International Journal for Pharmaceutical Research Scholars (IJPRS)

V-2, I-4, 2013 ISSN No: 2277 - 7873

RESEARCH ARTICLE

Antibiotic Susceptibility Pattern of ESBL Producing *Escherichia Coli* from Various Clinical Samples in a Tertiary Care Hospital, Kanchipuram, Tamilnadu

Senthamarai S[*1], Sivasankari S[1], Kumudhavathi MS[1], Somasunder VM[1], Suneel Kumar Reddy[1]

[1]*Dept. of Microbiology, Meenakshi Medical College, Kanchipuram, Tamilnadu, India.*

Manuscript No: IJPRS/V2/I4/00216, Received On: 27/11/2013, Accepted On: 30/11/2013

ABSTRACT

The incidence of Extended Spectrum of Beta Lactamase (ESBL) producing strains among clinical isolates has been steadily increasing with variable sensitivity rates for fluoroquinolones, aminoglycosides and 4th generation is a matter of concern. To determine the prevalence and the antibiotic susceptibility pattern of ESBL producing *E.coli* from various clinical samples in a tertiary care hospital, Kanchipuram, Tamilnadu. Total of 172 Escherichia coli isolates were isolated from different clinical specimens, during the period March 2012 to August 2013.They were evaluated for the presence of ESBL enzyme by combined disc diffusion test. The antibiotic sensitivity pattern of ESBL producing *E.coli* against various classes was determined. Out of 172 *E.coli* isolates 64 (37.2 %) were ESBL producers. It was found maximum in Urine sample (81.3%) and Female (67.2%) was commonly affected. More no of cases 38 (59.4%), were seen between 21-40 years 100% susceptibility was observed for imipenem, Nitrofurantoin (urine isolates), Netilmycin, Levofloxacin, and Ticarcillin/Clavulanic Acid showed 96.9%, 84.4%, 81.3% & 67.2 % sensitivity, respectively. This study highlights the prevalence of ESBL production in *E.coli* in our area. The quick detection is very important, because the ESBL strains can pass the gene to other clinical strains.

KEYWORDS

E.coli, Extended Spectrum of Beta Lactamase (ESBL), antibiotic susceptibility

INTRODUCTION

E.coli is one of the most common gram negative bacilli (GNB) from our hospital settings. Resistance to GNB is a matter of great concern, because of growing numbers of reports of the resistance to all available antibiotics used in therapy. Among the antibiotics, β-lactams are the most widely used agents accounting for over 50% of all systemic antibiotics in use[1].

Mechanisms by which clinical isolate of Gram negative bacteria resist β-Lactam antibiotics are through production of β- Lactamase, modification of cell wall and modification of target sites with reduced affinity for β-Lactam antibiotics. Among these the production of beta-lactamase appears to be of primary concern and one of the most rapidly developing and clinically significant antimicrobial resistance mechanisms.

ESBL are plasmid mediated enzymes capable of hydrolyzing oxyaminocephalosporin and are inhibited by betalactam inhibitors conferring broad spectrum resistance to penicillin, 3rd generation cephalosporin and monobactam but not to carbapenem[2].

***Address for Correspondence:**
Senthamarai S.
Department of Microbiology,
Meenakshi Medical College,
Kanchipuram, Tamilnadu, India.
E-Mail Id: thamaraimicro@gmail.com

The incidence of ESBL producing strains among clinical isolates has been steadily increasing over the past several years, resulting in limitation of therapeutic options. These strains have variable sensitivity rates for fluoroquinolones, aminoglycosides and 4th generation is a matter of concern. The selective pressure imposed by the use and overuse of new antibiotics in the treatment of patients has resulted in the emergence of new variants of beta lactamases. The ESBL production varying from 28% to 84% in India.[3]

E.coli being the commonest organism among GNB to exhibit ESBLs, complicates the problem, unless a definitive policy of detecting ESBL producing clinical isolates and reporting will institute the appropriate treatment and reduce the hospital stay, morbidity, mortality and health care expenses.[2,4]

Aim of the Study

Considering the burden, we have undertaken this study to determine the prevalence of ESBL producing *E. coli* isolated from different clinical samples and its drug susceptibility pattern in our tertiary care hospital.

