylcoccus aureus usually produces a golden yellow pigment on blood agar. Staphylococcus saprophticus produces either bright yellow or white colonies. Staphylococcus epidermidis produces white colony. Staphylococcus aureus is usually, but not always

beta-hemolytic. Staphylococcus epidermidis and saprophyticus are always non-hemolytic.

CLED agar

Cystiene Lactose Electrolyte Deficient Medium

It is used for the isolation and differentiation of urinary pathogens. An attempt to prevent swarming of Proteus by the use of an electrolyte-deficient medium, by Sandys in 1960 led to the development of a new medium. On subsequent modification by Mackey and Sandys with addition of cystine resulted in CLED agar media.

Beef extract and casein peptone provide nitrogen, vitamins, minerals and aminoacids for growth. Lactose is the fermentable carbohydrate providing carbon and energy. L-cystine is added as a growth supplement. Bromothymol blue is a pH indicator which differentiates lactose fermenters and non lactose fermenters. Organisms which ferment lactose will change the colour from green to yellow. It has the advantage of supporting the growth of Staphylococci, Streptococci and Candida.[37]

Of late **Chromogenic media** has been introduced and available which allows direct identification and differentiation of uropathogens on the primary plates itself. This enhances the rapid

identification of the isolates. In 1979 Dr.Alain Rambach started the work on chromogenic medium. In 1989 Rambach agar- salmonella was discovered. Chromagar UTI is formulated on the basis of the work done by Pezzlo, Wilkie et al, Friedman et al, Murray et al and Merlini et al. This media can be used in place of MacConkey agar, for isolation and identification of microorganisms. Identification is done on the basis of different colony colors produced by genus or species specific enzymes reacting with the chromogenic substrate. The basal nutritive ingredients are peptone and glucose. Addition of chromogenic mixtures and other substances have contributed to the development of a series of media, specific for different organisms. Greater recovery of organisms and increased specificity are the benefits of these media. Limited shelf life and cost effectiveness are to be compared with other standard media.[38] This media acts both as a selective medium and a differential medium.[39]

Enzymes produced by Escherichia coli, Enterococcus species and other coliform bacteria cleave the chromogenic substrates incorporated in the medium. E.coli produce pink colonies due to the enzyme β-D-galactosidase which cleaves the chromogenic substrate. It is further confirmed by spot indole test using

dimethylamino cinnamaldehyde reagent. The enzyme β glucosidase, produced by the Enterococcus species produce blue colonies. The ingredients peptic digest of animal tissue, and casein hydrolysate provide a rich source of phenylalanine and tryptophan. Proteus spp, Morganella spp and Providencia spp can be identified by the tryptophan deaminase activity , appearing brown on addition of ferric chloride reagent. The Klebsiella Enterobacter-Serratia group of species produce both β glucosidase and β galactosidase enzymes and the colonies appear predominantly blue.[39]

Interpretation of urine cultures

Colony count on calibrated loop plates: If urine is undiluted and if 4 mm loop is used which delivers 0.01 ml of urine , multiply the number of colonies by 100. Multiply by 1000 if 1 in 10 dilution of urine is used.

Significant bacteriuria

Although the greater part of urinary tract is devoid of commensal flora ,spontaneously voided urine is apt to be contaminated with commensal bacteria from the urethra and perineum .E.coli and Staphylococcus are the commonest organisms to infect the urinary tract .They are also the commensals. Hence proof of a urinary tract infection requires the demonstration that the

pathogens are present in freshly voided urine in numbers greater than those resulting from contamination.

L Rantz and CS Keefer introduced the [31]the diagnostic criteria of 10^5organism per ml in 1940 .It was substantiated in 1957 by **EH Kass** who also demonstrated the importance of clean catch mid stream urine sample.In 1982, **Stamm et al** studied the diagnostic criteria in women with acute symptomatic lower urinary tract infections. The criteria of 10^2 CFU/ml provided a better sensitivity and specificity. The presence of pyuria in uncentrifuged urine was considered as a sensitive adjuant.[40]

General recommendations for reporting

Category1:

Fewer than 10^4 CFU/ml. Report as probable absence of UTI.(exceptions are supra pubic puncture, cystoscopy, symptomatic women, or the presence of leukocyturia.)

Category 2:

10^4-10^5CFU/ml .If the patient is asymptomatic request a second urine specimen. If the patient is symptomatic proceed with the susceptibility tests.[41]

Category 3:

More than 10^5 CFU per ml. Proceed with identification and susceptibility tests.

In addition to the above mentioned guidelines, pure culture of Staphylococcus, Enterococci and candida are significant regardless of the number of CFUs. Also in mixed cultures from a Clean catch midstream urine, when there is predominance of a single organism, identification and susceptibility testings are done for that organism.[42]

Interpretation of mixed cultures

Specimen with 3 or more uropathgens : It indicates probable contamination. The organism should not be identified and ask for a fresh Clean catch midstream urine specimen.

Specimen with 1 or 2 significant uropathogens :

Organisms with $\geq 10^5$ CFU / ml should be identified and susceptibility tests should be done.

Specimen with 1 or 2 organisms in small numbers :

Organisms with colony counts$\geq 10^2$ CFU / ml should be identified in acute urethral syndrome or with previous antibiotic therapy.

Microbiology of UTI :

In uncomplicated cases the organisms isolated are usually Escherichia coli or Staphylococcus saprophyticus. In complicated cases along with Enterobacteriaceae, Gram positive organisms such as Enterococci are commonly encountered. Polymicrobial infections are common in catheterized patients. Pyelonephritis is commonly caused by Escherichia coli, Staphylococcus aureus and Proteus mirabilis.

In nosocomial infections, next to Escherichia coli ,Enterococci are most commonly isolated. Bacteremia is usually associated with urinary tract infections caused by Enterococci.

Prevalence of common organisms causing urinary tract infections[43]

Organism	**Community acquired infections**	**Hospital acquired infections**
Escherichia coli	70-80%	50%
Proteus mirabilis	10%	1-5%
Klebsiella	1-5%	5-10%
Staphylococcus saprophyticus	10-15%	0%
Staph.epidermidis	1-5%	10-20%
Enterococci	1-5%	10-20%
Other coliforms	< 1%	5-10%
Pseudomonas aeruginosa	1-2%	5-10%

Treatment of UTIs :[10,11,33]

Un complicated cystitis:

Drug of choice is cotrimoxazole and fluroquinolones, which are usually given for three days.. During pregnancy cotrimoxazole and quinolones are contraindicated. In such situations β- lactam antibiotics such as amoxycillin, cephalosporins or urinary antiseptics such as nitrofurantoin may be used.

Complicated UTI :

Occurs in patients with structural and functional abnormalities of the urinary tract. The treatment should be guided by urine culture results.The empirical therapy should be based on the previous culture data.

Pyelonephritis :

In uncomplicated pyelonephritis ,the choice of oral therapy includes Ciprofloxacin and Cotrimoxazole. For parenteral therapy fluroquinolones, an aminoglycoside with or without ampicillin ,an extended spectrum cephalosporin with or without an aminoglycoside or a carbapenem can be used. For complicated cases combination of β lactam and a β lactamase inhibitor or imipenem can be used depending on the culture results.

Asymptomatic bacteriuria:

In pregnancy and in persons undergoing urologic surgeries treatment should be based on the culture reports.

Nitrofurantoin and methenamine are urinary antiseptics They are concentrated in the urinary tract and are active at a lower pH.

Prophylaxis

Vaccines : [44,45]

Attempts have been made in developing vaccines against infections caused by Escherichia coli and Proteus. Vaccines based on single virulence factor or whole cell killed vaccine have been tried in animal models, but because of many virulence factors associated, this is not sufficient to give protection.

An antibiotic prophylaxis is indicated in patients with frequent UTI

Mechanism of antibiotic resistance:

Today every class of antibiotic is associated with the emergence of significant resistance. Two major factors are associated with the emergence of antibiotic resistance; evolution and clinical practices. Pathogens evolve to develop resistance to survive under stress. Indiscriminate use of antibiotics and poor clinical practices that fail to incorporate the pharmacological properties of

antimicrobials, amplify the speed of development of drug resistance. In Gram negative bacteria the mechanisms of antibiotic resistance are as follows:

Efflux of antibiotics from bacteria:

Efflux pumps play a key role in antibiotic resistance and also serve other functions in bacteria such as uptake of essential nutrients and ions, excretion of metabolic end products and deleterious substances as well as the communication between cells and the environment. This mechanism of resistance is a major problem because a single efflux pump can produce a simultaneous resistance to a number of antibiotics.

Outer membrane permeability:

The outer membrane of Gram negative bacteria is a barrier to hydrophobic and hydrophilic compounds. The outer membrane acts as a selective barrier by combining the hydrophobic lipid layer with pore forming proteins which have specific size and exclusion properties. Small hydrophobic antibiotics such as beta -lactams, use the pore forming proteins embedded in the outer membrane to enter into the bacteria, while macrolides and other antibiotics diffuse across the lipid bilayer. The existence of antibiotic resistant strains

in bacteria due to alteration in lipid or protein composition of the outer membrane shows the importance of outer membrane barrier .

Target modification:

The mechanism is based on alteration of bacterial sites that are targeted by antibiotics and thus preventing the antibiotic from binding to its site of action. For example fluroquinolone resistance is attributed to mutations within the drug ‘s target.(DNA gyrase and topoisomerase).

Enzymatic modification of the antibiotic:

There are two methods

1) β-lactamases that degrade antibiotics.

2) chemical transformation to render the antibiotic inefficient.(macrolide and aminoglycoside modifying proteins).

Acquisition and spread of antibiotic resistance in bacteria:

Inherent resistance:

Inherent resistance in bacteria may be due to lack of transport system for an antibiotic or lack of target to the antibiotic molecule. The outer membrane in Gram negative bacteria acts as a permeability barrier.

Acquired resistance:

All require either modification of existing genetic material or the acquisition of new genetic material from another source. In vertical transfer the resistance genes is transferred to bacterial progeny during DNA replication. In horizontal or lateral gene transfer , the genetic material is contained in small pockets of DNA and can be transferred to different bacteria of same species or between different species. The modes of transfer are **conjugation,** through plasmids **,transduction and transformation**.[46]

Beta lactam antibiotics: β lactam antibiotics are commonly used for the treatment of Gram positive , Gram negative and anerobic organisms.

They consist of four major groups**: Penicillins. cephalosporins, monobactum and carbapenems.**

They all have a beta lactum ring, which can be hydrolysed by betalactamases. They differ from each other by additional rings. e.g., Thiazolidine ring for penicillin, cephem nucleus for cephalosporin,double ring for carbapenem, and none for monobactum.At first they inhibit the action of transpeptidase responsible for the completion of cell wall. Secondly, they attach to the penicillin binding proteins which causes cell lysis.

Classification of cephalosporins

First generation cephalosporins: They include cefadroxyl, cefazolin, cephalexin, cephalothin, cephapirin and cephradine.

Second generation cephalosporins: include cefaclor, cefamandole, cefuroxime, loracarbef, and cephamycins such as cefmetazole, cefotetan and cefoxitin.

Third generation cephalosporins: cefaperazone, cefotaxime, ceftazidime, ceftizoxime, ceftriaxone, cefixime, cefpodoxime and cefibuten.

Fourth generation cephalosporins: Cefepime and cefpirome.

Beta lactamases are enzymes that catalyze the hydrolysis of beta lactam ring. Gram negative bacteria releases this enzyme into the periplasmic space, which gives individual protection. The genes encoding these beta lactamases are present on the bacterial chromosomes. These enzymes are constitutively expressed and inducible. The first report of a plasmid encoded beta lactamase was reported from Greece in 1965. Now there are more than 500

beta lactamases found.[47] Classification of betalactamases are based on two approaches, the first one is based on the biochemical and functional characteristics of the enzyme and the second approach is based on the molecular structure of the enzyme.

Evolution of functional classification of βlactamases

Initially in 1968, Sawai et al used cephalosporins vs penicillins as substrates, which was modified by Richmond and Sykes in 1973, by expanded substrate profile.

Sykes and Mathew in 1976, differentiated plasma mediated β lactamases on the basis of isoelectric focusing, which was modified in 1981, by Mitsuhachi and Inove who added cefuroxime hydrolyzing β lactamases category. Bush expanded substrate profile reaction with EDTA (1989) and correlated between functional and molecular classification.

Finally, in 1995, Bush Jacoby and Medeiros expanded the scheme using biochemical properties, molecular structure and nucleotide sequences.

Molecular classification;

It is based on the nucleotide and aminoacid sequences in these enzymes. Accordingly A-D four classes are recognized. The

action of Class A,D and C are based on serine based mechanism and the action of class B or metallo βlactamases are based on zinc.

Bush-Jacoby –Medeiros functional classification with correlation to the Ambler molecular classification[48,49]

Group 1: Cephalosporinases: , they are not inhibited by clavulanic acid. Corresponds to Molecular class C

Group 2a : Penicillanase: Molecular class A, inactivating penicillns and inhibited by clavulinic acid,

Group 2b: Broad spectrum: Molecular classA, capable of inactivating penicillins and cephalosporins; also inhibited by clavulanic acid.

Group 2be: Extended spectrum:molecular class A, capable of inactivating monobactems and third generation cephalosporins but inhibited by clavulanic acid.

Group 2br:Inhibitor resistant, Molecular class A –diminished inhibition by clavulanic acid.

Group 2c:Carbenicillinase Molecular class A, inactivate carbenicillin, with some effect on cloxacillin and inhibited by clavulanic acid.

Group2d:Cloxacillinase Molecular class A &D, inactivate cloxacillin, poorly inhibited by clavulanic acid.

Group2e: Cephalosporinase,Molecular class A,hydrolyse monobactams,inhibited by clavulanic acid.

Group 2f: Carbapenemase Molecular class A ,serine based carbapenemases.

Group 3: Metalloenzyme, Molecular class B ,not inhibited by clavulanic acid, able to hydrolyse penicillins, cephalosporins and carbapenems, act by the metal ion zinc.

Group 4:Penicillinase ,no molecular class, inhibited by clavulanic acid

Beta lactamase inhibitors :

These compounds resemble beta lactam antibiotics. They can bind to beta lactam antibiotics protecting them from destruction and serve as suicide bombers utilizing all enzymes. These compounds have weak anti bacterial activity but are potent inhibitors of many plasmid encoded and some chromosomal beta lactamases. The important beta lactamase inhibitors are clavulanic acid, tazobactum and sulbactum.

Clavulanic acid shows low level of antibacterial action but when combined with beta lactam antibiotics it potentiates the beta lactam antibiotics.

Sulbactam has broader spectrum of inhibition but less potent.

Tazobactam is as potent as clavulanic acid.

Extended spectrum betalactamases:

Extended spectrum beta lactamases are enzymes capable of hydrolyzing third and fourth generation cephalosporins and monobactams, but inactive against cephamycins and imipenem. They are inhibited by betalactam inhibitors such as clavulanic acid,sulbactam and tazobactum.[50,51]The ESBLs have amino acid serine at their active site and they attack amide bond in the lactam ring of these beta lactam antibiotics, causing subsequent lysis. They belong to Bush-Jacoby-Medeiros group 2be, 2d and Ambler class A.

History and evolution

The first plasmid mediated beta lactamase TEM-1 was described in 1960.[52]In early years the most common beta lactamases were TEM1,2 andSHV1 varities. In 1983, the first mutant of SHV-1 is called SHV-2 in a Klebsiella.pneumoniae strain was reported from Germany. It was capable of hydrolyzing oximinocephalosporins . These extended spectrum beta lactamases differ from the parent enzyme by aminoacid substitution . The pressure exerted by the use of expanded spectrum beta lactam antibiotics ,resulted in the development of newer TEM and SHV varieties.

The type of ESBLs are TEM, SHV, CTX-M, OXA, Amp C but majority are derived from TEM or SHV enzymes. These enzymes are commonly found in E.coli and Klebsiella pneumoniae. Sometimes more than one gene may be present in the same organism.[53]

Prevalence

Prevalence of ESBLs vary in different countries and in different areas. In India the prevalence varies from 8 to 80%.[54]The prevalence of TEM and SHV are 56% and 60% respectively.

Gene encoding ESBL

TEM:

TEM-1 enzyme was first reported from E.coli in 1965 and now it is the commonest betalactamase found in Enterobacteriaceae. The name TEM is derived from Temoniera , a patient from Greece from whom the strain was isolated. Based on the combination of different aminoacid changes ,currently 195 TEM type enzymes have been found.

SHV :

The name SHV denotes for sulfhydryl variable. About 68% of aminoacid is shared with TEM-1 .SHV -1 is most commonly found in Klebsiella pneumoniae. ESBLs in this family have amino

acid changes at 238 positions. There are about 60 SHV variants are known. SHV-5 was found to be responsible for outbreaks of nosocomial infections in several countries.

CTX-M :

CTX-M beta lactamases were first isolated from Munich in Japan in 1986.E.coli producing CTX-M have emerged as an important cause of community onset urinary tract infections. These enzymes have greater activity against cefotaxime. Change at 236 position enhances resistance to cefotaxime and change at 102 position enhances resistance to ceftazidime. There are about 80 CTX-M enzymes which are divided into 5 clusters on the basis of amino acid sequence.[55] CTX-M producing Escherichia coli are emerging as an important pathogen causing community onset and hospital acquired infections.

In India CTX-M -15 beta lactamase, is the most prevalent. The bowel provides a rich environment for genetic exchange between commensal enterobacteriaceae. Conditions such as overcrowding, poor sanitation and inappropriate use of antibiotics contribute to the widespread prevalence of CTX-M- 15 beta lactamases.[56] Some organisms may harbor more than one type of ESBLs which may alter the antibiotic resistance phenotype.

Other beta lactamases : OXA beta lactamases, PER, VEB, GES

Inhibitor resistant beta lactamases or Amp C beta lactamases

These beta lactamases are also derived from TEM and SHV type enzymes but they differ from ESBL s. They are not inhibited by beta lactamase inhibitors. There are about 19 inhibitor resistant TEM beta lactamases. They are present on the chromosome of Gram negative bacteria such as Serratia , Citrobacter and Enterobacter.

Carbapenamases:

They are beta lactamases that are active against the oxyimino cephalosporins , cephamycins and also against carbapenems.

Organisms producing ESBLs :

Eschrichia coli ,Klebsiella pneumonia, Proteus spp, Enterobacter,Citrobacter, and Pseudomonas

Risk factors associated with ESBL production[65]

1. Prolonged stay in intensive care units.
2. Poor nutritional status.
3. Severe illness.

4. High rate use of ceftazidime and other third generation cephalosporins.
5. Long term use of antibiotics.
6. Use of cental venous lines,urinary catheters, endotracheal tubes,arterial lines,nasogastric tubes.
7. Hemodialysis
8. Administration of total parenteral nutrition.
9. Decubitus ulcers.

Laboratory methods of identification of extended spectrum beta lactamases

1.Phenotypic methods

2. Genotypic methods:

Phenotypic methods are commonly performed by the laboratories because they are easy to perform and cost effective also. These tests depend on detecting synergy between clavulanic acid and the indicator cephalosporins.

Screening tests

The current Clinical and laboratory standard institute guidelines for detection of extended spectrum beta lactamases in Klebsiella spp. and E. coli includes an initial screening test with any two of the following beta lactam antibiotics : cefpodoxime ,

ceftazidime, aztreonam, cefotaxime or ceftriaxone. The use of more than one of the five beta lactam antibiotics will improve the sensitivity of detection. Cefpodoxime and ceftazidime show the highest sensitivity for ESBL detection. There is also a possibility of missing some strains if the inoculum is very low.

Disc diffusion method

According to the CLSI guidelines ,isolates showing inhibition zone size of the test results as follows

Zone of inhibition size in disc diffusion method	**MIC break point by broth dilution method**
Cefpodoxime(10µg) ≤17 mm	Cefpodoxime ≥4 µg/ml
Ceftazidime (30µg) ≤ 22 mm	Ceftazidime ≥ 1µg/ml
Ceftriaxone (30µg) ≤ 25 mm	Ceftriaxone ≥ 1µg/ml
Cefotaxime (30µg) ≤ 27 mm	Cefotaxime ≥1µg/ml
Aztreonam (30µg) ≤ 27 mm	

Screening by dilution methods: Ceftazidime, aztreonam, cefotaxime, or ceftriaxone can be used at a screening concentration of 1µg/ml. Growth at this screening antibiotic concentration is suspicious of ESBL production and an indication for the organisms to be tested by phenotypic confirmatory methods.

For cefpodoxime MIC >2 µg/ml is used as a criteria to perform phenotypic confirmatory tests.

Phenotypic confirmatory tests:

Double-disk synergy test :

This method of phenotypic confirmation was proposed in 1980 explained by Jarlier et al .Organism to be tested is placed on the Mueller –Hinton agar. An antibiotic disc containing one of the beta lactam antibiotics is placed 30 -15 mm apart (centre to centre) from amoxicillin- clavulanic acid disc. Following overnight incubation in air at 37◦c ,the discs are read. Enhancement of zone of inhibition of the beta lactam antibiotic caused by synergy of clavulanate indicates a positive result. Sensitivity is increased by reducing the distance between the discs to 20 mm. Addition of clavulanate to the Muller-Hinton agar increases the sensitivity.[57]

Disc strength : Cephalosporin (3rd generation) - 30µg/disc.

Coamoxyclav - 20µg amoxicillin+10µg clavulanic acid

Combined disc method :

In this test the organism to be tested is swabbed on a plate of Muller –Hinton agar. Ceftazidime10µg disc alone and in combination with clavulanic acid (ceftazidime 30µg +clavulanic acid 10µg) are placed 20 mm apart. Similarly cefotaxime 30µg and

cefotaxime 30µg+ clavulanic acid 10µg are also placed 20 mm apart. The plates are incubated overnight aerobically at 37◦c. An increase of ≥ 5mm of the zone diameter of either of the antimicrobial agent which are tested in combination with clavulanic acid compared to that of the individual discs alone are interpreted as positive for ESBL producers. Escherichia coli ATCC 25922 is the Control strain to validate the susceptibility test. [58]

Vitek ESBL cards:

The Vitek ESBL test utilizes ceftazidime and cefotaxime (0.5 µg/ml) discs alone and these discs combined with clavulanic acid (0.4 µg/ml). Once the growth control well reached a set threshold, automated analysis of the wells are performed. A predetermined reduction in the growth of cephalosporin wells containing clavulanic acid, compared with the level of growth in the wells with the cephalosporin alone ,indicates a positive result.[59]

BD Phonex automated microbiology system

Becton Dickinson Biosciences found out BD phoenix system.This test has a short incubation period. The principle of this test is the comparison of the growth response to cefpodoxime, ceftazidime, ceftriaxone and cefotoxime with or without clavulanate . Results are available within 6 hours.

The E test method (Epsilometer test)

It is a plastic drug-impregnated strip, one end of which generates a stable concentration gradient of cephalosporin,(i.e, ceftazidime, cefoaxime, cefepime) and the remaining end of which generates a gradient of cephalosporin plus clavulanate in a constant concentration. The MIC ratio of cephalosporin alone/ cephalosporin plus clavulanate MIC ≥ 8 indicates ESBL positive strains..For Enterobacter species cefepime/clavulanate E strip is preferred.[66]

Agar supplemented with clavulanate:

In this method Mueller –Hinton agar is supplemented with 4µg/ml of clavulanate.Antibiotic discs containing the beta lactam antibiotics are placed on this agar and the agar without clavulanate. A difference of ≥ 10 mm in the width of the zone diameter of the discs on the two media is considered positive for ESBL production. The clavulanate containing agar has to be freshly prepared, as the potency of clavulanate decreases after 72 hours.

Disc replacement method :

In this method three amoxicillin/clavulanate discs are placed on a Mueller-Hinton agar which is inoculated with the test organism. After 1 hour these discs are taken away and are replaced

by discs containing cefotaxime, ceftazidime and aztreonam on the same spot. Control discs of these drugs are placed 30 mm from these sites. An increase in the zone size of ≥ 5 mm in the discs which are used for the replacement of amoxicillin/clavulanate discs compared to the control discs indicate ESBL production.

Three dimensional test :

This test developed by Thomson, gives the phenotypic evidence of ESBL induced inactivation of the antibiotics. In this test it is not necessary to demonstrate the inactivation of beta lactamase by the beta lactamase inhibitor. Here the surface of the agar is inoculated with the test organism by standard methods for disk diffusion testing. A circular hole is cut in the agar. This hole is filled with heavy inoculam of the test organism. Beta lactam antibiotics are placed on the agar 3mm outside the hole. A distortion of the zone size at the point of cut indicates ESBL.[47,60]

Phenotypic ESBL confirmation tests are based on the in vitro inhibition of these enzymes by clavulanic acid. These tests are applicable to Enterobacteriaceae such as Klebsiella, Escherichia coli and Proteus mirabilis which have little or no chromosomal activity β lactamase activity. False negative results can be obtained in strains that coproduce an inducible or plasmid mediated Amp C

beta lactamase . Amp C enzymes may be induced by clavulanate and may then attack cephalosporins masking synergy which is due to inhibition of extended spectrum beta lactamases.

False positive results can be obtained using inhibitor based ESBL detection tests in isolates which have hyperproducing chromosomal beta lactamases.

The phenotypic detection of these enzymes in organisms such as Enterobacter and Citrobacter remains a problematic and controversial issue. The reason for this that the clavulanate effect present in ESBL producing organisms is not always present in these species.

Genotypic detection [61]:

Many of the research laboratories use genotypic methods for the identification of the specific genes responsible for the production of ESBLs. They also have the ability to detect ESBLs which are not detected by phenotypic methods. They also have the advantage that they can be done directly on clinical specimens without culturing bacteria, with subsequent reduction of the turnaround time.

