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Prevalence of ESBL and MBL Producing *Acinetobacter* species Isolated from Various Clinical Samples in Tertiary Care Hospital in North-West Region of Rajasthan

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A B S T R A C T

This study was conducted with an objective to find the prevalence of extended spectrum betalactamase (ESBL) and metallo betalactamase (MBL) in *Acinetobacter species.* It was conducted in the Department of Microbiology, Sardar Patel Medical college, Bikaner from Feb 2018 to Feb.2019. in various clinical specimens including urine, pus, blood, vaginal swabs, respiratory samples, and various body fluids were processed *Acinetobacter species* isolates were identified by standard protocols. Antibiotic sensitivity testing for all isolates was done using Kirby-Bauer disc diffusion method. Disc potentiation test was performed to check ESBL and MBL production in these bacteria. Maximum ESBL and MBL positive isolates of *Acinetobacter species* were observed among E.T. tube samples. Early detection, stringent antibiotic policies, and compliance towards infection control practices are the best defenses against this organisms.

Introduction

Resistant bacteria are emerging world wide as a threat to the favourable outcome of common infections in the community and hospital setting. β-lactamases production by several gram negative and gram positive organisms is perhaps the most important single mechanism of resistance to penicillin cephalosporin, monobactams and carbapenems[1,2,3]. Among the wide array of antibiotics, beta-lactams are the most widely used agents. The most common cause of resistance to beta-lactam antibiotics is the production of beta-lactamases. Extended spectrum beta-lactamases (ESBLs) and metallo beta-lactamases (MBL) represent a major group of beta-lactamases currently being identified world-wide in large numbers, most commonly produced by *Acinetobacter* species but also occur in other gram negative bacteria.[4,5] One of the most important mechanisms of

microbial resistance to beta-lactam antibiotics (penicillins, cephalosporins, monobactams and carbapenems) is hydrolysis by beta-lactamases. The accurate identification and reporting of ESBL and MBL producing *Acinetobacter* species will be helpful to infection control practitioners in preventing the spread of these multidrug resistant isolates.[6,7] Till date no enough study have been undertaken on prevalence of ESBL and MBL producing *Acinetobacter* species in the state of rajasthan, therefore the present study is undertaken to find the prevalence of ESBL and MBL producing *Acinetobacter* species isolated in the north west region of rajasthan.

Materials and Methods

The present study was conducted in Department of Microbiology, Sarder Patel Medical College, Bikaner from Feb 2018 to Feb 2019 to detect prevalence of ESBL and MBL producing *Acinetobacter* species in various clinical specimens such as blood, urine, pus, CSF, throat swab, vaginal swab, sputum, pleural fluid, broncho-alveolar lavage, bronchial aspirate samples etc. received from patients admitted in different wards at P.B.M. hospital and associate group of hospitals were included in this study.

Medical and demographic data of the patients were collected using a questionnaire. Data recorded were: demographic characteristics (age, gender); immune suppression, septicaemia, burn, malignancy, recent surgery (within one month), previous treatment with broad-spectrum antibiotics, use of invasive devices, fecal colonization with *Acinetobacter*, and prolonged hospital (> one week) or ICU stays.

After collection the samples were processed for the identification of organisms by the conventional biological tests.[8] All isolates were cultured on Mac conkey agar and blood agar and urinary isolates on Hi-chrome UTI media (obtained from Hi-Media, Mumbai, India) also incubated at 37°C for 24 hours. They are identified to species level by their characteristic appearances on the media, Gram's staining, catalase test, oxidase test, motility test, nitrate reduction test and *Acinetobacter* species were confirmed by indole test, methyl red test, citrate utilization test, urease production test, triple sugar Iron reaction, gelatin liquification test and by sugar fermentation test.

The isolates were tested for antimicrobial susceptibility, on Muller hinton agar by Kirby Bauer disk diffusion method with a inoculums matched with 0.5 McFarland turbidity standard as per CLSI recommendation[9]. The zone of inhibition was measured and reported as susceptible, intermediate or resistant according to standard zone size. For statistical purposes data were categorized as susceptible and non-susceptible (including intermediate and resistant groups).[9,10]

For Control *Acinetobacter* spp. (Ac. Baumanii) ATCC 19606 were used to check the potency of the disc and were used with every batch of antibiotic sensitivity testing.

