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Review Article

β-LACTAMASE PRODUCING ENTEROBACTERIACEAE: A GROWING CONCERN IN COMMUNITY ACQUIRED INFECTIONS

MIRZA S.B., MISRA R.N., GANDHAM N.R., ANGADI K.M., DAS N.K. AND JADHAV SAVITA*
Department of Microbiology, Dr D.Y. Patil Medical College Hospital and Research Centre, Dr D.Y. Patil Vidyapeeth, Pimpri, Pune, 411018, India
*Corresponding Author: Email - patilsc78@gmail.com

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Abstract- Background: The rising rate of antimicrobial drug resistance in Enterobacteriaceae reduces the number of reliably effective drugs that can be used to treat infections. Gram negative bacteria producing β-lactamases that are resistant to many other antibiotics and very few antimicrobial agents remain effective as treatment option. Presence of these enzymes can result in treatment failure when cephalosporins or Carbapenems are used. Due to extensive use of β-lactam and carbapenems over the last several decades in the clinical practice, various β-lactamases have emerged. B-lactamase producing bacteria have been increasingly reported as causal agents of not only nosocomial infection but also community acquired infection. The widespread use of ceftriaxone and/or cefotaximine has been proposed as a reason for the emergence of CTX-M enzymes. The increased frequency of isolation & reporting of CTX-M ESBLs is alarming and is likely to represent only the tip of iceberg for the underdeveloped continents where molecular technology for the analysis of ESBL enzymes is scare. The loss of oxyaminocephalosporins for the treatment of infections represents a serious problem that seems to reach unprecedented level globally. We investigated the clinical isolates positive for β-Lactamase producing bacteria in our institution, a tertiary care hospital in Pune (India), during a 2-year period (2014–2016). **Aim**: To isolate and identify the Extended spectrum β lactamase producer (ESBL), Metallo-β-lactamase (MBLs), AmpC β-lactamases in Enterobacteriaceae among community acquired infections in a tertiary care hospital and to find out antibiotic sensitivity pattern of these organisms. **Methodology**: Screening for all β-lactamase producers (ESBL`s, MBL`s, AmpC) done by Kirby-Bauer sensitivity testing as per CLSI guidelines and followed by confirmatory tests like combined disk diffusion, double disk diffusion, Modified Hodge test and E–strip testing. **Results**: Total 581 isolates from *Enterobacteriaceae* were isolated, 417 were MDR strains which were screened for these enzymes, where 293 isolates came out to be positive for either one of the three enzymes. Screening tests for ESBLs resulted in 283(67.86%) isolates out of the 417 MDR resistant to Ceftazidime. 269 (95.05%) of these were ESBL producers which were confirmed by Double Disk Diffusion Method (DDDT). *E.coli* 154(54.41%) and *K. pneumoniae* 83(29.32%)were the two most common isolate producing this enzyme. 15 (3.59%) isolates out of the 417 MDR were resistant to Imipenem when screened for carbapenemases. Out of which all 15 were carbapenemase producers confirmed by MHT, while 12 were MBL producers confirmed by CDT and E-strip test. 53 out of 114 screen (cefoxitin) positive were AmpC producers, which was confirmed by CC-DDS AmpC disc test and E-strip test. 269 ESBL producers, 53 AmpC producers and 12 MBL producers were isolated. **Conclusion**: The study emphasizes the high prevalence of multidrug-resistant Enterobacteriaceae organisms producing β-lactamase enzymes of diverse mechanisms in community acquired infections.

Keywords- *Enterobacteriaceae, MDR GNB, β-lactamases, ESBL`s, MBL`s, AmpC,*

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Introduction

Resistance to broad spectrum β-lactams and carbapenems, mediated by β-lactamases enzymes is an increasing problem worldwide [1]. Presence of these enzymes in clinical infections can result in treatment failure if one of the second- or third-generation cephalosporin is used. The scenario worsens in cases of MBL production where the drugs of last resort the carbapenems are rendered ineffective [2]. Due to extensive use of β-lactam antibiotics over the last several decades in the clinical practice, various β-lactamases have emerged. The emergence of ESBL producing organisms, particularly *E. coli* and *K. pneumoniae*, is now a critical concern for the development of treatment against bacterial infections. The major ESBL producer was *K. pneumoniae* before 2000, but now *E. coli* has become an important ESBL producer [3-5]. Because of the fact, the ESBL genes are usually found in large plasmids, they also contain other antimicrobial resistant genes. Therefore, most ESBL producing organisms also show resistance to aminogylcosides, fluororquinolones, tetracyclines, chloramphenicol and sulfonamides. Carbapenems are the mainstay of therapy for infections caused by ESBL producing organisms [6]. Therefore, resistance against these agents poses therapeutic challenge.

