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Original Research Article

# Detection of ESBL & MBL producing E. Coli From Urine Samples In A Tertiary Care Hospital In Jaipur, Rajasthan

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**Abstract:** Urinary tract infections typically occur when bacteria enter the urinary tract through the urethra and begin to multiply in the bladder. Although the urinary system is designed to keep out such microscopic invaders, these defenses sometimes fail. When that happens, bacteria may take hold and grow into a full-blown infection in the urinary tract. The most common UTIs occur mainly in women and affect the bladder and urethra. The objective is to isolate and identify E.coli from urine samples. To perform antibiotic sensitivity of E.coli by Kirby bauers disc diffusion method. To detect ESBL and MBL producing E.coli by phenotypic method and study their antibiotic sensitivity pattern so as to provide basic guideline in the treatment. Patients included in the study were given a sterile, dry, test tube and request for 10-20ml specimen. A mid-stream urine specimen (MSU) collected preferably prior to administering antibiotics. Uncentrifuged urine is inoculated on CLED agar Culture plates are incubated at 37°C for 24 hours. Detail of methodology is mentioned in material and method section. Out of the 71 isolates of E. Coli 36 (50.70%) were ESBLs producing. Out of the 71 isolates of E. coli 5 (7.04%) were MBL producing. In ESBLs producers group maximum susceptibility is seen to imipenem, meropenam, (100%, 91.66% respectively) followed by Cefoperazone salbactum (77.77%), Nitrofurantoin (77.77 %), Amikacin (58.33 %), Ceftazidime clavulanate (55.55 %), Piperacillin tazobactum (52.77%), Aztreonam (33.33%) followed by Cotrimoxazole (33.33%) and ceftazidime (00.00%). *E.coli 71* (47.33%) was the most common organism isolated from urine samples followed by *Klebsiella spp. 31*(21.33%), *Citrobacter spp.17* (11.33%), *Pseudomonas spp.15* (10*%), Aceinetobacter 5*(3.33%), *Proteus spp.4 (*2.66%) *Enterobacter spp*.3 (2%) staphylococci 2(1.33%) and candida albicans 1(0.66%) was the least common organism isolated.
**Keywords:** ESBL, MBL, E Coli

## INTRODUCTION:

Enterobacteriaceae is the most common cause of community acquired urinary tract infections (UTI) worldwide. These organisms are frequently resistant to many of the antimicrobial agents, leading to recurrent UTIs, especially in the high risk population. Urinary tract infections, either community or hospital acquired are extremely prevalent and difficult to eradicate. In the recent past, there are alarming reports about the emergence and spread of resistant strains from all around the world [1].

Antimicrobial resistance is associated with high morbidity, mortality, increased length of hospitalization and cost of health care. Bacterial resistance to third-generation cephalosporins, poses a great challenge, in a developing country like India.(1) Among tropical countries, India has emerged as the epicentre of antimicrobial resistant gram negative bacterial strains. Enterobacterial resistance to third-generation cephalosporins is typically caused by production of extended-spectrum beta lactamases (ESBL). Extended-spectrum β-lactamase (ESBL) - producing Escherichia coli, frequently resistant to many of the antimicrobial, show significant local variations. The majority of ESBLs are derived from the widespread plasmid mediated broad-spectrum β-lactamases TEM-1 and SHV-1, found in E. coli and other pathogens. Clinically orientated studies elaborating newer mechanism of resistance in *E. coli* causing UTI are scarce [2].

## MATERIAL & METHODS:

This cross sectional study was conducted in the Department of Microbiology, NIMS Medical College, NIMS University, Jaipur, during period of January 2015-May 2015 collected from hospital at NIMS Medical College & Hospital, Jaipur. A Performa was used to collect medical and demographic data of the patients. Data recorded were : demographic characteristics (age, gender),type of specimen collected ,brief relevant clinical history about underlying disease, recent surgery, immunocompromised status and the investigations to be done etc. Each specimen culture was assigned a unique number that links a specimen to patient. Baseline data was collected, enclosed Performa was filled.

**Collection of Sample:-**

Patients included in the study were given a sterile, dry, test tube and request for 10-20ml specimen. A mid-stream urine specimen (MSU) collected preferably prior to administering antibiotics. Specimen collected in sterile container. Before collecting a sample, genitalia should be cleaned with soap and water and men instructed to retract foreskin of glans penis where as women should keep labia apart. The first portion of urine is allowed to pass then without interrupting the urine flow, mid-stream of urine collected (clean catch, mid-stream). The male patients were advised to wash the hands before collecting the specimen.

**Culture media:** uncentrifuged urine is inoculated on CLED agar Culture plates are incubated at 37°C for 24 hours.

1) **Inoculation of samples:** All urine samples were processed immediately and routinely cultured as per standard protocol on CLED agar. These plates were routinely incubated at 37°C aerobically and after overnight incubation, they were checked for bacterial growth. The organisms were identified by their colony morphology, gram staining characters, motility and other relevant biochemical tests as per standard laboratory methods of identification. ESBLs and MBL isolates were identified by conventional phenotypic identification.

**Identification & Isolation:**

**2) Culture Characteristic:** It is an aerobe and facultative anaerobe and grow on CLED media culture medium at optimum temperature $37^0$ C.

***Colony morphology:*** colonies of some strains shows on CLED media show yellowish lactose fermentation. In general colonies are circular, moist and smooth with entire margin and non mucoid unlike colonies of Klebsiella which are mucoid. In liquid medium, growth occurs as uniform turbidity.

