Publisher homepage: www.universepg.com, ISSN: 2663-7529 (Online) & 2663-7510 (Print)
https://doi.org/10.34104/ejmhs.024.044049
European Journal of Medical and Health Sciences
Journal homepage: www.universepg.com/journal/ejmhs

Multidrug-Resistant ESBL-Producing *Enterobacteriaceae* Associated with Clinical Samples in a Tertiary Care Hospital, Sirajganj

Summiya Shamima Prity[1], Kakoli Akter[1], Md. Babul Aktar[1], Kaniz Mehzabin[1], Laila Jarin[2], Rasheda Yasmin Shilpi[2], Md. Farukh Faisal Ashrafi[3], Abdullah Akhtar Ahmed[1], Jafrul Islam[4], and Mohammad Zakerin Abedin[1,2]*

[1]Department of Microbiology, Khwaja Yunus Ali University, Bangladesh; [2]Microbiology Laboratory, Department of Botany, Jahangirnagar University, Savar, Dhaka, Bangladesh; [3]Department of Microbiology, Asgar Ali Hospital, Bangladesh; and [4]Department of Microbiology, Uttara Cresent Hospital, Bangladesh.

*Correspondence**:** akerin.abedin.mb@kyau.edu.bd (Mohammad Zakerin Abedin, Assistant Professor & Head, Department of Microbiology, Khwaja Yunus Ali University, Sirajganj, Bangladesh).

ABSTRACT

Extended-spectrum β-lactamase (ESBL) microorganisms have been shown to increase multidrug resistance globally, which is a great concern. The prevalence of ESBL-producing clinical pathogens and their antimicrobial resistance patterns were identified in 66 isolates from patients in Khwaja Yunus Ali Hospital with several clinical infections cultured on blood agar and MacConkey agar media. The most prevalent bacteria were *Escherichia coli* (80.3%), which were identified by the BD-Phonex automated identifier machine, followed by *Pseudomonas spp*. (6%), *Klebsiella spp. (12.1%),* and others (4.4%). This research was conducted from January 2023 to June 2023. Generally, a large number of antibiotic resistance patterns and ESBL-producing common bacterial isolates were found in this study, where most of the resistant percentage was found in third-generation cephalosporin antibiotics, which increases the public health problem. In this study, the most prevalent bacteria *E. coli* isolates were mostly resistant to penicillin (100%), ceftazidime, cefotaxime, and cefuroxime (98.4%). Besides, 95.4% resistance was shown against ceftriaxone. The double-disc synergy test was done to determine the presence of ESBL-producing bacterial strains. The most widely ESBL-positive isolate was *Escherichia coli* (83%). Among the 66 sample strains produced, the ESBL maximum (53.03%) belonged to female patients, while 46.97% belonged to male patients. This study focuses on the prevalence and patterns of clinical pathogens and the antimicrobial susceptibility profiles of ESBL-producing bacterial infections in a tertiary-level health service center in Bangladesh. Generally, a large number of antibiotic resistance patterns and ESBL-producing common bacterial isolates were found in this study, which increases the public health risk. Therefore, to save human life, we ought to be taking appropriate action against the threat.

Keywords: Extended-spectrum β-lactamase, Bacterial profiles, Multidrug-resistant, and Antibiotics.

INTRODUCTION:

Extended-spectrum beta-lactamase (ESBL)-producing bacteria have expanded dramatically globally, and they are one of the leading causes of morbidity and mortality in hospital-acquired infections (Kumar *et al*., 2014). This could be explained by the presence of the multidrug resistance in ESBL-producing isolates. Resistant bacteria are growing around the world as a challenge to the successful treatment of common diseases in both hospitals and the community (Prestinaci *et al.*, 2015). The most frequent infections contracted in hospitals caused by *Enterobacteriaceae* include urinary tract, gastrointestinal, and pyogenic infections *(*Atici *et al.,*

2016). *Escherichia coli* is the most commonly isolated species among the *Enterobacteriaceae* (Karlowsky *et al.,* 2003; Shahen *et al*., 2019).