MATERIALS AND METHOD

A total of 172 non repetitive clinical isolates of *E. coli* were collected. The samples included were Urine, Sputum, Blood, Fluids, Pus and wound swab. This descriptive study was carried out in Microbiology department at Meenakshi Medical College Hospital & Research Institute (MMCH&RI), Kanchipuram. The study period was from March 2012 to August 2013. Demographic, clinical history and co morbidities were recorded from all the patients and informed oral consent obtained.

The samples were inoculated on nutrient agar, MacConkey and blood agar for culture and incubated at 37^{o}C for 24 hours. Suspected colonies were subjected for biochemical reactions according to standard laboratory protocol for *E. coli*. The antibiogram was performed by Kirby Bauer disc diffusion technique with commercially available discs (Hi-Media) on Muller Hinton Agar and zone diameters were interpreted according to CLSI-2012. *E. coli* ATCC 25922 was used as control strain for test interpretation.

ESBL Screening Method (CLSI-2012)

Isolates exhibiting zone size ≤ 25mm with ceftriaxone (30µg), ≤ 22mm for ceftazidime (30µg), and ≤ 27mm with Cefotaxime (30µg), were considered as ESBL producer.

Phenotypic Confirmatory Test for ESBL: (Combined Disc Diffusion Method) (CLSI-2012)

They were further confirmed by combined disc diffusion test. 0.5 McFarland's turbidity standard suspension was made from the colonies of *E. coli* isolate. Using this inoculum, lawn culture was made on Muller Hinton Agar plate. Discs of Ceftazidime, Ceftriaxone and Cefotaxime alone and in combination with Clavulanic acid (30 mcg /10 mcg) were placed aseptically on the surface of MHA. The distance of 15mm was kept between the disc and overnight incubation was done at 37˚C. An increase of ≥ 5mm in zone diameter with Clavulanic acid in comparison to the zone diameter of 3GC alone confirmed the ESBL production by the organisms.

Antibiotic Susceptibility Test for ESBL Producing E. coli

These ESBL strains of *E. coli* were tested for the susceptibility pattern for the following drugs; Gentamycin , Amikacin , Ciprofloxacin, Norfloxacin, Ofloxacin, Cotrimoxazole, Ampicillin, Cephalexin, Piperacillin, Piperacillin Tazobactam, Amoxyclav, Ticarcillin-Clavulanic-acid, Cefoperazone-Sulbactam, Imipenum, Nitrofurantoin(for urinary isolates). Results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI-2012) guidelines.

RESULTS

A total of 172 *E. coli* isolates were isolated from different clinical specimens like urine, blood, sputum, pus and other body fluids from both in and outpatients of the hospital, during the period March 2012 to August 2013. All of them were

subjected to screening by using ceftazidime, cefotaxime and ceftriaxone for ESBL.

Prevalence of ESBL Producing *E. coli* Isolates

Among 172 *E. coli*, 64 (37.2 %) showed ESBL production in the combined disc diffusion test. (Table-1).

Table 1: Distribution of the prevalence of ESBL producing *E. coli* isolates

Isolates (n=172)	No. of Isolates	Percentage (%)
ESBL producers	64	37.2
Non-ESBL producers	108	62.8

Distribution of ESBL Producing *E. coli* in Various Clinical Isolates

They were identified from various clinical specimens: urine, blood, sputum, fluids and pus (Table-2).

Table 2: distribution of ESBL producing *E. coli* isolates in various clinical isolates

Clinical samples	No of isolates (n=64)	Percentage (%)
Urine	52	81.3
Pus	6	9.3
Sputum	3	4.7
Blood	2	3.1
Fluids	1	1.6

Age and Sex Distribution

Female 43(67.2%) were commonly affected than Male 21(32.8%).

