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Phenotypic Detection of Metallo-Beta Lactamases in *Acinetobacter baumanni* in a Tertiary Care Hospital of Western Rajasthan, India

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A B S T R A C T

Acinetobacter spp. is a gram negative cocco-bacilli bacteria, correlated with an important opportunist pathogen in a health care institutions worldwide. Its multiple drug resistance (MDR) is of great concern because of its intrinsic and acquired resistance mechanisms, limiting the treatment options. *Acinetobacter* spp. produces carbapenamases like metallo-β-lactamases (MBLs), which hydrolyze a varieties of β-lactams antibiotics including penicillin, cephalosporins and carbapenems. Present study was designed to determine the MBL production in *Acinetobacter baumanni* and to evaluate the phenotypic test methods for thedetection of MBL production in imipenem-resistant clinical isolates of *Acinetobacter baumanni.* Present study was conducted from September 2022 to December 2024 at the department of Microbiology, Dr. S. N Medical College and attached hospitals, Jodhpur, Rajasthan. Present study was a hospital based prospective study. Totally 178*Acinetobacter baumanni i* isolates were collected from different clinical specimens. Imipenem resistant isolates were subjected to 4 different phenotypic test methods for the detection of Metallo-beta –lactamases (MBL), Imipenem + EDTA combined disc test, double disc synergy test, Modified Hodge test and MBL E-test. Totally 178 *Acinetobacter baumanni i* isolates were collected out of which Imipenem resistant were 104 (58. 42%). Amongst these 104 (58. 42%) isolates, 57 (67. 05%) were MBL positive by CDT, 100 (96. 15%) DDST 26 (25%) MHT56 (53. 84%) and MBL E-test method 104 (100%). The present study showed that E-test was the most sensitive and specific phenotypic method followed by the combined disc test. Strict antibiotic stewardship programe and infection control measures can decreases the positivity rate of the superbugs in the hospital settings.

Introduction

Acinetobacter spp. is a gram negative cocco-baccillibacteria correlated mostly with with an important opportunist pathogen in a health care institutions worldwide, multiple drug resistance (MDR), is of great concern because of its intrinsic and acquired resistance mechanisms, limiting the treatment options. *Acinetobacter* spp. produces carbapenamases like metallo-β-lactamases (MBLs), which hydrolyze a variety

of β-lactams including penicillin, cephalosporins and carbapenems (1).

The bacterium is a gram-negativecocco-bacilli, non-motile, non-lactose fermenter.

Carbapenems were the only hope to for infections with these multi drug resistant (MDR) bacteria (2), However this last barrier was also broken by carbapenemases enzymes that started emerging worldwide and were able to destroy Carbapenems.

Metallo- beta lactamase (MBL) has ability to hydrolyze a wide variety of beta lactam drugs, such as Penicillin, Cephalosporins and Carbapenems. MBL are inhibited by metal chelators, such as ethylene demine tetra acid (EDTA) and thiol-based compounds (3). There is an alarming increase of antibiotic resistance in bacteria. Therefore, the treatment of *Acinetobacter*spp. has become challenges due to acquiring resistance to various antibiotics through the inherent and acquired mechanism (4).

Present study was designed to determine the MBL production in *Acinetobacter baumanni* and to evaluate the phenotypic test methods for thedetection of MBL production in imipenem-resistant clinical isolates of *Acinetobacter baumanni.*

Materials and Methods

Present study was conducted from September 2022 to December 2024at the department of Microbiology, Dr. S. N Medical College and attached hospitals, Jodhpur, Rajasthan. Present study was a hospital based prospective study.

Institutional Ethical Clearance was taken. The multi-drug resistant (MDR) isolates which were resistant to Imipenem were selected and tested for MBL production by the following phenotypic test methods.

