ORIGINAL RESEARCH PAPER

Medical Microbiology

# EVALUATION OF EXTENDED SPECTRUM BETA LACTAMASE (ESBL) PRODUCING ENTEROBACTERIACEAE FAMILY IN ICU SETTINGS IN TERTIARY CARE CENTRE IN SOLAPUR

**KEY WORDS:** Extended spectrum ß-lactamases (ESBLs), Enterobacteriaceae, antibiotic resistance, ICU.

**Dr. Shaikh Najmeen** Service Resident, Department of Microbiology, Dr. V.M.G.M.C. Solapur, Maharashtra, India.

**Dr. Suresh Kandle** Professor and Head

**Dr. Prakash Waghmare*** Assistant Professor. *Corresponding Author

**ABSTRACT**

**Introduction:** Resistant bacteria are emerging worldwide as a threat to favorable outcome of common infections in community and hospital settings1. Beta lactamase production by several gram negative and gram-positive organisms is perhaps the most important single mechanism of resistance to penicillins and cephalosporins1. Among the wide array of antibiotics, beta-lactams are most varied and widely used agents accounting for over 50 % of all systemic antibiotics in use2. The incidence of these beta lactamases ranges from 1.8-74% worldwide3 and in India the prevalence ranges from 6.6- 68% 4. So, it is necessary to know the prevalence of resistance pattern of bacterial strains and to formulate a policy of empirical therapy and to take preventive measures in hospital settings. **Materials and Methods:** The present study was aimed at knowing the prevalence of Extended Spectrum β-Lactamase (ESBL) in the isolates belonging to Enterobacteriaceae family which were obtained from ICU patients. A total 220 gram negative isolates from 1050 clinical samples which were received over a period of one year were processed for their identification and their antimicrobial susceptibility pattern was determined. They were then screened for the ß-lactamase production. **Results:** Among 220 isolates, ESBL production was obtained in 88 (40%) strains. Major ESBL producing Enterobacteriaceae bacteria was Klebsiella spp. (37%) followed by E. coli (34.54%). **Conclusion:** The high prevalence of the ESBL in the ICU isolates emphasizes the need for a continuous surveillance in the ICUs to detect the resistant strains, strict guidelines for the antibiotic therapy and the implementation of infection control measures to reduce the increasing burden of antibiotic resistance.

## INTRODUCTION

Enterobacteriaceae, a large group of facultative gram-negative rods, are common pathogens of healthcare and community associated infection worldwide. Emergence of multidrug resistance in Enterobacteriaceae is a major public health threat which poses a great challenge to combat infections[1].

B lactam antibiotics are among the most commonly prescribed antimicrobials in ICUs which is due to their broad spectrum, efficacy and less toxicity. In ICU, the incidence of hospital acquired infections and antibiotic resistance are in rise, due to their clinical disease with altered immunity and use of invasive procedures and indiscriminate use of empirical antibiotics[2]. The emergence of antimicrobial drug resistance is a major public health issues and threat to treatment failure. Now the increasing frequency of ESBL producing organisms are of concern due to the treatment failure and it may lead to complication, morbidity and mortality[2].

Infection by ESBL producing Enterobacteriaceae are the most important among the causes of infections in community and hospital in the recent years and are in rising trends[1]. ESBL is the most important mechanism of resistance to penicillin's and cephalosporins (B-lactam antibiotics). ESBLs are enzymes secreted by bacteria and these are capable of hydrolyzing all b-lactam drugs except cephamycin and carbapenem. This resistance is encoded by transferrable conjugative plasmid. Such organism producing these enzymes become multidrug resistant leading to limited therapeutic options for infection[3]. ESBL detection is important as knowledge about its prevalence is helpful to formulate infection control measure and to prevent its spread. This study was undertaken to document the prevalence and resistance pattern of ESBL producing Enterobacteriaceae and to helps in implementing an effective antibiotic policy.