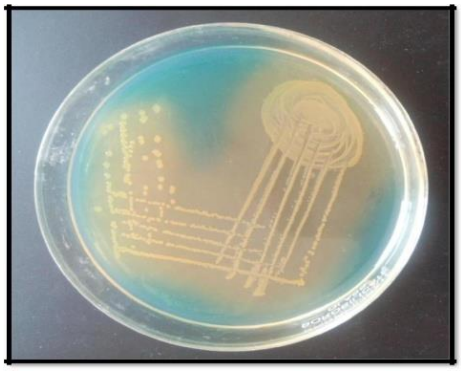

**Fig 1: E. coli colonies seen on CLED media**

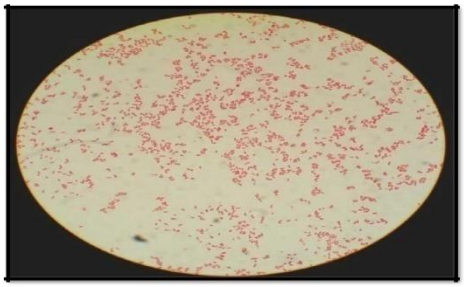

**Fig 1a: Microscopic image of E. coli 100X oil immersion**

**BIOCHEMICAL REACTION**

**(1)Catalase test:**

**Procedure (3):**

**A. Slide Method:** A small number of colony to be tested is picked from a nutrient agar with a clean sterile thin glass rod and is placed on a glass slide, than add 3% hydrogen peroxide solution and observe the result

**B.**

**C. Tube method**: A small amount of colony to be tested is picked from a nutrient agar plate with a clean sterile thin glass rod and this is inserted into 3% hydrogen peroxide solution held in a small clean test tube.

**Interpretation**: A positive catalase reaction produce gas bubbles immediately [3].

**2. Oxidase Test [4].**

**Principle: -** This test depends on the presence in the bacteria of certain Oxidase enzyme that will catalyze

the transport of electrons between electron donors in the bacterial and a redox dye- tetra methyl-p-phenylene-diamine. The dye is reduced to a deep purple color.

**Procedure:** freshly prepared solution of 1% tetra-methyl Para phenyl diamine dihydro-chloride (Oxidase reagent) is used. Filter paper strip soaked in the Oxidase reagent is smeared with test organism. Oxidase positive control (*Pseudomonas spp.*) should always be included to find out the working of Oxidase strip.

**Interpretation [5]**
Positive- Deep purple within 10 seconds. Eg: - *Pseudomonas spp.*
Negative- No change in color. E.g.:- members of *Enterobacteriace.*

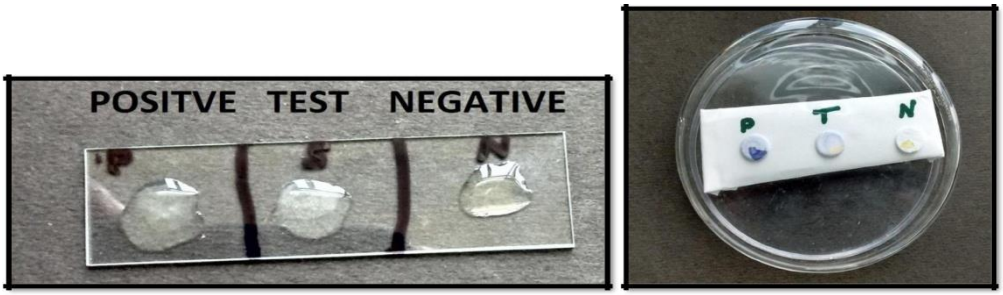


**Fig 2: Oxidase test with positive negative control and specimen**

**3. Indole** [6]
**Principle: -** Bacteria those possess the enzyme tryptophanase are capable of hydrolyzing and deaminating tryptophan with production of Indole. When Indole react with the aldehyde group of 4-dimethyl amino Benzaldehyde, iso amyl alcohol, hydrochloric acid known as Kovacs reagent result in formation of a red color ring complex

**Procedure**: - Test is done by inoculating broth with the test organism and incubates at 37°C for 18-24 hours. Add 15 drops of reagent down the inner wall of the tube and gently shaken.

**Interpretation:**
**Indole Positive:** A red color ring near the surface of the medium.
**Indole Negative**: Yellow color ring near the surface of the medium


**Fig 3: Indole test of E. coli**

**3. Methyl Red [7]**

Methyl red is a pH indicator with a range of between 6-4.PH at which methyl red detects acid is considerably lower (Fig 4)

**Principle: -** It is a qualitative test for acid production, requiring positive organism to produce strong acid from glucose through mixed acid fermentation pathway. Many species of *Enterobacteriaceae* produce efficient quantity of strong acid that can be detected by methyl produced indicator during the initial phase of incubation. Only organism that can maintain this low pH after prolong incubation, overcoming the pH buffering system of medium are MR positive.

**Procedure:** - The test organism is inoculated in a glucose phosphate broth and incubates at 37°C for 18-24 hours. Add 5 drops of reagent down the inner wall of the tube result in formation of a red colour (positive test).

**Interpretation: MR Positive:** Bright red color **MR Negative:** Yellow color.


**Fig 4: Methyl red (MR) test of E. coli**

**4. Voges – Proskauer [8]**

**Principle: -** Pyruvic acid, pivotal compound formed in fermentative degradation of glucose is further metabolized through number of pathway and result in production of acetoin. Acetyl- methyl-carbinol (acetoin) , neutral end product ( 2,3, butylene glycol) in presence of atmospheric oxygen and 40% KOH, acetoin is converted to diacetyl and α-napthanol serve as catalyst and bring out red color.

**Procedure: -** Test is done by inoculating glucose phosphate broth and incubates at 37°C for 18-24 hours. Add 1ml KOH and 3ml α- napthanol in ration of 1:3. Shake the tube gently for exposure to atmosphere O2 and leave the tube undisturbed for 10 – 15 minutes.

**Interpretation: VP Positive:** Development of pink color in 2-5 min., becoming crimson pink In 30 minutes. **VP Negative:** Colorless for 30 minute.


