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Prevalence of Metallo B-Lactamase Producing *Acinetobacter* in Clinical Specimens from S.S.G. Hospital, Vadodara, India

Jignasha Tadvi[1*], Jigna Karia[2], Rachna Bhavasar[3] and Hiral Patel[4]

[1]Department of Microbiology, Zydus Medical College & Hospital, Dahod, Gujarat, India
[2]Department of Microbiology, Baroda Medical College & SSG Hospital, Vadodara, Gujarat, India
[3]Dr. M.K. Shah Medical College & Research Centre, Ahmedabad, Gujarat, India
[4]GMERS Medical College, Valsad, India

**Corresponding author*

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A B S T R A C T

Acinetobacter species are the commonest pathogens causing nosocomial infections. *Acinetobacter* is essentially resistant to many antibiotics and they are known to produce extended spectrum beta lactamase and metalo beta lactamase. Aim of the study is to detect Metalo beta lactamase producing *Acinetobacter* spp. from clinical samples in tertiary care hospital. Between January 2015 and June 2015, total 368 *Acinetobacter* spp were isolated from different clinical samples. Antimicrobial susceptibility was done as per CLSI guideline. All imipenem resistant isolates were tested for MBL production by Imipenem - EDTA double disc synergy test (DDST) and Imipenem- EDTA combined disc test (CDT). Of 368 samples, majority of the *Acinetobacter spp* were isolated from blood 186(50.54%) followed by wound and pus samples 131 (35.6%). The isolation rate was highest from pediatric wards 192 (52.17%) and surgical wards 120 (32.61%). Total 368 samples, 14 (3.8%) isolates were MBL producer by CDST and DDST. Majority of the MBL producing *Acinetobacter spp.* were isolated from blood 5 (35.71%) followed by pus and wound 5 (35.71%). The isolation rate was highest from Pediatric wards 6 (42.90%) followed by the Surgical wards 5 (35.71%). Metallo-β-lactamase positive isolates of *Acinetobacter spp.* are important to identify because it poses therapeutic problems and serious concern for infection control management. There is also a need to emphasize on the rational use of antimicrobials and strictly adhere to the concept of "reserve drugs" to minimize the misuse of available antimicrobials.

Introduction

Acinetobacter species are the common pathogens causing nosocomial infections. Infections caused by *Acinetobacter* are either exogenous or endogenous origin, depending on several factors such as use of immunosuppressant agents, injudicious use of antimicrobial agents, prolonged surgical procedures and inadequate instrumentations. In recent years due to moderate and observed use of antibiotics, non-fermentative gram negative bacilli have emerged as an important health care associate pathogen. They have

been incriminated in infections such as septicemia, pneumonia, urinary tract infection and surgical site infection. *Acinetobacter* is essentially resistant to many antibiotics and they are known to produce extended spectrum beta lactamase and metallo-beta lactamase. Acquired drug resistance is frequent in nosocomial isolates of *Acinetobacter* spp. (Hemalatha *et al.*, 2005; Butt *et al.*, 2005) Acquired Metallo β-Lactamase (MBL) in *Acinetobacter* spp. have recently emerged as one of the most troublesome resistance mechanism because of their capability to hydrolyze all beta-lactam antibiotic including penicillins, cephalosporins and carbapenams, with the exception of Aztreonam. (Hemalatha *et al.*, 2005; Butt *et al.*, 2005; Ami Varaiya *et al.*, 2008; Debasrita Chakraborty *et al.*, 2010; Gian Maria Rossolini) Now a days resistance to Aztreonam producing Metallo β-Lactamase (MBL) is also revealed.

Currently, there are no recommendations available from CLSI (Clinical and Laboratory Standard Institute) for the detection of MBL. Several phenotypic methods are available for MBL detection. All these methods are based on the ability of metal chelators, such as EDTA and THIOI compounds to inhibit the activity of MBL.

