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# EXTENDED SPECTRUM BETA LACTAMASE (ESBL) PRODUCING BACTERIA: AN EMERGING THREAT TO SOCIETY

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**ABSTRACT** **Aims and objective**-Incidence of ESBL-producing Enterobacteriaceae in urine sample collected from suspected patients of UTI attending out patient department in a tertiary care center, sagar (M.P) **MATERIAL AND METHODS** This was a single centre, hospital (in-patient) based, Prospective cross-sectional study done in Department of Microbiology, Bundelkhand Medical College, and affiliated hospitals, Sagar, Madhya Pradesh. It is a tertiary care institute.The total duration of the study was four months; from 27/06/2022 to 27/10/2022.a total of 458 urine samples from the suspected patients of UTI attending out patient department in a tertiary care center ,sagar (M.P.).The isolates were identified by colony characteristics, gram staining and suitable biochemical tests. Assessment by laboratory culture and antibiotic sensitivity testing was done according to CLSI guidelines. **RESULTS AND OBSERVATIONS** Out of 458 samples, 275 samples were culture positive. Escherichia coli was the most predominant organism with 170 (62%) isolates followed by Klebsiella sp(25%), Citrobacter sp(8%), Proteus sp(5%). Of the total isolates, Escherichia coli were highest ESBL producers i.e 83 (49%) followed by klebsiella sp 32%, Citrobacter sp – 28%, Proteus sp – 19%. **CONCLUSION** There was a high prevalence of ESBL-producing Enterobacteriaceae and multi drug resistant isolates. The most frequent ESBL-producing Enterobacteriaceae were E. coli and Klebsiella sp . A higher level of resistance to multiple classes of antibiotics was observed among ESBL producers compared with non-ESBL producers.

**KEYWORDS :**

## INTRODUCTION

Extended-spectrum ß-lactamases (ESBLs) are group of plasmid-mediated, diverse, complex and rapidly evolving enzymes that are posing a major therapeutic challenge today in the treatment of hospitalized and community-based patients. Infections due to ESBL producers range from uncomplicated urinary tract infections to life-threatening sepsis.ESBLs are a rapidly evolving group of ß-lactamases which share the ability to hydrolyze third-generation cephalosporins and aztreonam but are inhibited by clavulanic acid. Extended-spectrum β-lactamase (ESBL) producing bacteria are the serious health problems due to their emerging resistance to antibiotics. ESBLs represent an impressive example of the ability of gram-negative bacteria to develop new antibiotic-resistance mechanisms in the phase of introduction of new antimicrobial agents.Thus there is need for efficient infection-control practices for containment of outbreaks; and intervention strategies, e.g. antibiotic rotation to reduce further selection and spread of these increasingly resistant pathogens.

Extended-spectrum β-lactamases (ESBLs) are a group of plasmid-mediated, diverse, complex and rapidly evolving enzymes that are posing a major therapeutic challenge today in the treatment of hospitalized and community-based patients. Infections due to ESBL producers range from uncomplicated urinary tract infections to life-threatening sepsis. Derived from the older TEM is derived from Temoniera, a patient from whom the strain was first isolated in Greece. β-lactamases, these enzymes share the ability to hydrolyze third-generation cephalosporins and aztreonam and yet are inhibited by clavulanic acid. In addition, ESBL-producing organisms exhibit co-resistance to many other classes of antibiotics, resulting in limitation of therapeutic option. Because of inoculum effect and substrate specificity, their detection is also a major challenge. At present, however, organizations such as the Clinical and Laboratory Standards Institute (formerly the National Committee for Clinical Laboratory Standards) provide guidelines for the detection of ESBLs in Klebsiella pneumoniae, K. oxytoca, Escherichia coli and Proteus mirabilis. In common to all ESBL-detection methods is the general principle that the activity of extended-spectrum cephalosporins against ESBL-producing organisms will be enhanced by the presence of clavulanic acid. Carbapenems are the treatment of choice for serious infections due to ESBL-producing organisms, yet carbapenem-resistant isolates have recently been reported. ESBLs represent an impressive example of the ability of gram-negative bacteria to develop new antibiotic-resistance mechanisms in the face of the introduction of new antimicrobial agents. Thus there is need for efficient infection-control practices for containment of outbreaks; and intervention strategies, e.g., antibiotic rotation to reduce further selection and spread of these increasingly resistant pathogens.

## MATERIAL AND METHODS

**STUDY DESIGN:** This was a single centre, hospital (in-patient) based, Prospective cross-sectional study done in Department of Microbiology, Bundelkhand Medical College, and affiliated hospitals, Sagar, Madhya Pradesh. It is a tertiary care institute.The total duration of the study was four months; from 27/06/2022 to 27/10/2022.

