

## Detection of ESBL Organisms in body fluids, a study from a tertiary care center

\*Linda Rose Jose<sup>1</sup>, Gogi Suresh<sup>2</sup>, Jisha P<sup>3</sup>, Deepthy BJ<sup>4</sup>, Hareesh PV<sup>5</sup>  
<sup>1,2,3,4,5</sup>(Department of Microbiology, DM WIMS, Wayanad)

### Abstract :

**Background of the study:** The Extended spectrum beta lactamase (ESBL) producing organisms are emerging as a serious threat to the community and its incidence especially from body fluids has been increasing rapidly over the past years. The aim of this study was to detect the ESBL producing organisms in body fluids in a tertiary care center.

**Materials and Methods:** This is a prospective study that was conducted in Department of Microbiology, DM WIMS, Wayanad. A total of 160 isolates were obtained from various exudates. Samples were obtained from both outpatients and inpatients between June 2016 and November 2016. The samples were cultured on BA, MA. The samples were processed based on standard laboratory techniques. Antibiotic susceptibility of the isolates was determined against various antibacterial agents by Kirby Bauer Disk Diffusion method.

**Result:** Among the 160 isolates, 68 (42.5%) were *E.coli*, and 35 (21.8%) were *K. pneumoniae*, 28 (17.5%) were MRSA, 14 (8.7%) were *Pseudomonas*, 8 (5%) were *Proteus spp.*, 4 (2.5%) were *Acinetobacter spp.* and 3 (1.8%) were *Citrobacter spp.* Of these 160 strains tested, 45 (28%) were found to be ESBL producers, of which 22 (48.8%) were *E.coli*, 18 (40%) were *K. pneumoniae*, 3 (6.6%) were *Acinetobacter spp.* and 2 (4.4%) were *Proteus spp.*

**Conclusion:** Restricted use of antibiotics will lead to a decreased selective pressure and the resistant strains can no longer sustain in such settings. Such studies might act as an eye opener to the health care providers and facilitate them to carry out better treatment strategies.

**Keywords:** ESBL, TEM, SHV, Enterobacteriaceae

Date of Submission: 08 -08-2017

Date of acceptance: 23-08-2017

### I. Introduction

ESBLs are plasmid mediated enzymes inactivating beta lactam antibiotics containing oxyimino group such as oxyimino- cephalosporins and oxyimino- monobactam, except cephamycins and carbapenems.<sup>1</sup> They are a group of enzymes that mediate resistance to extended spectrum antibiotics like cephalosporins ie: ceftazidime, cefotaxime and ceftriaxone and belong to Ambler molecular class A and Bush–Jacoby functional group 2be.<sup>2,3</sup> Many of the ESBL producers are resistant to many antimicrobial agents like aminoglycosides, trimethoprim and quinolones.<sup>3</sup> They are detected in various species of bacteria belonging to the Enterobacteriaceae family mostly *E. coli*, *Klebsiella spp*, *Citrobacter spp*, *Enterobacter spp*, *Proteus spp* and non-lactose fermenters like *Pseudomonas aeruginosa*, *Acinetobacter spp*.<sup>4</sup> Presently over 200 different types of ESBLs have been described.<sup>5</sup> Major outbreak involving such resistant organisms has been reported all over the world. If ESBL is suspected, it should be confirmed by standardized methods. The determination of inhibition by clavulanic acid is a common strategy used for the detection of ESBL producers and several methods have been developed till date to detect the presence of ESBL including double-disk synergy test (DDST) and double-disk diffusion test (DDDT), using cefotaxime and ceftazidime disks with or without clavulanic acid.<sup>5</sup> ESBL production whether generated by TEM, SHV or CTX-M gene identified in an isolate means that ESBL-producing organisms are often able to reduce the susceptibility of other non-β-lactamase antimicrobial classes, such as aminoglycosides, fluoroquinolones, trimethoprim-sulfamethoxazole, tetracyclines and nitrofurantoin; thus, leaving a limited option for the range of therapeutic agents.<sup>2</sup> ESBL strains have been associated with resistance to various other non β-lactam antibiotics like the aminoglycosides and chloramphenicol. The Clinical and Laboratory Standards Institute (CLSI) recommends the detection of ESBL in Gram-negative bacteria by recognizing their decreased susceptibility to the third generation cephalosporins such as ceftazidime, cefotaxime and ceftriaxone.<sup>4</sup>

ESBL producing strains are probably more prevalent than the currently available data because they often remain undetected by routine methods.<sup>6</sup> Another important property of these ESBL producing strains that has to be considered is that they might give a false sensitive zone of inhibition in the Kirby– Bauer disk diffusion method.<sup>2</sup>

