ECRONICON OPEN ACCESS

EC MICROBIOLOGY
Research Article

Prevalence of ESBL Producing *E. coli* in Tertiary Care Setting at Lahore, Pakistan

Faiza Habib[1]*, Samrin Habib[2] and Rabia Zulfiqar[3]

[1]*Medical Lab Technologist, Shoukat Khanum Memorial Cancer Hospital and Research Centre, Lahore, Pakistan*

[2]*Student MPhil, Lahore College Women University, Lahore, Pakistan*

[3]*Dental Technologist, King Edward Medical University, Mayo Hospital, Lahore, Pakistan*

***Corresponding Author:** Faiza Habib, Medical Lab Technologist, Shoukat Khanum Memorial Cancer Hospital and Research Centre, Lahore, Pakistan.

Received: January 09, 2019; **Published:** July 09, 2019

Abstract

Objectives

- To identify the prevalence of multidrug resistant *E. coli.*
- To assess the prevalence of ESBL producing *E. coli* in tertiary care hospital.

Background: Extended spectrum beta lactamases (ESBL) are enzyme that mediates resistance against extended spectrum of antibiotics. Now a day, ESBLs has been increasing as a community pathogen which have a multidrug resistance. It is a great challenge for clinician to treat these bacterial infections like UTI and other nosocomial infections. So, present study was carried out to detect the prevalence of ESBL producing *E. coli* by using different methods.

Methodology: This study was carried to detect the ESBL producing *E. coli* in urine sample collected from patients at Mayo Hospital Lahore. This non-probability convenient sampling technique was conducted in tertiary care setting of Mayo Hospital Lahore for a period of 6 months. The clean catch, mid-stream technique was used to collect the urine samples, which were inoculated at CLED agar. A total of 87 Gram negative isolates of *Escherichia coli* from urine samples were screened for resistance to beta lactam antibiotics ceftazidime, ceftriaxone, cefoxitin, imipenem by using standard method. Confirmation of ESBL was done by double disc diffusion test (DDDT).

Results: From 87 isolates of *E. coli*, 23 were ESBL producers and 64 were non-ESBL producers. All the isolates were tested against 7 antibiotics included in this study. Overall antimicrobial resistance patterns against different antibiotics were as follows: Augmentin 68.96%, ceftriaxone 70.11%, ceftazidime 73.56%, cefoxitin 71.26%, Gentamycin 66.67%, imipenem 37.93% and Aztreonam 77.01%.

Conclusion: From this study it is concluded that ESBL detection should be compulsory by combined methods. Ridiculous use of third generation cephalosporin and other beta lactam drugs must be depressed to reduce the multidrug resistant *Escherichia coli*.

Keywords: *ESBL; E. coli; Beta Lactam Antibiotics*

Introduction

Extended spectrum beta lactamases (ESBL) are rapidly evolving group of beta lactamase enzyme that mediate the resistance to extended spectrum of antibiotics [1]. They catalyse the hydrolysis of Beta lactam ring of the antibiotics and destroy the antimicrobial activity [2].

Most communal infections caused by *E. coli* (Gram negative bacteria), are the urinary tract infection (UTI) followed by wound infections and the other infections of blood stream, meningitis, septicaemia, pneumonia, intra-abdominal and gynaecological infections [3].

Beta lactam drugs assist in the release of autolytic enzymes which is a main cause of bacterial cell death. *E. coli* resistance to beta lactam antibiotics is due to the production of ESBL enzymes which inhibit the release of autolytic enzymes by modify the porin protein. These are a beta barrel protein that can cross cellular membrane and act as a pore through which molecule can diffuse. They arbitrate the resistance by flouting up the nitrogen-carbonyl bond in the beta-lactam ring [4].

Beta lactam antibiotics which used to treat the bacterial infections are the penicillins, cephalosporins, carbapenem and monobactams [5].

Antibiotics does not meet to the desired therapeutic effects by its inappropriate use. Resistance to each new drug has been increased due to lack of access, insufficient dose up, poor adherence, overuse and by using sub-standard antibiotics [6].

ESBL producing *E. coli* resistance to beta lactam increases significantly. It was first observed in 1983. UTI have been increased because of the increase production Of ESBL by *E. coli*. *E. coli* expressing ESBL hydrolyzing beta lactam not respond to therapy using beta lactam, which causes a lot of management and epidemiological issues [7].