The molecular methods in use are DNA probes, Polymerase chain reaction, Restriction fragment length polymorphism, Single

strand conformational polymorphism and Ligase chain reaction. Most of them are specific for gene family and detects TEM and SHV variants. Among these nucleotide sequencing is a *gold* standard, since it is capable of detecting all variants.

Treatment

The plasmid having the genes encoding for ESBLs also carry genes which encodes resistance to aminoglycosides and cotrimoxazole.

Porin loss was observed in strains producing ESBLs which leads to quinolone resistance. But for urinary tract infections quinolones can be prescribed if there is in vitro susceptibility.

β lactam/ β lactamase inhibitor combinations are active against organisms possessing a single ESBL only. Additionaly ESBL producing organisms may harbor parent enzymes(TEM-1 or SHV-1) .Hyper production of these enzymes and porin loss may contribute to reduction in the activity of beta lactamase inhibitors. Hence β lactam /β lactamase inhibitor combinations are not regarded as first line therapy for serious infections with ESBL producing organisms. But for urinary tract infections it can be a second line therapy.

Fosfomycin and Nitrofurantoin are the treatment of choice, for uncomplicated cystitis.[62,63]

Cefepime resistance is seen in strains producing CTX-M type ESBLs. Hence cefepime , if at all used for therapy should be used in high dosage.

Carbapenems have the most consistent activity against ESBL producing organisms. Hence should be regarded as the drugs of choice for serious infections with ESBL producing organisms.

MATERIALS & METHODS

MATERIALS AND METHODS

This study on , " Isolation and characterisation of uropathogens with conventional and chromogenic media and detection of extended spectrum beta lactamases." was conducted in the Department of Microbiology Coimbatore Medical College Hospital.

Study Period : August 2013 - July 2014

Type of study : Prospective .

Approval from the ethical committee was obtained prior to the conduct of the study.

During the study period, about 422 urine samples from inpatients and outpatients of various departments such as Medicine, Paediatrics, Surgery, Urology ,Nephrology and Obstetrics and Gynaecology were collected and processed.

Inclusion criteria :

1. Urine samples collected aseptically from the inpatients and out patients.
2. Patients with history of UTI s such as fever, abdominal pain, frequency and urgency in urination

Exclusion criteria :

1. Febrile conditions other than the urinary tract infections.

2. Any deviation in collection of urine samples, without proper aseptic precautions.

For all the patients detailed clinical history, symptoms , signs, any other investigations regarding urinary tract infection , history of antibiotic intake, and previous surgeries were taken according to the datas given in the worksheet. The name age, sex, address, occupation, date of admission, and inpatient number were noted. Complete systemic examination of the patient was done.

Collection of urine specimens:

As per the CLSI guidelines GP-16 A3, for urinalysis Clean widebore container with a capacity of about 50 ml was used. The containers were sterile and leak proof to avoid spillage.

Clean catch midstream urine[29]

Females were given instructions regarding the collection of urine. The periurethral area and perineum were first cleaned with gauze pads saturated with soap water using a forward to back motion. This was followed by a rinse using sterile saline or water. It was advised to held the labia apart during voiding. First few milliliters of urine were passed in the toilet or bedpan and the mid stream urine was collected in a sterile wide mouthed container.

For men simple cleaning of the urethral meatus was advised. Mid stream urine sample was collected in a wide mouthed sterile container.

Collection of samples from indwelling catheters

Urine samples were collected from an indwelling catheter using a no 28 needle and a syringe. The area where the needle puncture to be done was disinfected. Urine was aspirated through the soft rubber connector between the catheter and the connecting tubing It is advisible not to collect urine from catheter bags.

Transport of urine specimens

Urine specimens were sent to the laboratory without any delay and processed within two hours.

Macroscopic examination of the urine :

The color and presence of turbidty were examined.

Screening tests for bacterial products and pyuria were done by reagent impregnated dipstick method.

SD Urocolor test strips for urinalysis were used. These were plastic strips to which were attached several separate reagent areas. They provide tests for blood, bilirubin, urobilinogen, ketone, protein, nitrite, glucose pH, specific gravity, leucocytes and ascorbic acid in urine.[71]

Principle :

Nitrite One test strip contains p-arsanilic acid, 1, 2, 3, 4 tetrahydrobenzoquinolin-3-ol. The test is based on the diazotization reaction of nitrite with an aromatic amine to produce a diazonium salt. It is followed by an azo coupling reaction of this diazonium salt with an aromatic compound on the reaction membrane. The azo dye produced causes a color change from white to pink. Positive reaction reveals the presence of nitrite and hence indicates nitrite forming bacteria. Greater concentration of ascorbic acid in urine may cause a false negative reaction.

Leucocyte One strip contains derived phenylpyrrole, diazonium salt. The leucocytes contain esterases that catalyze the hydrolysis of the derived pyrrole aminoacid ester to liberate 3-hydroxy-5-phenyl pyrrole. This pyrrole reacts with a diazonium salt to produce a red purple product.

Procedure Mix well the collected urine, take the strip from the bottle, immerse test areas of the strips in urine and remove immediately. Place the strips on a gauze for 1-2 seconds to remove excess urine. Hold the strip in a horizontal position and compare the test areas to corresponding color chart on the bottle label .Read the results at sixty seconds.

Chromogenic agar was prepared inhouse with the readymade dehydrated powder.It is a HiCrome UTI agar,modified bought from Himedia. Composition and ingredients are given in the appendix -1.

Directions for preparation

Suspend 55.44 grams in 1000ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121◦C) for 15 minutes. Cool to 45-50◦C and pour into sterile petri dishes. The pH of the medium is between 7-7.4.

The media was stored at 2-8◦C for upto two weeks.

Each batch of media were tested for the growth of the following organisms. Staphlococcus aureus ATCC 25923, Escherichia coli ATCC 13883, Klebsiella pneumonia ATCC 13883, Enterococcus faecalis ATCC29212, Pseudomonas aeruginosa ATCC 27853, and Proteus mirabilis ATCC12453 . The characteristic growth and colony colour which were observed after incubation at 37◦C for 24 hours seem to match with the manufacturer's instructions.

Blood agar and MacConkey agar were prepared inhouse according to manufacturer's instructions.

Culture of the specimen : Fresh specimen (uncentrifuged urine) were inoculated directly on blood agar, MacConkey agar and chromogenic

agar plates and incubated overnight at 37◦c .Plates were read on the next day.

2mm or 4 mm loops were used and quadrant steaking method was followed.

Chromogenic agar plates were read after 24 hours .Presumptive identification of the organisms were made according to the colony color and typical morphology.

Organisms	**Colony color and appearance**
Eschrichia coli	Small purple or pink
Klebsiella	Mucoid metallic blue
Enterococcus	Dry,tiny blue
Proteus	Pale brown
Pseudononas	Green
Citrobacter	Metallic blue
Staphylococcus aureus	Small colourless to golden yellow colonies
Streptococcus	Small translucent opaque
CoNS	Colorless small differentiated
Candida	Creamy wet convex
Acinetobacter	Non transparent ,cream, white

Confirmation of E.coli was done by performing spot indole test using DMACA reagent. (Dimethyl amino cinnamaldehyde).

Spot indole test : Procedure:

Moisten a piece of Whatman's no 3 filter paper with a few drops of the reagent. Remove isolated colony from the plate, with a wooden stick and smear it on to the moistened filter paper. Observe blue color within two minutes. It indicates positive reaction.[67]

TDA reagent : This was done to identify Proteus organisms. The reagent is a ferric chloride solution which when applied on the colony growth produces a brownish discolouration around the organisms. Positive reaction indicates tryptophan deaminase activity.

Colonies from blood agar plate and MacConkey agar plates were subjected to biochemical reactions. These organisms were compared with the isolates identified from chromogenic agar.

Colony count and interpretation:

We used 4 mm loop to inoculate and streak the plates. This loop will carry about 0.01 ml of urine. So the colonies that appear on the plates were multiplied by 100.

Interpretation :

According to Kass concept significant bacteriuria indicates Colony count greater than 100000 in a single growth.

Colony count between 1000-100000 indicates doubtful growth.

Colony count less than 1000 indicates insignificant growth.

In mixed growth, the organism showing colony count greater than 100000 was considered significant and processed.

Even few colonies if present, from Staphylococcus aureus were processed.

Gram positive organisms were processed from blood agar plate. Gram negative organisms were processed from MacConkey agar plate.

Gram staining, motility, biochemical reactions like catalase, coagulase, oxidase, indole, Kligler Iron agar, citrate , and urease tests were done.

Tube coagulase test

0.5 ml of plasma was taken in a sterile tube. Few colonies were emulsified into the tube. The tube was incubated at 35◦C for 4 hours and observed for clot formation by gently tilting the tube. If there was no clot formation at that time ,reincubate at room temperature and read after 18 hours.

Positive control –Staphylococcus aureus

Negative control- Staphylococcus epidermidis

Indole test [68]:

Principle: This is the test to determine the ability of an organism to split indole from tryptophane molecule. Tryptophan present in peptones of the culture media are acted upon by the enzyme tryptophanase and converted into indole, pyruvic acid and ammonia. Indole reacts with aldehydes to produce coloured product.

Ehrlich's indole test:

Procedure : 1 ml of xylene was added to a 24 to 48 hour incubated broth culture. Shaked well , and allowed to stand for few minutes. Ehrlich reagent was added.

Interpretation : The development of bright red coloured ring at the interface of the medium and alcoholic layer within few seconds was indicative of positive reaction. This test was most useful in separating Escherichia coli (positive) from Klebsiella-Enterobacter-Hafnia-Serratia group (mostly negative).

Citrate utilization: Principle : The ability of an organism to utilize citrate as the sole source of carbon is used in this test. The utilization of citrate was indicated by the production of alkaline byproducts resulting in color change in the indicator.

Simmons citrate medium:

Procedure : A well isolated colony from the surface of the plate was picked up and inoculated on the slant surface of the citrate agar slope. The tube was incubated at 35◦C for 24-48 hours.

Interpretation : Positive test was detected by the growth /development of deep blue color. Each new batch of medium was tested with a positive and negative reacting organisms .Positive control-Enterobacter aerogenes. Negative control: Escherichia coli.

Urease test : Principle : This test was used to determine the ability of the organism to split urea, forming two molecules of ammonia by the action of the enzyme urease with resulting alkalinity.

Media used: Christensen's urea agar Procedure: The surface of the agar slant was streaked with a drop of 4-6 hour growth of bacterium in broth. Media were incubated at 35◦C for 18-24 hours.

Interpretation : Positive reaction was shown by the red color.

Each batch of media was tested with a positive and negative control organisms. Positive control- Proteus species, Negative control- Escherichia coli.

Kligler iron agar test: Principle :This test is based on the the ability of an organism to attack specific carbohydrates present in a

basal medium, with or without the production of gas or hydrogen sulphide.

Procedure : Organisms were picked up from the top of a single colony from primary isolation plate with a straight wire and inoculated by stabbing the butt, and then streaking the surface of the slant. The results were read only after 18-24 hours incubation at 35◦C.

Interpretation :A/A-Acid/ acid ,yellow color through out, Complete acidification of both the slant and the deep. It is produced by lactose fermenting organisms.

K/A –Alkaline/acid –Red/yellow color. Initial acidification of both the deep and slant of the medium by bacteria that ferment glucose, later the slant reverts back to alkaline pH as alkaline amines are formed from peptides near the surface.

K/K –Alkaline/alkaline- Red color in slant and butt. It shows peptones are utilized, both glucose and lactose are not fermented. The non fermenters are unable to produce acids from fermentation of glucose or lactose and there is no change in the medium.

Production of gas bubbles, indicate aerogenic organism and black color in the butt indicates, hydrogen sulphide production.

Bile esculin agar

It was prepared by dissolving 64.5 grams of dehydrated powder in 1000ml of distilled water, sterilized by autoclaving at 121◦C for 15 minutes. The test organism was inoculated on to the surface of the slope and incubated at 37◦ C for 48 hours. Blackening of the media indicated growth in the presence of 40% bile and esculin hydrolysis.

Positive control – Enterococcus faecalis

Negative control- Streptococcus viridians.

Antibiotic susceptibility testing

This test was done using modified Kirby-Bauer disc diffusion technique using Mueller Hinton agar[69]. A single colony from the plate was taken with a wire loop, and was inoculated into the peptone water. It was allowed to obtain turbidity that match with a McFarland standard.

Mueller-Hinton agar was prepared inhouse according to the manufacturer's instructions. Poured into 90mm diameter petri dishes to a depth of 4mm. Each batch was tested with a control strain of Enterococcus faecalis and cotrimoxazole disc. The zone of inhibition should be 20 mm or more in diameter.

Preparation of 0.5 Mcfarland standard

1. Prepare 1% w/v solution of sulphuric acid by adding 1 ml of concentrated sulphuric acid to 99 ml of water. Mix well.
2. Prepare 1% w/v solution of barium chloride by dissolving 0 .5 g of dehydrate barium chloride in 50 ml of distilled water.
3. Add 0 .6 ml of barium chloride solution to 99.4 ml of sulphuric acid solution and mix.

This can be stored at room temperature for upto six months. The 0.5 McFarland standard provides an optical density equivalent to the density of 1.5×10^8 colony forming units/ml. Matching was done by holding the bacterial suspension and McFarland tubes side by side and viewing them against a black-lined background.

Selection of antimicrobial agents for testing :

For Enterobacteriaceae ,Pseudomonas and Acinetobacter: Ampicillin, Ciprofloxacin, Cefotaxime, Amoxy clav, Norfloxacin, Cotrimoxazole, Nitrofurantoin, Amikacin ,Gentamycin,Meropenem and Cefoperazone sulbactum discs were used.

For Gram positive organisms :

For Enterococcus : Ciprofloxacin, Amikacin, Vancomycin, Nitrofurantoin, cotrimoxazole , Ampicillin, Norfloxacin and Amoxy clav discs were used.

For streptococcus : Mueller- Hinton agar supplemented with 10% sheep blood agar was used.

The plates were dried before streaking. A lawn culture was made with the organism to be tested. After allowing 3-5 minutes for the agar to dry, using sterile forceps antibiotic discs were placed. The discs should be about 15 mm from the edge of the plate and not closer than 25 mm from disc to disc. Within thirty minutes the plates were incubated at 37◦C . The plates were read on the nextday. The zone of inhibition around each antibiotic disc was measured . The sizes of zone of inhibition were interpreted by using Clinical and laboratory standard institute guidelines as shown below.[70]

Antimicrobial agent	**Disc content (mcg)**	**Resistant mm or less**	**Intermediate**	**Sensitive mm or more**
Amikacin (AK)	30	14	15-16	17
Gentamicin (G)	10	12	13-14	15
Nitrofurantoin (NIT)	300	14	15-16	17
Norfloxacin (Nx)	10	12	13-16	17
Ciprofloxacin (CIP)	5	15	16-20	21
Ampicillin(AMP)	10	13	14-16	17
Cefotaxime(CTX)	30	14	15-22	23
Amoxycillin/Clavulanic acid (AMC)	20/10	13	14-17	18
Cotrimoxazole (COT)	1.25/23.75	10	11-15	16
Meropenam (MRP)	10	13	14-15	16
Vancomycin (VA)	30	14	15-16	17
Cefoperazone Sulbactum (CFS)	75/10			27-33
Novobiocin(NV)	30	17	18-21	22

Screening for extended spectrum betalactamase : According to CLSI guidelines, those organisms showing zone inhibitory size less than 27mm for cefotaxime was considered as potential ESBL

producers and were subjected to confirmatory tests. Only the organisms such as Escherichia coli, Klebsiella and Proteus were subjected to confirmatory test.

Double disc synergy test :

Test organism was inoculated in peptone water and lawn culture was made on Mueller-Hinton agar as recommended for a standard disk diffusion susceptibility test. Discs containing 30µg of ceftazidime and 30µg cefotaxime were placed 15 mm apart (centre to centre) to the disc containing Amoxicillin/Clavulanic acid (20/10µg). Plates were incubated at 37◦ C overnight. Enhancement of zone of inhibition between the clavulanate discs and any one of the β lactam antibiotic disc was interrupted as an indication for ESBL production.

E test : EM079A

It is a phenotypic ESBL detection strip which is coated with mixture of three different cephalosporins with and without clavulanic acid on a single strip in a concentration gradient manner. The upper half has Ceftazidime, Cefotaxime and Cefepime + Clavulanic acid and Tazobactum with concentration gradient tapering downwards. The lower half is coated with Ceftazidime, Cefotaxime and Cefepime in a concentration gradient in reverse direction.

Procedure:

1. An inoculam was prepared from the pure culture of the test organism . It was standardized with that of the 0.5 McFarland standard.
2. Mueller-Hinton agar was prepared inhouse according to manufacturer's instructions.
3. A sterile cotton swab was dipped into the standardized inoculam and was rolled to express excess fluid.
4. Entire agar surface was streaked with the swab three times turning the plate at 60◦ angle between each streaking.
5. Strip was removed from the container with an applicator and was placed on the agar plate.
6. Plates were kept in the incubator and read on the next day.
7. Values were read where the ellipse intersects the scale on the strip.

Interpretation : The following criteria were used for interpretation

It was interrupted as ESBL positive When the ratio of the value obtained for (Mix/ Mix+) was greater than or equal to 8, or no zone was obtained for MIX and zone obtained in Mix +.

It was interrupted as ESBL negative strain when the ratio of the value obtained for (Mix/ Mix+) was less than eight.

It was considered, non conclusive for ESBL when no zone of inhibition was obtained on either side. In such cases resistance may be due to some other mechanisms other than ESBL production.

Quality control of the strips were carried out by testing the strips with the standard ATCC cultures.

Positive control – Klebsiella pneumonia ATCC 700603

Negative control – Escherichia coli ATCC 25922.

The information collected regarding all the selected cases were recorded in a Master Chart. Data analysis was done with the help of computer using **Epidemiological Information Package (EPI 2002).**

Fig. 1 Urine Screening Test

Fig. 2 MacConkey agar plate showing lactose fermenting colonies

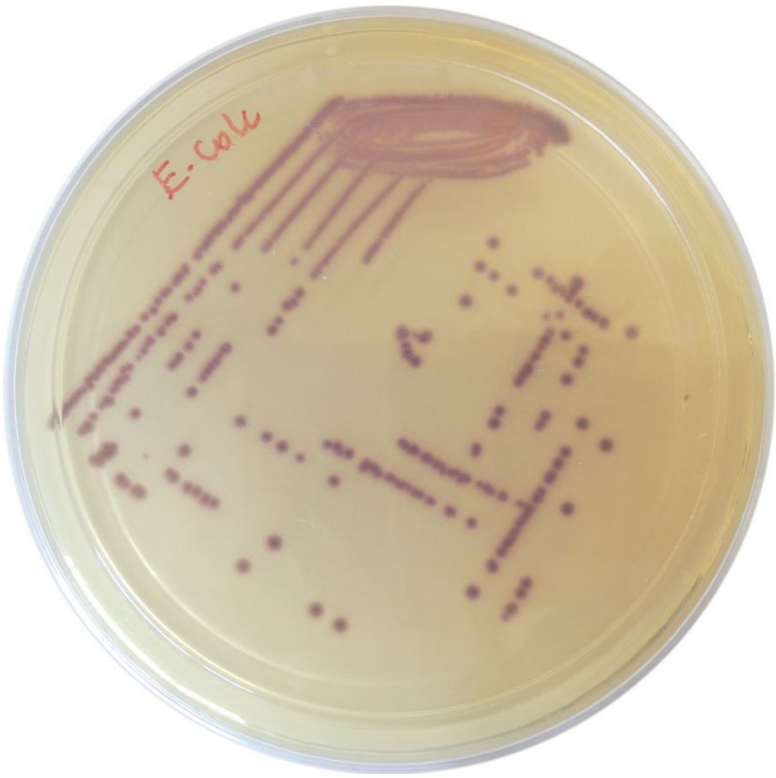


Fig. 3 Chromagar plate showing Escherichia Coli

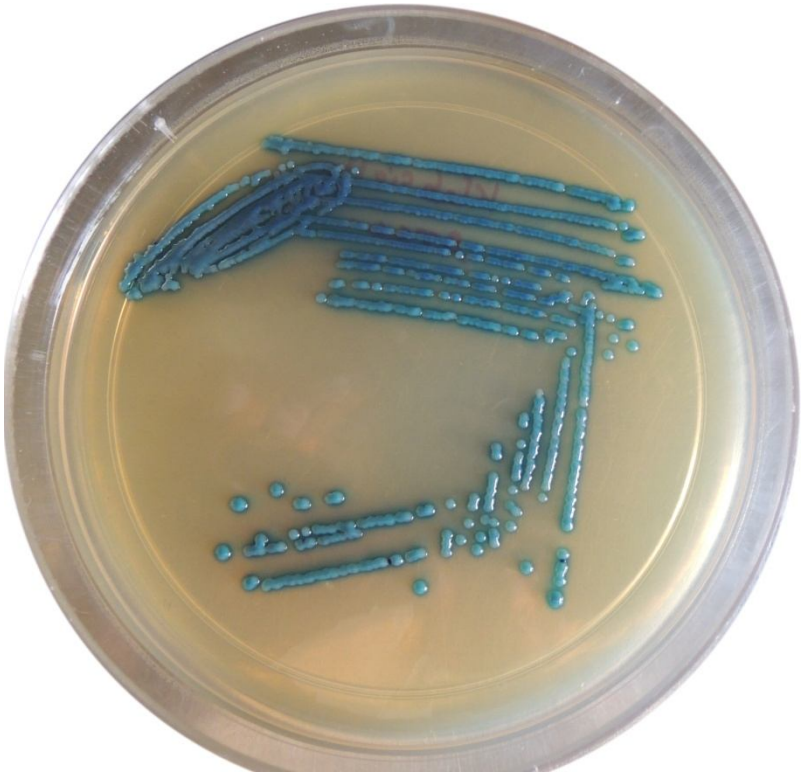


Fig. 4 Chromagar plate showing Klebsiella pneumoniae

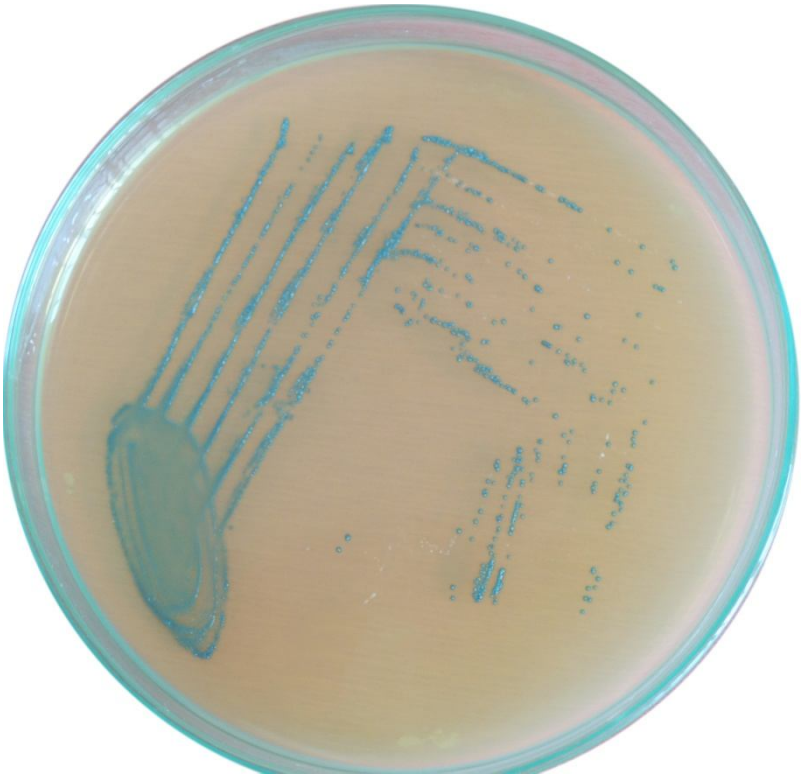


Fig. 5 Enterococcus faecalis on chromagar plate

Fig. 6 Staphylococcus aureus on chromagar plate

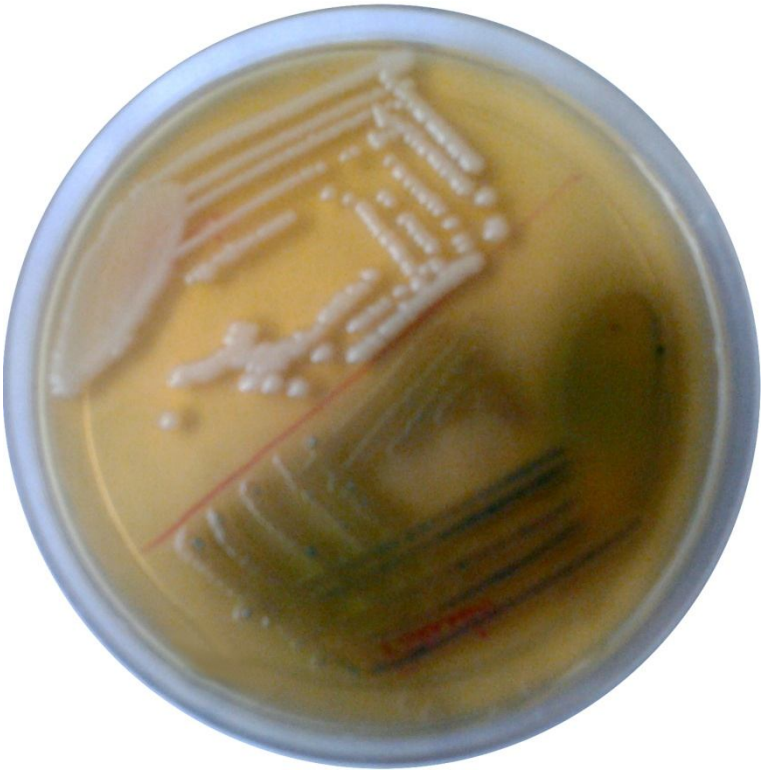
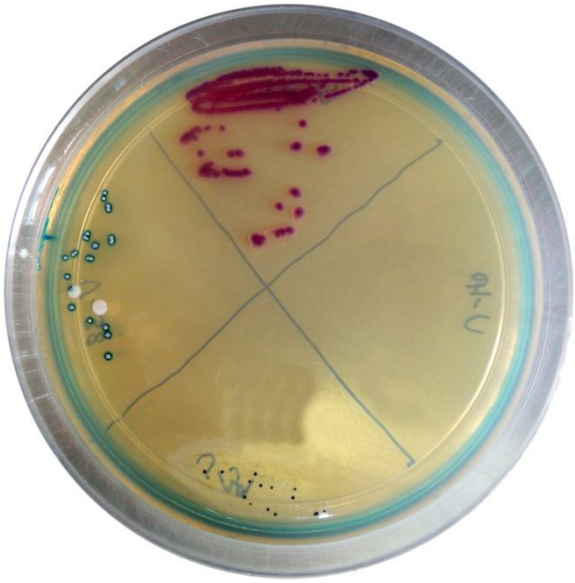


