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# Identification, Isolation and Detection of Metallo Beta Lactamase Resistance in *Acinetobacter* Species from Various Clinical Samples in a Tertiary Care Hospital

**Sundararajan Thangavel, Gomathi Manian* and Neelaveni Ramasamy**

*Department of Microbiology, Govt. Mohan Kumaramangalam Medical College, Salem, Tamil Nadu, India*

**Corresponding author*

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**A B S T R A C T**

Non fermenting Gram Negative Bacilli (NFGNB) once considered as contaminants, now emerged as a major cause of life threatening nosocomial infections and as multidrug resistant pathogens. *Acinetobacter* species are the opportunistic pathogens with increasing prevalance in the nosocomial infections. Community acquired infections are also common in *Acinetobacter*. It accounts for 10% of all community-acquired bacteremic pneumonias.

To isolate, identify and detect Carbapenem resistance producing *Acinetobacter spp.*, and confirm Metallo Beta Lactamase ( MBL) production by phenotypic methods. This cross sectional study conducted in a tertiary care hospital for a period of 6 months from various clinical samples were identified using standard protocol. The MBL resistant strains of *Acinetobacter species* were identified by Kirby-Bauer disc diffusion methods and confirmed by phenotypic methods. Out of clinically significant isolates of *Acinetobacter spp.*, 50 (67%) were *Acinetobacter baumannii* and 25 (33%) were *Acinetobacter lwoffi*. The antimicrobial susceptibility pattern revealed maximum resistance to Gentamycin (64%), Cotrimoxazole, Amikacin & Ciprofloxacin (40%) and Cefotaxime and Ceftazidime (36%). Sensitivity to Polymyxin B (100%) followed by Imipenem and Meropenem (90%). Among them 7 (9.3%.) isolates were MBL producers. Among the 7 isolates, CDDT was positive in 5(71%) isolates, DDST was positive in 3(43%) isolates. *Acinetobacter baumannii* were the most common isolate in this study. Difference in antimicrobial susceptibility poses a great problem in treating these infections. MBL production by these organisms leads to high morbidity and mortality and left with the only option of treating them by potentially toxic drugs like Colistin and Polymyxin B.

## Introduction

Non Fermenting Gram Negative Bacilli (NFGNB) are aerobic, non-spore forming organisms that do not utilize carbohydrates as a source of energy (or) degrade them through metabolic pathways other than fermentation [1,2,3]. These are ubiquitous in nature and frequently considered as contaminants, most of them have emerged as important nosocomial pathogens causing opportunistic infections which account for about 15% of all

bacterial isolates from a clinical microbiology laboratory [3] This group includes organisms from genera like *Pseudomonas, Acinetobacter, Stenotrophomonas, Burkholderia, Alcaligenes, Weeksella* and many more. Currently, *Pseudomonas aeruginosa and Acinetobacter baumanii* are the most commonly isolated nonfermenters pathogenic for humans whereas infections caused by other species are relatively infrequent [4].

*Acinetobacter* species are the opportunistic pathogens with increasing prevalence in the nosocomial infections [5]. It accounts for 10% of all community-acquired bacteremic pneumonias [6]. *Acinetobacter spp.,* have been reported to cause high mortality rate of 32% to 52% in blood stream infections. Similarly mortality rate up to 70% have been reported in ICU acquired pneumonia[7]. Different *Acinetobacter* species have differences in their antimicrobial susceptibility pattern, hence it is important to identify *Acinetobacter* isolates at species level[8]. *A. baumannii* is the most common species isolated from clinical specimens and they developed 70% of resistance to third generation cephalosporins, aminoglycosides and quinolones. 87% of *Acinetobacter* isolates were Multidrug resistant[9]. For ESBL and AmpC producers, carbapenem remain the drug of choice, whereas in carbapenem resistant strains we are left with Tigecycline and polymyxins which have started developing resistance to many Gram negative bacilli [10]. Carbapenem resistance in *Acinetobacter* may be due to oxacillinases, metallobeta lactamases, AmpC beta lactamases or due to porin deficiency [11]. Also metallo beta lactamases are more potent (100-1000 fold) hydrolysers of carbapenems when compared to OXA type carbapenamases which contribute to the carbapenem resistance to a greater extent [12].