In present study, two phenotypic methods are used for the detection of MBL producing *Acinetobacter* species. The present study includes the Imipenem- EDTA combined disc synergy test (CDST) and Imipenem- EDTA double- disc synergy test (DDST). (Ami Varaiya *et al.*, 2008; Debasrita Chakraborty *et al.*, 2010; Gian Maria Rossolini; Horieh Saderi *et al.*,)

The aim of this study to determine MBL producing *Acinetobacter* spp. from clinical isolates in a tertiary care hospital setting.

The objectives of this study include, to isolate and identify *Acinetobacter* from various clinical specimens (Blood, Body fluids, Sputum, Throat swab etc.)

To determine antibiotic sensitivity of the isolates to various antibiotics by Kirby-Bauer disc diffusion method

To screen for MBL producing isolates by detecting resistance to Imipenem (IPM).

To confirm MBL production in MBL screen test positive by:

Imipenem EDTA combined disc synergy test

Imipenem EDTA double disc synergy test

To study sensitivity and resistance pattern among isolates of *Acinetobacter* species from patients admitted in hospital.

Materials and Methods

Study Design: Cross- sectional
Study Setting: Department of Microbiology

Study Subject

The study was carried out over a period of 6 months from January 2015 to June 2015. A total non-repetitive of 368 *Acinetobacter* spp were isolated from different clinical samples like blood, pus and wound swabs, urine, body fluids, sputum, endo tracheal tube and secretions from the patients attending the hospital.

Antimicrobial susceptibility test of all the isolates was performed by the disc-diffusion (Kirby Baur disc diffusion method) according to CLSIs guidelines. All imipenem resistant isolates were tested for MBL production by Imipenem - EDTA double- disc synergy test (DDST) and Imipenem- EDTA combined disc test (CDT).

Phenotypic method for detection of Metallo- ß- Lactamases: (Lee *et al.*, 2001; Noyal *et al.*, 2009; Bashir *et al.*, 2011; Ejikeugwu Chika *et al.*, 2014)

Preparation of 0.5 M EDTA solution

A 0.5 M EDTA solution was prepared by dissolving 186.1 g of disodium EDTA.$2H_2O$ in 1,000 ml of distilled water. The pH was adjusted to 8.0 by using NaOH and was sterilized by autoclaving. The solution has to be stored at -20^0C.

Combined disk test (CDT): (Dongeun Yong *et al.*, 2002; Seema Bose *et al.*, 2012)

The strains resistant to carbapenems were screened for MBL by CDT. Test was done for detection of metallo- ß- Lactamases in the imipenem resistant isolates. 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. 10 µg imipenem disk and IMP (10 µg) + 5µl- 0.5 M EDTA (750 µg) was placed on the agar. An increase of 7mm or more in zone diameter in the presence of EDTA compared to those with IMP, tested alone was considered to be a positive test for the presence of an MBL (Fig. 1).

Double disk synergy (DST) test: (Lee *et al.*, 2001; Noyal *et al.*, 2009; Vasundhara Devi *et al.*, 2015; Lee *et al.*, 2003; Marufa Nasreen *et al.*, 2015; Sowmya *et al.*, 2015)

Test was done for detection of metallo- ß- Lactamases in the imipenem resistant isolates. 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. A 10 µg imipenem disk was placed on the agar. A blank disk (6 mm in diameter, Whatmann filter paper no. 1) was kept on the inner surface of the lid of the MHA plate and 10 µl of 0.5 M EDTA is added to it. This EDTA disk was then transferred to the surface of the agar and was kept 10 mm edge-to-edge apart from the imipenem disk. After incubating overnight at 37^0C, the presence of an expanded growth inhibition zone between the two disks was interpreted as positive for MBL production (Fig. 2).

Results and Discussion

In this study, majority of the *Acinetobacter spp* were isolated from age group of 1 to 10 years 124 (33.69%) followed by less than 1 year 56 (15.22%). Isolation was more from male patients 240 (65.22%) as compared to female patients 128 (34.78%) [Table- 1].