Attempts have been made to decrease the prevalence of ESBL by replacing 3rd generation cephalosporin to 4th generation. Parrantly administered carbapanamase is one of the few safe treatment choice left. ESBL producing *Escherichia coli* show less resistant to Carbapanamase antibiotic as compared to the third generation of cephalosporin [8].

Materials and Methods

Before the start of research a pre-research planning was done in which included selection of research site, target population, sample size, self-designed Performa, sampling method, research methodology, organizational issues and work plan. Logistics and ethical implications were thoroughly discussed with the supervisor at Department of Pathology, King Edward Medical University Lahore. Analytical cross-sectional study design. Study was conducted in Microbiology department of king Edward Medical University Lahore. Study had lasted for the duration of 6 months starting after the approval of synopsis. Sample size of 87 patients is estimated by using 95% confidence level, 9% absolute precision with expected percentage of *E. coli* in tertiary care as 24.2%.

$$n = \frac{Z^2_{1-\alpha/2}.p.q}{d^2}$$

$Z_{1-\alpha/2}$ = confidence level 95% =1.96
P = prevalence 24.2%
q = 1-p
d = absolute precision 9%

Non-probability, convenient sampling technique was done. Gender (Both male and female), Mid-stream clean-catch specimen of urine transported immediately to lab were included in the study. Urine in unsterilized containers, improper labeling and Delayed specimen excluded from the study. Informed consent was taken from all patients. Urine samples were taken aseptically from patients and analyzed in the Microbiology section of Pathology Department of King Edward Medical University. For the collection of sample at Mayo hospital the patient was provided a sterile, dry, test tube and appeal for the 10 - 20 ml of urine. The first urine at the beginning of the day was collected for examination (clean catch, mid-stream).

Mid stream clean catch centrifuged urine was cultureed on CLED agar with the help of sterilized wire loop and incubate it for 24 hours at 37°C. Next day, Antimicrobial sensitivity testing was done by using modified Kirby-Bauer sensitivity testing technique and further ESBL detection by double disk diffusion method.

E. coli was identified presumptively by the methods of colony morphology (On CLED agar *E. coli* produced small 0.5 - 1 mm, lactose fermenting yellow colour colonies), Gram staining (Gram staining from isolated culture shows the Gram negative rods. They were predominantly non-motile), Antimicrobial Sensitivity Testing (Antimicrobial sensitivity was performed by using modified Kirby-Bauer sensitivity testing technique. Inoculum was prepared according to McFarland 0.5 turbidity standard. Inoculation was done on nutrient ager by using cotton swab. After putting appropriate antimicrobial discs, the plate readed after the incubation of 16 - 18 hours at 35°C), Confirmatory test for ESBLs (Phenotypic confirmation for ESBL producers was done by double disc diffusion test (DDDT) as per CLSI 2010 guidelines). A disc of ceftazidime (30ìg), cefotaxime (30 µg) alone and another disc of ceftazidime and cefotaxime in combination with clavulanic acid (30/10ìg) was used *for E. coli* isolates. Both antimicrobial discs were placed 25 mm apart from each other, center to center, by using a lawn culture technique of the test isolate on Nutrient agar plate and incubate it for the whole night at 37°C. A ≥ 5 mm increase in zone diameter for either antimicrobial drug tested in combination with clavulanic acid versus its zone when tested alone was designated as ESBL positive). Data was entered in SPSS-21 quantitative variables presented as mean ± SDualitative variable presented as frequency and percentage.

Results

A Total of 87 cases of different age and sex those who fulfilled the inclusion criteria were included in the study. Out of 87 the 40 (46%) were females and 47 (54%) were male.

Out of 87 isolates of *E. coli* 23 (26.43%) were detected as ESBL producers and 64 (73.56%) were non ESBL producer as shown in table 1.

Serial No		Total isolates	N	Percentage
1	ESBL producer	87	23	26.43%
2	Non ESBL producer	87	64	73.56%

Table 1: *Frequency of ESBL producer vs non producer.*

All isolates were tested against 7 antibiotics included in this study. The sensitivity pattern of imipenem these antibiotics were as Augmentin 25 (28.73%), Ceftriaxone 23 (26.43%), Ceftazidime 21 (24.13%), cefoxitin 23 (26.43%), Gentamycin 26 (29.88%), imipenem 54 (62.06%), Aztreonam 20 (22.98%).