In the age distribution, more no of cases 38(59.4%), were seen between 21-40 years which is followed by 13(20.3%) in the age group 61-80 years (Table-3).

The Antibiotic Susceptibility Patterns ESBL Producing *E. coli*

The antibiotic susceptibility patterns were observed using Kirby Bauer disc diffusion method for ESBL producing *E. coli* isolates (Table-4).

100% susceptibility was observed for imipenem. Nitrofurantoin (urine isolates), netilmycin, Levofloxacin, and Ticarcillin/Clavulanic Acid showed 96.9%, 84.4%, 81.3% & 67.2 % sensitivity, respectively.

Maximum resistances (100%) were observed for both ampicillin and cephalexin, followed by, gentamycin (96.9%), norfloxacin (93.7%), ciprofloxacin (92.2%) and Cefoperazone-Sulbactam (81.3%).

DISCUSSION

ESBL producing organisms, being the significant nosocomial organism, it is very important to detect and treat them as early as possible. In 1999, 1st report of outbreak in nursing homes, were limited with high risk areas like ICU, oncology units, etc.[5] But, recent studies on ESBL production among gram negative bacteria from various clinical samples showed an increase in the occurrence of ESBL producers in all the health care set up.[6]

The prevalence of ESBL in our study was 37.2%. In the study of Tankhiwale *et al*, (2004)[6], at Nagpur, 48.3% were ESBL producer .41 % were the ESBL producer in E.coli, in the study of Baby Padmini et al. 2004[7]. In a similar study, Mathur et al, 2005[8] reported 62% ESBL producer in E.coli. The rate vary greatly in different geographical areas and different institutes, could be due to the study environment under which the study was performed. Various studies from India have reported ESBL production, from 28% to 84 %.[3]

Table 3: Showing sex distribution of ESBL producing *E. coli* isolates

Age in years (n=64)	No of isolates in female(n=43)	Percentage	No of isolates in male(n=21)	Percentage	Total	Percentage
0-20	5	11.7	0	0	5	7.8
21-40	32	74.5	6	28.6	38	59.4
41-60	2	4.7	3	14.3	5	7.8
61-80	3	6.8	10	47.6	13	20.3
>80	1	2.3	2	9.5	3	4.7

Table 4: The antibiotic susceptibility patterns ESBL producing *E. coli* isolates

Antimicrobial Agent(Mcg)	Sensitive	(%)	Resistance	(%)
Beta lactam drugs				
Ampicillin	0	0	64	100
Piperacillin	24	37.5	40	62.5
Cephalexin	0	0	64	100
Imipenem	64	100	0	0
Betalactam inhibitors				
Amoxyclav	21	32.8	43	67.2
Piperacillin-Tazobactam	26	40.6	38	59.4
Ticarcillin/Clavulanic Acid	43	67.2	21	32.8
Cefoperazone-Sulbactam	12	18.7	52	81.3
Aminoglycosides				
Gentamycin	2	3.1	62	96.9
Amikacin	16	25	48	75
Netilmycin	54	84.4	10	15.6
Fluoroquinolones				
Ciprofloxacin	5	7.8	59	92.2
Norfloxacin	4	6.3	60	93.7
Ofloxacin	26	40.6	38	59.4
Levofloxacin	52	81.3	12	18.7
Nitrofurantoin	62	96.9	2	3.1
Co-trimoxazole	24	37.5	40	62.5

Urinary Tract Infection (UTI) is the most common infection encountered in sexually active young female and E.coli is the predominant pathogen in causing UTI. E.coli was the predominant isolate of UTI, with the studies of Heuch & Hoban *et al* 2011, Iraj *et al.*[10] (2010), Zohreh *et al.*[11] (2013), Tijjani *et al.*[12] *(*2012).

Male: female ratio observed in our study was 1: 2.04. In this present study, 81.3% of isolates of E.coli was from urine. The commonest age group affected in female was between 21-40 years (74.5%) and in male, it was 61-80 years (47.6%).