The MBLs belong to group B of carbapenemases which are enzymes requiring divalent cations as cofactors for enzyme activity, being inhibited by the action of a metal ion chelator [7]. The MBLs efficiently hydrolyze all β-lactams, except aztreonam in vitro [8]. AmpC-mediated β-lactam resistance in *E. coli* and *Klebsiella* spp. is an emerging problem. High level AmpC production is typically associated with in vitro resistance to all β-lactam antibiotics except for carbapenems and cefepime. In addition, treatment failures with broad-spectrum cephalosporins have been documented [9,10]. The production of β-lactamases is the single most prevalent mechanism responsible for resistance to β-lactams among clinical isolates belonging to the family of Enterobacteriaceae [11]. Hence the present study was undertaken to study the prevalence of β lactamase resistance in Enterobacteriaceae. Vital inputs generated from the study will assist in guiding the present infection control guidelines in this setting.

Materials and Methods

A cross-sectional study was conducted after approval from Institute Ethical committee to determine the antibiogram of Enterobacteriaceae, screen and confirm using various tests for presence of β lactamases in these organisms from

community acquired infections in our institution, a tertiary care hospital in Pune (India), during a 2-year period (2014–2016).

Samples

All continuous, non-duplicate, clinically significant and pure isolates of Enterobacteriaceae obtained from various samples like urine, blood, pus, sputum from out-patients and freshly admitted patients to wards and ICU`s. Isolates obtained from patients already under treatment or patients with prior antibiotic administration history and with chronic illness were excluded. All the samples were collected using strict aseptic precautions, were transported in sterile containers and processed immediately as per standard protocols. Detailed clinical history, co-morbidities and predisposing factors were recorded, prior antibiotic and hospitalization history was taken. Isolates were identified on the basis of colony morphology and biochemical reactions as per conventional isolation and identification procedure. Bact/Alert 3D system and VITEK 2 were used where required. All isolates were subjected for antibiotic susceptibility testing using Kirby Bauer Disk diffusion method as per CLSI 2013 guidelines [12].

Processing of samples

The clinical samples were processed in various media as per protocol followed in our laboratory *e.g.* Blood agar and MacConkey agar for blood, sputum, pus sample, CLED agar for urine sample. Special media were used whenever required [13]. These plates were routinely incubated at 37°C aerobically and growth was observed after 24 hours of incubation and colony characteristics were noted.

Isolation and identification of organisms

After performing gram stain, all gram-negative bacteria that were isolated were further tested by various biochemical reactions characteristic of Enterobacteriaceae [14]. Antibiotic susceptibility testing was done along with screening for ESBL production done by Kirby-Bauer sensitivity testing as per CLSI 2013 guidelines [12].

Table-1 Antibiotic discs with their corresponding concentration.

SN	Antibiotic	Concentration/disc
1.	Imipenem(IPM)	10 µg
2.	Amikacin (AMK)	30 µg
3.	Gentamicin (GEN)	10 µg
4.	Norfloxacin(NX)	10µg
5.	Ciprofloxacin (CIP)	5 µg
6.	Ampicillin(AMP)	10µg
7.	Cefotaxime (CTX)	30 µg
8.	Ceftazidime (CAZ)	30 µg
9.	Colistin (CI)	10 µg
10.	Cefoxitin(CX)	30µg
11.	Chloramphenicol(C)	30 µg
12.	Cotrimaxazole(COT)	25 µg
13.	Nitrofurantoin(NIT)	300µg
14.	Nalidixic Acid(NA)	30µg

All Isolates were screened for ESBL production by using disc diffusion test for of Enterobacteriaceae. Ceftazidime (30µg), Cefotaxime (30µg) placed on inoculated plates containing Muller Hinton agar according to the CLSI 2013 recommendations. For Positive control *K. pneumoniae* ATCC 700603 and for Negative *E. coli* ATCC 25922 were used.

