International Journal of Medical Science and Clinical Research
Online-ISSN: 2664-7761; Print ISSN: 2664-7753
Received: 26-12-2020; Accepted: 16-01-2021; Published: 05-02-2021
www.journalofmedical.com
Volume 3; Issue 1; 2021; Page No. 16-21

Detection of metallo beta lactamases production in gram negative organisms by different phenotypic methods and their antimicrobial susceptibility pattern at a tertiary care hospital, Jaipur

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Abstract

Title: Detection of Metallo beta lactamases production in Gram Negative organisms by different phenotypic methods and their antimicrobial susceptibility pattern at a Tertiary care Hospital, Jaipur.

Introduction: The production of metallo beta lactamases is an important mechanism responsible for the increased Carbapenem resistance. Carbapenems are often regarded as last resort antibiotics in the treatment of clinical conditions due to MDR organisms. Current study was done to detect the prevalence of MBL producing organism in this hospital and antimicrobial susceptibility pattern of MBL producing organisms.

Material and Methods: This study was done on all clinical samples received in m Department of microbiology, National Institute of Medical Sciences and Research, Jaipur over a period of 6 months from June 2018 to November 2018. Samples were received and processed as per the standard guidelines and antimicrobial susceptibility testing was done according to the standard CLSI Guidelines. Detection of MBL production in Imipenem resistant strains was done by using Modified Hodge test, Double Disc synergy test, Disc Potentiation test and Thiol based compound test.

Result: Out of the 584 Gram Negative isolates, 159 isolates were found to be resistant to Imipenem. Majority of the MBL producing organisms were found to be multidrug resistant organisms. Only Polymixin B and Colistin showed better sensitivity towards MBL producing isolates. DDST method showed better result as compared to other methods.

Conclusion: MBL production in organisms is a leading cause of treatment failure as these enzymes inhibits the action of Carbapenems. High prevalence of MBL producing organisms is alarming sign and strict specific measures must be implemented to avoid the adverse outcomes

Keywords: MBL, modified hodge test, double disc synergy test, disc potentiation test, thiol based compound test

Introduction

Microorganisms can develop resistance against most of the Beta – lactam antibiotics by producing Beta – Lactamases. Heavy intake of antibiotics induces mutation which results in formation of Beta – Lactamases. Among all these Beta – lactamases, metallo beta lactamases is the most important one because this result in resistance towards all drugs of that class including Carbapenems. Mutation in porins, efflux mechanisms, loss of certain outer membrane proteins can result in development of resistance towards carbapenems by Beta – lactamases [1]. MBL producing strains shows resistance towards all Beta – lactams as well as frequently towards aminoglycosides & fluoroquinolones also. However they are sensitive towards polymixins [2]. Drugs of class carbapenems were introduced in 1980's and used as drug of last choice in treating serious infections caused by Gram Negative bacteria. These drugs are effective towards extended spectrum beta lactamases, AmpC produced by Gram Negative Bacteria [3]. MBL, belongs to the Ambler Class B owing to their capacity to hydrolyze penicillins, cephalosporins, carbapenems except aztreonam [4]. MBL production can be detected by many phenotypic and genotypic methods. Genotypic methods include polymerase chain reaction, DNA probes, and cloning and sequencing methods. These methods are very accurate and reliable except the cost factor. That's why these are available only in selected laboratories [5]. Phenotypic methods are mainly based on the action of metal chelators and thiol based compound which inhibit the MBL production. Modified Hodge test, Double disc synergy test, combined disc diffusion test, thiol based compound test are the common phenotypic methods for detection of MBL production in clinical isolates [5]. The current study was done to detect the prevalence of

MBL production in clinical isolates derived from various samples at a tertiary care center in Jaipur, Rajasthan.

Material and Methods

This was a prospective observational study conducted in Department of Microbiology in National Institute of Medical Sciences & Research, Jaipur over duration of 6 months from June 2018 to November 2018.

All samples were received and processed as per the standard laboratory procedures according to standard guidelines. Cultures were done on 5% Sheep blood agar, Cystiene lactose electrolyte deficient agar, Mac-Conkey agar. Gram staining was done followed by standard biochemical test to identify and differentiate the isolates. Antimicrobial Susceptibility testing was done on Mueller Hinton Agar by Kirby Bauer disc diffusion method [6].

All the isolates were screened for Imipenem resistance by Kirby Bauer disc diffusion method according to Clinical Laboratory Standard Institute [7].