Name of antibiotic	n (%) = 87		
	S	I	R
Augmentin	25 (28.73)	2 (2.29)	60 (68.96)
Ceftriaxone	23 (26.43)	3 (3.44)	61 (70.11)
Ceftazidime	21 (24.13)	2 (2.29)	64 (73.56)
cefoxitin	23 (26.43)	2 (2.29%)	62 (71.26)
Gentamycin	26 (29.88)	3 (3.44)	58 (66.67)
imipenem	54 (62.06)	0 (0)	33 (37.93)
Aztreonam	20 (22.98)	0 (0)	67 (77.01)

Table 2: *Antimicrobial susceptibility testing for 8 different antibiotics for 87 E. coli isolates.*

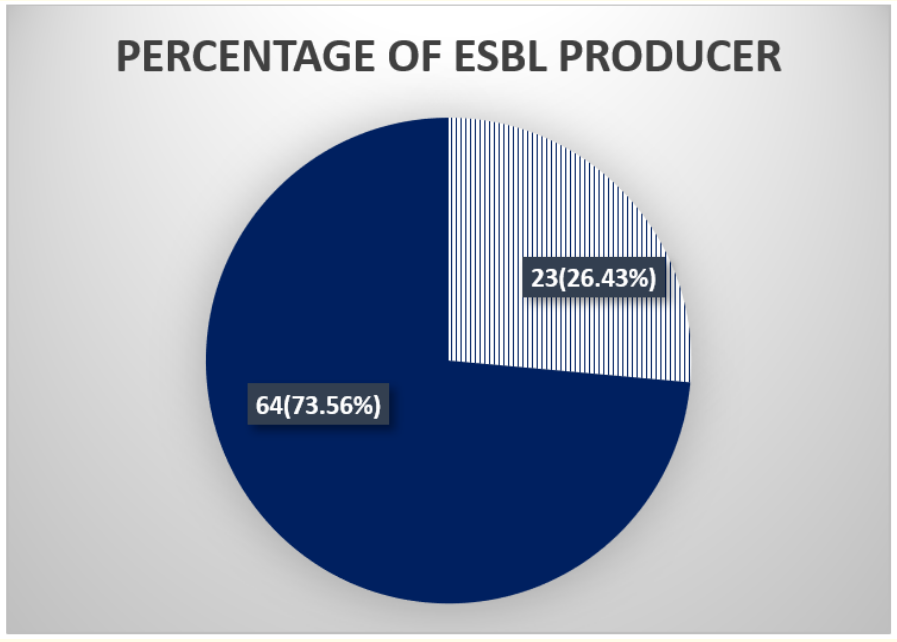

Figure 1: *The pie graph, showing a percentage of ESBL producing E. coli.*

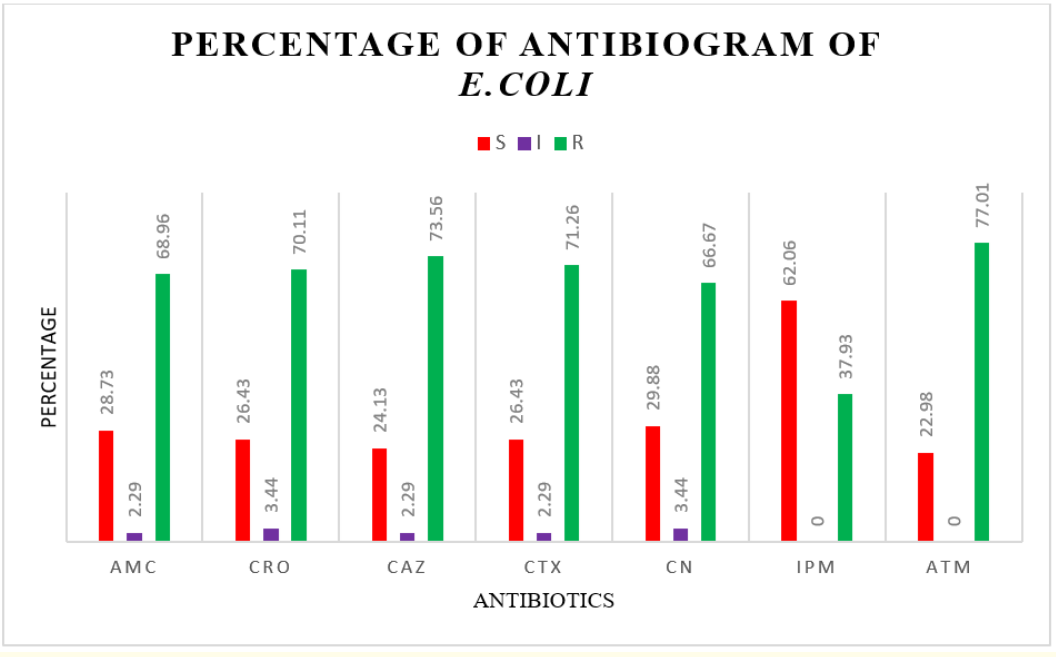

Figure 2: *The bar graph showing antibiogram of all E. coli strains isolated from urine specimens.*

Discussion

Antibiotics have been used widely and the excessive use of beta-lactam antibiotics created a significant problem leading to increased morbidity and mortality [9].

Proper use of antimicrobial drugs is very important for the treatment of infections. The main focus of research is the increase of bacterial resistance against the newer antibiotics [10].

ESBLs are the product of the excessive use of antibiotics like third generation cephalosporins. Our results clearly shows that ESBL are present in all of the farmers studies, and that they are mainly carried by *E. coli* strain. Their resistance increases with the passage of time Extended spectrum beta lactamases (ESBL) are promptly progressing group of beta lactamase enzyme that mediate resistance to extended spectrum of antibiotics [1].

They catalase the hydrolysis of Beta lactam ring of the antibiotics and destroy the antimicrobial activity [2].

Uncentrifuged urine was cultured on CLED agar with the help of sterilized wire loop and incubate it for 24 hours at 37°C Next day, Antimicrobial sensitivity was done by modified Kirby-Bauer sensitivity testing technique.

