JPSIM Journal of Pakistan Society of Internal Medicine

Original Article

Detection of Extended Spectrum Beta Lactamase Producing Enterobacteriaceae and Their Antibiotic Susceptibility Testing

Javairia Saeed,[1] Asghar Javaid,[1] Rashda Shabbir,[2] Romah Ashfaq,[3] Blossom Neelam,[1] Sumera Malik[2]

[1]Nishtar Medical University Multan, [2]CMH Multan Institue of Medical Sciences Multan, [3]Multan Medical and Dental College Multan

Abstract

Objective: To determine the antibiotic susceptibility pattern of Enterobacteriaceae and incidence of extended-spectrum beta-lactamases-producing Enterobacteriaceae.

Methods: This descriptive study consisted of a sample size of 200 Enterobacteriaceae isolates, was conducted in the Microbiology section of the Pathology department of Nishtar Medical University Multan from November 2020 to November 2021 . Blood, urine, and pus specimens were collected from patients of all age groups and either sex, after taking informed written consent. Identification was carried out by conventional biochemical tests and confirmed by Analytical Profile Index 20 E. Kirby-Bauer disc diffusion method was used to evaluate the sensitivity of bacterial isolates against different antibiotics. Identification of Extended Spectrum Beta Lactamase (ESBL) production was carried out by double-disc diffusion phenotypic confirmatory method.

Results: Among 200 culture-positive samples for Enterobacteriaceae, 77(38.5%) were from pus samples, 51(25.5%) were from blood and 72(36.0%) were from urine samples. The highest frequency of 111(55.5%) was identified as Eschercia coli. ESBL positive strains of enteric bacteria revealed in 29.5% of organisms. Patterns of drug susceptibility in ESBL negative showed highest resistance against Cefuroxime and Cefotaximeas 125/141 and 118/141 respectively while 59/59 for each in ESBL positive group.

Conclusion: A high proportion of ESBL- producing isolates belonging to Enterobacteriaceae were obtained from pus samples considering wound infections followed by urine and blood. The frequency of Eschercia coli remained the highest among ESBL and non-ESBL producer Enterobacteriaceae followed by Proteus and Enterobacter in this study. Meropenem and amikacin remained highly effective drugs for both groups followed by chloramphenicol and piperacillin/tazobactam.

Keywords: Resistance, Antibiotic, ESBL, β-lactamases, E. coli, Enterobacteriaceae.

How to cite this:

Saeed J, Javaid A, Shabbir R, Ashfaq R, Neelam B, Malik S. Detection of Extended Spectrum Beta Lactamase Producing Enterobacteriaceae and Their Antibiotic Susceptibility Testing.J Pak Soc Intern Med. 2024;5(1): 427-432

Corresponding Author: Dr. Javairia Saeed **Email:** :javariasaeed98@yahoo.com

Introduction

Enterobacteriaceae are non-sporing gram negative bacteria. Other than non-motile *Klebsiella* and *Shigella*, all have uniformly distributed flagella over the body. The pathogenic genera such as *Escherichia coli(E. coli), Citrobacter, Shigella, Serratia, Proteus, Enterobacter, and Klebsiella,* are a member of the large family *Enterobacteriaceae*.[1] Multidrug resistance is a well-known trait of *E.coli*. Long-term antibiotic exposure, hospitalization, severe sickness, first-time use of third-generation cephalosporins and increase usage of intravenous devices or catheters are all risk factors for infection with multidrug-resistant *E. coli*. Resistant bacteria are becoming more frequent around the world, posing a danger to the successful treatment of common diseases in both community and hospital settings. The most common hospital-acquired infections caused by members of the *Enterobacteriaceae* family are infections of the urinary tract, gastrointestinal tract, and pyogenic infections.[1]

The development of β-lactamase is arguably the single most important mechanism of penicillin and cephalosporin resistance.[2] A naturally occurring chromosomally mediated β-lactamase or plasmid-mediated -lactamase can be found in *E. coli*. Penicillin-binding proteins are considered to have evolved into these enzymes. This change was most likely caused by selective pressure

from β-lactam-producing soil microbes in the environment.[3] Extended-spectrum β-lactamases (ESBLs), are enzymes that demonstrate enhanced hydrolysis of oxyimino-β-lactams.[4] Over 200 distinct ESBLs have been identified so far.[5] These enzymes have been found in a variety of E. coli strains and have been identified in huge numbers from various places. They've also been detected in *Klebsiellaspp, Citrobacterspp, Enterobacterspp, Proteusspp,* and non-lactose fermenters like *Pseudomonas*.[6]

Antibiotic resistance to penicillin, cephalosporin, and the monobactam (aztreonam) is spread by Gram-negative bacteria that manufacture the ESBL enzymes.[7] Enterobacteriaceaebacteria are found to be the primary source of these antibiotic resistance determinants, making it more difficult to treat infections caused by these pathogens[8]. The use of antibiotics in excess has been linked to the acquisition of ESBL-producing microbes. Resistance to routinely used antibiotics, such as ampicillin, cotrimoxazole, gentamicin, erythromycin, tetracycline, and third-generation cephalosporins, has been reported to be on the rise[9].