Totally, 178 isolates of *Acinetobacter baumanni.* isolated from various clinical samples were collected. The isolates were identified by standard laboratory techniques. All these isolates were screened for imipenem resistant by Kirby-Bauer disc diffusion method as per Clinical and Laboratory Standard Institute guidelines. Antibiotic Susceptibility testing for all the isolates of *Acinetobacter baumanni.* was performed by Kirby-Bauer disc diffusion method on Mueller-Hinton agar (MHA) plates as per clinical laboratory standards institute guidelines.

The isolates that were resistant to imipenemwere subjected to MBL production test by following phenotypic methods.

1. Imipenem-EDTA combined disc test (IMP EDTA CDT): In this method, the test organism was lawn culture on Muller Hinton Agar (MHA). Two disks were placed on MHA plate. One was 10 ugimipenem, another was imipenem + EDTA (10/750 ug) combined disk. The increase in more than 7mm in the inhibition zone of the imipenem + EDTA disk over than imipenem disk alone, was considered as MBL positive.

2. Double disk synergy test (DDST): An imipenem (10 µg) disc was placed in the center of the plate a top of the lawn culture of the test organism, and a sterile blank disc was placed 20 mm away from it. Then, the blank disc was covered with 10 µL of EDTA (750 µg). Enhancement of the zone of inhibition in the area between the imipenem and EDTA discs in comparison with the zone of inhibition on the far side of the drug was interpreted as a positive result for MBL production.

3. Modified Hodge Test: *Escherichia coli* ATCC 25922 (an indicator organism sensitive to carbapenems) was cultured in peptone water to achieve 0. 5 McFarland opacity standard and was lawn cultured onto a Mueller-Hinton agar plate using sterile cotton swab. After drying, 10 µg Imipenem disc was placed at the centre of the plate on the lawn culture and an overnight growth of test strain was heavily streaked from the edge of the Imipenem disc outwards, to the periphery of the plate in four different directions. The plates were incubated at 37ºC overnight. The presence of a distorted zone (Clover-leaf shaped zone of inhibition) was considered as positive test.

4. E-test Metallo -β-lactamases strips: Single strips impregnated with a concentration gradient of imipenem (4–256 µg/mL) on one end and that of imipenem (1–64 µg/mL) fused with a stable concentration of EDTA on the other end were placed a top lawn cultures of the test organisms in culture plates. An 8-fold increase in the ellipse of imipenem and EDTA indicated the presence of MBL.

Result and Discussion

MBL-producing Gram-negative bacilli have now been

reported in many geographic areas. In the present study, prevalence of MBL production in *Acinetobacter baumanni* was 58. 42%, while, other studies have reported 14–62. 69% (5). The emergence of MBL-producing non fermenter gram negative bacilli (GNB) is challenge to the microbiology laboratories because there are no standardized guidelines available to detect them. MBLs disperse easily via plasmids, so they swiftly circulate within an institution and lead to poor consequences when infection occurs (6). The detection of MBL-producing GNB is of crucial importance to limit the spread of MBL genes among bacteria as well as to early start of appropriate treatment (7).

A total of 178 *Acinetobacter baumanni* isolates were obtained from clinical specimens collected from the patients suffering from various infections. Out of 178 *A. baumannii* was frequently isolated from Pus 47 (26. 40%) followed by urine 44 (22. 71%) and least was isolated from vaginal swab 04 (2. 24%). Maximum number of *Acinetobacter baumanni* were isolated from 51-60 yr (21. 15%). and 31-40 yr (19. 23%) age group patients followed by 41-50 yr (15%). 81 (77. 88%) /104 (58. 40%) isolates of *Acinetobacter baumanni* were from male patient and 51 (68. 91%) /74 (41. 57%) isolates from female patient.

