EXCELLENT PUBLISHERS

International Journal of Current Research and Academic Review

ISSN: 2347-3215 Volume 4 Number 2 (February-2016) pp. 170-175

Journal home page: http://www.ijcrar.com

doi: http://dx.doi.org/10.20546/ijcrar.2016.402.021

Molecular Characterization of TEM, SHV and CTX M Genes among ESBL Producing Gram Negative Isolates in ICU

A. Vasumathi[1], P.R. Thenmozhi Valli[2], S. Senthamarai[2*], C.P. Ramani[3] and Raja Jagadeesan[4]

[1]Government Stanley Medical College, Chennai, Tamilnadu, India
[2]Meenakshi Medical College and Research Institute, Enathur, Kanchipuram, Tamilnadu, India
[3]Institute of Microbiology, Madras Medical College, Tamilnadu, India
[4]Senior Assistant professor, King institute, Guindy, Tamilnadu, India

KEYWORDS

ESBL,
TEM,
SHV,
CTX-M

A B S T R A C T

ESBL(extended spectrum β lactamase) producing GNBs are continue to be the great challenge in clinical setups worldwide. The spread of ESBLs producing GNB has been rapidly increasing.T EM, SHV & CTX-M are the prevalent gene responsible for ESBL production. A total of 95 gram negative isolates were isolated from various clinical samples from ICU patients and subjected for ESBL screening and confirmed by combined disc diffusion method and MIC tests and 37 were ESBL producers. These strains were subjected for molecular analysis TEM, SHV and CTX-M genes. Among the 37 ESBL producers, CTX- M (48.3%) were the predominant gene of isolation in our study. SHV and TEM were 41.4 % and 17.2 % respectively. 68.7% of *E.coli* & 23.1% of *Klebsiella* spp had CTX-M genes, 92.3% of *Klebsiella* spp showed SHV genes and 12.5% *E.coli* & 23.1% of *Klebsiella* spp showed TEM genes. ESBL strains should be confirmed with the molecular analysis to know the prevalent gene and for epidemiological analysis.

Introduction

Antimicrobial resistance is a threat to public health. Among the GNB, extended spectrum beta Lactamase are the major cause of resistance. These enzymes causes lysis of the beta lactam ring which is a serious health concern and implicated in therapeutic failure and complications ESBLs were 1st reported in 1980s, and found out to be point mutations of TEM & SHV enzymes, which mediates resistance in the beta lactam group of antibiotics. (1,2,3)

The mutant of *temoneiea* (TEM) and *sulfhydryl* variable (SHV) are the classical ESBLs. The *cefotaximase* (CTX-M) is another type of beta lactamase which is originated from the environmental genus *Kluvera*. This hydrolyses cefotaxime and

ceftriaxone but has very weak action on ceftazidime.(4)

ESBLs are primarily produced by enterobacteriaceae family, in particular *Escherichia coli* and *Klebsiella* spp.. These species are playing major role in various types of both hospital and community acquired infections such as bacteremia, CNS infections, Urinary tract infections, respiratory tract infections, wound infections and diarrhoea.(5,6)

The aim of the study is to characterize the beta lactam resistant genes (TEM, SHV,CTX-M) in GNB isolated from various clinical samples from ICU patients.

Materials and Methods

A total of 95 gram negative bacilli were isolated from various clinical samples such as urine, blood, body fluid, sputum, pus, drainage tubes and aspirates, collected from ICU patients from tertiary care hospital, Chennai. All the samples were processed according to standard protocol. Antibiotic susceptibility testing was done by Kirby Bauer disc diffusion method. Isolates showing reduced susceptibility to indicator cephalosporins, ceftriaxone (30µg), ceftazidime (30µg) and cefotaxime (30µg) were further confirmed with combined disc diffusion method. (7,8,9)

In vitro susceptibility was determined using double disk synergy test (DDST), as recommended by CLSI guidelines E. coli (ATCC-25922) and K. pneumoniae (ATCC-700603) were used as reference strains for quality control of in vitro susceptibility testing.

