International Journal of Allied Medical Sciences and Clinical Research (IJAMSCR)

ISSN:2347-6567

IJAMSCR |Volume 4 | Issue 1 | Jan – Mar - 2016
www.ijamscr.com

Research article *Medical research*

Prevalence of multiple beta lactamases producing gram negative bacilli from various clinical samples in a tertiary care center at Kancheepuram.

Divya. G, Karthika Jayakumar, Aarya.V.Sankar.

Department of Microbiology, Shri Sathya Sai Medical College & Research Institute, Sri Balaji Vidyapeeth University, Thiruporur, Tamil Nadu, India.

***Corresponding author: Divya. G**

ABSTRACT

Introduction

The increasing numbers of multiple beta lactamases produced organisms leave very limited treatment options for clinicians. Single organism expressing multiple beta lactamase enzymes further complicated the treatment option. Hence this study investigated the co-existence of multiple beta lactamase enzymes in clinical isolates of gram negative bacteria.

Materials and methods

A total of 435 consecutive, non-repetitive, gram negative isolates were collected from various clinical samples included in this study. Antimicrobial susceptibility testing was performed as per CLSI. All the bacterial strains were subjected for detection of ESBL, AmpC, and MBL enzymes as recommended by CLSI.

Results

Out of 435 gram negative bacilli, 105 (24%) were ESBL producers, 40 (9%) were AmpC enzyme producer and 5 (1%) were MBL producers. *E.coli* was the predominant isolate accounting for (34.3%) of ESBL production, followed by *Pseudomonas aeruginosa* (31.5%), *Klebsiella sps* (19%) and *Acinetobacter baumannii* (31%). The highest incidence of AmpC was seen in *E.coli* 15.9%, followed by *Pseudomonas aeruginosa* 10.8%, *Klebsiella sps* 6.6% and *Acinetobacter baumannii* 6.8% respectively. While MBL production was only seen in 5 (1%) isolates. Co-existence of ESBL and AmpC was observed in 11 (2.5%), ESBL and MBL coproduction was detected in 4 (1%) and the coproduction of AmpC and MBL was observed in one isolate (0.2%).

Conclusion

Rapid identification of these enzymes along with routine sensitivity reports will help the clinicians in prescribing proper antibiotics and implementing infection control measures to prevent the dissemination of such resistance strains.

Keywords: Drug resistance, Extended spectrum beta lactamase (ESBL), AmpC beta lactamase (AmpC), Metallo beta lactamase (MBL).

INTRODUCTION

Emergence of antimicrobial resistant bacteria is the growing threat worldwide and a major reason for the increase in infections among community and health care settings. Resistance is mainly due to antibiotic abuse and over the counter drug delivery. Resistance

mechanisms have been found for almost every class of antibiotics. Persistent exposure of bacterial strains to antibiotics causes mutation which leads to the emergence of newer resistance mechanism and rapid clonal spread. One of such mechanism is production of beta lactamase [1].

β- Lactam antibiotics represent the most common treatment for a broad spectrum of gram positive and gram negative bacteria. One of the most important resistance mechanisms in gram negative bacteria against β-lactam antibiotics is induced by the production of β – lactamase enzymes. β – Lactamase enzymes are classified into four main group's viz., A, B, C and D. This is mainly due to the occurrence of point mutation in the sequence of the primary β – lactamase enzyme genes [2].

Commonly reported beta lactamases among gram negative organisms are extended spectrum beta lactamase (ESBL), AmpC and Metallo beta lactamase (MBL).

In the last decade the prevalence of ESBLs, MBLs and AmpC β-lactamases are gradually increasing in various parts of India and throughout the world; pose a challenge to the clinician in treating infections caused by such virulent strains. At the same time, these organisms became common nosocomial agents along with MRSA, VRE and Pseudomonas. The routine susceptibility tests fail to detect these strains, which may result in unsuccessful treatment. Consequently, it is necessary to report these ESBLs, MBLs and AmpC β-lactamases along with the routine sensitivity report so that the clinician can choose proper antibiotic for therapeutic purpose in right time.

Hence the present study is carried out to engender data on the prevalence of β-lactamase producing gram negative bacilli among hospitalized patients.