Detection of MBL production in Imipenem resistant strains was done by using Modified Hodge test, Double Disc synergy test, Disc Potentiation test and thiol based compound test.

Modified Hodge Test

On Mueller Hinton Agar, lawn culture of ATCC 25922 Escherichia coli (1:10 dilution of 0.5 McFarland's standard) was done. After placing a Imipenem disc in the center, Imipenem resistant test strain was streaked from edge of the disc to the periphery of the plate in four directions. After overnight incubation, plates showing "Clover-leaf" shaped zone of inhibition were interpreted as MHT positive [5].

The Double Disc Synergy Test

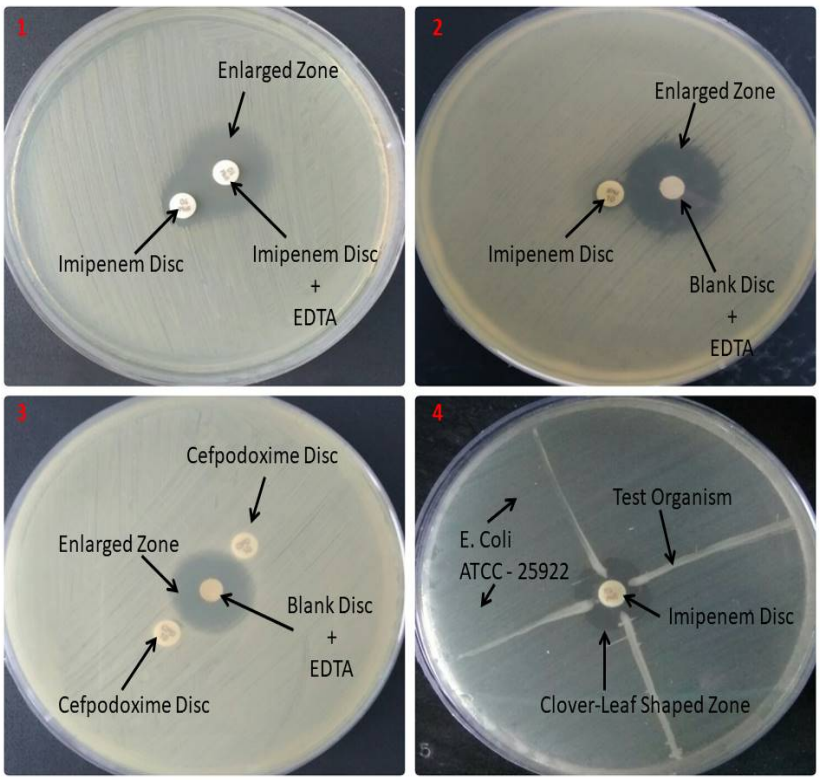
Suspension of 0.5 McFarland is prepared with test strain which is then inoculated on Mueller Hinton Agar plate. After drying place a Imipenem disc and place a blank disc 10 mm apart. Now add 10 micro liter of 0.5 M EDTA(by dissolving 186.1 gm of disodium EDTA.2H_2O in 1000 ml of distilled water and adjusting it to ph 8.0 using NaOH) solution on blank disc. After 24 hrs. of incubation note the zone of inhibition near EDTA disc. Enlarged zone of inhibition around the blank disc is interpreted as Double disc synergy test positive [8].

Disc Potentiation test

Prepare suspension of test organism and adjust to 0.5 McFarland standard. Inoculate test organism on MHA plate. After drying put 2 Imipenem disc on the plate. Add 5 microliter EDTA solution on one of the disc. Plates were compared after 16-18 hours of incubation. An increase in zone of inhibition more than7 mm around the disc with EDTA was recorded as positive result [8].

