International Journal of Pharmacy and Pharmaceutical Sciences

Print ISSN: 2656-0097 | Online ISSN: 0975-1491 Vol 17, Issue 6, 2025

Original Article

BURDEN OF *KLEBSIELLA PNEUMONIAE* AND *ESCHERICHIA COLI* ISOLATES THAT PRODUCE EXTENDED-SPECTRUM BETA-LACTAMASES AT THE TERTIARY CARE HOSPITAL IN UTTAR PRADESH, LUCKNOW

NEETI MISHRA*, DAYAVANTI KUMARI, KHYATI TIWARI

Department of Microbiology, T. S. Misra Medical College and Hospital Uttar Pradesh, India
***Corresponding author: Neeti Mishra; *Email: neetitripathi26@yahoo.com**

Received: 07 Apr 2025, Revised and Accepted: 08 May 2025

ABSTRACT

Objective: To ascertain the prevalence of *Escherichia coli* isolates and *Klebsiella pneumoniae* that generates Extended-Spectrum Beta-Lactamases (ESBLs) in the tertiary care hospital in Lucknow, Uttar Pradesh.

Methods: Screening tests have been developed by the Clinical Laboratory Standard Institute (CLSI) to identify the *Klebsiellla pneumoniae* and *Escherichia coli* that generate ESBLs. Finally, performing combination disk test on probable ESBL-producing isolates, ESBL phenotypic confirmation was established.

Results: 94 (62.7%) of the 150 ESBL-positive isolates were female, while 56 (37.3%) were male. The largest concentration of *E. coli* which was positive for ESBL production was from urine, and it also shows the lowest concentrations coming from sputum, blood, and CSF samples and the largest concentration of ESBL-positive *Klebsiella pneumoniae* was found in sputum, followed by urine, pus, and blood, while the lowest concentration was found in a CSF sample. *E. coli* had 158 of the 267 isolates, and of them, 80 were ESBL positive. Similarly, 70 of the 109 *Klebsiella pneumoniae* isolates tested were ESBL positive.

Conclusion: *E. coli* had an ESBL prevalence of 53.34% in the current study, while *K. pneumoniae* had 46.7% prevalence. *Klebsiella pneumoniae and Escherichia coli* that produce ESBLs were found in relatively high numbers in the urine, sputum, pus, ET aspirate, and blood samples. The doctors must follow stringent infection control procedures at the hospital and judicious antimicrobial usage guidelines.

Keywords: Extended-spectrum beta-lactamase, Beta-lactamase

DOI: https://dx.doi.org/10.22159/ijpps.2025v17i6.54499 Journal homepage: https://innovareacademics.in/journals/index.php/ijpps

INTRODUCTION

The most typical method of treating bacterial infections is using β-lactam antimicrobials, which also continue to be the main source of β-lactam antibiotic resistance among Gram-negative bacteria globally. Numerous beta-lactams have been continually exposed to different bacterial strains, resulting in dynamic, on-going creation and mutation of enzymes in these bacteria. This has increased these bacteria's activity even against recently developed beta-lactam antibiotics. Extended-spectrum β-lactamases (ESBLs) are the name given to these enzymes [1-3]. The various drug resistances of these organisms make treatment difficult. Due to a number of factors, including the difficulties in detecting ESBL production and inconsistent reporting, it is challenging to quantify the occurrence of ESBL-producing organisms at the level of a larger geographic scale [4]. Recent surveys revealed a marked rise in the rate of ESBLs everywhere in the world [5-12]. The Clinical Laboratory Standard Institute (CLSI) recommends routine reporting and testing for the main infections that continue to be found worldwide that produce ESBLs: *Klebsiella pneumoniae* and *Escherichia coli* [13, 14]. ESBL prevalence varies from institution to institution. Previous studies from India and internationally suggest that there might be anywhere from 8 to 80% production of ESBL. However, the antimicrobial resistance patterns and the frequency of ESBL production in *Klebsiella pneumoniae* isolates have not been extensively studied. Therefore, the primary goal of the current investigation is to determine the prevalence of *Klebsiella pneumoniae* and *Escherichia coli* isolates that produce ESBLs in the tertiary care hospital.