Drug resistance is the leading cause of antimicrobial ineffectiveness, increase mortality, and high treatment cost. The scarcity of data available from Pakistan indicates the utmost need for future research to understand the ESBL burden, further antibiotic susceptibility pattern by confirmatory testing and reporting. Therefore aim of this study is to identify isolates of Enterobacteriaceae, to determine the antibiotic susceptibility pattern of Enterobacteriaceaeand to determine the incidence of extended-spectrum beta-lactamases-producing Enterobacteriaceae.

Methods

This descriptive study was conducted in the Microbiology section of the Pathology department of Nishtar Medical University Multan, a teaching tertiary care hospital during November 2020 to November 2021. A sample size of 200 Enterobacteriaceae isolates was statistically calculated.A non-probability convenient sampling technique was used to collect data from patients of all age groups and either sex who were asked by physician to provide clinical samples of blood, urine, and pus from inpatients.Repetitive clinical isolates from various clinical samples of a same patient were excluded from the study.

Sample Collection and Transportation

Blood, urine, and pus specimens were collected from patients of all age groups and either sex, after taking informed written consent. If a delay of more than 2 hrs was expected then pus specimens were transferred to a container of Amie's transport medium following aseptic measures. Blood specimens were collected in the blood culture bottle before starting antibiotic treatment by using aseptic measures. Midstream urine specimens were taken in a sterile, leak-proof container containing boric acid. After labeling, the specimens of blood, urine, and pus were transported without delay to the microbiology section of the Pathology department of Nishtar Medical University, Multan.

Processing of Samples

Specimens of blood, urine, and pus were processed as per standard microbiological guidelines. The blood specimens were kept in a VersaTREK automated blood culture machine for identifying culture-positive bottles. The specimens of blood and pus were initially inoculated on Blood agar and MacConkey`s agar after Gram staining. Urine specimens were cultured on cysteine-lactose electrolyte-deficient (CLED) media with calibrated loop technique to determine colony-forming unit (CFU) after Gram staining. The specimens were incubated at 37°C under aerobic conditions for 24 hours.

Bacterial Identification

Identification was carried out based on colonial characteristics, morphology, motility, and conventional biochemical tests and isolates were confirmed biochemi-

Table 1: *Antibiotics Sensitivity test protocol*

Sr no	Antibiotic disc	Disc concentration	Susceptible	Intermediate	Resistant
1	Amikacin	30µg	≥17mm	15-16mm	≤14mm
2	Meropenem	10 µg	≥23mm	20-22mm	≤19mm
3	Ciprofloxacin	5 µg	≥21mm	16-20mm	≤15mm
4	Aztreonam	30µg	≥21mm	18-20mm	≤17mm
5	Trimethoprim/sulfamethoxazole	1.25/23.75 µg	≥16mm	11-15mm	≤10mm
6	Chloramphenicol	30 µg	≥18mm	13-17mm	≤12mm
7	Fosfomycin	200 µg	≥16mm	13-15mm	≤12mm
8	Azithromycin	15 µg	≥13mm	_____	≤12mm
9	Cefuroxime	30 µg	≥23mm	15-22mm	≤14mm
10	Cefotaxime	30 µg	≥26mm	23-25mm	≤22mm
11	Cefepime	30 µg	≥25mm	19-24mm	≤18mm
12	Piperacillin/Tazobactam	100/10 µg	≥21mm	18-20mm	≤17mm

cally with the help of the Analytical Profile Index 20 E.

Antibiotic Susceptibility Testing

Kirby-Bauer disc diffusion method was used to evaluate the sensitivity of bacterial isolates against different antibiotics. Antibiotic discs were applied according to the protocol described in table 1. These plates were then incubated for 24 hours at a temperature of 37o C.After the completion of the incubation period, the antibiotic zone of inhibition was measured according to Clinical and Laboratory Standard Institute (CLSI, 2020) guidelines.

