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PREVALENCE OF ESBL IN *ESCHERICHIA COLI* AND ITS ANTIBIOTIC RESISTANCE PATTERN FROM VARIOUS CLINICAL SAMPLES IN A TERTIARY CARE HOSPITAL

Pooja Prakash Chavan[1], Shanu Braj Kishor Sharma[1] and Dr. G. S. Karande[2]*

[1]Department of Microbiology, Krishna Institute of Medical Sciences, "Deemed To Be University", Karad, Malkapur, Karad - 415539 (Dist. Satara), Maharashtra, India.

[2]Professor and Head, Dept. of Microbiology, Krishna Institute of Medical Sciences, "Deemed To Be University", Karad, Malkapur, Karad - 415539 (Dist. Satara), Maharashtra, India.

***Corresponding Author: Dr. G. S. Karande**
Professor and Head, Dept. of Microbiology, Krishna Institute of Medical Sciences, "Deemed To Be University", Karad, Malkapur, Karad - 415539 (Dist.Satara), Maharashtra, India.

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ABSTRACT

Background: *E.coli* is commonest cause of urinary tract infection. ESBLs are increasingly reported worldwide and possessing great epidemic potential. Plasmids coding for ESBLs carry additional β -lactamase genes as well as genes extending resistance to other antimicrobial classes. Therefore, phenotypic detection of ESBLs among *Escherichia coli* is important for epidemiological purposes as well as for limiting the spread of resistance mechanisms. **Objective:** To isolate and identify *Escherichia coli* from various clinical samples, assess the antimicrobial susceptibility pattern of the organisms isolated and to study the different methods of ESBL resistance among these isolates. **Method:** A total of 140 isolates of *Escherichia coli* from clinical samples such as pus, sputum, urine, blood, body fluids were collected during the study. This isolates were cultured and identified by standard procedure. Antibiotic susceptibility testing of *Escherichia coli* was done including third generation of cephalosporins and resistant were done by screening for ESBL producing *Escherichia coli* by Double disc synergy test (DDST) and Combined disc diffusion test (CDDT). **Result:** By screening test, out of 140 isolates of *Escherichia coli* 111(79.29%) were suspected to be an ESBL producer and 29 (21.71%) were suspected as non-ESBL producers. Those 111 isolates obtained by screening test were further preceded for phenotypic confirmatory test. The ESBL positive isolates obtained by combine disc diffusion test was 70(63.06%), and by double disc synergy test 64(57.66%). **Conclusion:** Most of *Escherichia coli* ESBL positive isolates were observed in urine specimen. Combined disc diffusion test is more effective than double disc diffusion test. So, CDDT being simple and cheaper method should be included in the microbiology laboratories as a routine test for early deduction of ESBL producing organisms in specimen from critically ill patients.

KEYWORDS: *E. coli*, ESBL, Antibiotic susceptibility testing, UTI, OPD, IPD.

1. INTRODUCTION

Members of Enterobacteriaceae family causing infectious diseases are frequently treated with beta-lactam antibiotics. The beta- lactam antibiotics consists of four atom ring called as beta-lactam ring in their molecular structure. Beta-lactam is a broad spectrum antimicrobial agent and it inhibits cell wall synthesis in bacteria. Beta-lactam group includes penicillin, cephalosporins, oxyiminomonobactam and carbapenems monobactams, imipenem. e.g. cefotaxime, ceftriaxone, ceftazidime. *Escherichia coli* inactivate these antibiotics by hydrolysis of beta-lactam ring. The ESBLs are able to break down cephalosporins, monobactam and penicillin's.[1]

Escherichia coli is Gram negative, rod-shaped, and facultative anaerobic bacterium and member of Enterobacteriaceae family.[2] Most *E.coli* strains are commensal of gastrointestinal tract of humans and animals. Some strains are evolved into pathogenic *E. coli* by virulence factors through, bacteriophages plasmids, transposons. These pathogenic *E. coli* can be categorized based on clinical symptoms, pathogenicity mechanisms, serogroups and virulence factors.[3]

Escherichia coli is the most common isolated bacteria from microbiology laboratories. Some isolates of *Escherichia coli* are pathogenic and it cause diarrhea and extra-intestinal disorders in humans. *E. coli* is classified into three subtypes from human health perspective. First, it is commensal isolates in the gut of healthy individuals. The second is the diarrheagenic *E. coli* (DEC) isolates that cause diarrhea and differs according to their strain virulence. Third one is extraintestinal pathogenic *E. coli* (ExPEC) are similar to commensal in the human gut, but they can survive in extra-intestinal environments causing serious human diseases.[4]

1.1. Morphology

Escherichia coli is gram-negative, rod-shaped, facultative anaerobe, non-sporing coliform bacterium. It is about 2.0 µm long and 0.25–1.0 µm in diameter, with a cell volume of 0.6–0.7µm. Most of strains are motile, though motility is often feeble on primary isolation, and most strains are fimbriate. A few strains are capsulated and many others form abundant loose slime when grown on sugar-containing medium at 15-20°C. The flagella which allow the bacteria to swim have a peritrichous arrangement. It also attaches and effaces to the microvilli of the intestines via an adhesion molecule known as intimin.[5]

1.2. Cultural characters

On Mac Conkey agar**: -** Smooth, glossy and translucent, rose-pink in colour andit ferments lactose sugar.
On blood agar: - The colonies are smooth white in colour moist; some strains are surrounded by zones of hemolysis.

Growth is either impaired or totally inhibited on deoxycholate citrate agar; any colonies that do grow are small, pink and opaque.

E. coli is uninhibited by bile salt in Mac Conkey medium, but is inhibited by the citrate in Leifson's DCA medium and by sodium selenite, sodium tetrathionate, brilliant green and other substances used in media selective for salmonellae andshigellae. It is also inhibited by 7% NaCl in salt media used for isolation of *staphylococci*.[6]

1.3. Biochemical reactions

Identification of *Escherichia coli* based on the pattern of reactions the tests are usually considered sufficient: e.g. pink, moist colonies on Mac Conkey, motility, fermentation of glucose and lactose, indole production and negative for citrate and urease test.[7]

1.4. Infections caused by *Escherichia coli*

Escherichia coli commonly causes opportunistic infections in other parts of the body where there is some abnormality of defenses. They commonest cause of urinary tract infections (cystitis, pyelitis and pyelonephritis) and are commonly present in appendix abscess, peritonitis, cholecystitis, septic wounds and bedsores. They infect the lower respiratory passages these or cause bacteremia and endotoxin shock, particularly in surgical or otherwise debilitated patients being treated with antibiotics to which they are resistant and they occasionally cause meningitis in neonates. The commensal strains, however, do not act as primary pathogens in the intestine and are not known to cause gastroenteritis. Due to contamination with traces of faces, *E. coli* bacilli are commonly present on the skin and so come to contaminate many diagnostic specimens, such as midstream urines and wound exudates, in cases in which they have no pathogenic role.[8]

1.5. Urinary tract infection

E.coli is commonest cause of urinary tract infection. The *E.coli* serotype commonly responsible for community acquired UTI are normally found in guts of human, O group 1, 2,4,6,7 etc. Infection may be caused by urinary obstruction due to prostatic enlargement, calculi or pregnancy; primarily it causes asymptomatic bacteriuria, which if undetected and untreated, may leads to symptomatic infections. Later in pregnancy, pyelonephritis and hypertension in pregnant women, leading to prematurity and perinatal death of the fetus. Infection of lower urinary tract may be due to 'ascending infection'caused by gut flora, pyelonephritis is due to hematogenous spread. Strains carrying K antigens are more commonly responsible for pyelonephritis. The P pilli-positive E.coli are generally uropathogenic.[9]

1.6. Diarrhea

Escherich first isolated the bacillus from the faces of infants with enteritis.in 1945, Bray establish the causative role of specific type of *E.coli* during hospital outbreak of childhood diarrhea in London.
Six different types of diarrheagenic *E.coli*

- Enteropathogenic *E.coli* (EPEC)
- Enterotoxigenic *E.coli* (ETEC)
- Enteroinvasive *E.coli* (EIEC)
- Enterohemorrhagic *E.coli* (EHEC)
- Enteroaggregative *E.coli* (EAEC)
- Diffusely adherent *E.coli* (DAEC)

1.7. Pyogenic infections

E.coli causes intra-abdominal infections, such as peritonitis and abscesses resulting from spillage of bowel contents.

1.8. Septicemia

Bloodstream invasion by *E.coli* may lead to fatal conditions like septic shock and 'systemic inflammatory response syndrome (SIRS).

1.9. ESBL (Extended Spectrum Beta-Lactamase)

1.9.1. Definition

Enzymes capable of hydrolyzing major β – lactam antibiotics including third generation cephalosporins are called as extended spectrum beta lactamases. ESBLs are increasingly reported worldwide and possessing great epidemic potential. Subsequently, a group of β-lactamases were discovered in Germany that hydrolyzed extended spectrum cephalosporins and were named Extended Spectrum Beta- Lactamases (ESBL). There is no general agreement regarding the definition of ESBLs. ESBLs may be defined as a group of enzymes that are capable of conferring resistance to penicillins, first, second and third generation cephalosporins and aztreonam and render them ineffective. ESBLs are transmissible β-lactamases which are inhibited by clavulanic acid, tazobactam or sulbactam, and which are encoded by genes that can be exchanged between bacteria. Enterobacteriaceae and nowadays various

groups of ESBLs are produced by these microorganisms, such as CTX-M and SHV enzyme types. Plasmids coding for ESBLs carry additional β -lactamase genes as well as genes extending resistance to other antimicrobial classes. This can limit the chemotherapeutic options for ESBL- producing pathogens and facilitate the interspecies and intraspecies dissemination of ESBLs. Therefore, phenotypic detection of ESBLs among *Escherichia coli* is important for epidemiological purposes as well as for limiting the spread of resistance mechanisms. For detection of ESBL-producing *E. coli*, both CLSI and EUCAST recommend the use of cefotaxime and ceftazidime as indicator cephalosporins, and *E. coli* ATCC 25922 as the wild type quality control strain. In case of ESBL producing *E. coli* strains, a comparative analysis of double-disc synergy test, automated methods (VITEK 2 ESBL Test) and combined disc diffusion methods has shown combined disc diffusion method as the best choice for ESBL detection. It is important to consider that the validity of combined disc diffusion method is subject to invalidity when the strain under consideration co-produces AmpC β-lactamases. Therefore, it is recommended that confirmation of ESBL production should be done by one or more additional tests.[10]

1.9.2. CLASSIFICATION OF ESBLS

The classification of β-lactamases was first introduced by Jack and Richmond in 1970, on the basis of phenotypic characters such as Iso-electric focusing, molecular size, substrate profile, susceptibility to inhibitors, and immunological identity. It divides the β-lactamases from gram-negative bacilli into five major classes.[11]

Class 1: Primarily cephalosporinases.
Class 2: Penicillinases.
Class 3: Enzymes are active against a broad spectrum of penicillins and cephalosporins, and sensitive to cloxacillin and resistant to inhibition by p-CMB.
Class 4: Enzymes have substrate profile similar to Class 3 but are resistant to inhibition by cloxacillin and sensitive to p-CMB.
Class 5: Enzymes are penicillinases and have a spectrum, which is broader than that of Class 2.

Two major groups of enzymes arose from this classification scheme:

1. Chromosomally mediated β-lactamases.
2. Plasmid mediated β-lactamases.

Plasmid-mediated enzymes in gram-negative rods are constitutively expressed while chromosomal enzymes are inducible. Most plasmid-mediated enzymes are susceptible to inhibitors while most of the chromosomal enzymes are not. The phenotypic classification faces the problem that point mutation can greatly alter substrate specificity and inhibitor susceptibility of the enzyme. Therefore, the β-lactamases are now classified by amino acid and nucleotide sequence. Such classification is stable and cannot be distorted by mutations. This scheme separates β- lactamases into four major classes, A, B, C, and D. Classes A, C, and D comprise evolutionarily distinct groups of serine enzymes and class B contains Zn2+ types. Majority of ESBLs contain a serine at the active site and belong to Ambler's Molecule. Class A enzymes are characterized by an active site serine, a molecular mass of 29,000 Dalton and hydrolysis of penicillin. ESBLs contain a number of mutations that allow them to hydrolyse ESBL antibiotics. While TEM and SHV type ESBLs retain their ability to hydrolyse penicillin, but they are not catalytically as efficient as the parent enzymes. ESBLs are not active against cephamycin and most strains expressing ESBLs are susceptible to Cefoxitin and Cefotetan. But it has been reported that ESBL producing strains can become resistant to cephamycin due to the loss of an outer membraneporin protein.[12]

1.9.3. CHARACTERISTICS OF ESBLs

1) They are mostly class- A cephalosporinases carried on plasmids
2) They are more common in *Klebsiella* species followed by *Escherichia coli*
3) Described first in Germany and France.
4) All enzymes active against Cephalothin.
5) Imipenem and Cefoxitin not hydrolyzed.
6) Comparative activity against Cefotaxime and Ceftazidine varies with enzymes.
7) Some enzymes active against Aztreonam.
8) Resistance may not be detected by standard susceptibility tests.
9) Inhibition of activity by β-lactamase inhibitors can be demonstrated.