Thiol based compound test

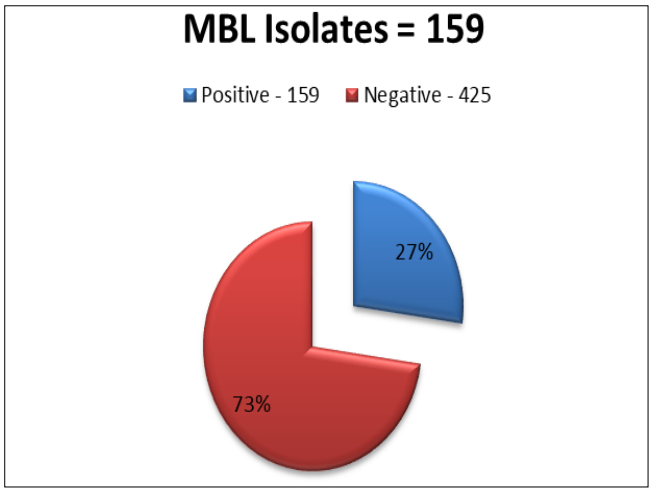
Dilute suspension of test strain is prepared in Mueller Hinton broth which is spread on MHA plate according to standard procedure. 2 commercially available cephalosporin disc were placed keeping 4 c.m. to 5 c.m. distance apart. Put a blank disc in the center of 2 disc. Add 10 microliter of EDTA on blank disc. Presence of enlarged zone near EDTA disc is considered as positive [8].



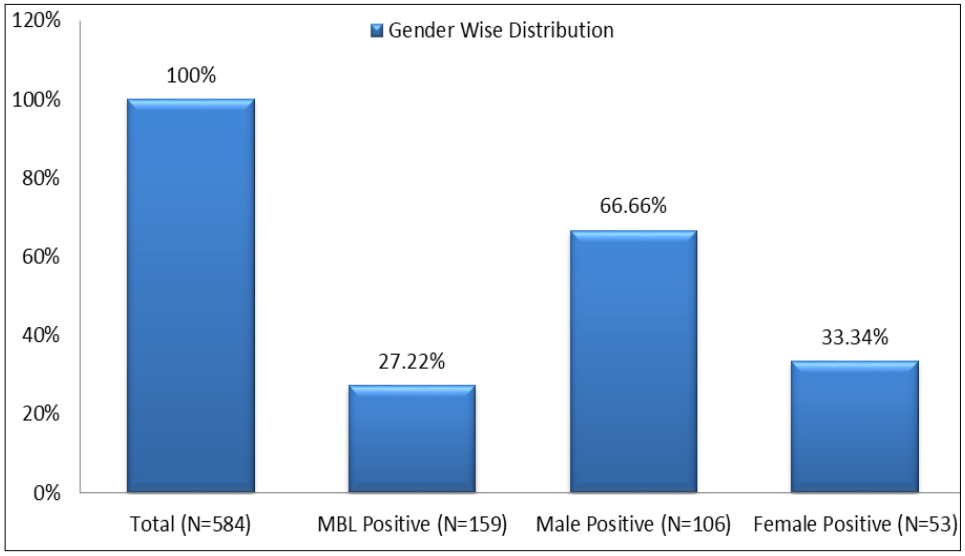
(**Fig 1:** Disc Potentiation test, 2- Double Disc Synergy test, 3- Thiol Based Compound test, 4- Modified Hodge test)

Results

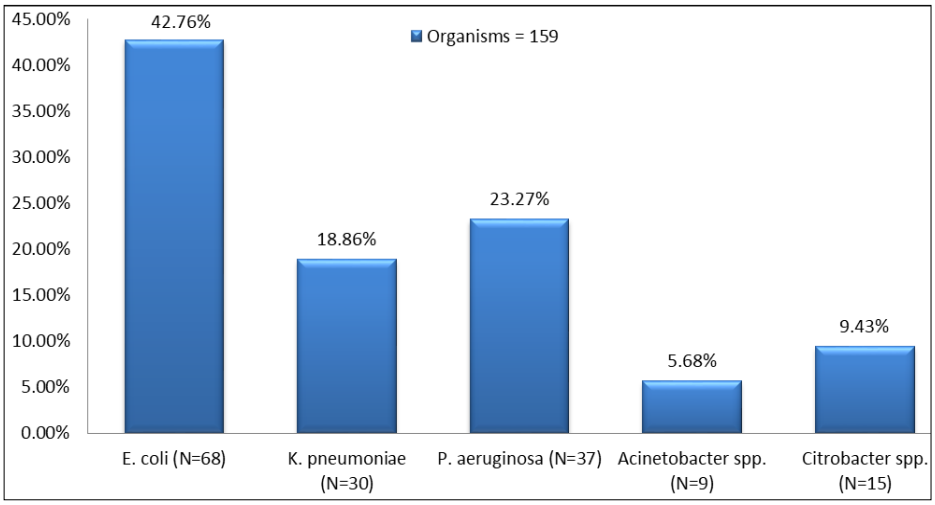
A total of 584 Gram Negative isolates were collected from various clinical samples over the study period. These samples include Urine, Blood, Pus, Sputum, Semen, CSF, Wound Swab, Ear Swab, ET Tube, Stool etc. Out of the 584 isolates 159 isolates were found to be resistant to Imipenem. These 159 isolates were tested for MBL production using 4 different methods: Modified Hodge test, Disc Potentiation test, Thiol based compound test, Double Disc synergy test. MBL production was detected in all 159 isolates. Among these 4 methods 100% (159) isolates gave positive result for Double Disc Synergy test. 95.5% (152) isolates gave positive Modified Hodge test result. 54% (86) isolates gave positive result for Disc Potentiation test and 49% (78) isolates gave positive result for Thiol based compound test. Out of 159 total isolates 106 isolates were obtained from male patients and 53 isolates from female patients which indicates the higher percentage of MBL production in male as compare to female. Among the tested antimicrobial agents, Polymixin B and Colistin were 100% sensitive. Most of the antimicrobial Agents were resistant among the MBL positive isolates.