Hence the detection of carbapenem resistance is important in the treatment of patients and also preventing the spread of resistant strains, as we have to go a long way for newer antibiotics. The present study was therefore taken to identify the *Acinetobacter spp.,* from various clinical specimens and to detect the MBL production.

To identify, isolate and detect Metallo Beta Lactamase (MBL) resistance in *Acinetobacter* species and confirmed by phenotypic methods from various clinical samples in a tertiary care hospital

## Materials and Methods

This Cross sectional study was conducted in the Department of Microbiology in a tertiary care hospital over a period of 6 months. Samples were collected from patients attending Out patient Department (OPD) and wards who satisfied the inclusion criteria. Inclusion Criteria included were hospitalized patients of all age groups undergoing treatment in ICU, medical, surgical and paediatric ward, patients affected with burns, Patients with non-healing ulcer, diabetic patients with ulcers, septicemia and pneumonia, peritonitis, patients with indwelling urinary catheter and on ventilators. Exclusion criteria included patients on prior antibiotic therapy, isolates of repeated samples from the same patient, patient who do not give consent.

Isolation and identification is mainly based on the Gram staining, motility, colony morphology on Nutrient Agar, MacConkey Agar and Blood Agar. All the catalase positive, oxidase negative, non-lactose fermenting colonies on MacConkey agar were provisionally identified by colony morphology and biochemical reactions. *Acinetobacter* species is a Gram negative, non-motile, encapsulated coccobacillus. The colonies which failed to acidify the TSI agar were considered as nonfermenters and subjected to the following tests. Indole, Citrate, Urease,

Nitrate reduction, growth at 42˚C. Sensitivity to Polymyxin B and following special biochemical tests and grouped according to Schreckenberger scheme.[1,10]

Since there are no CLSI guidelines for the detection of Metallobetalactamase (MBL), different studies used different methods. Despite PCR being highly accurate and reliable, its accessibility is limited only to reference laboratories. The present study was therefore taken to identify the *Acinetobacter spp.,* from various clinical specimens and to determine their antimicrobial susceptibility pattern and also to detect the MBL resistance by different phenotypic methods among *Acinetobacter species*, in the same isolates.

## Antimicrobial susceptibility testing:

### Disc diffusion method

Antimicrobial susceptibility was performed for all the isolates by modified Kirby -Bauer disc diffusion method. The panel of drugs used for antimicrobial sensitivity testing was as follows: Cefotaxime (30 µg), Ceftazidime (30 µg), Amikacin (30µg), Gentamycin (10µg), Ciprofloxacin (5µg), Piperazillin /Tazobactum (100/10µg), Trimethoprim/ Sulfamethoxazole (1.25/23.75µg), Imipenem (10µg), Meropenem (10µg), Polymyxin B (300U). Interpretations were made using the Clinical and Laboratory Standards Institute, USA guidelines (January 2016, M100-S24-Volume 34 No.1, Table 2B-2, Page 62/63)[14]. Journal reference was used for Polymyxin B and Colistin Disc diffusion standards as no CLSI guidelines exist for the same.[9,13]

### Detection of metallo betalactamase production in *Acinetobacter spp.,* by phenotypic methods

The *Acinetobacer* isolates which were found to be resistant to Imipenam, Meropenem subjected to various phenotypic detection methods such as Combined disc diffusion Test and Double disc synergy test.

### Combined Disc Diffusion Test (CDDT)

The strain to be tested was inoculated onto MHA plate as suggested by the CLSI. Two (10µg) Imipenem or Meropenem discs were placed on the plate at the distance of 20mm and 10 µl of 0.5 M EDTA solution was added to one of them to obtain the desired concentration (750 µg ). After 18 hours of incubation, the increase in inhibition zone with Imipenem EDTA, Meropenem with EDTA disc ≥5mm than the Imipenem, Meropenem disc alone was considered as MBL positive.

