Original Article

Phenotypic methods for detection of various β-lactamases in Gram-negative clinical isolates: Need of the hour

Abstract

Background: Many clinical laboratories have problems detecting various β-lactamases. Confusion exists about the importance of these resistance mechanisms, optimal test methods, and appropriate reporting conventions. It is more imperative to use various phenotypic methods for detection of various β-lactamases in routine microbiology laboratory on day-to-day basis to prevent antimicrobial resistance by evidence-based judicious use of antimicrobials. **Aims:** In view of the multidrug-resistant organisms being reported world over, we planned a cross-sectional prospective analytical study to determine resistance mechanism by various β-lactamases in Gram-negative clinical isolates using various phenotypic methods. **Materials and Methods:** All nonrepeat, nonenteric clinical isolates of Gram-negative bacilli, resistant to at least two third-generation cephalosporins, were first screened by Novel disc placement method, and isolates showing multiple mechanisms of resistance and reduced zone of inhibition for imipenem were further confirmed for AmpC and metallo β-lactamases. **Statistical Analysis:** All the data was managed and analyzed in Microsoft Excel. **Results:** Out of 807 isolates tested, as many as 795 (98.51%) revealed the presence of extended-spectrum β-lactamases (ESBLs). Only 10 isolates of *Escherichia coli* and 2 of *Klebsiella pneumoniae* did not show production of ESBL. A total of 450 (55.76%) isolates produced single enzyme,while 345 (42.75%) strains revealed multiple enzyme production simultaneously. Only ESBL production was seen in 315 (39.03%) strains, only AmpC in 75 (9.29%) and only MBL in 60 (7.44%) strains, while ESBL and AmpC together were seen in 219 (27.14%) and AmpC plus MBL in 92 (11.40%) strains. However, ESBL plus MBL were never observed together. All three enzymes were simultaneously detected in 34 (4.21%) strains. **Conclusion:** This innovative method of disc placement makes it easy, affordable, and reliable method for routine use by basic microbiology laboratories for detection of various β-lactamases, pending confirmation for AmpC and metallo β-lactamase by three-dimensional test and double disc potentiation test, respectively.

Key words:
AmpC β-lactamase, extended-spectrum β-lactamase, multidrug-resistant organisms, phenotypic detection, metallo β-lactamase

Introduction

Infections due to Gram-negative bacilli are on rise world over. The rampant use of broad-spectrum antibiotics can lead to colonization with resistant strains with an increase in morbidity, mortality, and significant economic loss. Multidrug-resistant organisms (MDRO) by virtue of production of various β-lactamases confer resistance to many classes of antibiotics, particularly cephalosporins.[1] Extended-spectrum β-lactamases (ESBLs), have the ability to hydrolyze and cause resistance to various types of the newer β-lactam antibiotics, including cephalosporins like cefotaxime, ceftriaxone, ceftazidime, and monobactams (e.g., aztreonam), but not the cephamycins (e.g., cefoxitin and cefotetan) and carbapenems (e.g., imipenem, meropenem, and ertapenem).[2]

The gene coding for β-lactamases constantly mutates under heavy antibiotic pressure to produce resistant

Access this article online	
Website: http://www.cysonline.org	Quick Response Code
DOI: 10.4103/2229-5186.103098	

Neena V. Nagdeo, Navinchandra M. Kaore[1], Vilas R. Thombare

Department of Microbiology, NKP Salve Institute of Medical, Sciences & RC, Digdoh Hills, Nagpur, Maharashtra, [1]Peoples College of Medical, Sciences & RC, Bhopal, Madhya Pradesh, India

Address for correspondence:
Dr. Navinchandra M. Kaore,
HIG, Block A – 13, Peoples College of Medical Sciences & RC,
PCMS Campus, Bhanpur Road, Bhopal – 462 037, Madhya Pradesh, India.
E-mail: navinnmk@gmail.com

strains with broad spectrum of activity. Plasmid-mediated AmpC β-lactamases have arisen through the transfer of chromosomal genes for the inducible AmpC β-lactamase onto plasmids. This transfer has resulted in plasmid-mediated AmpC β-lactamases in isolates of *Escherichia coli, Klebsiella pneumoniae*, *Salmonella* spp., *Citrobacter freundii*, *Enterobacter aerogenes*, and *Proteus mirabilis*.[3]

Metallo β-lactamases (MBLs) are important because of their ability to hydrolyze almost all drugs including carbapenems as well as aminoglycosides and fluoroquinolones and an ability to rapidly disseminate as they are plasmid mediated.[4]

Although transmission of MDROs is most frequently documented in acute care facilities, all healthcare settings are affected by the emergence and transmission of antimicrobial-resistant microbes. The severity and extent of disease caused by these pathogens vary by the populations affected and by the institutions in which they are found.