Multidrug resistance in the *E. coli* is extensively documented (Karlowsky *et al*., 2003). Prolonged antibiotic exposure, hospitalization, severe sickness, unprecedented usage of third-generation cephalosporin (Kumar *et al.*, 2014; Tewari *et al*., 2018) and increased use of intravenous devices or catheters are all risk factors for multidrug-resistant *E.coli* infection. Lactamase synthesis is possibly the single most important mechanism of penicillin and cephalosporin resistance (Tang *et al*., 2014). *E. coli* naturally produces chromosomally-mediated or the plasmid-mediated lactamases (Poirel *et al*., 2002). Penicillin-binding proteins are considered to have evolved into these enzymes (Hakenbeck, 1998). This evolution was most likely caused by the selection pressure applied by lactamase-producing soil organisms in the environment (Allen *et al*., 2009). Extended-spectrum beta-lactamases (ESBLs), enzymes that accelerate the hydrolysis of oxyimino-lactams such as cefotaxime, ceftriaxone, ceftazidime, and aztreonam, have recently been described (Jacoby, 1997).

They are members of the Ambler molecular class A and the Bush-Jacoby functional group 2be (Sawa *et al*., 2020). These enzymes have been identified in considerable quantities in diverse places and have been found to be abundant in several *E. coli* strains (Sawa, Kooguchi *et al.,* 2020). They have also been discovered in other *Enterobacteriaceae* members, including *Klebsiella spp, Citrobacter spp*, *Enterobacter spp*, *Proteus spp*, and non-lactose fermenters such as *Pseudomonas aeruginosa* (Abbott, 2011). Over 200 distinct ESBLs have been described to date (Kumari, 2017). Major epidemics of these resistant organisms in the several members of the *Enterobacteriaceae* and *Pseudomonas spp*. have been documented all over the world, resulting in a lack of therapeutic choices (Chaudhary, 2004).

ESBL-producing bacteria are likely more common than is currently known because they frequently go unnoticed by standard susceptibility testing methods (MP *et al*., 2010). Resistance to other non-lactam antibiotics, such as aminoglycosides and chloramphenicol, has been linked to ESBL strains (Pitout *et al*., 1997). Another characteristic of these ESBL strains is that they may exhibit a falsely sensitive zone of inhibition when tested using the Kirby-Bauer disk diffusion method (Kumar *et al*., 2014). To comprehend the disease burden and take the appropriate precautions to avoid its spread, current knowledge of the prevalence of ESBL generation by commonly isolated pathogens such as *E. coli* is required (Kumar *et al*., 2014). As a result, the current investigation was carried out with the goal of determining the prevalence of ESBL-producing *Enterobacteriaceae* and their antimicrobial resistance profile in order to develop an effective antibiotic strategy and plan.

MATERIALS AND METHODS:

Sample collection

Bacterial isolates from clinical samples such as pus, urine, blood, wound swab, throat, sputum, ear swab, and other bodily fluids were obtained in the department of microbiology at Khwaja Yunus Ali Medical College and Hospital (KYAMCH). The research was conducted from January 2023 to June 2023. Khwaja Yunus Ali University's Ethics Committee granted ethical permission.

Isolation and identification

20 ml of urine samples collected in a universal container and one loopful (0.002 ml) were inoculated into the chromogenic UTI agar, MacConkey agar, and blood agar plates. For the blood sample, we used a Bactec^{TM} BD-M15 (USA) automated machine along with a positive signal, which was then sub-cultured on MacConkey and Blood Agar media. Other specimens, such as sputum, body fluids, and swabs, were collected in sufficient amounts and then inoculated on the blood agar and MacConkey agar plates using an inoculating loop. All inoculated media were incubated aerobically overnight at 37°C. On the basis of colony morphology, the organisms were identified, and biochemical analyses were performed using oxidase test, Kligler Iron Agar stain, motility indole urea, and Simon citrate reaction, etc.

Antimicrobial Susceptibility Test

Antimicrobial susceptibility testing (AST) for all isolates was conducted on commercially available common antibiotics disc. All ESBL and non-ESBL producing clinical pathogen were studied for antimicrobial sensitivity using disc diffusion technique by "Kirby-Bauer method" on the culture medium of Mueller-Hinton agar (Himedia, India) and interpretations were recorded according to the guidelines of clinical and laboratory standard institute.