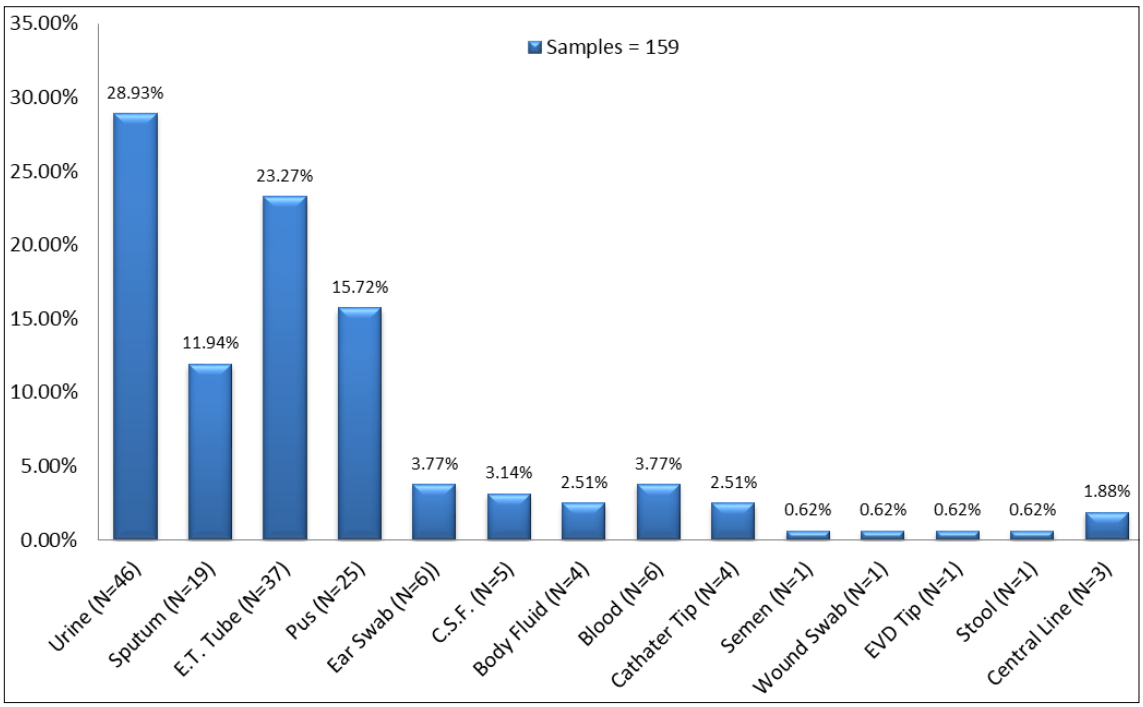
Graph 1: Out of the total isolated 584 strains, 159 were MBL producers.