Many clinical laboratories have problems in detecting various β-lactamases. Confusion exists about the importance of these resistance mechanisms, optimal test methods, and appropriate reporting conventions.

In view of need of cheap and easy methods for the diagnosis of various β-lactamases in basic microbiological laboratories ensuring an evidence-based medicine as prescribed by CDC, we planned a cross-sectional prospective analytical study to determine resistance mechanism by various β-lactamases in Gram-negative clinical isolates using various phenotypic methods.

Materials and Methods

The study was a cross-sectional prospective analytical study between November 2009 and July 2010. The study included 807, nonrepeat, nonenteric clinical isolates of Gram-negative bacilli collected over a period of 9 months from tertiary care hospital catering to rural population in central India. The isolates were from varied specimens obtained from patients of any age and either sex from IPD and OPD of various departments and showed resistance to at least two or more of the third-generation cephalosporins on routine antimicrobial susceptibility testing by disc diffusion method.[3] The isolates comprised 303 (35.54%) *E. coli*, 202 (25.03%) *K. pneumoniae*, 217 (26.88%) *Pseudomonas aeruginosa*, 50 (6.19%) nonfermenters, 20 (2.47%) *Proteus* species, and 15 (1.85%) *Citrobacter* species.

Disc placement method

All the selected strains were tested by disc placement method described earlier.[5] The lawn culture of test organism was made on Muller–Hinton agar (MHA) as done for disc diffusion antimicrobial susceptibility test. In the center of the plate, imipenem (10 μg) (Inducer) disc was applied. At the distance of 20 mm, the disc of cefotaxime (30 μg) was placed. From this disc, in a circular manner, clockwise, the discs of cefoxitin (30 μg) (Inducer), ceftriaxone (30 μg), ceftazidime (30 μg), ceftazidime + clavulinic acid (30/10 μg), and aztreonam (30 μg) were placed such that any two adjacent discs were 20 mm apart from center to center [Figure 1]. On overnight aerobic incubation at 37°C, the diameters of zones of inhibition were measured and interpreted as follows:

Extended-spectrum β-lactamase

(i) Zone diameter for aztreonam ≤27 mm, cefotaxime ≤27 mm, ceftazidime ≤22 mm, and ceftriaxone ≤25 mm.[6,7]
(ii) Susceptible to cefoxitin.[8]
(iii) Increase in zone size with addition of inhibitor (ceftazidime + clavulanic acid) by 5 mm or more [Figure 2].[8]

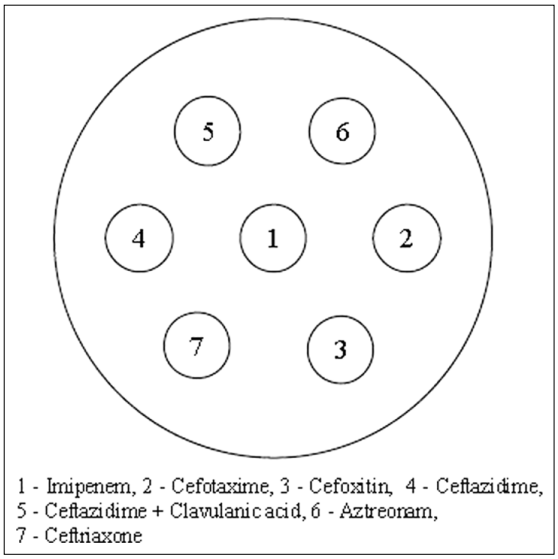

Figure 1: Novel disk placement method

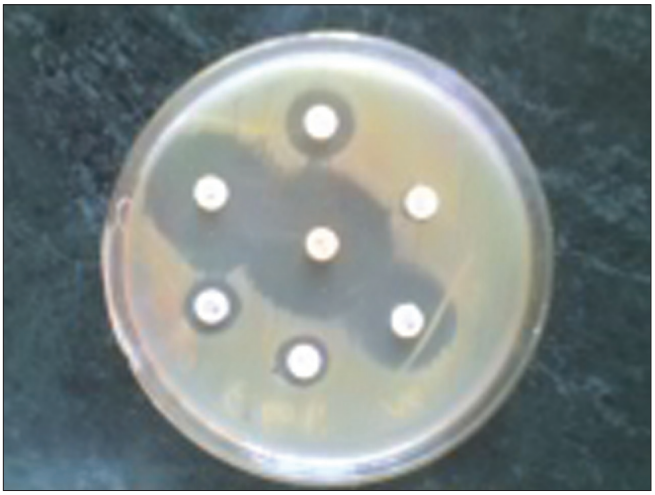

Figure 2: Novel disk placement method indicating ESBL-producing strain having increased zone size with ceftazidime + clavulunic acid and sensitive to cefoxitin

AmpC

(a) Inducible
  (i) Blunting of zone toward inducer [Figure 3]
  (ii) No increase of zone size with addition of inhibitor
(b) Derepressed mutants (DM)
  (i) Resistant to cefoxitin and cefotaxime
  (ii) No increase of zone size with addition of inhibitor

Metallo β-lactamases

Strains showing resistance to imipenem.