MATERIALS AND METHODS

The Institutional Ethics Committee (IEC) gave its clearance for the study, which was carried out in the Microbiology Department at T. S. M. Medical College, Lucknow, U. P., India, from January 2018 to February 2020. (Reference No. TSMMC and H/STC/952/2021)

Inclusion criteria

Only those patients who gave their permission and agreed to take part in the study.

Exclusion criteria

Individuals who are not interested in participating in the study and those who have systemic illness.

Data collection

The prospective investigation was conducted by the Microbiology Department of T. S. Misra Medical College and Hospital located in Amausi, Lucknow, Uttar Pradesh. A total of 384 different clinical samples were gathered in sterile containers that came from various departments. Samples that included isolated *Klebsiella pneumoniae* and *E. coli* were used in this experiment. All samples-whether from outpatient or inpatient—were included during the study period. Each patient's Performa was filled out after a thorough history was gathered, including information on age, sex, and medical history. According to CLSI recommendations, ESBL detection was carried out.

Processing of clinical samples

All clinical samples were cultured on routine culture media and then processed after an overnight incubation period at 37 °C. For the blood sample, brain heart infusion broth was incubated at 37 °C overnight. A drop of the broth from the Brain Heart Infusion was employed as an inoculum on routine culture media. These agars were then incubated overnight at 37 °C. The blood sample was deemed negative if bacterial colonies were not visible after seven days. Only isolates of *Klebsiella pneumoniae* and *Escherichia coli* that were collected from clinical specimens were taken into account in this experiment as a pure and predominant growth. The biochemical reactions and colony morphology of the organisms were used to identify them [15].

Screening for ESBL production

Screening tests have been developed by the CLSI to identify the *Klebsiella pneumoniae* and *E. coli* that generate ESBLs [14]. If a strain showed an inhibitory zone of ≤22 mm for Ceftazidime, ≤27 mm for Cefotaxime, and ≤25 mm for Ceftriaxone, it was chosen for confirmatory ESBL testing based on CLSI criteria.

ESBL production confirmation test

Phenotypic confirmation by combination disk test [16]. By performing phenotypic tests on probable ESBL-producing isolates, ESBL production was established. A lawn culture of the inoculum was created, and 25 mm-apart discs of Ceftazidime (30 µg) and Ceftazidime+Clavulanic acid (30 µg+10 µg) were used. Ceftazidime+Clavulanic acid, as ESBL makers, demonstrated about a 5 mm increase in the zone of inhibition in comparison to Ceftazidime alone. Isolates of ESBLs that were Cefoxitin-resistant were not considered for the investigation. This is done in order to eliminate related Amp C beta-lactamases [17, 18].

RESULTS

From January 2019 to December 2021, 384 clinical samples in total were examined for culture and sensitivity. Out of these, 267 samples demonstrated growth of *Klebsiella pneumoniae* and *E. coli.* Table 1 shows the gender-wise distribution of ESBL producers and ESBL non-producers in clinical isolates. 94 (62.7%) of the 150 ESBL-positive isolates were female, while 56 (37.3%) were male.

Table 1: Gender wise distribution

Gender	ESBL producer	Non-ESBL producer	Total
Female	94	72	166
Male	56	45	101
Total	150	117	267

Table 2: Dispersal pattern of the clinical isolates

Type of samples	Total samples	*Escherichia coli*	*Escherichia coli* positive for ESBL	*Klebsiella pneumoniae*	*Klebsiella pneumoniae* positive for ESBL	Total
Mid-stream urine	64	28	16	22	12	50
Sputum	79	38	14	19	14	57
Pus	58	16	09	16	10	32
CSF	15	02	01	06	05	08
Blood	47	23	12	11	09	34
Endotracheal aspirate	38	18	10	10	07	28
Body fluids	44	19	08	13	08	32
High vaginal swab	39	14	10	12	05	26

Table 2 indicates that the largest concentration of *E. coli* that was positive for ESBL production was from urine, and it also shows the lowest concentrations coming from sputum, blood, and CSF samples. The largest concentration of ESBL-positive *Klebsiella pneumoniae* was found in sputum, followed by urine, pus, and blood, while the lowest concentration was found in a CSF sample.