1.9.4 ACTION OF β-LACTAMASES

β-lactamases are enzymes produced by bacteria, which can destroy β-lactam ring of antibiotics. The β-lactam agent becomes so changed in its chemical structure that it is no longer recognized by the enzymes responsible for making the peptidoglycan layer of the bacterial cell wall Enzymes of class A, C, and D has their active site at serine residue. These serine-based enzymes first associated non- covalently with the antibiotic to yield the non-covalent Michaelis complex. The β- lactam ring is then attacked by the free hydroxyl on the side chain of a serine residue at the active site of the enzyme, yielding a covalent acyl ester. The hydrolysis of the ester finally liberates active enzyme and the hydrolyzed inactive drug. This mechanism is followed by β-lactamases of molecular classes A, C, and D but class B enzymes utilize a Zn^{2+} ion to attack the β-lactam ring.[13]

1.9.5. Types of ESBLs

Most ESBLs are derivatives of TEM & SHV. Now > 90 TEM type and > 25 SHV types are there. A few point mutations at selected loci with in the gene give rise to the extended spectrum phenotype. TEM & SHV type ESBLs are most often found in *Escherichia coli* and *Klebsiella. pneumoniae* but also found in *Proteus* species, *Providentia* species and other genera of

Enterobacteriaceae.[14]

1. TEM type ESBL (Temoneira)

TEM-type β-lactamases are responsible for ampicillin resistance among Enterobacteriaceae. TEM-1 β-lactamases are plasmid and transposon- mediated. This location has facilitated their spread to other bacterial species worldwide. They can be found in different members of the family Enterobacteriaceae, and in *P. aeruginosa*, *Haemophilus influenzae* and *Neisseria gonorrheae*. The majority of TEM β-lactamases are ESBLs. Some of the TEM derivate have reduced affinity for β-lactamase inhibitors and are called inhibitor-resistant TEM. They also have negligible activity against extended-spectrum cephalosporins, and therefore they are not considered as ESBLs.[15]

2. SHV type ESBL (sulfhydryl variable)

The probable ancestor of the enzyme SHV (sulfhydryl reagent variable) is a chromosomal penicillinase of *Klebsiella pneumoniae*. The first ESBL SHV enzyme was isolated from *Klebsiella ozaenae* in 1983, and, since then, various SHV types responsible for resistance to third generation cephalosporins have been described. SHV β-lactamases can be subdivided into three subgroups based on their molecular characteristics or functional properties. Members of the subgroup 2b hydrolyze penicillins and early generation cephalosporins, and are inhibited by clavulanic acid and tazobactam; members of the subgroup 2br are broad-spectrum β-lactamases that are resistant to clavulanic acid; and members of the subgroup 2be hydrolyze one or more oxyimino β-lactams (ceftazidime, cefotaxime, and aztreonam). Many of those are associated with resistance to third generation cephalosporins as well as monobactam and carbapenems.[16]

3. CTX-M type ESBL (Cefotaximases)

CTX-M-type β-lactamases hydrolyze cefotaxime, and there are over 220 different CTX-M-type enzymes characterized so far.CTX-M-type enzymes are susceptible to β-lactamase inhibitors as well as to the new non β-lactam-derived β-lactamase inhibitor avibactam.[17]

4. OXA

- Belongs to Class d and functional group 2d.
- Characteristics feature is that they are able to hydrolyse ampicilin, cephalosporin, cloxacillin, oxacillin and are poorly inhibited by clavulanicacid.
- OXA-type ESBLs are found mainly *in pseudomonas aeruginosa*.

OTHER ESBLs

1. PER-1 β-lactamase 18f isolated from *Pseudomonas aeruginosa* in Turkey.
2. PER-2 found in *Salmonella enterica* from Argentina.
3. VEB-1 1st found in *E-coli* isolate from Vietnam.
4. CME-1
5. TLA-1 identified in *Escherichia coli* isolate from Mexico
6. SFO-1 cannot hydrolyse cephamycin and inhibited by clavulanic acid.
7. GES-1 resembles carbenicillinas

1.9.6. MEDICAL SIGNIFICANCE OF DETECTION OF ESBL

Patients having infections caused by ESBL – producing organisms are at increased risk of treatment failure with expanded spectrum β-lactam antibiotics. So it is recommended that if an organism was confirmed to produce ESBL it is considered as resistant to all 3rd Generation cephalosporins. Many ESBL isolates will not be phenotypically resistant; even through their MIC is so high. ESBL producing strains have been established in many hospitals producing epidemic diseases especially in Intensive Care Units.[19] Failure to control outbreaks has resulted in new mutant types in some institution.

1.9.7. Major risk factors for ESBL production

1. Prolonged stay in ICU
2. Long term use of antibiotics
3. Nursing home residency
4. Severe illness
5. High rate use of ceftazidime and other Third Generation Cephalosporins
6. Use of lifelines and catheters

Presence of ESBL producing *E.coli* in hospital environment is usually complex phenomenon and it involve in many different mechanisms. Specific risk factors include urinary or arterial catheterization, intubation, prolonged hospital stay, ICU, severity of illness and mechanical ventilation. ESBL producers commonly occur in surgical wards and most other areas of the hospital and frequently from patient in extended care facilities.[6]

Infections caused by ESBL producing *E.coli* are associated with an increase of morbidity, mortality, and health care costs. To ensure patient safety, optimal treatmentand control of the spread of ESBL are essential. Isolation of colonized patients is one of the most important control measures and should be instituted as soon as possible. This requires accurate and rapid laboratory diagnosis. The objective of the study was to detect ESBL producing *E.coli* among a collection of clinically relevant samples by confirmatory tests.[20]

2. MATERIALS AND METHODS

2.1. STUDY SETTING

Study design: Prospective, Observational study.
Study period: 2 years (November 2019 to November 2021)

2.2. INCLUSION CRITERIA

- All clinical samples from patients related.
- Male and female patients were included.

2.3. EXCLUSION CRITERIA

- Individuals other than infected with *E.coli* isolate were excluded.

2.4. SAMPLE SIZE

As per the reference Dr. Assudani H *et al*, showed that 50.53%prevalence. Thus the sample size is being calculated by the formula, $4pq/l^2$

Where, p = prevalence (50.53), q = 100 – Prevalence (100 – 50.53 = 49.47)l = allowable error (10)

Therefore, Sample size = 4x 50.53 x 49.47/ 10^2 = 99.98 ≅ 100 samples.

So, the sample size taken was 140 samples.

2.5. COLLECTION OF DATA

- The study was undertaken on patients admitted with clinical infection at KrishnaHospital, Karad.
- Details such as name, age, sex, address, IPD no., date of admission, clinical data like personal history, high risk factors, immunocompromised status and details ofclinical diagnosis were collected

2.6. SAMPLE COLLECTION

- Total 140 samples received in the department of Microbiology from Krishna Hospital, Karad, were included in the present study.
- Samples such as pus, sputum, urine, blood, body fluids, from both gender and all groups of patients were included in the study.

2.7. SAMPLE PROCESSING

2.7.1. Microscopy: Gram stain

The heat fixed smear was stained by Gram stain technique and examined under oil immersion objective of light microscope. The smear was examined for the presence of Gram negative bacilli. The size, shape, arrangement of bacteria was noted

2.7.2. Culture of specimens

The specimens were cultured on Mac Conkey agar, Blood agar and Chocolate agar as culture media and the plates were incubated at 37°C for minimum 24 hrs.

2.7.3. Interpretation of the Culture

The plates were read after 24 hours of incubation for any growth. The plates with no growth or tiny colonies were incubated for an additional 24 hours before discarding and the plates with growth of organism were preceded for identification.

2.7.4. Identification of isolates

The isolates were identified based on colony morphology in Mac Conkey agar, Bloodagar and Chocolate agar and gram stain of the smear was made from the colonies. Further biochemical reaction was carried out as described in Practical Microbiology of Mackie McCartney 14th edition.

2.7.5. Motility

Bacterial colony was inoculated into peptone water broth and incubated at 37°C for 2-3 hrs. With a wire loop place a drop of suspension on a cover slip laid on the bench. With the help of petroleum jelly, ring or square (according to the shape and size of the cover slip) is outlined round the concavity. Invert the slide over the cover slip, allowing the glass to adhere to the jelly, and quickly turn round the slide so that the cover slip is uppermost. The drop should than be 'hanging' from the cover slip in the centre of the concavity. Place the slide on the microscope, observe with a low power objective and then with a high power objective.

2.8. Biochemical test performed for identification of *E.coli*

2.8.1. Indole test

Test culture was inoculated in peptone water broth. It is then incubated for 24 hours at37^0c. Kovac's reagent 0.5 ml was added and shaken gently. 10 g paradimethyl-aminobenzaldehyde was dissolved in 150 ml of amyl alcohol and then 50 ml concentrated hydrochloric acid was added slowly to prepare kovac'sreagent.

Interpretation- Appearance of red color in the alcohol layer indicated that test was positive.

2.8.2. Methyl red test

The test organism was inoculated in glucose phosphate peptone water further it was incubated at 37^0c for 24 hours. Five drops of methyl red reagent was added and mixed. 0.1g methyl red was dissolved in 300 ml of ethanol and in 200 ml of distilled water to prepare methyl red solution.

Interpretation- Bright red color appearance was taken as positive. Yellow color appearance was taken as negative.

2.8.3. Voges- Proskauer test

Test organism was inoculated in glucose phosphate peptone water. Then it was incubated at 37^0c for 24 hours. 1 ml of 40% potassium hydroxide, 3 ml of 5% solutionof α-naphthol in was added in ethanol.

Interpretation- A positive reaction was indicated by the development of pink color in 2-5 minutes, becoming crimson in 30 minutes.

2.8.4. Citrate utilization test

Suspension of the test organism was inoculated in Simmons' citrate medium. Further it was incubated at 37^0c for 24 hours.

Interpretation- A positive reaction was indicated by blue color on medium indicated that reaction was positive. Appearance of original green color indicated that test was negative.

2.8.5. Urease test

Test organism was heavily inoculated over of the entire slope surface of Christensen'smedium. It was incubated at 37°C and observed after 24 hours.

Interpretation- Urease positive culture changes the colour of the indicator to purple pink.

2.8.6. Triple Sugar Iron agar test

Triple sugar iron agar medium contains 10 parts lactose, 10 parts sucrose, 1 part glucose. As indicators of acidification, Phenol red and ferrous sulphate were used as indicator. With straight wire loop, well-isolated colony was inoculated in TSI media. Test organism inoculated by stabbing to the bottom of the tube through medium. Then streaked over surface of the agar slant. It was incubated 35°C for 24 hours.

The results were interpreted as follows:

1. Alkaline slant/ No change in butt (K/ No change) - glucose, lactose and sucrose Non-utilizers.
2. Alkaline slant / Acid butt (K/A) non-utilizers- glucose fermentation only.
3. Acid slant /Acid butt (A/A) - glucose, sucrose and/or Lactose fermenter.

A black precipitate in the butt indicated production of ferrous sulphide and H_2 S gas. Bubbles or cracks in the tube indicated the production of CO_2 or H_2.

2.8.7. Sugar fermentation test

Sugar fermentation medium contains Peptone, Andrade's indicator, Sugar s (Lactose, Sucrose, Glucose, Maltose, Mannitol) and distilled water. Andrade's indicator was prepared by adding sufficient I M sodium hydroxide to 0.5% aqueous acid fuchsine to turn the colour of the solution yellow.

Peptone and Andrade's indicator were dissolved in distilled water and the sugar to be tested was added. This media was distributed in standard test tubes containing an inverted Durham tube. It was sterilized by steaming at 100°C for 30 minutes on three consecutive days.

Each tube was inoculated with 1 drop of 18-24 hours broth culture and incubated at 35°C for up to 7 days in ambient air. The tubes were examined for acid (indicated bya pink colour) and gas production. Negative test was indicated by growth, but no change in colour.

2.8.8. Nitrate reduction test

Nitrate broth was inoculated with test culture and incubated for 96 hours. Immediately before use, mix equal volumes of solution a (sulfanilic acid) and B (α-naphthylamine) to give test reagent. Add 0.1 ml of the test reagent to the test culture. A red colour developing within 3 minutes indicated the presence of nitrate and hence the ability of the organism to reduce nitrate.

2.9. Antimicrobial susceptibility

2.9.1. Disc diffusion method

The antimicrobial susceptibility was done by Kirby Bauer disc diffusion method as recommended by CLSI guidelines 2020.[5] Control strains used were *Escherichia coli* ATCC 25922.