**Fig 5**: **Voges – Proskauer (VP) test of E. coli**


**Fig 6: IMViC reaction interpretations with urease, TSI and SIM (sulphide indole motility detection test)**

**6. Citrate utilisation test**

**Principle: -** This test is used to determine the ability of an organism to utilize sodium citrate as its only carbon source and inorganic ammonium salts as its only nitrogen source. Bacteria that can grow on this medium turn the bromo thymol blue indicator from green to blue.

**Procedure: -** Solid (Simmon's) media is used. A bacterial colony is picked up by a straight wire and inoculated into media. These inoculated media is incubated at $37^0$ C for overnight. Simmons's citrate medium contains agar, citrate and bromo thymol blue as an indicator. Original color of the medium is green(9).

**Interpretation:-Positive-** growth with an intense blue color on the slant**. Negative-** No growth with no change in color (green)


**Fig 7: Citrate utilisation test of E. coli**

**7. Urease Production (Christensen's Urease):-**

**Principle:** To determine the ability of an organism to produce an enzyme urease which splits urea to ammonia? Ammonia makes the medium alkaline and thus phenol red indicator changes to pink/red in color.

**Procedure: -** Test is done by heavily inoculating test organism into slant contain
Christensen's urea agar, incubate at 37°C for 24 hours.

**Interpretation: Urease Positive**: Pink color. **Urease negative**: No color change (Colorless)(10).


**Fig 8: urease production test**

**8. Triple Sugar Iron Agar:**

**Principle:** Triple sugar iron agar (TSI) is used to determine whether a gram-negative rod utilizes glucose and lactose or sucrose fermentative and forms hydrogen sulphide (H2S).

**Procedure:**

TSI is a composite medium which contains three carbohydrates namely glucose, lactose, sucrose and also ferric salts for testing H2S production. The concentration of lactose and sucrose is 10 times that of glucose in the medium and phenol red is incorporated as an indicator. This medium is widely used and is in the form of a butt and slant in the test tube. Slant and butt should be equal in length 1.5inch or 3cm to preserve two chamber effects. Test is done by inoculating the tube with slant and butt by stabbing made of 3-5mm of bottom and slant is streak with back & forth motion, tube is incubated at 37°C for 24 hour [11].

**Fig 9: Triple sugar iron (TSI) test of E. coli**

**PHENYL PYRUVIC ACID MEDIUM:**

**Principle:** This test is used to determine the ability of an organism to oxidatively deaminate phenylalanine to phenyl pyruvic acid. The phenyl pyruvic acid is detected by adding a few drops of 10% ferric chloride; a green colored complex is formed between these two compounds [12].

**Procedure:**

Inoculate with fairly heavy inoculums. Incubate for 4 hours or, if desired, up to 24 hours at 37 $^{0}$C. Add a few drops of a 10% solution of ferric chloride to run down over the growth on the slope.

**Interpretation:-**

***Positive***- Green color will develop in the fluid and in the slope. ***Negative***- No green color [33].

**Fig 10: PHENYL PYRUVIC ACID (PPA) test**

**5 Antimicrobial Susceptibility testing: [13-15]**

Antibiotic susceptibility testing was performed by modified Kirby-Bauer disc diffusion method. An inoculums with a turbidity equivalent to that of a 0**.**5 McFarland standard and Muller Hinton agar plates with commercially available antibiotic discs (Hi-Media, Mumbai) were being used**.** Plates were read after incubation of at 37°C for 24 hr**,** and the zone of inhibition obtained was measured and recorded as compared to that of the manufacturer interpretation charts according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI)**.** All the antibiotic disc and media were used of Hi-media, Mumbai, India

***Antibiotic sensitivity testing:***

Antibiotic susceptibility testing profile of E.coli by Kirby bauers disc diffusion method on Mueller hinton agar (MHA) media.

**Table 1: Antibiotic used in the study is among from these antimicrobial agents (AMAs)**

| S. No. | Antibiotic | Potency | Abbreviation |
|---|---|---|---|
| 1. | Ampicillin | 30µg | AMP |
| 2. | Amikacin | 30µg | AK |

| | | | |
|---|---|---|---|
| 3. | Gentamicin | 10µg | GEN |
| 4. | Norfloxacin | 10µg | NO |
| 5. | Ciprofloxacin | 05µg | CIP |
| 6. | Cefuroxime | 30µg | CXM |
| 7. | Ceftazidime | 30µg | CAZ |
| 8. | Cefpodoxime | 30µg | CPD |
| 9. | Ceftriaxone | 30µg | CTR |
| 10. | Cefepime | 30µg | CPM |
| 11. | Cefoxitin | 30µg | CPX |
| 12. | Amoxicillin/clavulanate | 10µg | AMC/CA |
| 13. | Ceftazidime/clavulanate | 30/10µg | CTX/CA |
| 14. | Piperacillin/tazobactum | 75µg+10µg | PIT |
| 15 | Ticarcillin/clavulanate | 75µg | TC |
| 16 | Cefoperazone/sulbactum | 75µg+30µg | CFS |
| **17** | Imipenem | 10µg | I |
| 18 | Meropenam | 10µg | MRP |
| 19 | Aztreonam | 30µg | AT |
| 20 | Nitrofurantoin | 30µg | NT |
| 22 | Co-trimoxazole | 25µg | COT |
| 23 | Imipenam / EDTA | 10µg/750µg | IE |

