(International Print/Online Journal)

SJIF IMPACT FACTOR: 5.565
PUBMED-National Library of Medicine ID-101739732

ISSN (Print): 2209-2870
ISSN (Online): 2209-2862

International Journal of Medical Science and Current Research (IJMSCR)
Available online at: www.ijmscr.com
Volume 4, Issue 2, Page No: 360-367
March-April 2021

Prevalence of Extended Spectrum Beta-Lactamase and Ampc Beta-Lactamase in Gram Negative Bacilli Isolated From Various Clinical Samples in a Tertiary Care Hospital

Diego Edwin[1], Arunava Kali[2], Kalaivani R[2], Seetha KS[2]

[1]Department of Microbiology, Trichy SRM Medical College Hospital and Research Centre (Affiliated to The Tamilnadu Dr. M.G.R. Medical University, Chennai), Tiruchirapalli, India

[2]Department of Microbiology, Mahatma Gandhi Medical College and Research Institute (Affiliated to Sri Balaji Vidyapeeth), Puducherry, India

Corresponding Author
Diego Edwin
Department of Microbiology, Trichy SRM Medical College Hospital and Research Centre (Affiliated to The Tamilnadu Dr. M.G.R. Medical University, Chennai), Tiruchirapalli, India

Type of Publication: Original Research Paper
Conflicts of Interest: Nil

ABSTRACT

Background: The prevalence of extended spectrum β-lactamases (ESBL) and AmpC producing gram negative bacilli are posing a problem to the clinicians, rendering all β-lactams, except carbapenems ineffective in the treatment of infections related to these organisms. Also, ESBL and AmpC producing organisms exhibit resistance to many other classes of antibiotics resulting in limitation of therapeutic options. Awareness about their prevalence is vital to guide the appropriate antibiotic treatment of severe infections in hospitalized patients. The main objective of this study is to understand the prevalence of ESBL and AmpC β-lactamase among Gram negative bacilli isolates. **Materials and Methods:** Prospectively, 400 isolates of gram negative bacilli from various clinical samples with reduced susceptibility to 3rd generation cephalosporins were selected. The isolates were subjected to ESBL and AmpC production by Double disc synergy test, phenotypic confirmatory disk test, Disk antagonism test and AmpC Disk test respectively. **Results:** Out of the 400 isolates tested, 293 (73.25%) were found to be ESBL Producers and 42 (10.5%) were found to be AmpC producers. *E. coli* was the predominant isolate observed in both groups (217 out of 293 ESBL producers and 24 out of 42 AmpC producers). Majority of the isolates were sensitive to imipenem, polymyxin-B and colistin. **Conclusion:** A high prevalence of ESBL and AmpC positive isolates were found in the study area thereby the Good Hospital Infection Control practices, formulation of antibiotic policy and rational prescribing practices of antibiotics are essential.

Keywords: Gram negative bacilli, ESBL, AmpC

INTRODUCTION

Antimicrobial resistance among bacterial isolates is rapidly increasing and considered as the major health related issue globally. With injudicious use of antibiotics, non-compliance to antibiotic regimen, inadequate surveillance and infection control measures, antibiotic resistance in bacteria has increased rapidly over past decades [1,2]. Among several antibiotic classes, β-lactam antibiotics are most frequently used in clinical practice. Enzyme hydrolyzing β-lactam ring of penicillin appeared shortly within few years in Staphylococcus aureus [2]. However, broad spectrum penicillins and cephalosporins remained as first line of defense against gram negative bacterial infections for over 20 years until the emergence of bacteria producing extended spectrum β-lactamase (ESBL).

The expression of ESBL is associated with resistance to penicillin, oxyimino-cephalosporins and monobactams but not to cephamycins and carbapenems [3]. Furthermore, agents like clavulunic acid, tazobactum and sulbactum inhibit these enzymes and restore the antimicrobial action of β-lactam antibiotics when used in combination. ESBLs are plasmid coded enzymes, capable of causing widespread dissemination of multidrug resistance among different gram negative bacterial pathogens. In contrast to ESBL, AmpC beta-lactamase confers resistance to oxyimino cephalosporins as well as cephamycins and it is not amenable to inhibition by β-lactamase inhibitors [3,4].

