ORIGINAL ARTICLE

Detection of Extended Spectrum Beta Lactamases (ESBLs) Producing Pathogenic Enterobacteriaceae in Khwaja Yunus Ali Medical College Hospital in 2016

Dr. Abdullah Akhtar Ahmed[1], Md. Shakhaowat Hossain[2] Md. Babul Aktar[3], Nusrat Akhtar Juyee[4], S.M. Ali. Hasan[5]*

ABSTRACT

Extended spectrum β-lactamase (ESBL)-producing organisms pose unique challenges to clinical microbiologists, clinicians, as well as infection control professionals. It has been found that ESBL producing pathogens of Enterobacteriaceae group are causing worldwide outbreaks of infection which is a challenging issue of infection control. The present study was undertaken to investigate the rate of ESBLs production and their antibiotic susceptibility pattern in Khwaja Yunus Ali Medical College Hospital which is a tertiary level health care delivery center of excellent standard, established in the rural area of Bangladesh in Sirajgonj district. A total of 215 Gram negative isolates from various clinical samples were studied and ESBLs production was detected by double disc synergy test. Antibiotic susceptibility test was done for commonly used antibiotics. Among the total isolates, 51.2% (110) were ESBL producers, and the rate of ESBLs positivity was 54.1% for E. coli (92 out of 170), 46.7% for Pseudomonas sp. (14 out of 30), 25% for Klebsiella sp. (3 out of 12), 33.3% for Acinetobacter sp. (1 out of 3). ESBLs producing organisms were resistant to most of the antibiotics but 83.7% was sensitive to antibiotics like nitrofurantoin and 82.6% to amikacin. This study suggests that ESBLs detection practice should be carried out in all the clinical diagnostic laboratories routinely to suggest physicians for proper antibiotic therapy..

***Key Words:* Beta Lactamase, Enterobacteriaceae, Antibiotic, Susceptibility**

1. INTRODUCTION

Multidrug-resistant bacteria expressing extended-spectrum β-lactamases (ESBLs) are emerging worldwide in hospitals and community as through plasmids the genes are transferred easily from one to another bacterium.

In addition, ESBL producing organisms exhibit co-resistance to many other classes of antibiotics such as fluroquinolones, trimethoprim- sulphamethoxazole, aminoglycosides and metronidazole [1]. In 1983, ESBL was first detected in Germany and later in France (1985) in isolates of *Klebsiella pneumoniae* [2]. Later these have been reported worldwide in many different genera of Enterobactericeae and *Pseudomonas* sp. [3]. Subsequently the reports of ESBL producing pathogens were published from the different parts of the world and rate of ESBL producers are found very high in Asia than in Europe [4].

In Bangladesh, no systemic study on ESBL producers was carried out until 2004. The first report of ESBL positive 43.2% and 39.5% *E. coli* and *K. pneumoniae* isolated from clinical samples respectively was reported in 2004 [5] and in 2010 the isolation rate was found increased to 57.89% [6]. The recently carried out study report of prevalence of ESBL production by commonly isolated organism such as *E. coli* is necessary to understand the

1 Professor & Head, *Department of Microbiology, Khwaja Yunus Ali Medical College & Hospital, Enayetpur, Sirajgonj-6751, Bangladesh.*
2 Assistant Professor & Head, *Department of Microbiology, Khwaja Yunus Ali University, Enayetpur, Sirajgonj-6751, Bangladesh.*
3 Lecturer, *Department of Microbiology, Khwaja Yunus Ali University, Enayetpur, Sirajgonj-6751, Bangladesh.*
4 Medical Officer, *Alamdanga Upazila Health Complex, Alamdanga, Chuadanga.*
5 Medical Officer, *Residency Course on Gastroenterology, BSSMMU, Dhaka.*
*** Correspondence to:** *E-mail: adullah_micro49@yahoo.com*

disease burden and to take necessary action to prevent the spread.

This study was carried out with the objective to detect the prevalence of ESBL producing *E. coli* and its antimicrobial sensitivity pattern to design proper antibiotic therapy for the physicians and plan a scientific modern preventive technique for controlling hospital infection.