Antimicrobial sensitivity testing was performed by using modified Kirby-Bauer sensitivity testing technique. Inoculum was prepared according to McFarland 0.5 turbidity standard. Inoculation was done on Muller-Hinton agar (MH agar) by using cotton swab. After putting appropriate antimicrobial discs, the plate readed after incubation of 16 - 18 hours at 35°C.

In the present study a total of 87 Gram negative *Escherichia coli* obtained from urine samples were screened for ESBL production. A disc of ceftazidime (30ìg), cefotaxime (30 µg) alone and a disc of ceftazidime and cefotaxime in combination with clavulanic acid (30/10ìg) was used for *E. coli*. Both the discs was placed 25 mm apart to each other, center to center, on a lawn culture of the test isolate on Muller Hinton agar plate and incubate it for the whole night at 37°C. A ≥ 5 mm increase in zone diameter for either antimicrobial disk tested in combination with clavulinic acid versus its zone when tested alone was designated as ESBL positive.

In the present study, 87 Gram negative *E. coli* were isolated from various clinical specimens of the organisms were isolated from the urine sample. A Total of 87 cases of different age and sex were those who fulfilled the inclusion criteria were included in the study. Out of 87, 40 (46%) were females and 47 (54%) were male. From 87 isolates of *E. coli*, 23 were confirmed as ESBL producers and 64 were non-ESBL producers.the percentage of ESBL producer was 26.43% and the percentage of non ESBL producing *Escherichia coli* was 73.56% resembling with the older study conducting in the Health Protection Agency's Antibiotic Resistance Monitoring and Reference Laboratory which having a 24% ESBL producer [11].

Results resemble with the past studies as in the studies done in in the southern eastern part of Europe and Egypt have ESBL frequencies of 10 - 60% [12].