Fig. 7 Acinetobacter and Pseudomonas on chromagar plate

Fig. 8 Proteus and candida on chromagar plate

Fig. 9 Enterococci on chromagar plate

Fig.10 E.coli on chromagar plate



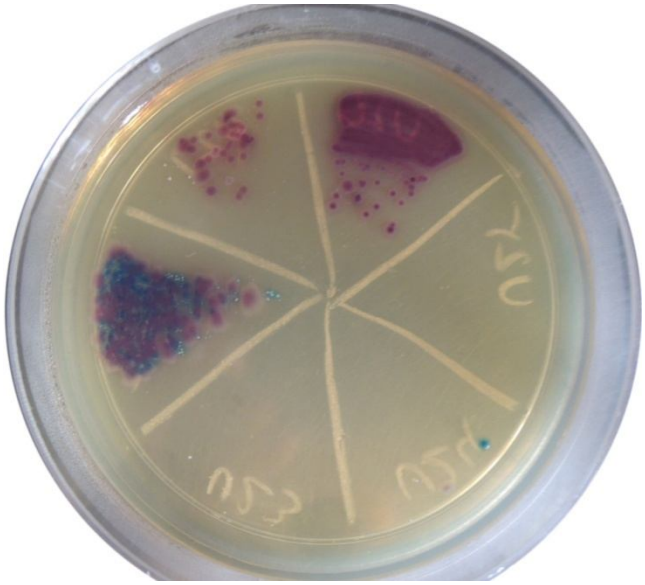
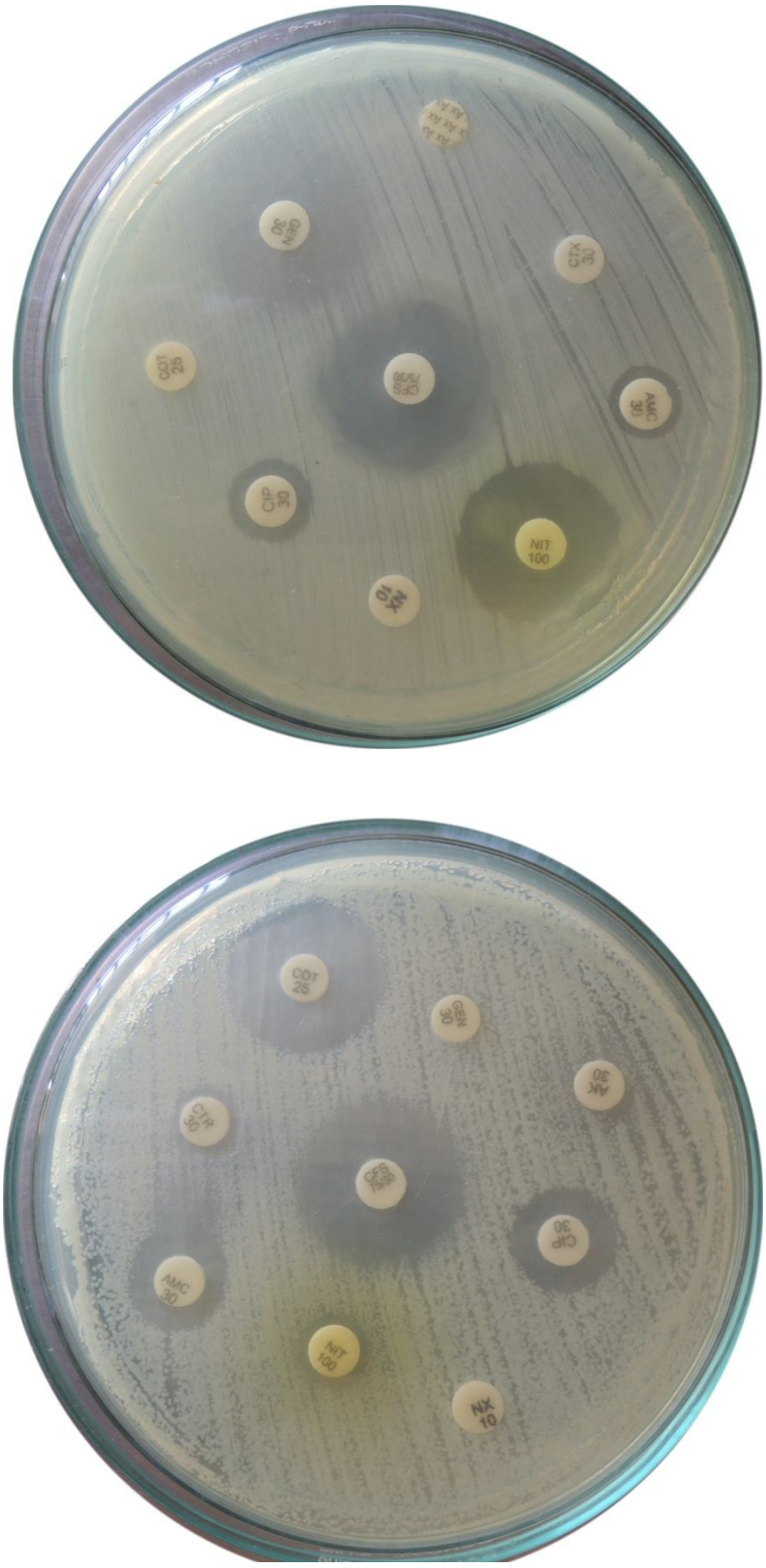


Fig. 11 Chromagar plate showing mixed culture

Fig. 12 AST pattern of ESBL Isolate



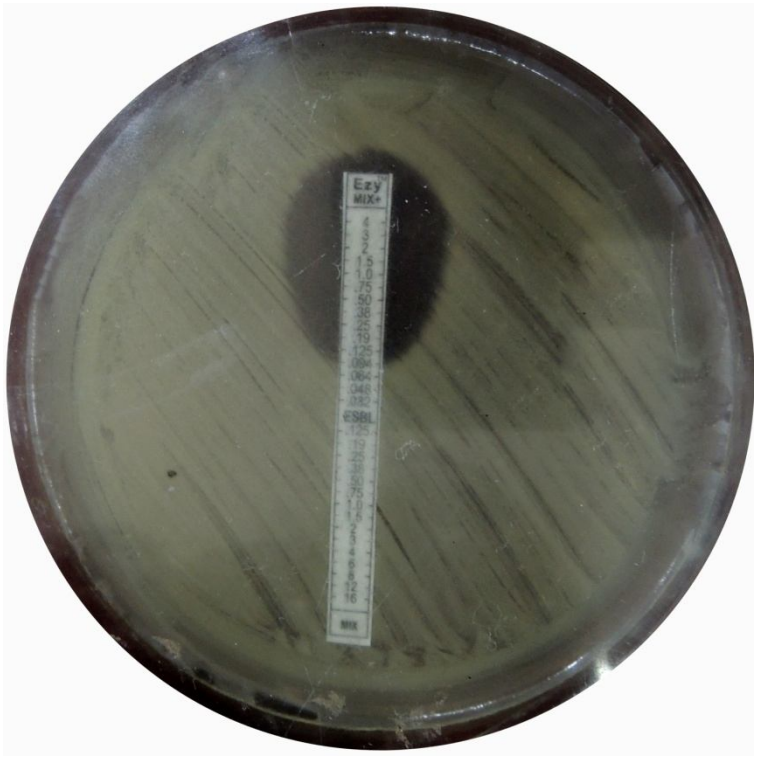


Fig. 13 Estrip with ESBL positive isolate

Fig. 14 Estrip with ESBL negative isolate

Fig. 15 DDST with ESBL positive isolate

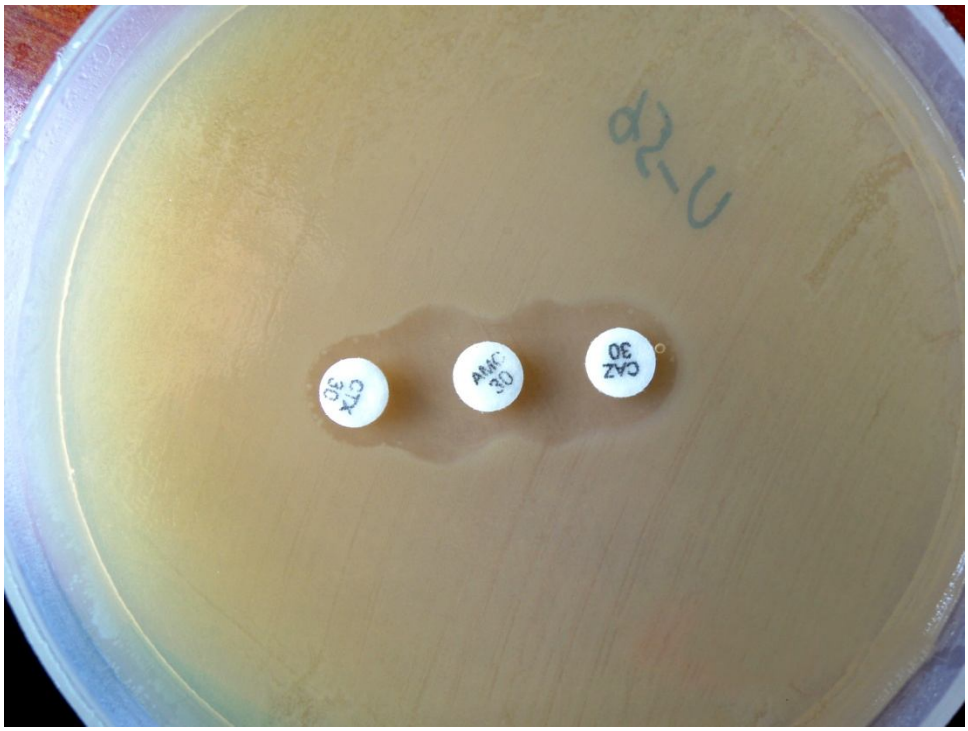
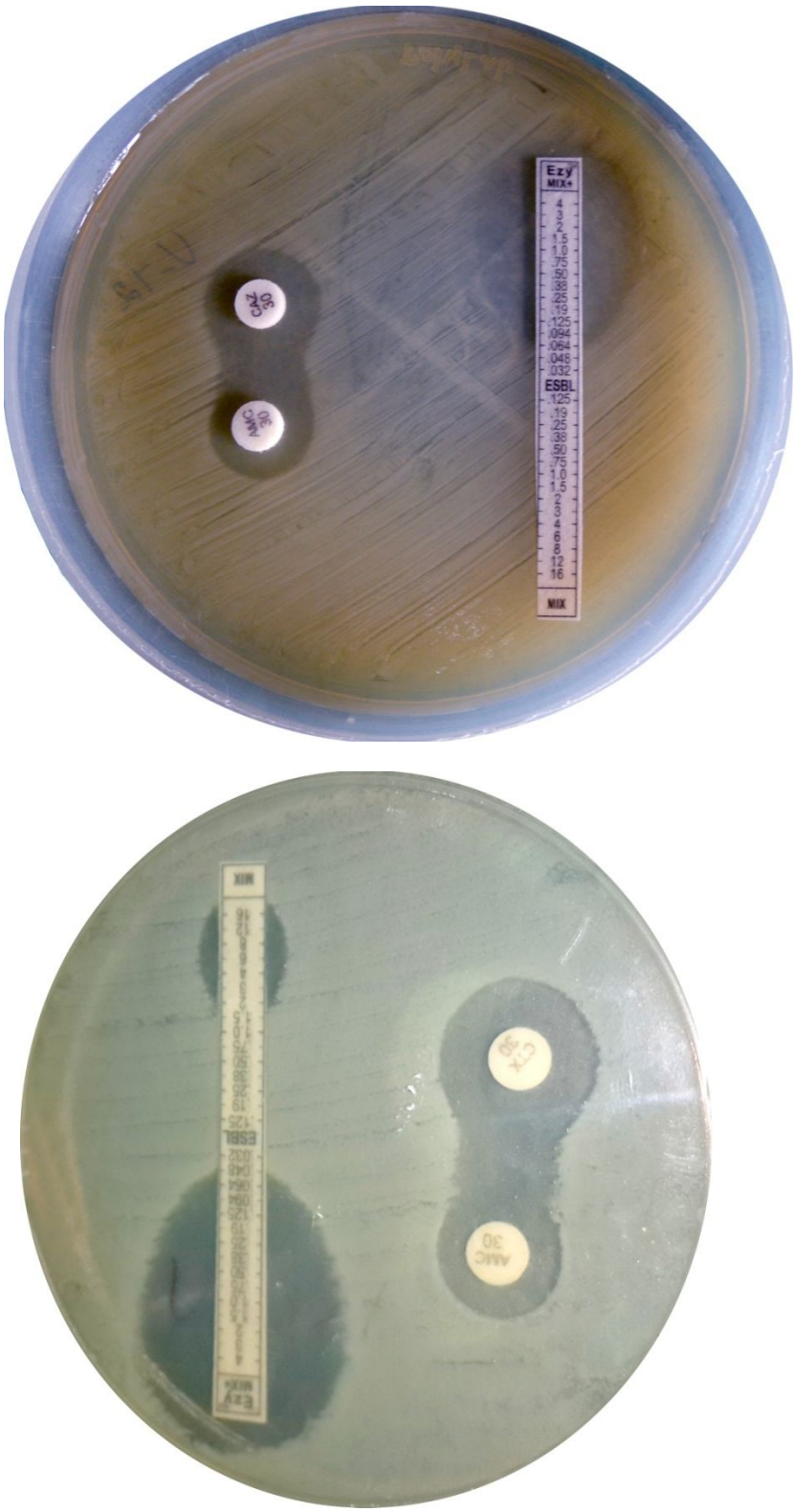


Fig. 16 Estrip and DDST with ESBL positive isolate



RESULTS

RESULTS

The 422 urine samples collected from clinically suspected UTI cases from various departments such as Medicine, surgery, urology, Paediatrics, Intensive care units , and outpatients were processed in Blood agar, MacConkey agar and Chromogenic agar. Out of this 206 were collected from males and 216 were from females .

1. Urine culture result among study population (n=422)

Culture result	**No**	**Percentage**
Positive	141	33.4
Negative	281	66.6
Total	422	100

2.Type of growth among positive isolates(n=141)

Growth	No	Percentage
Unimicrobial	131	92.9
Polymicrobial	10	7.1
Total	141	100

3. Distribution of Gram positive , Gram negative & other isolates

Organisms	No	Percentage
Gram negative	108	76.6
Gran positive	20	14.2
Fungus	13	9.2
Total	141	100

4. Distribution of organisms isolated from cases of urinary tract infection (n=141)

Name of the organism	No	Percentage
Escherichia coli	77	54.6
Klebsiella pneumoniae	15	10.6
Enterococcus species	14	9.9
Candida species	13	9.2
Proteus mirabilis	7	5
Staphylococcus saprophyticus	4	2.8
Acinetobacter baumanii	3	2.1
Proteus vulgaris	2	1.4
Pseudomonas aeruginosa	2	1.4
Enterobacter aerogenes	1	0.7
Streptococcus species	1	0.7
Citrobacter freundi	1	0.7
Staphylococcus aureus	1	0.7
Total	141	100

5.Organisms isolated from mixed growth in various media (n=10)

Organism	Number	BAP	MAC	Chromagar
E.coli +Enterococci	O+6	4	0	6
Klebsiella +E.coli	0+1	1	1	1
E.coli +Enterobacter	0+1	0	1	1
E.coli+Candida	0+2	2	0	2
Total	10(100%)	7(70%)	2 (20%)	10 (100%)

6. Sexwise Distribution of the organisms n=141

Sex	**No**	**Percentage**
Male	66	46.8
Female	75	53.2
Total	141	100

7. Age and sex distribution of the positive isolates (n=141)

Age	M		F		Total (M+F)	
	No	%	No	%	No	%
< 10	26	39.4	13	17.3	39	27.7
11-20	2	3.1	6	8	8	5.7
21-30	1	1.5	14	18.7	15	10.6
31-40	3	4.5	11	14.7	14	9.9
41-50	6	9.1	17	22.7	23	16.3
51-60	8	12.1	4	5.3	12	8.5
Above 60	20	30.3	10	13.3	30	21.3
Total	66	100	75	100	141	100

8. Total number of isolates from Various Departments (n=141)

Department	No of organisms isolated	Percentage
Medicine	40	28.4
Urology	23	16.3
Intensive care units(ICU)	19	13.5
Obstetrics & Gynaecology	17	12.1
Paediatrics	16	11.4
Surgery	14	9.9
Outpatient (O.P)	12	8.5
Total	141	100

9.Organisms isolated from various culture plates(n=141)

Name of the organism	Number of organism	BAP		MAC		Chrome agar	
		No	%	No	%	No	%
Escherichia coli	77	77	100	76	98.7	77	100
Klebsiella pneumoniae	15	15	100	15	100	15	100
Enterococcus species	14	12	85.7	5	35.7	14	100
Proteus mirabilis	7	7	100	7	100	7	100
Proteus vulgaris	2	2	100	2	100	2	100
Pseudomonas aeruginosa	2	2	100	2	100	2	100
Staphylococcus saprophyticus	4	4	100	0	0	4	100
Enterobacter aerogenes	1	0	0	1	100	1	100
Citrobacter freundi	1	0	0	1	100	1	100
Streptococcus species	1	1	100	0	0	1	100
Acinetobacter baumani	3	3	100	3	100	3	100
Candida species	13	13	100	9	69.2	13	100
Staphylococcus aureus	1	1	100	0	0	1	100
Total	141	137	97.2	121	85.8	141	100

10. Comparison of isolation of organisms in various media n=141

Name of the media	Number of isolates	Percentage
Blood agar	137	97.2
MacConkey agar	121	85.8
Chrome agar	**141**	100

11. Comparison of Chromagar with blood agar

Chrom agar	**Blood agar**		**Total**
	P	**N**	
P	137	4	141
N	0	0	0
Total	137	4	141

Sensitivity = 100

Accuracy = 97

PPV = 97

12. Comparison of Chromagar with MacConkey agar

Chrom agar	Mac Conkey agar		Total
	P	**N**	
P	121	20	141
N	O	O	0
Total	121	20	141

Sensitivity = 100

Accuracy = 86

PPV = 86

13. Overall sensitivity and resistance pattern of uropathogens to antibiotics (n=128)

Antibiotics	Sensitivity		Resistance	
	No	%	No	%
Ampicillin(A)	21	16.4	107	83.6
Gentanycin(G)	89	69.5	39	30.5
Ciprofloxacin(CIP)	63	49.2	65	50.8
Amikacin(AK)	119	93.0	9	7.0
Cotrimoxazole(COT)	32	25	96	75
Nitrofurantoin(NIT)	111	86.7	17	13.3
Norfloxacin(NX)	44	34.4	84	65.6
Cefotaxime(CTX)	35	27.34	93	72.7
Cefoperazone sulbactum(CFS)	105	82.0	23	18
Amoxyclav(AMC)	60	46.9	68	53.1
Meropenem (MRP)	128	100	0	0

14. Percentage of ESBL organisms(n=67)

Name of the organism	Total number	ESBL positivity	Percentage
E.coli	77	52	67.5
Klebsiella pneumoniae	15	10	66.7
Proteus species	9	5	55.6

15. Comparison of ESBL detection using the DDST and Etest

Organisms positive in ESBL screening	Organisms positive in double disc approximation test	Organisms positive in E test
E.coli (54)	48	52
Klebsiella (10)	10	10
Proteus (6)	5	5
Total (70)	63 (90%)	67 (95.7%)

16. Comparison of E test with DDST test

E test	DDST test		Total
	P	**N**	
P	63	4	67
N	0	3	3
	63	7	70
Degrees of freedom	132		
Fisher's exact chi square	18.7286		
'p'	0.0006		

Sensitivity = 100

Accuracy = 94

PPV = 94

17. Sexwise distribution of ESBL positive isolates (n=67)

Sex	**No**	**Percentage**
Male	36	53.7
Female	31	46.3
Total	67	100

18. Age and sex distribution of ESBL positive isolates (n=67)

Age group	No of positives		Total
	M	F	M+F
< 10	14 (38.9%)	8(25.8%)	22 (32.8%)
11-20	2(5.6%)	2(6.5%)	4(6.0%)
21-30	1(2.8%)	5(16.1%)	6(9%)
31-40	1(2.8%)	2(6.5%)	3(4.5%)
41-50	3(8.3%)	7(32.3%)	10(14.9%)
51-60	6(16.7%)	3(9.7%)	9(13.4%)
Above 60	9(25%)	4(12.9%)	13(19.4%)
Total	36(100%)	31(100%)	67 (100%)

19. Isolation of ESBL producing bacteria from different wards (n=67)

Name of the Department	**No of ESBL producing bacteria**	**Percentage**
Medicine	15	22.4
Urology	11	16.4
Outpatient (O.P)	10	14.9
Intensive Care unoits(ICU)	9	13.4
Paediatrics	9	13.4
Surgery	8	11.9
Obstetrics & Gynaecology	5	7.5
Total	67	100

20.Antibiotic sensitivity and resistance pattern of Gram negative isolates

Antibiotics	E.coli (n= 77)		Klebsiella (n= 15)		Proteus (n=9)		Acinetobacter (n=3)		Pseudomonas (n=2)	
	S	R	S	R	S	R	S	R	S	R
Ampicillin	15 (19.5%)	62 (80.5%)	1 (6.7%)	14 (93.3%)	1 (11.1%)	8 (88.9%)	-	-	-	-
Gentamycin	62 (80.5%)	15 (19.5%)	12 (80%)	3 (20%)	7 (77.8%)	2 (22.2%)	2 (66.7%)	1 (33.3%)	2 (100%)	-
Ciprofloxacin	30 (39%)	47 (61%)	11 (73.3%)	4 (26.7%)	8 (88.9%)	1 (11.1%)	2 (66.7%)	1 (33.3%)	2 (100%)	-
Amikacin	73 (94.8%)	4 (5.2%)	13 (86.7%)	2 (13.3%)	9 (100%)	0	3 (100%)	0	2 (100%)	-
Cotrimoxazole	18 (23.4%)	59 (76.6%)	4 (26.7%)	11 (73.3%)	2 (22.2%)	7 (77.8%)	0	3 (100%)	0	2 (100%)
Nitrofurantoin	76 (98.7%)	1 (1.3%)	11 (73.3%)	4 (26.7%)	-	-	1 (33.3%)	2 (66.7%)	1 (50%)	1 (50%)
Norfloxacin	17 (22.1%)	60 (77.9%)	6 (40%)	9 (60%)	4 (44.4%)	5 (55.6%)	0	3 (100%)	0	2 (100%)
Cefatoxime	23(29.9%)	54(70.1%)	5(33.3%)	10(66.7%)	3 (33.3%)	6 (66.7%)	2(66.7%)	1(33.7%)	1(50%)	1 (50%)
Cefoperazone sulbactum	74 (96.1%)	3 (3.9%)	15 (100%)	-	9 (100%)	0	3 (100%)	0	2 (100%)	-
Amoxycillin Clavulanic acid	38 (49.4%)	39 (50.6%)	7 (46.7%)	8 (53.3%)	5 (55.6%)	4 (44.4%)	-	-	-	-
Meropenam	77 (100%)	0	15 (100%)	0	9 (100%)	-	3 (100%)	-	2 (100%)	-

21.Antibiotic sensitivity and resistance pattern of Gram positive isolates

Antibiotics	Enterococci (n=14)		Staph saprophyticus (n=4)		Staph aureus (n=-1)	
	S	R	S	R	S	R
Ampicillin	1 (7.1%)	13 (92.9%)	1 (25%)	0	1 (100%)	0
Gentamycin	-	-	3 (75%)	1 (25%)	0	1 (100%)
Ciprofloxacin	3 (21.4%)	11 (78.6%)	3 (75%)	1 (25%)	1 (100%)	0
Amikacin	12 (85.7%)	2 (14.3%)	4 (100%)	0	1 (100%)	0
Cotrimoxazole	0	14(100%)	2(50%)	2(50%)	1(100%)	0
Nitrofurantoin	14 (100%)	0	4 (100%)	0	1 (100%)	0
Norfloxacin	0	14 (100%)	1 (25%)	3 (75%)	0	1 (100%)
Cefatoxime	13 (92.9%)	1 (7.1%)	2 (50%)	2 (50%)	1 (100%)	0
Cefoperazone sulbactum	14 (100%)	0	4 (100%)	0	1 (100%)	0
Amoxycillin Clavulanic acid	3 (21.4%)	11 (78.6%)	4 (100%)	0	1 (100%)	0
Vancomycin	14 (100%)	0	-	-	-	-

22. Comparison of resistance pattern of ESBL and non ESBL E.coli

Antibiotics	Total no of resistant organisms	ESBL E. coli(n=52)	Non –ESBL E. coli(n=25)
Ampicillin	62	50 (96.2%)	12 (48%)
Gentamycin	15	12 (23.1%)	3 (12%)
Ciprofloxacin	47	34 (65.9%)	13 (52%)
Amikacin	4	4 (7.7%)	0
Cotrimoxazole	59	44 (84.6%)	15 (60%)
Nitrofurantoin	1	1 (1.9%)	0
Norfloxacin	60	43 (82.7%)	17 (68%)
Cefotaxime	54	52 (100%)	2 (8%)
Cefoperazone Sulbactum	3	3 (5.8%)	0
Amoxicillin clavulanic acid	39	31 (59.6%)	8 (32%)

23.Comparison of resistance pattern of ESBL and non ESBL Klebsiella

Antibiotics	Total number of resistant organisms	ESBL Klebsiella (n=10)	Non ESBL Klebsiella (n=5)
Ampicillin	14	10 (100%)	4 (80%)
Gentamycin	3	2 (20%)	1 (20%)
Ciprofloxacin	4	4 (40%)	0
Amikacin	2	2 (20%)	0
Cotrimoxazole	11	9 (90%)	2 (40%)
Nitrofurantoin	4	3 (30%)	1 (20%)
Norfloxacin	9	6 (60%)	3 (60%)
Cefotaxime	10	10 (100%)	0
Cefoperazone Sulbactum	-	-	-
Amoxicillin clavulanic acid	8	7 (70%)	1 (20%)

Table 1 indicates that among the 422 (100%) samples, 281 (66.6%) samples yielded no growth in any of the media and growth was obtained from 141(33.4%) samples.