In present study total 178 *Acinetobacter baumanni* were isolated. Imipenem resistant isolates was 104 (58. 42%) and Imipenem sensitive was 74 (41. 57%). E-test is a quantitative technique for determining the minimum inhibitory concentration (MIC) of antibiotic against bacteria and for the detection of resistance mechanisms. E-test MBL strip based on a combination of a β-lactam substrate and a β-lactam or MBL inhibitor was specifically designed to detect clinically relevant MBLs (1). The E-test MBL strips (Imipenem-Imipenem + EDTA) has the ability to detect MBLs. MBL E-test to be very sensitive for detection of MBLs. Since in the current study sensitivity of the E –test is 100%, which is similar to the study done by ShivaprasadA *et al.,* (1) i.e 100%. After E test combined disc test is most sensitive phenotypic method i.e 96. 15% is similar to the study done by Shivaprasad A *et al.,* i.e 100% (1).

Several studies have reported the use of DDST as one of the convenient methods for the MBL production and the positivity percentage varies from 14. 8% - 72% (5, 6) which is in accordance with the present study i.e 25%. According to them, DDST was found to be more reliable method (8, 9). However, in the present study DDST showed the lowest positivity and current study result was similar to the study conducted by Behra *et al.,* (4)

Modified Hodge test is a simple method for screening MBL producing bacterial isolates. It was originally described by the Centre for Disease Control for Carbapenemase detection in *Enterobacteriaceae* (8-10). Previously published articles has shown low positivity of MHT technique when compared to other tests, which vary from 14. 8% - 56. 16% (11-15). However, in the present study positivity rate is 53. 84% which is similar to the study done by Shivaprasad A *et al.,* (1).

Table.1 Distribution of clinical samples and Acinetobacter baumanni isolates (N=178) in various clinical specimens.

S. NO	Sample type	Number of Isolates (%)
1	Pus	47 (26. 40%)
2	Urine	44 (24. 71%)
3	Burn wound	07 (3. 93%)
4	Sputum	28 (15. 73%)
5	Blood	16 (8. 98%)
6	Endotracheal tube	15 (8. 42%)
7	Bronchoalveolar lavage	09 (5. 05%)
8	Fluids and effusions	08 (4. 49%)
9	Vaginal swab	04 (2. 24%)
	Total	178 (100 %)

Table.2 Distribution of imipenem–resistant and imipenem-sensitive isolates of *Acinetobacter baumanni* (N=178)

S. No	Bacteria	No of IMP-Resistant Isolates (%)	No of IMP-Sensitive Isolates (%)	Total
1	*Acinetobacter baumanni*	104 (58. 42%)	74 (41. 57%)	178 (100%)

Table.3 Distribution of MBL and Non MBL producers according to Gender wise.

S. No	Gender	MBL producer (n=104)	MBL non producer (n=74)	Total isolates (N=178)
1	Male	81 (77. 88%)	23 (31. 08%)	104 (58. 42%)
2	Female	23 (22. 11%)	51 (68. 91%)	74 (41. 57%)
Total		104 (58. 42%)	74 (41. 57%)	178 (100%)

Table.4 Distribution of MBL and Non MBL producers according to Age wise.

S. No.	Age group	MBL producer (n=104)	MBL non producer (n=74)	Total (N=178)
1	<10 year	09 (8. 65%)	01 (1. 35%)	02 (1. 12%)
2	11-20 year	17 (16. 34%)	04 (5. 40%)	11 (6. 17%)
3	21-30 year	03 (2. 88%)	07 (9. 45%)	10 (5. 61%)
4	31-40 year	20 (19. 23%)	14 (18. 97%)	24 (13. 48%)
5	41-50 year	13 (12. 5%)	18 (24. 32%)	21 (11. 79%)
6	51-60 year	22 (21. 15%)	09 (12. 16%)	13 (7. 30%)
7	61-70 year	09 (8. 65%)	11 (14. 86%)	12 (6. 74%)
8	71-80 year	06 (5. 76%)	05 (6. 75%)	06 (3. 37%)
9	> 81 year	05 (4. 80%)	05 (6. 75%)	06 (3. 37%)
Total		104 (58. 42%)	74 (41. 57%)	178 (100%)

Table.5 Results of CDT, DDST, MHT and E test for the detection of MBL producers among imipenem – resistant bacterial isolates (n=104)