As can be seen from Table 2, majority of the *Acinetobacter spp* were isolated from the Blood 186(50.54%) followed by the wound swabs and pus samples 131 (35.6%). The isolation rate of *Acinetobacter spp.* was highest from pediatric wards 192 (52.17%) as well as the Surgical wards 120 (32.61%).

In the present study, Of the 368 *Acinetobacter spp.* isolates, 14 (3.8%) isolates were MBL producer by CDST and DDST [Table- 3].

As can be seen from Table 4, majority of the MBL producing *Acinetobacter spp.* were isolated from blood 5 (35.71%) followed by pus and wound swabs 5 (35.71%). The isolation rate of the MBL producer *Acinetobacter spp.* was highest from Pediatric wards 6 (42.90%) followed by the Surgical wards 5 (35.71%).

Acinetobacter spp. are the most frequent nosocomial pathogen and the infections due to these are often difficult to treat because of antibiotic resistance. Acquired drug resistance is frequent in nosocomial isolates of *Acinetobacter spp*. Acquired Metallo-β-lactamases (MBL) in *Acinetobacter spp.* have

recently emerged as one of the most worrisome resistance mechanism because of their capacity to hydrolyze all beta-lactam antibiotics including penicillins, cephalosporins and carbapenems, with the exception of aztreonam. For many years, these MBL producing isolates were restricted to Japan, but now it has disseminated worldwide. (Noyal *et al.,* 2009) *Acinetobacter species* accounted for 1.4% of all nosocomial infections during a 10-year period (1971 to 1981) in a university hospital in the United States with the principal sites and types of infection including the respiratory tract, bacteremia, peritoneum, urinary tract infection, surgical wounds, meningitis, and skin or eye infection (Lahiri, 2004). It almost exclusively infects hospitalized patients with lowered host resistance and is the most frequent pathogens isolated from nosocomial infections in ICU.

In the present study, total 368 isolates from *Acinetobacter* spp. from different clinical samples were studied for their susceptibility or resistance to the antibiotics by (Kirby Baeur disc diffusion method) according to CLSIs guidelines.

Isolation of *Acinetobacter spp* from various clinical wards and various clinical samples

Isolation of *Acinetobacter* spp. from blood cultures was found to be highest 186 (50.54%) followed by wound swabs and pus 131 (35.6%) in our study. Contrary to these studies carried out by (Sinha *et al.,* 2013) and (Mindolli *et al.,* 2010) report only 22.85% and 14% of the isolates from blood cultures while a maximum was from pus samples 37.14% and 30.5%. (Lais Lisboa Correa *et al.,* 2012) have reported an isolation rate of 23.5% from blood cultures but maximum from lower respiratory tract samples (37%). *Acinetobacter* are part of the human skin flora. In an epidemiological survey performed to investigate the colonization of human skin and mucous membranes with *Acinetobacter* species, up to 43% of non-hospitalized individuals were found to be colonized with these organisms. (Peleg *et al.,* 2008)

Fig.1 Combined Disk Test (CDT): positive strain shows a ≥ 7mm zone around the Imipenem + EDTA disk