Collection of sample/specimen for assessment:a total of 458 urine samples from the suspected patients of UTI attending out patient department in a tertiary care center ,sagar (m.p) were collected and processed. The isolates were identified by colony characteristics, gram staining and suitable biochemical tests. Assessment by laboratory culture and antibiotic sensitivity testing was done according to CLSI guidelines.

1. Primary smear examination: A smear is prepared and stained by Gram-staining method.
2. Isolation and identification: The isolates are identified by colonial morphology, Gram's stain, and conventional biochemical test.
3. For bacteria identification: Specimen is streaked onto the surface of Blood agar and McConkey's agar. The plates incubated at 37°C overnight for 18-24 hours aerobically and growth is examined.

## IDENTIFICATION OF THE CAUSATIVE AGENT:

1. Secondary smear examination;The smear made from bacterial culture is air dried and then heat fixed and Gram staining was done.
2. Biochemical tests:Based on the type of organisms observed in secondary gram staining, appropriate biochemical tests are

employed.Initially, catalase and oxidase tests are done on all the types of colonies grown on the media.

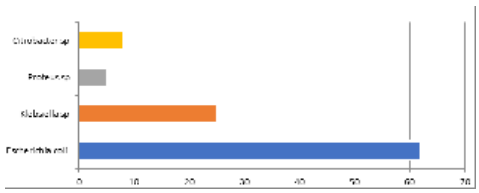
For gram-negative bacilli: Common biochemical tests routinely done are: Indole test, Citrate utilization test, Urea hydrolysis test, Triple sugar iron test (TSI), MR (methyl red) test, OF test (oxidation-fermentation test), Nitrate reduction test.

## DETECTION OF ESBL PRODUCERS

Antimicrobial susceptibility testing was performed using Kirby Bauer disc diffusion method. Screening of ESBL production was done using ceftriaxone and confirmation of ESBL production was done by three different 3rd generation cephalosporin/clavulanate combinations. inoculum of 0.5 McFarland dilutions of all test strains, negative control and positive control were prepared in 3 ml of normal saline. Streak a lawn of all dilution of all test strains, negative control and positive control to a Mueller Hinton agar plate and allow to dry 3–5 minutes. After drying, an amoxicillin-clavulanic acid (20/10 mg) disc was placed at the center of the plate. A disc of ceftazidime (30 mg) and ceftriaxone (30 mg) were placed one on either side of amoxicillin-clavulanic acid (20/10 mg) disc at a distance of 15 mm from center to center. All plates were incubated at 35°C ± 2°C in incubator for 16–24 hours. E.coli (ATCC 25922)and K.pneumoniae (ATCC 700603) producing beta-lactamase were used as Negative control and positive control. After the incubation of 24 hr the culture plate was studied for zone of inhibition in an area between ceftriaxone and amoxicillin clavulanic acid in comparison with the zone of inhibition on the far side of these drugs was taken as a positive.

## RESULTS AND OBSERVATIONS

Out of 458 samples, 275 samples were culture positive. Escherichia coli was the most predominant organism with 170 (62%) isolates followed by Klebsiella sp(25%), Citrobacter sp(8%), Proteus sp(5%). Of the total isolates, Escherichia coli were highest ESBL producers i.e 83 (49%) followed by klebsiella sp 32%, Citrobacter sp – 28%, Proteus sp – 19%. Of the three different 3rd generation cephalosporin/clavulanate combinations used, ceftazidime/clavulanate combination was found to be most effective for phenotypic confirmation of ESBL producers.




## DISCUSSION

In our study, rate of ESBL production was similar to the rates of ESBL production in other studies and maximum ESBL production was detected by CAZ/ CAZ+CV combination which correlated with other studies.

The magnitude of ESBL-producing Enterobacteriaceae in our study was comparable with a studies in Bahir-Dar-Ethiopia (57.6%) [1], Burkina Faso (58.0%) [2], Uganda (62.0%) [3], Ghana (49.3%) [4], and Karnataka-India (57.5%) [5]. One of the most important factors in the emergence of ESBLs production is the selective pressure caused by the use of 3rd generation cephalosporins [6]. Lack of antibiotic surveillance, antibiotics misuse, and weak infection control measures may also contribute to the high magnitude of ESBL.

## CONCLUSION

There was a high prevalence of ESBL-producing Enterobacteriaceae and multi drug resitant isolates. The most frequent ESBL-producing Enterobacteriaceae were E. coli and Klebsiella sp . A higher level of resistance to multiple classes of antibiotics was observed among ESBL producers compared with non-ESBL producers.

The rise of MDR and ESBLs necessitates the strengthening of clinical bacteriology research and the diagnostic capacity of laboratory professionals for the detection and surveillance of antibiotic resistance. We recommend routine screening of ESBLs production of Enterobacteriaceae along with strong infection prevention strategies.

We recommend to use ceftazidime/clavulanate combination for phenotypic confirmation of ESBL producers. Routine ESBL testing for uropathogens along with conventional antibiogram would be useful for proper early management of all the cases of urinary tract infections.

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