Testing for the ESBL Production

The identification of ESBLs production by 66 positive clinical bacterial pathogens was conducted by a modified double-disc synergism test (Ahmed *et al*., 2017). Bacterial suspension of 0.5 McFarland standards was plated in Muller-Hinton agar with the Amoxycillin+clavulanic acid (30 µg) disc in between and 20 mm apart from Ceftazidime (30 µg) and Ceftriaxone (30 µg) discs. Expansion of the zone of inhibition around Ceftriaxone and/or ceftazidime disc towards the amoxicillin-clavulanic acid disc was considered ESBL production.

Statistical analysis

Chi-square test is used for statistical analysis of the data. A 'P value' less than 0.05 were considered statistically significant.

RESULTS:

During the research period, 66 clinical pathogens were detected in various clinical samples of inpatients. In 66 sample isolates, phenotypic identification of ESBL production revealed that *E. coli* was the most common (80.3%), followed by *Klebsiella spp*. (12.1%), *P. aeruginosa* (3%), and others (4.5%). The bulk (45%) of the 66 sample strains developed belonged to female patients, while the other 55% belonged to male patients. In terms of patient age groups, females over the age of 49 had the highest prevalence (47%), followed by those aged 13-24 years (3%), and those aged 37-48 years (19%). In men, the age group was 37-48 years (33%), the age group was 25-36 years (17%), and the remaining 13% were in the age group of 13-24 years (**Table 1).**

ESBL Prevalence and the Antimicrobial Susceptibility Profiles

Number and frequency of ESBL producing bacteria, *E.coli, Klebsiella spp* as well as *Pseudomonas spp* found highest percentage in urine sample presented in **Table 2**. Overall antibiotic resistant pattern for ESBL-producing bacteria among all the 15 tested antibiotics. Where Amikacin (22.7%), Amoxicillin (42.4%), Imipenem (46.9%), Meropenem (33.3%), and Nitrofurantoin (40.9%) showed less resistance than the other tested antibiotics. The rest of the antibiotics were mostly above 90% resistant as well as.

In this study, the most prevalent bacteria, namely *E. coli* isolate, showed mostly resistance to penicillin (100%), ceftazidime, cefotaxime, and the cefuroxime (98.4%). Besides, the 95.4% resistance was shown against ceftriaxone presented in the **Table 3**. The resistance rates for the aminoglycoside and carbapenem groups of antibiotics were quite less than those for the third generation of antibiotics, where amikacin, gentamicin, imipenem, meropenem, and nitrofurantoin were 16.7%, 57.5%, 39.4%, 33.3%, and 31.8%, respectively.

Table 1: Demographic data of study population.

Age range	Female (%)	Male (%)
0-12	3	7
13-24	3	13
25-36	28	17
37-48	19	33
>49	47	30
Total	100	100

Table 2: Number and Frequency (%) of ESBL producing bacteria.

ESBL producing bacteria	Biological samples				
	Urine	Pus	Blood	Sputum	Swab
E. coli	33(50%)	11(16.7%)	3(4.5%)	1(1.5%)	5(7.6%)
Klebsiella spp.	4(6%)	-	-	2(3%)	2(3%)
Pseudomonas spp.	2(3%)	-	-	-	-
Salmonella enterica	-	-	1(1.5%)	-	-
Staphylococcus spp.	-	-	-	-	1(1.5%)
Streptococcus spp	-	-	-	-	1(1.5%)

Table 3: Results of Antibiotic Susceptibility pattern of bacteria.

Bacterial isolate	Pattern	AK	AMC	CPM	AMP	CAZ	CTR	CXM	CTX	ATM	GEN	IPM	MEM	NIT	P	SXT
E. coli (n=53)	S	79.25	75.48	1.9	9.44	1.89	5.66	1.89	3.78	18.36	56.6	51	58.5	56.4	0	26.42
	R	20.75	24.52	98.1	90.56	98.11	94.34	98.11	96.22	81.14	43.4	49	41.5	43.4	100	73.58
Klebsiella spp. (n=8)	S	87.5	25	25	12.5	12.5	25	0	0	75	75	62.5	62.5	63	0	50
	R	12.5	75	75	87.5	87.5	75	100	100	25	25	37.5	37.5	38	100	50
Salmonella (n=1)	S	0	0	0	100	0	0	0	0	100	0	100	100	100	0	0
	R	100	100	100	0	100	100	100	100	0	100	0	0	0	100	100