MATERIALS AND METHODS

Bacterial isolates

The study was conducted at Shri Sathya Sai Medical College and Research Institute, Chennai, over a period of January 2014- March 2015. A total of 435 consecutive, non-repetitive, gram negative isolates from various clinical samples such as urine, pus, ear swab, draining tube tip (DTT), sputum, bronchial wash, endotracheal tube, throat swab and conjunctival swab which were received in the clinical bacteriology laboratory, were included in the study. All the isolates were processed and identified as per standard protocol [3]. The reference strains, ESBL positive *Klebsiella pneumonia* ATCC 700603 and ESBL negative *Escherichia coli* ATCC 25922 were included in the study.

Antimicrobial susceptibility testing

The isolates were subjected to antibiotic susceptibility testing by Kirby-Bauer disc diffusion method as per CLSI guidelines [4], using commercially available antibiotic discs procured from (HIMEDIA, Mumbai, India) Cefazoline (30µg), Cefoxitin (30µg), Cefotaxime (30µg), Ceftazidime (30µg), Cefipime (30µg), Imipenem (10µg), Amoxycillin/clavulinate (20/10µg), Amikacin (30µg), Gentamicin (10 µg), Ampicillin (10µg) and Ciproploxacin (5µg) on Mueller Hinton agar plate.

Criteria for the selection of the esbl, ampc and metallo beta lactamase producing strains

- The isolates were tested for their susceptibility to third generation cephalosporins (3GCs) e.g. ceftazidime (30 µg), cefotaxime (30 µg) and ceftriaxone (30 µg) by using the standard disc diffusion method as recommended by the CLSI [4]. If a zone diameter of < 22 mm for ceftazidime, < 27 mm for cefotaxime and < 25 mm for ceftriaxone were recorded, then the strain was considered to be "suspicious for ESBL production" [10]. Only those isolates which were resistant to one of the 3 GCs were selected for the study and were processed for ESBL production.
- Isolates showing resistance or reduced sensitivity to cefoxitin, cefotaxime, ceftriaxone, ceftazidime, cefpodoxime or aztreonam and sensitive to cefepime. No increase in zone size with addition of an inhibitor by 5 mm. Isolates showing blunting of zone of inhibition (ceftazidime or cefotaxime) adjacent to inducer (imipenem or cefoxitin) were considered as a screen positive AmpC producer and subjected to AmpC disk test [4].
- Gram negative organisms that showed reduced susceptibility to Imipenem (10µg) were selected for MBL production [4].

Tests for esbl production

Double disk approximation test for screening (DDAT)

The test organisms adjusted to 0.5 McFarland standards were lawn cultured on to a Mueller Hinton agar plate. Antibiotic discs of Amoxicillin/Clavulanic acid (20/10µg) and cefotaxime (30 µg) were placed at

a distance of 15 mm from centre to centre of the disc apart and incubated at 37^{0}C for 18-24hrs. Organisms that showed a clear extension of the cefotaxime inhibition zone towards the disc containing Clavulanate were considered as ESBL producer. The organisms which were screened and found positive for ESBL production were subjected to confirmatory test [5].

CLSI phenotypic confirmatory disc diffusion test (PCDDT)

Ceftazidime (30 µg) and ceftazidime plus Clavulanic acid (30/10 µg) were placed 20 mm apart on lawn culture of the test isolate on Mueller Hinton agar and incubated overnight at 37^{0}C. The organism was considered as ESBL producer if there was a ≥ 5mm increase in diameter of Ceftazidime plus Clavulanic disc and that of ceftazidime disc alone [4&6].

Amp c disk antagonism test

A lawn culture of *E. coli* ATCC 25922 was prepared on MHA plate. Sterile disks (6mm) was moistened with sterile saline (20µl) and inoculated with several colonies of test organisms. The inoculated disk was then placed 5mm beside a cefoxitin disc. Plates were incubated overnight at 35°C. A positive test was appeared as a flattening or indentation of the cefoxitin inhibition zone in the vicinity of the test disc [7]. A negative test had an undistorted zone.

Detection of the metallo- β- lactamases (MBLs)

Imipenem-EDTA Combined Disc Test (CDT)

The metallo- β- lactamase production was detected by the imipenem – EDTA double disc synergy test. Two 10µg imipenem disks were placed on the MHA plate inoculated with culture adjusted to 0.5 McFarland standards and 10 µl of sterile 0.5 M EDTA solution was added to one of the imipenem disk. The inhibition zones of the imipenem and imipenem plus EDTA disks were compared after inoculation. The organisms were considered to be MBL producers if the increase in the inhibition zone of the beta lactam+EDTA disk was ≥ 5 mm when compared to imipenem disk alone [8].

