Int.J.Curr.Microbiol.App.Sci (2017) *6*(8): 2857-2863

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 6 Number 8 (2017) pp. 2857-2863**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2017.608.341

Prevalence of Extended Spectrum Beta- Lactamase Producers among Various Clinical Samples in a Tertiary Care Hospital: Kurnool District, India

Suguneswari Giddi, Sarada Dasari* and C. Suryakumari

Department of Microbiology, Viswabharathi Medical College, Penchikalapadu, Kurnool District, A.P., India
**Corresponding author*

Keywords

ESBL producers, *Escherichia coli*, *Klebsiella*, Susceptibility pattern, Resistance pattern.

Article Info

Accepted:
21 June 2017
Available Online:
10 August 2017

A B S T R A C T

Extended spectrum beta lactamase producers are posing a major obstacle in the therapeutic outcome of patients. They cause enzymatic hydrolysis of the beta lactum ring, resulting in resistance to penicillins and cephalosporins. The rise in ESBL producers results in prolonged treatment which leads to increased hospital stay and financial burden on the patient. It is necessary for all the hospitals to monitor ESBL producers to formulate preventive and therapeutic measures accordingly. The study was conducted in a tertiary care hospital during the period from December 2015 to November 2016. A total of 500 isolates from *Enterobacteriaceae* which included 400 *Escherichia coli* and 100 *Klebsiella* species isolates were processed. ESBL producers were detected by phenotypic screening and confirmatory methods which included double disc synergy method, Cephalosporin Clavulanate combination disc. Out of 400 *Escherichia coli*, 220 were ESBL producers (55%) and 34 out of *Klebsiella* species were ESBL producers (34%). Overall ESBL production was 50.8% and it was found to be higher in inpatients (55.67%) than outpatients (41.28%). All ESBL producers are 100% susceptible to *Imipenem* and *Meropenem* and maximum resistance to penicillin and cephalosporins (80-90%). ESBL producers pose threat in hospitals; their detection can be made mandatory along with routine antibiogram.

Introduction

Multidrug resistant bacteria are emerging worldwide in the hospitals and cause treatment failures especially the *Enterobacteriaceae* group of bacteria. *Enterobacteriaceae* group of organisms cause various hospital acquired infections like gastrointestinal, urinary tract and pyogenic infections. Among *Enterobacteriaceae* the most important member that exhibits multiple drug resistance is *Escherichia coli* (Dinesh Kumar *et al.*, 2014). *Enterobacteriaceae* family possesses plasmid mediated β lactamases and which is the single most important cause of resistance to penicillins and cephalosporins. This resistance is due to mutations in genes like TEM, SHV, and CTX-M. These mutations cause alteration of aminoacid configuration which results in their ability to hydrolyze various beta lactum antibiotics like penicillins, cephalosporins and monobactum (Agarwal, 2004).

Among *Enterobacteriaceae* the major ESBL producers are *Escherichia coli* and *Klebsiella*. ESBL producers are worldwide in distribution and its rate and methods of isolation varies in different areas. ESBL are found in clinical samples like urine, blood, sputum, swabs,

body fluids, and catheter tips (Kenneth, 2008). Apart from resistance to broad spectrum cephalosporins and monobactums, ESBL also exhibit co-resistance to other groups of antibiotics such as quinolones, co-trimoxazole, and tobramycin, this leads to limitation of available therapeutic options. ESBL production is indicated by eight fold reduction in MIC and accentuation of zone of inhibition of third generation cephalosporins in the presence of clavulinic acid (Iraj Alipourfard, 2010).

High rate of ESBL producing organisms associated with fewer therapeutic options lead to longer hospital stay, increased cost, high rate of morbidity and mortality (Folasoge A *et al.*,, 2014). Therefore the present study was conducted to be able to study the prevalence, antibiotic resistance profile of ESBLs so that an effective antibiotic strategy can be planned to limit infections due to them.