Confirmatory test for ESBLs

Phenotypic confirmatory test for ESBL producers were done by double disc diffusion test (DDDT), for all the ESBL producing isolates as per CLSI 2013 guidelines [12-16].

Screening and Confirmation for AmpC β lactamase production

All isolates were subjected for screening for AmpC β-lactamase production using 30 µg cefoxitin disc (CX) by disc diffusion method as of CLSI guidelines. Confirmatory test for AmpC β–lactamases (cefoxitin-cloxacillin double disc synergy test)[13-15] and AmpC E-test [16-19].

Screening and Confirmation for MBL Production

Screening for carbapenem resistant GNB from the routine clinical samples was done by using 10µg imipenem discs (HiMedia). Modified Hodge test, Imipenem-EDTA Disc method Combined Disc test¬20 and E-test were performed on all imipenem resistant isolates for phenotypic detection of carbapenemases.

Statistical analysis

All the data in the present study was entered into spreadsheet (Excel 2007; Microsoft) as well as in S.P.S.S.20 (Statistical Package for Social Sciences, version 20) for analysis. Yate's correction was applied to the Chi-square test whenever frequency of variable was less than 5.

Results

A total number of 581 Enterobacteriaceae isolates were reported from various clinical specimens during the period from May 2014 to June 2016. Out of which 417 isolates were multi-drug resistant and were subjected to various tests to check for β-lactamases production. 293 out of these 417 showed resistance to any of the three drugs tested for β lactamases. Of these 293 isolates, 283 isolates were resistant to Ceftazidime which were tested for ESBL production, 15 were resistant to Imipenem, which were checked for carbapenemases production and 114 isolates were resistant to Cefoxitin which were checked for AmpC production.

Out of the 293 β-lactamase producing organisms 172 were isolated from females and 121 from males samples.

Table-2 Sample wise distribution of β-lactamases (n=293)

Samples	Frequency	Percent%	ESBL	MBL	AmpC
Urine	198	67.6	186	8	40
Sputum	40	13.7	35	2	6
Pus	31	10.6	27	2	3
Blood	24	8.2	21	0	4
Total	293	100.0			

From the total 293 β- lactamase producers, 21 organisms isolated from blood were ESBL producers and 4 AmpC producers, 27 isolates from pus were ESBLs, 2 MBLs and 3 AmpC producers, 35 isolates from sputum were ESBLs, 2 MBLs and 6 AmpC producers and 186 from urine were ESBLs, 8 MBLs and 40 were AmpC producers.

Table-3 Organism wise distribution of β-lactamases (n=293)

Organisms	Frequency	Percent(%)	ESBL	MBL	AmpC
Escherichia coli	161	54.9	154	6	32
K. pneumoniae	86	29.4	74	4	14
Citrobacter koseri	20	6.8	19	0	3
Citrobacter freundii	15	5.1	14	0	3
Proteus mirabilis	7	2.4	6	1	0
Proteus vulgaris	2	0.7	1	0	0
Enterobacter spp	1	0.3	1	0	1
Klebsiella oxytoca	1	0.3	0	1	0
Total	293	100.0			

About 65% of the bacteria showed ESBL positive by combination (CAZ/CAC). *E. coli* (57.2%) and *K. pneumoniae* (27.5%) showed maximum ESBL production. Also, when all the isolates were screened for carbapenemase producers it was found out that 15 isolates were Imipenem resistant organisms, 12 organisms were confirmed as MBL producers by Imipenem double disk synergy test and Imipenem-EDTA disc method (combined disc test). Remaining 3 of the Imipenem resistant isolates were MHT positive meaning they were not MBLs but some other carbapenemase producers. *E.coli* (40%) and *K. pneumoniae* (26.6%) were the 2 most common organisms exhibiting this phenomenon. Lastly, on screening for AmpC producers by Cefoxitin, 114 isolates showed resistance and 53 were confirmed by double disk synergy test. Table shows that majority of the AmpC also belonged to *E.coli* (60.37%)and *K. pneumoniae* (26.41%). Most of the isolates were MDR, meaning they were resistant to three or more group of antibiotics. Aminoglycoside like Amikacin (76.79%) was highly sensitive to majority of β-lactamases along with Choramphenicol(90.78%). Also most of the isolates were sensitive to Imipenem(94.88%). And all 293 MDR were sensitive to colistin.