RESULTS

Out of 435 Gram negative organisms included in this study, the predominant isolates was *E. coli* 119 (27.3%), followed by *Pseudomonas aeruginosa* 111 (25.5%), *Klebsiella pneumonia* 80 (18.9%), *Proteus mirabilis* 43 (9.8%), *Acinetobacter baumannii* 29 (6.6%), *Klebsiella oxytoca* 25 (5.7%), *Citrobacter Spp* 15 (3.4%), *Proteus vulgaris* 10 (2.2%) and *Enterobacter aerogenes* 3 (0.68%). Among the 435 gram negative isolates, a Multi drug resistant pattern was detected in 260/435 (59.7%) (**TABLE: 1)**.

DETECTION OF ESBL, AmpC AND MBL

Out of 435 gram negative bacilli, 105 (24%) isolates were confirmed as ESBL producers. AmpC disk test detected 40 (9%) isolates as AmpC enzyme producer and 5 (1%) isolates were found to be MBL producers.

DDAT detected only 100 (22.9%) ESBL producers and whereas PCDDT detected 105 (24%) isolates as ESBL producers. Two strains of *E.coli* and three strains of *Pseudomonas aeruginosa* that were not detected as ESBL producers by DDAT, were detected as ESBL producers by PCDDT (**Fig. 1,2**). The remaining 100 isolates were found to produce ESBL by both the methods.

Among 105 (24%) ESBL producers, *E.coli* was the predominant isolate accounting for about 41 (34.3%) of ESBL production, the second predominant isolate was *Pseudomonas aeruginosa* 35 (31.5%) followed by *Klebsiella sps* 20(19%) and *Acinetobacter baumannii* 9 (31%).

The highest incidence of AmpC was seen in *E.coli* (19) 15.9%, followed by *Pseudomonas aeruginosa* (12)10.8%, *Klebsiella sps* (7)6.6% and *Acinetobacter baumannii* (2)6.8% respectively (**Fig, 3,4**). While MBL production was only seen in 5 (1%) isolates. 4 *Pseudomonas aeruginosa* and 1 *Enterobacter aerogenes.* (**Fig. 5,6**).

Co-existence of ESBL and AmpC was observed in 11 (2.5%), it was found to be higher in *E.coli* (5%), *Pseudomonas aeruginosa* (1.6%), *Klebsiella sps* (2.8%), whereas ESBL and MBL coproduction was detected in only 4 isolates which is of 1%. All the four isolates were *Pseudomonas aeruginosa* (3.6%). The coproduction of AmpC and MBL was observed in one isolate (0.2%) of *Enterobacter aerogenes* (**CHART: 1**)

Except 5 isolates which showed reduced susceptibility to Imipenem, the remaining 98.8% of the isolates were sensitive to Imipenem.

TABLE: 1 Multi drug resistant gram negative isolates

Organisms	**No of Gram negative isolates (%) N=435**	**No. of Multi drug resistant isolates N=260**	**Percentage of Multi drug resistant isolates (%)**
E.coli	119(27.3%)	98	82.3
Klebsiella pneumonia	80(18.9%)	40	50
Klebsiella oxytoca	25(5.7%)	15	60
Pseudomonas aeruginosa	111(25.5%)	75	67.5
Acinetobacter baumannii	29(6.6%)	10	34.4
Proteus mirabilus	43(9.8%)	10	23.25
Proteus vulgaris	10(2.2%)	5	50
Citrobacter sps	15(3.4%)	7	46.6
Enterobacter aerogenes	3(0.68%)	-	-
Total	435	260	59.7