Double Disc Synergy Test

(Phenotypic Confirmatory Test) for ESBL**:**

From the colonies of gram negative bacilli, 0.5 McFarland's turbidity standard suspension was prepared. Lawn culture was made on Muller Hinton Agar plate with this inoculum. Discs of Ceftazidime and Ceftazidime + 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^{o}C. An increase of ≥ 5mm in zone diameter of Ceftazidime + Clavulanic acid in comparison to the zone diameter of Ceftazidime alone confirmed the ESBL production by the organisms.(9)

Minimal Inhibitory Concentration (MIC) Agar Dilution Method for ESBL Detection

Inoculate 1-2µl of 4 hours peptone water culture of the isolates were plated on Mueller Hinton Agar plates containing 2µg/mL to 0.125µg/mL concentration of serially diluted ceftazidime with and without clavulanic acid. A greater than or equal to 3 fold decrease in MIC for ceftazidime–clavulanic acid versus its MIC when tested alone indicates ESBL positive (CLSI)

Molecular Characterization of Gram Negative ESBL Strains

Determination of the Genetic Type of β-Lactamase by Polymerase Chain Reaction (PCR)

A total of 37 phenotypically confirmed (DDST & MIC), ESBL producing strains were investigated to determine the probable type of β-lactamase enzyme which was responsible for resistance. Extraction of DNA was performed using DNA extraction kit. PCR amplification of *bla* genes, including *bla*TEM, *bla*SHV and *bla*CTX-M was performed with *Taq* master mix DNA polymerase using primers listed in table,

under the following conditions:Initial denaturation step: 94°C for 5 min. 30 cycles consisting of: Denaturation: 94°C for 1 min. Annealing: 61°C for 1 min.DNA extension: 72°C for 1 min. Final extension: 72°C for 5 min. Further analysis of the CTX-M enzymes using specific primers listed in table was done to determine the specific group of CTXM under the same conditions. All PCR amplicons were verified by gel electrophoresis on a 2% agarose gel electrophoresis stained with ethidium bromide (1 µl/ml) for 30 min under 100V in 1X TAE buffer and visualized by ultraviolet transilluminator. PCR amplicon sizes were calculated by a comparison with 100 bp molecular weight DNA ladder. PCR primers for detection of *blaTEM*, *blaSHV* and *blaCTX* were blaTEM- F AAACGCTGGTGAAAGTA, R AGC GATCTGTCTAT –822bp, blaSHV - F ATGCGTTATATTCGCCTGTG, RTGC TTTGTTATTCGGGCCAA- 753 bp and blaCTX-M F CGCTTTGC GATGTGCAG, R ACCGCGATATC GTTGGT- 550 bp **(10, 11)**

Results and Discussion

Among the 95 isolates of gram negative bacilli, *Escherichia coli* and *Klebsiella* spp accounts the predominance of 37.9% & 30.5%. Among the 95 GNB,49 were ESBL producer with screening method. Among 49, 38.9% were found to be ESBL producers by DDST & MIC test . 43.3% of *E.coli* & 35.1% of *Klebsiella* spp were predominant ESBL producers. Urine was the predominant sample of isolation of ESBL producers (51.4).

Globally, patients in the ICUs have encountered an increasing emergence and spread of antibiotic resistant pathogens. Although ICUs generally comprise 5% of all hospital bed, they account for 20% to 25% of all nosocomial infections. The increased risk of infections is associated with the severity of the patient contact with healthcare personal and length of stay in the ICU [(12)]

Infection and antibiotic resistance are important public health issues and the impact of increased drug resistance are far-reaching beyond any doubt. Rise in antibiotic-resistant strains of bacteria is one of the major problem worldwide, mainly in hospitals, and also in the community which has proved difficult to control without effective resources and expenditure.

In our study, (38.9%) were ESBL producers. The ESBL production varies between 17% to 70% , reported with various studies.[(13, 14)]

By using phenotypic methods for ESBL detection, we can only confirm whether an ESBL is produced or not, but cannot detect the ESBL subtype. Definitive identification is possible only by molecular detection methods which is very essential for the epidemiological analysis. [(15)]

A study by Grover *et al.* on phenotypic and genotypic methods of ESBL detection concluded PCR to be a reliable method of ESBL detection [(16)]

Among the 37 ESBL producers, CTX- M (48.3%) were the predominant gene of isolation in our study. SHV and TEM were 41.4 % and 17.2 % respectively.

68.7% of *E.coli* & 23.1% of *Klebsiella* spp had CTX-M genes, 92.3% of *Klebsiella* spp showed SHV genes and 12.5% *E.coli* & 23.1% of *Klebsiella* spp showed TEM genes.