2.9.2. Preparation of Inoculum

Tip of 3-5 representative colonies were picked up and put in 4-5ml of broth and incubated at 37oC for 2– 6 hrs to attain 0.5 Mc Farland's standard which corresponds to 150 million organisms/ml. If it was more turbid then some more broth was added and adjusted to 0.5 Mc Farland's standard by comparing against a card with white background and contrasting black lines.

2.9.3. Inoculation on MHA Plates

After standardization of bacterial suspension, immerse a sterile cotton swab in it and make a lawn on the surface Muller-Hinton agar plate. Place the appropriate antimicrobial impregnated discs on the surface of the agar using sterile forceps. Invertthe plates and incubate at 35^0C-37^0C for 16-18 hours. After incubation, the zone of inhibition was measured by using zone measuring scale and interpreted as per the CLSI standards 2020.

2.9.4. Application of discs to Inoculated agar plates

The predetermined battery of antimicrobial discs which included Co- trimoxazole, Amikacin, Ciprofloxacin, Cefotaxime, Ceftazidime, Ceftriaxone and Ampicillin, Amoxycillin-Clavulanic acid, Levofloxacin, Cefuroxime, Nitrofurantoin, Imipenem, Cefixime, Cefepime, Piperacillin-Tazobactam, Colistin were dispensed onagar plates and pressed down to ensure completes contact with the agar surface. Discs were distributed evenly so that they were not closer than 24mm from centre to centre. Not more than 6 discs for a 90 mm plate. Plates were inverted and incubated at 37oC for 16-18 hrs.

2.9.5. Reading plates and interpretation of results

After16-18 hrs of incubation each plate was examined. The diameter of the zones of complete inhibition was measured, including the diameter of the disc. Zones were measured to the nearest whole millimeter using a ruler which was held on the back of the inverted Petri plate.

Control strains

Positive control: *Escherichia coli* ATCC 25922 Negative control: *Klebsiella pneumonia* ATCC 700603.

2.9.6. Screening of ESBL production

Multi drug resistant *Escherichia coli* was subjected to various test for the detection of ESBL. *Escherichia coli* isolated was suspected to be an ESBL producer if it is resistant to the following drugs: Aztreonam (30ug)=27mm, Cephotaxime (30ug)=27mm, Cefodoxime (10ug)=21mm, Ceftazidime (30ug)=22mm, and Ceftriaxone (30ug)=25mm.

2.9.7 Phenotypic detection of esbl producing *Escherichia coli*

Combined disc diffusion test (cddt)

All the strains which were screened out for ESBL production were also subjected to confirmation by using the CDDT, as recommended by the CLSI.[4] The ceftazidime (30 µg) and Cefotaxime (30µg) discs without clavulanic acid and the disc withclavulanic acid were applied on Mueller Hinton Agar (MHA) plate The zone of inhibition of Ceftazidime +clavulanic acid and Cefotaxime +clavulanic acid was increased by ≥ 5mm

than the zone of ceftazidime and Cefotaxime without clavulanic acid disc was considered to be a marker for ESBL production.

Double-disc synergy test

Organism was swabbed onto a Muller – Hinton agar plate. An antibiotic disc containing one of the oxyimino β-lactam antibiotics e.g. ceftazidime and Cefotaxime was placed 30mm (centre to centre) from the amoxicillin – clavulanic acid disc. Enhancement of zone of inhibition of the ceftazidime and Cefotaxime Amoxy-clav disc was a positive result. This test is more reliable. Sensitivity is increased by reducing the distance between the discs to 20mm. Addition of clavulanate (4µg/ml) to the MHA increases the sensitivity.

Disc strength: - 3rd generation cephalosporins 30µg/ disc, Amoxyclav - 20µg amoxicillin + 10µg clavulanic acid, Ceftazidime (30 µg) and Cefotaxime(30µg) was the best sentinel antibiotic for suspecting resistance to 3rd generation cephaloporins.

3. OBSERVATION AND RESULTS

Over a period of 2 year, bacterial isolates obtained from patients admitted in various medical, surgical, and intensive care units were studied in the Department of Microbiology, Krishna Institute of Medical Sciences, Deemed to be University, Karad.

Table No.1: Biochemical test performed for identification of *E.coli.*

Biochemical tests	Result
Indole test	Positive
MR Test	Positive
VP Test	Negative
Citrate Test	Negative
Urease Test	Negative
TSI Test	Acid/Acid, gas present
Nitrate Reduction Test	Positive
Lactose	Acid + Gas
Sucrose	Variable
Glucose	Acid + Gas
Maltose	No fermentation
Mannitol	Acid + Gas

Table No.2: Antibiotics used for Escherichia coli.

Antibiotics	Disc content	Sensitive (mm)	Intermediate (mm)	Resistant (mm)
Ampicillin	10 µg	≥17	14-16	≤13
Amikacin	30 µg	≥17	15-16	≤14
Amoxy-clave	(20/10 µg)	≥18	14-17	≤13
Nitrofurantoin	300 µg	≥17	13-16	≤14
Cotrimoxazole	(1.25/23.75 µg)	≥16	11-15	≤10
Piperacillin-Tazobactam	(100/10 µg)	≥21	18-20	≤17
Imipenem	10 µg	≥23	20-22	≤19
Colistin	10 µg	≥18	11-17	≤10
Cefepime	30 µg	≥25	19-24	≤18
Levofloxacin	5 µg	≥21	17-20	≤16
Cefuroxime	30 µg	≥18	15-17	≤14
Ceftazidime	30 µg	≥21	16-20	≤17
Cefotaxime	30 µg	≥26	23-25	≤22
Ciprofloxacin	5 µg	≥26	22-25	≤21
Cefixime	5 µg	≥19	16-18	≤15

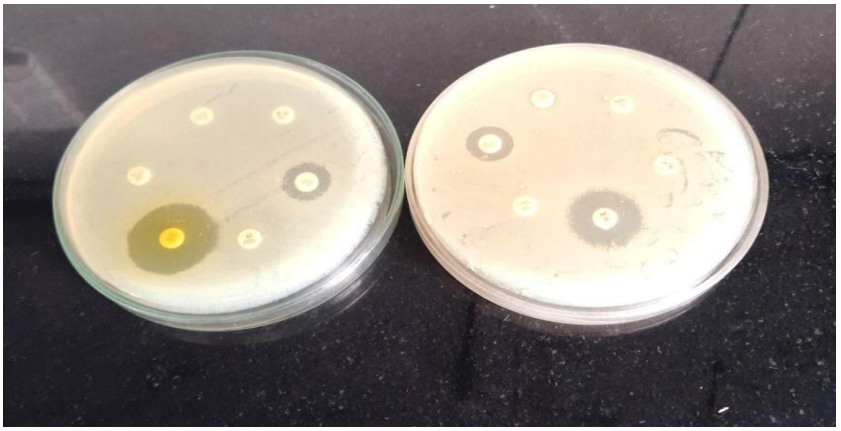
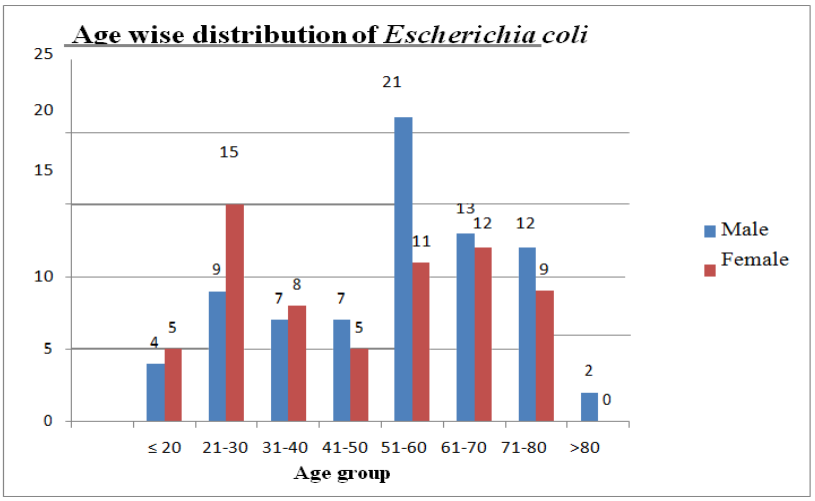


Figure 1: Antimicrobial susceptibility testing byKirby Bauer disc diffusion method.

Table No. 3: Age and Gender Wise Distribution of Escherichia Coli.

Age group(year)	Male(n)	Female(n)	Total(n)	Percentage(%)
≤ 20	4(5.33%)	5(7.69%)	9	6.43
21-30	9(12%)	15(23.07%)	24	17.14
31-40	7(9.33%)	8 (12.30%)	15	10.72
41-50	7(9.33%)	5 (7.69%)	12	8.57
51-60	21(28%)	11(16.92%)	32	22.86
61-70	13(17.33%)	12(18.46%)	25	17.86
71-80	12(16%)	9(13.85%)	21	15
>80	2(2.66%)	0(0%)	2	1.42
Total (n)	75 (53.57%)	65 (46.43%)	140	100



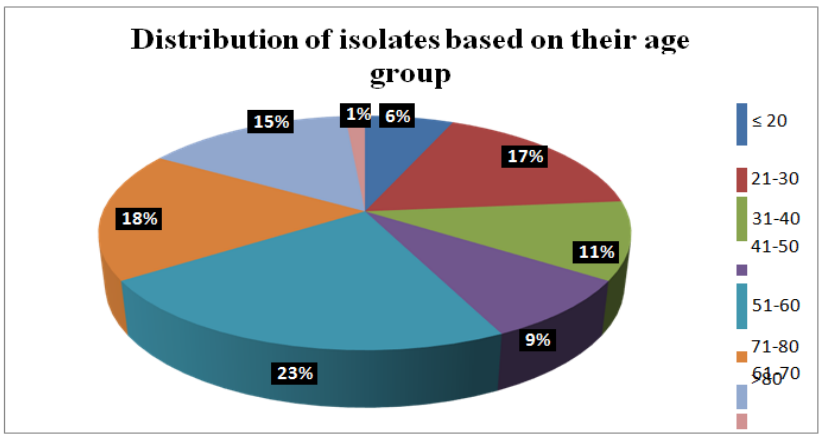
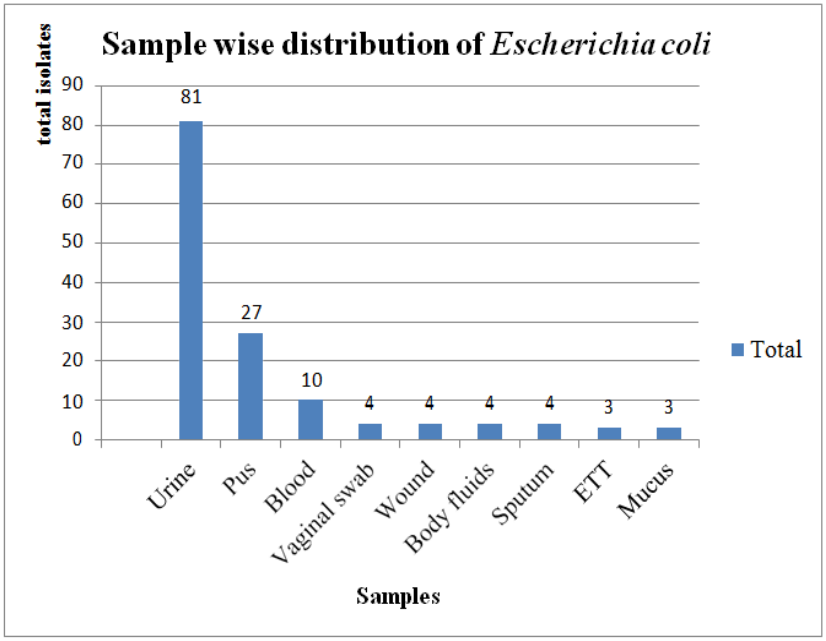


Table No. 3 shows age and gender wise distribution of *Escherichia coli*. Maximum isolates were from 51-60 age group 22.86% followed by 61-70 age group 17.86%, 21-30 age group 17.14%, 71-80 age group 15%, 31-40 age group 10.72%, 41-50 age group 8.57%, ≤20 age group 6.43%, ≥80 age group 1.42%. In female maximum isolates were from 21-30 age group 23.07% followed by 61-70 age group 18.46%, 51-60 age group 16.92%, 71-80 age group 13.85%, 31-40 age group 12.30%, 41-50 age group 7.69%, ≤20 age group 7.69%. In male maximum isolates were from 51-60 age group 28%, followed by 61-70 age group 17.33%, 71-80 age group 16%, 21-30 age group 12%, 31-40 age group 9.33%, 41-50 age group 9.33%, ≤20 age group 5.33%, ≥80 age group 2.66%.