TEM or SHV beta lactamases are derived from the point mutation of plasmid as per the studies conducted.<sup>1,2,7</sup> ESBLs are the most evolving mechanism of antibiotic resistance among the family Enterobacteriaceae due to the inappropriate use of third generation cephalosporins, and this is most commonly encountered in ICU settings.<sup>7</sup> ESBL producers may have spread through communities, especially in those with poor hygienic and sanitary conditions, through fecal contamination of soil and water, since most patients with ESBL producers may have had their gastrointestinal tracts colonized for a longer period of time by the organisms as was reported by Paterson and Bonomo (2005).<sup>4</sup> ESBLs are enzymes secreted by bacteria which make several antibiotics ineffective. This makes it a serious threat to the community. ESBL isolates were first detected in Western Europe in the mid- 1980s. Since then the incidence of such cases are increasing rapidly. ESBLs are able to hydrolyze 3 and 4 generation cephalosporins and monobactams.<sup>8</sup> These strains are inhibited by  $\beta$ -lactamase inhibitors. Prevalence of ESBLs varies with different areas and also it differs in various clinical samples.<sup>9</sup>

Reliable detection of ESBL production by clinical microbiology laboratory is essential to guide the clinicians to select appropriate treatment modality. With the spread of ESBL positive strains in ICUs, there is a need to create a policy of empirical therapy in a high risk unit where infection due to resistant organisms is higher. Hence this study was designed to know the presence of ESBL organisms in body fluids isolated at DMWIMS, Meppadi, Wayanad and to know the antibiotic susceptibility pattern among ESBL producers.

## II. Materials And Methods

The study was conducted in Department of Microbiology, DM WIMS Medical College, Wayanad. A total of 160 isolates were obtained from various exudates. Samples were obtained from both outpatients and inpatients between June 2016 and November 2016. The samples were cultured on Blood Agar and MacConkey Agar. The samples were processed for isolation and identification based on standard laboratory techniques. Antibiotic susceptibility of the isolates was determined by Kirby Bauer Disk Diffusion method. They include ceftriaxone, cefotaxime, ceftazidime, gentamicin, ampicillin, tobramycin, amikacin, netilmicin, nalidixic acid, ciprofloxacin, imipenem, cefaperazone- sulbactam, co-trimoxazole, piperacillin-tazobactam and chloramphenicol. The results were recorded and interpreted according to the standard guidelines. This was a laboratory based study which has no directly involvement with the concerned patients. The specimen sources and patient information such as sex, age and setting, were carefully recorded from laboratory request forms.

## III. Results

Among the 160 isolates, 68 (42.5 %) were *E.coli*, and 35 (21.8 %) were *K. pneumoniae*, 28 (17.5 %) were MRSA, 14 (8.7%) were *Pseudomonas spp.*, 8 (5%) were *Proteus spp.*, 4 (2.5%) were *Acinetobacter spp.* and 3 (1.8%) were *Citrobacter spp.* Of these 160 strains tested, 45 (28%) were found to be ESBL producers, of which 22 (48.8%) were *E.coli*, 18(40%) were *K. pneumoniae* , 3 (6.6%) were *Acinetobacter spp.* and 2 (4.4%) were *Proteus spp.* Among these 94 % showed resistance to atleast one of the three third generation cephalosporins (ceftazidime,, cefotaxime, ceftriaxone), 22% showed resistance to all the three third generation cephalosporins. *E.coli* , *Acinetobacter* and *Proteus spp.* showed 100 % sensitivity to Piperacillin-tazobactam (Pt) and Cefaperazone- sulbactam (Cfs). The resistance pattern of each isolate to the various antibiotics used in this study is shown in graph 1. In our study, prevalence of ESBL among in-patients and out-patients was 37.5% and 14 % , respectively. The incidence of ESBL producers were more in in-patients compared to out-patients. The male to female ratio was 1:1 and higher incidence of ESBL producers were seen in the age group 60-70. Although the prevalence of ESBL in out-patients is less than in-patients, ESBL producers are common in communities as well. Distribution of ESBL producers in in-patients and out- patients is given in table:1. Age wise and sex wise distribution of patients who were confirmed to be infected with ESBL producers is given in Table 2 and Table 4.