In this study conducted in our area, CTX-M was the predominant gene among *E.coli,* and SHV gene were prevalent in *Klebsiella* spp. SHV and TEM type ESBLs were dominant

all over the world in members of the family *Enterobacteriaceae* during the 1990s, but now appear less important than the widely distributed CTX-M enzymes

Table.1 Distribution of ESBL Producers among the GNB

Species	Total number	ESBL producers	Percentage (%)
E.coli	36	16	43.3
Klebsiella spp	29	13	35.1
Proteus spp	17	4	10.8
Pseudomonas aeruginosa	11	4	10.8
Acinetobacter spp	2	0	0
Total	**95**	**37**	**38.9 %**

Table.2 Distribution of ESBL and the Specimens

Specimens (n=37)	ESBL producers	Percentage (%)
Urine	19	51.4
Drainage tube tips	6	16.2
Wound swab	5	13.5
Trachea bronchial aspirates	4	10.8
Blood	3	8.1

Table.3 Distribution of ESBL Genes among the Species

Isolates	CTX-M	SHV	TEM
E.coli (n=16)	11(68.7%)	-	2(12.5%)
Klebsiella pneumonia (n=13)	3(23.1%)	12(92.3%)	3(23.1%)
Total (n=29)	**14 (48.3%)**	**12(41.4%)**	**5(17.2%)**

68.7% of *E.coli* harboured a CTX-M gene which is followed by TEM (12.5%) and none had SHV. The predominance of CTX gene in *E.coli* in our study is correlates with, the study of Vaida *et al.* [17]

In the study of Amany *et al*, CTX- M was the main type of beta Lactamase, followed by TEM, then SHV. [18] CTX-M gene is the most prevalent ESBL encoding gene found worldwide and now it is replacing TEM and SHV types as the predominant ESBL in many European and Asian countries. [19]

In our study, 92.3% of *Klebsiella pneumoniae* had SHV gene, 23.1% had CTX-M & 23.1% had TEM gene. 72% Klebsiella harboured SHV gene, was reported with the study of Sharma J *et al.* **[15]**

In a study conducted at Chennai, the bla CTXM to be predominantly isolated in *E.coli* and Enterobacter species and bla SHV to be predominantly found in *Klebsiella* and *Enterobacter* species, which is also concordant with our study.[20]

CTX-M type β lactamases may be the most frequent type of ESBL producing strains worldwide, and they were predominantly found in three geographic areas such as South America, the Far East and Eastern Europe, which has been also reported in China, Japan, India, North America and Western Europe. **(21)**

ESBL continue to be the major challenge in clinical setups worldwide. The spread of ESBLs producing GNB has been rapidly increasing. This indicates that continuous monitoring system and effective infection control measures absolutely required. **(1)**

Conclusion

E.coli and *Klebsiella* spp are the commonest ESBL producers and they were maximum in urine samples. Among the ESBL producers, CTX- M were the predominant gene of isolation followed by SHVand TEM. Infection among ICU patients might be hospital or community acquired and intensive care is a multi–disciplinary team effort lead by the microbiologist and supported as and when required by associated medical & surgical personnel's. A sustained part the intensivist and the clinical microbiologist are essential for improving patient outcome & also for resource utilization. The possibility of minimising resistance by controlling the use of antibiotics is a rational approach, but the implementation of effective policies need, a combined approach of appropriate antibiotic use, effective surveillance and good infection control practices is essential to tackle the problem of antibiotic resistance.

References

1.Saroj Kumar Sah ,Hemalatha S. ESBL mechanisms of antibiotic resistance and epidemiology. Int J Pharm Tech Research,vol 7,2, 303-309.

2. Fam N., Leflon-Guibout V., Fouad S., Aboul-Fadl L., Marcon E., Desouky D., CTX-M-15-producing *Escherichia coli* clinical isolates in Cairo (Egypt), including isolates of clonal complex ST10 and clones ST131, ST73, and ST405 in both community and hospital settings, Microb. Drug. Resist., 2011, 17,6773.
3. Meeta Sharma, Sati Pathak, and Preeti Srivastava Prevalence and antibiogram of Extended Spectrum β-Lactamase (ESBL) producing Gram negative bacilli and further molecular characterization of ESBL producing Escherichia coli and Klebsiella spp.J Clin Diagn Res. 2013 Oct; 7(10): 173–2177.
4. Bonnet R. "Growing group of extended-spectrum β-lactamases: the CTX-M enzymes," *Antimicrobial Agents and Chemotherapy*. 2004; 48(1):1–14.
5.Paterson D.L., and Bonomo R.A., Extended-spectrum beta-lactamases clinical update, *Clini.* Microbiol.Rev., 2005, 18, 4, 657–686.
6.Foxman B, the epidemiology of urinary tract infections, Nat Rev Urol. 2010,7,12:653-660
7. Mackey and McCartney practical medical microbiology (14th edition)
8. Bailey & Scott's diagnostic microbiology (12th edition). Laboratory methods and strategies for antimicrobials susceptibility testing
9. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial testing, Twenty-First Informational Supplement M100-S21. CLSI, Wayne, Pennsylvania, USA, 2011.
10. Paterson DL, Hujer KM, Hujer AM, Yeiser B, Bonomo MD, Rice LB, Bonomo RA, and the International Klebsiella Study Group: Extended-spectrum β-lactamases in Klebsiella pneumoniae bloodstream isolates from seven countries: Dominance and widespread prevalence of SHV- and CTX-M-type β-lactamases. Antimicrob Agents Chemother 2003, 47(11):3554–3560.