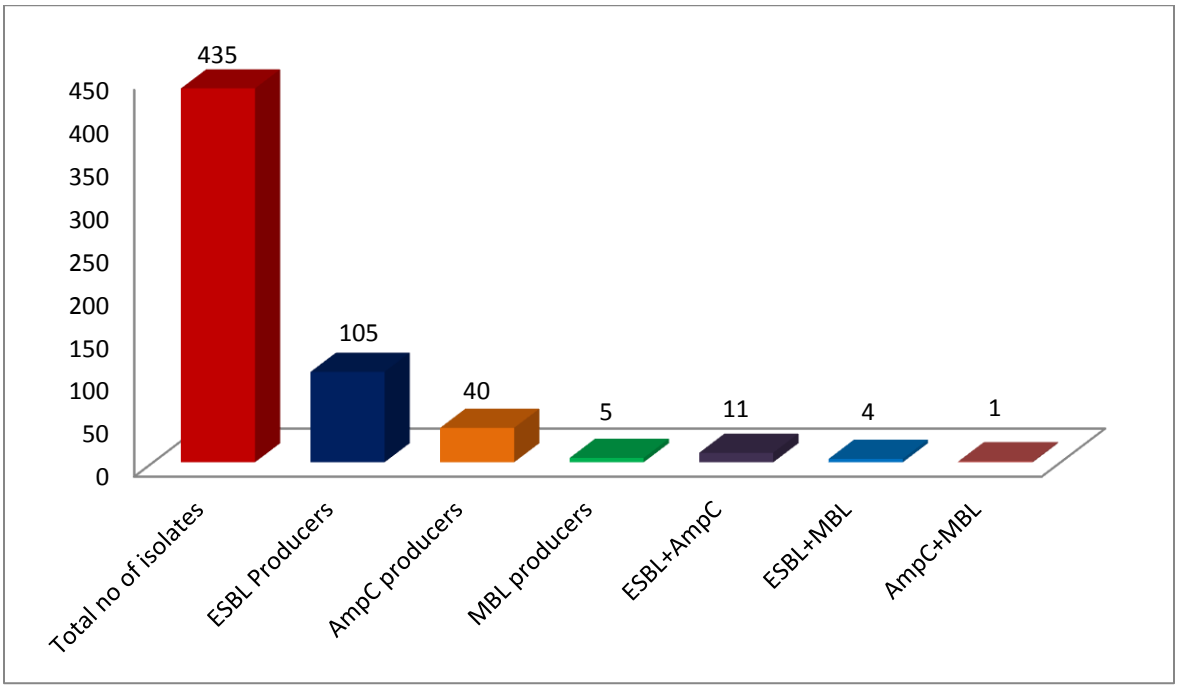

CHART:1 Distribution of β-lactamases and its co-production.

Fig: 1 A >5mm increase in zone of inhibition for ceftazidime/ clavulanic acid CAC versus ceftazidime alone confirmed ESBL production.

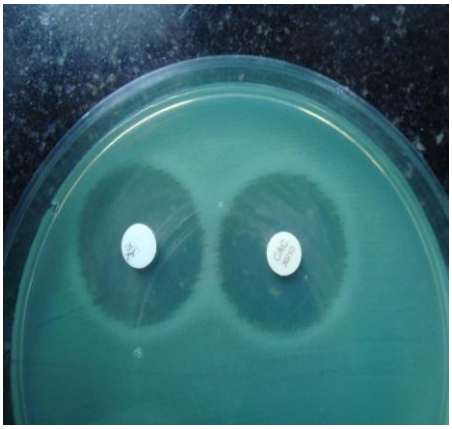

Fig: 2 No increase in zone of inhibition negative for ESBL production

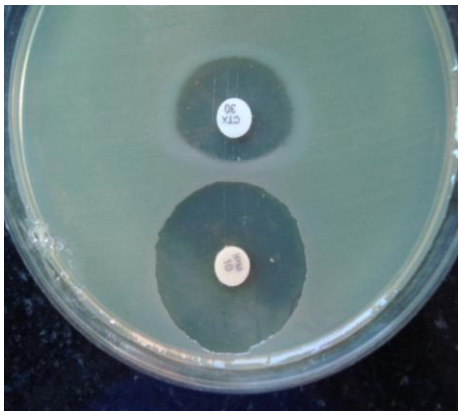

Fig: 3 Disk antagonism test showing AmpCβ lactamase production showing blunting of the cefotaxime disc adjacent to the Imipenem disk.

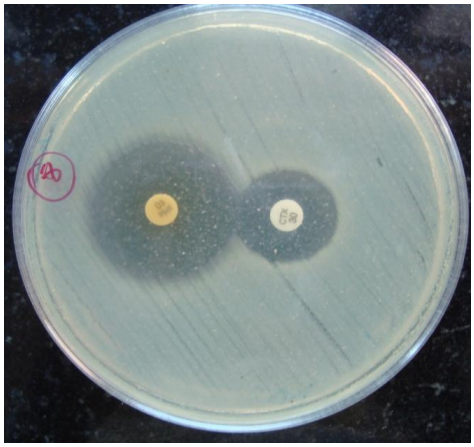

Fig: 4 No blunting of zone AmpC negative

Fig: 5 EDTA/ imipenem combined disk test showing increase in the zone of inhibition with EDTA/Imipenem disk compared with Imipenem disk alone- MBL positive