Table No. 4: Sample Wise Distribution of *Escherichia coli.*

Specimens	Total	Percentage
Urine	81	57.86
Pus	27	19.28
Blood	10	7.14
Vaginal swab	4	2.86
Wound	4	2.86
Body fluids	4	2.85
Sputum	4	2.85
ETT	3	2.15
Mucus	3	2.15
Total (n)	140	100

Table No.4 shows sample wise distribution of *Escherichia coli*. Majority of the isolates were from urine 81 (57.86%) followed by pus 27 (19.28%), blood 10 (7.14%), vaginal swab 4 (2.86%), wound swab 4 (2.86%), body fluid 4 (2.85%), sputum 4 (2.85%), ETT 3 (2.15%), mucus 3 (2.15%).



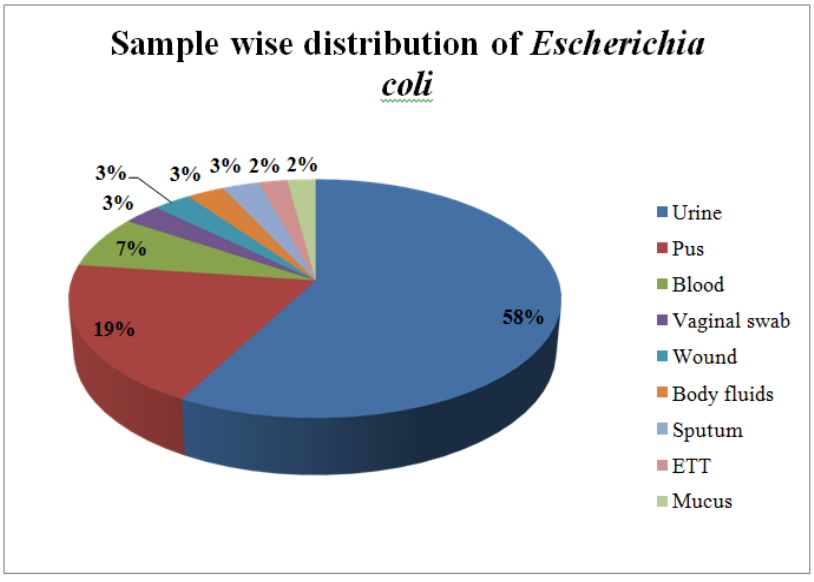


Table No.5: Distribution of Samples Obtained From Different Indoor Departments of Hospital.

IPD Wards	Samples (n)	Percentage (%)
Medicine	25	19.53
Surgery	15	11.72
OBG	2	1.56
Ortho	2	1.56
Pediatric (NICU)	2	1.56
ICU	80	62.5
CVTS	1	0.78
ENT	1	0.78
Total	128	100

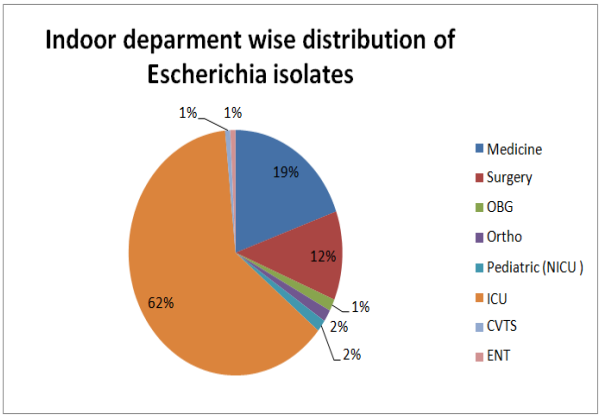


Table No. 5: Shows distribution of samples obtained from different indoor departments of hospital. Maximum isolates were from ICU 80 (62.5%) followed by Medicine 25 (19.53%), 15 (11.72%), OBG 2 (1.56%), Ortho 2 (1.56%), Pediatric (NICU) 2 (1.56%), CVTS 1 (0.78%), ENT 1 (0.78%).

Table No.6: Distribution of Samples Obtained FromDifferent Outdoor Departments of Hospital.

OPD Wards	Samples (n)	Percentage (%)
Medicine	0	0
Surgery	2	16.67
OBG	7	58.33
Ortho	1	8.33
Pediatric (NICU)	2	16.67
Total	12	100

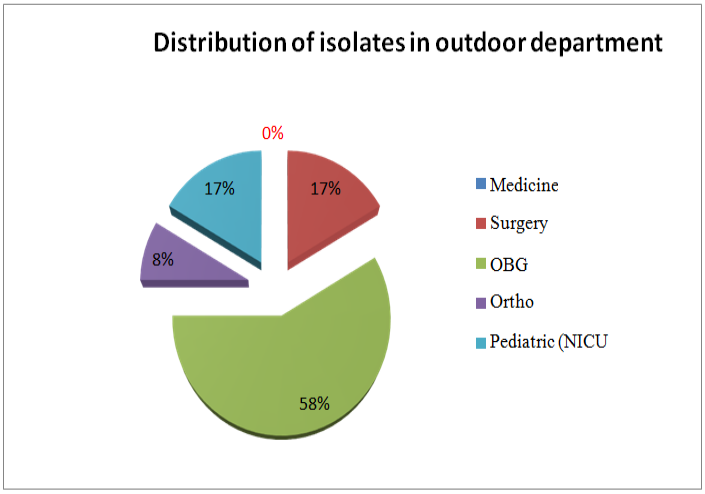


Table No. 6: Shows distribution of samples obtained from different department of OPD section of hospital. Maximum isolates were from OBG 7 (58.33%) followed by Surgery 2 (16.67%), Pediatric (NICU) 2 (16.67%), Ortho 1 (16.67%).

Table No.7: Distribution of *Escherichia Coli* From IcuWards.

ICU Ward	Samples (n)	Percentage (%)
Medicine	66	80.49
Surgery	9	10.97
OBG	3	3.66
Ortho (SICU)	2	2.44
NICU	2	2.44
Total	82	100

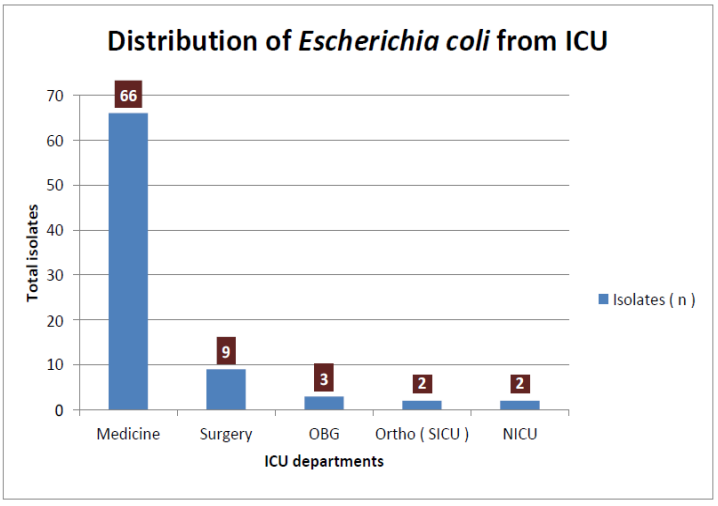


Table No. 7: Shows distribution of *Escherichia coli* from ICU. Maximum isolates were from medicine 66 (80.49%), followed by Surgery 9 (10.97%), OBGY 3 (3.66%), Ortho (SICU) 2 (2.44%), NICU 2 (2.44%).

Table No. 8: Sample Wise Distribution of *E.Coli* in OpdPatients.

Samples	Patients (n)	Percentage (%)
Urine	5	41.66
Pus	6	50
Wound	1	8.34
Total	12	100

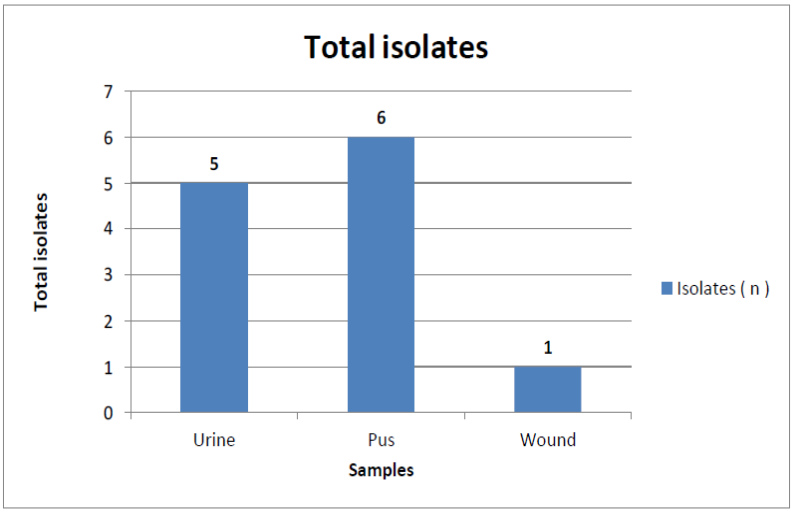


Table No. 8 shows sample wise distribution of *E.coli* in OPD patients. Majority of the isolates were from Pus 6 (50%), followed by Urine 5 (41.66%), wound 1 (8.34%).

Table No. 9: Sample Wise Distribution of *E. Coli* Isolatedfrom Different Ipd Departments.

IPD wards	Patients (n)	Percentage (%)
Blood	10	7.81
ETT	3	2.34
Mucus	3	2.34
Body fluid	4	3.13
Pus	21	16.40
Sputum	4	3.13
Urine	76	59.38
V. swab	4	3.13
Wound	3	2.34
Total	128	100

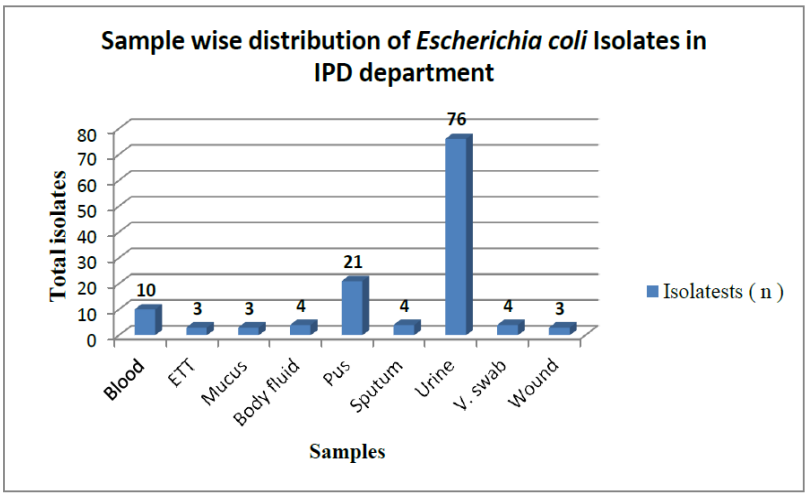
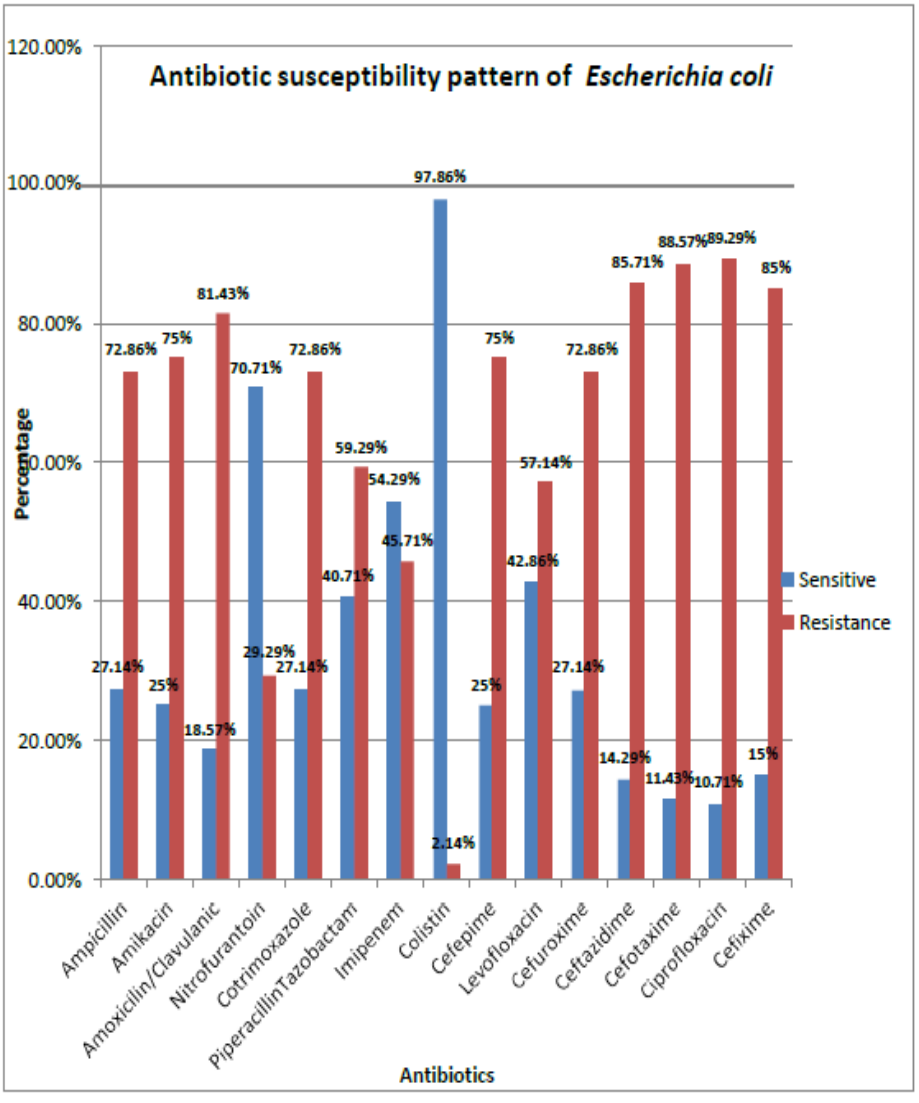


Table No. 9 shows sample wise distribution of *E.coli* isolates from different department of IPD patients. Maximum isolates were from Urine 76 (59.38%), followed by Pus 21 (16.40%), Blood 10 (7.81%), Sputum 4 (3.13%), Vaginal swab 4 (3.13%), Wound 3 (2.34%), ETT 3 (2.34%), Mucus 3 (2.34%).