### Double Disc Synergy Test (DDST)

Lawn culture of the test organism was prepared over Mueller-Hinton agar plate as per CLSI guidelines. A plain sterile disc was kept 20 mm apart from either Imipenem or Meropenem (10µg) disc. 5 µl of EDTA was added to plain disc and incubation was done at 37˚C overnight. Presence of an extended zone from Imipenem or Meropenem disc towards EDTA was interpreted as positive.

## Results and Discussion

All the isolates of *Acinetobacter spp.,* were characterised to the species level and the results were analysed. During the study period, of the 75 *Acinetobacter spp*., isolated, 50(67%) were *A.baumannii* and 25(33%) were *A.lwoffi*. Age distribution of *Acinetobacter spp*., was analysed which showed, majority of the patients were from the age group of more than 50 years of age 21(28%), followed by <10 years 18(24%) and 21-30yrs 17(23) years of age (Table 1). Of the 75 isolates, 46(61%) were males and 29 (39%) were females. Majority of isolates of *Acinetobacter spp.,*

were from Surgical ward (27%) followed by NICU (20%). (Table 2). The disk diffusion susceptibility testing of the isolates shows the percentage of sensitivity of the isolates. Among all the isolates maximum resistance was recorded for Gentamycin (64%), Amikacin, Cotrimoxazole and Ciprofloxacin (40%) followed by Cefotaxime and Ceftazidime (36%) (Table 3). Among the 75 isolates of *Acinetobacter* spp., screened for Meropenem resistance by Kirby-Bauer disc diffusion method, of which 7(9.3%) isolates were found to be resistant to Meropenem. Out of 7 isolates of *Acinetobacter spp*., 5(6.7%) isolates of *A.baumanii* and 2(2.6%) *A.lwoffi* were MBL producers. Of the 7 MBL resistant strains of *Acinetobacter spp.*, 2 (29%) were from pus, ET swab and Blood and 1(13%) from sputum (Table 4). The meropenem resistance by Kirby -Bauer disc diffusion method was taken as the indicator for carbapenamase production and was further tested for their mechanisms of carbapenam resistance confirmed by phenotypic methods.

Among the 7 isolates, CDDT was positive in 5(71%) isolates, DDST was positive in 3(43%) isolates. Of the 7 isolates both CDDT, DDST was positive in 3 (43%) isolates and CDDT alone was positive in 4(57%) isolates (Table 5).

**Table.1** Age wise distribution (n=75)

| Age in years | Number of patients | Percentage (%) |
|---|---|---|
| **< 10** | 18 | 24 |
| **11-20** | 3 | 4 |
| **21-30** | 17 | 23 |
| **31-40** | 8 | 11 |
| **41-50** | 8 | 11 |
| **>50** | 21 | 28 |
| **TOTAL** | 75 | 100 |

**Table.2** Ward wise *Acinetobacter* spp., isolation (n=75)

| Ward | Number of patients | Percentage (%) |
|---|---|---|
| **NICU** | 13 | 20 |
| **PICU** | 3 | 4 |
| **Medicine** | 10 | 13 |
| **IMCU** | 1 | 1 |
| **Surgery** | 20 | 27 |
| **SICU** | 1 | 1 |
| **O&G** | 7 | 9 |
| **Burns** | 5 | 7 |
| **Urology** | 6 | 8 |
| **Orthopaedics** | 4 | 5 |
| **ENT** | 3 | 4 |
| **Paediatricsward** | 2 | 3 |
| **TOTAL** | 75 | 100 |

Majority of isolates of *Acinetobacter* spp., were from Surgical ward (27%) followed by NICU (20%).