**Table 2: Zone size interpretative table accordance to CLSI**

| S. No. | **antimicrobial agents (AMAs)** | Disc Conc.(µg) | Resistant | intermediate | Sensitivity |
|---|---|---|---|---|---|
| 1 | Ampicillin | 30µg | ≤ 13 | 14-16 | ≥ 17 |
| 2 | Amikacin | 30µg | ≤ 14 | 15-16 | ≥ 17 |
| 3 | Gentamicin | 10µg | ≤ 12 | 13-14 | ≥ 15 |
| 4 | Norfloxacin | 10µg | ≤ 12 | 13-16 | ≥ 17 |
| 5 | Ciprofloxacin | 05µg | ≤ 15 | 16-20 | ≥ 21 |
| 6 | Cefuroxime | 30µg | ≤ 14 | 15-17 | ≥ 18 |
| 7 | Ceftazidime | 30µg | ≤ 17 | 18-20 | ≥ 21 |
| 8 | Cefpodoxime | 30µg | ≤ 17 | 18-20 | ≥ 21 |
| 9 | Ceftriaxone | 30µg | ≤ 19 | 20-22 | ≥ 23 |
| 10 | Cefepime | 30µg | ≤ 14 | 15-17 | ≥ 18 |
| 11 | Cefoxitin | 30µg | ≤ 14 | 15-17 | ≥ 18 |
| 12 | Amoxicillin/clavulanate | 10µg | ≤ 24 | 25-29 | ≥ 30 |
| 13 | Ceftazidime/clavulanate | 30/10µg | ≤ 27 | 28-33 | ≥ 34 |
| 14 | Piperacillin/tazobactam | 75µg+10µg | ≤ 17 | 18-20 | ≥ 21 |
| 15 | Ticarcillin/clavulanate | 75µg | ≤ 14 | 15-19 | ≥ 20 |
| 16 | Cefoperazone/sulbactum | 75µg+30µg | ≤ 27 | 28-32 | ≥ 33 |
| 17 | Imipenam | 10µg | ≤ 19 | 20-22 | ≥ 23 |
| 18 | Meropenam | 10µg | ≤ 19 | 20-22 | ≥ 23 |
| 19 | Aztreonam | 30µg | ≤ 17 | 18-20 | ≥ 21 |
| 20 | Nitrofurantoin | 30µg | ≤ 14 | 15-16 | ≥ 17 |
| 22 | Co-trimoxazole | 25µg | ≤ 10 | 11-15 | ≥ 16 |
| 23 | Imipenam / EDTA | 10µg/750µg | ≤ 25 | 26-30 | ≥ 31 |

**Reading: -** After overnight incubation, the zone diameter (including the 6-mm. disk) was measured with a ruler (millimeter scale) on the undersurface of the Petri dish. [17, 69].

6. **For ESBLs Detection [16-19]:**

**Screening of ESBLs:-**

As per the Clinical and Laboratory Standards Institute (CLSI) guidelines screening for ESBL production will be done by using disk-diffusion method**.**

Antibiotic susceptibility testing can screen for ESBL production by noting specific zone diameters of the antibiotics (among these Ceftazidime**,** Cefpodoxime**,** Aztreonam**,** Cefotaxime or Ceftriaxone disks are used) which indicate a high level of suspicion for ESBL production**.** According to the CLSI guidelines , isolates showing inhibition zone of size of ≤ 22 mm with ceftazidime (30µg) and ≤ 27 mm with cefotaxime (30µg) and < 25 mm for ceftriaxone were recorded were identified as potential ESBL producers and shortlisted for confirmation of ESBL production.

**Phenotypic confirmatory test for detection of ESBLs (Cephalosporin/clavulanate Combination disks):-**

As per the CLSI guidelines use of ceftazidime (30µg) disks with or without clavulanate (10µg) for phenotypic confirmation of the presence of ESBLs production in Gram Negative Bacilli on the confluent growth on Mueller- Hinton agar**.** A disk of Ceftazidime (30µg) alone and a disk of Ceftazidime + Clavulanic acid (30 µg/10 µg) will be used.

Both the disks will be placed at least 30 mm apart, center to center, on a lawn culture of the test isolate on Mueller Hinton Agar (MHA) plate was made on the surface of medium by a sterile cotton swab with a inoculums matched with 0.5 Mc Farland Turbidity standard prepared by suspending few colonies of test strain in 0.9% sterile saline and incubated overnight at 37°C. Difference in zone diameters with and without clavulanic acid was measured.

**Interpretation: -** When there is an increase of ≥ 5 mm in inhibition zone diameter around combination disk of Ceftazidime + Clavulanic acid (CAC) versus the inhibition zone diameter around Ceftazidime (CAZ) disk alone, it confirms ESBL production.

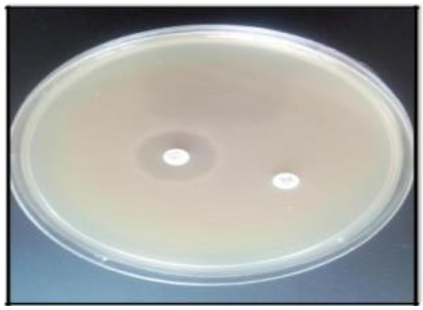

**Fig 11: ESBLs producing E. Coli image inoculated on Mueller Hinton agar (MHA) media**

***Detection of MBL***

All isolates resistant to Imipenem and Meropenem / Ertapenem & third generation cephalosporins were selected for test.

***Combined Disc Test*: -** An overnight broth culture of the test strain (opacity adjusted to 0.5 Mc Farland opacity standards) was used to inoculate a plate of Mueller-Hinton agar. (Hi- Media)An imipenem disc 10µg was initially placed on the MHA. Another imipenem disc to which EDTA (10 µl) {5 % EDTA (i.e. a concentration of 500 µg/disc)}, was added was also placed on the plate. After 24 hours incubation period at 37 0C and increase of zone of IMP-EDTA by 5mm or more as compared to IMP disc alone was considered to be an MBL producer [20].