Determination of ESBL production

Enterobacteriaceaeisolates were further processed for identification of ESBL production by standard laboratory methods using double-disc diffusion(DDDT) phenotypic confirmatory method for ESBL detection.[10] The data was analyzed using IBM SPSS version 25 to calculate the mean and standard deviation.

Results

Samples of a total of 387 patients were considered in this study consisting of 247(63.8%) males and 140 (36.2%) females. The highest number of patients at 231(59.7%) were observed in the department of medicine while the lowest numbers were observed at 4(1.0%) in each cardiology and ortho departments. The frequency of various types of samples, age groups, frequency of samples regarding age groups and growth status are also presented in Table 2.

Enterobacteriaceae were observed according to the type of samples and found that among 200 culture-positive samples for Enterobacteriaceae, 77(38.5%) were from pus samples, 51(25.5%) were from blood and 72(36.0%) were from urine samples. The highest frequency of 111(55.5%) was identified as E. coli while the lowest frequency of 5(2.5%) was identified as Salmonella. Similarly 59 (29.5%) were ESBL positive. Distribution of ESBL positive strains of enteric bacteria revealed 29.5% of organisms. Highest frequency of 26 (33.8) ESBL positive was found in pus samples followed by 21(29.2%) in urine and 12(23.5%) in blood. Type of organism and sample wise distribution ESBL positive (+) and negative (-) is presented in table 3.

Table 2: *Characteristics of patients and samples (n=387)*

Characteristics	Category	Frequency	
		n	%
Gender	Male	247	63.8
	Female	140	36.2
Inpatient's Department	Medicine	231	59.7
	Paeds	63	16.3
	Surgery	47	12.2
	Chest	16	4.1
	Gynae	12	3.1
	ICU	10	2.6
	Cardiology	4	1.0
	Ortho	4	1.0
Type of Specimen	Pus	164	42.4
	Urine	152	39.3
	Blood	71	18.3
Age Groups	< 30 years	208	53.7
	30-60 years	130	33.6
	>60 Years	49	12.7
Growth Status	No growth	98	25.3
	Insignificant bacteria	89	23.0
	Enterobacteriaceae	200	51.7

Table 3: *Enterobacteriaceaeand type of sample along ESBL status (n= 200)*

Organism Identified	Type of Specimen					
	Pus		Blood		Urine	
	ESBL+ n=26 (%)	ESBL- n=51(%)	ESBL+ n=12 (%)	ESBL- n=39(%)	ESBL+ n=21 (%)	ESBL- n=51(%)
E. coli	17 (65.4)	24 (47.0)	4 (33.3)	7 (17.9)	18 (85.7)	41 (80.4)
Proteus	5 (19.2)	19 (37.3)	1 (8.3)	1 (2.6)	1 (4.8)	5 (9.8)
Enterobacter	2 (7.7)	7 (13.7)	1 (8.3)	21 (53.8)	0 (0.0)	3 (5.9)
Klebsiella	2 (7.7)	1 (2.0)	2 (16.7)	1 (2.6)	2 (9.5)	1 (2.0)
Salmonella	0 (0.0)	0 (0.0)	0 (0.0)	5 (12.8)	0 (0.0)	0 (0.0)
Citrobacter	0 (0.0)	0 (0.0)	4 (33.3)	4 (10.3)	0 (0.0)	1 (2.0)

Patterns of drug susceptibility were also observed in ESBL positive group and the highest resistance was observed against cefuroxime, and cefotaxime, further drug resistance patterns are presented in table 4.

Table 4: *Susceptibility pattern of ESBL positive Enterobacteriaceae*

Drug Sensitivity Results		ESBL Positive Enterobacteriaceae					
		E. coli	Proteus	Enterobacter	Salmonella	Klebsiella	Citrobacter
		n	n	n	n	n	n
Amikacin	Sens	34	5	1	0	5	4
	Res	5	2	2	0	1	0
Meropenem	Sens	39	6	3	0	4	4
	Res	0	1	0	0	2	0
Ciprofloxacin	Sens	13	3	3	0	3	2
	Res	26	4	0	0	3	2
Aztreonam	Sens	6	2	0	0	0	2
	Res	33	5	3	0	6	2
Trimethoprim/ sulfamethoxazole	Sens	6	1	0	0	1	3
	Res	33	6	3	0	5	1
Chloramphenicol	Sens	26	4	2	0	2	2
	Res	13	3	1	0	4	2
Fosfomycin	Sens	15	3	2	0	2	3
	Res	24	4	1	0	4	1
Azithromycin	Sens	-	-	-	0	-	-
	Res	-	-	-	0	-	-
Cefuroxime	Sens	0	0	0	0	0	0
	Res	39	7	3	0	6	4
Cefotaxime	Sens	0	0	0	0	0	0
	Res	39	7	3	0	6	4
Cefepime	Sens	16	3	0	0	0	3
	Res	23	4	3	0	6	1
Piperacillin/Tazobactum	Sens	26	6	2	0	3	4
	Res	13	1	1	0	3	0