**Table.3** Antimicrobial susceptibility pattern of *Acinetobacter* spp., (n=75)

| Drugs | A.baumaniii (n=50) | | A.lwoffii (n=25) | |
|---|---|---|---|---|
| | **S** | **%** | **S** | **%** |
| **Gentamycin** | 19 | 38 | 9 | 36 |
| **Amikacin** | 32 | 64 | 15 | 60 |
| **Ciprofloxacin** | 23 | 46 | 15 | 60 |
| **Ceftazidime** | 34 | 68 | 16 | 64 |
| **Cefotaxime** | 34 | 68 | 16 | 64 |
| **Pip - Taz** | 32 | 64 | 17 | 68 |
| **Cotrimoxazole** | 10 | 20 | 15 | 60 |
| **Imipenem** | 45 | 90 | 23 | 92 |
| **Meropenem** | 45 | 90 | 23 | 92 |
| **Polymyxin - B** | 50 | 100 | 25 | 100 |

The disk diffusion susceptibility testing of the isolates shows the percentage of sensitivity of the isolates. Among all the isolates maximum resistance was recorded for Gentamycin (64%), Amikacin, Cotrimoxazole & Ciprofloxacin(40%) followed by Cefotaxime & Ceftazidime (36%).

**Table.4** Sample distribution of MBL isolates (n=7)

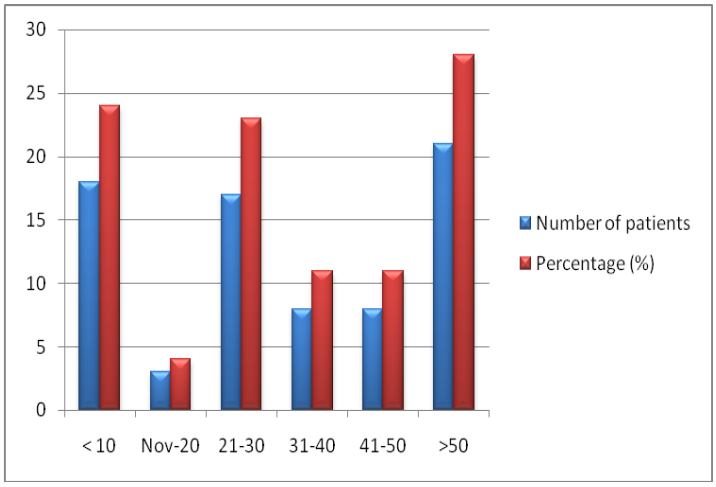
| Clinical samples | No. of MBL | Percentage (%) |
|---|---|---|
| **Pus** | 2 | 29 |
| **ET swab** | 2 | 29 |
| **Sputum** | 1 | 13 |
| **Blood** | 2 | 29 |
| **Total** | 7 | 100 |

Of the 7 MBL resistant strains of *Acinetobacter* spp., 2 (29%) from pus, ET swab and Blood and 1(13%) from sputum

**Table.5** Comparison of MBL detection by different methods

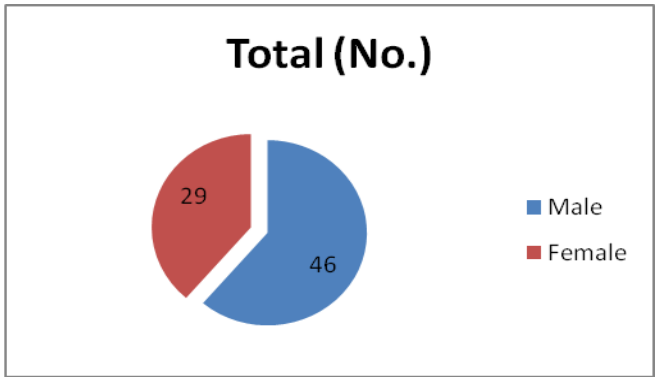
| Organism | No. | Double disc Synergy test | | Combined disc test | |
|---|---|---|---|---|---|
| | | **+ve** | **-ve** | **+ ve** | **- ve** |
| **A.baumanii** | 5 | 3 | 2 | 5 | - |
| **A.lwoffii** | 2 | - | 2 | 2 | - |
| **Total** | 7 | 3 | 4 | 7 | |

**Fig.1** Age wise distribution (n=75)




Age distribution of *Acinetobacter* spp., was analysed which showed, majority of the patients were from the age group of more than 50 years of age 21(28%), followed by <10 years 18(24%) and 21-30yrs 17(23) years of age.