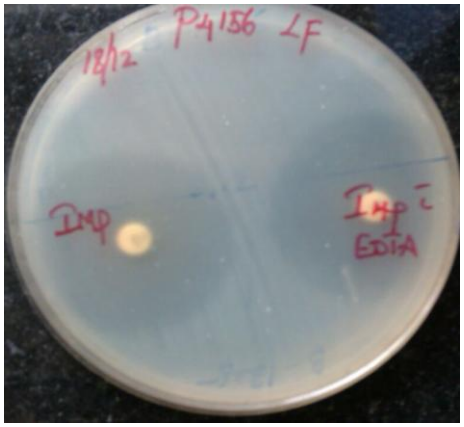

Fig: 6 no increase in zone for imipenem/ EDTA disk- negative for MBL

DISCUSSION

β-lactams are the drug of choice for the various infections caused by Gram positive and negative organisms. Among which cephalosporins are currently used to treat enterobacterial infections. The selective pressure of misuse and overuse of third generation cephalosporins in the hospitals has resulted in increased emergence of ESBL, plasmid mediated AmpC and MBL producing bacteria in many areas of hospitals.

Undiscerning administration of β-lactam antibiotics to the hospitalized patients increases the chance of colonization of beta-lactamase producing organisms that results in cross contamination of resistant strains between patients as well as health care workers.

The infections which are caused by multidrug-resistant beta lactamase enzymes producing gram negative bacilli have been reported with an increasing frequency, from various tertiary care centers and they are associated with a significant morbidity and mortality [9]. The numerous beta lactamases are encoded either by the chromosomal genes or by the transferable genes, which are located on the plasmids or the transposons [10].

Initially, these enzymes are commonly found in the *Klebsiella* species and in *E.coli*, [11] but now these enzymes are produced by all members of enterobacteriaceae and other gram negative bacilli [12]. Infections caused by such resistant strains can limit the therapeutic option and also pose challenge for the microbiologist and clinicians in identifying and treating them. Hence it has become necessary to detect such resistant pattern along with routine sensitivity testing.

In our study, the prevalence of multidrug pattern among gram negative bacteria, including the enterobacteriaceae and the non-fermenters was 59.7%, which is quiet high in a rural setting. The ESBL production in our analysis was found to be maximum (24%) as compared to the other beta-lactamase tested. Among which *E.coli* was the predominant isolate accounting for 41(34.3%) of ESBL production, the second predominant isolate was *Pseudomonas aeruginosa* 35(31.5%) followed by *Klebsiella sps* 20(19%) and *Acinetobacter baumannii* 9(31%).

Similarly a report from Punjab by Loveena oberoi *et al.,* [13] showed high prevalence of ESBL (35.16%) producer among all beta-lactamases tested in ICU patients which is comparable to our study. A study which was done by Nachimuthu Ramesh *et al.* (2008) [14] and Kumar *et al.* (2006) [12] also reported high prevalence of ESBLs among *E.coli*.

The incidence of ESBL in major hospitals of India has been reported as high as 60-80% [11,15]. US hospitals have reported 40% [16] of ESBL producers among GNB tested whereas report from Taiwan [17] showed 94% of ESBL production. A study done by Harakuni *et al* [18] reported a high prevalence of ESBLs (74%) in ICU patients. Whereas, Laghawe *et al* [19] and Menon *et al* [20] have reported lower percentage of ESBL producers (19.67%) and (20%) respectively when compared to our study. Hence it has been proved that the prevalence of the ESBLs among clinical isolates is not uniform and varies from country to country and institution to institution within the same country.

Among two tests done to detect ESBLs production, PCDDT detected more ESBLs than DDAT. Hence Correct identification of ESBL positive enterobacteriaceae in due time is mandatory, not only for optimal patient management but also for immediate institution of appropriate infection control measures, to prevent the spread of these organisms. [21] The double disc approximation test (DDAT) lacks sensitivity because of problem of optimal disc space and the correct storage of the clavulanic acid containing discs. Hence addition of single CAC disk at 20mm distance of CAZ along with routine diagnostic sensitivity testing would screen all gram negative bacteria in the diagnostic laboratory for ESBL production. This method is technically simple and inexpensive [22].

In our study low prevalence (9%) of AmpC production was observed when compared with the other study that had reported high prevalence of the AmpC producers rangings from 10.95% - 50.9% [23,24,25,26] in different parts of India.