Streptococcus (n=1)	S	100	100	0	0	0	0	0	100	0	100	100	100	0	100	0
	R	0	0	100	100	100	100	100	0	100	0	0	0	0	0	100
Staphylococcus (n=1)	S	0	100	0	0	0	0	0	100	100	100	100	100	100	0	0
	R	100	0	100	100	100	100	100	0	0	0	0	0	0	100	100
Pseudomonas (n=2)	S	50	100	0	0	0	0	0	0	50	100	0	50	50	0	0
	R	50	0	100	100	100	100	100	100	50	0	100	50	50	100	100

- ❑ Ceftriaxone(CTR)
- ❑ Amoxiclave (AMC)
- ❑ Amikacin (AK)
- ❑ Cefuroxime(CXM)
- ❑ Nitrofurantoin(NIT)
- ❑ Imipenem(IPM)
- ❑ Cephradine(CTX)
- ❑ Ampicillin(AMP)
- ❑ Ceftazidime(CAZ)
- ❑ Aztreonam (ATM)
- ❑ Cefotaxime(CPM)
- ❑ Gentamycin(GEN)
- ❑ Meropenem(MEM)
- ❑ Penicillin(P)
- ❑ Sulfamethoxazole (SXT)

DISCUSSION:

For physicians around the world, extended-spectrum β-lactamase-expressing multidrug-resistant clinical pathogens provide major obstacles to the therapeutic management of clinical cases of urinary tract infection. The goal of the current investigation was to show that an ESBL-producing bacterium could be isolated from clinical samples of patients in a major hospital in Bangladesh. It was found that 66% of pathogens produced ESBLs. *E. coli* (80.3%) produced ESBLs more frequently than *Psedomonas spp.* (3%), or *Klebsiella spp.* (12%). This figure was reported in a study completed in Khartoum State hospitals by Mekki *et al*. (2010) who recorded ESBL production among *E. coli* and *Klebsiella* species isolates as 66%. Similarly, a few numbers of ESBL-producing *E. coli* (36%), Egypt, occurred during 2013-14 (ElSayed *et al*., 2023). The high ranges of 41.0 to 63.6 percent in *E. coli* were reported for the prevalence of ESBL production in other studies in India (Grover *et al.,* 2006). The most well-known pathogens, *E. coli*, are thought to be part of the normal flora in the genitourinary and digestive tracts. However, they can ascend the urethra and enter the urinary tract. *E. coli* has explicit virulence characteristics that allow it to adhere to and attack host cells, create toxins, consume supplements, and evade the host's immune system *(*Abedin *et al*., 2020).

ESBLs pose a significant risk to β-lactam treatment. Many of these bacterial isolates have been incorrectly reported to be susceptible to the widely used broad-spectrum beta-lactams because they are difficult to detect using existing clinical procedures (MacKenzie *et al.,* 2002). We found such an associated resistance with gentamicin (36%) and the flouroquinolones (67%). Gupta *et al*. (2007) reported 91.17% and 94.91% resistance, respectively, to gentamicin and ciprofloxacin in the ESBL producers. Compared with our previous studies done at Khwaja Yunus Ali Medical College & Hospital, the current investigation found lower resistance rates for the majority of the isolated *E. coli*, which were resistant to Meropenem (50%) and Amikacin (66%), followed by gentamicin (34%), amoxiclav (40%), and Ciprofloxacin (57.8%) (Ahmed *et al.,* 2016). This decreased drug resistance indicates successful coordinated monitoring of drug activity and usage. Overall, these results show that ESBL production in bacterial species varies significantly over the world and changes quickly over time and space.

Study limitations

This study carries several basic limitations. This study analyzed only a few ESBL producing bacteria and the use limited b-lactam antibiotics. The small sample size was also a limiting factor in performing fully powered statistical analyses. However, our results were generated from a Resource - limited setting and maintained internal validity by repeating independent experiments where necessary.