S. No	Bacterial isolates	Resistant to Imipenem (n=104)	CDT		DDST		MHT		E-test	
			Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
1	*Acinetobacter baumanni*	104	100 (96. 15%)	04 (3. 84%)	26 (25%)	78 (75%)	56 (53. 84%)	48 (46. 15%)	104 (100%)	00

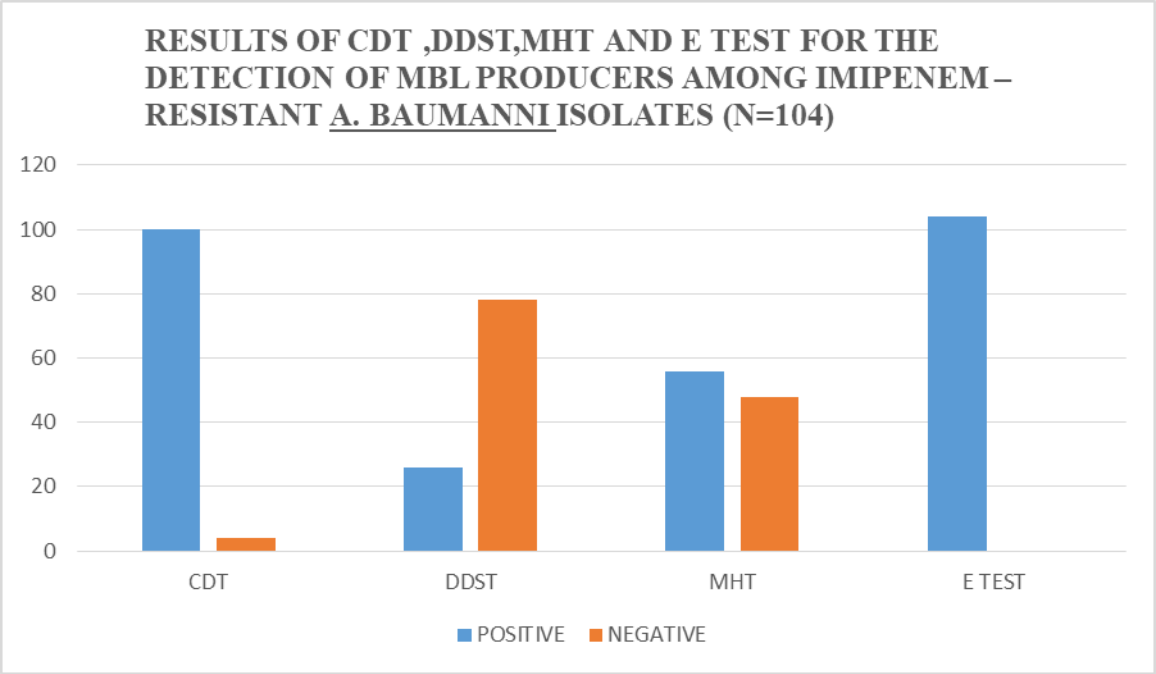
RESULTS OF CDT ,DDST,MHT AND E TEST FOR THE DETECTION OF MBL PRODUCERS AMONG IMIPENEM – RESISTANT A. BAUMANNI ISOLATES (N=104)
120
100
80
60
40
20
0
CDT
DDST
MHT
E TEST
POSITIVE
NEGATIVE