Multiple mechanisms

(i) Resistant to cefoxitin
(ii) Blunting of zone toward inducer
(iii) Increase of zone size with addition of inhibitor by 5 mm or more [Figures 4 and 5].

All isolates showing AmpC β-lactamases (inducible and DM) were further confirmed by modified three-dimensional test.

Modified three-dimensional test for AmpC β-Lactamase

Briefly, fresh overnight growth from MHA was transferred to a preweighed sterile microcentrifuge tube. The tube was weighed again to ascertain the weight of the bacterial mass. The technique was standardized so as to obtain 10–15 mg of bacterial wet weight for each sample. The growth was suspended in peptone water and was pelleted by centrifugation at 3000 rpm for 15 min. Crude enzyme extract was prepared by repeated freeze–thawing seven times. Lawn cultures of *E. coli* ATCC 25922 were prepared on MHA plates and cefoxitin (30 μg) discs were placed on the plate. Linear slits (3 cm) were cut using a sterile surgical blade 3 mm away from the cefoxitin disc. Small circular wells were made on the other end of the slits at 5 mm distance, inside the outer edge of the slit, by stabbing with a sterile pasture pipette on the agar surface. The wells could easily be loaded with the enzyme extract in 10 μL increments until the well was filled to the top. Approximately 20–30 μL of extract was loaded in the wells. The plates were kept upright for 5–10 min until the solution dried and were then incubated at 37°C overnight [Figure 6].[9]

The isolates showing resistance to imipenem were tested by Hodge test for MBL production and further confirmed by double disc potentiation test.[10,11]

Modified hodge test

An overnight culture suspension of *E. coli* ATCC 25922 adjusted to 0.5 McFarland standard was inoculated using a sterile cotton swab on the surface of a MHA. After drying, 10 μg imipenem disc was placed at the center of the plate and the test strain was streaked heavily from the edge of the disc to the periphery of the plate. The plate was incubated overnight at 37°C. Indentation produced in the zone of inhibition produced by the imipenem indicates a positive

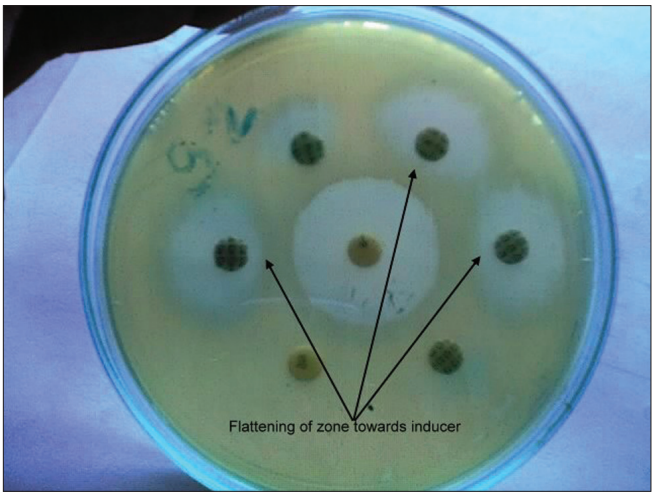

Figure 3: Novel disk placement method indicating group 1-inducible β-lactamase-producing strain having flattening of zone of inhibition toward inducer (imipenem)

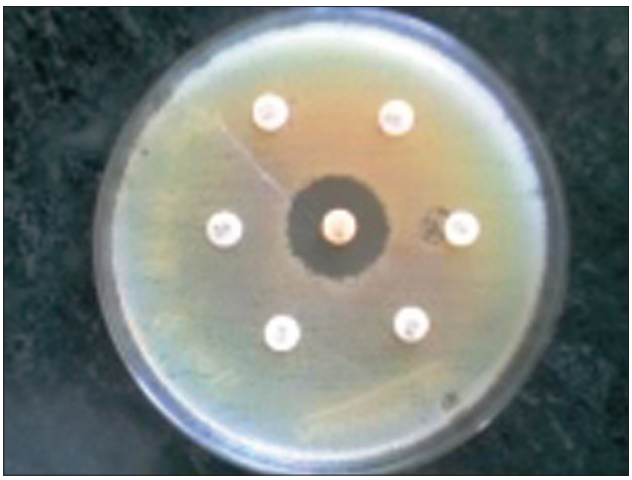

Figure 4: Novel disk placement method indicating presence of multiple mechanism without metallo β-lactamase as strain is sensitive to imipenem