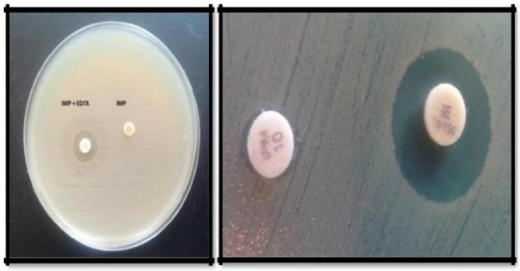

**Fig 12: MBLs producing E.Coli image inoculated on Mueller Hinton agar (MHA) media**

**RESULTS:**

During the study period 300 mid-stream urine (MSU) urine samples with proper aseptic condition of patients of all ages and both sexes were collected attending various departments, outpatients, Inpatients and ICUs at NIMS hospital. Out of 300 urine samples 150 samples were sterile and remaining 150 showed growth, in which *E.coli 71* (47.33 %) was the most common organism isolated followed by *Klebsiella spp.32* (21.33%) and *Citrobacter spp.17 (*11.33%), *Pseudomonas spp. 15 (*10*%), Aceinetobacter* (3.33%), *Proteus spp. 4 (*2.66%), *Enterobacter spp*. 3 (2%), staphylococci spp. 2 (1.33%) and Candida albicans 1(0.66%) were the least common organism isolated. (Table 3-5)

During the study period E.coli isolated from urine specimen of patients of all ages and both sexes were processed further for detection of ESBL and MBL production (Table 6-9). Among *E.coli* the common resistance was given by Cefuroxime (71.84%), Cotrimoxazole (64.78%), followed by norfloxacin (71.84), ceftazidime (71.84%), Aztreonam (57.74%), Ampicillin (52.12%), ceftriaxone (50.71%), Cefepime (38.03%). (Table 10-12)

Minimum resistance was show against Ceftazidime clavulanic acid (30.99%), Amikacin (36.61%), Nitrofurantoin (21.13%), Piperacillin tazobactum (33.81%), Meropenam (15.49%), Cefoperazone salbactum (22.53%) and (7.04%) resistance was given by imipenem. In ESBLs producers group maximum susceptibility is seen in imipenem, meropenam, (100%, 91.66%) followed by Cefoperazone salbactum (77.77%), Nitrofurantoin (77.77 %), Amikacin (58.33 %), Ceftazidime clavulanate (55.55 %), Piperacillin tazobactum (52.77%), Aztreonam (33.33%) followed by Cotrimoxazole (33.33%) and ceftazidime (00.00%).

In non ESBLs producer's group maximum susceptibility is seen in imipenem (85.71%), followed by Ceftazidime clavulanate (82.85%), Nitrofurantoin (80%), piperacillin tazobactum (80%), meropenam (77.14%), Cefoperazone salbactum (77.14%), Cefepime (71.42%). In the comparative study among ESBL producers and Non-ESBL producers it reflected that multidrug resistance was significantly ($P < 0.05$) higher in ESBL producers than in Non-ESBL producers. Prevalence of MBL production in relation with the sex are equally (40% & 60%) in both sexes. A total 5 isolates are found in which 2 are male and 3 are female among total 71 E. coli isolates in urine samples. Prevalence of MBL are slightly more in females than in males that's 60:40 consecutively.

**DISCUSSION:**

Increasing prevalence of infections caused by multiresistant gram-negative bacilli due to synthesis of extended-spectrum β-lactamase (ESBL) and metallo β-lactamase (MBL) is a major concern in the hospitalized patient population throughout the world. ESBL confer resistance to third-generation cephalosporins and monobactams and infections with these multi-resistant gram negative bacilli prolong hospital stay and mortality. The prevalence of ESBLs and MBL among clinical isolates varies greatly worldwide and in geographic areas they are rapidly changing over time.

The present study was conducted in the department of microbiology, NIMS Medical College and hospital, Jaipur Rajasthan, from January 2015 to May 2015. During the study period, 71 E.coli isolate from 300 urine specimens of patients of all ages and both sexes attending various outpatients, Inpatients and ICUs at NIMS hospital were processed in which 150 samples (50%) were sterile. Remaining 150 samples are *E.coli 71* (47.33%) was the most common organism isolated followed by *Klebsiella spp.32* (21.33%) and *Citrobacter spp.17* (11.33%), *Pseudomonas spp. 15* (10*%). Aceinetobacter 5* (3.33%), *Proteus spp. 4 (*2.66%) *Enterobacter spp*. 3 (2%) staphylococci 2 (1.33%) and Candida albicans 1(.66%) was the least common organism isolated. In comparison of K.usha, e kumar, dvr. Sai gopal et al 2013 total of 121 Gram negative bacilli were isolated and identified by standard biochemical tests *E.coli* was predominant (37.19%) followed by *Pseudomonas* spp. (22.31%),

*Klebsiella* spp. (19.83%), Non-fermentative gram negative bacilli (NFGNB) (10.74%), *Enterobacter* spp. (6.61%) and others (3.30 %). In the present study, 50.704% of the patients were female, and 49.295% were male patients. Male to Female ratio was 0.97**:**1 In a similar study from Sharma A *et al.;* [21] in 2012 a total of 230 isolates were obtained. 45.21% isolates were from males and 54.78% were from females with a male to female ratio of 0.82:1. Among *E.coli* the common resistance was given by Cefuroxime (71.84%), Cotrimoxazole (64.78%), followed by norfloxacin (71.84%), ceftazidime (71.84%), Aztreonam (57.74%), Ampicillin (52.12%), ceftriaxone (50.71%), Cefepime (38.03%), Minimum resistance was shown against Ceftazidime clavulanic acid (30.99%), Amikacin (36.61%), Nitrofurantoin (21.13%), Piperacillin tazobactum (33.81%), Meropenam (15.49%), Cefoperazone salbactum (22.53%) and (7.04%) resistance was given by imipenem.