In 2003, 20.7% of AmpC producers were reported from Delhi and 37% were from Chennai [27,28]. The number of AmpC producers has been increasing over the past few years. Similar to our finding Loveena oberoi *et al*. [13] reported low prevalence of AmpC (5.4%), this low prevalence could be suggestive of difference in the geographical distribution, which may have given varied resistance patterns [13].

The only β-lactam active against co AmpC and ESBL producers are carbapenems, however, recently resistance to carbapenems has been on the rise, which is mainly due to the production of metallo-β-lactamases [29,30]. Production of MBL has

tremendous therapeutic consequences since these organisms also carry multidrug resistance genes and the only viable option left are the potentially toxic polymyxin B and Colistin [31].

Prevalence of MBLs in different regions in India ranges from 2.9% - 19.67% among all gram negative isolates [32,33,34]. In our study, MBL production was observed in 5 (1.1%) isolates, with maximum production in *Pseudomonas aeruginosa* (4) followed by *Enterobacter aerogenes* (1). This was lower than that reported by (Shobha et al., 2009 & Varun goel et al., 2013) [35,36] and comparable (3.4%) to that reported by Vidya Pai et al., [37] among gram negative bacteria. The lower resistance to imipenem in our study may probably be due to the reserved use of these anitibiotics. To our interest one isolate of *Pseudomonas sps* that was sensitive to imipenem by routine disk diffusion technique showed MBL production by CDT. This varying result may be due to the presence of hidden MBL genes, which may spread unnoticed and may lead to untoward infection control problems. Hence it is recommended to follow CDT by CLSI as proved by other studies.

[13] In our study Co-existence of ESBL and AmpC was observed in 2.5% isolates, whereas ESBL and MBL coproduction was detected in 4 (1%) isolates. The coproduction of AmpC and MBL was observed in only 1 (0.2%) isolate. A study conducted by Loveena oberoi et al., [13] reported that 8.79% isolates showed ESBL and MBL coproduction of about, 3.67% of isolates showed AmpC and MBL coproduction and 3.67% of isolates showed AmpC and ESBL coproduction.

Recently, the co-existence of both AmpC β-lactamase, ESBL and MBL in some gram negative bacilli has also been reported. This could be because of plasmid mediated AmpC β-lactamase which has been disseminated among the Enterobacteriaceae. These strains in combination with ESBL may give false negative tests in the detection of ESBL as they may mask the recognition of the ESBLs and it may result in a fatal and an inappropriate antimicrobial therapy [13].

CONCLUSION

The present study emphasizes the prevalence of multiple β-lactamase producing gram negative bacilli in a rural setting tertiary hospital. High prevalence of β-lactamase is definitely alarming and there is an urgent need for evidence-based medicine particularly in rural settings, where laboratory facilities are lacking and antibiotics is being rampantly used by the quacks. Hence regular monitoring of the incidence of the β-lactamase production along with routine antibiotic susceptibility testing is necessary. As early detection of beta lactamase producing isolates would be important for the reduction of mortality, morbidity and avoid the intra hospital dissemination of such strains. To prevent the spread of the β-lactamase producing strains, hospitals must have functional hospital infection control committee with an appropriate hospital antibiotic policy, with regular updates.

ACKNOWLEDGEMENT

We wish to express our thanks and gratitude to Management and the Department of Microbiology, Shri Sathya Sai Medical College & Research Institute for their encouragement and support given during the study.

REFERENCES

[1]. Gaurav Dalela. Prevalence of Extended Spectrum Beta Lactamase (ESBL) Producers among Gram Negative Bacilli from Various Clinical Isolates in a Tertiary Care Hospital at Jhalawar, Rajasthan, India. *Journal of Clinical and Diagnostic Research*. 2012; 6 (2): 182-187.

[2]. Yazdi M, Nazemi A, Mirinargasi M, Jafarpour M and Sharifi S H. Genotypic versus Phenotypic methods to detect Extended-Spectrum Betalactamases (ESBLs) in Uropathogenic Escherichia coli. *Annuals of Biological Research*. 2012; 3 (5): 2454-2458.

[3]. Washington Winn, Jr., Stephen allen, Elmer Koneman *et al*., Koneman's color atlas and Textbook of Diagnostic Microbiology, 6th Edition, Publisher- Lippincottot Williams and Wilkins: Page 213-351.

