Int.J.Curr.Microbiol.App.Sci (2019) *8*(9): 1678-1684

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 8 Number 09 (2019)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2019.809.190

Detection of ESBL and MBL Producing Gram Negative Bacilli from various Clinical Samples at a Tertiary Care Hospital

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Keywords

ESBL, MBL, CDDT, Modified Hodge Test.

Article Info

Accepted:
18 August 2019
Available Online:
10 September 2019

A B S T R A C T

Antimicrobial resistance pattern and resistance to third generation cephalosporins has become increased worldwide. The detection of extended spectrum lactamases (ESBLs) and metallobetalactamases (MBLs) among members of Enterobacteriaceae family guide us to use Beta lactam, if not tested leading to treatment failure. One year prospective study, our aim is to detect ESBL and MBL producing gram negative bacilli from various clinical samples. A total of 100 Gram negative bacilli were processed. ESBL was detected by phenotypic confirmatory disc diffusion test (PCDDT) using Ceftazidime / Cefotaxime alone and in combination with clavulanic acid. MBL detection was done by Imipenem EDTA combined disc diffusion test (CDDT), double disc synergy test (DDST) and Modified Hodge test. Out of 100 isolates, 47 (47%) were ESBL producers, 23 (23%) were MBL producers and 13(19%) isolates were both ESBL and MBL producers. ESBL production was observed in *Escherichia coli, K.pneumoniae, Pseudomonas spp* and *Citrobacter spp* and MBL production was observed in *Pseudomonas aeurginosa, Acinetobacter spp, E.coli, Klebsiella spp* from various clinical samples. Among the 47 ESBL producers, 97% of the isolates showed resistance to any one of the third generation cephalosporin (ceftazidime, cefotaxime, ceftriaxone) and 3% showed resistance to all the three third generation cephalosporin. Among 23 MBL producers, 15 (65%) were MBL producers by CDDT whereas 8 (35%) by DDST methods. Introduction of simple, reliable and reproducible screening tests for early detection and identification of ESBL and MBL producing gram negative bacilli in routine diagnostics is of crucial important to prevent nosocomial dissemination of resistance. In our study we found that CDDT is the most sensitive method among three phenotypic methods (CDDT, DDST, and Modified Hodge Test) for detection of MBL.

Introduction

Antibiotic resistance is a major problem for clinicians in treating infections. The organism's ability to mutate or acquire or transmit mobile genetic elements encoding resistance gene to other susceptible organisms.[1] Carbapenems are betalactam antibiotics, which are used as the most potent agents for treating multidrug resistant gram negative bacterial infections because of their high rate of permeation through bacterial outer membranes.[2] The newer betalactamases, including extended spectrum betalactamases

(ESBLs), Amp C betalactamases (Amp C) and Metallo betalactamases (MBLs) have emerged worldwide as a cause of antimicrobial betalactamase mediated resistance in gram negative bacteria.[3]

ESBLs are plasmid mediated enzymes that shows resistance to penicillins, first, second and third generation cephalosporins and aztreonam (but not cephamycins and carbapenems). ESBLs are transmissible betalactamases which are inhibited by clavulanic acid, tazobactum and sulbactum.[4] Being plasmid mediated, they are easily transmitted thus facilitating the dissemination of resistance to beta lactams.[5]They also exhibit resistance to other classes of drugs such as aminoglycosides, cotrimoxazole, tetracycline and fluoroquinolones.[6,7]

Resistance to carbapenem is predominantly mediated by metallo betalactamases (MBL), a class B type of betalactamases that recognize bivalent metal ions.[8]MBL can hydrolyze all metallo beta lactams except monobactams and are not inactivated by beta lactamase inhibitors like clavulanic acid, sulbactam and tazobactam.[9] They may be chromosomally or plasmid mediated and therefore poses a threat of spread of resistance by gene transfer among gram negative bacteria.[10]

The multidrug resistant isolates that are present in the ICU and in the hospital environment pose not only therapeutic problems but also serious concerns for infection control management.[11]Early detection of MBL and ESBL producing organisms is crucial to establish appropriate antimicrobial therapy and to prevent their interhospital and intrahospital dissemination of infections.[12]So the present study was undertaken to detect ESBL and MBL production in gram negative bacilli from various clinical samples.