CONCLUSION:

In this investigation, the incidence and trends of ESBL and the non-ESBL-producing uropathogens against routinely prescribed antibiotics in clinical isolates from tertiary institutions in Bangladesh were examined. This discovery emphasizes how crucial it is to continuously monitor and program antibiotic resistance in our hospitals. It also demonstrated the necessity of creating strategies to lessen the occurrence of clinical infections that produce ESBLs. Patients infected with ESBL-producing bacteria must be treated with the proper antibiotics since ESBLs are clinically significant. Finally, the results of numerous studies differed depending on location and time, raising concerns about their validity and

making it challenging to draw comparisons between them. In order to reduce the overall rate of resistance, it is necessary for observation methods and routine surveillance to be normalized throughout the nation. These results call for immediate surveillance and action to prevent the introduction of ESBL-producing bacteria on a national and international scale.

ACKNOWLEDGEMENT:

We are grateful to the Chairman of the Trusty Board and the Director of Khwaja Yunus Ali Medical College Hospital for carrying out this study. We express gratitude toward Md. Abdul Karim and Mazharul Haque for assisting in laboratory work at the Department of Laboratory Services of Khwaja Yunus Ali Medical College & Hospital, Sirajganj, Bangladesh.

CONFLICTS OF INTEREST:

There is no conflict of interest among the authors.

REFERENCES:

1) Ahmed, A. A., Hasan, S. A., & Abedin, M. Z. (2021). Patterns of extended-spectrum β-lactamase producing uropathogens detection in tertiary Hospital of Bangladesh. *Am. J. Pure Appl. Biosci*, **3**(2), 29-34. https://doi.org/10.34104/ajpab.021.029034
2) Ahmed, Abdullah Akhtar, Shakhaowat Hossain, and SM Ali Hasan. (2016). "Prevalence of Methicillin Resistant *Staphylococcus Aureus* In Khwaja Yunus Ali Medical College Hospital." *KYAMC Journal,* **7**(1), 673-677.
3) Abbott, S. L. (2011). *Klebsiella*, enterobacter, citrobacter, serratia, plesiomonas, and Other Enterobacteriaceae. *Manual of clinical microbiology*, 639-657.
4) Allen, H. K., Moe, L. A., & Handelsman, J. (2009). Functional metagenomics reveals diverse β-lactamases in a remote Alaskan soil. *The ISME journal*, **3**(2), 243-251.
5) Abedin, M Z., *et al*. (2020). Predominance of nosocomial pathogens among patients with post-operative wound infections and evaluation of the antibiotic susceptibility patterns in rural hospitals in Bangladesh. *Recent adv biol med*, **6**(4), 17990.
6) Atici, S., Soysal, A., & Bakır, M. (2016). Healthcare-associated infections in a newly opened pediatric intensive care unit in Turkey: Results of four-year surveillance. *The Journal of Infection in Developing Countries*, **10**(03), 254-259.
7) 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.
8) ElSayed, N., El-Attar, L., & Amine, A. (2023). Prevalence of antimicrobial-resistant bacteria in conventional vs. organic livestock farms in Egypt: a cross-sectional comparative study. *Letters in Applied Microbiology*, **76**(1). https://doi.org/10.1093/lambio/ovac048
9) Hakenbeck, R., & Coyette, J. (1998). Resistant penicillin-binding proteins. *Cellular and Molecular Life Sciences CMLS*, **54**, 332-340.
10) Grover, S. S., Sharma, M., & Singh, G. (2006). Phenotypic and genotypic detection of ESBL mediated cephalosporin resistance in *Klebsiella pneumoniae*: emergence of high resistance against cefepime, the fourth generation cephalosporin. *J. of Infection*, **53**(4), 279-288.
11) Gupta, V., Singla, N., & Chander, J. (2007). Detection of ESBLs using third & fourth generation cephalosporins in double disc synergy test. *Indian J. of Medical Research,* **126**(5), 486-487.
12) Jacoby, G. A. (1997). Extended-spectrum β-lactamases and other enzymes providing resistance to oxyimino-β-lactams. *Infectious disease clinics of North America*, **11**(4), 875-887. https://doi.org/10.1016/s0891-5520(05)70395-0
13) Karlowsky, J. A., Jones, M. E., & Sahm, D. F. (2003). Trends in antimicrobial susceptibilities among Enterobacteriaceae isolated from hospitalized patients in the United States from 1998 to 2001. *Antimicrobial agents and chemotherapy*, **47**(5), 1672-1680.
14) Kumar, D., Singh, A. K., and Chander, Y. (2014). Antimicrobial susceptibility profile of extended spectrum β-lactamase (ESBL) producing *Escherichia coli* from various clinical samples. *Infectious Diseases: Research and Treatment*, **7**, IDRT-S13820.
15) Kumari, S., & Sharma, S. (2017). Extended-spectrum Beta-lactamases in Urinary Isolates of *Escherichia coli*, *Klebsiella Pneumoniae* and other Gram-negative Bacteria in Tertiary Care. *Inter. Journal of Contemporary Microbiology*, **3**(2).