2. Materials and methods

The study was held in Khwaja Yunus Ali Medical College Hospital, a tertiary care hospital situated in the rural area of Bangladesh in Sirajgonj district. MDR *E. coli*, *Klebsiella* sp., *Pseudomonas* sp., and *Acenatobacter* sp. strains were isolated from the clinical specimens in 2016.

The study was carried out in microbiology section of the Department of Laboratory Services of the hospital. Sterile technique was applied to collect throat swab, nasal swab, and wound swab from abscess or infection either by disposable syringe or by sterile swab stick and inoculated in Blood agar and MacConkey agar media containing culture plates. After overnight incubation at 37^0C, plates were checked for the growth of any suspected pathogenic microorganism. The identification and antimicrobial sensitivity tests of the isolated organisms were done as per standard laboratory methods.

2.1 ESBL detection

The method recommended by Clinical Laboratories Standard Institute (CLSI) requires a two steps approach of initially screening for ESBL production and then performing confirmatory tests on screen positive isolates [7].

2.1.1 Screening for ESBL producing microbes by Agar dilution method:

The screening for ESBL producers was done by agar dilution method as recommended by Clinical and Laboratory Standard Institute (CLSI).

Any of the isolated organisms found to be grown at this stated screening antibiotics concentration (That is Minimum Inhibitory Concentration [(MIC)] of third generation cephalosporins, namely ceftriaxone, ceftazidime and cefotaxime >2µg/ml) according to CLSI, 2007 was considered as possible ESBL producers and spelled for the confirmatory tests. The use of more than one antibiotic disc for screening improves the sensitivity of detection [7].

2.1.2 Antimicrobial sensitivity test:

All the ESBLs producing pathogens were tested for antimicrobial sensitivity using disc diffusion technique by "Kirby-Bauer method" against Carbapenem and different non-beta-lactam antimicrobial agents [11]. These included Cotrimoxazole 1.25/23.75µg (COT), Ciprofloxacin 5µg (CIP), Nitrofurantoin 300µg (NF), Gentamicin 10µg (CN), Amikacin 30µg (Ak), Imipenem 10µg (IMP), and Netilmicin 30 µg (NET).

Antimicrobial sensitivity or resistance was determined on the basis of interpretative criteria which is recommended by the Clinical and Laboratory Standards Institute. A total of 12 *E. coli* ATCC 25922 was used as the quality control strain [8].

2.1.3 Detection of ESBLs by the confirmatory tests

Phenotypic confirmatory test: The confirmation of the ESBL producing microbes was done by the phenotypic confirmatory test according to CLSI recommendation. In this test, third generation cephalosporin i.e. ceftazidime (30 µg) and cefotaxime (30 µg) disc alone and in combination with clavulanic acid (10µg) were used. Ceftazidime, cefotaxime discs were placed on one side and ceftazidime, cefotaxime discs combined with clavulanic acid (30/10 µg) were placed on other side of the inoculated plate. After overnight incubation at 37°C, diameter of zone of inhibition was measured. A 5 mm or more increase in diameter of zone of inhibition for ceftazidime and cefotaxime tested in combination with clavulanic acid versus its zone when ceftazidime and cefotaxime tested alone confirms an ESBLs producing organism [7].

2.1.4 Reference strain for quality control used for ESBL detection

E. coli BB-32327 (CTX-M9) was used as positive control and *E. coli* ATCC (American Type Culture Collection)

25922 was used as negative control of ESBL detection test.

3. Results

In the present study, a total of 215 Gram negative bacteria were isolated from 11 types of clinical samples from the hospitalized patients. The majority of isolates were from urine 139 (64.6%), followed by pus 35 (16.3%), stool 11 (5.1%), throat swab 8 (3.7%) and wound swab 6 (2.8%) (Table-1).