Materials and Methods

The present study was undertaken at the Department of Microbiology, Vijayanagara Institute of Medical Sciences, Ballari. Total 100 Gram negative bacilli were isolated from various clinical samples (urine, pus, blood, sputum, ET secretions etc). They were identified by standard microbiological procedures.[13] The antibiotic susceptibility of isolates was determined by Kirby- Bauer disc diffusion method using Clinical and Laboratory Standard Institute guidelines (CLSI).[14] Antibiotics included were cetazidime (30 µg), aztreonam (30 µg), cefipime (30 µg), cefotaxime (30 µg), cefoxitin (30µg), imipenem (10 µg), meropenem (10 µg), cefoperazone/ sulbactam (75/30 µg), piperacillin/ tazobactam (100/10 µg), gentamicin (10 µg), amikacin (30 µg), ciprofloxacin (5 µg), cotrimoxazole (1.25/23.75 µg), polymyxin B (300U) and colistin (10 µg). Isolates which are resistant to third generation cephalosporins were tested for ESBL production and isolates showed resistance to imipenem were tested for MBL production

Detection of ESBL

The double disk diffusion method was used for detection of ESBL as recommended by CLSI guidelines. [14] The suspension was streaked onto Mueller-Hinton agar plates.

A disc of either ceftazidime (30 µg) or cefotaxime (30µg) alone in combination with clavulanic acid (30µg/10 µg) was placed at the distance of 20 mm (centre to centre).

After overnight incubation at 35°C, a positive test result was considered as a 5 mm increase in inhibition zone of the combination discs compared with a disk alone was considered to be ESBL producer.

Detection of MBL

Imipenem resistant isolates were screened for production of MBL by Imipenem EDTA combined disc diffusion test (CDDT), Double disc synergy test (DDST) and Modified Hodge test and strains showed positive results by three methods were considered as MBL producers.

Imipenem (IMP) - EDTA Combined Disc Diffusion Test (CDDT): Test organisms were inoculated on to Muller Hinton agar plates as recommended by CLSI guidelines. 0.5 M EDTA solution was prepared by dissolving 18.61g in 100ml of distilled water. The mixture was sterilised by autoclaving. Two 10µg imipenam discs were placed on the plate and appropriate amounts of 10µl of EDTA solution were added to one of them to obtain the desired concentration of 750µg. The inhibition zones of the imipenam and imipenam-EDTA discs were compared after 16-18hrs of incubation at 35°C.If the increase in inhibition zone with the imipenam and EDTA disc was more than 7mm than the imipenam disc alone, it was considered as MBL producer. [15]

IMP- EDTA Double Disc Synergy Test (DDST)

Test organisms were inoculated on to plates with Muller Hinton agar as recommended by CLSI. Imipenam (10µg) disc was placed 20mm center to center from a blank disc containing 10µl of 0.5 M EDTA (750µg). Enhancement of zone of inhibition in the area between Imipenam and EDTA disc more than 5mm is considered as MBL producer. [15]

Modified hodge test: An overnight culture of *E. coli* ATCC 25922 was adjusted to 0.5 McFarland standards and lawn culture was made on Muller Hinton agar plates (Hi-media, Mumbai). 10µg imipenem disc was placed on the centre of the plate and test culture was streaked from the edge of the disc to the periphery of the plate. After 16-18 hours of incubation at 37°C, the presence of a cloverleaf zone or distortion of inhibition around the imipenem disc was interpreted as positive for MBL production. [14]

Results and Discussion

Out of 100 Gram negative isolates, *Escherichia coli* 41(41%) was the most common organism isolated, followed by *Klebsiella pneumoniae* 24(24%) and *Pseudomonas spp* 17(17%), *Acinetobacter spp* 9(9%), *Citrobacter spp* 4(4%), *Proteus spp* 3(3%) and *Enterobacter spp* 2(2%)(Table 1). In this study 34(72%) ESBL producers were from IPD patients and 13(28%) were from OPD patients. Amongst the MBL producers 14(60%) were from IPD patients and 9(40%) were from OPD patients (Table 2).Out of 100 Gram negative bacilli, 47 were ESBL producers and 23 were MBL producers. Highest ESBL production was seen in *Escherichia coli* 28(60%) followed by *Klebsiella pneumoniae* 17(36%). Highest MBL production was seen in *Klebsiella pneumoniae* 7(30%) and *Pseudomonas spp* 5(22%) (Table3). Highest percentage of ESBL and MBL production was seen in urine and pus isolates (Table 4). Combined disc diffusion test shows higher positivity (65%) than Double disc synergy test (35%) (Table5).

In this study maximum ESBL producers 34 (72%) and MBL producers 14 (60%) were from IPD patients which is similar to findings done by other authors. [16,17,18] These results correlates with the fact that most of the risk factors are associated with infections which are present in both indoor and ICU patients like cross transmission, immune compromised patients, patients with indwelling devices, heavy use of broad spectrum antibiotics and frequent contamination of the hands of health care workers while taking care of patients.