The reason for age distribution and female preponderance could be due to the teeming of enteric flora in the periuretheral region, short urethra and sexual activity further drive the bacteria into urinary tract. Whereas in male, prostatic enlargement in the later age group may be the reason for UTI.

All the isolates in various samples were sensitive to imipenem (100%). Similar observations were made by Umadevi *et al.*[13], (2011) & Baby Padmini *et al.*[7] (2004).

In our study, the drugs which were found to be effective against the ESBL producers were nitrofurantoin, netilmycin, levofloxacin and Ticarcillin-Clavulanic acid and these can be used as reserve drugs for ESBL producers.

Maximum resistance was noted for ampicillin (100%), cephalexin (100%), gentamycin (96.9%), norfloxacin (93.7%), ciprofloxacin (92.2%) and Cefoperazone-Sulbactam (81.3%).Similar kind of resistance to commonly used antibiotics, were reported by Aminzadeh *et al.*[11] & Iraj Alipourfard *et al.*[10] Inappropriate and indiscriminate use of these drugs at health care set up could be the reason for resistance.

ESBL producing bacteria are also frequently resistant to many other classes of antibiotics which include aminoglycosides and fluoroquinolones. This resistance could be attributable to possible coexistence of genes encoding drug resistance to other antibiotics on the plasmids which encode ESBL[14]. This fact was noted in our study. Among the fluoroquinolones, norfloxacin, ciprofloxacin and ofloxacin showed 93.7%, 92.2% and 59.4% resistance respectively, in this present study. The ESBL strains showed 96.9% & 75% resistance to gentamycin and amikacin respectively. Iraj Alipourfard *et al.*[10], Akyar *et al.*[16], and Grandesso *et al.*[17], reported related findings about amikacin and ciprofloxacin which is concordant with our study.

The quick detection of these strains in microbiology laboratories is very important, because the ESBL strains are resistant to available antibiotics and they can pass the gene to other clinical strains. Careful antimicrobial drug selection and appropriate use has an important role in the treatment of human bacterial infections, but the drug resistance that has emerged in the treatment of bacterial infections due to ESBL enzymes degrades all beta lactam antibiotics and thus bacteria become multidrug resistant.

CONCLUSION

Presence of ESBL is of main concern as it is now come to the alert of the physician that ESBL is spreading fast in the community and responsible for outbreak status spreading among patients and locals, perhaps owing to particular pathogenicity traits. Many ESBL producers are multi -resistant to non-beta lactam antibiotics such as quinolones and amino glycosides, narrowing treatment options. It is essential to report ESBL production along with the routine sensitivity reporting, which will help the clinician in prescribing the proper antibiotics. Imipenem is the most active and reliable agents for the treatment of infections which are caused by ESBL producing organisms in this study. The drugs which were found to be effective against the ESBL producers were nitrofurantoin, netilmycin, levofloxacin and Ticarcillin-Clavulanic acid and these can be used as reserve drugs for ESBL producers.

ACKNOWLEDGEMENT

Dean of Meenakshi medical college and research institute, and MAHER University.