Out of 71 E. coli isolates, 36 were ESBL producers and 5 MBL producers in which *E. coli* showed maximum ESBL production (50.70%) and very few numbers of MBL producing E. Coli that's are 5 (7.04%). It's also finding in mita D wadekar *et al.;* [22] Out of 100 (56). Enterobacteriaceae isolates, 43 were ESBL producers and 18 MBL producers. *E. coli* showed maximum ESBL production in urine sample's 11(42.3%) and MBL producing E. coli was 3(11.3%). In pandya NP et al study shows 2.87% E. coli MBL producing [23].

The worldwide emergence of multi-drug, resistant bacterial strains is a growing concern which is usually found in those hospitals where antibiotic use is frequent and the patients are in critical condition. Broad resistance spectrum is a cause for concern and necessitates the restricted use of extended-spectrum cephalosporins, and a trial of other suitable alternatives [24]. Therapeutic options for the infections which are caused by the ESBL producers have also become increasingly limited [25]. Recent studies on ESBL production among the members of Enterobacteriaceae which were isolated from clinical specimens, showed an increase in the occurrence of ESBL producers. In Umadevi *et al*., 2011 study show out of the 100

Enterobacteriaceae isolates, 43 were ESBL producers. ESBLs were predominantly present among *E. coli* 26(50%) [26]. Our findings are similar to that of Nachimuthu Ramesh *et al.;* in 2008 [27] who reported a high prevalence of ESBLs among *E. coli*. Correct identification of ESBL positive Enterobacteriaceae in due time is mandatory not only for optimal patient management but also for immediate institution of appropriate infection control measures to prevent the spread of these organisms [28]. Early detection will definitely help in controlling hospital infections which are caused by this group of organism. The double disc synergy test (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 currently testing the sensitivity for ceftazidime by using the disc diffusion test and it required only one disc to be added to the sensitivity plate by phenotypic confirmatory disc diffusion test and would screen all gram negative bacteria in the diagnostic laboratory for ESBL production. This method is technically simple and inexpensive. The only β-lactam active against co-AmpC and ESBL producers are carbapenems; however, recently resistance to carbapenems has been increasing, which is mostly due to the production of metallo-β-lactamases [29].

Metallo-β -lactamases (MBLs) are enzymes belonging to Ambler s class B that can hydrolyze a wide variety of Beta-lactams, Including penicillins, cephapimes and carbapenems except aztreonam [30]. Although, PCR method is simple to use in detecting MBL producing isolates, it has become more difficult with the increased number and types of MBL. Combined disc test is simple to perform and highly sensitive in differentiating MBL-producing isolates. Thus, implementation of simple method using Imipenem- EDTA disk for MBL detection is quick, specific, sensitive and reproducible (Uma Chaudhary *et al*., 2008). This study showed MBL production in 18 Isolates with maximum production in *Klebsiella* spp (33.3%), *Enterobacter* spp. (16.6%) and *E.coli* (13.4%) which is consistent with studies by Varun goel *et al*.; in 2013 and Pandya *et al.;* in 2011.

Production of MBL has tremendous therapeutic consequences since these organisms also carry multidrug resistance genes and the only viable option remains the potentially toxic polymyxin B and colistin. The early detection of beta lactemase producing isolates would be important for the reduction of mortality rates for patients and also to avoid the intra hospital dissemination of such strains. Simple phenotypic screening tests are proved to be rapid and convenient for their detection in the clinical laboratory. To overcome the problem of emergence and the spread of multidrug resistant organisms, a combined interaction and cooperation between the microbiologists, clinicians and the infection control team is needed [31].

In this study, multidrug resistance was observed. The possible mechanism of resistance may be:

i) Intracellular degradation of antibiotic.
ii) Hyper production of chromosomal class C enzymes.
iii) Presence of multidrug efflux system.
iv) Low outer membrane permeability.
v) Resistance factor with Resistance transfer factor in plasmid.
vi) Poor binding with cell surface receptor.

The prevalence of ESBLs (50.26%) in the present study was approximate comparison to reports from different parts of the country (17% to 70%). It has been proved that the prevalence of the ESBLs among the clinical isolates varies from country to country and institution to institution within the same country. This might be due to judicious usage of extended spectrum cephalosporins and adopting appropriate infection-control measures in our hospital (32).

**Table 3: Percentage of ESBLs positive Isolates in different studies**

| S. no | **Study groups** | **Place of study & Year** | **Total No. of samples** | **ESBls isolated** |
|---|---|---|---|---|
| 1. | Bithika Duttaroy *et al.;* [35] | Baroda, (2005) | 187 | 53(28.34%) |
| 2. | Ananthan S *et al.;* [68] | Chennai, (2005) | 98 | 24(24.50%) |
| 3. | MS Kumar *et al.;* [38] | Hyedrabad,(2006) | 1699 | 14(20%) |

| | | | | |
|---|---|---|---|---|
| 4. | T Menon *et al.;* [37] | Chennai ,(2006) | 70 | 336 (19.8%) |
| 5. | S Hoşoğlu *et al.;* [39] | Turkey ,(2007) | 104 | 83(79.83) |
| 6. | Ami Y. Varaiya *et al.;* [40] | Mumbai,(2008) | 134 | 31(23.13) |
| 7. | Husam S. Khanfar *et al.;* | Saudi Arabia, (2009) | 6750 | 409 (6%) |
| 8. | Dinesh S.Chandel *et al.;* [43] | Rourkela ,(2010) | 252 | 42(16.66%) |
| 9. | Helene Garrec *et al.;* [28] | France ,(2011) | 107 | 52(49%) |
| 10. | Bandekar N *et al.;* [58] | Devangere, (2011) | 83 | 33(39.8%) |
| 11. | Ritu Nayar *et al.;* [61] | Delhi ,(2012) | 50 | 20(39.6%) |
| 12. | Nibedita Das *et al.;* [45] | Assam (2012) | 171 | 42(24.56%) |
| 13. | Mita D.Wadekar *et al.;* [69] | Mysore, (2013) | 100 | 43(43%) |
| 14. | Meeta Sharma *et al.;* [47] | Jaipur ,(2013) | 722 | 379 (52.49%) |
| 15. | Mohd Nasir khan *et al.;* | Jaipur ,(2014) | 200 | 87(43.5%) |
| 16. | **Present Study** | **Jaipur ,(2015)** | **71** | **36(50.26)** |