Materials and Methods

The present study was conducted in Viswabharathi medical college, a tertiary care hospital in Kurnool, during the period from December 2015 to November 2016. The isolates were collected from various clinical samples which include urine, blood, sputum, swabs and body fluids. Samples from patients attending both outpatient and inpatient departments were included in the study without applying any selection criteria. Sufficient amount of various clinical samples were collected under aseptic conditions and were inoculated on to blood agar and MacConkey agar. Blood samples were collected under aseptic conditions and inoculated in brain heart infusion agar. They were subcultured on to blood agar and MacConkey agar after overnight incubation at 37ºC for 24hrs. All the inoculated samples were incubated at 37ºC for 24 hrs and were processed further for identification of the organism by studying colony morphology, motility, and gram staining and biochemical reactions.

Isolated organisms were subjected to antimicrobial susceptibility testing by Kirby-Bauer disc diffusion method on Mueller Hinton agar following clinical laboratory standards institute guidelines CLSI guidelines (CLSI, 2014). Commercially available antibiotic discs from Hi- media were used. The antibiotic discs used were *Ampicillin* (10µg), *Cephalexin* (30µg), *Cefoxitin* (30 µg), *Ceftazidime* (30 µg), *Cefotaxime* (30µg), *Ceftriaxone* (30µg), *Aztreonam* (30µg), *Imipenem* (10µg), *Gentamicin* (10µg), *Amikacin* (30µg), *Ciprofloxacin* (5µg), *Norfloxacin* (10µg), *Nitrofurantoin* (300µg), *Meropenem* (10µg), *Amoxyclav* (20/10µg), *Ticarcillin-Clavulinic* acid (75/10ug), *Piperacillin-Tazobactum* (100/10 µg). ESBL detection was detected by phenotypic screening and confirmatory tests.

Phenotypic screening test

Ceftazidime, *Aztreonam*, *Cefotaxime* and Ceftriaoxone antibiotic discs were used for antibiotic sensitivity testing as a routine method. The zone of inhibition was measured and it was ≤ 22mm for *Ceftazidime*, ≤ 27mm *Aztreonam*, ≤ 25 *Ceftriaxone*, and ≤ 27mm for *Cefotaxime*. The above zone sizes indicate suspected ESBL producers. They were confirmed by confirmatory tests.

Phenotypic confirmatory tests 2 methods

Double disk synergy test (DDS)

It is a diffusion test in which 30 µg of *Ceftriaxone*, *Ceftazidime*, *Cefotaxime* and *Aztreonam* antibiotic discs are subjected to antibiotic susceptibility testing on Mueller Hinton agar with lawn culture of the isolate about 30mm from Amoxicillin Clavulanic

acid disk. After overnight incubation extension of zone of inhibition of antibiotic disc towards Clavulanic acid containing disc indicates ESBL (Dinesh Kumar, 2014).

Combination disc containing clavulanic acid

Cefotaxime and *Ceftazidime* disc with and without Clavulanic acid were placed at appropriate distance on Mueller Hinton agar plate with isolate and read after overnight incubation. A difference of more than 5mm of zone size between cephalosporin disc and their clavulanate containing disc indicates ESBL production (Dinesh Kumar *et al.*, 2014).

Results and Discussion

This study was conducted in a tertiary care hospital at Kurnool district during the period December 2015 to November 2016. Samples from inpatients and outpatients attending various clinical departments are included in the study. Various clinical samples included in the study were urine, sputum, blood, pus, swabs and fluids. Maximum ESBL productions was seen in urine (65.35%) samples followed by swabs (12.59%), blood (8.66%), fluid (7.87%), sputum (5.51%) (Table 1).

Among 254 total ESBL producers *Escherichia coli* was the dominant organism from urine (96.38%), swab (81.25%), blood (81.81%), fluids (70%), where as in *Klebsiella* maximum ESBL production was seen in sputum samples (85.71%) (Table 2).