Table 1 - Pattern of ESBL producing Enterobacteriaceae pathogen in clinical sample

Sample	*E. coli*		*Klebsiella sp.*		*Pseudomonas sp.*		*Acenatobacter sp.*		Total
	ESBL +	ESBL -	ESBL +	ESBL -	ESBL +	ESBL-	ESBL +	ESBL -	
Urine	66	55	02	03	04	08	00	01	139
Stool	05	06	00	00	00	00	00	00	11
Pus	13	13	00	00	04	05	00	00	35
Wound swab	01	03	00	00	02	00	00	00	06
Sputum	02	00	00	00	01	01	00	01	05
Throat swab	00	00	01	06	00	01	00	00	08
Nasal swab	00	01	00	00	00	01	00	00	02
Blood	00	00	00	00	02	00	01	00	03
Catheter tip	03	00	00	00	01	00	00	00	04
Vaginal swab	01	00	00	00	00	00	00	00	01
Endo Cervical swab	01	00	00	00	00	00	00	00	01
Total	92	78	03	09	14	16	01	02	215

The distribution of different types of Gram negative bacteria isolated among the total 215 isolates were as follows: *Escherichia coli* 170 (79.0%), *Klebsiella* sp. 12 (5.6%), *Pseudomonas* sp. 30 (14.5%) and Acinetobacter spp 3 (1.4%). All the 215 Gram negative isolates were tested for ESBLs production by double disc synergy test and it was found that total 110 (51.2%) isolates were ESBL producers. Among these ESBLs producers 92 (54.1%) were *Escherichia coli*, 03 (25.0%) *Klebsiella* sp., 14 (46.7%) *Pseudomonas* sp, and 1 (33.3%), *Acinetobacter* sp. (Table-2).

Table 2- Rate of ESBL producing Enterobacteriaceae pathogen

Enterobacteriaceae pathogen	ESBL producer		ESBL non-producer	
	Frequency	Percentage	Frequency	Percentage
Escherichia coli (n=170)	92	54.1	78	45.9
Klebsiella sp. (n=12)	03	25	09	75
Pseudomonas sp. (n=30)	14	46.7	16	53.3
Acenatobacter sp. (n=03)	01	33.3	02	66.7
Total (215)	110	51.2	105	48.8

The details of antibiotic sensitivity pattern of the *Escherichia coli*, *Klebsiella* sp., *Pseudomonas* sp, and *Acinetobacter* sp. isolates are given in Table 3.

Table 3. Frequency of Antibiotic sensitivity to 110 ESBL positive pathogens isolated from KYAMCH in 2016

Name of Antibiotics	Name of ESBL positive pathogens' antibiotic sensitive in %			
	E. coli (n=92)	*Klebsiella sp.* (n=03)	*Pseudomonas sp.* (n=14)	*Acenatobacter sp.* (n=01)
Amikacin	82.6	66.7	93.0	100
Cotrimoxazole	3.2	00	7.1	00
Cefixim	5.4	33.3	00	00
Cefotaxime	4.3	33.3	14.2	00
Ceftazidime	9.8	00	14.2	100
Ceftriaxone	8.7	33.3	14.2	100
Ciprofloxacin	18.4	33.3	50	100
Gentamicin	50	33.3	64.2	00
Pipercilin	1.0	00	78.5	00
Imipenem	24.1	00	71.4	00
Meropenem	79.3	100	50	00
Nitrofurantoin	83.7	100	50	100
Azithromycin	2.1	00	00	00
Aztreonam	24.1	66.7	57.1	100

About 50 - 93% isolated *Escherichia coli, Klebsiella* sp., *Pseudomonas* sp, and *Acinetobacter* sp. were sensitive to Amikacin and Nitrofurantoin. The third-generation cephalosporin such as ceftriaxone, ceftazidime and cefotaxime were less (4.3 - 14.2%) effective to *Escherichia coli* and *Pseudomonas* sp. The aztreonam is found moderately sensitive to Klebsiella spp., *Pseudomonas* spp. and *Acinetobacter* sp. (57.1 - 100%). However, most isolates were sensitive to meropanem (50-100%) (Table-3).