As seen in the table a very high variability is observed in the percentage of ESBLs producing strains throughout India. The causative reasons may be as follows for this could be ---

- Prevalence of different ESBL producing strains in different areas of country,
- Different clinical samples being included in different studies
- (C) Studies being conducted on different age groups of patients,
- Better infection control measures at some places and
- Most importantly, difference in the methods used for detection of ESBL production.
- ESBL producing strains was (50.70%) in the present study which correlates well with the study of Wadekar DM *et al [22]*.

In the present study MBL production was found to be 7.04 %. On the contrary in various other studies production rate varies from 2% to 50%.

**Table 4: Percentage of MBL positive Isolates in different studies**

| S.NO | Study groups | Place of study & Year | Total No. of samples | MBL isolated |
|---|---|---|---|---|
| 1. | Haque S F | Aligarh 2011 | 170 | 20 (12.20%) |
| 2. | Pandya N | Gujarat 2011 | 27 (MDR) | 26(96.30%) |
| 3. | Datta S | New Delhi 2012 | 77,618(10 yr) | 2.4-52 % |
| 4. | Ejikeugwu P.C., | Nigeria 2012 | 79 | 2 (2.25%) |
| 5. | Enwuru V.N *et al.;* | Lagos Nigeria 2011 | 14 (MDR) | 7 (50%) |
| 6. | *Sai Gopal et al.;* | Karnataka 2013 | 13 (MDR) | 6 (46.15%) |
| 7. | Patwardhan N.S | Maharashtra 2013 | 135 | 13(9.63%) |
| 8. | Tellis R | Mangalore 2013 | 100 (MDR) | 74(74%) |
| 9. | Altun S | Turkey 2013 | 78 | 0 |
| 10. | Chakraborty A | Karnataka 2013 | 300 | 15(5%) |
| 11. | Wadekar *et al.;* | Karnataka 2013 | 100 | 16(16%) |
| 12. | Present study | Jaipur 2015 | 71 | 5 (7.04%) |

**Table 5: - Comparison of resistance pattern of the ESBLs and Non-ESBL producing strains to various antibiotics to other study**

| S. No | **Study Group** | **Sai Gopal *et al.;* (2013)** | | | **Present study (2015)** | | |
|---|---|---|---|---|---|---|---|
| | **Antimicrobials** | **ESBL (n=37)** | **Non ESBLs (143)** | P-value | **ESBL (n36)** | **Non-ESBLs (35)** | p-value |
| 1. | ***AMIKACIN*** | 27.03% | 22.12% | **<0.05** | **41.61%** | **31.42 %** | **<0.05** |
| 2. | ***AZTREONAM*** | 94.59% | 38.94% | **>0.05** | **66.66 %** | **42.85%** | **<0.05** |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 3. | ***CEFOPERAZONE/ SALBACTUM*** | - | - | - | **22.22 %** | **22.85 %** | **>0.05** |
| 4. | ***COTRIMOXAZE*** | 97.30% | 61.06% | **<0.05** | **66.66%** | **62.85 %** | **<0.05** |
| 5. | ***IMIPENEM*** | 0.00% | 0.00% | **<0.05** | **0%** | **14.28%** | **>0.05** |
| 6. | ***CEFEPIME*** | 97.30% | 2.65 % | **<0.05** | **47.23%** | **28.58 %** | **<0.05** |
| 8. | ***CEFTAZIDIME*** | 94.59% | 33.63% | **<0.05** | **100 %** | **42.86%** | **<0.05** |
| 9. | ***CEFTAZIDIME/ CLAVULANATE*** | - | - | - | **44.55 %** | **17.15 %** | **<0.05** |
| 10. | ***CEFUROXIME*** | - | - | - | **75 %** | **68.58 %** | **>0.05** |
| 11. | ***AMPICILLIN*** | - | - | - | **58.34 %** | **45.72 %** | **<0.05** |
| 12. | ***NITROFURANTOIN*** | - | - | - | **22.23 %** | **20 %** | **>0.05** |
| 13. | ***NORFLOXACIN*** | - | - | - | **72.23 %** | **71.43 %** | **>0.05** |
| 14. | ***CEFTRIAXONE*** | 91.89% | 37.17% | <0.05 | **58.34 %** | **42.86 %** | **<0.05** |
| 15. | ***MEROPENAM*** | - | - | - | **8.34%** | **22.86%** | **>0.05** |
| 16. | ***PIPERACILLIN/ TAZOBACTUM*** | 91.89% | 39.82% | <0.05 | **47.23 %** | **20 %** | **<0.05** |

Among the 50 carbapenem resistant isolates, 39 (78%) were found to be MBL positive by the E-test strip method. Nineteen (38%) of the MBL producers were sensitive to the cefoperazone-sulbactam combination and the aminoglycosides. Only seven (14%) MBL-producers were sensitive to the ampicillin-clavulanic acid combination. Thirteen percent of the MBL producers were sensitive to piperacillin-tazobactum combination and only 11% showed sensitivity to tetracycline group.