Out of 500 isolates, 400 isolates were *Escherichia coli* and 100 isolates were *Klebsiella* spp. Among 400 isolates of *Escherichia coli* 220 (55%) were ESBL producers and 60 (21.42%) were ESBL non producers. Among 100 isolates of *Klebsiella* species 34 (34%) were ESBL producers and 36 (48.57%) were non ESBL producers (Table 3).

The overall ESBL producers from total 500 samples were 50.8% and non ESBL producers were 19.2 % (Table 4).

All the isolates exhibited 100% sensitivity to *Imipenem* and *Meropenem*. Among all the isolates 80.31% showed sensitivity to *Pipercillin-Tazobactum*, 77.16% to *Amikacin* and *Amoxyclav*, followed by 76.37% to *Ticarcillin-Clavulanic* acid, 58.26% to *Gentamicin* and 50.39% isolates were sensitive to *Nitrofurantoin*. All the isolates showed maximum resistance (80% to 90%) to all 4- generation Cephalosporins.

The resistance pattern for other drugs were 93.7% to *Ampicillin*, 90.55% to *Aztreonam*, 72.64% to *Norfloxacin*, and 70.07% isolates were resistant to *Ciprofloxacin* (Table 6).

Table.1 Frequency of samples yielding ESBL producers

Source	Frequency	Percentage
Urine	166	65.35%
Swabs	32	12.59%
Blood	22	8.66%
Fluids	20	7.87%
Sputum	14	5.51%
Total	254	

ESBL- Extended spectrum betalactamase

Table.2 ESBL prevalence among various clinical samples

Sample	No of isolates	ESBL Positive			
		Escherichia coli		*Klebsiella*	
		No	Percentage	No	Percentage
Urine	166	160	96.38%	6	3.61%
Swabs	32	26	81.25%	6	18.75%
Blood	22	18	81.81%	4	18.18%
Fluids	20	14	70%	6	30%
Sputum	14	2	14.28%	12	85.71%
Total	254	220		34	

ESBL- Extended spectrum betalactamase

Table.3 ESBL production in *Escherichia coli* and *Klebsiella*

	Escherichia coli				*Klebsiella*			
	E.coli isolates	**Screening positives**	**ESBL producers**	**Non-ESBL producers**	***Klebsiella* isolates**	**Screening positives**	**ESBL producers**	**Non-ESBL producers**
Urine	250	193	160	33	17	14	6	8
Swabs	85	37	26	11	13	9	6	3
Blood	30	27	18	9	10	8	4	4
Fluids	25	19	14	5	16	11	6	5
Sputum	10	4	2	2	44	28	12	16
	400	280	220 (55%)	60 (21.42%)	100	70	34 (34%)	36 (48.52%)

E.coli- *Escherichia coli*, ESBL-Extended spectrum betalactamase, Non-ESBL- Non Extended spectrum betalactamase

Table.4 ESBL and Non ESBL producers in *Escherichia coli* and *Klebsiella*

Organisms	**Total isolates**	**ESBL producers & %**	**ESBL non-producers & %**
Escherichia coli	400	220 (55%)	60 (21.42%)
Klebsiella	100	34 (34%)	36 (48.57%)
Total	500	254 (50.8%)	96 (19.2%)
			P- Value <0.01

ESBL- Extended spectrum betalactamase, Non-ESBL- Non Extended spectrum betalactamase

Table.5 Number of *Enterobacteriaceae* isolates in in-patients and out-patients

	Escherichia coli			*Klebsiella*	
Specimen	**IP**	**OP**	**Specimen**	**IP**	**OP**
Urine (n=250)	147	103	Urine (n=17)	10	7
Swabs (n=85)	44	57	Swabs (n=13)	7	6
Sputum (n=30)	2	8	Sputum (n=44)	28	16
Blood (n=30)	20	10	Blood (n=10)	8	2
Fluids (n=25)	4	11	Fluids (n=16)	12	4
400	217	183	100	65	35
				P- Value- < 0.05	