## MATERIALS AND METHOD:

This study was prospective study carried out in microbiology dept at tertiary care center in Solapur done over a period of 18 month from Dec 2018 to May 2020. All the specimens collected according to standard procedures and transported without delay to microbiology dept for routine culture and sensitivity test from medicine ICU. Clinical isolates belonging to enterobacterales were included in this study. All the isolates were identified by standard microbiological tests. Antimicrobial susceptibility testing of isolates was determined by Kirby Bauer disc diffusion method according to CLSI guidelines (2018).

### Detection of ESBL[12]:

**Screening Test for ESBLs:** An inoculum of 0.5 Mc Farland standard turbidity was prepared on a nutrient broth from an isolated colony taken from 18-24 hour agar plates. A sterile swab was dipped into the nutrient broth within 15 minutes of preparing the inoculum and inoculated onto a dried and sterile Mueller Hinton Agar (MHA) plate. The antibiotics were applied to the surface of the plate after 3-5 minutes of inoculation. The discs were pressed firmly against the surface of the plate and distributed evenly so that the minimum distance between the discs was 24 mm. The plates were inverted and incubated aerobically at 37°C overnight. If any of the isolates had zone of inhibition for 3rd generation cephalosporins i.e., Cefpodoxime (10µg)≤ 17mm, ceftazidime (30 µg) ≤ 22mm, aztreonam (30µg) ≤ 27mm, cefotaxime (30µg) ≤27mm and ceftriaxone (30µg) ≤ 25mm (ESBL breaking point) as well as strains which were resistant were taken as screen positive for ESBL. All strains found to be ESBL screen test positive were subjected to further confirmation by CLSI recommended phenotypic confirmatory tests.

### Phenotypic Confirmatory Disc Diffusion Test (PCDDT)[12]:

The strains screened positive for ESBL production were tested by PCDDT for confirmation. Discs of ceftazidime (CAZ-30µg) and ceftazidime with clavulanic acid (CAC-30/10µg) and cefotaxime (CTX- 30µg) and cefotaxime-clavulanic acid (CTX/C-30/10µg) were dispensed at a minimum distance of 24mm on an MHA agar plate inoculated with the lawn culture of the isolate screened positive for ESBL production and

incubated aerobically at 37°C overnight. If there was an increase in zone size by ≥ 5mm with ceftazidime/clavulanic acid and cefotaxime-clavulanic acid in comparison to ceftazidime or cefotaxime alone, then the strain was confirmed to be an ESBL producer.

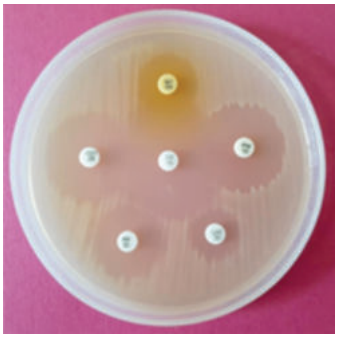

**Photo 1: Screening test for ESBL : Antibiotic susceptibility testing by Kirby Bauer Disc Diffusion method**

**Photo 2: ESBL detection by Phenotypic Confirmatory Disc Diffusion Test**

**RESULTS:**

Total 220 isolates were recovered from different clinical specimens. Majority of the isolates were from respiratory specimens (51.36%) followed by urine 50 (22.7%), blood 42 (19%), CSF 12 (5.4%), 7(3.18%) from wound swab and pus, and 6 (2.7%) isolates from other samples (peritoneal fluid, Drain fluid, Ascitic fluid, Catheter tip.

The commonly isolated gram negative bacteria were *Klebsiella spp*. 82(37%), followed by *E.coli* which constituted for 76 (34.54%), *Citrobacter spp*. - 36 isolates (16.36%), *Enterobacter spp*. - 24 (10.09%), and *Proteus spp*-2 (0.9%) of total isolates.