Discussion

The emerging antimicrobial resistance is a global challenge and failure to the identification of causative agent may prolong the ailment as well as render the complications to the patient. The distribution of Enterobacteriaceaein present study was comparable to a study with a total of 426 isolates.[11] Concomitant frequency of 31% pus samples in a study while a higher proportion of 58% urine samples presented the Enterobacteriaceae.[12]

Comparable results are presented by a recent study showing 51% isolates of E. coli, 4% Enterobacter, 19% Proteus and Klebsiella each[12]. Another study explored the highest numbers of Klebsiella at 53.8%, followed by E. coli at 30.8%, Enterobacter at 14.1%, and Proteus at 1.3% among Enterobacteriaceae isolates 13 thus, not comparable with present findings. However a study from Uganda 14 and an African study 15 also presented similar findings.

Presently 29.5% of organisms were ESBL, which agrees with the study which reported ESBL production in 30.24% of Enterobacteriaceae isolates.[16] Present findings are not concomitant to the study which revealed 55.4% ESBL positive cases.[17] Another study presented ESBL producing E. coli in 42.7% and Klebsiella in 33.7%. Overall ESBL production of Enterobacteriaceae also increased from 23.8% to 38.4% in the same period as above.[18] A study also presented variable degrees of microorganisms in ESBL positive and negative groups with the prevalence of E. Coli, Klebsiella, Citrobacteras 64.6%, 30.2%, 4.2% respectively for ESBL positive group while 66.1%, 21.6%, 2.9% respectively for ESBL negative group[17] and a high degree of relevance is obtained to present findings.

Results of ESBL negative Enterobacteriaceae are comparable with the study which presented 18.2% resistant isolates against cefotaxime in non-ESBL producers.[14] A study presented resistance of non-ESBL Enterobacteriaceae against ciprofloxacin at 12%, piperacillin/ tazobactam at 77%, ceftazidime &cefepime at 3% each,

and fosfomycin at 85%.[19] An Indian study presented 89.9% and 54-90% resistance of Enterobacteriaceae against aminopenicillin, and cephalosporins.[16] Thus, inconstant degrees of resistance patterns are presented with or without an agreement to present findings.

Trimethoprim and tetracycline showed the highest resistance of 85% and were comparable to the present study. An Ethiopian study showed resistance against trimethoprim at 77.0% following amoxicillin/clavulanic acid at 71.6%, cefotaxime, cefepime& ceftazidime at 62.2%, 60.3% & 60.8% respectively[11] hence classically comparable with the present findings. A Polish study concluded that patients suffering from non-urinary chronic disorders and urogenital disease are more prone to infections with these pathogens.[20] These are few aspects which may be considered the limitations of this study as were not considered during data collection.

Conclusion

A high proportion of ESBL- producing isolates belonging to Enterobacteriaceaewere were obtained from pus samples considering wound infections followed by urine and blood. The frequency of E. coli remained the highest among ESBL and non-ESBL producer Enterobacteriaceae followed by Proteus and Enterobacter in this study. Meropenem and amikacin remained highly effective drugs for both ESBL and non-ESBL producers followed by chloramphenicol and piperacillin/ tazobactam. E.coli remained the most dominant microorganism among Enterobacteriaceae and consisted more than half of the load alone.