Prolonged stay in intensive care unit (ICU), exposure to multiple antibiotics, total parenteral nutrition, instrumentation, catheterization, mechanical ventilation and presence of other co-morbidities like burns, renal failure are the risk factors reported to be associated with infections caused by ESBL or AmpC producing organisms [1,5,6]. Although high antibiotic selection pressure facilitates development and colonization of these multidrug resistant (MDR) strains in hospital settings, community acquired infections are also increasingly becoming more frequent. Recurrent urinary tract infections (UTI), diabetes mellitus, indwelling catheters, previous antibiotic exposure and old age are well-recognized predisposing factors for community-acquired ESBL infections [6].

The occurrence of ESBL and AmpC β-lactamase show a discrepancy globally amongst various gram negative isolates. The maximum rate of ESBL was detected among the isolates of K. pneumoniae from Latin America (44%), Pacific region of Asia (22.4%), North America (7.5%) and Europe (13.3%) [7]. In India, the prevalence of AmpC and ESBL producers are 8 - 59.4% and 18.7 - 64% correspondingly. The co-expression of both ESBL and AmpC β-lactamase has been reported in 25% Enterobacteriaceae and 20% Pseudomonas spp. Infections caused by MDR gram negative bacilli (GNB) resulted in treatment failure, hospital outbreak, longer hospital stay, increase in cost of treatment, high mortality and morbidity. In sight of observing various differences in virulence, pathogenicity and drug resistance, β-lactamase producing GNB are highly variable in different geographical areas [5,6,7].

Even within a country, there is local variation in predominant MDR GNB strains in various hospitals with difference in their resistance patterns. This fact clearly highlights the need of characterization of these organisms at regular basis and at various levels for formulating the antibiotic policy and to facilitate infection control in health care settings. Although there are several Indian studies on ESBL and AmpC β-lactamase producers, no proper prevalence data encompassing all clinical samples is available. Hence, this study was conducted to detect the prevalence of these enzymes in GNB isolates from various clinical samples in a tertiary care teaching hospital in South India.

MATERIALS AND METHODS

All the clinical specimens were microbiologically processed and GNB isolates were identified by standard techniques [8]. Antimicrobial susceptibility testing was done Kirby Bauer disc diffusion method using commercially available discs (HiMedia, Mumbai, India) as per Clinical and Laboratory Standard Institute (CLSI) guidelines [9,10]. The antibiotic discs used in this study were ampicillin (10mcg), cotrimoxazole (25mcg), ciprofloxacin (5mcg), gentamicin (30mcg), amikacin (30mcg), ceftriaxone (30mcg), cefotaxime (30mcg), ceftazidime (30mcg), cefoxitin (30mcg), Piperacillin/tazobactam (100/10mcg), Imipenem (10mcg), Polymyxin-B (50mcg) Colistin (10µg) were included in the study.

ESBL detection by double disk synergy test (DDST)[11,12]

The isolates which showed resistance or intermediate susceptibility to any of the third generation cephalosporin namely ceftazidime (≤22mm), cefotaxime (≤27 mm) by disk diffusion method were subjected to ESBL screening by double disk synergy test. The Amoxycillin+clavulanic acid (ACA) (20µg+10µg) disk was placed in the centre and the ceftazidime (30µg) and cefotaxime (30mcg) discs were placed on either sides at a distance of 15mm centre to centre from the ACA disc. Plates were incubated at 37^{o} C for 18 to 24 hours and pattern of zone of inhibition was noted.

Phenotypic confirmatory disk diffusion test (PCDDT) [11,12]

Volume 4, Issue 2; March-April 2021; Page No 360-367
© 2021 IJMSCR. All Rights Reserved

A lawn culture of test organism was made on Mueller Hinton Agar (MHA) plate. Sensitivity disks containing third generation cephalosporins with or without clavulanic acid were used as follows: ceftazidime 30mcg, Ceftazidime (30µg)+clavulanic acid (10mcg), cefotaxim 30µg and cefotaxim (30µg) + clavulanic acid 10mcg. Disk Diffusion assay was carried out as per CLSI guidelines and the plates were then incubated aerobically at 37°C for 18 to 24 hours. After incubation, plates were examined and the differences in zone diameter between disks with and without clavulanic acid were recorded.