All Isolates of *E. coli* were tested against the.7 antibiotics included in this study. The sensitivity pattern of these antibiotics were as Augmentin 25 (28.73%), Ceftriaxone 23 (26.43%), Ceftazidime 21 (24.13%), cefoxitin 23 (26.43%), Gentamycin 26 (29.88%), imipenem 54 (62.06%), Aztreonam 20 (22.98%). The intermediate pattern of these antibiotics were as follows. Augmentin 2 (2.29%), Ceftriaxone 3 (3.44%), Ceftazidime 2 (2.29%), Cefoxitin 2 (2.29%), Gentamycin 3 (3.44%), imipenem 0 (0%), Aztreonam 0 (0%).

These results are agreed with the result of sensitivity pattern of Gentamycin 59% and Imipenem 96%. Their are a little discripencies with the result of this study done in Botany Department, Benha University, Qalubia, Egypt. Because it was performed only in UTI patients [13].

ESBL identification was done by double disc diffusion test (DDDT). In my own study ESBL confirmation was detected by double disk diffusion method (DDDT) as given in the following figure 3.

Conclusion

Results of this study concluded that all antibiotics shows very high degree of resistance as compared to previously reported studies. The prevalence of ESBL producing *E. coli* is 26.43% and the resistance profile of all the antibiotics against *E. coli* shows a high degree of resistance to it. Resistance is increases day by day to all of the antibiotics mostly due to the overuse or misuse of antibiotics. Patients infected with ESBL-producing *E. coli* experience a poor outcome if they are treated with inappropriate antibiotics. So, the appropriate usage of antibiotics are highly necessary to overcome the diseases.

Recommendations

Antibiotics should be used in a proper way to eradicate all issues. ESBL detection should be made compulsory by combined methods. Ridiculous use of third generation cephalosporins and other beta lactam drugs must be depressed to reduce the multidrug resistant bacteria.

Bibliography

1. Ewers C., *et al*. "Extended-spectrum β-lactamase-producing and AmpC-producing Escherichia coli from livestock and companion animals, and their putative impact on public health: a global perspective". *Clinical Microbiology and Infection* 18.7 (2012): 646-655.
2. Bush K. "Proliferation and significance of clinically relevant β-lactamases". *Annals of the New York Academy of Sciences* 1277.1 (2013): 84-90.
3. Kang C-I., *et al*. "Epidemiology and risk factors of community onset infections caused by extended-spectrum β-lactamase-producing Escherichia coli strains". *Journal of Clinical Microbiology* 50.2 (2012): 312-317.
4. Blair JM., *et al*. "Molecular mechanisms of antibiotic resistance". *Nature Reviews Microbiology* 13.1 (2015): 42-51.
5. Page MG. "Beta-lactam antibiotics. Antibiotic Discovery and Development". Springer (2012): 79-117.
6. Ventola CL. "The antibiotic resistance crisis: part 1: causes and threats". *Pharmacy and Therapeutics* 40.4 (2015): 277-283.
7. Neuner EA., *et al*. "Experience with fosfomycin for treatment of urinary tract infections due to multidrug-resistant organisms". *Antimicrobial Agents and Chemotherapy* 56.11 (2012): 5744-5748.
8. Myat TO., *et al*. "ESBL-and carbapenemase-producing enterobacteriaceae in patients with bacteremia, Yangon, Myanmar, 2014". *Emerging Infectious Diseases* 23.5 (2017): 857-859.
9. Weinstein RA. "Controlling antimicrobial resistance in hospitals: infection control and use of antibiotics". *Emerging Infectious Diseases* 7.2 (2001): 188-192.
10. Kolář M., *et al*. "Antibiotic selective pressure and development of bacterial resistance". *International Journal of Antimicrobial Agents* 17.5 (2001): 357-363.
11. Woodford N., *et al*. "Community and hospital spread of Escherichia coli producing CTX-M extended-spectrum β-lactamases in the UK". *Journal of Antimicrobial Chemotherapy* 54.4 (2004): 735-743.
12. Canton R., *et al*. "Prevalence and spread of extended-spectrum beta-lactamase-producing Enterobacteriaceae in Europe". *Clinical Microbiology and Infection* 14.1 (2008): 144-153.

13. European Antimicrobial Resistance Surveillance System (EARSS) (2008).

14. Elsayed TI., *et al*. "The Occurrence of Multidrug Resistant E. Coli which Produce ESBL and Cause Urinary Tract Infections". *Journal of Applied Microbiology and Biochemistry* 2 (2017): 1.

Volume 15 Issue 8 August 2019