Following antibiotic discs (obtained from Hi-Media, Mumbai, India) were used for antimicrobial sensitivity testing- amikacin (30µg), piperacillin/tazobactum (100/ 10µg), tobramycin (10µg), ceftazidime (30µg), ampicillin/sulbactum(10/10µg), ceftriaxone (30µg), ciprofloxacin (5µg), levofloxacin (5µg), imipenem (10µg), meropenem (10µg), co-trimoxazole(25 µg), & colistin(10 µg). The inhibition zone diameter was measured in mm with the help of a special measuring scale and results recorded for each isolate separately as Sensitive, Resistant, Intermediate(S,R,I) according to the given standard zone size.

Extended Spectrum Beta Lactamases (ESBLs)

Detection was done by Initial screening tests[1,10]. According to the CLSI guidelines, isolates showing inhibition zone size of ≤22mm with Ceftazidime(30µg) and ≤27mm with Cefotaxime (30µg) were identified as potential ESBL producers and shortlisted for confirmation of ESBL production. After screening Phenotypic confirmatory test was done with combination disk[1,10]. Metallo Beta Lactamases Detection was done by Initial Screening Tests:[11] The isolates showing inhibition zone size of ≤16mm with imipenem(10µg), Meropenem(10µg) and with ceftazidime(30µg) were identified as MBL producers and shortlisted for confirmation of MBL production. For confirmation, phenotypic confirmatory test with combination disks[12] was carried out.

Results and Discussion

Out of 100 positive cases for *Acinetobacter* species, 45(45%) cases were ≤40 years of age and 55(55%) cases were above 40 years of age,60(60%) were males and 40(40%) were females.

Out of 100 isolates of *Acinetobacter* species, 30 (30%) were found to be ESBL producers and 44 (44%) were MBL producers. ESBL producing *Acinetobacter* species were most frequently recovered from E.T. tube 40% (12/30) followed by blood 16.67% (5/30), urine 13.33% (4/30), pus 13.33% (4/30), sputum 10% (3/30), ascitic fluid 6.67% (2/30) respectively.

ESBL producing *Acinetobacter* species were frequently recovered from E.T. tube 40%(12/30) followed by blood 16.67% (5/30), urine 13.33% (4/30), pus 13.33% (4/30), sputum 10% (3/30), ascetic fluid 6.67% (2/30) respectively.

MBL producing *Acinetobacter* species were most frequently recovered from E.T. tube 36.36% (16/44) followed by blood 20.45%(9/44), urine 20.45%(9/44), pus 9.09% (4/44), sputum 6.81% (3/44), wound swab 6.81% (3/44) respectively.

From clinical specimens some isolates were more sensitive while others were more resistant.

In ESBL positive (30) isolates maximum cases were from ICU 13(43.33%) and Intubation & Mechanical ventilation 12 (40%). In MBL positive (44) isolates maximum cases were also from same- ICU (19; 43.2%) and Intubation & Mechanical ventilation (16; 36.4%).

In the present study, out of 100 isolates for *Acinetobacter* species 55(55%) were belonged to age group >40 years and 45(45%) cases were belonged to group ≤40 years. This predominance was due to a weak immune system, so more risk of nosocomial infections by opportunistic microorganisms. The results are similar to other studies[13,14,15,] which showed that 53% to 71% isolates were belonged to >40 year age and 47% to 29% isolates were of <40 years old age group.

In the present study higher indices of *Acinetobacter* infection in male 60 (60%) as compared to female 40 (40%). The results are in accordance with some other studies[13,15,16,17] that also showed higher incidence in males than females.