Screening for AmpC β-lactamases [10]

The isolates which showed resistance or intermediate susceptibility to cefoxitin (≤18mm) by disk diffusion were subjected to AmpC screening test.

Disk antagonism test [13,14]

Disk of inducing agent cefoxitin and cephalosporins were placed on a lawn of bacterial culture of the suspected inducible AmpC beta-lactamase producers on MHA plate. A 0.5 McFarland standard of test isolates was swabbed on MHA plates and a disk of cefotaxim (30mcg) and ceftazidime (30mcg) were placed adjacent to cefoxitin (30mcg) disk at a distance of 20mm from each other from centre to centre.

Detection of AmpC betalactamase [13,14]

AmpC β-lactamase production was further confirmed by AmpC disk test. A lawn culture of 0.5 McFarland's suspension of E. coli ATCC25922 was prepared on MHA plate. A sterile plain disk (6mm) was moistened with sterile saline (20µl) and inoculated with several colonies of test organism. The inoculated plain disk was placed just beside the 30mcg cefoxitin disk (almost touching) on the inoculated plate with the inoculated disk space in contact with the agar surface and was incubated overnight at 37°C.

RESULTS

Out of the 400 isolates tested, 293 (73.25%) were found to be ESBL Producers and 42 (10.5%) were found to be AmpC producers and the details were depicted in table 1.

Out of 400 isolates, 333 (83.2%) were from inpatients and 67 (16.7%) from outpatients. The distribution of various specimens in our study shows that the maximum number of these isolates were from urine sample (57.5%) and exudate samples (32.2%), followed by sputum (6%), blood (3.5%) and stool (0.75%) samples and these samples were mainly received from Medicine, Surgery and Obstetrics & Gynecology departments. Majority of the isolates selected for this study comprised of E. coli (69.5 %, n=278) and K. pneumoniae (18.25%, n=73) isolated from patients in Medicine, Surgery, Obstetrics & Gynecology wards and ICU. Whereas, Citrobacter spp., Pseudomonas spp., Proteus spp. and Acinetobacter spp. accounted for only 5.75%, 4%, 1.75% and 0.75% isolates respectively. A relatively higher isolation of Citrobacter spp. and Proteus spp was found from surgical wards and Acinetobacter spp. from NICU.

The Enterobacteriaceae isolates were mainly from urine samples, followed by pus and exudates In contrast, all Acinetobacter isolates were from respiratory samples (3 of 3) and Pseudomonas isolates were mostly from pus (8 of 16) and urine (6 of 16).These clinical patterns of GNB infection are correlating with other current reports. In a multi-centric surveillance study from northern India, the common samples were pus (56%), urine (14%), blood (10%), respiratory (10%), and vaginal samples (10%) and the predominant clinical GNB isolates were E. coli, P. aeruginosa and K. pneumonia (50).

In the present study, all 400 isolates were subjected to DDST and PCCDT for ESBL and DAT and AmpC disk test for AmpC beta-lactamase detection. 330 (82.5%) and 293 (73.25%) of our isolates were ESBL producers by DDST and PCCDT respectively. Whereas, AmpC beta-lactamase production by DAT and AmpC disk test was noticed in 43 (10.75%) and 42 (10.5%) isolates respectively. Unlike ESBL tests, both phenotypic tests for AmpC production used in this study had strong concordance in their results. However, sensitivity and specificity of these tests could not be estimated since we have not used molecular methods (gold standard) for confirmation.