Table No. 10: Antibiotic Susceptibility Pattern of Escherichia Coli.

Antibiotics	Sensitive		Resistance	
Ampicillin	38	27.14%	102	72.86%
Amikacin	35	25%	105	75%
Amoxicilin/Clavulanic	26	18.57%	114	81.43%
Nitrofurantoin	99	70.71%	41	29.29%
Cotrimoxazole	38	27.14%	102	72.86%
PiperacillinTazobactam	57	40.71%	83	59.29%
Imipenem	76	54.29%	64	45.71%
Colistin	137	97.86%	3	2.14%
Cefepime	35	25%	105	75%
Levofloxacin	60	42.86%	80	57.14
Cefuroxime	38	27.14%	102	72.86%
Ceftazidime	20	14.29%	120	85.71%
Cefotaxime	16	11.43%	124	88.57%
Ciprofloxacin	15	10.71%	125	89.29%
Cefixime	21	15%	119	85%



The bacterial isolates were tested against antimicrobial agents, and their resistance pattern was observed. *Escherichia coli* showed maximum sensitivity to Colistin (97.86%) followed by Nitrofurantoin (70.71%) Imipenem (54.29%) whereas maximum resistance was to Ciprofloxacin(89.29%) followed by Cefotaxime (88.57%), Ceftazidime (85.71%)

Table No.11: Distribution of Total Isolates of *E.Coli* By Screening Method.

Screening test	Total (n)	Percentage (%)
Positive screen test	111	79.29
Negative screen test	29	20.71
Total	140	100

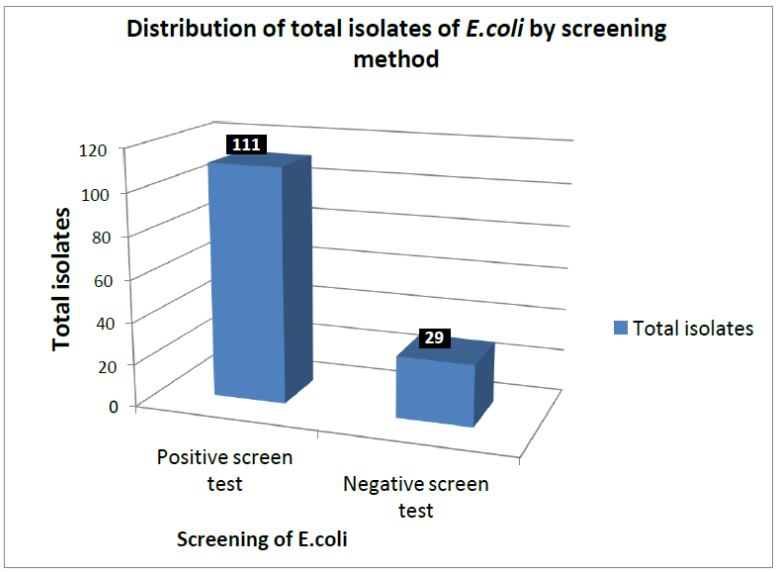
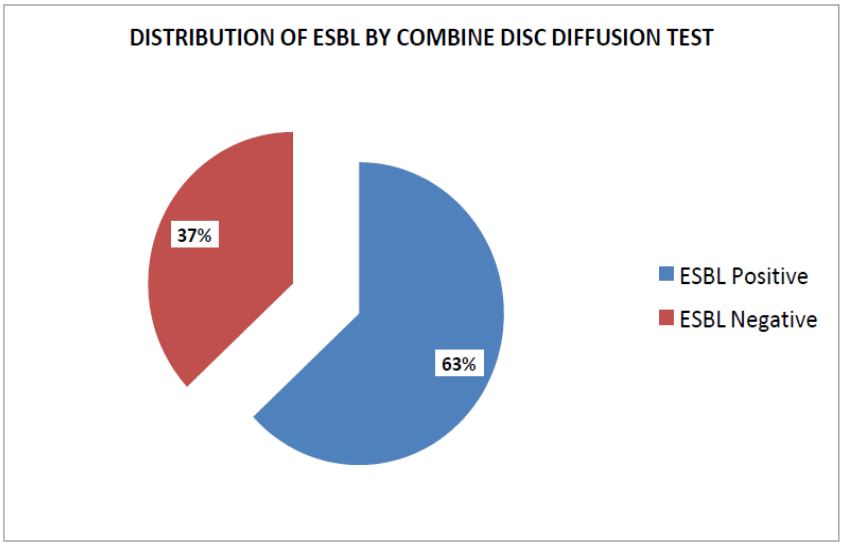


Table no. 11 shows distribution of *Escherichia coli* based on its screen test. Out of 140 isolates of *E.coli* 111(79.29%) were suspected to be an ESBL producer and 29 (21.71%) were suspected as non-ESBL producers.

Table no. 12: Distribution of Esbl By Combined Disc Diffusion Test.

Test	Numbers (n)	Percentage (%)
ESBL Positive	70	63.06
ESBL Negative	41	36.94
Total	111	100



By this method of detection of ESBL producing *Escherichia coli*, out of 111 isolates, 70(63.06%) were ESBL producers and 41 (36.94%) were non ESBL.

Table No.13: Gender Wise Distribution of Esbl By CombinedDisc Diffusion Test.

Gender	ESBL Positive	ESBL Negative
Male	42 (60%)	18 (43.90%)
Female	28 (40%)	23 (56.10%)
Total	70 (100%)	41 (100%)

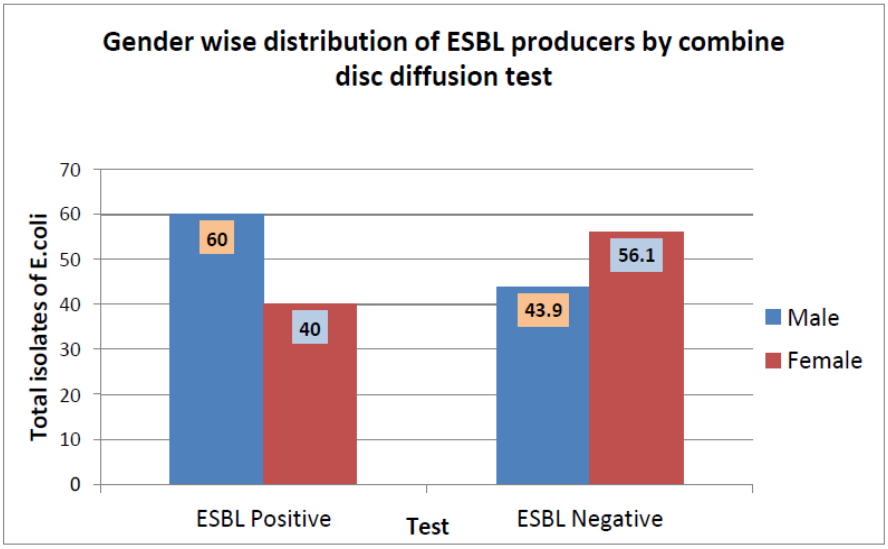
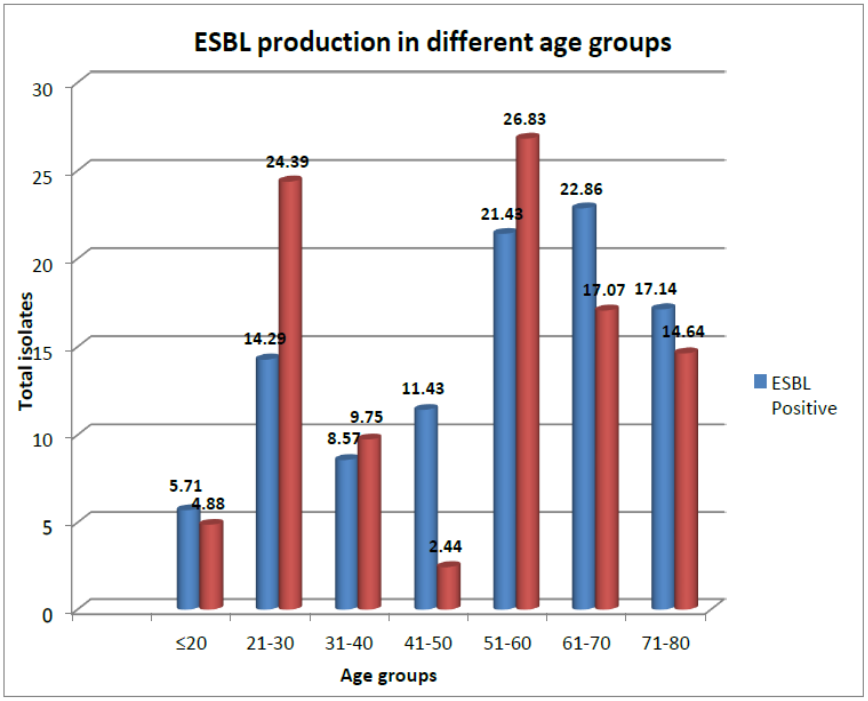


Table no. 13 shows gender wise distribution of *Escherichia coli*. Highest number of ESBL production is observed in male i.e. 60% followed by in female i.e. 40%. Out of 41 non-ESBL producer 23 (56.10%) in female and in male 18(43.90%).

Table No.14: Age Wise Distribution of Esbl By Combine DiscDiffusion Test.

Age group	ESBL Positive		ESBL Negative	
	Number (n)	Percentage (%)	Number (n)	Percentage (%)
≤20	4	5.71	2	4.88
21-30	10	14.29	10	24.39
31-40	6	8.57	4	9.75
41-50	8	11.43	1	2.44
51-60	15	21.43	11	26.83
61-70	16	22.86	7	17.07
71-80	12	17.14	6	14.64
Total	70	100	41	100

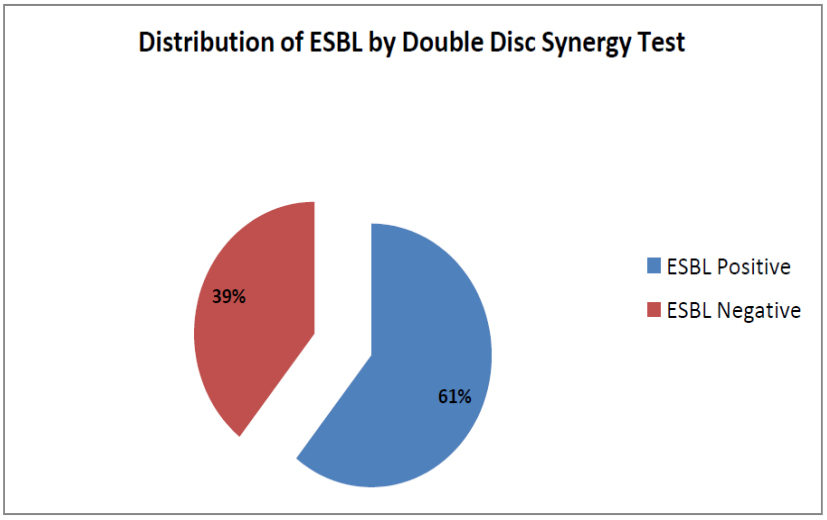


Out of 70 isolates of ESBL positive, highly affected age group was 61-70 showed 16(22.86%) ESBL positive, followed by 51-60 age group showed 15 (21.43%) ESBL positive, 71-80 age group showed 12(17.14%), in 21-30 the percentage is 10 (14.29%), 41-50 age group showed 8 (11.43%) ESBL positive 31-40 age group showed 6(8.57%) ESBL positive, in age group ≤20 showed 4(5.71%) ESBL positive

Out of 41 ESBL negative isolates 11 (26.83%) were observed in age group 51-60, followed by 21-30 age group showed 10(24.39%) ESBL negative, 61-70 age group showed 7(17.07%) ESBL negative, the lowest ESBL negative isolates were observed in age groups 41-50 showed 1(2.44%) and ≤20 showed 2(4.88%) ESBL negative

Table No. 15: Distribution of Esbl By Double Disc Synergy Test.