IP- In patient, OP- Outpatient

Table.6 Susceptibility and resistance pattern of ESBL producers

Antibiotic	Sensitivity		Resistance	
	Number	**Percentage**	**Number**	**Percentage**
Ampicillin	16	6.24%	238	93.7%
Cephalexin	42	16.53%	212	83.46%
Cefoxitin	50	19.68%	204	80.3%
Ceftazidime	37	14.56%	217	85.43%
Cefotoxime	24	9.44%	230	90.55%
Ceftriaxone	30	11.81%	224	88.18%
Cefaperazone	32	12.59%	222	87.40%
Aztreonam	24	9.44%	230	90.55%
Imipenem	254	100%	0	0
Gentamicin	148	58.26%	106	41.73%
Amikacin	196	77.16%	58	22.83%
Ciprofloxacin	76	29.92%	178	70.07%
Norfloxacin	84	33.07%	170	72.64%
Nitrofurantoin	128	50-39%	126	49.60%
Meropenem	254	100%	0	0
Amoxyclav	196	77.16%	58	22.83%
Ticarcillin-Clavulinic acid	194	76.37%	60	23.62%
Pipercillin-Tazobactum	204	80.31%	50	19.16%

ESBL- Extended spectrum betalactamase

A total of 500 isolates from *Enterobacteriaceae* were processed for ESBL production in our one year study period. Although other members of enterobacteriacae also produce ESBL, *Escherichia coli* and *Klebsiella* species were taken into consideration.

Out of the 400 *Escherichia coli* isolates 220 (55%) were ESBL producers, 34 (34%) among 100 *Klebsiella* species were ESBL producers. Overall ESBL production was seen in 254 isolates (50.84%) which matches with the study by Hima bindu (2015) (58.8%). ESBL production in *Escherichia coli* and *Klebsiella* was 55% and 34% respectively in our study which is consistent with studies by Hima bindu (2015) (56.4%, 62.3%) and Sasirekha (52.8%, 45.1%). Study by Dinesh Kumar *et al.,* (2014) showed 55.55% of ESBL production in *Escherichia coli* which is highly similar with our study (55%). ESBL production in *Klebsiella* species in our study was 34% which is similar to the study by Shukla *et al.,* (30.18%) and slightly less than the study done by Baby Padmini *et al.,* (2004) (40%).

Overall percentage of ESBL producers in India varies between 22- 75% (Aruna *et al.,* 2012). In our study ESBL production was found to be high among inpatients (55.67%) than out patients(48.28%) this can be compared to the study by Dinesh Kumar *et al.,* (2014) (IP-60.95%, OP-48%) and Rinki *et al.,* (2016) (IP-71.3%, OP-50.5%). The present study showed highest number of ESBL producers from urine and *Escherichia coli* was found to be the dominant organism, this observation is in agreement with the study by Folasoge.A.*et al.,*, In our study highest susceptibility was found for *Imipenem* and *Meropenem* (100%) followed by *Piperacillin-Tazobactum* (80%), *Amikacin*,

Amoxyclav (77.16%) and *Ticarcillin-Clavulanate* (76%). Maximum resistance was seen for *Ampicillin*, all 4-generations of Cephalosporins and *Aztreonam* (80-90%). This susceptibility and resistance pattern is in accordance with other studies (Pooja Shakya *et al.,* 2017; Akila *et al.,* 2016 and Dinesh Kumar, 2014).

The present study was done in a tertiary care hospital at Kurnool district on ESBL production among *Enterobacteriaceae* group of organisms. *Escherichia coli* emerged as a dominant ESBL producer when compared to *Klebsiella*. All 4 generations of Cephalosporins, and other drugs like *Ampicillin*, *Aztreonam* and *Ciprofloxacin* showed maximum resistance; only *Imipenem* and *Meropenem* exhibited 100% susceptibility to ESBL producers. Indiscriminate usage of board spectrum antibiotics as an option of empirical choice of treatment leads to rise of ESBL producers complicating treatment options. Strict implementation of hospital infection control measures along with restricting inadvertent use of cephalosporins may contribute in preventing ESBL producers.