Table 3: ESBL prevalence

Organism	Total	ESBL
Klebsiella pneumoniae	109	80 (53.34%)
E. coli	158	70 (46.66%)
Total	267	150

Prevalence of ESBL is described in table 3. *E. coli* had 158 of the 267 isolates, and of them, 80 were ESBL positive. Similarly, 70 of the 109 *Klebsiella pneumoniae* isolates tested were ESBL positive.

DISCUSSION

Many scientific studies have examined the incidence of *K. pneumoniae* strains that produce ESBL, for example, in India, where many groups have documented a significant burden of these pathogenic strains. In the current investigation, the prevalence of ESBLs in *E. coli* was 53.34%, while that in *Klebsiella pneumoniae* was 46.66%. The percentage of bacteria that produce ESBLs in India varied from 4% to 83% [19, 20]. Maharashtra has a lower proportion of ESBL producers, according to Rodrigues *et al.* [21]. Out of 47 *K. pneumoniae* isolates, four (8.5%) were positive ESBL producers, according to their findings. At this moment, the production of ESBL should have increased in the same location, and this ratio most likely indicates their early stages. This makes sense because there are a number of variables that affect the frequency of extended-spectrum beta-lactamase producers in hospitals, such as the institution's antibiotic policies, the infection rates among staff, and the types of disinfectants used, particularly in the intensive care unit [22]. ESBL isolates were more prevalent in females in the current study. Gupta S. *et al.* provides similar results [23]. Studies by Mendelson G *et al.* and Bazzaz B *et al.* indicate that males are more susceptible to ESBL production [24, 25] ESBL-producing *Klebsiella* species have also been observed in recent years in the USA (42-44%) and Canada (4.9%), Turkey (78.6%), Spain (20.8%), Taiwan (28.4%), Algeria (20%), and China (51%) [26]. There are significant geographic variations in the occurrence of ESBLs when targeting the epidemiology in Europe. According to recent research involving 1,610 E. coli and 785 *K. pneumoniae* isolates from 31 sites across ten European countries, the incidence of ESBL in these organisms varied significantly, ranging from 1.5% in Germany to 39–47% in Russia, Poland, and Turkey [27]. *E. coli* had an ESBL prevalence of 53.34%, and *K. pneumoniae* had 46.7% prevalence. In comparison to an Indian investigation [28], this found that about 40% of urine isolates of *E. coli* and *K. pneumoniae* were ESBL positive, this value was noticeably higher. Latin America (54.4%), the western Pacific (24.6%), and Europe (22.6%) were the regions with the highest reported isolation rates of *K. pneumoniae* generating ESBLs. 8.5%, 7.8%, and 5.3%, respectively, of *E. coli* in these regions were observed to produce ESBLs.

LIMITATIONS OF THE STUDY

ESBL production's molecular characterisation could not be investigated because of the lack of resources. The participants in this study were restricted to one hospital. The results might therefore not be generalizable to other regions.

CONCLUSION

E. coli had an ESBL prevalence of 53.34% in the current study, while *K. pneumoniae* had 46.7% prevalence. *Klebsiella pneumoniae and Escherichia coli* that produce ESBLs were found in relatively high numbers in the urine, sputum, pus, ET aspirate, and blood samples. The doctors must follow stringent infection control procedures at the hospital and judicious antimicrobial usage guidelines. Clinical laboratories must regularly and routinely monitor the presence of clinical isolates that produce ESBLs.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

Drs. Neeti and Daya were responsible for creating the conceptual framework, the draft, and the data analysis. As Dr. Khyati wrote the manuscript and oversaw the final round of editing, Dr. Daya also helped with data collection and analysis.

CONFLICT OF INTERESTS

Declared none

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