Fig.1 E-test

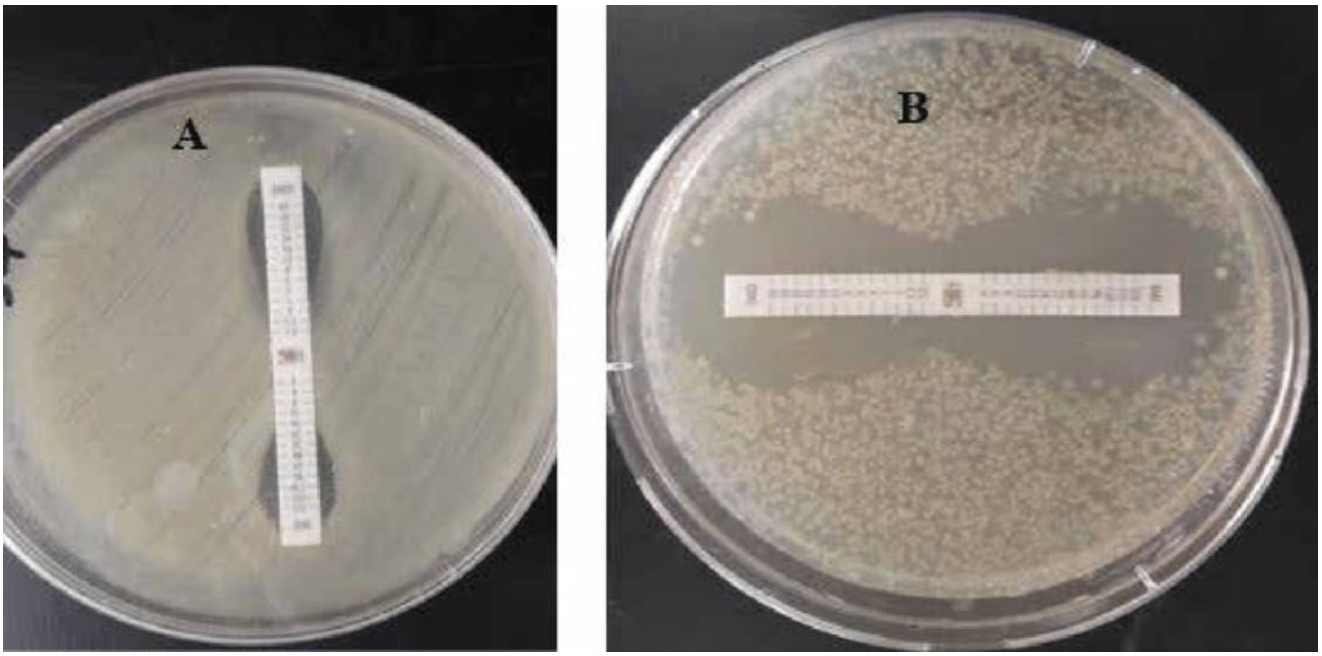
A
B

Fig.2 (a) Combined disc test and (b) Double disc synergy test

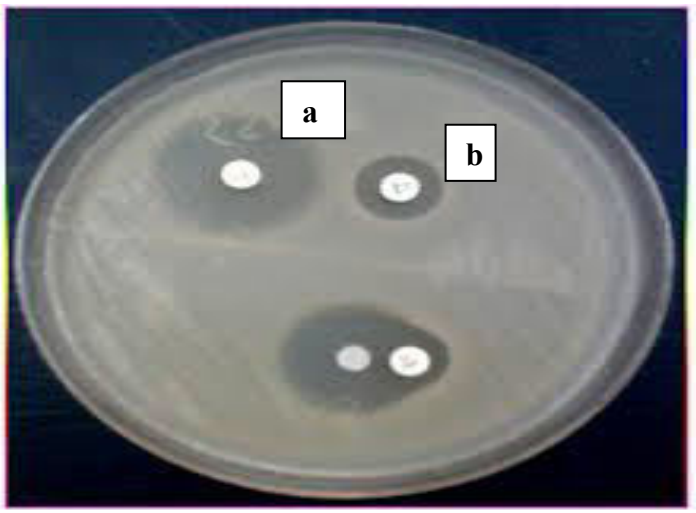

Fig.3 Modified Hodge test

In conclusion, the present study showed that E-test was the most sensitive phenotypic method followed by combined disc test. Strict antibiotic stewardship policy and infection control measures can decreases the positivity rate in the hospital settings as well as in community also. Early and easy detection techniques of MBLs are required in routine clinical labs.

Author Contributions

Vishakha Ashopa: Investigation, formal analysis, writing—original draft. Prabhu Prakash: Validation, methodology, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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