11. Hujer KM, Hujer AM, Hulten EA, Bajaksouzian S, Adams J, Donskey CJ, Ecker DJ, Massire C, Eshoo MW, Sampath R, Thomson JM, Rather PN, Craft DW, Fishbain JT, Ewell AJ, Jacobs MR, Paterson DL, Bonomo RA: Analysis of antibiotic resistance genes in multidrug resistant Acinetobacter sp. isolates from military and civilian patients treated at the Walter Reed Army Medical Center. Antimicrob Agents Chemother 2006, 50(12):4114–4123.
12. (Eggimann ,P., Pittet D, Infection control in the ICU, Chest;2001;120:2059-2093.
13. Gopalakrishnan R, Sureshkumar D: Changing Trends in Antimicrobial Susceptibility and Hospital Acquired Infections Over an 8 Year Period in a Tertiary Care Hospital in Relation to Introduction of an Infection Control Programme. *J Assoc Physicians India.* 2010 December; 58 Suppl: 25-31.
14. Mubarak SA, Abida AE, Hajar MA, Adeel IA. Molecular Characterization and Epidemiology of Extended-Spectrum Beta-Lactamase-Producing *Escherichia coli* and *Klebsiella pneumoniae* Isolates in the United Arab Emirates. *Med Princ Pract.* 2011; 20:177-80.
15. Sharma J, Sharma M & Ray P: Detection of TEM & SHV genes in *Escherichia coli* & *Klebsiella pneumoniae* isolates in a tertiary care hospital from India. *Indian J Med Res.* 2010 September; 132: 332-36.
16 Grover SS, Sharma M, Chattopadhya D, Kapoor H, Pasha ST, Singh G. Phenotypic and genotypic detection of ESBL mediated cephalosporin resistance in Klebsiella pneumoniae: emergence of high resistance against cefepime, the fourth generation cephalosporin. J Infect 2006; 53(4): 279-88.
17. Vaida S, Marius L, Aurelija B, Justas P, Rita P, Agne G et al., Molecular characterization of extended-spectrum b-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* isolates from hospitals in Lithuania. *Journal of Medical Microbiology*. 2010; 59 (10): 1263-65.
18. Amany G. Thabit ,Tharwat R, Elkhannissy, Maggie A, Ibrahim and Adel E Attia. Detection of ESBL production by E.coli urinary pathogens at Assiut University Hospital...
19. Livermore DM, Canton R, Gniadkowski M, Nordmann P, Rossolini GM, Arlet G, et al., CTX-M: changing the face of ESBLs in Europe. *J Antimicrob Chemother*. 2007 Feb; 59:165–74.
20. Jemima SA, Verghese S. Multiplex PCR for *bla*CTX-M & *bla*SHV in the extended spectrum beta-lactamase (ESBL) producing Gram-negative isolates. Indian J Med Res 2008; 128: 313-7.
21. Asma Al-Jasser M. Extended-Spectrum Beta-Lactamases (ESBLs): A Global Problem. Kuwait Medical Journal. 2006; 38 (3): 171-185.

How to cite this article:

Vasumathi, A., P.R. Thenmozhi Valli, S. Senthamarai, C.P. Ramani and Raja Jagadeesan. 2016. Molecular Characterization of TEM, SHV and CTX M Genes among ESBL Producing Gram Negative Isolates in ICU. *Int.J.Curr.Res.Aca.Rev.* 4(2): 170-175.
doi: http://dx.doi.org/10.20546/ijcrar.2016.402.021