**Fig.2** Gender distribution (n=75)




Of the 75 isolates, 46(61%) were males and 29 (39%) were females.

**Fig.3** Ward wise *Acinetobacter* spp., isolation (n=75)

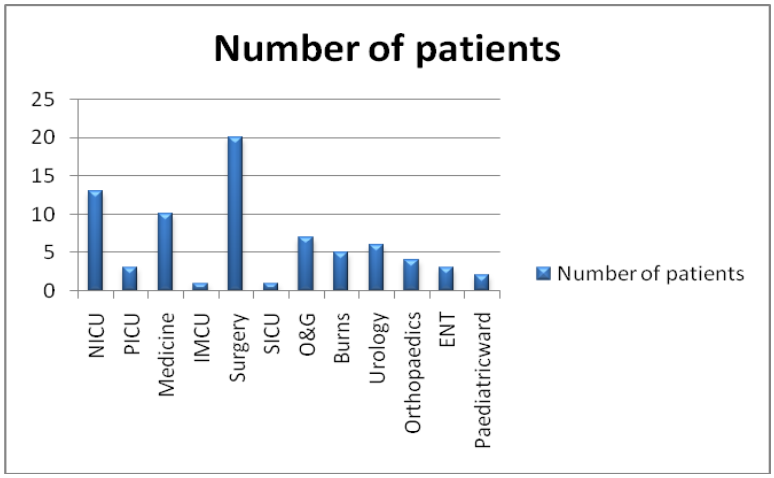
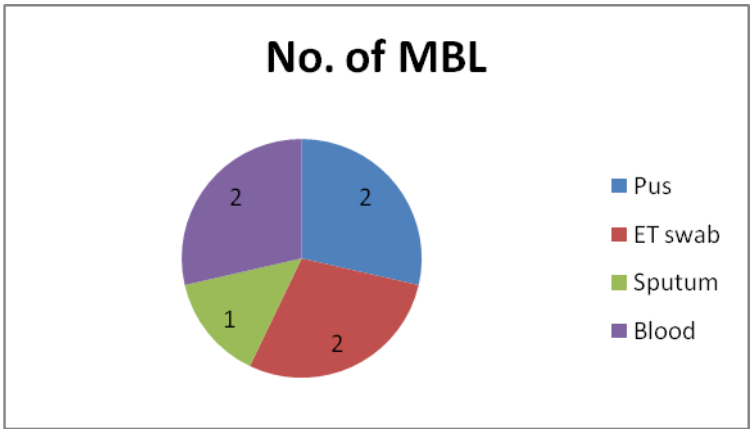
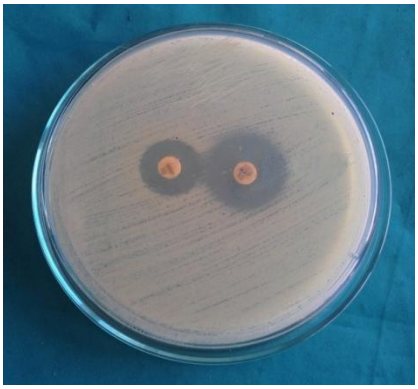