REFERENCES

1. Kumar MS, Lakshmi V, Rajagopalan R. et al., "Occurrence of extended spectrum beta lactamases among Enterobacteriaceae spp. isolated at a tertiary care institute", Indian Journal of Medical Microbiology, 2006, 24(3), 208-211.
2. Mehrgan H, Rahbar M, "Prevalence of Extended spectrum beta lactamases producing E.coli in a tertiary care hospital in Tehran, Iran", International Journal of Antimicrobial Agents, 2008, 31, 147-151.
3. Narayanaswamy A, Mallika M, "Prevalence and susceptibility of extended spectrum beta lactamases in urinary isolates of E.coli in a tertiary care hospital, Chennai-south India". IJM, 2010-2011, 6(1), 39-43.
4. Wani KA, Thakur MA, Fayaz SA, Fomdia B, Gulnaz B, Maroof P, et al., "Extended spectrum beta lactamases mediated resistance in Escherichia coli in a tertiary care hospital", International Journal of Health Sciences, Qassim university, 2009, 3(2), II1430H.
5. Wiener J, Quinn JP, Bradford PA, Goering RV, Nathan C, Bush K et al, "Multiple antibiotic-resistant Klebsiella and Escherichia coli in nursing homes", Journal of American Medical Association, 1999, 281, 517-523.
6. Tankhiwale SS, Jalgaonkar SV, Ahamad S, Hassani U. et al., "Evaluation of extended spectrum beta lactamase in urinary isolates", Indian Journal of Medical Research, 2004, 120, 553-556.
7. Babypadmini S, Appalaraju B, "Extended spectrum –lactamases in urinary isolates of Escherichia coli and Klebsiella pneumoniae - prevalence and susceptibility pattern in a tertiary care hospital", Indian Journal of Medical Microbiology, 2004, 22, 172-174.
8. Singhal S, Mathur T, Khan S, Upadhyay DJ, Chugh S, Gaind R, et al.,. Evaluation of methods for AmpC beta-lactamase in gram negative clinical isolates from tertiary care hospitals. Indian Journal of Medical Microbiology, 2005, 23, 120-124.
9. Hsueh PR, Hoban DJ, Carmeli Y, et al., "Consensus review of the epidemiology and appropriate antimicrobial therapy of complicated urinary tract infections in Asia-Pacific region", Journal of Infection, 2011, 63(2), 114-23.
10. Alipourfard I, Nili NY, "Antibiogram of Extended Spectrum Beta-lactamase (ESBL) producing Escherichia coli and Klebsiella pneumoniae isolated from Hospital Samples", Bangladesh Journal of Medical Microbiology, 2010, 04(01), 32-36.
11. Aminzadeh Z, Yadegarynia D, Fatemi A, Saieed Azad Armaki, Bahram Aslanbeygi, et al., "Prevalence and Antimicrobial Susceptibility Pattern of Extended Spectrum Beta Lactamase (ESBL) and non-ESBL Producing Enteric Gram-Negative Bacteria and Activity of Nitrofurantoin in the era of ESBL", Jundishapur Journal of Microbiology, 2013, 6(7), e6699.
12. Tijjani J., Arzai, AH and Sadiq NM et al., "Antimicrobial Susceptibility Pattern of Extended Spectrum Betalactamase Producers In Gram-Negative Urogenital Isolates In Kano, Nigeria", Bayero Journal of Pure and Applied Sciences, 2012, 5(1), 20 – 25.
13. Umadevi S, Kandhakumari G, Joseph N M, Kumar S, Easow J M, Stephen S, Singh U K. et al., "Prevalence and antimicrobial susceptibility pattern of ESBL producing Gram Negative Bacilli", Journal of Clinical and Diagnostic Research, 2011, 5(2), 236-239.
14. Nathisuwan S, Burgess DS, Lewis JS et al., "Extended spectrum beta lactamases: Epidemiology, detection, and treatment", Pharmacotherapy, 2001, 21, 920-928.
15. Aminzadeh Z, Yadegarynia D, Fatemi A, Armaki SA, Bahram Aslanbeygi et al., "Prevalence and Antimicrobial Susceptibility Pattern of Extended Spectrum Beta Lactamase (ESBL) and non-ESBL

Producing Enteric Gram-Negative Bacteria and Activity of Nitrofurantoin in the era of ESBL", Jundishapur Journal of Microbiology, 2013, 6(7), e6699.

16. Akyar I, "Antibiotic resistance rates of extended spectrum beta-lactamase producing Escherichia coli and Klebsiella spp. strains isolated from urinary tract infections in a private hospital", Mikrobiyol Bul., 2008, 42(4), 713-715.
17. Grandesso S, Sapino B, Mazzucato S, Alessandrini R, Solinas M, Gion M. et al., "Study on *in-vitro* susceptibility of ESBL-positive Escherichia coli isolated from urine specimens", Infez Med., 2010, 18(3), 162-168.