Among the 141 samples which were culture Positive, 131 of them showed a single isolate (92.9%). Ten (7.1%) of the samples showed more than one type of organism and was considered as mixed growth. (**Table 2).**

Table 3 shows the distribution of Gram positive and Gram negative bacteria and other isolates. Out of 141 positive isolates , Gram negative organisms predominate showing 108 (76.60%) . The Gram positive organisms and Candida species were isolated at a rate of 20 (14.18%) and 13 (9.22%) respectively.

The isolation rate of uropathogens were shown in **Table 4.** Among the isolates Escherichia coli was the predominant organism. 77(54.60%) . Other organisms isolated in decreasing order were Klebsiella pneumoniae 15(10.63%), Enterococcus faecalis14(9.92%), Candida albicans13(9.21), Staphylococcus saprophyticus 4(2.83%) Proteus mirabilis 7(5%),Proteus vulgaris2(1.4%), Acinetobacter baumanii 3(2.12%), Pseudomonas aeruginosa 2(1.41%), Enterobacter aerogenes

1(0.70%), Staphlococcus aureus 1(0.70%), and Streptococcus species1(0.70%) .

Table 5 shows the isolation of predominant organism from mixed culture in various media. All the predominant isolates from mixed culture(100%) were isolated in chromagar whereas 20% and 70% of isolates were identified from MacConkey agar and blood agar.

Table 6 depicts the sexwise distribution of the isolates. Among the 141 positive isolates, growth from samples collected from males were 66 (46.8%) and from females were 75 (53.2%) .Females predominate with female to male ratio of 1.2:1.

Age and sex wise distribution of the positive isolates were shown in the **Table 7**. In males the common age group affected were below 10 years and above 60 years. In females, 21-50 years were the age group commonly affected.

The rate of isolation of organisms from different wards were shown in **Table 8**. Among these Medicine wards40 (28.4%) showed highest percentage of positive isolates. The isolation rate among other wards were urology ward 23(16.3%), Intensive care units 19 (13.5%), Obstetrics and Gynaecology ward 17(12.1%), Paediatrics

ward 16(11.4%), Surgery ward 14(9.9%) and out patients 12(8.5%).The positive culture was less from outpatient department.

The rate of isolation of organisms in blood agar, MacConkey agar and chromogenic agar were depicted in **Table 9** . All the 141 isolates were identified in chromogenic agar whereas from Blood agar and MacConkey agar 137 and 121 organisms were identified respectively. All the pathogens were isolated in chromagar 141(100%). In blood agar all the organisms were isolated (100%) except Enterococcus, Citrobacter and Enterobacter. In MacConkey agar all other organisms showed 100% isolation rate except E.coli (98.7%), Enterococci (35.7%) and Candida (69.2%). Most of the organisms identified in chromogenic agar which were not identified from blood agar were from mixed cultures.

The isolation rate in various media were compared in **Table 10** which showed statistically significant difference. The isolation of organisms from Chromogenic agar was 100%. whereas it was 97.2% from blood agar and 85.8% from MacConkey agar. Chromogenic agar showed maximum number of organisms.Escherichia coli from chromagar were confirmed by spot indole test.

Table 11 shows, the comparison of chromogenic agar with blood agar. Out of 141 isolates, 137 isolates were identified from

blood agar and 4 isolates were not identified from blood agar. All 141 isolates were identified from chromogenic agar. The sensitivity is 100% ,PPV is 97%, accuracy is 97%.

The comparison of chromogenic agar with MacConkey agar. Out of 141 isolates 121 were identified from MacConkey agar and 20 were not identified from MacConkey agar. (**Table 12**). The sensitivity is 100%, PPV is 86%, accuracy is 86%.

The overall sensitivity and resistance pattern of uropathogens were shown in **Table 13** .All the organisms were 128(100%) sensitive to Meropenem. The highest sensitivity was shown to Amikacin 119(92.97%), and the lowest sensitivity was shown to Ampicillin 21(16.41%). The sensitivity to all other antibiotics were , for Nitrofurantoin 111 (86.72%), Cefoperazone sulbactum 105 (82.03%), Gentamycin 89 (69.53%), Ciprofloxacin 63(49.22%), Norfloxacin 44(34.38%),, Cefotaxime 35 (27.34%), Cotrimoxazole 32 (25%) and Ampicillin 21 (16.41%).

All the Cefotaxime resistant isolates of E. coli , Klebsiella and Proteus were subjected to confirmatory tests by double disc approximation test and E tests. The results were shown in **Table 14 .** Among the 67 ESBL positive isolates , Escherichia coli 52 (67.5%) was the predominant ESBL producing organism . The ESBL positive

isolates among Klebsiella and Proteus were 10 (66.7%) and 5(55.6%) respectively.

The comparison of ESBL detection using double disc synergy test and E test was depicted in **Table 15**. Out of 70 isolates which were positive in screening tests, 63 were detected as ESBL producers from double disc synergy test and 67 were detected in E test. Out of these two tests E test detected maximum number of organisms (95.7%) compared to DDST (90%)

Out of 70 screening positive isolates , 67 were positive in Etest, whereas 63 were positive in DDST test. 7 organisms were negative in DDST test and only 3 organisms were negative in E test. There was statistically significant association between E test and DDST test. P value 0.0006 (**Table 16).**

The sex wise distribution of ESBL positive isolates were shown in **Table 17**. The isolation rate was more in males 36(53.7%) compared to females 31(46.3%).

ESBL isolates were more common in 0-10 years age group(32.8%) in both sexes. Another peak was shown in above 60 years age group.(19.4%).**Table 18**.

The isolation of ESBL organisms from various wards were shown in **Table 19** . ESBL strains were isolated predominantly from Medical ward (22.4%) followed by urology ward (16.4%).

The antibiotic sensitivity pattern of predominantly isolated Gram negative organisms were shown in **Table 20**. It revealed that apart from Meropenem (100%) the maximum sensitivity for Escherichia.coli was seen with Nitrofurantoin (98.7%), followed by Cefoperazone sulbactum(96.1%) Amikacin (94.8%), Gentamycin (80.5%) and amoxy clavulanate(49.4%). The maximum resistance was seen against Ampicillin (80.5%), Norfloxacin (77.9%), Cotrimoxazole (76.6%), Cefotoxime (70.1%) and Ciproflxacin(61%) .

Klebsiella pneumoniae was most sensitive to Meropenem (100%0 Cefoperazone sulbactum(100%) followed by Amikacin (86.7%), Gentamycin 12(80%),Ciprofloxacin 11(73.3%), Nitrofurantoin 11(73.3%) Maximum resistance was seen with Ampicillin (93.3%), Cotrimoxazole 11(73%), Cefotaxime 10(66.7%),Norfloxacin9(60%) and amoxyclav 8(53.3%).

Proteus species were most sensitive to Meropenem (100%) Amikacin and Cefoperazone sulbactum (100%).The highest percentage of resistance was shown for Ampicillin(88.9%).

Acinetobacter baumanii showed 100% sensitive to Meropenem, Amikacin and Cefoperazone sulbactum and 100% resistant to Cotrimoxazole and norfloxacin.

Pseudomonas aeruginosa were 100% sensitive to Meropenem, Cefoperazone sulbactum, and Amikacin and 100% resistant to norfloxacin and Cotrimoxazole.

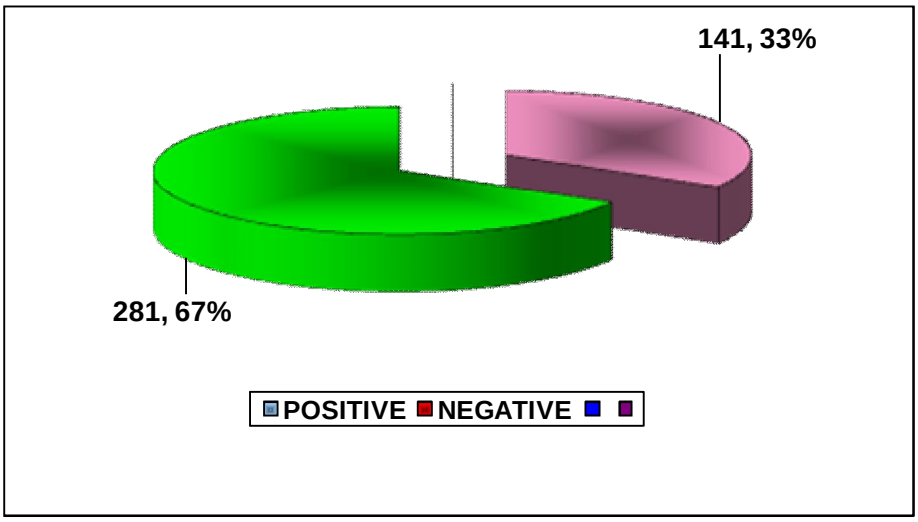
The antibiotic sensitivity pattern of Gram positive organisms which were isolated commonly were depicted in **Table 21**. The Enterococcus were most sensitive to Vancomycin 14 (100%), Cefoperazone sulbactum (100%) and Nitrofurantoin(100%) and were resistant to Ampicillin 1 (7.1%). Staphylococcus saprophyticus was most sensitive to Amikacin 4(100%), Amoxy clav 4 (100%), Cefopreazone sulbactum 4(100%) and Nitrofurantoin 4 (100%). Stapylococcus aureus was sensitive to Ampicillin, Ciprofloxacin, Amikacin, Nitrofurantoin, Cotrimoxazole , Cefoperazone sulbactum and Amoxy clav (100%) and resistant to Gentamycin and Norfloxacin(100%).

The resistance pattern of ESBL and non ESBL E. coli were compared in **Table 22**. The ESBL Escherichia coli isolates showed highest resistance towards Cefotaxime 52(100%), Ampicillin

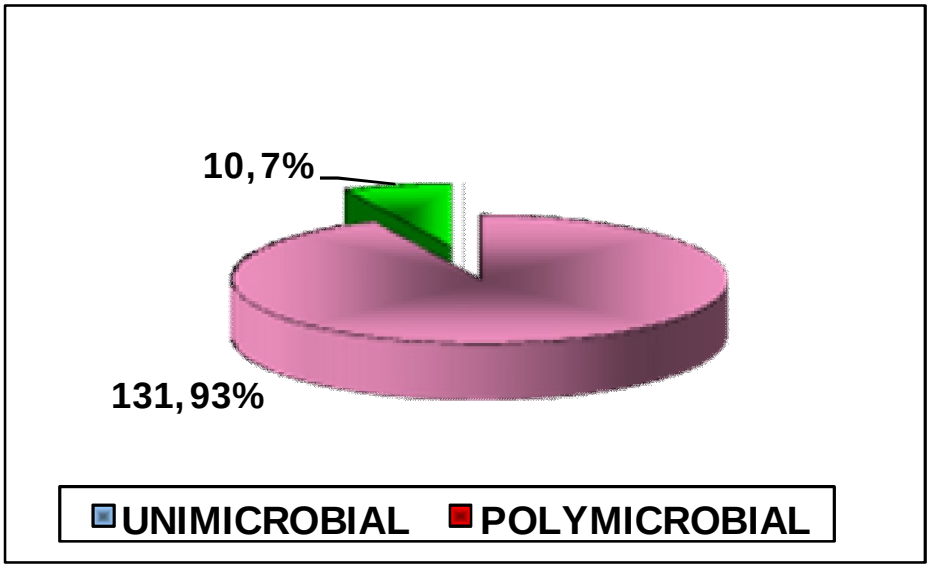
50(96.2%), Cotrimoxazole 44(84.6%), Norfloxacin 43(82.7%) and Ciprofloxacin 34(65.9%) compared to non ESBL stains.

The resistance pattern of ESBL and non ESBL Klebsiella were compared in **Table 23** . The ESBL Klebsiella strains were highly resistant towards Cefotaxime 10 (100%), Ampicillin 10(100%), Cotrimoxazole 9(90%), Amoxy clavulanic acid 7(70%) and Norfloxacin 6(60%)

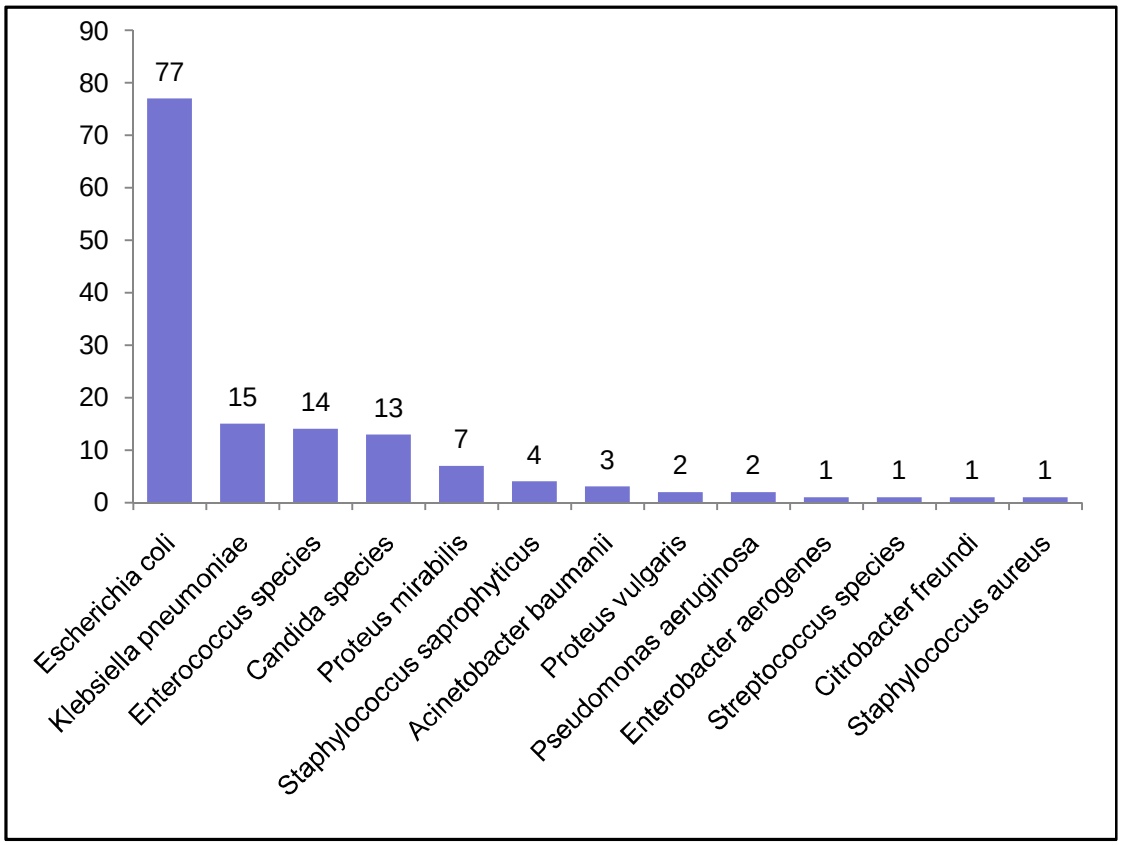
1. RESULT OF URINE CULTURE AMONG STUDY POPULATION



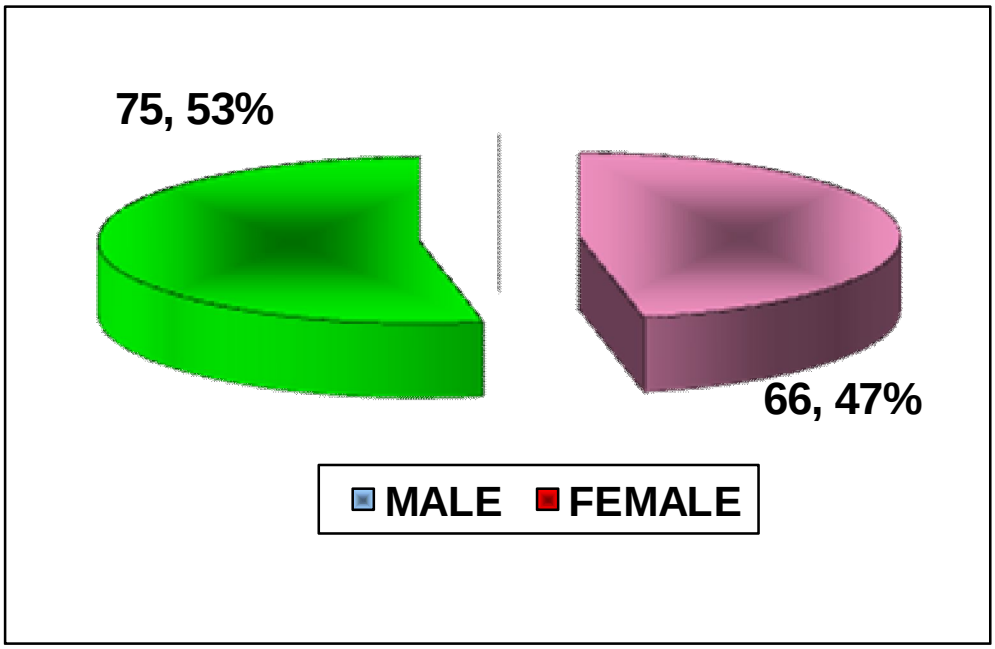
2. TYPE OF GROWTH AMONG POSITIVE ISOLATES



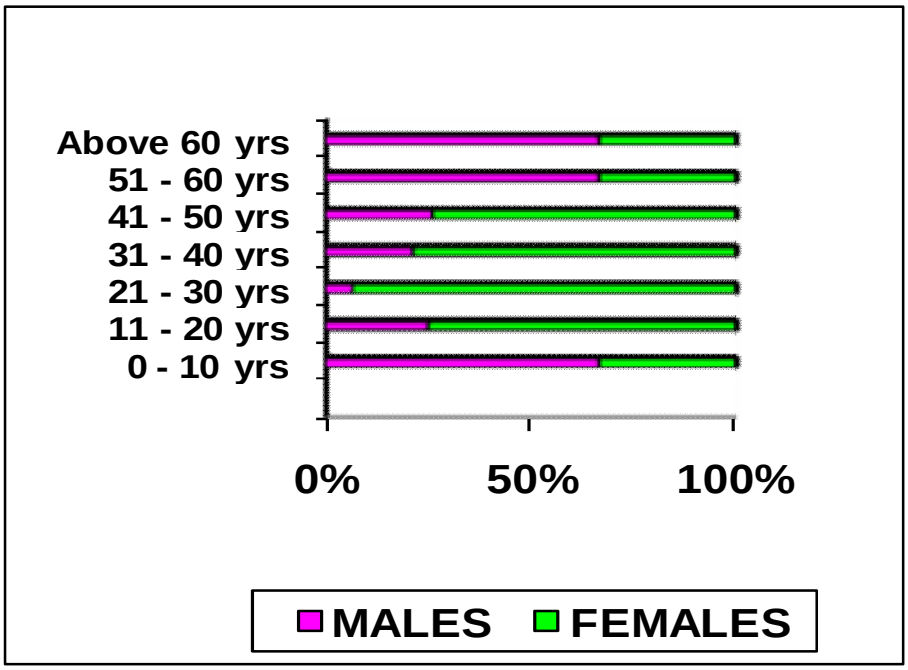
3. DISTRIBUTION OF ORGANISMS ISOLATED FROM CASES OF UTI



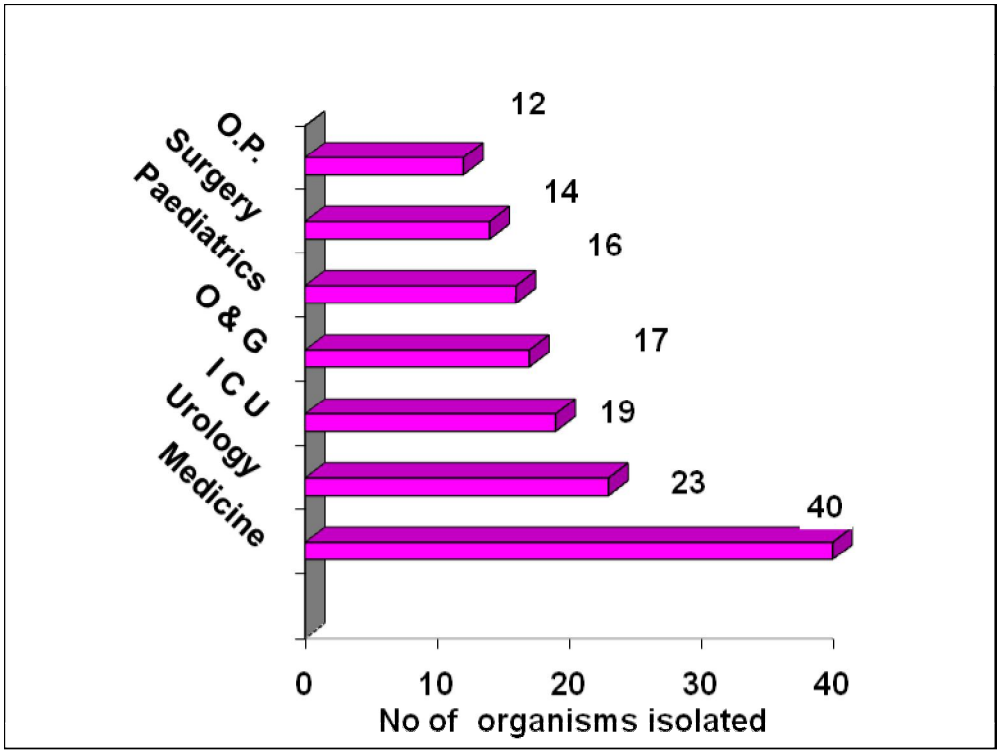
4. SEXWISE DISTRIBUTION OF THE ORGANISMS



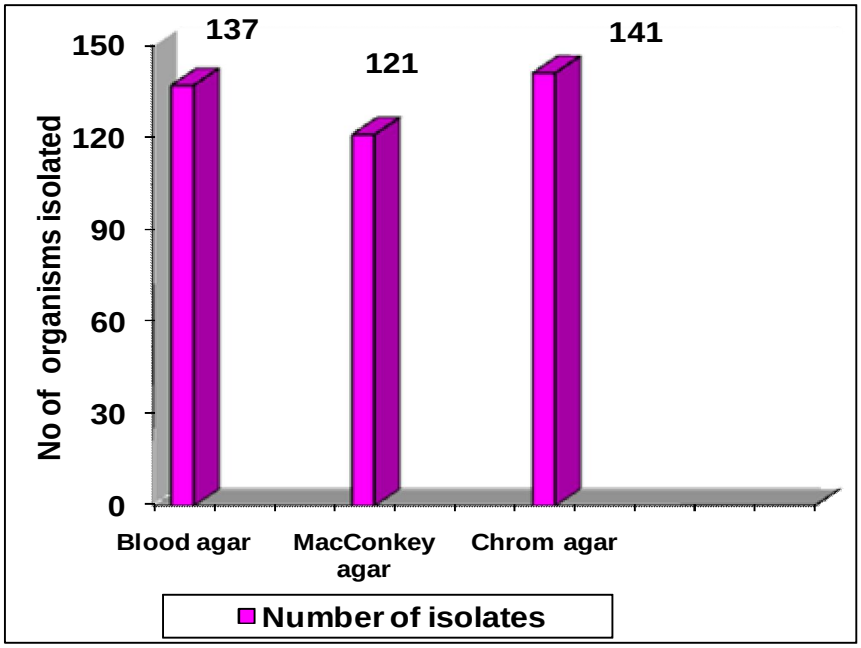
5. AGE AND SEX DISTRIBUTION OF THE POSITIVE ISOLATES



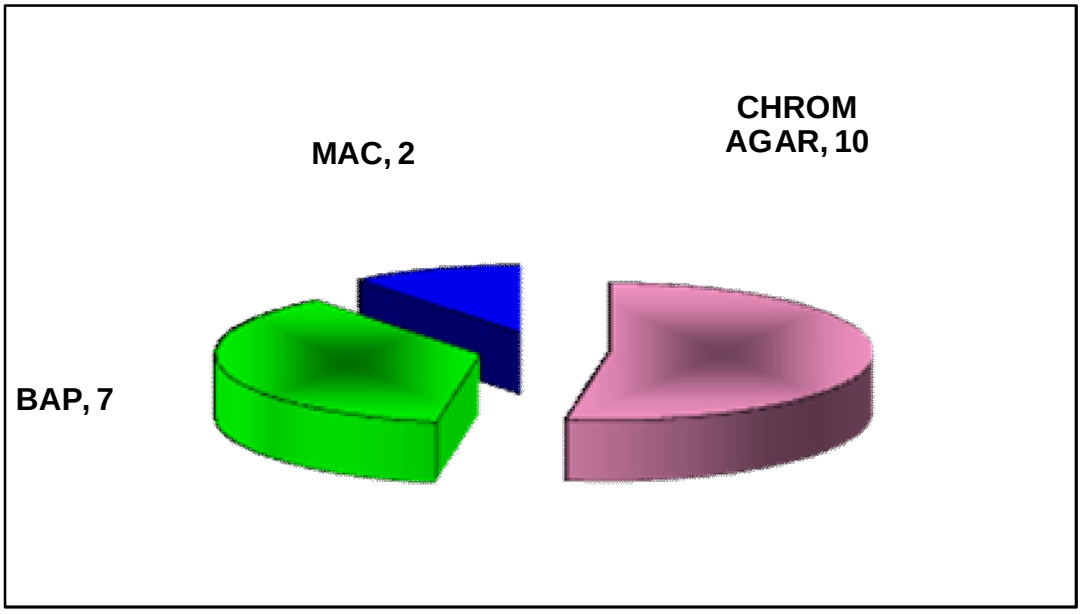
6. NO OF ISOLATES FROM VARIOUS DEPARTMENTS