Table.1 Distribution of ESBL producing and non-ESBL producing *Acinetobacter species* isolates from various clinical specimens

S.No.	Clinical samples	Total *Acinetobacter* sp. Isolates (%)	ESBL positive isolates (%)	ESBL negative isolates (%)
1.	E.T. tube	32(32%)	12(40%)	20(28.57%)
2.	Blood	19(19%)	5(16.67%)	14(20%)
3.	Urine	17(17%)	4(13.33%)	13(18.57%)
4.	Pus	11(11%)	4(13.33%)	7(10%)
5.	Sputum	9(9%)	3(10%)	6(8.57%)
6.	Wound swab	7(7%)	-	7(10%)
7.	Ascitic fluid	5(5%)	2(6.67%)	3(4.29%)
8.	Total	100	30	70

Table.2 Distribution of MBL producing and non-MBL producing *Acinetobacter species* isolates from various clinical specimens

S.No.	Clinical samples	Total *Acinetobacter* sp. Isolates (%)	MBL positive isolates (%)	MBL negative isolates(%)
1.	E.T. tube	32(32%)	16(36.36%)	16(28.57%)
2.	Blood	19(19%)	9(20.45%)	10(17.86%)
3.	Urine	17(17%)	9(20.45%)	8(14.29%)
4.	Pus	11(11%)	4(9.09%)	7(12.5%)
5.	Sputum	9(9%)	3(6.81%)	6(10.71%)
6.	Wound swab	7(7%)	3(6.81%)	4(7.14%
7.	Ascitic fluid	5(5%)	-	5(8.93%)
8.	Total	100	44	56

Table.3 Antimicrobial susceptibility pattern of *Acinetobacter species*

S.No.	Antibiotics	Sensitive isolates (%)	Resistant isolates (%)
1.	Amikacin(AK)	55%	45%
2.	Piperacillin/tazobactam(PIT)	62%	38%
3.	Tobramicin(TOB)	37%	63%
4.	Ampicillin/Sulbactam(AMS)	47%	53%
5,	Ceftazidime(CAZ)	37%	63%
6.	Ceftriaxone(CTR)	17%	83%
7.	Ciprofloxacin(CIP)	55%	45%
8.	Levofloxacin(LE)	50%	50%
9.	Imipenem(IPM)	52%	48%
10.	Co-trimoxazole(COT) (trimethoprim/sulphamethoxazole)	52%	48%
11.	Colistin(CL)	98%	2%
12.	Meropenem(MRP)	65%	35%

Table.4 Association of risk factors with ESBL production and MBL production

S.No	Risk factors	ESBL positive isolates(%)	ESBL negative isolates(%)	MBL positive isolates(%)	MBL negative isolates(%)
1.	ICU stay	13(43.33%)	30(42.86%)	19(43.18%)	24(42.86%)
2.	Recent surgery	08(26.67%)	14(20%)	09(20.45%)	13(23.21%)
3.	Use of invasive devices (urinary catheterization)	05(16.67%)	12(17.14%)	09(20.45%)	08(14.28%)
4.	Use of broadspectrum antibiotics	02(6.67%)	06(8.57%)	02(4.54%)	06(10.71%)
5.	Intubation & Mechanical ventilation	12(40%)	20(28.57%)	16(36.36%)	16(28.57%)
6.	Prolonged hospital stay	02(6.67%)	05(7.14%)	04(9.09%)	03(5.36%)
7.	Immune suppression	02(6.67%)	01(1.42%)	-	03(5.36%)
8.	Old age	10(33.33%)	15(21.43%)	10(22.72%)	15(26.78%)

Fig.1 Antimicrobial susceptibility of ESBL and MBL producing strain on MHA showing resistance to ceftazidime (CAZ) and imipenem (IPM)