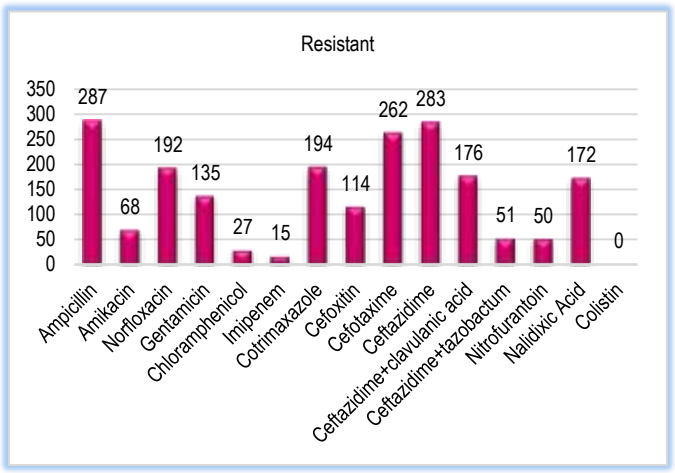

Fig-1 Resistance pattern of β-lactamases

283(67.86%) isolates out of the 417 MDR were resistant to Ceftazidime. *E.coli* and *K. pneumoniae* were the two most resistant ones. All the Gram negative organisms belonging to Enterobacteriaceae were subjected to screening tests using Ceftazidime for ESBL production. 283 isolates were resistant to Ceftazidime and 269 (95.05%) of these were ESBL producers which were confirmed by Double Disk Diffusion Method (DDDT). *E.coli* 154(54.41%) and *K. pneumoniae* 83(29.32%) were the two most common isolate producing this enzyme. 114(27.33%) isolates out of the 417 MDR were resistant to Cefoxitin which were confirmed by Cefoxitin-cloxacillin Double Disk Synergy Test (CC-DDS) and by AmpC E-strip Test showing 53(46.49%) as AmpC producers. *E.coli* and *K. pneumoniae* were the two most common organisms with 32 (28.07%) and 14 (12.2%) isolates respectively. Whereas, by AmpC disk test this was found out to be 48 (39.47%) cases concluding that CC-DDS and E-strip Test to be a more sensitive test. 15 (3.59%) isolates out of the 417 MDR were resistant to Imipenem, 12 of these were MBL producers by Imipenem E-strip test and Imipenem-EDTA disc method (combined disc test) and all 15 positive by Modified Hodge Test. These 3 isolates were negative for CDT combined disk test but were positive for MHT explaining some other carbapenemase enzyme being responsible for Imipenem resistance. Hence, these 3 isolates were carbapenemase producers but not MBLs which can be confirmed by genotypic methods.