Conflict of Interest: *None*

Funding Source: *None*

References

1. Janda JM, Abbott SL. The changing face of the family enterobacteriaceae (order:"Enterobacterales"): New members, taxonomic issues, geographic expansion, and new diseases and disease syndromes. Clin Microbiol Rev. 2021;34(2):e00174-20.
2. Bush K, Bradford PA. Interplay between β-lactamases and new β-lactamase inhibitors. Nat Rev Microbiol. 2019;17(5):295-306.
3. Kumar D, Singh AK, Ali MR, Chander YJIDR, Treatment. Antimicrobial susceptibility profile of extended spectrum β-lactamase (esbl) producing escherichia coli from various clinical samples. 2014;7:IDRT. S13820.
4. Castanheira M, Simner PJ, Bradford PA. Extended-spectrum β-lactamases: An update on their characteristics, epidemiology and detection. JAC-Antimicrob Resist. 2021;3(3):dlab092.
5. Rezaei M, Akya A, Elahi A, Ghadiri K, Jafari S. The clonal relationship among the citrobacter freundii isolated from the main hospital in kermanshah, west of iran. Iranian journal of microbiology. 2016;8(3):175.
6. Paudel P, Shrestha S, Poudel S, Tiwari BR. Multi-drug resistant bacteria isolated from pus and phenotypic detection of metallo β-lactamase activity of pseudomonas aeruginosa. Nepal J Health Sci. 2021;1(1):8-15.
7. Ghafourian S, Sadeghifard N, Soheili S, Sekawi ZJCIiMB. Extended spectrum beta-lactamases: Definition, classification and epidemiology. 2015;17(1):11-22.
8. Rottier WC, Ammerlaan HS, Bonten MJJoAC. Effects of confounders and intermediates on the association of bacteraemia caused by extended-spectrum β-lactamase-producing enterobacteriaceae and patient outcome: A meta-analysis. 2012;67(6):1311-20.
9. Mshana SE, Matee M, Rweyemamu MJAocm, antimicrobials. Antimicrobial resistance in human and animal pathogens in zambia, democratic republic of congo, mozambique and tanzania: An urgent need of a sustainable surveillance system. 2013;12(1):1-10.
10. Farzana R, Shamsuzzaman S, Mamun K, Shears P. Antimicrobial susceptibility pattern of extended spectrum (beta-lactamase producing gram-negative bacteria isolated from wound and urine in a tertiary care hospital, dhaka city, bangladesh. Southeast Asian Journal of Tropical Medicine and Public Health. 2013;44(1):96-103.
11. Teklu DS, Negeri AA, Legese MH, Bedada TL, Woldemariam HK, Tullu KD. Extended-spectrum beta-lactamase production and multi-drug resistance among enterobacteriaceae isolated in addis ababa, ethiopia. Antimicrob Resis Infect Control. 2019;8(1):1-12.
12. Qadi M, Alhato S, Khayyat R, Elmanama AA. Colistin resistance among enterobacteriaceae isolated from clinical samples in gaza strip. Canad J Infect Dis Med Microbiol. 2021; https://doi.org/10.1155/2021/ 6634684.
13. Tang H-J, Hsieh C-F, Chang P-C, Chen J-J, Lin Y-H, Lai C-C, et al. Clinical significance of community-and healthcare-acquired carbapenem-resistant enterobacteriaceae isolates. PLoS One. 2016;11(3):e0151897.
14. Kateregga JN, Kantume R, Atuhaire C, Lubowa MN, Ndukui JG. Phenotypic expression and prevalence of esbl-producing enterobacteriaceae in samples collected from patients in various wards of mulago hospital, uganda. BMC Pharmacol Toxicolo. 2015;16(1):1-6.
15. Kengne M, Dounia AT, Nwobegahay JM. Bacteriological profile and antimicrobial susceptibility patterns of urine culture isolates from patients in ndjamena, chad. Pan Afr Med J. 2017;28(1):258.
16. Nanoty VV, Agrawal GN, Tankhiwale SS. Evaluation of antibiotic resistance and β-lactamase production in clinical isolates from a tertiary care hospital in central india. J Clin Basic Res. 2018;2(1):1-5.

17. Yousefipour M, Rasoulinejad M, Hadadi A, Esmailpour N, Abdollahi A, Jafari S, et al. Bacteria producing extended spectrum β-lactamases (esbls) in hospitalized patients: Prevalence, antimicrobial resistance pattern and its main determinants. Iran J Pathol. 2019; 14(1): 61.
18. Caron Y, Chheang R, Puthea N, Soda M, Boyer S, Tarantola A, et al. Beta-lactam resistance among enterobacteriaceae in cambodia: The four-year itch. Int J Infect Dis. 2018;66:74-9.
19. Ouedraogo A-S, Sanou M, Kissou A, Sanou S, Solaré H, Kaboré F, et al. High prevalence of extended-spectrum ß-lactamase producing enterobacteriaceae among clinical isolates in burkina faso. BMC Infect Dis. 2016; 16(1):1-9.
20. Będzichowska A, Przekora J, Stapińska-Syniec A, Guzek A, Murawski P, Jobs K, et al. Frequency of infections caused by esbl-producing bacteria in pediatric ward–single center five-year observation. Arch Med Sci. 2019;15(3):688-93.