Table.1 Distribution of gram negative bacilli (GNB)

(n=100)

GNB	No. (%)
Escherichia coli	41(41%)
Klebsiella pneumoniae	24(24%)
Pseudomonas spp	17(17%)
Acinetobacter spp	9(9%)
Citrobacter spp	4(4%)
Proteus spp	3(3%)
Enterobacter spp	2(2%)
Total	100 (100%)

Table.2 Distribution of ESBL and MBL producers

Producers	OPD	IPD
ESBL (n=47)	13 (28%)	34 (72%)
MBL(n=23)	9 (40%)	14 (60%)
Total (70)	22	48

Table.3 ESBL and MBL producers among organisms

*Organisms	No. (%)	ESBL producers	MBL producers
Escherichia coli	41(41%)	28 (60%)	4(17%)
Klebsiella pneumoniae	24(24%)	17 (36%)	7(30%)
Pseudomonas spp	17(17%)	1 (2%)	5(22%)
Acinetobacter spp	9(9%)	1 (2%)	3(13%)
Citrobacter spp	4(4%)	0	2(9%)
Proteus spp	3(3%)	0	1(4%)
Enterobacter spp	2(2%)	0	1(4%)
Total	100 (100%)	47 (100%)	23 (100%)

*13 (19%) -organisms were both ESBL and MBL producers

Table.4 Distribution of ESBL and MBL in various clinical samples

Sample	No. of isolates	ESBL producers	MBL producers
Urine	30	15 (32%)	4 (17%)
Pus	27	12 (26%)	6 (26%)
Blood	18	8 (17%)	5 (21%)
Sputum	25	12 (26%)	8 (35%)
Total	100	47 (100%)	23 (100%)

Table. 5 Comparison of DDST and PCDT methods

Tests	No. of MBL Positive	Percentage (%)
(IMP and IMP with EDTA) CDDT	15	65%
(IMP and EDTA) DDST	8	35%
Total	23	100

In our study maximum ESBL production was observed in *Escherichia coli* 28 (60%) followed by *Klebsiella pneumoniae* 17 (36%) which is similar to other studies [19,20,21,22]. In the present study highest MBL production was seen in *Klebsiella pneumoniae* 7 (30%) and *Pseudomonas spp* 5 (22%) which is similar to other studies. [3,16,23]

In this study maximum ESBL and MBL production was observed in urine and pus isolates which correlates with other studies. [16,18] Early detection of ESBL and MBL positive isolates is necessary not only for management of the patient but also for appropriate infection control measures to prevent the spread of resistance. [23] In the present study for MBL detection, combined disc diffusion test showed higher positivity (65%) when compared to other two tests and this fact correlates with the findings done by other authors. [24] The double disc synergy test (DDST) less sensitivity because of the problem of optimal disc space. Phenotypic methods are technically simple and inexpensive when compared with other methods. [25]

The only beta lactam active against Amp C and ESBL producers are carbapenems but in recently resistance to carbapenems has been increased due to the production of metallo-lactamases.[26] Metallo beta lactamases (MBLs) are enzymes belonging to Ambler s class B that can hydrolyze a wide variety of beta lactams, including penicillins, cephimes, and carbapenems except aztreonam. [27,28] Although, PCR is a simple but costly method to use in detecting MBL producing isolates, it has become more difficult with the increased number and types of MBL. [29] Combined disc test is simple to perform and highly sensitive in differentiating MBL producing isolates. [30]

Thus, implementation of simple method using Imipenem-EDTA disk for MBL detection is quick, specific, sensitive and reproducible.[27] Production of MBL has tremendous therapeutic consequences since these organisms also carry multidrug resistance genes and the only viable option remains the potentially toxic Polymyxin B and colistin. [31]

Hence, the early detection of beta lactamase producing isolates would be important for the reduction of mortality rates for patients and also to avoid the intra hospital dissemination of such strains. Simple phenotypic screening tests are proved to be rapid and convenient method for their detection in the clinical laboratory. Monitoring and judicious usage of cephalosporins and imipenem, periodic surveillance of antibiotic resistance patterns and efforts to decrease empirical antibiotic therapy would solve some of the problems which are associated with ESBL an MBL producer. To overcome the problem of emergence and the spread of multidrug resistant organisms, a combined interaction between the microbiologist, clinicians and infection control team is needed.

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How to cite this article:

Iswarya, M. and Narayan Shrihari. 2019. Detection of ESBL and MBL Producing Gram Negative Bacilli from various Clinical Samples at a Tertiary Care Hospital. *Int.J.Curr.Microbiol.App.Sci.* 8(09): 1678-1684. doi: https://doi.org/10.20546/ijcmas.2019.809.190