References

Akila. K., *et al.,* 2016. Prevalence of ESBL producing *Klebsiella* species and their in-vitro antimicrobial susceptibility pattern in a tertiary care hospital. *IOSR Journal of Dental and Medical Sciences*, 5-10.

Aruna. K., *et al.,* 2012. Prevalence of ESBL production among uropathogens in South Mumbai and its Antibiogram pattern. EXCLIJ. 11:363-372.

Baby Padmini, *et al.,* 2004. Extended spectrum beta-lactamases in urinary isolates of *Escherichia coli* and *Klebsiella* pneumoniae - prevalence and susceptibility pattern in a tertiary care hospital. Indian J Med Microbiol, 22, 172–174

Chowdary U., *et al.,* 2004. Extended spectrum β-alactamases (ESBL);An Emerging threat to clinical therapeutics. *Indian journal of medical microbiology, 22(2):* 75-80.

CLSI, 2014. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement. CLSI document M100-S24. Wayne, PA: Clinical and Laboratory Standards Institute.

Dalela. G., 2012. Prevalence of ESBL produces among Gram negative bacilli from various clinical isolates in a tertiary care hospital at Jhalawar, Rajasthan, India. *Journal of Clinical Diag.Res*. 6(2):182-187.

Dinesh Kumar. A., *et al.,* 2014. Antimicrobial susceptibility profile of extended spectrum betalactamse producing *Escherichia coli* from various clinical samples. *Infectious diseases reserch and treatment*.

Folasoge, A., *et al.,* 2014. A multicenter study of Beta Lactamase resistant *Escherichia coli* and *Klebsiella* pneumoniae reveals high level chromosome mediated extended spectrum β Lactamase resistance in Ogun State, Nigeria. *Interdisciplinary perspectives on infectious diseases*.

Hima Bindu. M., *et al.,* 2015. Prevalence of ESBL production in *Escherichia coli* and *Klebsiella* Spp from different clinical samples-A study in a teaching hospital in Telangana, India. *International Journal of Current Microbiology and Applied Sciences*, 4(3):236-243.

Iraj Alipourfard, *et al.,* 2010. Antibiogram of extended spectrum Beta-lactamase producing *Escherichia coli* and *Klebsiella* pneumoniae isolated from hospital samples. *Bangladesh journal of*

medical mecrobiology, vol 4. (01):32-36.

Kenneth. T., 2008. *Bacterial Resistance to Antibiotics*. Todars Online Textbook of Bacteriology.

Pooja Shakya, *et al.*, 2017. ESBL production among E.coli and *Klebsiella* Spp causing urinary tract infections: A hospital based study. *The Open Microbiology Journal*, 11, 23-30.

Rinki R., *et al.*, 2016. The detection of extended spectrum Beta-Lactamases (ESBLs) producing *Escherichia coli* isolated from clinical samples. International journal of advance research in biological sciences, 3(5):9-15.

Sasirekha. B., 2013. Prevalence of ESBL, Amp C β-Lactamases and MRSA among uropathogens and its antibiogram. EXCLIJ, 12:81-88.

Shukla. R., 2004. Prevalence of extended spectrum beta-lactamase producing *Klebsiella* pneumoniae in a tertiary care hospital. Indian J Med Microbiol, 22, 87–91.

How to cite this article:

Suguneswari Giddi, Sarada Dasari and Suryakumari, C. 2017. Prevalence of Extended Spectrum Beta- Lactamase Producers among Various Clinical Samples in a Tertiary Care Hospital: Kurnool District, India. *Int.J.Curr.Microbiol.App.Sci.* 6(8): 2857-2863.
doi: https://doi.org/10.20546/ijcmas.2017.608.341