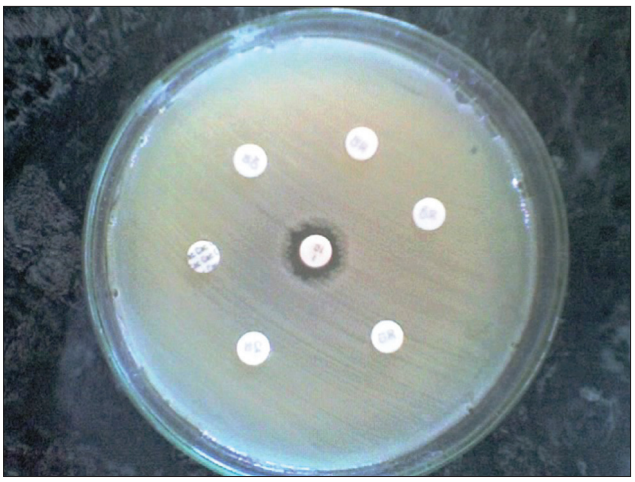

Figure 5: Novel disk placement method indicating presence of multiple mechanism with MBL as strain is resistant to imipenem

test. Maximum four strains can be tested at a time (all four directions) which gives a presence of a "cloverleaf shaped" zone of inhibition if all four test strains are positive for MBL production [Figure 7].[10]

Double disc potentiation test

A 0.5 M EDTA solution was prepared by dissolving 186.1 g of disodium EDTA. $2H_2O$ (REACHEM, Chennai, India) in 1000 ml of distilled water. The *p*H was adjusted to 8.0 by using NaOH (HI-MEDIA) and was sterilized by autoclaving. An overnight liquid culture of the test isolate was adjusted to a turbidity of 0.5 McFarland standard and spread on the surface of a MHA plate. Two 10 µg imipenem discs were placed on the agar 15 mm apart (center to center). 10 µl of 0.5 M EDTA is added to one of the imipenem disc to get a desired concentration of 750 µg. After incubating overnight at 37°C, the presence of an expanded growth inhibition zone between the two discs or increase of zone size of more than 7 mm in the disc potentiated with the EDTA (chelating agents) was interpreted as positive for MBL production [Figure 8].[11,12]

All the tests and their interpretations are according to the CLSI guidelines and well-accepted methods by various authorities.[9-12]

Results

Out of 807 isolates tested, as many as 795 (98.51%) revealed the presence of ESBLs. Only 10 isolates of *E. coli* and 2 of *K. pneumoniae* did not show production of ESBL.

The ESBL-positive isolates were found to be producing three different enzyme classes, i.e., ESBL, AmpC, and MBL, either single or in combinations with varying frequencies. The frequency distribution of these three enzymes in various strains is shown in Table 1.

A total of 450 (55.76%) isolates produced single enzyme, while 345(42.75%) strains revealed multiple enzyme production simultaneously. Table 2 depicts this frequency among different species tested.

Only ESBL production was seen in 315 (39.03%) strains, only AmpC in 75 (9.29%), and only MBL in 60 (7.44%) strains. while ESBL and AmpC together were seen in 219 (27.14%) and AmpC plus MBL in 92 (11.40%) strains. However, ESBL plus MBL were never observed together. All three enzymes were simultaneously detected in 34 (4.21%) strains. The distribution of production of ESBL, AmpC, and MBL, either single or in combination by different species, is presented in Table 3.

The detection of production of AmpC and MBL by disc placement method used in this study had an absolute correlation with the confirmatory tests done, respectively, for both these enzymes.

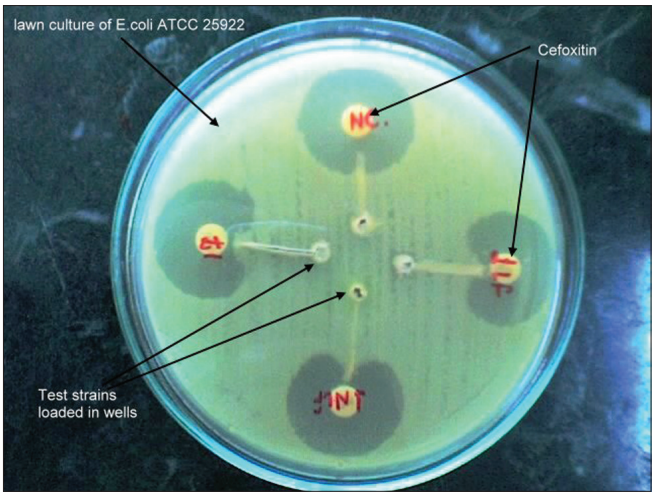

Figure 6: Three-dimensional test for detection of AmpC β-lactamase with negative control