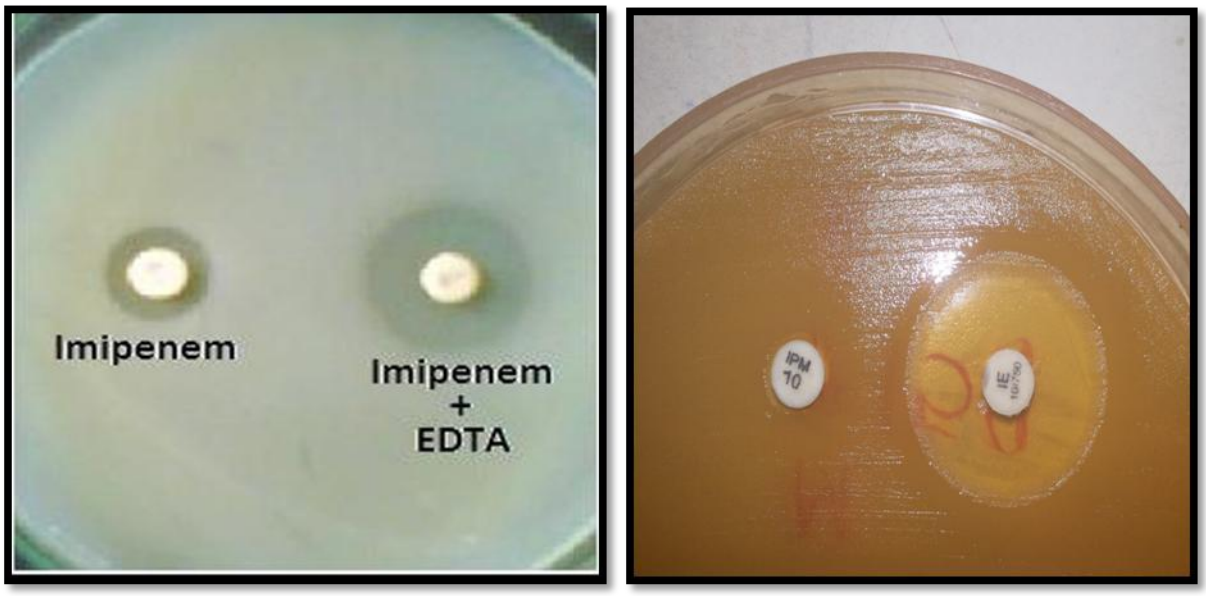

Fig.2 Double Disk Synergy Test (DDST)/ EDTA Disk Synergy Test: Positive strain shows a synergistic zone of inhibition between Imipenem and EDTA disc