**Table no. 1 :**

| sr. no. | Organism | Number | Percentage |
|---|---|---|---|
| 1 | *E.coli* | 76 | 34.54% |
| 2 | *Klebsiella spp.* | 82 | 37.27% |
| 3 | *Citrobacter spp.* | 36 | 16.36% |
| 4 | *Enterobacter spp.* | 24 | 10.00% |
| 5 | *Proteus spp.* | 2 | 0.90% |

Among 220 isolates, 88 strains were ESBL producers (40%)

The major ESBL production was observed in *Klebsiella* species (n=39) were followed by *E.coli* (n=35).

Table no. 3 Shows antibiotic resistance pattern of ESBL producers. These strains were also showing resistance to ciprofloxacin

All ESBL producing isolates were resistant to 3rd generation cephalosporins. And all were sensitive to Imipenem, 61.3 % sensitive to piperacillin-tazobactam, 60 % to amikacin, and 51.1% to Ciprofloxacin

**Table 2: ESBL production in various organisms**

| | **ESBL** |
|---|---|
| *E.coli* | 35(39.7%) |
| *Klebsiella spp.* | 39 (44.3%) |
| *Enterobacter spp.* | 8 (9%) |
| *Citrobacter spp.* | 5 (5.6%) |
| *Proteus spp.* | 1(1.13%) |
| Total | 88 |
| Statistics applied | Chi square: 73.13, Df=4, P<0.01 |

**Table no 3: Sensitivity among ESBL producer**

| Sensitivity among ESBL producer | N= 88 | % |
|---|---|---|
| AMK | 53 | 60.2% |
| GEN | 30 | 34% |
| CIP | 45 | 51% |
| COT | 36 | 40.9% |
| PI | 27 | 30.68% |
| PIT | 54 | 61.3% |
| IPM | 88 | 100% |
| CTX | 0 | 0 |
| CAZ | 0 | 0 |
| CTX | 0 | 0 |

**DISCUSSION:**

Bacteria from Enterobacteriaceae family are often associated with different human infections like urinary tract infections, respiratory tract infections, blood stream infections, meningitis, endocarditis, skin, soft tissue and bone infections, etc[10].

Bacteria are the main cause of health care associated infections, among them most frequently isolated are gram-negative bacteria. Many of them are responsible for serious infections, and are difficult to treat, not only because serious patients are hospitalized but also because of the overuse of antibiotics and development of resistance among bacteria.

Among the most important pathogens, the frequently isolated are from the Enterobacteriaceae family. Members of the Enterobacteriaceae family are part of the microbiota of the gastro-intestinal tract of mammals. The main bacteria isolated from this family being Escherichia coli which is responsible mainly for urinary tract infections. Among others, *Klebsiella spp*. and *Enterobacter spp*. are frequently isolated from patients with pneumonia. The remaining members of Enterobacteriaceae are associated with the blood stream, soft tissue and intra-abdominal infections. The emergence of antibiotic resistance and spread of antibiotic resistance in Enterobacteriaceae complicates the treatment of serious infections[15].

In present study, an attempt has been made to find out the occurrence of ESBL in the isolates belonging to Enterobacteriaceae family and their antibacterial susceptibility pattern.

Various clinical specimens from the patients admitted in medicine ICUs were analyzed for the microbiological investigation. Respiratory specimens like sputum, ETT, tracheal secretion, pleural fluid (51.36%) were the most frequently analyzed specimens followed by Urine (22.7%) and blood (19%). Other specimens analyzed were CSF (5.4%), wound swab and pus (3.1%). In Sah et al[10], Shahzad et al[16], maximum analyzed specimens were urine followed by blood and respiratory specimens. In Loveena O et al[11], Muneesh et al[14], maximum analyzed specimens were urine followed by respiratory specimens.