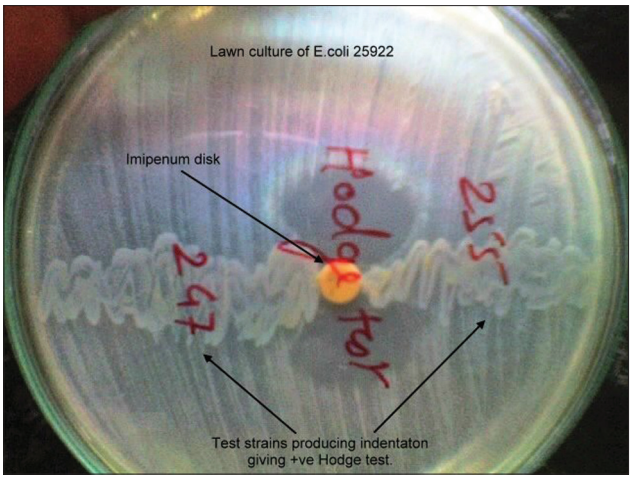

Figure 7: Hodge test for screening of metallo β-lactamase where the positive strain is producing indentation in zone of inhibition of imipenem to *E. coli* ATCC 25922

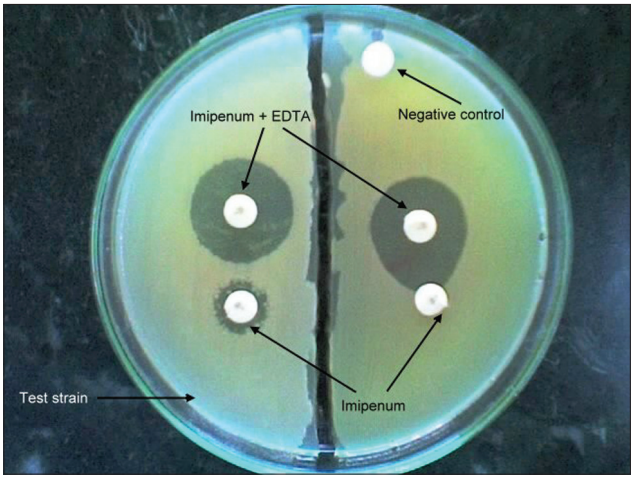

Figure 8: Double disk potentiation test showing increase in zone size with imipenem disk potentiated with EDTA to test strain when compared with plain imipenem disk

Table 1: Shows frequency distribution of ESBL. AmpC & MBL in various strains

Organism	Number tested	ESBL producers (%)	AmpC producers (%)	MBL producers (%)
E. coli	303	237 (78.22)	147 (48.52)	30 (9.91)
Klebsiella	202	128 (63.37)	170 (84.16)	62 (30.69)
Pseudomonas	217	155 (71.43)	67 (30.88)	62 (28.57)
Non fermenters	50	17 (34.00)	30 (60.00)	29 (58.00)
Proteus sp.	20	20 (100.0)	1 (05.00)	0 (0.00)
Citrobacter sp.	15	11 (73.33)	5 (33.33)	3 (20.00)
Total	807	568 (70.38)	420 (52.05)	186 (23.05)

ESBL - Extended-spectrum β-lactamase; MBL - Metallo β-lactamases

Table 2: Depicts frequency of single or multiple β-lactamases amongst different species tested

Organism	Total no of strains	No ESBL activity	Number tested	Single enzyme (%)	Multiple enzymes (%)
E. coli	303	10	293	174 (57.43)	119 (39.27)
Klebsiella	202	02	200	65 (32.18)	135 (66.83)
Pseudomonas	217	00	217	155 (71.43)	62 (28.57)
Non fermenters	50	00	50	26 (52.0)	24 (48.0)
Proteus sp.	20	00	20	19 (95.0)	1 (05.0)
Citrobacter sp.	15	00	15	11 (73.33)	4 (26.67)
Total	807	12	795	450 (55.76)	345 (42.75)

ESBL - Extended-spectrum β-lactamase; MBL - Metallo β-lactamases

Table 3: The distribution of production of ESBL, AmpC and MBL, either single or in combination by different species

Organism (n)	ESBL (%)	AmpC (%)	MBL (%)	ESBL+ AmpC (%)	AmpC+ MBL (%)	ESBL+ AmpC+ MBL (%)
E. coli (293)	125 (41.25)	28 (9.24)	21 (6.93)	110 (36.30)	7 (2.31)	2 (0.66)
Klebsiella (200)	25 (12.38)	35 (17.33)	5 (2.48)	78 (38.61)	32 (15.84)	25 (12.38)
Pseudomonas (217)	128 (58.99)	5 (2.30)	22 (10.14)	22 (10.14)	35 (16.13)	5 (2.30)
Non fermenter (50)	10 (20.0)	6 (12.0)	10 (20.0)	5 (10.0)	17 (34.0)	2 (4.0)
Proteus (20)	19 (98.0)	0 (0.00)	0 (0.00)	1 (5.0)	0 (0.00)	0 (0.00)
Citrobacter (15)	8 (53.33)	1 (6.67)	2 (13.33)	3 (20.0)	1 (6.67)	0 (0.00)
Total (795)	315 (39.03)	75 (9.29)	60 (7.44)	219 (27.14)	92 (11.40)	34 (4.21)