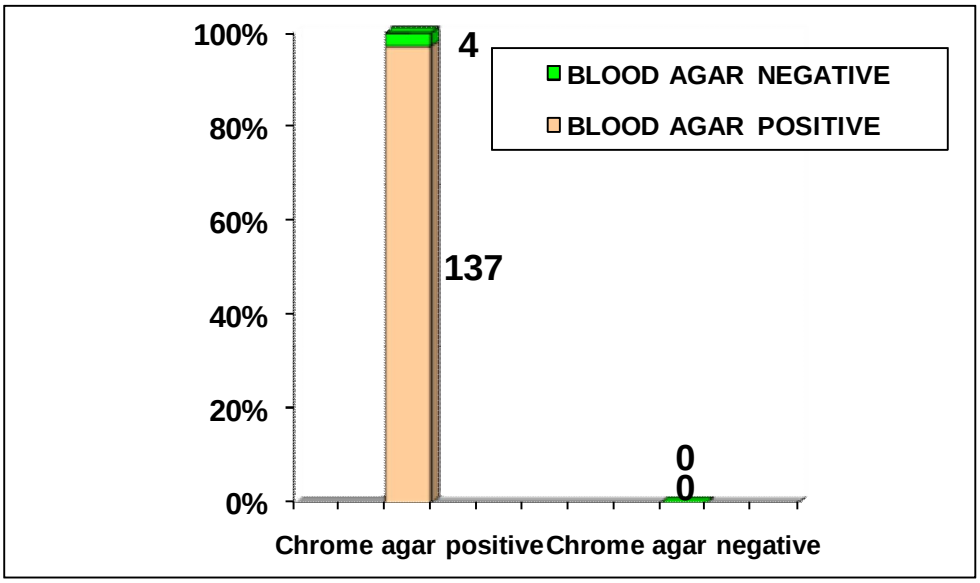
7. ISOLATION OF ORGANISMS IN VARIOUS MEDIA



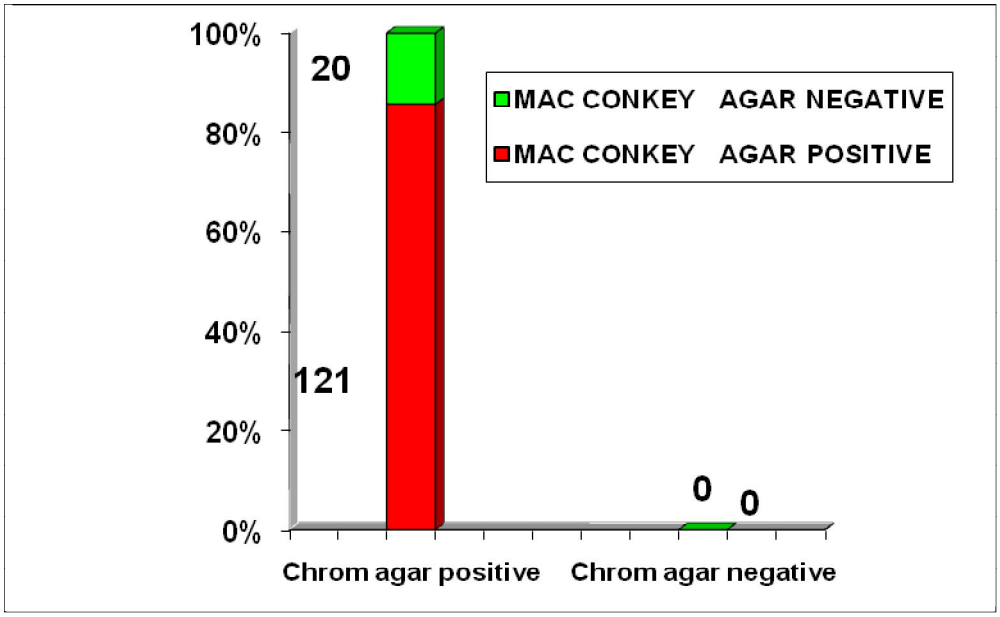
8. ORGANISMS ISOLATED FROM MIXED GROWTH IN VARIOUS MEDIA



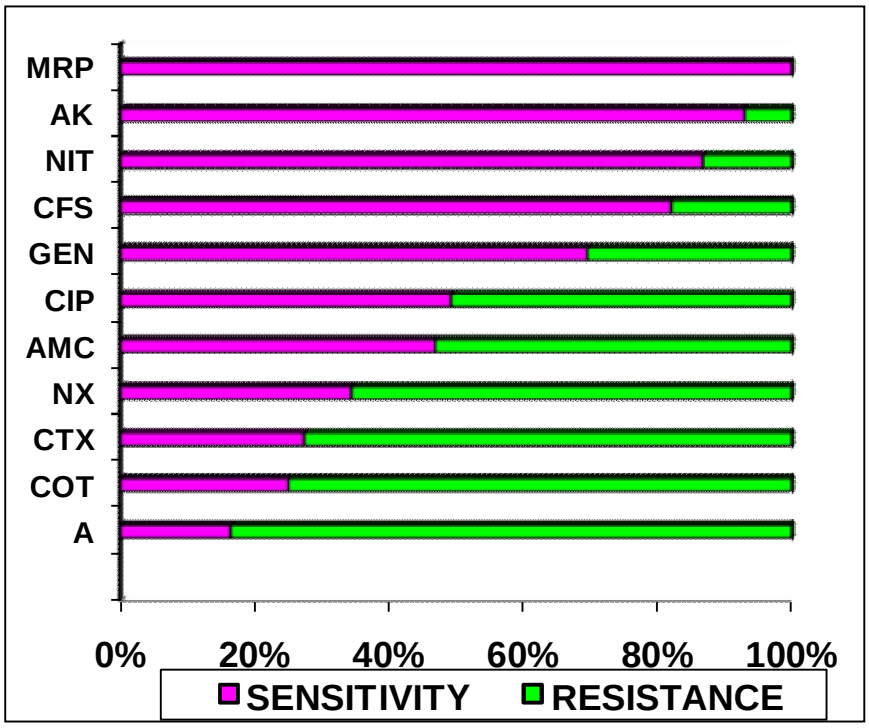
9. COMPARISON OF CHROM AGAR WITH BLOOD AGAR



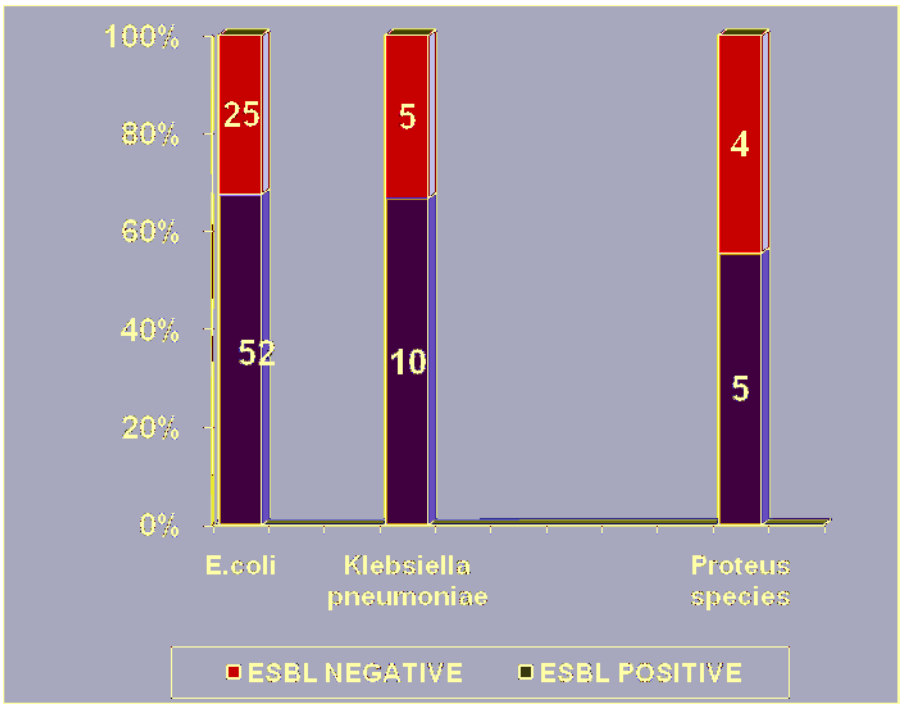
10. COMPARISON OF CHROM AGAR WITH MAC CONKEY AGAR



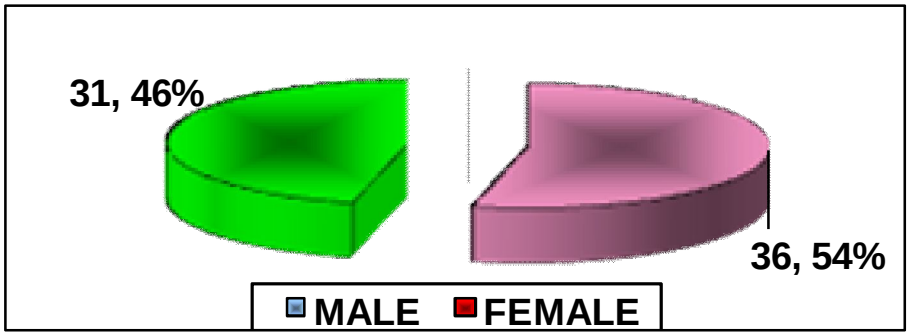
11. SENSITIVITY AND RESISTANCE PATTERN OF UROPATHOGENS TO ANTIBIOTICS



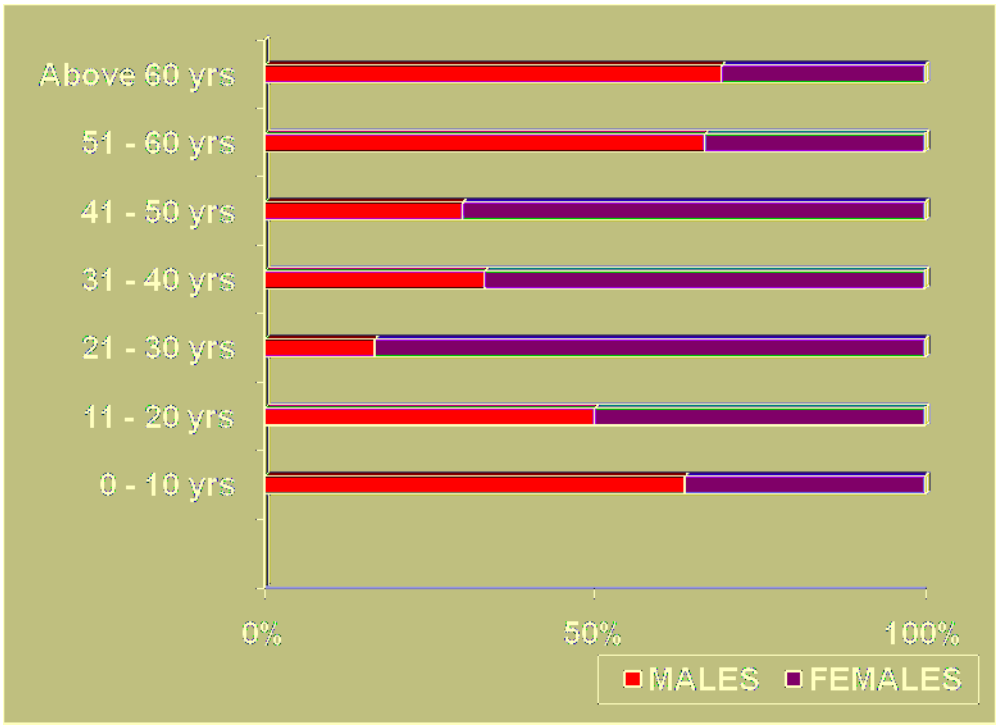
12. PERCENTAGE OF ESBL ISOLATES



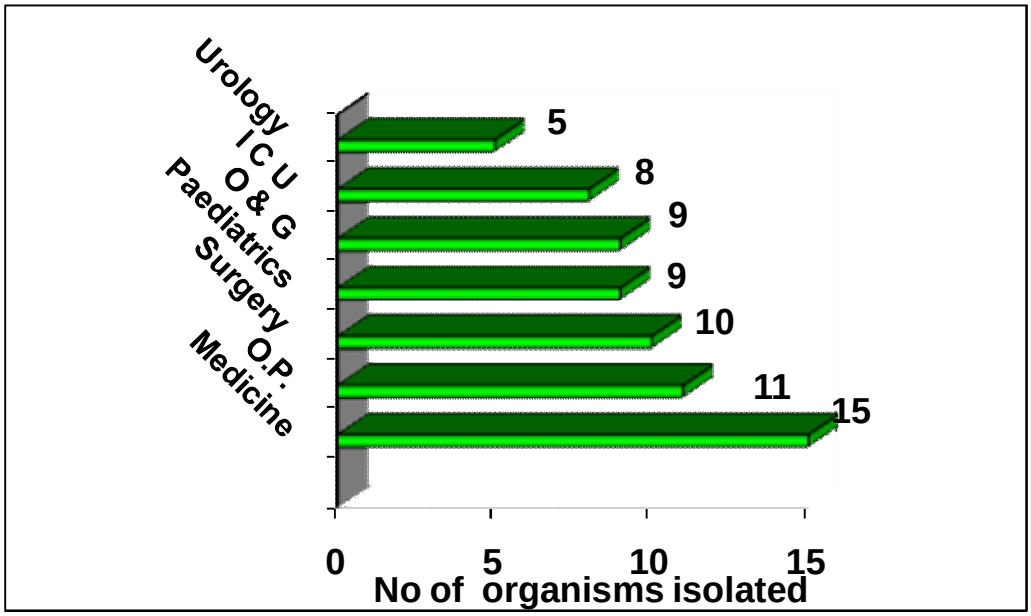
13. SEXWISE DISTRIBUTION OF ESBL POSITIVE ISOLATES



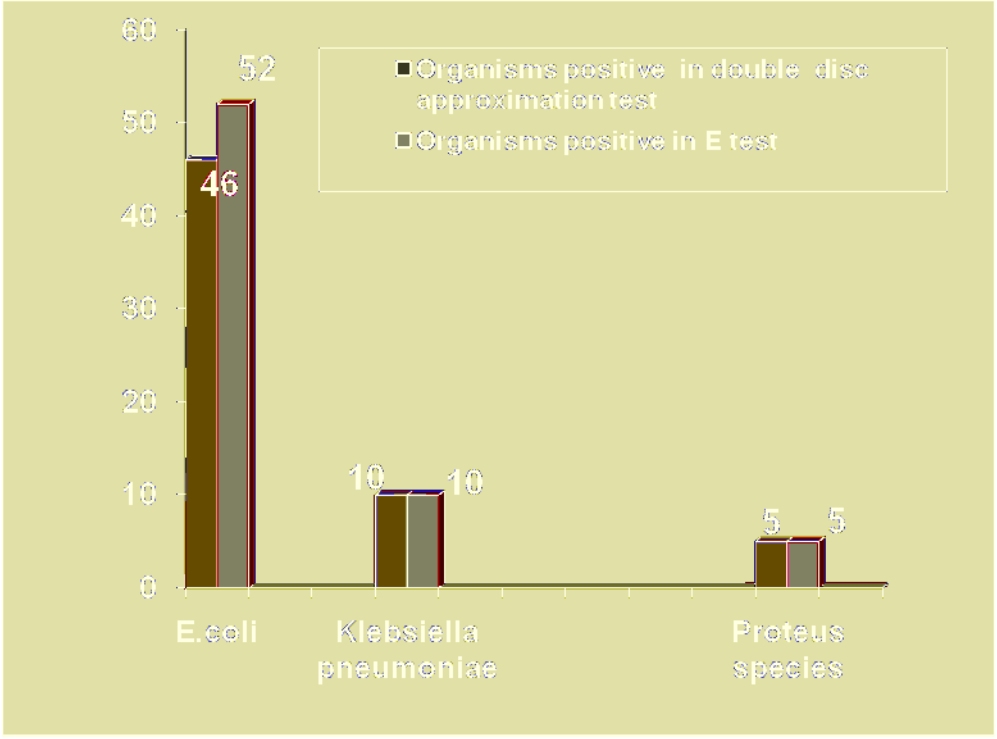
14. AGE AND SEX DISTRIBUTION OF ESBL POSITIVE ISOLATES



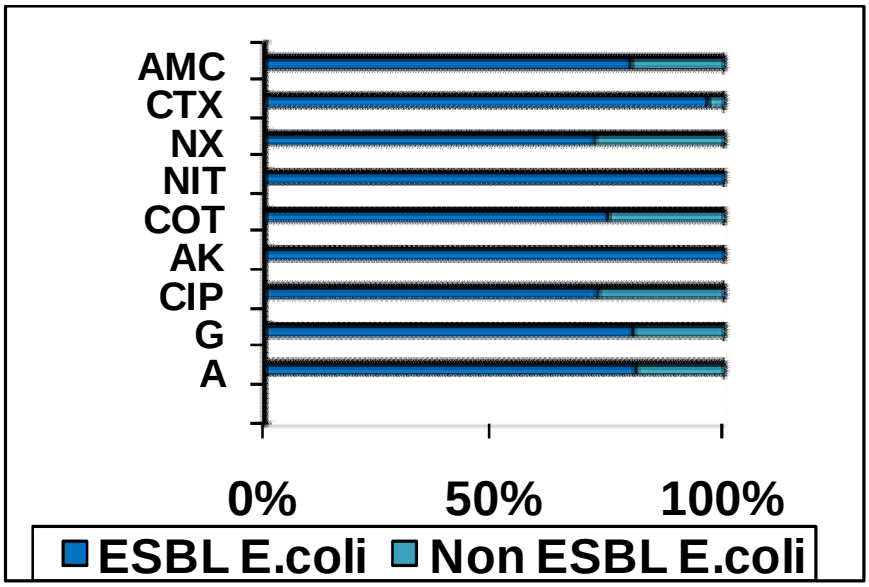
15. ISOLATION OF ESBL PRODUCING BACTERIA FROM DIFFERENT DEPARTMENTS



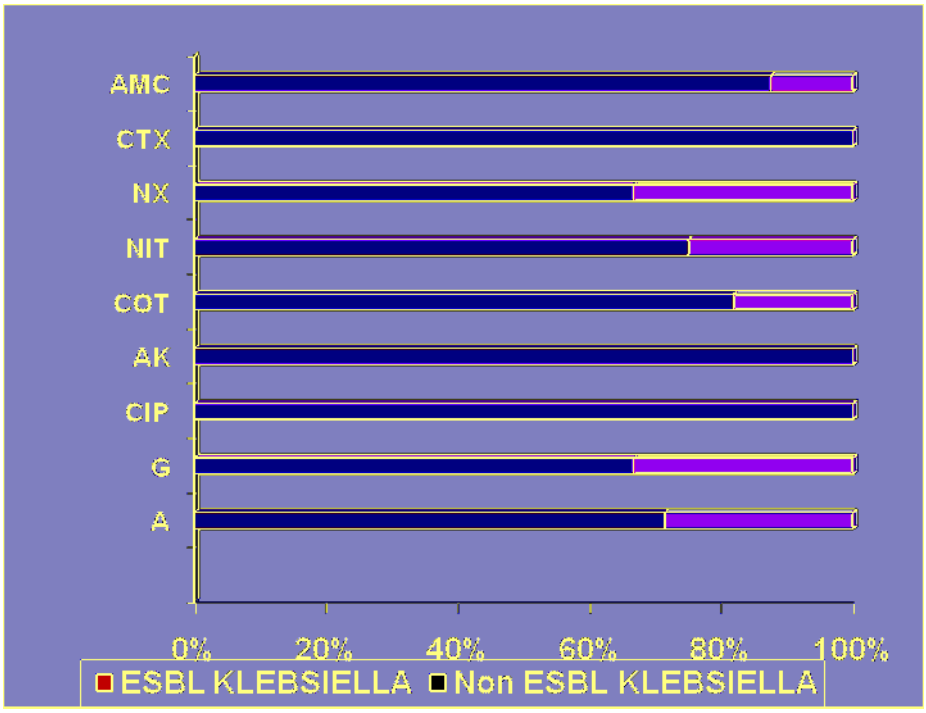
16. COMPARISON OF ESBL DETECTION USING DDST AND E TEST



17. RESISTANCE PATTERN OF ESBL AND NON ESBL E.COLI



18. RESISTANCE PATTERN OF ESBL AND NON ESBL KLEBSIELLA



DISCUSSION

DISCUSSION

The gold standard for the diagnosis of UTIs, is the culture of urine samples on solid media. Blood agar and MacConkey are the conventional media used in our laboratory. The present study , evaluated chromogenic media , for its effectiveness as a medium for direct isolation of organisms from urine samples. Previous studies have demonstrated equal or superior performance of chromogenic media compared to conventional media for the isolation and identification of uropathogens. The results from this study were in agreement with prior reports.

In case of most of the urinary tract infections , antibiotics are given empirically before the laboratory results are available. Current knowledge of the organisms that cause urinary tract infection and their antibiotic susceptibility pattern is mandatory, to ensure appropriate therapy.

Prevalence of the organisms

From the 141 (33.4%) positive growth , 131 (92.9%) were unimicrobial and 10(7.1%) were polymicrobial. This was slightly higher the study done by **Leeela rani et al.,** [72], which revealed 95.8% were unimicrobial and 4.12% were polymicrobial growth. The study done by **V.Lakshmi et al.,**[73] , obtained 95.12% unimicrobial

and 4.87% polymicrobial growth. Other study by **Soley Sharmin.[6],** showed 90.6% unimicrobial and 9.4% polymicrobial growth which was similar to our study. This study had slight difference with the study done by **R Praveen et al.,**[5] which showed 31.67% unimicrobial and 2% polymicrobial growth.

In the present study Gram negative organisms were responsible for 76.6% of UTI s and Gram positive organisms were responsible for 14.2% of UTI s and the remaining 9.2% were caused by candida. The studies by **Arwa M Abdullah et al.,** [74] showed 75.33% of UTI were caused by Gram negative and 24.66% by Gram positive bacteria. Another study by **Z. Samra et al.,** [3] showed 70% of Gram negative organisms , 26% Gram positive organisms and 4% of Candida species were responsible for UTI s.. The present study showed slight decrease in isolation of Gram positive organisms, but greater recovery of candida. The Candida species in our study were commonly isolated from diabetic and patients aged above 60 years.

All the organisms isolated from chromogenic agar were consistent with the manufacturer's description. Escherichia coli 77 (54.6%) was the predominant organism isolated. This is similar to the study done by **Chaudhary Navin kumar et al.,** [75] which

showed 52.4% of E.coli and another study by **Sohely sharmin et al.,**[6] which showed 53.2% of E.coli.

The isolation rate of Klebsiella pneumoniae(10.6%) and Enteococcus (9.9%) in our study, were also similar to the study by **Sekikawa Elly 2011.,**[76] which showed the isolation rate of Escherichia coli (55.4%), Klebsiella pneumoniae (9.5%), and Enterococcus (9.5%). Another study by **Sohely Sharmin et al** .,[6] the isolation rate of Klebsiella was 10.7% and Enterococcus was 10.7%. In all these studies chromogenic media were used for the isolation of urinary tract pathogens. In a study by **Tambekar et al.,** [77]Pseudomonas aeruginosa was the second common isolate. In all other studies , similar to our study Klebsiella pneumoniae was the second common isolate.

In the present study isolation of organisms from mixed cultures were 100% in chromogenic agar. This was similar to the study done by **R Parveen et al.,**[5] and **Leela Rani et al**[72] which also showed similar results.

Comparison of chromagar with Blood agar and MacConkey agar

The isolation of organisms from Blood agar(97.2%), MacConkey agar (85.8%) and Chromogenic agar (100%) in our study , showed similar isolation rates as that of the study by

Leela Rani et al 2012.,[72] (Blood agar (97.5%), MacConkey agar (88.1%)). Enterococcus produced characteristic tiny blue appearance which was more easily identified by Chromogenic agar. Due to the characteristic color and morphology they can be easily identified from mixed cultures. Klebsiella, Enterobacter and Citrobacter produced similar colony colors which were further identified by biochemical reactions. This was also observed in a similar study by Lakshmi et al.[73] In the studies by **Sekikawa Elly**[76] **and Lakshmi et al .,**[73] they observed a few strains of Escherichia coli and Klebsiella strains did not produce proper colors , and were identified by further biochemical reactions. No such discrepancy was noted in our study.