[4]. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Twenty first informational supplement ed. CLSI document M100-S21. 2011: Pennsylvania CLSI (31).

[5]. Jarlier V, Nicolas MH, Fournier G, Philippon A. ESBLs conferring transferable resistance to newer b-lactam agents in Enterobacteriaceae: hospital prevalence and susceptibility patterns. *Rev Infect Dis* 1988; 10:867-878.

[6]. Bhattacharya S. Extended spectrum β-lactamases from petridish to the patient. *Indian J Med Microbial*. 2006; 24(1):20-24.

[7]. Singhal S, Mathur T, Khan S, Upadhyay DJ, Chugh S et al., Evaluation of methods for AmpC β lactamase in gram negative clinical isolates from tertiary care hospitals. *Ind J Med Microbiol*. 2005; 23:120-124.

[8]. Varaiya A, Kulkarni N, Kulkarni M, Bhalekar P, Dogra J Incidence of metallo beta lactamase producing Pseudomonas aeruginosa in ICU patients. *Indian J Med Res*. 2008; 127: 398-402.

[9]. Itokazu G, Qinn U et al., The antimicrobial resistance rates among aerobic the gram negative bacilli which were recovered from the patients in the intensive care units: the evaluation of national post marketing surveillance program. *Clin infect dis*. 1996; 23:779-84.

[10].] Mary VJ, Kandathi AJ, Balaji V. Comparison of the methods for the detection of the carbapenemase and the Metallo-beta-lactamases production in the clinical isolates. *Ind J Med Res*.2005; 121: 780-83.

[11]. Mathur P, Kapil A, Das B et al., The prevalence of ESBL producing gram negative bacteria in a tertiary care hospital. *Ind J Med Res*.2002; 115: 153-57.

[12]. Kumar MS, Lakshmi V, Rajagopalan R. The occurrence of extended spectrum beta lactamases among the enterobacteriaceae species which were isolated at a tertiary care institute. *Ind J Med Microbiol*.2006; 24 (3):208-11.

[13]. Loveena oberoi, Nachhatarjit sigh, Poonam Sharma, Aruna aggarwal. ESBL, MBL and AmpC β lactamases producing superbugs- Havoc in the intensive care units of Punjab India. *Journal of Clinical and Diagnostic Research*. 2013; 7(1):70-73.

[14]. Nachimuthu ramesh, chettipalayam samiappan sumathi et al., Urinary tract infection and antimicrobial susceptibility pattern of extended spectrum beta-lactamase producing clinical isolates. *Adv.Biol. Res*. 2008; 2(5-6): 78-82.

[15]. Hansotia JB, Agarwal V, Pathak AA, Saoji AM. Extended spectrum beta-lactamase mediated resistance to third generation cephalosporins in *Klebsiella pneunoniae* in Nagpur, central India. *Indian J Med Res*. 1997; 105: 158-61.

[16]. Jacoby GA, Munoz-price LS. The new beta lactamase. *N Eng J Med*. 2005; 352: 380-91.

[17]. Yan JJ, Hsueh PR, Chang FY et al., Extended spectrum beta lactamases and plasmid mediated AmpC enzymes among clinical isolates of *E.coli* and *Klebsiella pneumonia* from seven medical centres in Taiwan. *Antimicro Agents chemother*. 2006; 50(5): 1861-1864.

[18]. Harakuni S, Karadesai SG, Mutnal MB et al., the prevalence of the extended spectrum β-lactamase producing clinical isolates of Klebsiella pneumonia in the intensive care unit patients of a tertiary care hospital. *Annals of tropical medicine and health*. 2011; 4:96-98.

[19]. Laghawe A, Jaitly N, Thombare V. The simultaneous detection of the ESBL and the AmpC β-lactamase in gram negative bacilli. *Journal of Clinical and Diagnostic Research.* 2012; 6:660-63.

[20]. Menon T, Bindu D, Kumar CPG, Nalini S, Thirunarayan MA. Comparision of double disc and three dimensional methods to screen for ESBL producers in a tertiary care hospital. *Ind J Med Microbiol*.2006; 24(2): 117-120.

[21]. Irith Wiegand, Heinrich K. Geiss, Dietrich Mack, Enno sturenburg and Harrald Seifert. Detection of extended spectrum-β-lactamases among enterobacterriaceae by use of semiautomated microbiology systems and manual detection procedures. *J.Clini.Microbiol*. 2007; 45(4):1167-1174.