(Excluding a total of 12 isolates without β–lactamases)

Discussion

Cephalosporins are currently the drugs of choice for infections caused by the Enterobacteriaceae. The extensive use of third-generation cephalosporins has resulted in the increased prevalence of ESBL and plasmid-mediated AmpC among these organisms.

An indiscriminate administration of betalactams also increases the risk of colonization of hospitalized patients with ESBL-producing Enterobacteriaceae. Such organisms are usually derived from colonized healthcare settings. Recent studies showed an increased prevalence of community-acquired infections with ESBL-producing organisms.[13]

The occurrence of MDRO not only limits the therapeutic options but also poses a challenge for microbiology laboratories to identify them. The detection of the coproduction of various β-lactamases singly or in combinations is essential for enhanced infection control and effective antimicrobial therapy. Although ESBL detection and reporting is recommended routinely by CLSI, it lacks guidelines for the AmpC or MBL or combination of various β-lactamases .Several studies have been done for phenotypic detection of AmpC and metallo β-lactamses.[1-6,9-12,14-19]

Our study was planned to look for simple phenotypic methods to detect organisms producing ESBL, group 1-inducible AmpC β-lactamases, plasmid-mediated AmpC β-lactamases, MBL, and or combinations of the above at one go.

All the isolates that were showing resistance to third-generation cephalosporins (zone of inhibition less than prescribed in CLSI guidelines 2010) were taken for the study as they are potential isolates showing either ESBL or AmpC β-lactamases. In a recent study, Aarestrup FM *et al* studied the efficacy of eight different cephalosporins and showed that cephalosporin-resistant strains predict the presence of various β-lactamases.[20] In our study, out of 807 isolates tested, as many as 795 (98.51%) revealed the presence of ESBLs. Only 10 isolates of *E. coli* and 2 of *K. pneumoniae* did not show production of ESBL, thus indicating that

screening for resistance with at least two third-generation cephalosporins indicate presence of ESBL in a majority of isolates.

A very simple method of placement of discs was used for detection of various β-lactamases. Imipenem disc in the center and cefoxitin disc acts as an inducer. Side by side placement of ceftazidime and ceftazidime + clavulanic acid disc around imipenem will show the ESBL-producing organisms as ESBLs are inhibited by clavulanic acid. The blunting of zone of inhibition of other cephalosporin discs (cefotaxime and ceftriaxone) toward inducers indicates the presence of inducible β-lactamases. The disc antagonism method given by Sander's *et al* is created here by placing the ceftriaxone and cephotaxime discs adjacent to cefoxitin.[21]

Resistance to cefoxitin with non inhibition of β-lactamase by clavulanic acid indicates constitutive production of AmpC β-lactamase. Decreased zone size of imipenem indicates presence of MBL. Resistance to cefoxitin, blunting of zone toward inducer, and increase of zone size with addition of inhibitor by 5 mm or more indicate multiple mechanisms involved.

The results of this study indicate that ESBL production is a major mechanism of resistance to cephalosporins among Gram-negative bacteria. Our study of phenotypes indicates that these organisms produce ESBL, AmpC, and MBL, but with varying frequencies. Over two third strains produce ESBLs, half will produce AmpC and about a quarter MBL. While most of the Gram-negative organisms tend to produce ESBL more frequently, *Klebsiella* produces AmpC and nonfermenters tend to produce AmpC and MBLs.

In over half (55.76%) strains, organisms conferred resistance by producing a single class of enzyme, but in 42.75% instances multiple resistance enzymes operate, which certainly is a matter of concern. *Klebsiella* again tends to produce multiple enzymes more frequently (66.83%).

The distribution of the three enzyme classes among all the species studied presented a characteristic pattern. When resistance was conferred by producing a single enzyme, it was commonly the ESBL producer but AmpC and MBLs were more commonly seen when the multiple enzyme production was observed. In this study, 4.21% of the total isolates produced all the three, viz, ESBL, AmpC, and MBL. Although, prima facie the number appears to be small, but it sounds an alarm for existence of pathogens likely to be highly resistant.