Discussion

A total number of 581 Gram negative bacteria belonging to Enterobacteriaceae were reported from various clinical specimens during the period from May 2014 to June 2016. Most isolates were reported in urine samples (70.56%), followed by sputum (15.31%) pus (7.04%) and blood (6.71%) similar results were seen in two different studies done by Kateregga, *et al* and Shrikant, *et al* [21,22]. Among the isolated organisms *E. coli* was the most prevalent 58.69%, followed by *K. pneumoniae* 24.61% [Table-3]. Similar studies carried out by Grover *et al*., and Agrawal *et al*., also isolated *E. coli* as most common isolate 69.08% and 50.70% respectively from India [23]. The most effective antibiotic was colistin which should be reserved for MDR cases, followed by Imipenem. Least effective of them was Ampicillin. Aminoglycosides like gentamicin was sensitive only in 218 cases (37.52%). But, amikacin was more effective in 484 (83.3%) cases and this was similar to a study done by Sasirekha, *et al* [24]. Chloramphenicol was promisingly sensitive in 534 cases *i.e*. is 91.2%, whereas Baral, *et al* showed it to be 73 % [25]. The antibiotic susceptibility pattern of β-lactamases. This study observed that resistance to Quinolones (norfloxacin) was 66%. Where it was about 68%. Aminoglycosides have good activity against clinically important gram-negative bacilli [26]. In the present study 76.79% isolates were susceptible to amikacin, followed by 53.92% to gentamicin. Several studies showed that amikacin was more effective than gentamicin but if it is over used than more organisms may become resistant. In 2010 resistance gentamicin was 59% in India and 55.5% in Bangladesh [24-28]. These variations may be due to increased use of gentamicin, caused by selection pressure of aminoglycosides in different regions [29]. Majority of the isolates were resistant to ampicillin (97.9%) and cotrimoxazole (66.21%) Our isolates also showed resistance to antibiotics such as amikacin upto (23.20%) and chloramphenical (9.21%). Likewise, Baral, *et al*. also reported similar resistance rates of ciprofloxacin (92.6%), ampicillin (94.1%), cotrimoxazole (86.8%), amikacin (6.2%) and chloramphenicol (27.1%)[25-30]. Low level of resistance to amikacin and chloramphenicol meant that they can still be used clinically in MDR cases with β-lactamase production. In our study imipenem was sensitive to 94.88% *i.e*. 5.12% resistance for all MDR isolates. Originally ESBLs were most commonly reported to be a hospital based problem but it is now common among community acquired isolates, especially *E.coli* [31-32]. Denholm and associates found *E. coli* to be the most common community acquired isolates among the ESBLs [33]. All the 417 Gram negative organisms belonging to Enterobacteriaceae which were MDR were subjected to screening test for β-lactamase production. 293 isolates were positive after screening for three enzymes ESBL, MBL and AmpC. Out of which 283 isolates which were resistant to Ceftazidime and 269 of these were ESBL producers which were confirmed by Double Disk Diffusion Method (DDDT). *E.coli* 154(54.41%) and *K. pneumoniae* 83(29.32%)were the two most common isolate. Similar studies carried out by Grover *et al*., and Agrawal *et al*. Our study also shows 114 isolates to be screen positive for cefoxitin *i.e*. 38.9% of the total 293 screened β-lactamase producers. On confirmation by AmpC Disc Test showed only 48(42.10%) as AmpC producers of the cefoxitin resistant ones. These isolates were also subjected to cefoxitin-cloxacillin Double Disk Synergy Test (CC-DDS) and AmpC E-Strip test showed 53 as AmpC producers. Yilmaz *et al* reported 39.56% isolates to be AmpC producers. We found higher number of AmpC producers by cefoxitin-cloxacillin disk diffusion test (CCDDS) test and E-strip test as compared to AmpC disc test. 417 MDR gram negative organisms belonging to Enterobacteriaceae were subjected to screening tests using Imipenem. Out of which 15(3.59%) isolates were resistant to Imipenem and 12(2.87%) of these were MBL producers by Imipenem-EDTA disc method (combined disc test) and Imipenem E-strip test showing perfect agreement between the two tests, but all 15 positive by Modified Hodge Test. Most MBL producing isolates belonged to *E.coli* followed by *K. pneumoniae*. 3 isolates were negative for CDT combined disk test (CDT) but were positive for MHT explaining some other carbapenemase enzyme being responsible for imipenem resistance. Hence, these 3 isolates were carbapenemase producers but not MBLs which can be confirmed by genotypic methods. Our previous on prevalence and antimicrobial susceptibility showed high incidence of *K. pneumonia* infections [34].

Conclusion

This study re-enforces the importance of continuous surveillance, especially of MDR *E.coli* and *K. pneumoniae* in the community so that appropriate treatment can be administered. In the present study it was seen that production of β-lactamases like ESBL and MBL are actually species dependent and statistically significant which was proved by chi square tests. Hence, whenever such organisms are isolated they should be screened for all β-lactamases like (ESBL and MBL) and dealt with proper antibiotics.

Application of research: The dissemination of ESBL-producing Enterobacteriaceae is a consequence of the clonal expansion of a few epidemic strains and the spread of resistance plasmids among bacterial organisms which has been associated with community as well as hospital acquired. Since the resistance displayed by bacteria reflects the environment in which the organism thrives, immediate action, including reinforcement of infection control measures, should be taken to prevent further spread of the resistant bacteria.

Review Category: Medical microbiology

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***Principle Investigator or Chairperson of research: Dr Shahzad Beg Mirza**
University: Dr D.Y. Patil Vidyapeeth, Pimpri, Pune, 411018, India
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