4. Discussion

The infections which are caused by multidrug-resistant gram-negative bacteria that produce various β-lactamase enzymes have been reported with an increasing frequency in the hospitalized patients and they are associated with a significant morbidity and mortality [9]. The numerous β-lactamases are encoded either by the chromosomal genes or by the transferable genes which are located on the plasmids or the transposons [10]. These enzymes were commonly found in *Klebsiella* and *E. coli* species. However, recently these enzymes are reported to be produced by all members of Enterobacteriaceae and other Gram-negative bacilli [11, 12].

In this study, an important step was made to detect Extended Spectrum Beta Lactamases (ESBLs) producing pathogenic Enterobacteriaceae. This study was based on laboratory findings of the patients attending the in-patient departments of Khwaja Yunus Ali Medical College Hospital in 2016.

ESBL producing *E. coli* were isolated from all sites of the body from which samples were obtained namely, blood, urine, sputum, throat swab, nasal swab, wound swab, pus, stool, and catheter tip. More than 50% of the isolates from urine, and pus were ESBL producers. This observation is of serious concern because of the severity of urinary tract and wound infections. On screening with third generation cephalosporin, a total of 92 *Escherichia coli*, *Klebsiella* sp., *Pseudomonas* sp. and *Acenatobacter* sp. clinical isolates were selected and studied for their antimicrobial susceptibility and β-lactamase productions such as ESBL. Highest susceptibility was found to nitrofurantoin (83.7%) followed by amikacin (82.6%) and meropanem (79.3%). *E. coli* were resistant to most of the drugs used as first line drugs. A low susceptibility was observed with third generation cephalosporin (cefotaxime, ceftazidime, and ceftriaxone) (4.3 and 9.8, 8.7%, respectively), monobactam (aztreonam) (24.1%), penicillin (piperacillin) (1.0%), cotrimoxazole (3.2%), and cefixime (5.4%), Carbapenem (imipenem) (24.1%).

Almost similar significantly high degree of susceptibility patterns were observed by Kibret *et al* to nitrofurantoin (96.4%), and gentamicin (79.6%) ([3]. Bamford et al also demonstrated a significant decline in susceptibility to β-lactam antibiotics and fluoroquinolones, while susceptibility to amikacin and gentamicin remained significantly high [14].

In the present study, out of 170 *E. coli*, 54.1% were ESBL producers by phenotypic confirmatory methods. The prevalence of ESBL producing *E. coli* varies from country to country and from center to center. In the United States, ESBL producing *E. coli* ranges from 0 to 25% with the average being around 3% [15]. In Japan, the prevalence of ESBL producing *E. coli* is <0.1% [16]. In Asia, the rate of ESBL production in *E. coli* is 4.8%, 8.5%, and up to 12% in Korea, Taiwan, and Hong Kong, respectively [17,19]. In India, the percentage of ESBL producers ranges from 22 to 75% [20,23].

In a study conducted by Ankur *et al* on clinical isolates of ESBL producing *E. coli*, resistance found to amikacin was 14.7%, gentamicin 66.7%, and ciprofloxacin 93.8% [24]. Maina et al documented a higher proportion of isolates resistant to ciprofloxacin, and approximately 100% sensitivity to carbapenems [25].

5. Conclusion

The ESBL producing pathogenic Enterobacteriaceae are a cause of concern to the microbiologist as well as to the clinicians, particularly the multi drug resistant strains. Correct choice of antimicrobial agents according to the sensitivity profile is essential for appropriate empirical treatment. In our study, the amicacin, nitrofurantoin, and meropanem retained good activity and would thus be the drugs of choice for empiric therapy in life threatening infections and non-complicated urinary tract infections respectively. It is required to carry out more scientific work to determine the epidemiology of ESBL related infections and the associated clinical burden locally.

Place of Study:

Department of Laboratory Services, Khwaja Yunus Ali Medical College Hospital, Enayetpur Sharif, Sirajgonj.

Acknowledgment

We are grateful to the Chairman of the Trusty Board and Director of Khwaja Yunus Ali Medical College Hospital to conduct this study. We thank Mr. Md. Abdul Karim, Medical Technologist and Mr. Md. Mazharul Islam, Medical Technologist of the department of Laboratory Services of Khwaja Yunus Ali Medical College Hospital for assisting in laboratory work.

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