Out of 293 ESBL positive GNB, 60.8% were urinary isolates, followed by 28.7% from exudates, 6.1% from sputum, 3.4% from blood and 1.0%from stool. Likewise, 50%, 38.1% and 11.9% of AmpC positive isolates (n=42) were from urine, exudate and sputum respectively. Distribution of these isolates shows high

Volume 4, Issue 2; March-April 2021; Page No 360-367
© 2021 IJMSCR. All Rights Reserved

occurrence of both ESBL and AmpC producers in patients admitted in Medicine, Surgery and Obstetrics and Gynaecology wards. This finding may be explained by the fact that higher number of clinical samples was received from these wards. A similar finding was noted in GNB strains from inpatient and outpatient. Isolates obtained from the inpatient department had the most number of ESBL and AmpC producers, with a percentage of 84% and 85% respectively compared to outpatient department (15%ESBL and 14 % AmpC producers.

These reports are in accordance with our findings. We found 76.2% (212 of 278) E. coli, 71.2% (52 of 73) K. pneumoniae, 43.4% (10 of 23) Citrobacter spp and 68.75 (11 of 16) Pseudomonas spp isolates were ESBL producer; whereas, AmpC production was detected in only 8.6%, 11%, 13%and 12.5 % E. coli, K. pneumoniae, Citrobacter spp and Pseudomonas spp respectively. Although the number was less, 66.6% (2 of 3) Acinetobacter isolates showed ESBL as well as AmpC production and 85.7% (6 of 7) Proteus spp showed ESBL production. In this study, the organism which has the highest ESBL production rate has the least AmpC production and vice versa.

The overall resistance pattern of GNB selected in this study showed 100% resistance to Ampicillin and Cefotaxime, followed by 93% resistance to ceftriaxone, 65% resistance to Gentamicin, 58% resistance to ciprofloxacin, 50% resistance to Piperacillin tazobactam and 48.5% resistance to Cotrimoxazole .In contrast, the resistance was least against Colistin (17.5%), Polymyxin-B (17%) and Imipenem (5%). When we analysed the resistance profile of 293 ESBL producers and 42 AmpC producers, the latter group had much higher resistance to all antibiotics. ESBL producers had 62.8, 59 and 57% susceptibility to Imipenem, Polymyxin-B and Colistin respectively. However, these three drugs were susceptible only in 8.4, 11.7 and 10 AmpC producing isolates respectively.

Isolates that exhibited a distinct shape or size with potentiation towards amoxicillin+clavulanic disc were considered potential ESBL producers and short listed for confirmation of ESBL producers (Figure 1).

The zone of inhibition of the antibiotic alone (ceftazidime was compared with the zone of inhibition in combination with clavulanic acid (CAC). A difference of ≥ 5mm increase in zone diameter between the two agents (zone of CAC is more than CAZ) confirmed the presence of ESBL (Figure 2).

Isolates showing blunting of ceftazidime or cefotaxim zone of inhibition adjacent to cefoxitin disk was reduced susceptibility to each of the above test (ceftazidime or cefotaxim) and to cefoxitin were considered screening positive and selected for confirmation of AmpC beta lactamase. The organisms were considered to produce inducible AmpC β lactamase (Figure 3).

A positive test appeared as flattening or indendation of the cefoxitin inhibition zone in the vicinity of the test disk indicating enzymatic inactivation of cefoxitin. A negative test had an undistorted zone indicating no significant inactivation of cefoxitin (Figure 4).

E. coli was the predominant isolate among both the groups including 217 out of 293 ESBL producers and 24 out of 42 AmpC producers. Majority of the isolates were sensitive to Imipenem, Polymyxin-B and Colistin

DISCUSSION

Antibiotics are the most frequently prescribed medicine and the most potent weapon against bacterial infections. Among several antibiotics, β-lactam agents are of utmost importance. Pertaining to wide and diverse spectrum of activity, low toxicity profile and safety during pregnancy and lactation, β-lactam drugs have significant advantages over the other antibiotic families for clinical use. However, inadvertent use, lack of awareness, noncompliance to antibiotic regimen, inadequate infection control has led to development of multiple resistance mechanisms against β-lactam drugs. β-lactamase enzymes have limited the therapeutic benefits of these antibiotics to a great extent and the problem of ESBL and AmpC production in GNB has emerged as a global concern. These enzymes are implicated in treatment failure and increased morbidity and mortality when treated with penicillins and first three generations of cephalosporins [15,16].