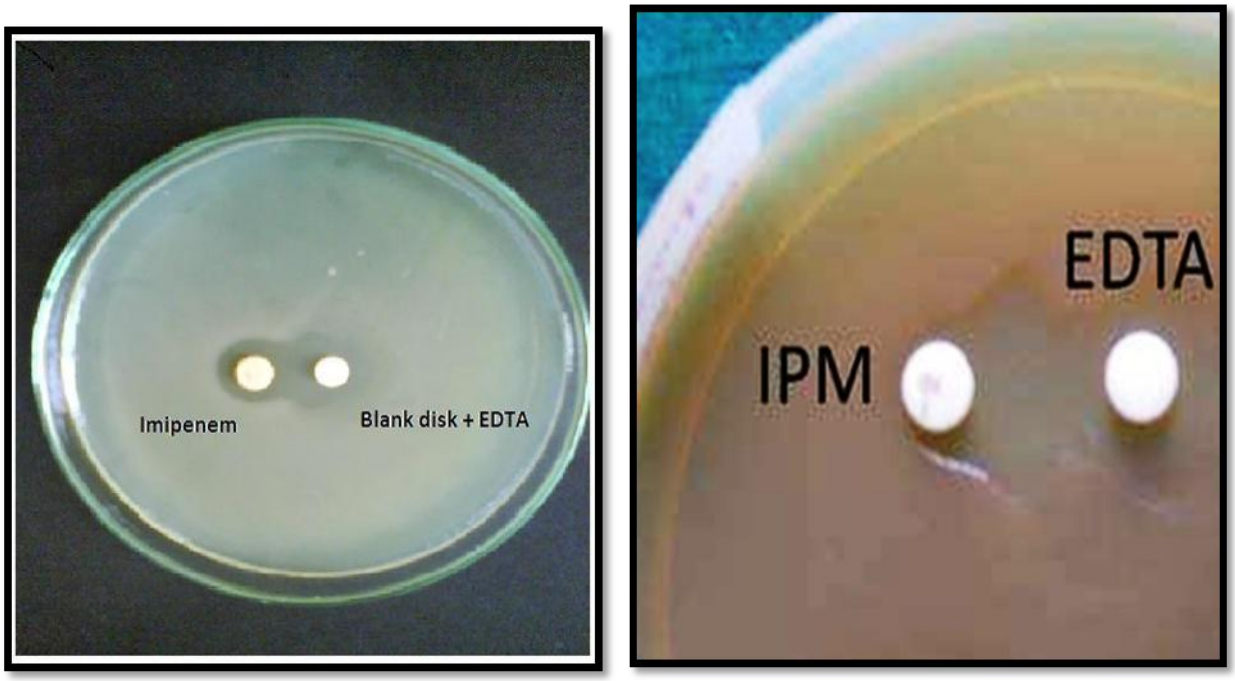

Table.1 Age and Sex distribution of isolated *Acinetobacter spp.* (n=368)

Age in Years	Male	Female	Total
< 1	31	25	56 (15.22%)
1 to 10	84	40	124 (33.69%)
11 to 20	17	16	33 (8.97%)
21 to 30	20	19	39 (10.60%)
31 to 40	26	9	35 (9.51%)
41 to 50	26	6	32 (8.69%)
51 to 60	19	5	24 (6.52%)
61 to 70	12	4	16 (4.35%)
71 to 80	5	4	9 (2.45%)
Total	240 (65.22%)	128 (34.78%)	368

Table.2 Isolation of *Acinetobacter spp*. from various clinical wards and various clinical samples

CLINICAL SAMPLES	Blood	Body fluid	Pus/ Wound	Sputum	ET/ TT	Urine	Total
Surgical	4	4	101	4	6	1	120 (32.61%)
Medical	5	2	0	3	0	0	10 (2.72%)
Pediatrics	176	0	2	1	11	2	192 (52.17%)
Orthopedic	0	0	6	0	0	0	6 (1.63%)
Obs & Gynec	0	0	2	0	0	0	2 (0.54%)
Burns	0	0	17	0	0	0	17 (4.62%)
ENT/ Eye	0	0	1	0	0	0	1 (0.27%)
Others (Ward 22,23)	1	9	2	8	0	0	20 (5.44%)
Total	186 (50.54%)	15 (4.07%)	131 (35.6%)	16 (4.35%)	17 (4.62%)	3 (0.82%)	368

Table.3 Prevalence of MBL producer and MBL non-producer of *Acinetobacter spp*

ISOLATES	MBL producer N (%)	MBL non producer N (%)	TOTAL N (%)
Acinetobacter spp.	14 (3.8%)	354 (96.2%)	368 (100%)

Table.4 Prevalence of MBL producing *Acinetobacter spp.* From various clinical samples and various clinical wards

CLINICAL SAMPLES	Blood	Body fluid	Pus/ Wound	Sputum	ET/ TT	Urine	Total
Surgical	0	0	4	0	1	0	5 (35.71%)
Medical	0	0	0	1	0	0	1 (7.14%)
Pediatrics	5	0	0	0	1	0	6 (42.90%)
Orthopedic	0	0	0	0	0	0	0 (0%)
Obs & Gynec	0	0	1	0	0	0	1 (7.14%)
Burns	0	0	0	0	0	0	0 (0%)
ENT/ Eye	0	0	0	0	0	0	0 (0%)
Others (Ward 22,23)	0	1	0	0	0	0	1 (7.14%)
Total	**5 (35.71%)**	**1 (7.14%)**	**5 (35.71%)**	**1 (7.14%)**	**2 (14.30%)**	**0 (0%)**	**14**

Table.5 Antibiogram pattern of isolates of *Acinetobacter spp.* to different Antibiotics are as follow