**Table:1: Resistance pattern of isolates**

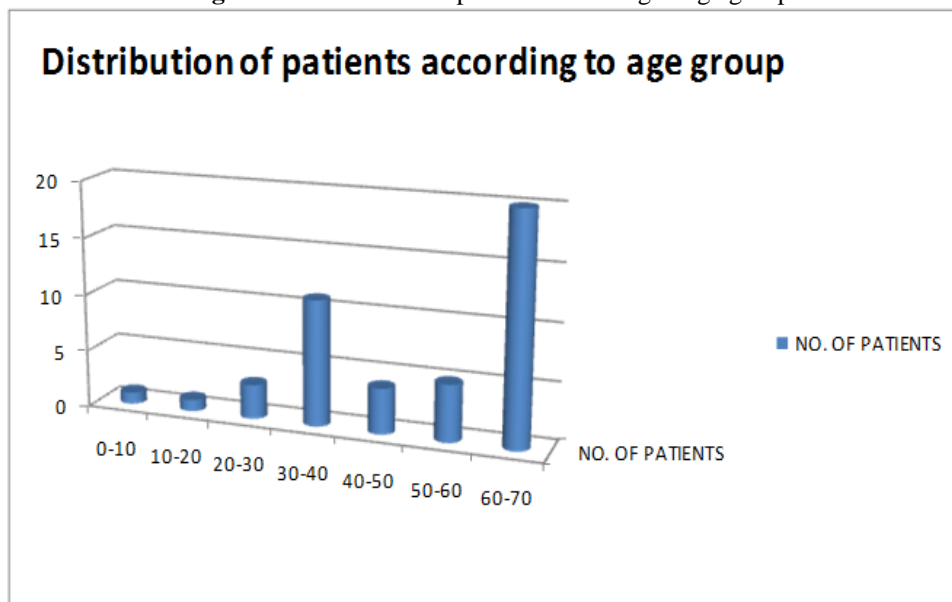
Sl.No	Antibiotics	Percentage of resistance			
		E.coli	K.pneumoniae	Acinetobacter spp.	Proteus spp.
1	Imipenem	0	0	0	0
2	Cefaperazone-sulbactam	0	21	0	0
3	Piperacillin- tazobactam	0	6	0	0
4	Amikacin	32	57	10	13
5	Chloramphenicol	43	80	0	22
6	Co- trimoxazole	61	86	0	3
7	Nalidixic acid	78	88	0	13
8	Gentamicin	70	91	0	70
9	Ciprofloxacin	95	68	3	0
10	Netilmicin	78	93	0	6
11	Ampicillin	91	64	90	0

12	Tobramycin	89	64	2	28
----	------------	----	----	---	----

**Table:2** Distribution of ESBL producers in in-patients and out-patients sample.

	In-patients	Out- patients
ESBL producers	36(37.5%)	9(14%)
Non-ESBL producers	60(62.5%)	55(85.9%)
<b>Total</b>	96	64

**Figure:1** Distribution of patients according to age group



**Table: 3** Distribution of patients according to age group

AGE GROUP	NO. OF PATIENTS
0-10	1
10-20	1
20-30	3
30-40	11
40-50	4
50-60	5
60-70	20

**Table: 4** Sex wise distribution of patients

Sex	Number Of Patients From Which Esbl Producers Were Isolated
Males	24
Females	21

#### IV. Discussion

The isolation of ESBL producers from various clinical samples has been steadily rising over the past years and it is a major threat to the clinical treatment strategy. High degree of antibiotic co-resistance among ESBL producers emphasizes the fact that antimicrobials should be used judiciously. Many studies from India have reported the incidence of ESBL producers to be high, to be upto 6.6 to 68% .<sup>10</sup> The frequency of ESBL producers in our study is 28% and it is similar to many other studies from India. The frequency of ESBL producers in this study can be considered low when compared to many other studies from India and abroad, but ESBL producers in its lowest occurring frequency also should be considered a serious threat to the community. Several studies have reported a very high incidence of ESBL producers.<sup>11,12,13</sup>

Prolonged antibiotic exposure, overstay in hospitals, severe illness, unprecedented use of third generation cephalosporins, and increased use of intravenous devices or catheters are important risk factors for infection with multidrug resistant *E. coli*.<sup>13</sup> *Klebsiella pneumoniae* and *Escherichia coli* remain the major ESBL producing organisms' isolated worldwide.<sup>14,15</sup> In this study also both these organisms were the predominant ones, 48.8 % were *E.coli*, 40% were *K. pneumonia* ,6% were *Acinetobacter* and 4% were *Proteus spp.* Shiju MP et.al. in 2010 reported ESBL *E.coli* incidence to be 51.47% and ESBL *K. pneumonia* to be 48.53% which

is in concordance with our study.<sup>9</sup> Latifpour et al. reported the prevalence of ESBL-producing *K. pneumoniae* isolates as approximately 38.1%.<sup>15</sup> Several worldwide studies have reported the ESBL producers as emerging pathogens.<sup>15</sup>

In the wake of the increasing resistance rates, the 100 % carbapenem sensitivity advocates the use of carbapenem as the therapeutic alternative to this menace.<sup>16</sup> Antimicrobial susceptibility testing should be performed for each strain before prescribing antibiotics which has to be done routinely in a laboratory. The carbapenem should be used as a reserve drug only in cases of multi drug resistant strains. It is important to keep information on an isolate to avoid the misuse of extended spectrum cephalosporins, which still remain as an important component of antimicrobial therapy in high risk wards.<sup>17</sup>

## V. Conclusion

A detailed analysis of the resistance pattern of different strains of bacteria in a geographical area will help in appropriate and judicious antibiotic use by the clinicians. Rather than adapting a screening and confirmatory strategy for ESBL production, direct phenotypic confirmatory test along with routine antibiotic susceptibility testing will help to report ESBL production within 48 hours. Restricted use of antibiotics will lead to a decreased selective pressure and the resistant strains can no longer sustain in such settings. The medical professionals should make an attempt to educate the general public regarding the misuse of antibiotics.