Similar to our study , studies **by Leela Rani et al .,** [72] **V.Lakshmi et al.,**[73] **and J D Perry et al.,** [78] observed easy identification of organisms from mixed cultures in Chromogenic agar and also in identifying contaminated samples.

In a study done by **Jeyshri Pethani et al.,** [79], the antimicrobial susceptibility testing was done from the Chromogenic agar which revealed equivalent susceptibility results as compared to the susceptibility tests done from Blood agar or MacConkey agar.

Swarming of Proteus was limited in chromogenic agar , in our study and all other studies conducted by others.

Since the presumptive identification of the common organisms were possible in the primary plate itself , it was observed that the turn around time of reporting the urine culture could be reduced. This would help in starting early and appropriate antibiotic therapy.

Cost is an important issue, if a laboratory is introducing a new method. Chromogenic media though more expensive , can be cost effective due to easier recognition of significant isolates from culture plates and more accurate detection of mixed cultures. The study by **Retelj and Harlander.,**[80] have concluded that chromogenic media for urinary tract pathogens is cheaper than conventional methods only when there is high rate of isolation of organisms such as Escherichia coli, Enterococci Klebsiella and Proteus.

Age and sex distribution of UTI

In our study more pathogens were isolated from females(53.2%) than males (46.8%). This was in correlation with the study conducted by **Sabrina J Moyo et al.,** [81] in which the isolation of pathogens from females were at a rate of 54.4% and from males were at a rate of 45.6%. Another study by **B.Sasirekha 2013.,**[82] , also showed that females (51.3%) were affected more commonly

than males (48.6%). Anatomic factors like short urethra , hormonal factors and pregnancy contribute to the female preponderance of the UTI s.

Our study demonstrated that the highest incidence of urinary isolates were from 21-50 (36.9%) years age group. This is in coincident with the study by **S.Shafiyabi et al 2014.,**[83]**.** In this study there is a peak incidence of UTI was noted in the age group between 21-50 years. (55%).

This result proves that among females the sexually active and child bearing age group were more vulnerable to UTIs. 0-10 years were the age group commonly affected in both sexes in our study.. This is similar to the study by **Dr.Sumer singh.,** [84], in which children were the second predominant group (24%) . More number of samples from paediatric wards which could be the possible reason were attributed to uncircumcision and vesicouretral reflux. Acute diarrohea , respiratory tract infections and malnutrition are also some of the causes for UTIs in children. Another small peak incidence in the age group of above 50 years (29.8%) were noted . This proves that, this age group is more vulnerable to UTIs because of the risk factors like poor emptying of the bladder due to neurogenic causes and urinary tract obstruction .

Antibiotic susceptibility pattern

The overall antibiotic sensitivity pattern of the uropathogens in our study showed higher sensitivity to Meropenem(100%) ,Amikacin (93%) and Nitrofurantoin(86.7%). There was a higher degree of resistance towards Ampicillin(83.6%) and Cotrimoxazole (75%). This was in accordance with the study of **Sandhiya R et al.,**[85] where the uropathogens showed a higher degree of sensitivity towards nitrofurantoin and Amikacin. **Chaudhary U et al .,**[86.] have proved that uropathogens were 100% sensitive to Meropenem.Another study by **Durgesh D et al.,**[87] showed that there was a high degree of resistance towards Ampicillin(79%), similar to our study.

Another study by **Gaurav Dalela et al.,** [88] showed that there was a high degree of resistance towards Cotrimoxazole (78.9%), Cefotaxime (78.2%), Amoxy-clavulanate (82.4%) and Norfloxacin (67.6%). This is similar to our study where the resistance was 75%, 72.7% ,53.1% and 65.6% for Cotrimoxazole, Cefotaxime, Amoxy-clavulanate and Norfloxacin respectively.

ESBL detection

In our study , ESBL sceening was done using Cefotaxime disc which was similar to the study done by **N M Suryawanshi et al.,[89].** In this, they have proved that all the ESBL producers were uniformly resistant to all three third generation cephalosporins (Cefotaxime, Ceftriaxone and Ceftazidime). Confirmation was done by double disc synergy test and Etest.[90] .Out of 70 isolates which were positive ESBL producers in screening test , 63(90%) were positive in DDST test and 67 (95.7%) were positive in Etest. Two Escherichia strains and one proteus strain produced inconclusive results. This could be due to some other resistance mechanism other than ESBL production such as AmpC.[91]production. Our study shows that E test was more sensitive compared to double disc synergy test which was also proved in a study by **Martin G Cormican et al.,[66]**.A study by **Anandakumar et al.,** [111] showed that E test was 100% sensitive and 97.6% specific with 97.3% positive predictive value. Another study by **Dr. Pavani et al** .,[112] also showed 100% sensitivity for DDST and 87.5% specificity for DDST.

Prevalence of ESBL in E.coli , Klebsiella and Proteus

According to our study 67.5% of Escherichia coli, 66.7% of Klebsiella and 55.6% of Proteus were confirmed as ESBL producers. A a study by **Anbumani Narayanasamy et al.,**[92]

indicated that the prevalence of ESBL varies from 28% to 84%. In our study high rate of ESBL was seen in Escherichia coli isolates followed by Klebsiella and Proteus. Our study correlates with the Studies **by S Baby padmini et al., Rugved Kulkarni et al . , S.Krishna kumar et al. , Venkatadri Babu et al., Ramesh Kumar M.R et al .,** and **Umadevi S et al 2011.,** which showed that Escherichia coli was the commonest ESBL producer.[93,94,95,96,97,98] Other studies by **Venkatadri Babu et al 2014.,**[96] and **Omar B Ahmed et al.,** [99] 2013 showed that Klebsiella pneumoniae was the common ESBL producers.

The ESBL percentage of Escherichia coli in our study coincides with the study by **Omar B Ahmed et al.,**[99] which showed 65% of Escherichia coli were ESBL producers. The study by **Rugved Kulkarni et al** [94]., and **S.Krishnakumar et al.,** [95] showed 40.7% and 44.4% of ESBL producing Escherichia coli respectively. This is lower than the isolation of ESBL Escherichia coli in our study. Other study by **Umadevi S et al** .,[98] showed a higher prevalence of ESBL Escherichia coli (81%). The same study also showed that the prevalence of ESBL producing Klebsiella were 74%, which was higher than the rate of ESBL Klebsiella in our study. Other studies by **Rugved Kulkarni et al.,**[94] (15.9%) and

S.Krishnakumar et al .[95], (37.0%) isolated lower percentage of ESBL producing Klebsiella. The study in Coimbatore by **BabyPadmini et al.,**[93] during 2004, showed 41% of ESBL E.coli and 40% of ESBL producing Klebsiella . This is lower than the ESBL isolates in our study. The rate of ESBL production in Proteus was 48.9% and 33.3% respectively by the studies conducted by **Jitendra Kumar Pandy.,**[100] **and Omar B Ahmed** .,[99] which was slightly lower than our study.

Age and sex prevalence of ESBL isolates

In our study the ESBL organisms were more commonly isolated from males (53.7%) than females (46.3%). This is in similarity to the studies by **Pankaj Baral et al** .,[101] which showed males were most commonly affected than females. Males are usually less prone to UTI s as compared to females because of the longer course of urethra and bacteriostatic prostatic secretions. ESBL producing strains can overcome this barrier and hence there is higher incidence in males. This fact was also proved in the study by **E Mahesh et al.,**[102] This is in contrast to the study by **Datta P et al.,**[103] which showed female preponderance.

Majority of ESBL isolates were from Medicine wards. This is in similarity with the study by **Vipul M Khakhkhar et al.,**[104] where the isolation was more common from Medicine ward.

In our study the most common age group affected were children below ten years and above 60 years age group. This is in similarity with the studies by **Mehrgan et al.,** [105] and **Sabrina J Moyo et al .,**[81] This could be explained by the fact that, immune system is not well developed in children below one year and also congenital abnormalities like posterior urethral valves and uncircumcision contribute to the infection in children. In the elderly due to instrumentation the local immunological barrier would have been destroyed which favours the spread of infection and also diabetes , menopause and other immunocompromised states contribute to infection by resistant organisms.

Antibiotic susceptibility pattern of ESBL and non ESBL organisms

The sensitivity pattern of Escherichia coli revealed that maximum sensitivity was shown towards Nitrofurantoin, Cefoperazone sulbactum and Amikacin. Other than beta lactum antibiotics ESBL strains showed maximum resistance towards Gentamycin, Ciprofloxacin, Norfloxacin and Cotrimoxazole. Compared to non ESBL isolates this resistance was statistically significant.

This showed that ESBL strains exhibit co resistance for fluroquinolones , aminoglycosides and cotrimoxazole.. The study by

Swaminathan ranjan.,[106] also showed that ESBL E.coli strains were highly resistant to Gentamycin, Norfloxacin, Cotrimoxazole and Ciprofloxacin. ESBL E.coli strains showed high sensitivity towards Nitrofurantoin and Amikacin.

The resistance pattern of ESBL Klebsiella showed high resistance towards Cotrimoxazole and Norfloxacin apart from betalactam antibiotics. A study by **Anil Chander et al.,** [107] showed similar results. The study by **S.Shafiyabi et al .,**[83] showed that there was an increased resistance towards Amikacin . This was contradictory to our study and many other studies which showed that there was high sensitivity towards Amikacin. In a study conducted by **Mohammed Rashid et** al., [108] Escherichia coli and Klebsiella isolates were highly resistant against nitrofurantoin This is in contrast to our study where the sensitivity towards nitrofurantoin was high.

Carbapenems are the most effective and reliable beta lactams as they are highly resistant to the hydrolytic activity of all ESBL enzymes due to trans hydroxyl ethyl group. Since they are the only option in case of highly resistant strains they should be kept as a reserve drug for treating complicated cases.

All though ESBL activity is inhibited by beta lactamase inhibitor, UTIs are the only infections that may be treated with beta lactam - beta lactamase inhibitor combination. In this instance beta lactamase inhibitor concentration is high enough to counteract the hydrolytic activity of ESBLs.They exert in vitro pressure on ESBL s their by facilitating their reverse mutation.[86]

Our study suggests Nitrofurantoin as the first line drug against UTI before culture and sensitivity is done. It is cost effective and readily available. It is also safer in pregnancy. The high level susceptibility of uropathogens to nitrofurantoin may be influenced by its narrow spectrum of activity, limited indication, narrow tissue distribution and limited contact with bacteria outside the urinary tract[109]. It has been proved that since nitrofurantoin has multiple mechanisms of action , thereby requiring organisms to develop more than a single mutation in order to develop resistance.[110].

Considering aminoglycosides and fluroquinolones, Gentamycin and Norfloxacin were highly resistant. Ciprofloxacin also showed resistance in ESBL isolates compared to non ESBL isolates. This can be explained due to co-resistance of ESBLs with

aminoglycosides and fluroquinolones because of plasmid mediated gene transfer.[91]

Nitrofurantoin can be considered in case of un complicated UTI cases and Amikacin along with cefoperazone sulbactum can be used in treating hospitalized patients with complicated UITs with Meropenem as a reserve drug where response to all other drugs are inadequate.

SUMMARY

SUMMARY

- A total of 422 aseptically collected urine specimens were studied during the period of one year from August 2013 to July 2014 in Coimbatore Medical College Hospital Coimbatore.
- Out of 422 samples, 206 were from males and 216 were from females.
- Among these samples 141 (33.4%) yielded positive growth and 278 (66.6%) samples yielded no growth.
- Among the positive isolates single organism was isolated from 131 (92.9%) samples and polymicrobial growth was present in 10 (7.1%) samples.
- Among the positive isolates Gram negative organisms 108 (76.6%) predominate .
- The predominant organisms from mixed culture was maximally isolated from chromagar.(100%) rather than from conventional media.
- E.coli was the most common isolate accounting for 54.6% followed by Klebsiella which accounts for 10.6% of the isolates. The isolation rate of Proteus was 6.4% . Among the Gram positive isolates Enterococcus 14 (9.9%) was the commonest organism isolated.

- Females were the commonly affected group with a male to female ratio of 1:1.2
- Among males, children less than 10 years and elderly above 60 years were commonly affected. Females in the age group of 21-50 were more commonly affected.
- The isolation of organisms from Medicine department (28.4%) was higher compared to others. From the out patient department the isolation rate was 8.5%.
- All the organisms were isolated from chromagar media141(100%). Blood agar showed 100% isolation rate of all the organisms except Enterococci ,Citrobacter and Enterobacter .MacConkey agar showed 85.8% isolation rate of organisms.
- Uropathogens were most sensitive to Meropenem (100%), Amikacin (93%) and least sensitive to Ampicillin (16.4%).
- Among the 70 isolates which were positive in screening test 67 were confirmed to be ESBL producers.
- Compared to Double disc approximation test E test was considered the most sensitive method for the confirmation of ESBL organisms.

- E.coli (67.5%)was the most common organism producing ESBLs.The ESBL positivity rate among Klebsiella and Proteus was 66.7% and 55.6% respectively.
- ESBL organisms were common in children below 10 years and elderly above 60 years. Males were commonly affected by ESBL strains.
- Medicine department was the commonest among other departments where ESBL organisms were isolated
- E.coli was most sensitive to Nitrofurantoin (98.7%) and least sensitive to Ampicillin(19.5%). Klebsiella was most sensitive to cefoperazone sulbactum and highly resistant to Ampicillin. Proteus was highly sensitive to Amikacin and cefoperazone sulbactum (100%) and was resistant to Ampicillin(93.3%) and Cotrimoxazole (73.3%).
- Pseudomonas and Acinetobacter were 100% sensitive to Meropenem and cefoperazone sulbactum . They were 100% resistant to Norfloxacin and Nitrofurantoin.
- Enterococci showed 100% sensitivity to Vancomycin and Nitrofurantoin.
- Among the ESBL positive organisms , coresistance to ampicillin, cotrimoxazole, and Ciprofloxacin were found.

- ESBL E.coli along with cefotaxime showed more resistance to Ampicillin, Cotrimoxazole ,Norfloxacin and Ciprofloxacin.
- ESBL Klebsiella showed more resistance towards Ampicillin, Cotrimoxazole and Ciprofloxacin.

CONCLUSION

CONCLUSION

Urinary tract infections are the most common infections and urine samples are among the most numerous specimens sent to the laboratory. The aim of the Microbiology laboratory is to reduce morbidity through accurate and timely diagnosis with appropriate antimicrobial sensitivity testing .

Our study compared the efficacy of chromogenic agar with conventional media. The chromogenic media supports the growth of all uropathogens. The media prevents the spread of swarming of Proteus. There is greater differentiation of Gram negative bacteria and Enterococci can be easily distinguished . Direct biochemical reactions and antibiotic susceptibility tests can be performed from the primary plate itself when mixed pathogens are present. This in turn also reduces the turnaround time. Hence chromogenic media can be used as a single medium for the isolation of uropathogens.

Escherichia coli is the predominant isolate from urine specimens. It is also the most common ESBL producer in our study. Multidrug resistance is significantly higher in ESBL positive isolates. Knowledge of the prevalence of ESBLs and resistance pattern of bacterial isolates are important in the prevention of the

emergence and spread of resistance pattern among bacterial pathogens.

An easy ,rapid and reproducible method has to be adopted in the laboratory to detect ESBL isolates. Our study suggests that even though E test is superior to DDST, because of its simplicity it can also be adopted as a method of ESBL detection.

Though ESBL isolates are more susceptible to Meropenem, for urinary isolates other drugs like Nitrofurantoin and Amikacin can be prescribed. Unnecessary usage of cabapenems will lead to its resistance which is an alarming global threat today. There is also associated coresistance of non beta lactam antibiotics like Norfloxacin, Cotrimoxazole and Gentamycin which has been observed among the ESBL positive isolates. Hence they can no longer be prescribed as an empirical therapy.

It is also concluded that ESBL detection for uropathogens along with antibiotic sensitivity testing is mandatory in all the laboratories to prevent morbidity and mortality .

Strict infection control policy in hospitals along with antibiotic stewardship programs can limit the spread of these multi drug resistant organisms. New antibiotic policy has to be formulated and adopted in the hospitals to prevent the spread of these

organisms. Various measures such as correct hand washing procedures, strict asepsis during any invasive procedures in hospitals can prevent the spread of these organisms. Since there are possibilities of occurrence of new variants, which may result in serious therapeutic problems in the future, all the control measures have to be followed strictly.

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ANNEXURES

APPENDIX -1

HiCrome UTI Agar, Modified M1418

Ingredients :	Gms/Litre
Peptic digest of animal tissue	18.00
Casein enzymic hydrolysate	4.00
Beef extract	6.00
Chromogenic mixture	12.44
Agar	15.00

Final pH 7.2 ∓0.2

Directions:

Suspend 56.94 grams of M 1418 in 1000ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15Ibs pressure (121◦C) for 15 minutes. Cool to 45-50◦C and pour into sterile petri plates.

LIST OF TABLES

LIST OF CHARTS

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14	Age and sex wise distribution of ESBL positive isolates
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16	Comparison of ESBL detection using DDST and Etest
17	Resistance pattern of ESBL and non ESBL E.coli
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LIST OF COLOUR PLATES

S.NO	COLOUR PLATES
1	Urine screening test
2	MacConkey agar plate showing lactose fermenting colonies
3	Chromagar plate showing Escherichia coli
4	Chromagar plate showing Klebsiella pneumoniae
5	Enterococcus fecalis on chromagar plate
6	Staphylococcus aureus on chromagar plate
7	Acinetobacter and pseudomonas on chromagar plate
8	Proteus and Candida on chromagar plate
9	Enterococci on chromagar plate
10	E.coli on chromagar plate
11	Chromagar plate showing mixed culture
12	AST pattern of ESBL isolate
13	Estrip showing ESBL isolate
14	E strip showing non ESBL isolate
15	DDST with ESBL positive isolate
16	Estrip and DDST with ESBL positive isolate

LIST OF ABBREVIATIONS

UTI	- Urinary tract Infections
I_g A	- Immunoglobulin A
I_gM	- Immunoglobulin M
E test	- Epsilometer test
ESBL	- Extended Spectrum Beta Lactamases
CFU	- Colony Forming Units
ATCC	- American Type Culture Collection
TEM	- Temoneria
SHV	- Sulph Hydryl Variant
CTX M	- Cefotaxime
TDA	- Tryptophan Deaminase Reagent
DDST	- Double Disc Synergy Test
CLSI	- Clinical and Laboratory Standards Institute
E.coli	- Escherichia coli
β lactamases	- Beta lactamases
CAUTI	- Catheter Associated Urinary Tract Infections

PROFORMA

Name DOA:

Age DOD

Sex Lab no

Ward Date of receipt of specimen

Occupation

Address

Present illness:

Fever

Abdominal pain

Dysuria/Frequency/Urgency

H/O Present illness:

H/O Comorbid conditions: SHT ,DM, CRF, BPH, Nephrotic syndrome

H/O Antibiotics intake

Past history: Urological surgeries, recurrent UTI,Nephrotic syndrome ,Stricture urethra, Circumcision

Personal history:

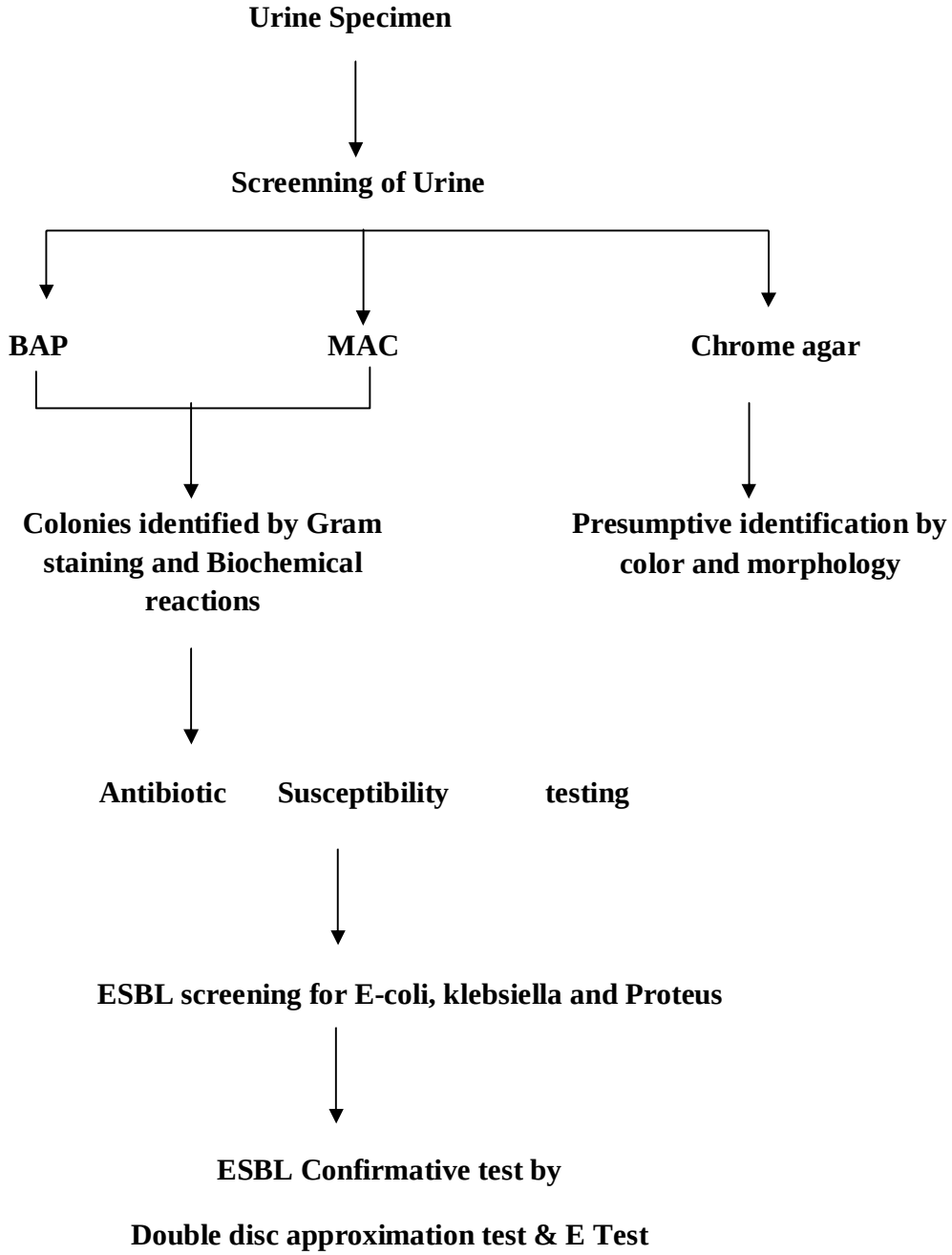
Systemic examination: CVS

RS

Abdomen

Investigations: Complete haemogram, Urine routine, Blood sugar, Serum urea, creatinine.