In India, percentage of ESBL producers ranges from 6% to 85%. 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. In our study, we found 40% isolates to be ESBL producers. Similar findings were seen in study by Loveena O et al[11] (35.16%.) Thongjum et al[7] found ESBL producers only in 26.3% of isolates. Shukla et al[9] found ESBL producers in 30.18% of isolates. This variation

in the prevalence of the ESBL production could be due to the differences in the geographical distribution, which may have produced variations in the prevalence of the β-lactamases and these variations may be present in the different organisms with varied resistance patterns. The only β-lactams which were active against the and the ESBL producers were the carbapenems. However, recently, the resistance to the carbapenems is increasing, which is mostly due to the production of the Metallo β-lactamases.

**ESBL production in various organisms:**

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. In present study, *Klebsiella species* (44.8%) was maximum ESBL producer followed by *E.coli* (39.4%). Percentage of ESBL in *E.coli* is higher in Sujata et al102 69%, 56.25% in Loveena et al[11]. While lower percentage of ESBL in *E.coli* is observed in Vasumati et al[6] 29.1%, Sujata et al[13] 30% Higher percentage ESBL in *Klebsiella spp*. was seen in Neema et al[17] 48.27%. and lower percentage of ESBL in *Klebsiella spp*. was observed in Loveena et al[11] 15.6%.

**Antibiotic Susceptibility pattern**

In present study, all ESBL producers were 100% resistance to 3rd generation cephalosporins, and 100% sensitive to Imipenem, 61.3% sensitive to piperacillin tazobactam, 60% sensitive to amikacin, 51% to Ciprofloxacin, 40.9% cotrimoxazole and 34% gentamycin. In study by Nipa S et al[8], ESBL producers were most sensitive to Imipenem followed by amikacin and piperacillin-tazobactam. Also, in study by Shukla et al[9], ESBL producers were 100% sensitive to Imipenem followed by amikacin and Ciprofloxacin. In the study by Vasumati et al[6] and Shukla et al[9], ESBL producers were resistance to 3rd generation cephalosporins.

From the present study, it is seen that there is an increased resistance for 3rd generation Cephalosporins like Cefotaxime, Ceftazidime and also Ceftriaxone. So, the increasing resistance to Cephalosporins in recent years forced us to search for Beta-Lactamase producers. The incidence of ESBL strains among clinical isolates have been steadily increasing over the past few years resulting in difficulty in treating the patient. Therefore, the regular detection of ESBLs should be carried out in every laboratory. Based on the prevalence rate of the ESBL producers in a healthcare facility, antibiotic policy of the institution can be tailored to achieve superior therapeutic outcome and reduction in the morbidity and mortality rate. It also minimizes misuse of conventional cephalosporins in a significant proportion of patients.

**CONCLUSION:**

High morbidity and mortality rates associated with severe infections in the critically ill patients continue to be a significant issue for the healthcare system. The incidence of infections due to organism resistance to Beta-lactam agents due to production of various enzymes has increased in recent years. Detection of ESBL production is of paramount importance both in hospital and community acquired infections. So, vigilance and timely recognition of the infections with resistance bacteria is necessary to start the appropriate antibiotic therapy. In this study, most of the isolates were resistance to beta lactams and non-beta lactam antibiotics (75 % of all the isolates). This is because of injudicious use of beta lactams antibiotics and other higher antibiotics for the treatment of infections caused by isolates belongs to Enterobacteriaceae family. So judicious use of antibiotics, formation of Hospital antibiotic policy, implementation of appropriate infection control measures in hospital are necessary in preventing the spread of these multidrug resistance bacteria in medical ICU unit.