[22]. Shukla I, Tiwari R, Agarwal M. Prevalence of extended- spectrum β- lactamase producting *Klebsiella pneumonia* in a tertiary care hospital. *Indian J Med Microbiol*. 2004; 22(2):87-91.

[23]. Upadhyay S, Malay R S,Bhattacharhee A. Presence of different beta lactamase classes among clinical isolates of *Pseudomonas aeruginosa* expressing Amp C betalactamase enzyme. *J.Infect Dev Cties*. 2010; 4(4): 239-42.

[24]. Basak S, Khodke M, Bose S et al., Inducibe AmpC beta lactamase producing *Pseudomonas aeruginosa* isolated in a rural hospital of central Inida. *Journal of Clinical and Diagnostic Research*. 2009; 3: 1921-7.

[25]. Chatterjee SS, karmacharya R, Madhup SK, Gautham V, Das A, Ray P. High prevalence of co-expression of newer beta-lactamases (ESBLs, AmpC- β-lactamases and metallo-beta-lactamases) in gram negatice bacilli. *Indian J Med Microbiol*. 2010; 28(3): 267-268.

[26]. Samantha P, Praveen kumar V. Prevalence of ESBL & AmpC beta-lactamases in gram negative clinical isolates. *J Biosci Tech*. 2011; 2(4): 353-7.

[27]. Manchand V, Sigh NP. Occurrence and detection of AmpC β lactamases among gram negative clinical isolates using a modified three dimensional test at guru Teghbahadur hospital, Delhi, India. *J Antimicrobial chemotherapy*.2003; 51:415-418.
[28]. Subha A, Devi VR, Ananthan: AmpC β lactamase producing multidrug resistant strains of *Klebsiella sps* and *E.coli* isolated from children under five in Chennai. *Ind J Med Res.* 2003; 117: 13-18.
[29]. Supriya Upadhaya, Malay Ranjan Sen and Amitabha Bhattacharjee. Presence of different beta-lactamase classes among clinical isolates of *Pseudomonas aeruginosa* expressing AmpC beta lactamase enzyme. *J.Infect.Dev.Ctries*. 2010; 4(4): 239-242.
[30]. Livermore DM, Woodford N: Carbapenemase a problem in waiting? *Curropinmicrobiol*. 2000; 3:489-95.
[31]. Veenu gupta, Deepinder Chhina and Amarjeet Kaur. Incidence of metaallo-beta-lactamase (MBL) producing non fermenters isolated from respiratory samples in ICU patients. *Int. J. Pharm. Bio. Sci*. 2013; 4(2): 580-585
[32]. Noyal M.J.C, Menezes G.A, Harish B.N, Sujatha S & S.C. Parija. Simple screening tests for detection of carbapenemases in clinical isolates of non-fermentative gram-negative bacteria. *Indian J Med Rs*. 2009; 129: 707-12.
[33]. Deshmukh DG, Damle AS, Bajaj JK, Bh, Patwardhan NS. Metallo-β-lactamase producing clinical isolates from patients of a tertiary care hospital. *JLP*.2011; 3(2):93-97.
[34]. John S, Balraghunathan R. Metallo-beta-lactamse producing *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. *Indian J Med Microbiol*. 2011; 29(3): 302-4.
[35]. Varun goel, Sumati A. Hogade, SG Karadesai. Prevalence of extended spectrum beta-lactamases, AmpC beta lactamase, and metallo beta-lactamase producing *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in an intensive care unit in a tertiary care hospital. *J. Scientific Soc*. 2013; 40(1): 28-31.
[36]. Shobha KL, Lenka PR, Sharma MK, Ramchandra L, Bairy I. Metallo-betalactamse production among *Pseudomonas* species and *Acinetobacter species* in coastal Karnataka. *Journal of clinical and diagnostic research*. 2009; 3: 1747-1753.
[37]. VidyaPai, Sunil Rao P, Bhaskaran Nair. Multiple Beta-lactamase enzymes producing clinical isolates of gram negative bacteria in a teaching hospital. *IJPBS*. 2013; 3(1): 590-595.

How to cite this article: Divya.G, Karthika Jayakumar, Aarya.V.Sankar, Prevalence of multiple beta lactamases producing gram negative bacilli from various clinical samples in a tertiary care center at kancheepuram. Int J of Allied Med Sci and Clin Res 2016; 4(1): 127-135.
Source of Support: Nil. **Conflict of Interest:** None declared.