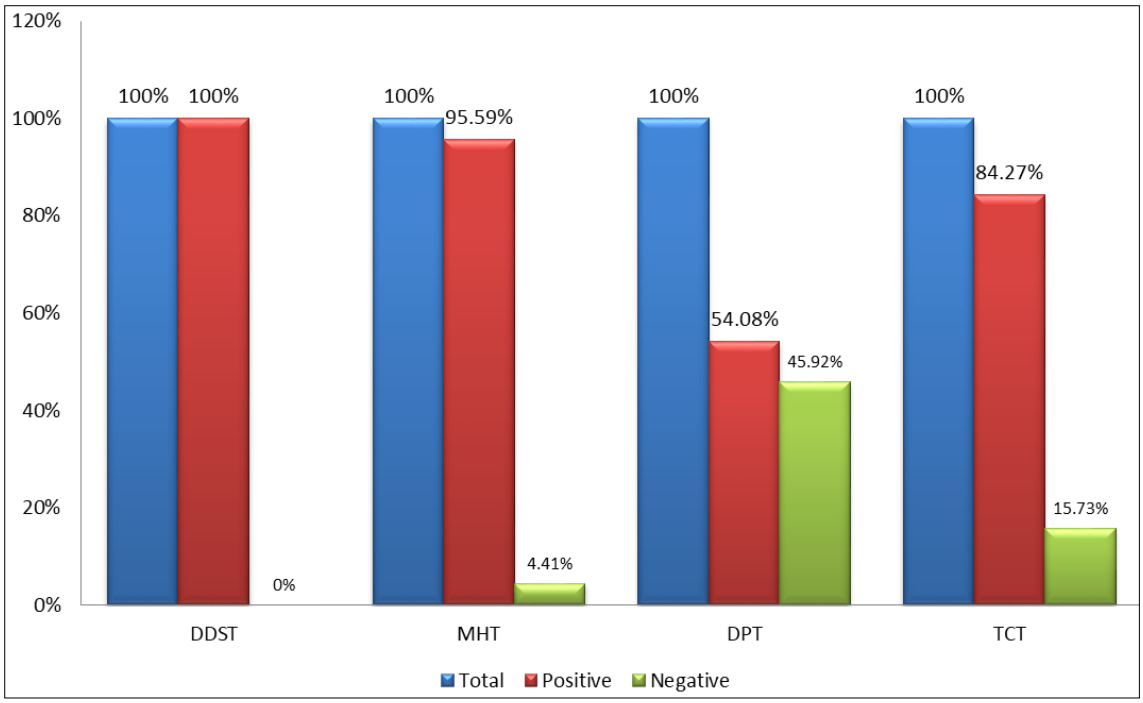
Graph 2: Out of the total 159 MBL producers, 106 isolates were obtained from male patients and 53 isolates were obtained from female patients.



Graph 3: Out of the total 159 MBL positive strains, 68 isolates were E. coli, 30 isolates were K. pneumoniae, 37 isolates were Pseudomonas aeruginosa, 9 isolates were Acinetobacter spp. and 15 were Citrobacter spp.



Graph 4: Most of the MBL producers were isolated from Urine followed by E.T. Tube. Many organisms were isolated from sputum and pus also.