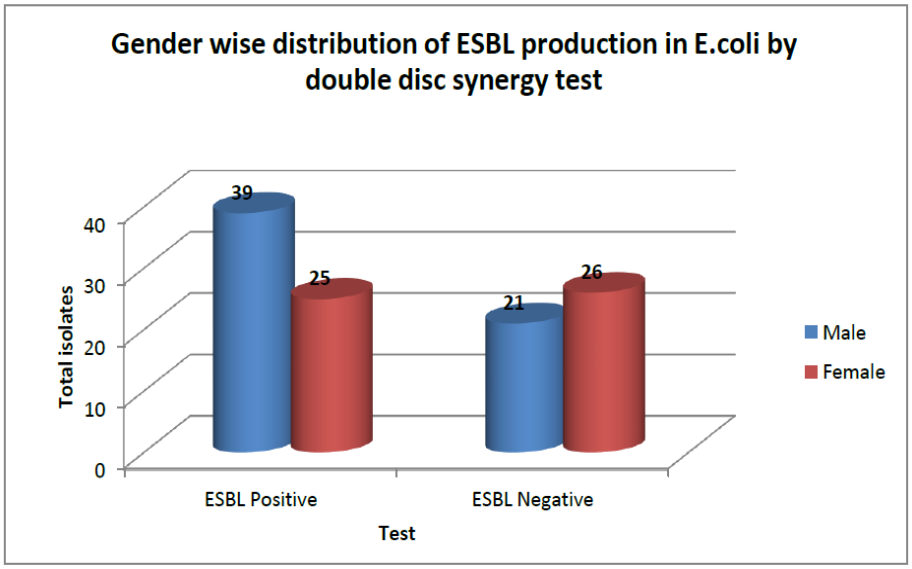
Test	Numbers (n)	Percentage (%)
ESBL Positive	64	57.66
ESBL Negative	47	42.34
Total	111	100



Out of 111 isolates of *Escherichia coli*, 64 (57.66%) were ESBL positive and 47 (42.34%) ESBL negative isolates. Maximum isolates were ESBL positive.

Table No. 16: Gender Wise Distribution of Esbl By DoubleDisc Synergy Test.

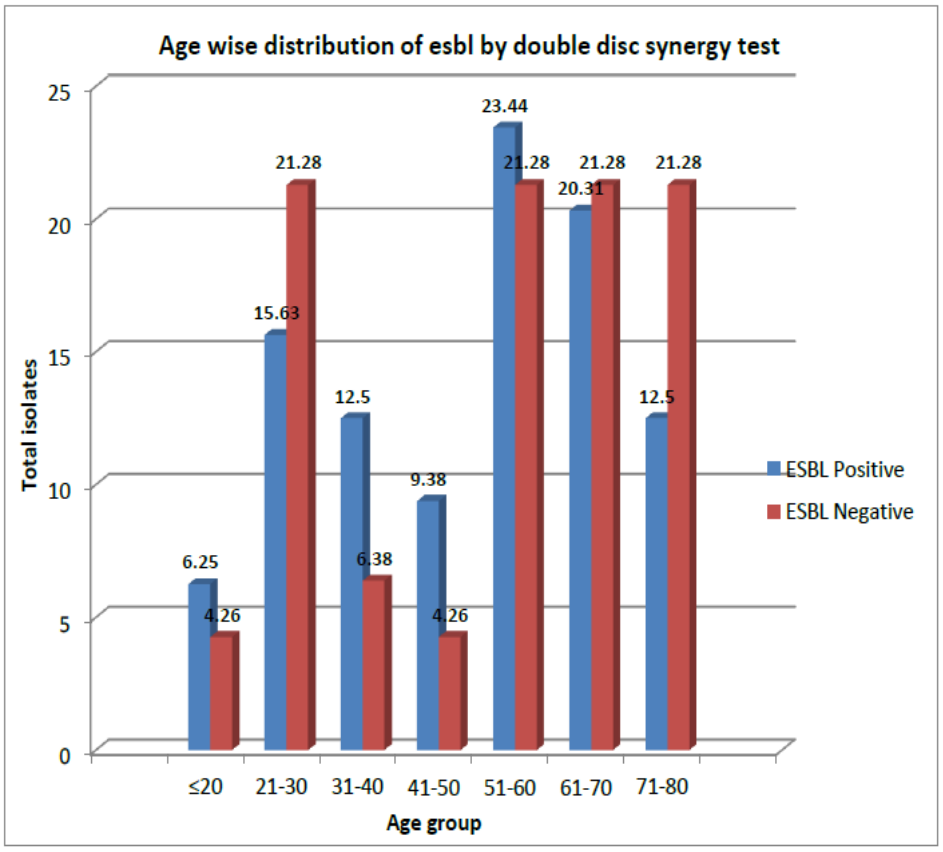
Gender	ESBL Positive	ESBL Negative
Male	39(60.94%)	21(44.68%)
Female	25(39.06%)	26(55.32%)
Total	64 (100%)	47(100%)



Out of 64 ESBL positive isolates, maximum ESBL positive isolates observed in male 39(60.94%) and in female 25(39.06%).

Table No.17: Age Wise Distribution of Esbl By Double DiscSynergy Test.

Age group	ESBL Positive		ESBL Negative	
	Number (n)	Percentage (%)	Number (n)	Percentage (%)
≤20	4	6.25	2	4.26
21-30	10	15.63	10	21.28
31-40	8	12.5	3	6.38
41-50	6	9.38	2	4.26
51-60	15	23.44	10	21.28
61-70	13	20.31	10	21.28
71-80	8	12.5	10	21.28
Total	64	100	47	100

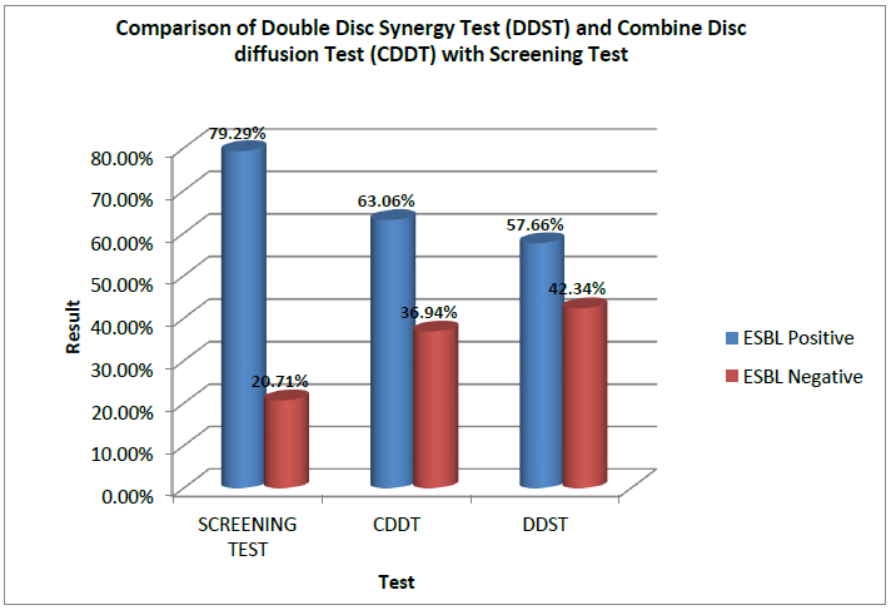


Maximum ESBL positive isolates were observed in age group 51-60,15(23.44%), followed by 61-70 age group showed 13(20.31%), 21-30 showed 10(15.63%) ESBL positive isolates,71-80 and 31-40 both showed 8(12.5%), minimum ESBL positive isolates were observed in age group ≤20 showed4(6.25%), followed by age group 41-50 showed 6 (9.38%) ESBL positive isolates.

Maximum ESBL negative isolates were observed in age groups 21-30,51-60,61-70,71-80 all showed similar rate 10(21.28%), minimum ESBL negative isolates were observed in age group ≤20 and41-50 both showed 2(4.26%), followed by 31-40 age group showed 3 (6.38%) ESBL negative isolates.

Table No.18: Comparison of Double Disc Synergy Test (Ddst) and Combine Disc Diffusion Test (Cddt) With Screening Test.

Result	Screening Test	Double Disc Synergy Test	Combine Disc Diffusion Test
ESBL Positive	111(79.29%)	64(57.66%)	70(63.06%)
ESBL Negative	29(20.71%)	47(42.34%)	41(36.94%)
Total	140 (100%)	111(100%)	111 (100%)



Out of 140 isolates of Escherichia coli 111 were obtained by screening test that was further proceeded for phenotypic confirmatory test. The ESBL positive isolates obtained by combine disc diffusion test was 70(63.06%), and by double disc synergy test 64(57.66%) ESBL negative obtained by combine disc diffusion test was 41(36.94%) and by double disc synergy test 47(42.34%).

Both confirmatory tests were compared to screening test and it was seen that the combined disc diffusion test was more effective than that of double disc diffusion test.

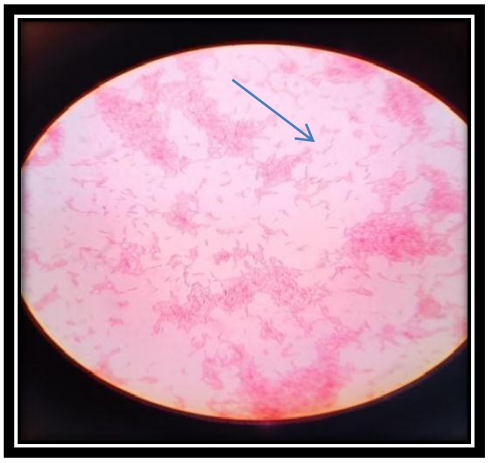


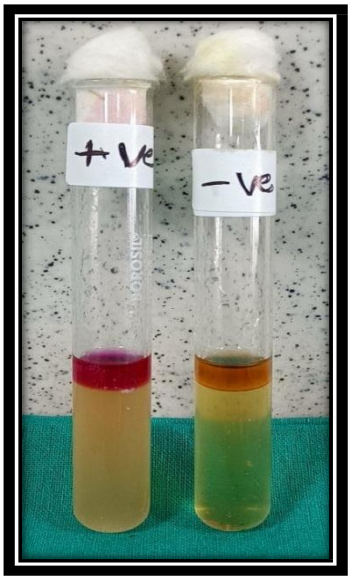
Figure 2: Gram Stain – Gram negative bacilli.

Figure 3:- Growth on Mac Conkey Agar

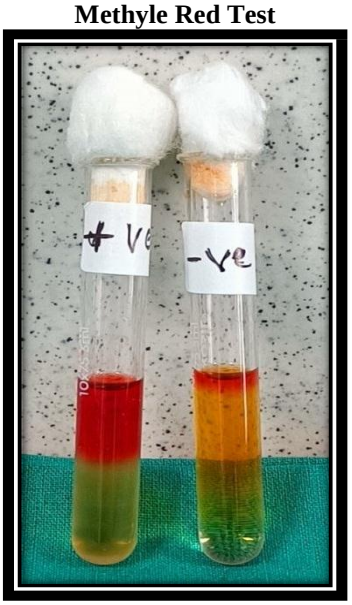
Figure 4:- Growth on Blood Agar

BIOCHEMICAL TESTS

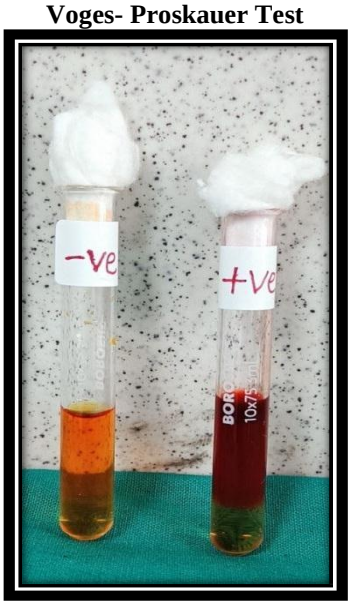
Indole Test



Methyle Red Test



Voges- Proskauer Test



Urease Test

Citrate Test

Nitrate Reduction Test

TSI Test

Sugar Fermentation Test

PHENOTYPIC TEST

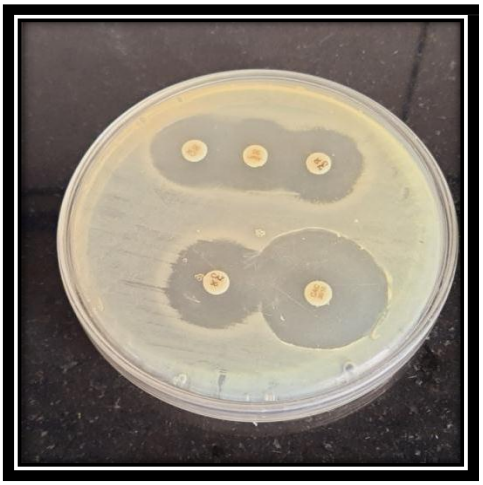


Figure 3: Double Disc Synergy Test

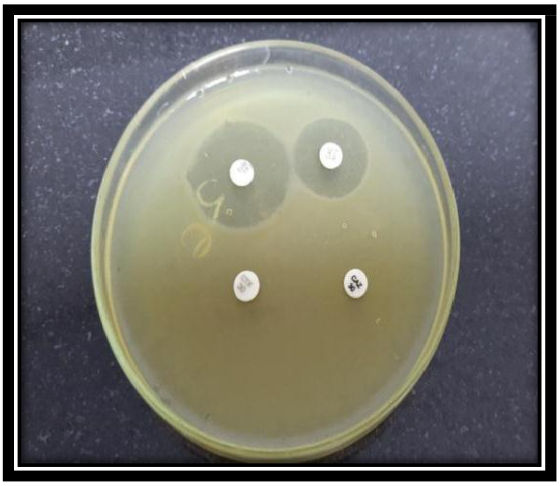


Figure 4: Combined Disc Diffusion Te

DISCUSSION

A number of nosocomial outbreaks which were caused by ESBL producing *Escherichia coli*, have been reported in the United States.[31,32] Although most of the outbreaks were limited to high risk patient care areas such as ICUs, the first report of an outbreak in nursing homes appeared in the literature in the year 1999.[33] Therefore, now- a-days, the threat of ESBL producing isolates is not limited to ICUs or tertiary care hospitals only.