A total of 400 GNB isolates displaying resistance to Cefotaxime or Ceftazidime or Ceftriaxone from various clinical specimens during the study period were considered as probable beta-lactamase producers and were included in the study. The

Volume 4, Issue 2; March-April 2021; Page No 360-367
© 2021 IJMSCR. All Rights Reserved

isolates were characterized for their identification, antibiogram and ESBL and AmpC beta-lactamase production.

Among various methods of ESBL and AmpC β-lactamase detection in GNB, phenotypic methods are widely employed for routine reporting because of their technical simplicity and cost-effectiveness. However, results of these phenotypic methods are essentially affected by several environmental and technical factors, such as, temperature, incubation period, quality and thickness of media, inoculum, distance between the antibiotic disks and subjective errors. Double disk synergy test (DDST) and PCCDT for ESBL and Disk antagonism test (DAT), Disk potentiation test, Modified three-dimensional test (MTDT) and AmpC disk test for AmpC β-lactamases have been frequently used in most laboratories which do not have molecular diagnostic facilities.

Several authors have evaluated results of these methods in comparison to genotypic tests to establish their diagnostic utility. However, the findings are inconsistent. Singh et al. reported PCCDT and DDST had 83 to 94% sensitivity and 100% specificity for ESBL detection which is comparable Vitek 2 using AST-GN25 panel (91.8% sensitivity and 97.2% specificity) and various commercially available ESBL E-tests (83.6 to 91.8% sensitivity and 99.31 to 100% specificity). Whereas, Bora et al. found PCR was superior over PCCDT method as it detected ESBL genes in isolates which were negative by CDT [17]. In another recent study, Htoutou et al. had compared DDST, E-test, modified microdilution method and Phoenix automated system and found that DDST (100%) and Phoenix system (99%) had highest sensitivity for detection of ESBL producers. However, the PCCDT and microdilution method are recommended Phenotypic Confirmatory Test as per CLSI guideline [9].

Although there is no CLSI recommendation for AmpC detection methods, various studies have successfully used phenotypic AmpC tests. Singhal et al. found concordant results between MTDT and AmpC disk test [9]. In another study, the sensitivity of Phoenix system was low (38%) compared to AmpC disk test and microdilution (95% sensitivity in both). A Disk potentiation test with AmpC β-lactamase inhibitors like boronic acid in combination with cephalosporins has been reported to detect chromosomal as well as plasmid-borne AmpC with high sensitivity and specificity [3].

High prevalence of ESBL among the Enterobacteriaceae members has been reported from Latin America, Asia, North America and Europe [6]. In India, ESBL and AmpC production among GNB varies from 8 - 59.4% and 18.7 - 64% correspondingly [7]. The prevalence of these enzymes is different in various GNB species. In a recent study, AmpC enzyme expressing strains were found among 9.9% E. coli and 31.1% K. pneumoniae. Much higher occurrence of AmpC (59.4%) among P. aeruginosa has been reported from north India [8]. Co-expression of both ESBL and AmpC β-lactamases has been reported in 25% Enterobacteriaceae and 20% Pseudomonas spp [7]. In case of ESBL enzymes, several authors have reported maximum prevalence among E. coli. However, Anandan et al. had found K. pneumoniae to be the most predominant ESBL producing bacilli, followed by E. coli and others. The prevalence of ESBL among E. coli ranges from 41.6% to 73.6% [17]. Among other members of Enterobacteriaceae, ESBL production also identified among clinical isolates of Serratia spp. (33%), Citrobacter spp (35.4%), non-typhoidal salmonellae (48%) and Shigella flexneri (46%) [18].