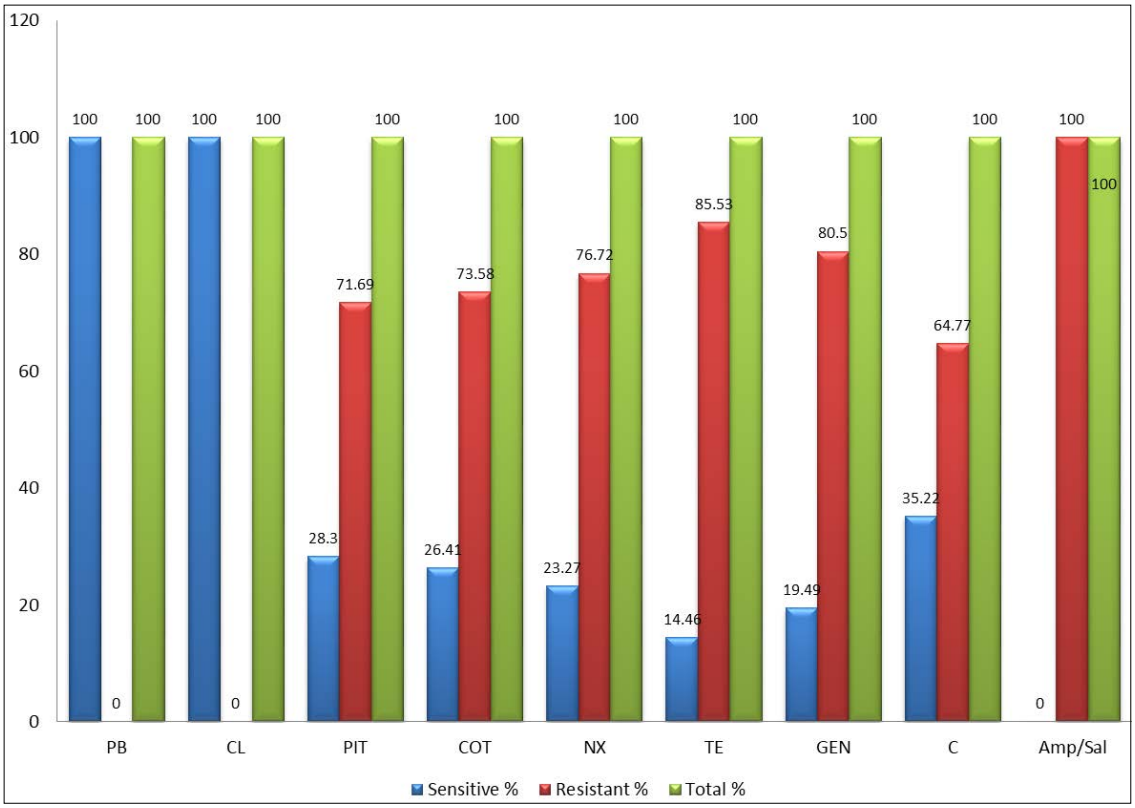


Graph 5: Comparison of result of different methods for detection of MBL production in 159 clinical isolates.

Table 1: Comparison of result of different methods for detection of MBL production in 159 clinical isolates.

Method	Total	Positive	Positive %	Negative
DDST	159	159	100	0
MHT	159	152	95.59	7
DPT	159	86	54.08	73
TCT	159	134	84.27	25

(DDST – Double Disc Synergy Test, MHT – Modified Hodge Test, DPT – Disc Potentiation Test, TCT – Thiol Based Compound Test)



Graph 6: Antimicrobial susceptibility test was performed according to C.L.S.I. 2018 guidelines.

Table 2: Antimicrobial susceptibility pattern of MBL producing organisms.

Antimicrobial Agents	Abb.	Sensitive Isolates	Sensitivity%	Resistant isolates	Resistant%
PolymixinB	PB	159	100	0	0
Colistin	CL	159	100	0	0
Piperacillin Tazobactum	PIT	45	28.30	114	71.69
Cotrimoxazole	COT	42	26.41	117	73.58
Norfloxacin	NX	37	23.27	122	76.72
Tetracyclin	TE	23	14.46	136	85.53
Gentamycin	GEN	31	19.49	128	80.50
Chloramphenicol	C	56	35.22	103	64.77
Ampicillin/Salbactum	Amp/Sal	0	0	159	100

Discussion

Carbapenems are mainly used as drug of choice in treating multidrug resistant Gram Negative organisms because of their stability and sensitivity towards ESBL and AmpC beta lactamases [9]. However in past few time, there has been increase in the resistance seen in carbapenems [10]. This increase in resistance is due to increase in efflux mechanisms, alteration of penicillin binding protein, decreased membrane permeability and production of carbapenem hydrolyzing enzymes i.e. carbapenemases. Resistance by hydrolyzing enzymes such as Metallo Beta Lactamases may be chromosomally encoded or plasmid mediated and that's why poses great chances of spreading resistane by gene transfer in Gram Negative Isolates [11].

In our study prevalence of MBL production is 27.22% among all clinical isolates. Out of 584 clinical isolates, 159 were MBL producing isolates. Similarly Mita D. *et al* in her study got prevalence rate 18% among all clinical isolates [12]. Manish Bansal *et al* in his study got 29.39% prevalence rate of MBL producing organisms [13].

In our study isolation of MBL producing organisms was higher in male patients (66.66%) than in female patients (33.34%). Smita Sood *et al* in her study got 70% MBL producing organisms in male patients and 30% MBL producing organisms in female patients which indicates the higher rate of MBL production in male patients than in female patients [14].

In our study out of the 159 MBL producing isolates, 42.76% isolates were identified as Escherichia coli, 18.86% were identified as Klebsiella pneumoniae. Manish Bansal *et al* in his study got slightly different results from our study. He got 27% Escherichia coli, 23.94% Klebsiella pneumonia [13]. In our study 23.27% MBL producing isolates were identified as Pseudomonas aeruginosa while Sachdeva R *et al* in his study got 18.37% MBL producing Pseudomonas aeruginosa [5].