Serious problems created by ESBL producing *Escherichia coli*. It causes major problem in antibiogram therapy.[34] Excess use of antibiotics in hospitals was expensive and cause drug resistance in ICU patients. In various pathogenic infections ESBL producing *Escherichia coli* was more susceptible.

- **Comparative studies in different regions of India**

Author	Place	Year	Prevalence %
Gaurav Dalela *et al*[35]	Jhalawar	2011	61.1
Sridhar PN Rao *et al*[36]	Karnataka	2014	61.4
Kamlesh Kumar Yadav *et al*[37]	Nepal	2015	26.87
Nipa Singh *et al*[38]	Bhuvaneshwar	2015	61.1
Pooja Shakya *et al*[39]	Kathmandu	2016	21.4
Abdulaziz Alqasim *et al*[2]	Riyadh	2018	33
Assudani H. *et al*[29]	Gujarat	2019	50.76
Present study	Karad	2021	63.06

In India the prevalence of the ESBL producers was 20% to 70%. Sridhar PN Rao *et al* reported 61.4% ESBL producers in their study cohort. The ESBL production which was reported among *Escherichia coli* by Kamlesh Kumar Yadav *et al* was 26.87%.

Nipa Singh et al detected ESBL positivity in 61.1% isolates and Pooja Shakya *et al* reported 21.4% ESBL positivity among *E.coli*. Abdulaziz Alqasim *et al* shows 33% ESBL positive isolates of *Escherichia coli* and Assudani H. *et al* shows 50.76%.

Gaurav Dalela *et al* reported the prevalence of *Escherichia coli* causing ESBL production by (DDST) about 57.53% and by (PCDDT) 61.17%. Sridhar PN Rao *et al* show 61.4 % ESBL production. These findings correlated well with those of ourstudy.

In our study the prevalence of *Escherichia coli* causing ESBL production by Double Disc Synergy Test (DDST) about 57.66% and by Combine Disc diffusion Test (CDDT) 63.06%.

- **Comparative study showing age distribution in the ESBL positiveisolates:-**

Auther	Year	Age group mainly affected (year)
Roshan M. *et.al.*[40]	2011	>40 years
Rajan S. *et al*[41]	2012	>50 years
Kamlesh Kumar Yadav *et al*[37]	2015	>60 years
Pooja Shakya *et al*[39]	2016	>20 years
Abdulaziz Alqasim *et al*[2]	2018	>20 years
Present study	2021	>50 years

Abdulaziz Alqasim *et al* reported that the mainly affected age group was >20 years. In Pooja Shakya *et al* study it was >20 years. Rajan S. *et al* shows >50 years affected age group. Kamlesh Kumar Yadav *et al* shows highly affected age group was >60 years Roshan M. *et al* it was >40 years.

In present study mainly affected age group was >50 years similar to the study reportedby Rajan S. *et al*.

- **Comparative study showing gender wise distribution of ESBL positiveisolates.**

Auther	Year	Male (%)	Female (%)
Rajan S. *et al*[41]	2012	48.6	51.3
Nipa Singh *et al*[38]	2015	59.3	40.7
Pooja Shakya *et al*[39]	2016	18.2	81.8
Abdulaziz Alqasim *et al*[2]	2018	24	76
Assudani H. *et al*[29]	2019	69.51	30.49
Present study	2021	60.94	39.06

Previous study of Abdulaziz Alqasim *et al*; shows ESBL positive isolates in males 24% and in females 76%. In the study Assudani H. *et al* shows 69.51% in males and in females 30.49%. Pooja Shakya *et al* shows 18.2% ESBL positive in male and in female 81.8%. Rajan S. *et al* shows 48.6% in male and in female 51.3%

In our study, majority of the ESBL production was observed in males (60.94%) by DDST and in PCDDT (60%) and co-relates to study of Nipa Singh *et al* 59.3%.

- **Comparative study showing sample wise distribution of ESBL positiveisolates.**

Authors	Year	Sample	Percentage (%)
Gaurav Dalela *et al*[35]	2011	Urine	66.4
Sridhar PN Rao *et al*[36]	2014	Urine	61.4
Kamlesh Kumar Yadav *et al*[37]	2015	Urine	31.33
Nipa Singh *et al*[38]	2015	Urine	82.6
Assudani H. *et al*[29]	2019	Urine	32.08
Present study	2021	Urine	60.61

In the study of Gaurav Dalela *et al* 66.4% isolates were in urine specimen. Kamlesh Kumar Yadav *et al* showed 31.33% ESBL positive urinary isolates. Nipa Singh showed 82.6% and in the study of Assudani H. *et al* 32.08%.

In our study ESBL producing Escherichia coli is about 60.61% in urine, similarly has reported by Sridhar PN Rao *et al* shows that 61.4%. This is because of UTI and they are common infections in hospitalized patients.[42]

In present study, Medicine ICU shows highest prevalence of ESBL producing *Escherichia coli.*

- **Comparative study showing Screening test in different studies**

Authors	Year	Screen test positive(%)
Thakur S. *et al*[43]	2013	73.68
Sridhar PN Rao *et al*[36]	2014	74
Kamlesh Kumar Yadav *et al*[37]	2015	95.52
Pooja Shakya *et al*[39]	2016	85.02
Abdulaziz Alqasim *et al*[2]	2018	41
Present study	2021	79.29

Thakur S. *et al* shows 73.68 % screen test positive isolates. In the study of Kamlesh Kumar Yadav *et al* it was 95.52%. Abdulaziz Alqasim *et al* reported 41%. Pooja Shakya *et al* reported 85.02 % screen test positive isolates in theirstudy.

In present study the screen test positive isolates were 79.29 similar to the study reported by Sridhar PN Rao *et al* 74%.

Comparative study showing antimicrobial susceptibility testing in variousstudies

Authors	Sensitive (%)	Resistant (%)
Assudani H. *et al*[29]	Nitrofurantoin (89.2)	Ceftazidime (83.16) Cefotaxime (74.74)
MS Kumar *et al*[42]	Gentamicin (28)	Ceftazidime (37) Cefotaxime (95)
Abdulaziz Alqasim *et al*[2]	Imipenem (100) Nitrofurantoin (21)	Ceftazidime (85) Ciprofloxacin (76)
Kamlesh Kumar Yadav *et al*[37]	Imipenem (94.44) Nitrofurantoin (72.22) Amikacin (100)	Ceftazidime (100) Cefotaxime (100)
Present study	Colistin (97.86) Nitrofurantoin (70.71) Imipenem (54.29)	Ciprofloxacin (89.29) Cefotaxime (88.57) Ceftazidime (85.71)

Kamlesh Kumar Yadav *et al* reported most sensitive antibiotics were Imipenem (94.44), Nitrofurantoin (72.22), Amikacin (100) and most resistant antibiotics were Ceftazidime(100), Cefotaxime (100); Abdulaziz Alqasim *et al* shows most sensitive antibiotics Imipenem (100), Nitrofurantoin (21) most resistant antibiotic Ceftazidime (85),Ciprofloxacin (76).

In our study, the more effective antibiotic for ESBL producers was Colistin (97.86%) sensitive followed by Nitrofurantoin (70.71%), Imipenem (54.29%). most resistance observed in Ciprofloxacin (89.29%) followed by Cefotaxime (88.57%), Ceftazidime (85.71%). This study was co-related to Abdulaziz Alqasim *et al* were resistantantibiotics was similar to present study.

The PCDDT test was compared with DDST and it was found to be an inexpensive alternative for the DDST, for the detection of ESBL producers. The DDST lacks sensitivity because of the problem of optimal disc space and the correct storage of the clavulanic acid containing discs. Assuming that a laboratory is currentlytesting the sensitivity for Ceftazidime by using the disc diffusion test and it required only one disc to be added to the sensitivity plate by PCDDT and would screen *Escherichia coli* isolates in the diagnostic laboratory for ESBL production. Thismethod is technically simple and inexpensive. The Clinical and Laboratory Standards Institute (CLSI) therefore, also recommended the use of PCDDT for the phenotypic confirmation of the ESBL producer among *E. coli*. Many clinical laboratories are not fully aware of the importance of the ESBL producers and of methods to detect them. Laboratories may also lack the resources which are needed to curb the spread of these resistance mechanisms. This lack of understanding or resources is responsible for a continuing failure to respond appropriately to prevent the spread of ESBL producing Escherichia coli. The consequence of this has been avoidable therapeutic failures (sometimes fatal) in patients who received inappropriate antibiotics and outbreaks of infections which were caused by multidrug-resistant, gram negative pathogens that required expensive control efforts.

Hence, their detection must be quick, for formulating an antibiotic policy and containment measures to solve the issue of antibiotic resistance. Therefore, the regular detection of ESBLs by conventional methods should be carried out in every laboratory where molecular methods cannot be performed, as genotyping is used only for the detection and confirmation of ESBLs and as it is not informative for selecting the right treatment. However, the techniques which are required for the task of identifying the exact ESBL subtype (e.g. DNA probing, polymerase chain reaction, restriction fragment length polymorphism and isoelectric focusing) are available only at research facilities.

CONCLUSION

Most of *Escherichia coli* ESBL positive isolates were observed in urine specimen. All ESBL producers showed resistant to Cefotaxime, ciprofloxacin, ceftriaxone and Ceftazidime. Colistin, Iimipenem and Nitrofurantoin were most effective antibiotics. In the present study 79% isolates were screen test positive and 21% screen test negative. Phenotypic methods for detection and confirmation of ESBL producing *Escherichia coli* were studied and it was observed that, by DDST 57.66% isolates were ESBL positive and CDDT 66.06%. In hospitals use of third generation Cephalosporins has been cited as a contributing factor in the development of ESBL mediated resistance along with prolonged stay and intubations. So by following proper infection control and giving correct antibiotics after proper screening ESBL production can be minimized.

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ETHICAL CONSIDERATION

Ethical and research clearance was approved by Ethics Committee of Krishna Institute of Medical Sciences, Deemed to be University Karad.

DATA AVAILABILITY

The article contains the appropriate and proper data obtained during the experiment which supports the

result, discussion and conclusion of the research article.

CONSENT FOR PUBLICATION
Not applicable.

CONFLICTS OF INTEREST
The authors declare that they have no conflict of interest.