In a similar study, Gupta *et al* from Chandigarh has reported 69% ESBL producers among 100 cephalosporin resistant strains, more commonly in *E. coli*.[22] while in their study of 228 isolates involving *E. coli* and *Klebsiella Shiju*, MP *et al* reported 59.65% ESBL production in cephalosporin-resistant strains.[23] Similarly in their comparison of methods for detection of ESBL, Giriyapur RS has reported a upto 63.89% ESBL production in strains initially screened by third-generation cephalosporins.[24]

Rodrigues C. *et al* have evaluated 286 isolates resistant to third-generation cephalosporins and reported 151 (53%) ESBL producers, of which 131 (46%) were also showing AmpC β-lactamase, 40 (14%) were plain AmpC, while inducible AmpC β-lactamase production was seen in 19 (7%) isolates; the majority of isolates being *E. coli* and *Klebsiella*.[5]

Mohamudha PR *et al* in 2010 evaluated a total of 235 strains from tertiary care hospital in south India with 134 strains resistant to third-generation cephalosporins, of which 63 (47%) were plasmid-mediated AmpC β-lactamase producers.[25]

In our study, expressions of various β-lactamases either singly or in combination are in agreement with the other studies. The findings in the study are from tertiary hospital in the rural settings and high level of single or multiple ESBL mechanism in MDRO is definitely alarming necessitating an urgent need for evidence-based medicine particularly in rural settings where laboratory facilities are lacking and antibiotics are being rampantly used by the quacks. This also calls for the concerted efforts to bring all laboratories under the umbrella of quality practice to meet national standards to combat antimicrobial resistance. This can be best achieved through creating awareness and training in good clinical laboratory practice, to follow standard procedures, quality control, and quality assurance. Standard practice on antimicrobial testing must be strictly adhered to in the laboratory.

The automated methods used for antimicrobial sensitivity testing fail to discriminate between various β-lactamases identifying only the ESBL and requiring manual methods for confirmation.[26,27] The genotypic methods can give a confirmed evidence of presence β-lactamases but are costly and not available at grass root levels. So in a resource-poor developing countries, this innovative disc placement method is easy and interpretation is also very easy. This is an affordable and reliable method for detection of various β-lactamases pending confirmation for AmpC and MBLs by three-dimensional test and DDPT, respectively. In our study, 100% of the isolates indicating AmpC and MBLs production were further confirmed by these confirmatory tests. These phenotypic methods are easy and are able to discriminate between various mechanisms which even the automated methods fail to do.

A large-scale multicentric study involving private microbiology laboratories for detection of β-lactamases with this cheap method which is easy to interpret is need of the hour so as to know the exact burden of these ESBL-producing MDROs in the community and also for evidence-based antibiotic policy initiation by the treating physicians, to curb the menace of MDRO prevailing in the society.

Studies should also be undertaken to compare the results with genotypic and automated methods to substantiate the findings of this simple phenotypic method.

This will be positive step forward toward WHO's World Health Day theme this year to combat drug resistance "Antimicrobial resistance- No action today, No cure tomorrow."

Acknowledgment

We acknowledge Dr. Yagnesh Thakar for constant encouragement and constant support of Dr. Umesh Hassani and Dr. Nutan Bhongle. We also thank all the staff members of our department for their support.