In our study we applied 4 different phenotypic methods for detection of MBL production in isolates. These methods were Double Disc Synergy Test, Modified Hodge Test, Disc

Potentiation Test, Thiol Based Compound Test. All isolates gave positive Double Disc Synergy test, 95.59% isolates gave positive Modified Hodge Test, 54.08% isolates gave positive Disc Potentiation test and 84.27% isolates gave positive Thiol Based Compound Test. Smita Sood *et al* in her study evaluated Double Disc synergy test, Disc Potentiation test, Thiol Based Compound test and reported that all the methods were equally effective in detection of MBL production [8]. In another study Rohit Sachdeva *et al* reported that 62.5% isolates were positive for MHT, 82.3% isolates were positive for DDST and 97.9% isolates were positive for DPT [5].

All the MBL producing isolates in our study were multidrug resistant organisms. Only Polymixin B and Colistin were 100% sensitive among all the antimicrobial agents. Sensitivity pattern of other antimicrobial agents was in range of 15%-35% only. Similar results were observed in the study of Smita Sood *et al* in Jaipur [14].

Conclusion

Increasing prevalence of MBL production in various isolates is an alarming sign and indicates excessive use of Carbapenem. Also, it is very important to follow strict antimicrobial stewardship policies to avoid the over use of Carbapenem and other broad spectrum drugs. From this study we can conclude that use of simple test like DDST will be a crucial step toward large scale monitoring of these emerging resistant determinants.

References

1. Walsh TR, Bolmstrom A, Qwarnstrom A, Gales A. Evaluation of a new Etest for detecting metallo-β-lactamases in routine clinical testing. J Clin Microbiol. 2002; 40:2755-9.
2. Walsh TR, Toleman MA, Poirel L, Nordmann P. Metallo-β- lactamases: the quiet before the storm? Clin Microbiol Rev. 2005; 18:306-25.
3. Gladstone P, Rajendran P, Brahmadathan KN. Incidence of carbapenem resistant non fermenting Gram negative bacilli from patients with respiratory infections in the Intensive Care Units. Indian J Med Microbiol. 2005; 23:189-91.
4. Livermore DM, Woodford N. Carbapenemases: A problem in waiting? Curr Opin Microbiol. 2000; 3:489-95.
5. Sachdeva R, Sharma B, Sharma R. Evaluation of different phenotypic tests for detection of metallo-β-lactamases in imipenemresistant Pseudomonas aeruginosa. J Lab Physicians. 2017; 9:249-53.
6. Colle JG, Miles RS, Wan B. Tests for the identification of bacteria. In: Colle JG, Fraser AG, Marimon BP, Simmons A, editors. Mackei and McCartney Practical Medical Microbiology. 14th ed. Edinburg: Churchill Livingstone, 1996, 131-50.
7. Clinical Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. Twentieth Informational Supplement. CLSI Document M100-S20. Wayne, PA: CLSI , 2010.
8. Smita Sood. Phenotypic test for detecting incidence of Metallo-B-Lactamase (MBL) producing Pseudomonas aeruginosa in Jaipur. National Journal of Medicine. 2014; 3(2):22-27.
9. Payne DJ, Bateson JH, Gasson BC, Proctor D, Khushi T, Farmer TH, *et al*. Inhibition of metallo-β-lactamases by a series of mercaptoacetic acid thiol ester derivatives. Antimicrob Agents Chemother. 1997; 41:135-40.
10. Gupta E, Mohanty S, Sood S, Dhawan B, Das BK, Kapil A *et al*. Emerging resistance to carbapenems in a tertiary care hospital in North India. Ind J Med Res. 2006; 124:95-8.
11. Gladstone P, Rajendran P, Brahamanand KN. Incidence of carbapenem resistant nonfermenting gram-negative bacilli from patients with respiratory infections in the intensive care unit. Ind J Med Microbiol. 2005; 23:189-91.
12. Mita D Wadekar K, anuradha D. Venkatesha. Phenotypic detection of ESBL and MBL in clinical isolates of Enterobacteriaceae. Int. J. Curr. Res. Aca. Rev. 2013; 1(3):89-95.
13. Manish Bansal, Nitya Vyas, Babita Sharma RK. Maheshwari. Differentiation of Carbapenemase producing Enterobacteriaceae by triple disc test. IJBAMR. 2013; 3(1):314-320.
14. Smita Sood. Identification and Differentiation of Carbapenemases in Klebsiella pneumonia: A Phenotypic Test Evaluation Study from Jaipur, India. Journal of Clinical and Diagnostic Research. 2014; 8(7):DC01-DC03.