Among the 13 Imipenem resistant isolates [13] 100% were positive for the MBL production by the IMP+EDTA method, and in present study 5 (7.04%) were found resistant and these are tasted for MBL production and all were positive for MBL production (100%) like this study [33]. Interestingly, 5 of the reported MBL-producers had increased zone. These results showed that both methods are sensitive and specific in detecting MBL producer, this is in agreement with Clare *et al*.; in 2006, who reported on DDST and a combined disk test MBL detection methods in which they correctly detected 51 out of 52 PCR-confirmed MBL-negative isolates. Their report showed, using PCR that the sensitivity and specificity of the phenotypic MBL detection method was 100% and 98%, respectively [34]. As described above, no single method demonstrated absolute positive results for MBL detection, and therefore, we recommend the combination of both techniques. There are reports of MBL production among gram negative organisms from various countries like India, Pakistan [35] Brazil [36] and Korea [37]. This enzyme (MBL) was first reported as a zinc dependent enzyme in *Bacillus cereus* in mid-1960 [36]. A few decades later imipenem hydrolyzing metallo-enzymes was described in *Aeromonas hydrophila* [38] and *Bacteroides fragilis*. Our study has demonstrated a high level of resistance in *E.coli* to most of antibiotics tested within the environment studied [39]. The outcome of this study may be associated with excessive use of broad-spectrum antibacterial agents. Thus, early detection of the occurrence of this enzyme using this simple methodology replicated in this study will smack off a control strategy. The awareness of the existence of MBL initializes indication for the need for proper use of antibiotics to stem selective pressure and spread of MDR bacterial strains within these hospital and communities. Continued surveillance of MBL enzymes within these settings is a novel tool to provide the necessary information for care handlers and policy progenitors on the nature and spread of this type of resistance [40].

**CONCLUSION:**

The present study was undertaken for Detection of ESBL & MBL producing E. coli from urine samples in a tertiary care hospital, in the Department of Microbiology, NIMS Medical College Jaipur.

A Total of 71 E. Coli was isolated from urine samples which were included in our study .The study were carried out over period of six months from January 2015 to May 2015.

The maximum percentage of isolation was seen among the age group 21-30 years (21.12%) in females.

In the present study female patients show more prevalence of gram negative bacilli infections than male. *E.coli 71* (47.33%) was the most common

organism isolated from urine samples followed by *Klebsiella spp. 31*(21.33%), *Citrobacter spp.17* (11.33%), *Pseudomonas spp.15* (10*%), Aceinetobacter 5*(3.33%), *Proteus spp.4 (*2.66%) *Enterobacter spp*.3 (2%) staphylococci 2(1.33%) and candida albicans 1(0.66%) was the least common organism isolated.

Among *E.coli* the highest resistance was given by ceftazidime (71.84%) followed by Cefuroxime (71.84%), norfloxacin (71.84), Cotrimoxazole (64.78%), Aztreonam (54.92%), Ampicillin (52.12%), ceftriaxone (50.71%) and Cefepime (38.03%).

Minimum resistance was show against Ceftazidime clavulanic acid (30.99%), Amikacin (36.61%), Nitrofurantoin (21.13%), Piperacillin tazobactum (33.81%), Meropenam (15.49%), Cefoperazone salbactum (22.53%) and least (7.04%) resistance was given by imipenem.

Out of the 71 isolates of E. Coli 36 (50.70%) were ESBLs producing.

Out of the 71 isolates of E. coli 5 (7.04%) were MBL producing.

In ESBLs producers group maximum susceptibility is seen to imipenem, meropenam, (100%, 91.66% respectively) followed by Cefoperazone salbactum (77.77%), Nitrofurantoin (77.77 %), Amikacin (58.33 %), Ceftazidime clavulanate (55.55 %), Piperacillin tazobactum (52.77%), Aztreonam (33.33%) followed by Cotrimoxazole (33.33%) and ceftazidime (00.00%).

Among 5 MBL producing E. Coli showed 100% resistance to amikacin, aztreonam, ampicillin, norfloxacin, ceftazidime, cefuroxime, cefepime and Imipenem resistance. But some of them like Piperacillin tazobactam (20%), Nitrofurantoin (20%), Ceftazidime clavulanic acid 1 (20%) and Cefoperazone salbactum 1 (20%) were sensitive to MBL producing E. coli

Thus to conclude ESBL-producing E. coli is a growing risk for infection in the community. To control the emergence and spread of the new multidrug-resistant E. coli, it is essential for the public to practice good hygiene habits and to comply with recommendations on the proper use of antibiotics. The combined disc diffusion method which is simple, inexpensive method for the detection of ESBL can be used by laboratories for detection of ESBL along with implementation of appropriate infection control practices, and to prescribe appropriate chemotherapeutic agents. And also early detection of MBL-producing isolates would be important for the reduction of mortality rates for patients infected with MBL producing isolates and also to avoid the intra hospital dissemination of such strains 'Imipenem-EDTA combined disk test' (CDST-IPM) is the most sensitive method for detection of MBL

The present study shows higher rate of resistance to antimicrobials in a tertiary care hospital, which is the result of the irrational use of antibiotics and this irrational use of antibiotics bring us to a point, as frightening as the pre antibiotic era for patients infected with multidrug-resistant gram negative bacteria, where there will be no magic bullet available for treatment. The identification of the infective organism up to species level along with the mechanism of resistance in diagnosis helps in the judicious use of chemotherapeutic agents effective against them, which will withdraw the selection pressure and resistant bacteria will no longer have survival advantage in such settings.

In the end, it is felt that there is a need to formulate strategies to prevent and detect the emergence of resistance for an effective treatment of the infections which are caused by them. There is a need of concerted efforts on the part of academic researchers and their institutions, industry, and government is crucial if humans are to maintain the upper hand in this battle against bacteria - a fight with global consequences. Otherwise, we may reach to era, where "Bad bugs, there is no drugs".

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