References

1. Livermore DM. Bacterial resistance: origins, epidemiology, and impact. Clin Infect Dis 2003;36(suppl 1): S11-23.
2. Bradford PA. Extended-spectrum β-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clin Microbiol Rev 2001;14:933-51.
3. Thompson KS. Controversies about Extended-Spectrum and AmpC Beta-Lactamases. Emerg Infect Dis 2001;7:333-6.
4. Walsh TR, Toleman MA, Poirel L, Nordmann P. Metallo-β-lactamases: the quiet before the storm? Clin Microbiol Rev2005;18:306-25.
5. Rodrigues C, Joshi P, Jani SH, Alphonse M, Radhakrishnan R, Mehta A. Detection of lactamases in nosocomial gram negative clinical isolates. Indian J Med Microbiol 2004;22:247-50.
6. Blondeau JM. Extended sprectrum Beta lacyamases. Semin Respir Infect 2001;16:169-76.
7. Guidelines on susceptibility of antibiotic resistant Enterobacteriaceae due to extended spectrum beta lactames(ESBL). Canadian External Quality Assurance Advisory Groups on Antimicrobial resistance (CEQA-AGAR) and Bureau of Microbiology;Health Canada, December 1999.
8. National Committee for Clinical Laboratory Standards. (2010). Performance Standards for Antimicrobial Susceptibility Testing—Twentieth Informational Supplement: M100-S10. NCCLS, Wayne, PA, USA.
9. Manchanda V, Singh NP. Occurrence and detection of AmpC b-lactamases among Gram negative clinical isolates using a modified three-dimensional test at Guru Tegh Bahadur Hospital, Delhi, India. J Antimicrob Chemother 2003;51:415-8.
10. Lee K, Chong Y, Shin HB, Kim YA, Yong D, Yum JH. Modified Hodge test EDTA disk synergy tests to screen metallo- β-lactamase-producing strains of Pseudomonas and Acinetobacter species. Clin Microbiol Infect 2001;7:88-91.
11. Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, Chong Y. Imipenem-EDTA disk method for differentiation of metallo-β-lactamases producing clinical isolates of Pseudomonas spp. and Acinetobacter spp. J Clin Microbiol 2002;40:3798-801.
12. Pitout JD, Gregson DB, Poirel L, McClure JA, Le P, Church DL. Detection of Pseudomonas aeruginosa producing metallo-β-lactamases in a large centralized laboratory. J Clin Microbiol 2005;43:3129-35.
13. Paterson DL, Bonomo RA. Extended spectrum Beta lactamases: A clinical update. Clin Microbiol Rev 2005;18:657-86.
14. Black JA, Moland ES, Thomson KS. AmpC disk test for detection of plasmid mediated Amp C beta lactamases in enterobacteriocae lacking chromosomal AmpC beta lactamases. J Clin Microbiol 2005;43:3110-3.
15. Yagi T, Wachino J, Kurokawa H, Suzuki S, Yamane K, DoiY, *et al*. Practical methods using Boronic acid compounds for identification of class C beta lactamases producing Klebsiella pneumoniae and Escherichia coli. J Clin Microbiol 2005;43:2551-8.
16. Nasim K, Elsayed S, Pitout JD, Conly J, Church DL, Gregson DB. New method for laboratory detection of ampC beta lactamses in Escherichia coli and Klebsiella pneumoniae. J Clin Microbiol 2004;42:4799-802.
17. Coudron PE, Moland ES, Thomson KS. Occurrence and detection of AmpC beta-lactamases among Escherichia coli, Klebsiella pneumoniae, and Proteus mirabilis isolates at a Veterans medical center. J Clin Microbiol 2000;38:1791-6.
18. Shahid M, Malik A, Agarwal M, Singhal S. Phenotypic detection of extended spectrum and AmpC beta lactamases by a new spot inoculation method and modified three dimensional extract test: Comparison with conventional three-dimensional extract test. J Antimicrob Chemother 2004;54:684-7.
19. Singhal S, Mathur T, Khan S, Upadhyay DJ, Chugh S, Gaind R, *et al*. Evaluation method for AmpC beta lactamses ingram negative clinical isolates from tertiary care hospitals. Indian J Med Microbiol 2005;23:120-4.
20. Aarestrup FM, Hasman H, Veldman K, Mevius D. Evaluation of Eight Different Cephalosporins for Detection of Cephalosporin Resistance in Salmonella enterica and Escherichia coli. Microb Drug Resist 2010;4:253-61
21. Sanders CC, Sanders WE, Goering HV. In vitro antagonism of _β -lactam antibiotics by cefoxitin. J Antimicrob Chemother 1982;21:968-75.
22. Gupta V, Singla N, Chander J. Detection of ESBLs using third & fourth generation cephalosporins in double disc synergy test. Indian J Med Res 2007;126:486-7.
23. Shiju MP, Yashavanth R, Narendra N. Detection of Extended Spectrum Betalactamase Production and multidrug resistance in clinical isolates of E.coli and K.pneumoniae in Mangalore. J Clin Diagn Res 2010;4:2442-5.
24. Giriyapur RS, Nandihal NW, Krishna BV, Patil AB, Chandrashekhar MR. Comparison of disc diffusion methods for detection of extended spectrum beta lactamases producing enterobacterioceae. J Lab physicians 2011;3:33-6.
25. Mohamudha PR, Harish BN, Parija SC. Ampc Beta lactamases among gram negative clinical isolates from a tertiary hospital, South India. Brazilian J Microbiol 2010;41:596-602.
26. Wiegand I, Geiss HK, Mack D, Stu¨renburg E, Seifert H. Detection of Extended-Spectrum Beta-Lactamases among Enterobacteriaceae by Use of Semiautomated Microbiology Systems and Manual Detection Procedures. J Clin Microbiol 2007;45:1167-74.
27. Gagliotti C, Sarti M, Benini F, Cipolloni AP, Testa G, Venturelli C, *et al*. Laboratory detection of extended-spectrum beta-lactamase by an automated system. New Microbiol 2008;31:561-4.

How to cite this article: Nagdeo NV, Kaore NM, Thombare VR. Phenotypic methods for detection of various β-lactamases in Gram-negative clinical isolates: Need of the hour. Chron Young Sci 2012;3:292-8.

Source of Support: Nil, **Conflict of Interest:** None declared