16) MP, S., Yashavanth, R., and Narendra, N. (2010). Detection of extended spectrum beta-lactamase production and multidrug resistance in clinical isolates of *E. coli* and *K. pneumoniae* in Mangalore. https://doi.org/10.7860/JCDR/2010/.748

17) Mekki, A. H., Hassan, A. N., & Elsayed, D. E. M. (2010). Extended spectrum beta lactamases among multi drug resistant *Escherichia coli* and *Klebsiella* species causing urinary tract infections in Khartoum. *J. of Bacteriology research*, **2**(3), 18-21.

18) MacKenzie, F. M., Miller, C. A., & Gould, I. M. (2002). Comparison of screening methods for TEM-and SHV-derived extended-spectrum β-lactamase detection. *Clinical microbiology and infection*, **8**(11), 715-724.

19) Pitout, J. D., Sanders, C. C., & Sanders Jr, W. E. (1997). Antimicrobial resistance with focus on β-lactam resistance in gram-negative bacilli. *The American j. of medicine*, **103**(1), 51-59. https://doi.org/10.1016/s0002-9343(97)00044-2

20) Poirel, L., Kämpfer, P., and Nordmann, P. (2002). Chromosome-encoded Ambler class A β-lactamase of Kluyvera georgiana, a probable progenitor of a subgroup of CTX-M extended-spectrum β-lactamases. *Antimicrobial agents and chemotherapy*, **46**(12), 4038-4040. https://doi.org/10.1128/AAC.46.12.4038-4040.2002

21) Prestinaci, F., P. Pezzotti and A. Pantosti (2015). "Antimicrobial resistance: a global multifaceted phenomenon." *Pathogens and global health,* **109**(7), 309-318.

22) Sawa, T., K. Kooguchi and K. Moriyama (2020). "Molecular diversity of extended-spectrum β-lactamases and carbapenemases, and antimicrobial resistance." *J. of intensive care,* **8**, 1-13.

23) Shahen MZ, Mahmud S, Uddin ME and Alam MS. (2019). Effect of antibiotic susceptibility and inhibitory activity for the control of growth and survival of microorganisms of extracts of *C. officinalis*, *Eur. J. Med. Health Sci*. **1**(1), 1-9. https://doi.org/10.34104/ejmhs.0190109

24) Tang, S. S., A. Apisarnthanarak and L. Y. Hsu (2014). "Mechanisms of β-lactam antimicrobial resistance and epidemiology of major community-and healthcare-associated multidrug-resistant bacteria." *Advanced drug delivery reviews,* **78**, 3-13.

25) Tewari, R., S. D. Mitra, F. and B. R. Shome (2018). "Prevalence of extended spectrum β-lactamase, AmpC β-lactamase and metallo β-lactamase mediated resistance in *Escherichia coli* from diagnostic and tertiary healthcare centers in south Bengaluru, India." https://www.msjonline.org/index.php/ijrms/article/view/4604

Citation: Prity SS, Akter K, Aktar MB, Mehzabin K, Jarin L, Shilpi RY, Ashrafi MFF, Ahmed AA, and Abedin MZ. (2024). Multidrug-resistant ESBL-producing *Enterobacteriaceae* associated with clinical samples in a tertiary care hospital, Sirajganj, *Eur. J. Med. Health Sci.*, **6**(1), 44-49. https://doi.org/10.34104/ejmhs.024.044049