**REFERENCES:**


1. Chaudhary, U., & Aggarwal, R. (2004). Extended spectrum β-lactamases (ESBL)–An emerging threat to clinical therapeutics. *Indian journal of medical microbiology*, *22*(2), 75-80.
2. Agrawal, P., Ghosh, A. N., Kumar, S., Basu, B., & Kapila, K. (2008). Prevalence of extended-spectrum β-lactamases among Escherichia coli and Klebsiella pneumoniae isolates in a tertiary care hospital. *Indian Journal of Pathology and Microbiology*, *51*(1), 139.
3. Gupta, V. (2007). An update on newer beta-lactamases. *Indian Journal of Medical Research*, *126*(5), 417.
4. Menon, T., Bindu, D., Kumar, C. P. G., Nalini, S., & Thirunarayan, M. A. (2006). Comparison of double disc and three dimensional methods to screen for ESBL producers in a tertiary care hospital. *Indian Journal of Medical Microbiology*, *24*(2), 117-120.
5. Adhikari, R. P., Shrestha, S., Rai, J. R., & Amatya, R. (2018). Antimicrobial resistance patterns in clinical isolates of enterobacteriaceae from a tertiary care hospital, Kathmandu, Nepal. *Nepalese Medical Journal*, *1*(2), 74-78.
6. Vasumathi, A., PR, T. V., & Senthamarai, S. Extended Spectrum β Lactamase (ESBL) Producing Uropathogens in the Intensive Care Unit in A Tertiary Care Hospital, Tamilnadu.
7. Chanu, T. R., Shah, P. K., Soni, S., & Ghosh, A. N. Phenotypic detection of extended spectrum, AmpC, Metallo beta-lactamases and their coexistence in clinical isolates of commonly isolated gram negative bacteria in GKGH hospital, Bhuj.
8. Singh, N., Pattnaik, D., Neogi, D. K., Jena, J., & Mallick, B. (2016). Prevalence of ESBL in Escherichia coli isolates among ICU patients in a tertiary care hospital. *Journal of clinical and diagnostic research: JCDR*, *10*(9), DC19.
9. Shukla, I., Tiwari, R., & Agrawal, M. (2004). Prevalence of extended spectrum β-lactamase producing Klebsiella pneumoniae in a tertiary care hospital. *Indian journal of medical microbiology*, *22*(2), 87-91..
10. Sah, B. S., Aryal, M., Bhargava, D., & Siddique, A. (2017). Drug resistance pattern of bacterial pathogens of Enterobacteriaceae family. *Tribhuvan University Journal of Microbiology*, *4*, 15-22.
11. Oberoi, L., Singh, N., Sharma, P., & Aggarwal, A. (2013). ESBL, MBL and Ampc β lactamases producing superbugs–Havoc in the Intensive Care Units of Punjab India. *Journal of clinical and diagnostic research: JCDR*, *7*(1), 70.
12. CLSI, Performance standard for Antimicrobial susceptibility testing 28th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standard Institute;2018
13. Sujatha, R., Deepak, S., & Nashra, A. Detection of ESBL and Co-production of AmpC in various samples by DDST, PDCT, MDDST method at a tertiary care hospital in Kanpur.
14. Sharma, M. K., Bisht, D., & Pal, S. (2019). Detection of Metallo-β-lactamase producing Gram Negative Bacteria in clinical isolates in Tertiary care Hospital-A prospective study. *Int Arch Integ Med*, *6*(4), 107-11.
15. Mahaluca, F. A., Essack, S., Zimba, T., & Sacarlal, J. (2019). Profile of Antibacterial Resistance of the Enterobacteriaceae Family in Pediatric and Adult Patients. *Ann Clin Immunol Microbiol. 2019; 1 (2), 1007*.
16. Mirza, S., Jadhav, S., Misra, R. N., & Das, N. K. (2019). Coexistence of β-lactamases in community-acquired infections in a tertiary care hospital in India. *International journal of microbiology*, *2019*.
17. Shashwati, N., Kiran, T., & Dhanvijay, A. G. (2014). Study of extended spectrum β-lactamase producing Enterobacteriaceae and antibiotic coresistance in a tertiary care teaching hospital. *Journal of natural science, biology, and medicine*, *5*(1), 30.