WORK SHEET


Urine Specimen
Screenning of Urine
BAP
MAC
Chrome agar
Colonies identified by Gram staining and Biochemical reactions
Presumptive identification by color and morphology
Antibiotic Susceptibility testing
ESBL screening for E-coli, klebsiella and Proteus
ESBL Confirmative test by
Double disc approximation test & E Test

MASTER CHART

S.NO	Name	Age	Sex	Ward	Diagnosis	Comorbid condition	Screening test	BAP	MAC	chrome agar	Presumptive organism	AST Pattern		DDST test	E test
												Sensitive	Resistant		
1	Hasina Begam	7	Fch	Paed ICU	Fever		N	NG	NG	NG					
2	Malarvizhi	28	F	OG	Fever		N	NG	NG	NG					
3	Shalini	7	Fch	BMW	Fever		P	GWC	LF	Pink Colony	E.coli	AK,G,CFS,NIT ,MRP	CIP,NX,COT,AMC,A,CTX	N	P
4	Ragupathy	45	M	Medical ward	Fever		N	NG	NG	NG					
5	Veerammal	33	F	OG	UTI		N	NG	NG	NG					
6	Naveenkumar	10	Mch	BMW	Fever		N	NG	NG	NG					
7	Pachiammal	44	F	Fever OP	UTI		P	GWC	FLF	Pink Colony	E.coli	AK,G,CFS,NIT,COT,MRP	CIP,NX,CTX,AMC,A	P	P
8	Sanjay	9	Mch	Paed OP	Fever		P	GWC	FLF	Pink+BlueColony	E.coli	AK,G,CFS,NIT,COT,CTX,A ,CIP ,AMC,MRP	NX	N	N
9	Ainyram	4	Mch	Paed OP	Phimosis		P	GWC	FLF	Pink Colony	E.coli	AK,G,CFS,NIT,MRP	CIP,NX,COT,CTX,AMC,A	P	P
10	Sujithra	3	Fch	Paed OP	Fever		N	NG	NG	NG					
11	Seenu	2	Mch	Paed OP	Fever		P	NG	LF	E.coli+Tiny blue colony	MG/Enterococci	AK,NIT,VAN	NX,COT,CIP,AMC,A		
12	Mekala	6	Fch	Paed OP	Fever		N	NG	NG	NG					
13	Mariammal	72	F	Fever OP	UTI		N	NG	NG	NG					
14	Ashok kumar	17	M	Medicine OP	UTI		N	NG	NG	NG					
15	Nandhini	7	Fch	Paed OP	Fever		N	NG	NG	NG					
16	Jamila	55	F	Medicine OP	UTI		N	NG	NG	NG					
17	Anitha Rani	29	F	Medicine OP	UTI		N	NG	NG	NG					
18	Easwari	25	F	OG ward	UTI	PIH	P	GWC	NLF	Brownish tinged colony	Proteus mirabilis	AK,G,CFS,CIP,NX ,CTX,MRP	COT,AMC,A	N	N
19	Jagadeeswaran	1	Mch	PICU	Fever		N	NG	NG	NG					
20	Pugalendi	5	Mch	PICU	Fever		N	NG	NG	NG					
21	Indumathi	22	F	OG ward	Fever		N	WOC+GWC	NLF	Small Cream colony+ Pink colny	MG/Candida				
22	Soundarraj	10	Mch	BMW	Fever		N	NG	NG	NG					
23	Sandhya	19	F	OG ward	UTI		N	2 Types	FLF+MLF	Pink+BlueColony	E.coli	AK,G,CFS,NIT,CIP,NX ,CTX,AMC ,MRP	COT,A	N	N
24	Sijo	10	Mch	Paed OP	UTI		N	2 Types	FLF+MLF	Pink+BlueColony	E.coli	AK,G,CFS,NIT,CIP,AMC,MRP	CTX,COT,A,NX	N	P
25	Vasantha mallika	4	Fch	Paed OP	Fever		N	NG	NG	NG					
26	Suliman	43	M	Medicine OP	Fever		N	NG	NG	NG					
27	Raguman	10	Mch	BSW	Phimosis		N	NG	NG	NG					

28	Shanmugavel	10	Mch	BSW	Epididimoorchitis		P	WOC	NG	small Colourless colony	CONS	AK,G,NIT,AMC,A	NX,COT,CIP		
29	Rasidha parveen	12	Fch	BSW	Fever		N	NG	NG	NG					
30	Gopinath	9	Mch	BSW	Phimosis		N	NG	NG	NG					
31	Sekaran	47	M	Medicine OP	Fever		N	NG	NG	NG					
32	Pandian	55	M	Medical ward	Fever	COPD	P	GWC	LF	Blue Colony	Klebsiella	AK,G,CFS,MRP	NIT,AMC,NX,CIP,CTX,COT,A	P	P
33	Karuppusamy	60	M	Medicine OP	UTI	DM	N	WOC	NG	NG					
34	Sivakumar	9	Mch	BSW	Phimosis		N	NG	NG	NG					
35	Nagan	73	M	Medical ward	Fever	DM	P	GWC	NLF	Brownish tinged colony	Proteus mirabilis	AK,G,CIP,CFS ,MRP	NX,COT,AMC,CTX,A	P	P
36	Arusamy	65	M	Urology ward	Stricture urethra		P	GWC	LF	Pink colony	E.coli	AK,G,CIP,AMC,NX ,MRP	NIT,CFS,COT,CTX,A	P	P
37	Mareeswaran	30	M	Medicine OP			N	NG	NG	NG					
38	Balusamy	63	M	Urology op	UTI		P	GWC-2types	LF+NLF	Pink+BlueColony	Klebsiella	AK,G,CFS,CTX,CIP,NIT,AMC,MRP	A,COT,NX	N	N
39	Ramakrishnan	14	M	Medicine OP	Fever		N	NG	NG	NG					
40	Saravanavel	6	Mch	Paed op	UTI		N	NG	NG	NG					
41	Pushpalatha	43	F	Medicine OP	UTI		P	WOC-Micrococci	NG	NG					
42	Elachiappa Gr	70	M	Urology op	BPH		N	NG	NG	NG					
43	Ramasamy	32	M	Urology op	Stricture urethra		P	GWC	LF	Pink colony	E.coli	AK,G,NIT,CFS,MRP	NX,COT,AMC,CTX,CIP,A	P	P
44	Anjali	22	F	OG op	UTI	Anemia	N	NG	NG	NG					
45	Kaliammal	23	F	Medical ward	Pyelonephritis		P	WOC	NG	small Colourless colony	Staphylococcus aureus	AK.NIT,AMC,CIP,A,COT	NX,G		
46	Katheeja parveen	23	F	OG	Fever	Anemia	N	NG	NG	NG					
47	Durgeswari	3 mon	Fch	BSW	Fever		P	GWC	LF	Pink Colony	E.coli	AK,G,NIT,CFS,CTX,AMC,A,MRP	CIP,COT,NIT	N	N
48	Anitha	40	F	Medical ward	Fever	Recurrent UTI	P	GWC	NG	Tiny blue colony+pink colony	MG/Enterococci	AK,NIT,G,VAN	NX,COT,CIP,AMC,A		
49	Ragavendra	2	Mch	PICU	Fever		P	GWC+WOC	NG	Pink+BlueColony	E.coli	AK,G,NIT,CIP,CFS,MRP	CTX,COT,NX,AMC,A	P	P

50	Abdulla	46	M	Nephro ward	Fever		N	NG	NG	NG					
51	Nizam	24	M	Medical ward	Fever		N	NG	NG	NG					
52	Senthil	35	M	Surgery ward	Fever		N	NG	NG	NG					
53	Narayanan	73	M	Surgery ward	Fever		P	GWC 2 TYPE	LF	Pink+BlueColony	E.coli	AK,G,COT,NIT,NX,CTX,CIP,CFS,AMC,A,MRP		N	N
54	Lakshmipriya	19	F	OG	UTI		N	NG	NG	NG					
55	Vignesh	12	Mch	PICU	Ectopia vesicae		P	GWC	NLF	Pale colony with brownish tinge	Proteus mirabilis	AK,G,CIP,CFS,MRP	NX,COT,CTX,A,AMC	P	P
56	Eswaran	37	M	IMCU	Fever		N	NG	NG	NG					
57	Ayyasamy	45	M	Urology ward	UTI		P	Tiny WOC+GWC	NG	Pink+Tiny blue colony	MG+Enterococci	AK,NIT,VAN,CIP	NX,COT,AMC,A		
58	Sahana	2	Fch	PICU	UTI		N	NG	NG	NG					
59	Ganesh	29	M	Medicine OP	Fever		N	NG	NG	NG					
60	Saleema	10	Fch	Paed op	UTI		N	NG	NG	NG					
61	Bavani	10	Fch	Paed OP	Fever		N	NG	NG	NG					
62	Jeyalaxmi	41	F	Medical ward	Fever		P	GWC	NLF	Pink Colony	E.coli	AK,G,COT,NIT,NX,CTX,CIP,CFS,AMC,A,MRP		N	N
63	Safrene	2	F	PICU	Fever		N	NG	NG	NG					
64	Thebathammal	75	F	Medical ward	Fever	DM	P	WOC 2 Types	NG	Tiny blue + Colourless colony	E.coli	AK,G,CIP,AMC,CFS,NIT,MRP	COT,NX,A,CTX	P	P
65	Palany	65	M	Medical ward	Fever	DM	P	GWC	LF	Pink Colony	E.coli	AK,G,NIT,CFS,NX,MRP	CIP,CTX,COT,AMC,A	N	P
66	Antony	67	M	Medical ward	Fever	COPD	N	NG	NG	NG					
67	Madesh	60	M	Urology OP	Fever		N	NG	NG	NG					
68	Manjula	21	F	OG	Fever		P	GWC	NLF	Non transparent creamy white	Acinetobacter	AK,G,CIP,CTX,CFS,MRP	NIT,COT,NX.		
69	Pandian	67	M	Urology OP	Fever		N	NG	NG	NG					
70	Palani	50	M	Urology OP		Stricture urethra	N	NG	NG	NG					
71	Rubasree	20	F	OG	Fever		N	NG	NG	NG					
72	Marudhachalam	40	M	Urology OP	Fever		N	NG	NG	NG					
73	Ammasai	55	F	Medicine OP	Fever		N	NG	NG	NG					
74	Senthilvel	44	M	Urology OP	Fever		N	NG	NG	NG					
75	Rajagopal	74	M	Surgery ward	Fever		N	NG	NG	NG					
76	Pushpa	43	F	Gynaec ward	Fever		N	NG	NG	NG					
77	Palaniammal	65	F	Urology OP	UTI		P	GWC	LF	Pink Colony	E.coli	AK,G,CIP,CFS,NIT,AMC,MRP	CTX,COT,NX,A	P	P
78	Poornesh	3	Mch	Paed OP	Fever		N	NG	NG	NG					
79	Pradeep	31	M	IMCU	Fever		N	NG	NG	NG					

80	Kadheer	65	M	Surgery ward	UTI		P	GWC	LF 2TYPES	Pink+BlueColony	E.coli	AK,G,CIP,CFS,NIT,AMC,MRP	COT,NX,A,CTX	P	P
81	Sudha	27	F	Surgery ward	Fever		N	NG	NG	NG					
82	Safreena	2	Fch	BMW	Fever		P	GWC 2 TYPES	MLF+LF	Pink+tiny Blue Colony	MG				
83	Sindhu	23	F	OG/LW	Fever		N	NG	NG	NG					
84	Ravi	35	M	Medical ward	Fever		P	GWC+WOC	LF	WOC+Pink colony	E.coli	AK,G,CIP,CFS,CTX,NIT,COT,AMC,MRP	A,NX,	N	N
85	Dinesh	2	Mch	PICU	Fever		P	GWC	LF	Pink colony	E.coli	AK,G,CIP,NX,NIT,CFS,MRP	AMC,CTX,COT,A	P	P
86	Shanthi	22	F	OG/LW	Fever		N	NG	NG	NG					
87	Elizabath	24	F	OG OP	UTI		N	NG	NG	NG					
88	Sundari	32	F	Medicine OP	UTI		N	NG	NG	NG					
89	Yasararafath	5	Mch	Paed OP	Fever		N	NG	NG	NG					
90	Shanthanu	3	Mch	Paed op	UTI		P	Tiny WOC+GWC	NG	Pink+Tiny blue colony	MG/Enterococci	AK,NIT,VAN	NX,COT,CIP,AMC,A		
91	Hamu	8	Mch	Paed op			N	NG	NG	NG					
92	Soujanya	9	Fch	PICU			N	NG	NG	NG					
93	Raj kumar	47	M	Medical ward	Fever		N	NG	NG	NG					
94	B/O Meenakshi	2mon	Mch	BMW	Fever		P	GWC	FLF	Blue Colony	Klebsiella	NIT,NX,CIP,AK,CFS,MRP	COT,CTX,AMC,G,A	P	P
95	Vasudevan	62	M	Surgery ward	Fever		N	NG	NG	NG					
96	Kavitha	33	F	Nephro ward	Fever		N	WOC+GWC	NLF	Pink+small Colourless colony	MG/Candida				
97	Sanddep	3	Mch	BMW	Fever		N	NG	NG	NG					
98	Gowri	12	Fch	PICU	UTI	Bowel p	N	NG	NG	NG					
99	Darnika	18 Mon	Fch	BMW	Fever		P	GWC+WOC	NLF+MLF	Pink + Colourless colony	MG				
100	Ajmel	10	Mch	PICU	Fever		N	WOC	NLF	small Colourless colony	Candida				
101	Karpagam	25	F	OG/LW	Fever		N	NG	NG	NG					
102	Christoper	11	Mch	BMW	Fever		N	NG	NG	NG					
103	Savithri	35	F	OG	UTI		N	NG	NG	NG					
104	Kousik	3	Mch	BMW	Fever		N	WOC(Micrococci)	NG	NG					
105	Niswan	2 Mon	Mch	BSW	Post pyeloplasty		P	GWC	MLF	Blue Colony	Klebsiella	CFS,MRP	AK,G,COT,AMC,NIT,NX,CIP,CTX,A	P	P
106	Chinu	54	M	Urology ward	stricture urethra		N	NG	NG	NG					
107	Jithish Sriram	10	Mch	PICU			N	NG	NG	NG					
108	Divya	10	Fch	PICU	Fever		N	NG	NG	NG					

109	Arhhanari	75	M	Urology ward	BBH		N	NG	NG	NG					
110	Magudeswari	12	Fch	PICU	Meningitis		N	WOC	NLF	small Colourless colony	Candida				
111	Prakash	9	Mch	Surgery ward	Fever		N	NG	NG	NG					
112	Sabharish	5 Mon	Mch	PICU	Fever		P	GWC	MLF	Pink Colony	E.coli	AK,G,NIT,CFS,MRP	CTX,COT,NX,CIP,AMC,A	P	P
113	Saraswathi	42	F	Medical ward	UTI		P	GWC	NLF	Brownish tinged colony	Proteus mirabilis	AK,G,CIP,NX,CFS,CTX,AMC,A,MRP		N	N
114	Karthikeyan	7	Mch	PICU	Fever		N	NG	NG	NG					
115	Krithika	10	Fch	Paed OP	Fever		N	NG	NG	NG					
116	Gopal	52	M	Medicine OP	Fever		N	NG	NG	NG					
117	Thassama	5	Fch	Paed OP	UTI		N	NG	NG	NG					
118	Sundaram	58	M	Medical ward	Fever		N	NG	NG	NG					
119	Satish	9	Mch	PICU	Fever		N	WOC(Micrococci)	NG	NG					
120	Yasmin	5	Fch	Paed OP	Fever		N	NG	NG	NG					
121	Bhuvaneswari	32	F	OG/LW	Fever		N	NG	NG	NG					
122	Antony Daisy	26	F	Fever OP	Fever		N	WOC(Micrococci)	NG	NG					
123	Gayathri	4	Fch	Paed OP	Fever		N	NG	NG	NG					
124	Kanagaraj	29	M	Surgery ward	Fever		N	NG	NG	NG					
125	Asma	33	F	OG OP	Fever		N	NG	NG	NG					
126	Sudha	20	F	OG ward	Fever		N	WOC(Micrococci)	NG	NG					
127	Prabu	31	M	Medical ward	Fever		P	GWC 3 Types	LF + MLF	Pink + Blue Colony	MG				
128	Shanmugam	55	M	Urology OP	UTI		N	NG	NG	NG					
129	Thulasi	73	F	Medical ward	UTI		P	GWC	FLF	Pink colony	E.coli	AK,G,COT,NIT,CTX,CIPCFS,AMC,A,MRP	NX	N	N
130	Balu	50	M	Medicine OP	Fever		N	NG	NG	NG					
131	Keerthana	6	Fch	BMW	Fever		N	NG	NG	NG					
132	Mani	29	M	Medicine OP	Fever		N	NG	NG	NG					
133	Sahul	5	Mch	Paed OP	Fever		N	NG	NG	NG					
134	Aswin	4	Mch	Paed OP	Fever		N	NG	NG	NG					
135	Jeevitha	15 Mon	Fch	PICU	Fever		N	NG	NG	NG					
136	Jerina Begam	4	Fch	PICU	Fever		N	NG	NG	NG					
137	Meganathan	32	M	Medical ward	Fever		N	NG	NG	NG					
138	Periasamy	42	M	Urology OP	UTI	BPH	P	GWC	LF	Pink Colony	E.coli	AK,G,NIT,CFS,MRP	NX,CIP,COT,CTX,AMC,A	P	P
139	Danush	2	M	PICU	Fever		N	NG	NG	NG					
140	Anandhi	32	F	Surgery ward	UTI		N	NG	NG	NG					
141	Ganesan	49	M	Urology ward	UTI		N	NG	NG	NG					
142	Subbian	77	M	Urology ward	UTI		N	NG	NG	NG					
143	Mailammal	60	F	Urology ward	UTI		P	GWC	NLF	Pink Colony	E.coli	AK,G,CIP,NX,NIT,CFS,CTX,AMC,A,COT,MRP		N	N

144	Kanagavel	83	M	Urology ward			N	NG	NG	NG					
145	Ponnusamy	62	M	Surgery ward	UTI		P	GWC	FLF	Pink Colony	E.coli	AK,G,NIT,CFS,AMC,MRP	CIP,NX,COT,CTX,A	P	P
146	Jegadhambal	59	F	Medical ward	UTI		P	GWC	NLF	Green colour colony	Pseudomonas	AK,G,CIP,CTX,CFS,NIT,MRP	COT,NX		
147	Babu	23	M	Medical ward	Fever		N	NG	NG	NG					
148	Vishvesh	2	Mch	PICU	Fever		N	NG	NG	NG					
149	Mani	27	M	Medical ward	Fever		P	GWC	LF	Pink colony	E.coli	AK,G,NIT,CFS,MRP	NX,COT,CIP,CTX,AMC,A	P	P
150	Thejaswini	2	Fch	PICU	Fever	Nephrotic syndrome	P	GWC	MLF	Pink colony	E.coli	AK,G,NIT,CFS, CIP,MRP	NX,COT,CTX,AMC,A	N	P
151	Radhamani	50	F	Medical ward	Fever		P	GWC	LF	Pink Colony	E.coli	AK,G,NIT,CFS,MRP	CIP,NX,COT,CTX,AMC,A	P	P
152	Paramasivam	55	M	Urology ward	Fever		N	NG	NG	NG					
153	Archana	19	F	Medical ward	Fever		P	GWC	LF	Pink+BlueColony	E.coli	AK,NIT,CFS,MRP	COT,CTX,NX,CIP,AMC,A,G	P	P
154	Maheswari	21	F	OG ward	Fever		N	NG	NG	NG					
155	Praneeth	10	Mch	PICU	UTI		N	NG	NG	NG					
156	Babu	34	M	Medical ward	UTI		N	NG	NG	NG					
157	Shanthi	20	F	Medical ward	UTI		P	GWC	FLF	Pink Colony	E.coli	AK,G,CTX,NX,NIT,COT,CIP,CFS,AMC,A,MRP		N	N
158	Fathima	30	F	Medical ward	Fever		P	GWC	FLF	Pink Colony	E.coli	AK,G,CIP,NIT,NX,CFS,MRP	COT,AMC,CTX,A	P	P
159	Thangaraj	50	M	Surgery ward	UTI		N	NG	NG	NG					
160	Vidhya sri	6	Fch	BMW	Fever		N	NG	NG	NG					
161	Peria rasu	68	M	Urology ward	UTI		P	WOC+GWC	NG	Pink+Tiny blue colony	MG/Enterococci	AK,NIT,VAN	NX,COT,CIP,AMC,A		
162	Avinash	12	Mch	Paed OP	Fever		N	NG	NG	NG					
163	Lakshmi	37	F	OG Ward	UTI		N	NG	NG	NG					
164	Selvi	26	F	Medical ward			N	NG	NG	NG					
165	Jothi	13	Fch	Paed OP	Fever		N	NG	NG	NG					
166	Santhosh	20	M	Surgery ward	Fever		N	NG	NG	NG					
167	Rajeshwari	23	F	OG/LW	Fever		N	NG	NG	NG					
168	Subbulakshmi	20	F	OG/LW	UTI		P	GWC	? LF	Non transparent creamy white Colony	Acinetobacter	AK,G,CIP,CTX,CFS,MRP	NIT,COT.NX		
169	Natchal	71	F	Medical ward	Fever		N	NG	Tiny Whte colony	small Colourless colony	Candida				
170	Vijaya	61	F	Surgery ward	Fever		P	GWC	FLF	Pink Colony	E.coli	AK,G,NIT,CFS,A,MRP	CTX,AMC,CIP,NX,COT	P	P
171	Selvaraj	4	Fch	PICU	Fever		N	NG	NG	NG					
172	Divya	39	F	OG	Fever		N	NG	NG	NG					
173	Palanisamy	63	M	Urolgy ward	UTI		N	NG	NG	NG					

174	Swamynathan	56	M	Urology ward	UTI		P	GWC	FLF	Pink Colony	E.coli	AK,NIT,MRP	G,CFS,CIP,NX,COT,AMC,CTX,A	P	P
175	Divya	10	Fch	Paed OP	Fever		N	NG	NG	NG					
176	Jagannathan	33	M	Urology ward	Fever		N	NG	NG	NG					
177	Fathima	25	F	OG ward	Fever		N	NG	NG	NG					
178	Sadhasivam	61	M	Urology ward	UTI		N	NG	NG	NG					
179	Sundara valli	42	F	Nephro ward	CKD		P	GWC	MLF	Pink Colony	E.coli	AK,NIT,CFS ,MRP	G,CTX,AMC,CIP,NX,COT,A	P	P
180	Saraswathi	37	F	OG Ward	Fever		N	NG	NG	NG					
181	Tamilselvi	43	F	Urology ward	Fever		N	NG	NG	NG					
182	Veerathal	43	F	OG ward	Fever	Catheterised 2 months	P	GWC	NLF	Brownish tinged colony	Proteus vulgaris	AK,CFS,CIP,MRP	AMC,COT,A,NX,G,CTX	P	P
183	Alwin charles	7	Mch	BSW	Fever		P	GWC	NLF	Pink Colony	E.coli	AK,G,CIP,COT,NX,NIT,CFS,MRP	CTX,AMC ,A	P	P
184	Arusamy	50	M	Surgery ward	Fever		N	NG	NG	NG					
185	Rajesh kannan	40	M	Urology ward	Fever		N	NG	NG	NG					
186	Balu	50	M	Nephro ward	Fever	CKD	N	NG	NG	NG					
187	Shobana	21	F	OG/LW	Fever		N	NG	NG	NG					
188	Uchi kallai	45	M	Surgery ward	UTI		P	GWC	LF	Pink Colony	E.coli	AK,G,COT,CFS,NIT,MRP	CIP,CTX,NX,AMC,A	P	P
189	Murugajothi	35	F	OG/LW	Fever		N	NG	NG	NG					
190	Savior	62	M	Medicine OP	Fever		N	NG	NG	NG					
191	Surya	39	M	Medicine OP	Fever		N	NG	NG	NG					
192	Varadaraj	25	M	Medicine OP	Fever		N	NG	NG	NG					
193	Mariammal	50	F	Medical ward	Fever		P	GWC	LF	Pink Colony	E.coli	AK,G,NIT,CFS,MRP	CIP,CTX,NX,AMC,COT,A	P	P
194	Aiswarya	10	Fch	Medicine OP	Fever		P	GWC	LF	Pink Colony	E.coli	AK,CFS,NIT,G,MRP	CIP,CTX,NX,AMC,COT,A	P	P
195	Jayaprakash	6	Mch	BMW	Fever		N	NG	NG	NG					
196	Rithika	5	Fch	BMW	Fever		N	NG	NG	NG					
197	Chinnaraj	23	M	Medicine OP	Fever		N	NG	NG	NG					
198	Sathya	10	Fch	PICU	Fever		P	GWC	LF	Pink Colony	E.coli	CIP,NX,NIT,CFS,MRP	AK,G,CTX,AMC,COT,A	P	P
199	Nagendran	43	M	Medical ward	Fever		N	NG	NG	NG					
200	Palaniammal	25	F	OG/LW	Fever	Eclamps	N	NG	NG	NG					
201	Mailathal	40	F	Medical ward	Fever	Meningi	P	WOC	NG	WOC	CONS	AK,G,NIT,AMC	A,NX,COT,CIP		
202	Manojkumar	10	M	BMW	Fever		N	NG	NG	NG					
203	Palani	50	M	Medical ward	Fever	CKD	N	NG	NG	NG					
204	Sasikumar	11	M	BSW	Fever		N	NG	NG	NG					
205	Saravanan	12	M	Paed OP	Fever	Nephroti	N	NG	NG	NG					
206	Yasmin	29	F	OG ward	UTI		P	GWC	LF	Pink Colony	E.coli	AK,G,CFS,NIT,MRP	CIP,COT,NX,CTX,AMC,A	P	P
207	Sathyabama	46	F	Surgery ward	UTI		N	NG	NG	Tiny blue colony	Enterococci	AK,NIT,VAN	NX,COT,CIP,AMC,A		
208	Kanagapushpam	51	F	OG ward	Fever		N	NG	NG	NG					
209	Sumathi	43	F	OG OP	Fever	DUB	N	NG	NG	NG					

210	Muneeshwari	29	F	OG OP	Fever		N	NG	NG	NG					
211	Lakshmi	51	F	Medical ward	UTI		N	NG	NG	NG					
212	Prabu	25	M	Medical ward	Fever		N	NG	NG	NG					
213	Sundaraj	10	Mch	BSW	Eneuresis		N	NG	NG	NG					
214	Ramasamy	75	M	IMCU	Sepsis	CKD	P	GWC	NLF	Pink colony	E.coli	AK,G,NIT,CFS,CTX,AMC, MRP	NX,CIP,COT,A	N	N
215	Poonkodi	30	F	IMCU	Fever	DKA	N	NG	NG	NG					
216	Kaniammal	60	F	IMCU	Fever	DKA	P	GWC	LF	Pink Colony	E.coli	CIP,NIT,CFS,MRP	AK,G,CTX,AMC,COT,NX,A	P	P
217	Shanmugam	38	M	IMCU	Meningitis		N	NG	NG	NG					
218	Nallammal	65	F	Medical ward	Fever		N	NG	NG	NG					
219	Divya	20	F	OG /LW	Fever		N	NG	NG	NG					
220	Mailammal	45	F	Surgery ward	Fever		P	GWC	NLF	Brownish tinged +Pink+Blue colony	Proteus vulgaris	AK,CIP,CFS,AMC,MRP	COT,NX,G,CTX,A	P	P
221	Praneeth	6	Mch	BMW	Nephrotic syndrome		P	NG	NG	Tiny blue colony	Enterococci	NIT,AK,VAN	NX,CIP,AMC,A,COT		
222	Dheep	6	Mch	BSW	Fever		N	NG	NG	NG					
223	Ajay	6	Mch	BSW	Phimosis		N	NG	NG	NG					
224	Jayalakshmi	15	F	FM1/M1	Fever		N	NG	NG	NG					
225	Vasantha mani	50	F	Surgery ward	Fever		P	GWC	LF	Pink + Blue Colony	E.coli	NIT,NX,CIP,AK,G ,CFS,AMC,MRP	COT,CTX,A	P	P
226	Palani	55	M	Nephro ward	Fever		N	NG	NG	NG					
227	Parimala	28	F	OG ward	UTI		N	NG	NG	NG					
228	Karuppal	65	F	Nephro ward	Fever	CKD	N	NG	NG	Tiny blue colony	Enterococci	AK,NIT,VAN	NX,CIP,AMC,A,COT		
229	Balendra	12	Mch	BMW	Fever		N	NG	NG	NG					
230	Anandhapaul	35	M	Urology ward	Fever		N	NG	NG	NG					
231	Anuradha	42	F	Urology ward	Fever		N	NG	NG	NG					
232	Rajan	9mon	Mch	BSW	Fever		N	NG	NG	NG					
233	Deena	4	Fch	BSW	Fever		P	GWC	NLF	Pink colony	E.coli	AK,G,CIP,NIT,AMC,CFS,M RP	NX,COT,CTX,A	P	P
234	Senthil kumar	30	M	Medical ward	Cystocele		N	NG	NG	NG					
235	Palanisamy	72	M	Urology ward	Fever			3types	Pink+Blu e+woc colony	MG					
236	Subramani	55	M	Urology ward	Fever		N	NG	NG	NG					
237	Muthulakshmi	50	F	Fever OP	UTI		N	NG	NG	NG					
238	Gunaranjan	63	M	Urology ward	Fever		P	GWC	LF	Pink colony	E.coli	AK,G,NIT,CFS,AMC,CTX, MRP	NX,COT,CIP,A	N	N
239	Saleema	42	F	Medical ward	Fever		N	NG	NG	NG					
240	Dhanalakshmi	65	F	Medical ward	Fever		N	NG	NG	NG					