**Fig.4** Sample distribution of MBL isolates (n=7)

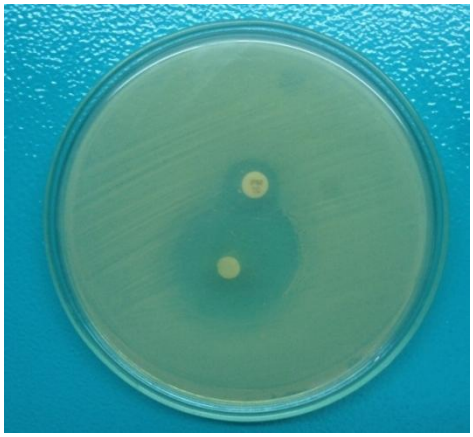



Imipenem-EDTA combined disc test for MBL detection



I – Imipenem IE – Imipenem EDTA

Double disk synergy test for MBL detection



The meropenem resistance by Kirby -Bauer disc diffusion method was taken as the indicator for carbapenamase production and was further tested for their mechanisms of carbapenam resistance confirmed by phenotypic methods.

Among the 7 isolates, CDDT was positive in 5(71%) isolates, DDST was positive in 3(43%) isolates. Out of the 7 isolates CDDT, DDST was positive in 3 (43%) isolates and CDDT alone was positive in 4(57%) isolates.

Non fermenting Gram Negative Bacilli (NFGNB) are being isolated with increasing frequency from clinical specimens and treatment failure due to their multidrug resistance in the recent years**.** In our study, we have isolated 75 *Acinetobacter spp*., over a period of 6 months from various clinical samples and were evaluated for their role in infections in hospitalized patients including the drug resistance and screening for MBL production revealed that 7 (9.3%) isolates were found to be resistant to Meropenem. The prevalence and sensitivity of nonfermenters often varies between communities among different patient populations in the same hospital. Faced these variations, the physician in the clinical practice has the responsibility to acess recent data on the prevalence and resistance pattern of commonly encountered pathogens[(15)].

The present study observed highest resistance of *Acinetobacter spp*., against first line antibiotics which are the commonly used drugs. This necessitates the judicious use of these antibiotics in empirical therapy. Maximum sensitivity was observed with newer agents like carbapenams and pipercillin-tazobactum and Polymyxin, Moderatly sensitive to Aminoglycosides and Fluroquinolones. Major risk of using monotherapy is the emergence of antibiotic resistance as observed in the present study which showed high rate of multidrug resistance and MBL producers.

Carbapenamase resistance was observed as emerging drug resistant mechanisms in the NFGNB from this hospital. Antibiotic therapy either empirical or documented is based upon antibiotic combination supplemented by the knowledge of local epidemiology of susceptibility pattern in choosing a suitable combination. Therefore combination therapy such as piperacillin-tazobactum, quinolones amikacin, imipenam-amikacin would be an ideal choice of therapy on the basis of antimicrobial susceptibility testing as observed in this study along with an adequate infection control measures especially in the surgical and ICU units.[(16,17)]

The treatment of *Acinetobacter* infections remains a great challenge because resistance to aminoglycosides, cephalosporins and quinolones has substantially increased worldwide. Carbapenems are the drug of choice for MDR *Acinetobacter* infections, for ESBL producing isolates, but resistance to carbapenems by the production of carbapenamases and various other mechanisms has limited the therapeutic options.[(18)] Because of increasing carbapenem resistance and limited therapeutic options available, the old antibiotic colistin is being used more extensively nowadays, but resistance to colistin has also been reported.[(19)]. In my study all the isolates were sensitive to Polymyxin B. Hence currently combination therapy like meropenem with tigecycline and colistin with sulbactum or rifampicin are being tried in the treatment of *Acinetobacter spp.,* infection[(20)].

The prevalence and sensitivity of nonfermenters often varies between communities. Faced these variations, the physician in clinical practice has the responsibility of making clinical judgments and should access to recent data on the

prevalence and antimicrobial resistance pattern of commonly encountered pathogens. It is therefore important to institute a system for the surveillance of antimicrobial resistance that will involve the collection of both clinical and microbiological data. Difference in antimicrobial susceptibility poses a great problem in treating these infections. Multidrug resistance by these organisms leads to high morbidity and mortality and left with the only option of treating them by potentially toxic drugs like Colistin and Polymyxin B. This warrants the judicious use of antimicrobial drugs after appropriate laboratory screening and confirmatory methods.

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