## References

- [1]. Rudresh SM and Nagarathnamma T. Extended spectrum  $\beta$ -lactamase producing Enterobacteriaceae & antibiotic co-resistance, Indian J Med Res. 2011 Jan; 133(1): 116–118.
- [2]. Kumar et al. Antimicrobial Susceptibility Profile of Extended Spectrum  $\beta$ -Lactamase (ESBL) Producing *Escherichia coli* from Various Clinical Samples. Infectious Diseases: Research and Treatment 2014;7; 1–8.
- [3]. Jonathan, N. (2005). Screening for extended-spectrum  $\beta$ -lactamase producing pathogenic Enterobacteria in district general hospitals. J Clin Microbiol. 2005; 43(3):1488–1490.
- [4]. Paterson DL, Bonomo RA. Extended-spectrum  $\beta$ -lactamases: a clinical update. Clin Microbiol Rev. 2005;18:657–686.
- [5]. Mutasim E I, Naser E B, Magzoub AM, Mohamed EH. Prevalence of Extended-spectrum  $\beta$ -Lactamases-producing *Escherichia coli* from Hospitals in Khartoum State, Sudan M, Oman Medical Journal. 2013;28( 2):116-120.
- [6]. Subha A, Ananthan S. Extended spectrum  $\beta$ -lactamase (ESBL) mediated resistance to third generation cephalosporins among *Klebsiella pneumoniae* in Chennai. Indian J Med Microbiol. 2002;20(2):92–95.
- [7]. Bradford PA. Extended-spectrum  $\beta$ -lactamases in the 21<sup>st</sup> century: Characterization, epidemiology and detection of this important resistance threat. Clin Microbiol Rev. 2001;14:933–51.
- [8]. Giraud-Morin C, Fosse TA. Seven-year survey of *Klebsiella pneumoniae* producing TEM-24 extended-spectrum-lactamase in Nice University Hospital (1994–2000) J. Hosp. Infect. 2003;54:25–31.
- [9]. Shiju MP, Yashavanth R, Narendra N, Detection Of Extended Spectrum Beta-Lactamase Production And Multidrug Resistance In Clinical Isolates Of *E.Coli* And *K. Pneumoniae* In Mangalore, 2010,Jun;4(3):2442-2445.
- [10]. Shukla I, Tiwari R, Agarwal M, Prevalence of extended spectrum  $\beta$ -lactamase producing *Klebsiella pneumoniae* in a tertiary care hospital. Indian J Med Microbiol 2004; 22:87-91.
- [11]. Sridher Rao PN, Basavarajappa KG, Krishna GL. Detection of extended spectrum beta-lactamase from clinical isolates in Davangere. Indian J Pathol Microbiol. 2008;51:497–9.
- [12]. Kumar MS, Lakshmi V, Rajagopalan R. Occurrence of extended-spectrum  $\beta$ -lactamases among Enterobacteriaceae spp. isolated at a tertiary care institute. Indian J Med Microbiol. 2006; 24:208–11.
- [13]. Chaudhary U, Aggarwal R. Extended spectrum  $\beta$ -lactamases (ESBL)—an emerging threat to clinical therapeutics. Indian J Med Microbiol. 2004;22(2):75–80.
- [14]. Jacoby G. A. and Munoz-Price L. S. “The new  $\beta$ -lactamases,” The New England Journal of Medicine, , 2005; 352( 4); 380– 391.
- [15]. Latifpour M, Gholipour A, Mohammad S D. Prevalence of Extended-Spectrum Beta-Lactamase-Producing *Klebsiella pneumoniae* Isolates in Nosocomial and Community-Acquired Urinary Tract Infections Jundishapur J Microbiol. 2016 March; 9(3).
- [16]. Abigral S, Mathai E, Jesudasan MV, John JJ, Ceftazidime resistance among *K.pneumoniae* in South India. Indian J Med Res, 1995; 102:53-55.
- [17]. Philipon A, Labia R, Jacoby G. Extended spectrum lactamases. Antimicrobial Agents Chemother 1989; 33:1131- 6.

\*Linda Rose Jose. "Detection of ESBL Organisms in body fluids, a study from a tertiary care center ." IOSR Journal of Dental and Medical Sciences (IOSR-JDMS) 16.8 (2017): 48-51