Name of Drugs	Sensitive	Resistance
Piperacillin + Tazobactam (100µg/ 10 µg)	331 (89.95%)	37 (10.05%)
Gentamicin (10 µg)	202 (54.89%)	166 (45.11%)
Levofloxacin (5 µg)	354 (96.19%)	14 (3.81%)
Cefepime (30 µg)	130 (35.33%)	238 (64.67%)
Cefotaxime (30 µg)	127 (34.51%)	241 (65.49%)
Amikacin(30 µg)	203 (55.18%)	165 (44.84%)
Imipenem (10 µg)	354 (96.19%)	14 (3.81%)

Table.6 Prevalence of MBL producers among *Acinetobacter spp*

Years of study	Place of study	Authors	MBL
			Producers
2008	Pakistan	S Irfan *et al.*, [25]	96.60%
2009	Puducherry	Noyel *et al.*,[8]	6.50%
2010	Mumbai	Anuradha S De [26]	36%
2011	Punjab	Mahajan G *et al.*,[27]	19%
2011	Tamil Nadu	John & Balagurunathan[28]	27.70%
2012	Maharashtra	Simit H kumar.[29]	14.29%
2012	South India	Dheepa M and Appalaraju [30]	10%
2013	kolkata	Rit K *et al.*, [31]	22%
Present study	Vadodara		3.8%

When distribution of clinical samples from which *Acinetobacter spp*. were isolated, was studied in different locations of hospital, it was found that majority of the samples were from Pediatric wards 192 (52.17%), followed by Surgical ward 120 (32.61%). A second important group of patients may consist of neonates. (Lahiri *et al.*, 2004) The predisposing risk factors for septicemia are low birth weight, previous antibiotic therapy, mechanical ventilation, and the presence of neonatal convulsions.

Acinetobacter spp. should be added to the list of organisms capable of causing severe nosocomial infection in neonatal ICUs. (Lahiri *et al.*, 2004) Studies carried out by different authors like (Lias Lisboa Correa *et al.*, 2012) (57.1%), (Sinha *et al.*, 2013) (22.14%) (Amudhan *et al.*, 2011) (95.68%), (Anupuraba *et al.*, 2005) (20.8%) and (Mindolli *et al.*, 2010) (27%) have all reported a majority of their *Acinetobacter* spp to be isolated from the ICUs followed by the pediatric wards. Contaminated hands and gloves of the wards staff seem to have an important role in patient-to-patient transmission of *Acinetobacter* spp. (Tankovic *et al.*, 1994). In our study, 3.8% isolates of *Acinetobacter* spp. were found to be MBL producers. It was low as compared to the other studies as shown in the following table:

Antibiotic resistance pattern of MBL producing *Acintobacter* spp.

All MBLs were resistant to important groups of antibiotics tested, including third

generation cephalosporins, Aminoglycosides and Quinolones – a feature of MBL producers. (De *et al.,* 2010; Kumar *et al.,* 2012) For MBLs, limited treatment options are available and the only therapeutic option may be polymyxin, but it should never be used as monotherapy. Combination therapy often employed in treatment of MBL – producing *Acinetobacter* spp. with imipenem/ Imipenem combined with Ampicillin-sulbactum. (Kumar *et al.,* 2012) In their study, (John and Balagurunathan, 2011) reported resistance to Amikacin (56.1%), Piperacillin (57.1%), Ciprofloxacin (72.1%), and Gentamicin (77%) among the *A.baumannii*.

In his study, (Kumar *et al.,* 2012) reported that all the MBL positive isolates of *A.baumannii* were resistant to all the major antibiotics except for Piperacillin/Tazobactum to which only 16.67% isolates were susceptible.

MBL (metallo-β-lactamase) positive isolates of *Acinetobacter spp.* are important to identify because it poses not only therapeutic problem, but also a serious concern for infection control management.

There is also a need to emphasize on the rational use of antimicrobials and strictly adhere to the concept of "reserve drugs" to minimize the misuse of available antimicrobials. In addition, regular antimicrobial susceptibility surveillance is essential.

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