241	Venkatachalam	52	M	Urology ward	stricture urethra	Bronchie	P	GWC	LF	Pink Colony	E.coli	AK,G,NIT,CFS,AMC,MRP	NX,CIP,COT,CTX,A	P	P
242	Varadharaj	70	M	Urology OP	UTI		P	GWC	NLF	Non transparent creamy white Colony	Acinetobacter	AK,NIT,CFS,MRP	NX,COT,CIP,CTX,G		
243	Kavin	7	Mch	BMW	UTI		P	GWC	MLF	Blue Colony	Klebsiella	AK,G,NX,CIP,NIT,CFS,AMC,MRP	COT,CTX,A	P	P
244	Ranganayagi	25	F	OG/LW	Fever		P	NG	NG	Tiny blue colony	Enterococci	AK,NIT,VAN	NX,CIP,AMC,A,COT		
245	Kumar	12	Mch	PICU	Fever		N	NG	NG	NG					
246	Jayaraj	62	M	Medical ward	Fever		N	NG	NG	NG					
247	Periasamy	40	M	Urology OP	UTI		P	GWC 2 Types	LF	Pink+tiny Blue Colon	E.coli	AK,G,NIT,COT,AMC,CFS,CTX,A,MRP	CIP,NX	N	N
248	Kandasamy	75	M	Urology OP	UTI		P	GWC	NLF	Pink Colony	E.coli	AK,G,NIT,CTX,CFS,A,MRP	COT,CIP,NX,AMC	N	N
249	Hussain	79	M	Urology OP	Fever		P	GWC	LF	Pink Colony	E.coli	AK,G,NIT,NX,AMC,COT,CFS,MRP	CTX,CIP,A	P	P
250	Goutham	7	Mch	Pead OP	UTI		P	GWC	LF	Pink Colony	E.coli	AK,G,NX,CIP,NIT,CFS,CTX,A,MRP	COT,AMC	N	N
251	Amudha	27	F	Gynaec ward	Fever		N	NG	NG	NG					
252	B/O Meena	3Mon	Mch	BSW	Fever		P	GWC	MLF+LF	Blue Colony + Pink colony	MG/Enterobacter	CIP,NIT,CFS,NX,AMC,MRP	AK,G,COT,CTX,A		
253	GaneshKumar	6	Mch	BMW	Fever		N	NG	NG	NG					
254	Glory	33	F	OG/LW	Fever		N	NG	NG	NG					
255	Sivashankari	23	F	OG/LW	Fever		N	NG	NG	NG					
256	Sahul hameed	45	M	Urology ward	UTI		N	NG	NG	NG					
257	Sarojini	63	F	Medical ward	Fever		N	NG	NG	NG					
258	Malayammal	30	F	Medical ward	Fever		P	GWC	MLF	Blue Colony	Klebsiella	AK,G,NX,CIP,NIT,CFS,AMC,MRP	CTX,COT,A	P	P
259	Malarkodi	45	F	Urology ward	UTI		P	GWC+WOC	LF	Pink+Tiny blue colony	MG/Enterococci	NIT,AMC,VAN	AK,G,CIP,NX		
260	Rathinam	75	M	Fever OP	Fever		N	NG	NG	NG					
261	Gandhimathi	36	F	OG ward	Fever		N	NG	NG	NG					
262	Sathya	10	Fch	PICU	UTI		N	NG	NG	Candida	Candida				
263	Maragadhamani	35	F	OG ward	Fever		P	NG	NG	Tiny blue colony	Enterococci	AK,NIT,VAN,A	CIP,NX,AMC,COT		
264	Nagaraj	51	M	Medical ward	Fever		N	NG	NG	NG					
265	Kavin	4Mon	Mch	BMW	Fever		P	GWC	MLF	Blue Colony	Klebsiella	AK,G,NX,CIP,NIT,CFS,MRP	CTX,COT,AMC,A	P	P
266	Asbia banu	4	Fch	Pead OP	Fever		P	GWC	MLF	Blue Colony	Klebsiella	AK,G,CFS,NIT.CIP,MRP	COT,CTX,AMC,A,NX	P	P
267	Jeeva	6	Mch	PICU	Fever		N	NG	NG	NG					
268	Mery matha	12	Fch	PICU	UTI		N	NG	NG	NG					
269	Jeenath	27	F	OG ward	UTI		P	NG	NG	Pink Colony	E.coli	AK,G,NIT,NX,AMC,CIP,CFS,MRP	COT,CTX,A	N	P

270	Marappan	45	M	Fever OP	UTI		P	GWC	LF	Pink Colony	E.coli	AK,G,NIT,CFS,AMC,MRP	NX,CIP,COT,CTX,A	P	P
271	Parvathi	37	F	OG ward	UTI		P	GWC	LF	Pink Colony	E.coli	AK,G,NIT,CIP,AMC,CFS,CTX,A	COT,CTX	N	N
272	Manikam	60	M	Urology ward	Fever		P	GWC	FLF	Pink Colony	E.coli	AK,G,NIT,CFS,AMC,MRP	NX,CIP,COT,CTX,A	P	P
273	Pandiaraj	23	M	Urology ward	UTI		N	NG	NG	NG					
274	Sourav	5	Mch	PICU	Fever		N	NG	NG	NG					
275	Ramaselvan	56	M	Medical ward	Urosepsis	DM	P	3 Types	MLF	Blue Colony	Klebsiella	AK,G,NIT,CIP,CFS,COT,MRP	CTX,NX,AMC,A	P	P
276	Chandrakala	23	F	OG ward	UTI		N	NG	NG	NG					
277	Mohamed	3	Mch	PICU	Fever		N	NG	NG	NG					
278	Shameema	20	F	OG/LW	Fever		N	NG	NG	NG					
279	Buvaneswari	26	F	OG/LW	UTI		N	NG	NG	NG					
280	Sekar	46	M	Nephro ward	UTI		N	NG	NG	NG					
281	Akash	6	M	Pead OP	UTI		N	NG	NG	NG					
282	Chinnasamy	64	M	Urology OP	Fever		P	GWC	LF	Blue Colony	Klebsiella	AK,G,CIP,CTX,CFS,MRP	COT,NX,NIT,AMC,A	N	N
283	Pusphaveni	38	F	Fever OP	Fever		N	NG	NG	NG					
284	Suganya	23	F	OG/LW	UTI		N	NG	NG	NG					
285	Indrani	57	F	OGward	Fever		P	GWC	FLF	Pink+tiny Blue Colon	E.coli	NIT,CFS,MRP	AK,G,CIP,COT,NX,CTX,AMC,A	P	P
286	Senthilkumar	46	M	Urology ward	Fever		N	NG	NG	NG					
287	Sharmila	23	F	OG ward	Fever		N	NG	NG	NG					
288	Sasi	50	F	Gynaec ward	Fever		N	NG	NG	NG					
289	Manimekalai	50	F	Gynaec ward	UTI		P	GWC	NLF	Brownish tinged colo	Proteus mirabilis	AK,G,CFS,AMC,MRP	COT,CTX,NX,CIP,A	N	Inconclusive
290	Divya	20	F	Medical ward	Fever		N	NG	NG	NG					
291	Balasubramani	67	M	IMCU	Urosepsis	DM	N	WOC	NG	Small colourless colo	Candida				
292	Balu	50	M	Nephro ward	UTI		P	WOC	NG	Streptococci	Streptococci	P,E,DO,COT,CTX,CIP,NIT			
293	Mohamed rafi	3	Mch	Nephro ward	UTI		P	GWC	MLF	Blue Colony	Klebsiella	AK,G,NIT,COT,CFS,CTX,AMC,A,MRP	CIP,NX	N	N
294	Devendran	28	M	Urology ward	Fever		N	NG	NG	NG					
295	Elavarasan	21	M	Urology ward	Fever		N	NG	NG	NG					
296	Baby Shalini	6	Fch	BMW	Fever		P	GWC	FLF	Pink Colony	E.coli	AK,G,NIT,CFS,MRP	CIP,NX,CTX,COT,AMC,A	P	P
297	Syed rafia	20	F	Surgery ward	Fever	Bronchia	N	NG	NG	NG					
298	Yadeesh	7	Mch	BMW	Fever	DKA	N	NG	NG	NG					
299	Vikash	5	M	BMW	Fever		N	NG	NG	NG					
300	Sujatha	32	F	Medical ward	UTI		P	GWC	FLF	Pink Colony	E.coli	AK,G,NIT,CFS,CTX,NX,CIP,A,MRP	COT,AMC	N	N
301	Binnathesna	20	F	Medical op			N	NG	NG	NG					

302	Baby	45	F	Urology ward	Renal calculi	P	GWC	FLF	Pink colony	E.coli	AK,G,NIT,CFS,COT ,AMC,MRP	CIP,NX,CTX,A	P	P
303	Muthusamy	45	M	Urology op		N	NG	NG	NG					
304	Kamatchi	67	F	Surgery ward	Cystitis	P	GWC	NLF	Pink colony	E.coli	AK,G,NIT,CFS,CTX,CIP,A, MRP	NX,COT,AMC	N	N
305	Krubakar	9Mon	Mch	PICU	Meningitis	N	NG	NG	NG					
306	Anusuya	8	Fch	BMW	Fever	N	NG	NG	NG					
307	Nithiya	23	F	OG/LW	UTI	P	GWC	LF	Blue Colony	Citrobacter	AK,G,CIP,NX,NIT,CTX,CF S,MRP	COT,AMC,A		
308	Kandasamy	48	M	Medical ward	Fever	N	NG	NG	NG					
309	Soumiya	12	Fch	PICU	Fever	N	NG	NG	NG					
310	Janaki	30	F	Surgery ward	Renal calculi	P	GWC	NLF	Brownish tinged colo	Proteus mirabilis	AK,G,CIP,COT,NX,CFS,A MC,MRP	CTX,A	P	P
311	Bannari	30	F	OG/LW	Fever	N	NG	NG	NG					
312	Sulochana	63	F	Surgery ward	Pyelonephritis	P	GWC	FLF	Pink colony	E.coli	AK,NIT,CFS,MRP	G,NX,CIP,AMC,CTX,COT,A	P	P
313	Nalini	65	F	OG/LW	Cystitis	N	NG	NG	NG					
314	Priyadharsini	11	Fch	PICU	Fever	N	NG	NG	NG					
315	Nirmala	22	F	OG/LW	Fever	N	NG	NG	NG					
316	Ananthi	46	F	OG ward	Fever	N	NG	NG	NG					
317	Muthuraj	69	M	Surgery ward	Pyelonephritis	P	Dry GWC	FLF	Pink Colony	E.coli	AK,NIT,CFS,MRP	G,CTX,NX,CIP,AMC,COT,A	N	P
318	Poonkodi	26	F	Medical ward	Fever	N	NG	NG	NG					
319	Nadhia	25	F	OG/LW	UTI	N	NG	NG	NG					
320	Salathmary	29	F	OG/LW	Chorioamnionitis	N	NG	NG	NG					
321	Mithra	1	Fch	PICU	Fever	N	WOC+GWC	FLF	Pink colony+WOC	CONS	AK,G,NIT,AMC,CIP	NX,COT,A		
322	Mohanbabu	62	M	Medical ward	UTI	N	NG	NG	Small colourless colo	Candida				
323	Rukmani	32	F	Nephro ward	CKD	N	GWC	FLF	Pink colony+tiny blue	E.coli	AK,NIT,COT,MRP	G,NX,CIP,AMC,CTX,CFS,A	N	In conc lusiv e
324	Manojkumar	10	Mch	PICU	Fever	N	NG	NG	Tiny blue colony	Enterococci	NIT,VAN	AK,CIP,COT,NX,AMC,A		
325	Sundaram	58	M	Nephro ward	Cystitis	N	NG	NG	NG					
326	Kamalanathan	55	M	Medical ward	UTI	P	Dry GWC	FLF	Pink colony	E.coli	AK,NIT,CFS,COT,MRP	G,CIP,NX,CTX,AMC,A	P	P
327	Sagayamery	32	F	Nephro ward	CKD	P	GWC	LF	Pink colony	E.coli	AK,NIT,CFS,MRP	G,CTX,COT,AMC,CIP,NX,A	N	In conc lusiv e
328	Poovathal	75	F	Surgery ward	Fever	N	NG	NG	NG					
329	Ashiya	48	F	OG ward	UTI	N	NG	NG	NG					
330	Pushpam	57	F	OG ward	UTI	N	NG	NG	NG					
331	Rajendran	60	M	Urology ward	Cystitis	N	NG	NG	NG					
332	Revathi	22	F	OG op	Fever	N	NG	NG	NG					
333	Gurshith	65	F	Medical ward	Fever	N	NG	NG	NG					
334	Jothi	28	F	Surgery ward	UTI	N	NG	NG	NG					

335	Sakthivel	8	Mch	Paed OP	Phimosis		N	NG	NG	NG						
336	Ponnammal	45	F	Medical ward	Fever		N	NG	NG	NG						
337	BhuvaneswarI	19	F	Fever OP	UTI		N	NG	NG	NG						
338	Lakshmi	38	F	Urology ward	UTI		P	GWC	FLF	Blue colony	Klebsiella	AK,G,CIP,COT,NX,NIT,CFS,AMC,CTX,MRP	A	N	N
339	Abiga	1	Fch	BSW		Neuroger	P	GWC	FLF	Pink colony	E.coli	AK,G,NIT,AMC,CFS,CTX	NX,CIP,COT,A	N	N
340	Yadeesh	7	Mch	PICU	Fever		P	NG	NG	Small colourless colo	Candida				
341	Kasiammal	34	F	Medical ward	Fever		P	GWC	FLF	Pink colony	E.coli	AK,G,COT,NIT,CFS,AMC,MRP	CIP,CTX,NX,A	P	P
342	Mena mery	20	F	Surgical ward	Fever		N	NG	NG	NG					
343	David	4	Mch	Paed op	UTI		N	NG	NG	NG					
344	Keerthana	12	Fch	Paed op	UTI		N	NG	NG	NG					
345	Sehenaas	4	Fch	Paed op	UTI		N	NG	NG	NG					
346	Rudhanya	2	Fch	PICU	Nephrotic syndrome		P	GWC	NLF	Pink colony	E.coli	AK,G,NIT,AMC,CTX,CFS,A,MRP	NX,COT,CIP	N	N
347	Nithyasree	10	Fch	PICU	Pyelonephritis		N	NG	NG	NG					
348	Rahmad nizha	45	F	Surgery ward			N	woc	NG	Small colourless colo	Candida				
349	Ranjitha	13	Fch	Paed O.P	Meningitis		P	GWC	MLF	Pink+tiny Blue Colon	E.coli	AK,G,NIT,CFS,MRP	NX,CIP,CTX,COT,AMC,A	P	P
350	Kalamani	37	F	Medical ward	UTI		P	GWC	FLF	Pink colony	E.coli	AK,G,NIT,AMC,CFS,MRP	NX,CIP,CTX,COT,A	P	P
351	Gurumurugan	2	Mch	Paed op	Fever		N	NG	NG	NG					
352	Selvi	35	F	Nephro ward	Fever		N	NG	NG	NG					
353	Shanthi	30	F	Medical ward	UTI		P	GWC	FLF	Pink+tiny Blue Colon	E.coli	AK,G,NIT,CFS,CTX,AMC,MRP	NX,CIP,COT,A	N	N
354	Swer Mohamed	67	M	BMW	UTI		N	NG	NG	NG					
355	Shanmuga Sakth	9	Mch	BMW	UTI	Nephroti	N	NG	NG	NG					
356	Shanmugam	45	M	Urologyward	Fever		N	NG	NG	NG					
357	Seema	22	F	OG ward	Fever		P	NG	NG	NG					
358	Harini	9	Fch	BMW	Fever		N	NG	NG	NG					
359	Aswin	17	M	BMW	Fever		N	NG	NG	NG					
360	Sivagami	18	F	OG ward	Fever		N	NG	NG	NG					
361	Mohandas	65	M	Nephro ward	Fever		P	GWC	FLF	Pink colony	E.coli	AK,G,CFS,NIT,CTX,AMC,MRP	NX,COT,CIP,A	N	N
362	Gangadaran	64	M	Medical ward	Fever		N	NG	NG	NG					
363	Syed basha	30	M	Medical ward	Fever		N	NG	NG	NG					
364	Manikkam	60	M	Medical ward	Fever	D.M	N	WOC	NG	Small colourless colo	Candida				
365	Mohamed Aness	9	Mch	Paed op	Fever		N	NG	NG	NG					
366	K.U.Muthu	60	M	Surgery ward	UTI		P	GWC	FLF	Pink colony	E.coli	AK,G,COT,CIP,NIT,NX,CTX,CFS,MRP	AMC,A	N	N
367	Devi	42	F	Medical ward	Fever		N	NG	NG	NG					

368	Palanisamy	75	M	Urology ward	Renal calculi	P	GWC	NLF	Pink colony	E.coli	AK,G,CIP,CFS,NIT,MRP	COT,NX,CTX,AMC,A	P	P
369	Shidenashree	3	Fch	Paed o.p	Fever	N	NG	NG	NG					
370	Ammalu	58	F	OGward	UTI	N	NG	NG	NG					
371	Jenneth	50	F	OG ward	UTI	N	NG	NG	NG					
372	Chandran	9	MCh	Paed op	Urinary incontinence	N	NG	NG	NG					
373	Vasanth	9	Mch	BSW	UTI	N	NG	NG	NG					
374	Gayathri	30	F	Medical ward	Fever	N	NG	NG	NG					
375	Amudha	48	F	OG ward	UTI	N	NG	NG	NG					
376	Asmika	9	Fch	PICU	Fever	N	NG	NG	NG					
377	Asif	7	Mch	BSW	Fever	N	NG	NG	NG					
378	Venkat raj	60	M	Surgery op	UTI	N	NG	NG	NG					
379	Sundarambal	80	F	Medical ward	UTI	N	WOC	NG	Small colourless colo	Candida				
380	Uma maheswari	50	F	Nephro ward	Urethral stricture	P	GWC	NLF	Green colour colony	Pseudomonas	AK,G,CIP,CFS,MRP	NIT,COT,CTX,NX		
381	Salman Ranuz	10	Mch	PICU	Fever	N	WOC	NG	WOC colony	CONS	AK,NIT,AMC	NX,G,COT,CIP,A		
382	Anandhi	37	F	Medical ward	Fever	N	NG	NG	NG					
383	Vellingiri ammal	28	F	OG/LW	Fever	N	NG	NG	Tiny blue colony	Enterococci	AK,CIP,NIT,VAN,AMC	COT,A,NX		
384	Shree Harini	4	Fch	PICU	Fever	P	GWC	FLF	Pink colony	E.coli	AK,CFS,NIT,AMC,MRP	G,CIP,COT,CTX,NX,A	P	P
385	Deepa	8	Fch	BSW	Fever	P	GWC	FLF	Pink colony	E.coli	AK,NIT,COT,CFS,AMC,CTX,MRP	CIP,NX,G,A	N	N
386	Shakeela	26	F	Medical ward	Fever	P	GWC	FLF	Blue colony	Klebsiella	AK,G,CIP,NX,NIT,COT,AMC,CFS,CTX,MRP	A	N	N
387	Rajeshwarai	28	F	OG ward	Fever	N	NG	NG	NG					
388	Thulasirvani	22	F	OG/LW	Fever	N	NG	NG	NG					
389	Selvi	45	F	OG/LW	Fever	P	GWC	LF	Pink colony	E.coli	AK,G,NIT,CFS,AMC,MRP	NX,CIP,COT,CTX,A	P	P
390	Rakshna	12	Fch	Paed op	Fever	N	NG	NG	NG					
391	Sanmathi	4	Fch	Paed op	Fever	N	NG	NG	NG					
392	Asiya	31	F	Surgical ward	Fever	N	NG	NG	NG					
393	Karthi	13	M	Fever OP	UTI	N	NG	NG	NG					
394	Silambarasan	12	Mch	Paed OP	UTI	P	GWC	FLF	Pink colony	E.coli	NIT,CIP,CFS,MRP	NX,COT,AK,G,CTX,AMC,A	P	P
395	Jogala	70	F	Urology ward	stricture urethra	N	NG	Tiny L.F	Tiny blue colony	Enterococci	AK,CIP,NIT,VAN,AMC	NX,COT,A		
396	Harini	3	Fch	BMW	Fever	N	NG	NG	NG					
397	Subash	3	Mch	PICU	Fever	P	GWC	FLF	Pink colony	E.coli	AK,NIT,CFS,AMC	NX,COT,G,CIP,CTX,A	P	P
398	Indrani	47	F	Medical ward	Fever	N	WOC	NG	Small colourless colo	Candida				
399	Paulraj	8	Mch	PICU	Fever	N	NG	NG	NG					
400	Vandana	3	Fch	PICU	Nephrotic syndrome	N	NG	NG	NG					
401	Darun	4	Mch	PICU	Fever	N	NG	NG	NG					

402	Tejaswani	2	Fch	PICU	Fever		N	NG	NG	NG						
403	Thilaga laxmi	21	F	OG ward	Fever		N	NG	NG	NG						
404	Gokila	24	F	OG ward	BOH		N	NG	NG	NG						
405	Fatric	36	M	Fever OP	UTI		N	NG	NG	NG						
406	Bakiyam	50	F	OG ward	Urinary incontinence	UV Prola	P	GWC	NLF	Brownish tinged colo	Proteus mirabilis	AK,G,CIP,NX,CFS,COT,CTX,AMC	A	N	N
407	Thulasimani	28	F	OG/LW	Fever		N	NG	NG	NG						
408	Krishnaveni	26	F	OG/LW	Fever		N	NG	NG	NG						
409	Ganeshwaran	55	M	Urology ward	stricture urethra		N	NG	NG	NG						
410	Subramani	67	M	Urology ward	stricture urethra		N	NG	NG	NG						
411	Ashiyabanu	4	Fch	Paed op	Recurrent UTI		P	GWC	FLF	Pink colony	E.coli	AK,G,CFS,NIT,AMC,MRP	NX,CIP,CTX,COT,A	P	P
412	.Sudha	2	Mch	Paed op	Fever		N	NG	NG	NG						
413	Majeeth	52	M	Surgery ward	Fever		N	NG	NG	NG						
414	Subramani	63	M	Surgery ward	Fever		N	NG	NG	NG						
415	Sadhasivam	61	M	Urology op	Fever		N	NG	NG	NG						
416	Chitra	38	F	Urology op	Fever		N	NG	NG	NG						
417	Sakthidaran	62	M	Urology ward	Fever		N	NG	NG	NG						
418	Jebaraj	42	M	Urology ward	Fever		N	NG	NG	NG						
419	Deva sundaram	85	M	Urology ward	Fever		P	GWC	FLF	Blue colony	Klebsiella	AK,G,CIP,NIT,CFS,MRP	NX,COT,CTX,AMC,A	P	P
420	Shankar	51	M	Urology ward	Fever		N	NG	NG	NG						
421	Nishanth	6mon	Mch	Paed ward	Fever		P	GWC	MLF	Blue colony	Klebsiella	CFS,AMC,MRP	AK,G,COT,CTX,NIT,NX,CIP,A	P	P
422	Abdul ahamed	63	M	Urology ward	Fever		N	NG	NG	NG						

KEY TO MASTER CHART

UTI	- Urinary tract infections
E.coli	- Escherichia coli
CONS	- Coagulase Negative Staphlococcus
MAC	- MacConkey agar
BAP	- Blood Agar Plate
FLF	- Flat Lactose Fermenting Colonies
NLF	- Non Lactose Fermenting Colonies
LF	- Lactose Fermenting Colonies
GWC	- Greyish White Colonies
WOC	- White Opaque Colonies
PICU	- Paediatric Intensive Care Unit
LW	- Labour Ward
BSW	- Baby Surgical Ward
GUS	- Genito Urinary Surgery
FM	- Female Medical Ward
MM	- Male Medical Ward
N	- Negative
P	- Positive
ESBL	- Extended Spectrum Beta Lactamases
AK	- Amikacin
G	- Gentamycin
NIT	- Nitrofurantoin
COT	- Cotrimoxazole
AMC	- Amoxy clavulanic acid

CIP	- Ciprofloxacin
NX	- Norfloxacin
CTX	- Cefotaxime
CFS	- Cefoperazone sulbactum
A	- Ampicillin
NG	- No growth
MG	- Mixed growth
DM	- Diabetes mellitus
CKD	- Chronic Kidney Disease
DKA	- Diabetic Keto Acidosis