In a study from Bhopal, ESBL producing Enterobacteriaceae which were highly resistant to Co-trimoxazole, fluoroquinolones and gentamicin showed good in vitro sensitivity to imipenem (100%), meropenem (87.5%), piperacillin-tazobactam (89.3%) and amikacin (83.9%). A similar finding was reported in P. aeruginosa isolates in patients with burn injury [19]. Beta-lactam/ β-lactamase inhibitor combinations have been evaluated by several authors to assess their role in treatment in MDR GNB infections. In one study, Ceftriaxone-sulbactam was found to have effective in vitro activity for ESBL and MBL producing isolates showing 64 to 100% susceptibility. Sood et al. compared activity of six beta-lactam/beta-lactamase inhibitor combinations against 278 Enterobacteriaceae and 106 non fermenters and found that the susceptibility of Cefepime- tazobactam (64%), Cefoperazone-sulbactam (89%) and Piperacillin – tazobactam (53.9 %) was reasonably good against Enterobacteriaceae; while the non-fermenters had 49.04% sensitivity to Cefepime-

Volume 4, Issue 2; March-April 2021; Page No 360-367
© 2021 IJMSCR. All Rights Reserved

tazobactam [20]. The variations of resistance profile in different studies may be attributed to regional variation of MDR genes among several GNB species, antibiotic usage patterns and infection control measures. Among various ESBL genes blaCTX-M-15 is most prevalent in India. Ensor et al. have reported 73% and 72% occurrence of this gene among E. coli and K. pneumoniae isolates respectively (21). In another study, 10.2% Citrobacter spp. was found to carry bla CTX-M gene. In the study, the most prevalent ESBL genotypes was bla CTX-M among E. coli (88.67%) and bla TEM in K. pneumoniae (77.58%) from Northeast India [21].

Conclusion

The major limitation of this study was low sample size. Consequently, the findings need further confirmation using a larger number of isolates. Since it is a hospital based study, bacterial isolates were mainly from hospitalized patients and outdoor patients represented only a minority. Likewise, the number of samples from different wards was not uniform throughout the study. Most clinical samples were received from departments like medicine and surgery. Hence, these wards might have the higher isolation of ESBL and AmpC producers. Lastly, the phenotypic methods are known for subjective errors, variations in results and lower sensitivity and specificity. We could not confirm the results of phenotypic methods using molecular tests because of cost constrain.

REFERENCES

1. Ramphal R, Ambrose PG. Extended spectrum beta-lactamases and clinical outcomes: current data. Clin Infect Dis 2006;15:164-72.
2. Vignaroli C. Methicillin-resistant Staphylococcus aureus USA400 Clone, Italy. Emerg Infect Dis 2009;15:995-6.
3. Harada S, Ishii Y, Yamaguchi K. Extended spectrum beta-lactamases: implications for the clinical laboratory and therapy. Korean J Lab Med 2008;28:401-12.
4. Jacoby GA. AmpC beta-lactamases. Clin Microbiol Rev 2009;22:161-82.
5. Azim A, Dwivedi M, Rao PB, Baronia AK, Singh RK, Prasad KN, et al. Epidemiology of bacterial colonization at intensive care unit admission with emphasis on extended-spectrum beta-lactamase and metallo-beta-lactamase-producing Gram-negative bacteria-an Indian experience. J Med Microbiol 2010;59:955-60.
6. Dhillon RHP, Clark J. ESBLs: A Clear and Present Danger? Crit Care Res Pract 2012;2012:11-6.
7. Rawat V, Singhai M, Verma PK. Detection of different beta-lactamases and their co-existence by using various discs combination methods in clinical isolates of Enterobacteriaceae and Pseudomonas spp. J Lab Physc 2013;5:21-5.
8. Collee J, Fraser A, Marmion B, Simmons A. Mackie and McCartney practical Medical Microbiology. 14th ed. London: Churchhill Livingstone; 1996.
9. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: 23rd informational supplement. CLSI document M100-S23. Wayne, Pennsylvania: Clinical and Laboratory Standards Institute; 2013.
10. Mohamudha PR, Harish B, Parija S. AmpC beta-lactamases among gram negative clinical isolates from a tertiary hospital, South India. Braz J Microbiol 2010;41:596-602.
11. 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.
12. Baby PS, Appala RB, Mani KR. Detection of Enterobacteriaceae producing CTX-M extended spectrum beta-lactamases from a tertiary care hospital in south India. Indian J Med Microbiol 2008;26:163-6.
13. Basak S, Mallick SK, Bose S. Extended spectrum beta-lactamase producing Escherichia coli and Klebsiella pneumoniae isolated in a rural hospital. J Indian Med Assoc 2009;107:855-8.