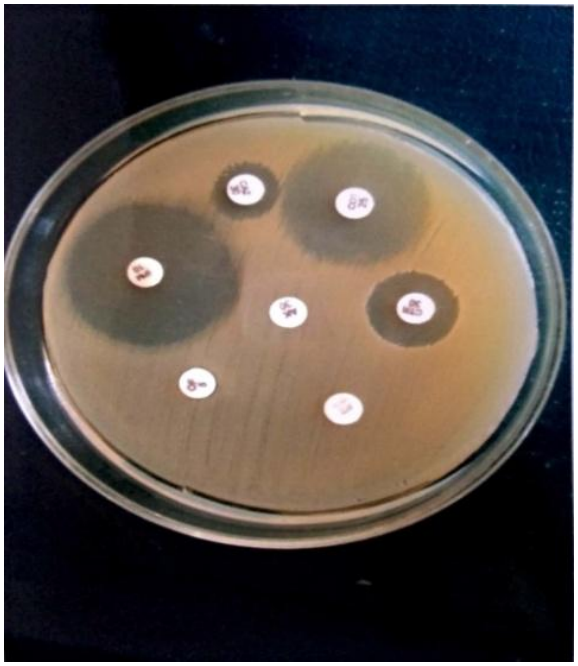


Fig.2 Combination disc method of MBL confirmation showing >7mm increase in zone size around imipenem + EDTA

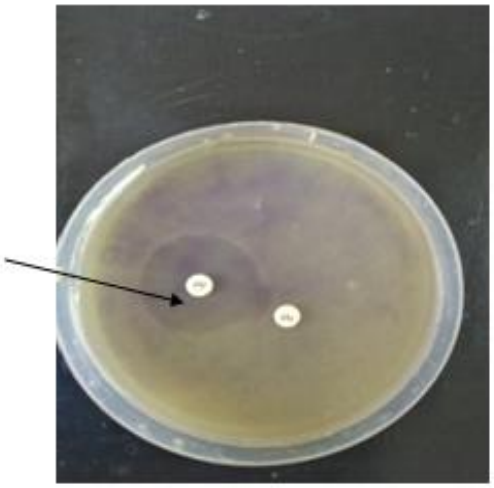
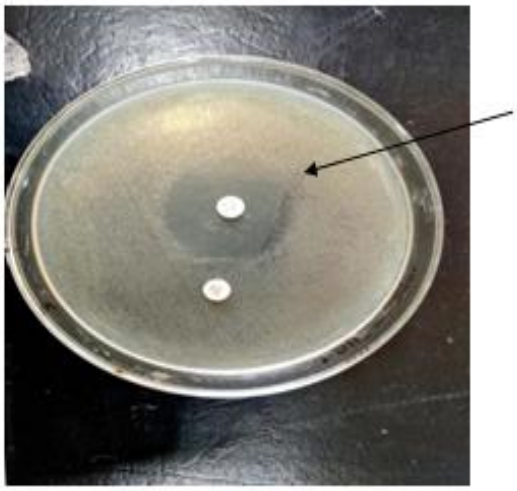


Fig.3 Combination disc method of ESBL confirmation showing >5mm increase in zone size around ceftazidime + clavulanic acid (CAC)



In the present study, 30% isolates of *Acinetobacter* species were positive for ESBL production and 44% isolates were positive for MBL production. These results are similar to study by Purti Tripathi *et al.,* (2013)[18] who had reported ESBL production in *Acinetobacter* species was 29% and MBL production was 37%, Harekrishna Nath *et al.,* (2016)[15] who had reported ESBL production was 32% and MBL production was 38%.

In the present study ESBL- producing *Acinetobacter* species were recovered most frequently from E.T.tube (40%) followed by blood (16.67%), similar observations were made by Molay Banerjee *et al.,* (2013)[14] who had reported ESBL producing *Acinetobacter* species were recovered most frequently from E.T. Tube (35.29%) followed by blood (14.70%), Amandeep Kaur *et al.,* (2018)[19] who had reported (37.5%) from E.T. tube followed by (9.3%) from blood, Nashwa M. *et al.,* (2017)[20] reported (39.3%) from E.T.tube.

In the present study MBL-producing

Acinetobacter species were recovered most frequently from E.T. tube (36.36%) followed by blood (20.45%) and urine (20.45%) similar observations were made by Molay banerjee *et al.,* (2013)[14] who had reported MBL producing *Acinetobacter* species were recovered most frequently from E.T.tube (43.75%) followed by blood (12.5%) and urine(12.5%), Amandeep kaur *et al.,*(2018)[19] who had reported (32.63%) from E.T.tube followed by blood (5.7%) and urine (11.53%), Muneeza Anwar *et al.,* (2016)[21] also reported (18.2%) from blood and (21.2%) from urine.