REFERENCES

1. Saroj Kumar Sah et al Extended Spectrum Beta Lactamase (ESBL) mechanism of antibiotic resistance and Epidemiology; Pharmatech Res., 2014-2015; 7(2): 303-309.
2. Abdulaziz alqasim *et al.* Prevalence of Multidrug resistance and extended- spectrum beta-Lactamase Carriage of clicnical Uropathogenic *Escherichia coli* isolates in Riyadh, SaudiArebia International Journal of Microbiology, 2018. Article ID 3026851.
3. Youn Lim, Jang W. Yoon, and Carolyn J. Hovde, A Brief Overview ofEscherichia coli O157:H7 and Its Plasmid O157 J Microbiol Biotechnol, January, 2010; 20(1): 5–14.
4. El-Shaer S, Abdel-Rhman SH, Barwa R, Hassan R. Genetic characterization of extended-spectrum β-Lactamase- and carbapenemase- producing Escherichia coli isolated from Egyptian hospitals and environments. PLoS ONE, 2021; 16(7): e0255219. https://doi.org/10.1371/ journal.
5. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory StandardsInstitute, 2020.
6. I. T. M. A. Overdevest,1,2 I. Willemsen,2 S. Elberts,2 C. Verhulst,2 and J. A. J. W. Kluytmans2,3; Laboratory Detection of Extended-Spectrum-Beta- Lactamase-Producing Enterobacteriaceae: Evaluation of Two Screening Agar Plates and Two Confirmation Techniques Journal Of Clinical Microbiology, Feb., 2011; 519–522: 0095-1137/11/$12.00 doi:10.1128/JCM.01953-10.
7. Zachary D Blount; The Natural History Of Model Organisms The unexhausted potential of E. coli Blount. eLife, 2015; 4: e05826. DOI: 10.7554/eLife.05826.
8. Goodmann & Gillman's the pharmacological basis of therapeutics 10th edition, 1189-1218.
9. David L. Paterson and Robert A. Bonomo Extended spectrum βlactamases, a clinical update. Clinical Microbiology Reviews (CMR), Oct, 2005; 18: 657-686.
10. Mandell, Douglas and Bennetts. Principles and Practice of Infectious Diseases,4th edition, 214-217.
11. Mackie and Mac Cartney Practical Medical Microbiology, 14th edition, 131-150.
12. Gazal LES, Medeiros LP, Dibo M, Nishio EK, Koga VL, Gonçalves BC, Grassotti TT, de Camargo TCL, Pinheiro JJ, Vespero EC, de Brito KCT, de Brito BG, Nakazato G and Kobayashi RKT Detection of ESBL/AmpC- Producing and Fosfomycin-Resistant Escherichia coli From Different Sources in Poultry Production in Southern Brazil. Front. Microbiol, 2021; 11: 604544. doi: 10.3389/fmicb.2020.604544.
13. Poulou, A., Grivakou, E., Vrioni, G., Koumaki, V., Pittaras, T., Pournaras, S., & Tsakris, A. Modified CLSI extended-spectrum β-lactamase (ESBL) confirmatory test for phenotypic detection of ESBLs among Enterobacteriaceae producing various β-lactamases. *Journal of clinical microbiology*, 2014; *52*(5): 1483–1489. https://doi.org/10.1128/JCM.03361-13
14. Bajaj P, Singh NS and Virdi JS Escherichia coli β-Lactamases: What Really Matters. Front. Microbiol, 2016; 7: 417. doi: 10.3389/fmicb.2016.00417
15. Bryan, L.E. General mechanisms of resistance to antibiotics, J. Antimicrob. Chemother, 1988; 22(A): 1.
16. Matthew M, Hedges RW, Smith JT. Types of beta-lactamase determined by plasmids in gram-negative bacteria. J Bacteriol, Jun, 1979; 138(3): 657-62. doi: 10.1128/jb.138.3.657-662. PMID: 378931; PMCID: PMC218087.
17. Livermore, D.M. β-lactamases in laboratory and clinical resistance.Clin. Microbiol. Rev., 1995; 8(4): 557.
18. Colour Atlas and Textbook of Diagnostic Microbiology –Fifth edition.
19. Frere, J.M. β-lactamases and bacterial resistance to antibiotics. Mol. Microbiol, 1995; 16(3): 385.
20. Bush, K.; Macielag, M.J. New β-lactam antibiotics and β-lactamase inhibitors. Expert Opin. Ther. Patents, 2010; 20: 1277–1293.
21. Bradford, P.A. Extended-Spectrum-Lactamases in the 21st Century: Characterization, Epidemiology, and Detection of This Important Resistance Threat. Clin. Microbiol. Rev., 2001; 14: 933–951.
22. Smet, A.; Martel, A.; Persoons, D.; Dewulf, J.; Heyndrickx, M.; Herman, L.; Haesebrouck, F.; Butaye, P. Broad-spectrum β-lactamases among Enterobacteriaceae of animal origin: Molecular aspects, mobility and impact on public health. FEMS Microbiol. Rev., 2010; 34: 295–316.
23. Kliebe, C.; Nies, B.A.; Meyer, J.F.; Tolxdorff-Neutzling, R.M.; Wiedemann, B. Evolution of plasmid-coded resistance to broad-spectrum cephalosporins. Antimicrob. Agents Chemother, 1985; 28: 302–307.
24. Liakopoulos, A.; Mevius, D.; Ceccarelli, D. A review of SHV extended- spectrum β-lactamases: Neglected yet ubiquitous. Front. Microbiol, 2016; 7.
25. Poirel, L.; He, C.; Podglajen, I.; Sougakoff, W.; Gutmann, L.; Nordmann, P. Emergence in Klebsiella pneumoniae of a Chromosome-Encoded SHV β- Lactamase That Compromises the Efficacy of Imipenem. Antimi, 2003; 47: 755–758.
26. Lagacé-Wiens, P.R.S.; Tailor, F.; Simner, P.; DeCorby, M.; Karlowsky, J.A.; Walkty, A.; Hoban, D.J.; Zhanel, G.G. Activity of NXL104 in combination with β-lactams against genetically characterized Escherichia coli and Klebsiella

pneumoniae isolates producing class A extended-spectrum β- lactamases and class C β-lactamases. Antimicrob. Agents Chemother, 2011; 55: 2434–2437.
27. Jarlier V, Nicolas MH, Fournier G, Philippon A. Extended broad-spectrum beta-lactamases conferring transferable resistance to newer beta-lactam agents in Enterobacteriaceae: hospital prevalence and susceptibility patterns. Rev Infect Dis., 1988; 10: 867–878.
28. Manual of Clinical Microbiology by Patrick R. Murray, 8th edition 693-696, 1108-1115: 1183-1185.
29. Assudani H , Jigar Gusani, Dr. N.D. Desai; Prevalence of extended spectrum B lactamase producing *E. coli & Klebsiella spp.* isolated in tertiary carehospital, Gujarat.
30. Forbes BA, Sahm DF, Weissfeld AS. Bailey and Scott's Diagnostic Microbiology.10th ed. Missouri; mosby, 1998.
31. Karas JA, Pillay DG, Muckart D, Sturm AW. Treatment failure due to extended spectrum beta-lactamase. J Antimicrob Chemother, 1996; 37: 203-04.
32. Rice LB et al. Outbreak of ceftazidime resistance caused by extended- spectrum beta-lactamases at a Massachusetts chroniccare facility. Antimicrob Agents Chemother, 1990; 34: 2193-99.
33. Wiener J, Quinn JP, Bradford PA, Goering RV, Nathan C, Bush K, et al. Multiple antibiotic-resistant Klebsiella and Escherichia coli in nursing homes. JAMA, 1999; 281: 517-23.
34. Harakuni S, Mutnal M, Karadesai S, Metgud S. Prevalence of extended spectrum β-lactamase-producing clinical isolates of Klebsiella pneumoniae in intensive care unit patients of a tertiary care hospital. Ann Trop Med Public Health, 2011; 4(2): 96-8.
35. Gaurav Dalela Prevalence of Extended Spectrum Beta Lactamase (ESBL) Producers among Gram Negative Bacilli from Various Clinical Isolates in a Tertiary Care Hospital at Jhalawar, Rajasthan, India ID: JCDR/2012/3537:1926
36. Rao SP, Rama PS, Gurushanthappa V, Manipura R, Srinivasan K. Extended Spectrum Beta-Lactamases Producing *Escherichia coli* and *Klebsiella pneumoniae*: A Multicentric Study Across Karnataka.J Lab Physicians, 2014; 6(1): 7-13.doi: 10.4103/0974-2727.129083.
37. Kamlesh Kumar Yadav, *et al;* Multidrug resistant Enterobacteriaceae and extended ssspectrum beta-lactamase producing Escherichia coli: a cross-sectional study in National Kidney Center, Nepal, 2015. doi: 10.1186/s13756-015-0085-0
38. Nipa Singh1 , Dipti Pattnaik2 , Dhruba kumar Neogi3 , Jagadananda Jena4 , Bandana Mallick Prevalence of ESBL in Escherichia coli Isolates Among ICU Patients in a Tertiary Care Hospital; Journal of Clinical and Diagnostic Research, Sep, 2016; 10(9): DC19-DC22.
39. Pooja Shakya *et al;* ESBL production Among *E.coli* and *Klebsiella* spp. Causing urinary tract Infection: A Hospital Based Study. Doi: 10.2174/1874285801711010023
40. Roshan M. Ikram A, Mirza IA, Malik N, Abbasi SA, Alizai SA. Susceptibility Pattern of Extended Spectrum B-Lactamase Producing Isolates in Various Clinical Specimens. J Call Physicians Surg Pak., 2011; 21: 342-6.
41. Rajan S, Prabavathy J. Antibiotic Sensitivity and Phenotypic Detection of ESBL producing E. coli Strains Causing Urinary Tract Infection In a Community Hospital, Chennai, Tamil Nadu, India. Webmed Central. PharmSci., 2012; 3.
42. MS Kumar, V Lakshmi, R Rajagopalan; Occurrence of Extended spectrum beta-lactamases among Enterobacteriaceae spp. Isolated at the tertiary care hospital2006;. Indian Journal of Medical Microbiology. 0255-0857
43. Thakur S, Pokhrel N. Sharma N. Prevalence of Multidrug Resistant Enterobacteriaceae and Extended Spectrum Lactamase Producing Escherichiacoli in Urinary Tract Infection. RJPBCS, 2013; 4: 1615-24.
44. Dutta P, Thakur A, Mishra B, Gupta V. Prevalence of clinical strains resistant to various β-lactams in a tertiary care hospital in India. Jpn J Infect Dis., 2004; 57: 146-49.
45. Shah AA, Hasan F, Ahmed S, Hameed A. Extended-spectrum beta-lactamases in Enterobacteriaceae: related to age and gender. New Microbiol, 2002; 25: 363-6.
46. Kandeel A. Prevalence and risk factors of extended-spectrum beta-lactamses producing Enterobacteriaceae in a general hospital in Saudi Arabia, JMID, 2014: 4-50-4. doi:10.5799/ahinjs.02.2014.02.0126
47. Shukla I, Tiwari R, Agarwal M. Prevalence of extended-spectrum β-lactamaseproducing Klebsiella pneumoniae in a tertiary care hospital. Indian J Med Microbiol, 2004; 22(2): 87-91.
48. Nagshetty, K., Shilpa, B.M., Patil, S.A., Shivannavar, C.T. and Manjula, N.G. An Overview of Extended Spectrum Beta Lactamases and Metallo Beta Lactamases. Advances in Microbiology, 11, 37-62. https://doi.org/10.4236/aim.2021.111004
49. Anne Marie Queenan *et al* Effects of Inoculum and -Lactamase Activity in AmpC- and Extended-Spectrum -Lactamase (ESBL)-Producing Escherichia coli and Klebsiella pneumoniae Clinical Isolates Tested by Using NCCLS ESBL Methodology DOI: 10.1128/JCM.42.1., 2004; 269–275.
50. Andrea J *et al B*rown Evaluation of Four Commercially Available Extended- Spectrum Beta-Lactamase Phenotypic Confirmation Tests doi:10.1128/JCM.43.3., 2005; 1081–1085.
51. A. A. Shah, F. Hasan, S. Ahmed, and A. Hameed Extended-Spectrum β- Lactamases (ESBLs): Characterization, Epidemiology and Detection; Microbiology, 2004; 30(1): 25–32.

52. Babypadmini S, Appalaraju B. Extended-spectrum β-lactamases in the urinary isolates of Escherichia coli and Klebsiella pneumoniae – prevalence and susceptibility pattern in a tertiary care hospital. Indian J Med Microbiol, 2004; 22(3): 172-74.
53. Mathur P, Kapil A, Das B, Dhawan B. Prevalence of extended spectrum β- lactamase producing gram negative bacteria in a tertiary care hospital. Indian J Med Res., 2002; 115: 153-57.
54. Rodrigues C, Joshi P, Jani SH, Alphonse M, Radhakrishnan R, et al. Detectionof β-lactamases in nosocomial gram negative clinical isolates. Indian J Med Microbiol, 2004; 22(4): 247-50.
55. Chatterjee M, Banerjee M, Guha S, Lahiri A, Karak K. Prevalence of ESBL producing urinary isolates and their drug resistance pattern in eastern part of India. Sri Lankan Journal of Infectious Diseases Sri Lankan J Infec Dis., 2012; 1(2): 36-41.
56. Das N, Borthakur A. Antibiotic coresistance among extended-spectrum betalactamase-producing urinary isolates in a tertiary medical center: A prospective study. Chronicles of Young Scientists Chron Young Sci., 2012; 3: 53-6.