Volume 4, Issue 2; March-April 2021; Page No 360-367

© 2021 IJMSCR. All Rights Reserved

14. 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 J Med Microbiol 2005;23:120-4.
15. Kalaivani R, Shashikala P, Sheela Devi C, Prashanth K, Saranathan R. Phenotypic assays for detected of ESBL and MBL producers among the clinical isolates of multidrug resistant Pseudomonas aeruginosa from a tertiary care hospital. Int J Curr Res Rev 2013;5:28-37.
16. Indra PR, Kavitha A, Reena R, Mathavi S, Rajesh KR. Prevalence of bla extended spectrum beta-lactamase gene in Enterobacteriaceae from critical care patients. J Lab Physc 2011;3:80-3.
17. Bora A, Hazarika NK, Shukla SK, Prasad KN, Sarma JB, Ahmed G. Prevalence of blaTEM, blaSHV and blaCTX-M genes in clinical isolates of Escherichia coli and Klebsiella pneumoniae from Northeast India. Indian J Pathol Microbiol 2014;57:249-54.
18. Menezes GA, Khan MA, Harish BN, Parija SC, Goessens W, Vidyalakshmi K, et al. Molecular characterization of antimicrobial resistance in non-typhoidal salmonellae associated with systemic manifestations from India. J Med Microbiol 2010;59:1477-83.
19. Kumar V, Sen MR, Nigam C, Gahlot R, Kumari S. Burden of different beta-lactamase classes among clinical isolates of AmpC-producing Pseudomonas aeruginosa in burn patients: A prospective study. Indian J Crit Care Med 2012;16:136-40.
20. Sood S. Comparative Evaluation of the in vitro activity of Six beta-lactam/ beta-lactamase inhibitor combinations against Gram negative bacilli. J Clin Diagn Res 2013;7:224-8.
21. Ensor VM, Shahid M, Evans JT, Hawkey PM. Occurrence, prevalence and genetic environment of CTX-M beta-lactamases in Enterobacteriaceae from Indian hospitals. J Antimicrob Chemother 2006;58:1260-3.

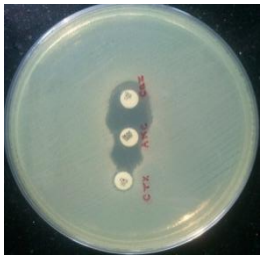
****

Table 1: Distribution of ESBL and AmpC producers by PCDDT and disk test

Enzyme production	ESBL	AmpC
Producer	293 (73.25)	42 (10.5)
Non-producer	107 (26.75)	358 (89.5)

[Figure in parenthesis denoted percentages]

Figure 1: ESBL double disk synergy test positive – CTX and CAZ showing potentiation towards AMC



Volume 4, Issue 2; March-April 2021; Page No 360-367
© 2021 IJMSCR. All Rights Reserved

Figure 2: ESBL Phenotypic confirmatory combined disk test positive with ceftazime and ceftazidime+clavulanic acid showing more than 5mm difference in zone size

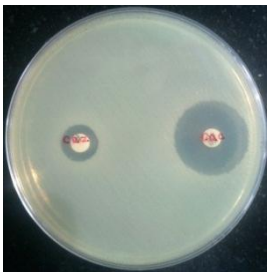


Figure 3: AmpC Disk antagonism test positive with cefoxitin, cefotaxime and cefatzidime showing blunting of zone of inhibition of CTX and CAZ

Figure 4: AmpC disk test positive with cefoxitin and sterile disk inoculated with test organism showing blunting towards cefoxitin

Volume 4, Issue 2; March-April 2021; Page No 360-367

© 2021 IJMSCR. All Rights Reserved