In the present study 55% isolates of *Acinetobacter* species were susceptible to Amikacin. The results are similar to Purti Tripathi *et al.,* (2013)[18] who had reported 55.14% susceptibility to amikacin, Harekrishna nath *et al.,*(2016)[15] who had reported 57.15% susceptibility, Amandeep kaur *et al.,* (2018)[19], S john *et al.,* (2011)[22] and Mahua sinha *et al.,* (2007)[23] who have reported 60.3%, 43.3% and 33% susceptibility respectively.

In the present study 62% isolates of *Acinetobacter* species were susceptible to piperacillin/tazobactam. The results are similar to Molay banerjee *et al.,* (2013)[14] who had reported 65.33% susceptibility to piperacillin/tazobactam, Amandeep kaur *et al.,* (2018)[19]and Gunjan Shrivastava *et al.,* (2013)[24] who have reported 64.6% and 62% susceptibility respectively.

In the present study sensitivity of other drugs like Tobramicin, Ampicillin/sulbactam, Ceftazidime, Ceftriaxone, Ciprofloxacin, Levofloxacin (LE), Imipenem (IPM), Co-Trimoxazole (COT), Colistin (CL), Meropenem (MRP) is almost similar to findings of Gunjan Shrivastava *et al.,* (2013)[24], Richa gupta *et al.,* (2014)[25], Amir peymani *et al.,* (2011)[26], Banerjee *et al.,* (2013)[14], Mahua Sinha *et al.,* (2007)[23] and Amandeep kaur *et al.,* (2018)[19], Harekrishna nath *et al.,* (2016)[15] etc.

In the present study total in 30% ESBL producing *Acinetobacter* species, 13(43.33%) were isolated from ICU patients followed by 08 (26.67%) in recent surgery patients, 05(16.67%) patients with invasive medical devices(urinary cathetrization), 02 (6.67%) patients with use of broad spectrum antibiotics, 12(40%) patients with intubation & mechanical ventilation, 02(6.67%) patients with prolonged hospital stay, 02(6.67%) patients with immune suppression, 10(33.33%) in old age patients. Similar observation were made by Mahua sinha *et al.,* (2007)[23] where 38% isolates were obtained from patients admitted in ICU followed by 22% in recent surgery patients, 18% patients with use of broad spectrum antibiotics respectively. Bhattacharyya *et al.,* (2013)[16] also reported 40% isolates were obtained from patients admitted from ICU followed by 18% in recent surgery patients, 13% patients with use of broad spectrum antibiotics respectively.

In the present study total in 44% MBL producing *Acinetobacter* species, 19(43.18%) were isolated from ICU patients followed by 09(20.45%) in recent surgery patients, 09(20.45%) patients with invasive medical devices (urinary cathetrization), 02(4.54%) patients with use of broad spectrum antibiotics, 16(36.30%) patients with intubation & mechanical ventilation, 04(9.09%) patients with prolonged hospital stay, 10(22.72%) in old age patients. Similar observation were made by Harekrishna nath *et al.,* (2016)[15] where 42% isolates were obtained from patients admitted in ICU followed by 18% in recent surgery patients, 34% patients with intubation and mechanical ventilation respectively. R moniri *et al.,* (2010)[17] also reported 86.7% isolates from ICU followed by 15 % in recent surgery patients, 22% patients with intubation and mechanical ventilation respectively.

A total of 100 clinical isolates of *Acinetobacter* species recovered from various clinical specimens from all ages and both sexes were studied, Among these isolates 30 were found to be ESBL producer and 44 were MBL producer. As ESBL and MBL producing isolates occur in large number of patients and show false susceptibility to extended spectrum cephalosporins and imipenem in standard disk diffusion method, therefore cephalosporins and aztreonam should not be given on the basis of routine susceptibility test results. *Acinetobacter* species were highest recovered from ICU patients followed by intubation and mechanically ventilated patients and least isolates were recovered from immune suppression and prolonged hospital stay patients.

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