

Original Research Article

<https://doi.org/10.20546/ijcmas.2017.609.132>

Study of *Acinetobacter* Species with Special Reference to Species Differentiation and Metallo-Beta Lactamase Production in a Rural Tertiary Care Hospital

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ABSTRACT

Acinetobacter has developed resistance against a number of broad spectrum drugs and is considered as multi-drug resistant bacteria. Metallo-beta-lactamase (MBL) production is important mechanisms of the resistance as they can hydrolyse almost all beta-lactam antimicrobial agents. To isolate *Acinetobacter* species from different clinical specimens and to determine their antimicrobial susceptibility pattern as well as to know the rate of MBL production. The study was done in Department of Microbiology, RMC, Loni. The clinical samples were subjected to Gram staining, aerobic culture using MacConkey and blood agar. *Acinetobacter* species were identified by standard microbiological identification methods. Antimicrobial susceptibility testing was done by Kirby Bauer disc diffusion technique and according to CLSI guidelines. The Imepenem resistant strains were subjected to MBL detection by Disk Potentiation test (DPT) and Epsilon meter test. A total of 101(2.18%) *Acinetobacter* species were isolated out of 4627 clinical samples. Rate of isolation was maximum from burn department (38) followed by ICUs (23). *A. baumannii* (71) was the commonest species isolated followed by *A. lowfii* (18). The isolates were highly resistant to cefotaxime (100%), Co-trimoxazole (87%), Ciprofloxacin (78%), Cefepime (77%), Gentamicin (73%). All isolates were sensitive to Colistin. The number of MBL producing strain by DPT and E-test were 42 & 40 respectively. MBL production was detected better by DPT than E-test, therefore can be used as better screening method. Emergence of MBL producers and multidrug resistance in hospitals is alarming and reflect excessive use of carbapenems. Therefore early detection and prompt infection control measures are needed.

Keywords

Acinetobacter,
Metallo-beta-lactamase,
Carbapenemase resistance.

Article Info

Accepted:
17 July 2017
Available Online:
10 September 2017

Introduction

Acinetobacter are opportunistic pathogens and are frequently present on normal skin. Though widely prevalent in nature and generally regarded as commensals of human skin and respiratory and genitourinary tracts, (Al-Khoja, 1979) (Glew *et al.*, 1977) they

have been implicated as the cause of serious infectious diseases such as meningitis, pneumonia, tracheobronchitis, endocarditis, wound infections, and septicemia, mostly involving patients with impaired host defences (Seifert *et al.*, 1993). They can

survive for long in hospital environment and colonize almost all patients on prolonged hospitalization.

The most alarming problem encountered is the ability of this species to acquire the different mechanisms of resistance and the emergence of strains that are resistant to all commonly used antibiotics coupled with the scarcity of development of new antimicrobial agents, this has resulted in a limited choice of antibiotics for treatment of multidrug resistant isolates of *Acinetobacter* (Ghaima *et al.*, 2016).

Carbapenems are many times used as last resort antibiotics for treating serious hospital acquired infections caused by multidrug resistant organisms (Shobha *et al.*, 2009). Although resistance to antibiotics is caused by many mechanisms, carbapenem resistance is due to carbapenem hydrolysing enzymes (Kabbaj *et al.*, 2013). According to molecular Ambler classification, Carbapenem hydrolysing enzymes belong to classes A, B, D and are called as carbapenemases. The carbapenemases in class B require one or two zinc ions for their complete catalytic activity and are therefore called Metallo-beta-lactamases (MBLs) (Shlesinger *et al.*, 2011). MBLs are considered to be more crucial than other mechanisms of the resistance as they can hydrolyse almost all beta-lactam antimicrobial agents. Till today, there are no clinically approved MBL inhibitors, making these enzymes a serious threat to health of mankind. MBL encoding genes are able to easily disseminated from one bacterium to other via mechanism of horizontal gene transfer (Anwar *et al.*, 2016).

Therefore, this study aims to isolate *Acinetobacter* species from different clinical specimens and to determine their antimicrobial susceptibility pattern as well as

to know the rate of MBL production from isolated species.

Materials and Methods

The study was done in Department of Microbiology, Rural Medical College, Pravara Institute of Medical College, Loni. All clinical specimens received from inpatient departments and outpatient departments from Pravara Rural Hospital at the Department of Microbiology were included in study. Repeat sample from a patient, the isolates other than *Acinetobacter* species were excluded.

All the samples were subjected to Gram staining and aerobic culture. The clinical specimens were inoculated on to blood agar & MacConkey agar plates and which were incubated at 37° C for 18 hours.

Acinetobacter species were identified by colony morphology, Gram stain, catalase, oxidase test and other standard microbiological identification methods (Washington *et al.*, 2006). Antimicrobial susceptibility testing were done by Kirby Bauer disc diffusion technique using Hi Media discs and the result were interpreted according to CLSI guidelines ('Clinical and Laboratory Standards Institute CLSI, 2017). E strips were used for determining susceptibility of Colistin. The Imepenem resistant strains were subjected to MBL detection by Disk Potentiation test and Epsilometer test.

Detection of MBL production

Disk potentiation test

A lawn culture of the 0.5 McFarland turbidity matched test strain was inoculated onto Mueller-Hilton agar. Two 10 µg Imipenem disks were placed wide apart with 5 µl of 0.5M EDTA solution added to one Imipenem

disk. After overnight incubation, an increase in zone size ≥ 7 mm of around the Imipenem EDTA disk as compared to Imipenem disk only was considered as positive for MBL production (Yong *et al.*, 2002).

Epsilometer test

The test is done using Ezy MIC strip (Hi Media, Mumbai) which is coated with mixture of Imipenem plus EDTA and Imipenem in a concentration gradient manner. The test procedure and interpretations are done according to manufacturer's instruction.

The antibiotic potency of the disks was standardized against the reference strains of *Escherichia coli* ATCC 25922 as the negative control and *A. baumannii* ATCC 19606 as the positive control.

All the patients' information details like age, gender, diagnosis, OPD/IPD were recorded. The appropriate statistics were applied wherever needed. The study was conducted after dual approval was obtained from the institutional ethical committee.

Results and Discussion

The study was conducted in Department of Microbiology for six month. During this study period, 4627 clinical samples received in the department, out of that, a total of 101 (2.18%) *Acinetobacter* species were isolated.

Maximum rate of isolation was seen in the age group of above 60 years old followed by those of below 10 years old (Graph 1). Rate of isolation was more common in males 55(54%) compared to those of females 46(46%).

Maximum number of *Acinetobacter* species were isolated from pus sample (41) followed by miscellaneous samples which included

drainage tubes, catheter tips, endotracheal tubes (35). Least isolation was from CSF (1) and sputum sample (1) (Graph 2).

Rate of isolation was maximum from the cases of burn department (38) followed by ICUs (23) and was least from Dept. of Obstetrics and Gynecology. Out of 101 *Acinetobacter* isolates, 96 were from Inpatient department, and 5 were from outpatient department. (Graph 3) *Acinetobacter baumannii* (71) was the commonest species isolated amongst the total followed by *A. lowfii* (18) and *A. haemolyticus* (12) (Table 1).

In the present study, we have found that the isolates were highly resistance to cefotaxime (100%), Co-trimoxazole (87%), Ceftazidime (85%), Ciprofloxacin (78%), Cefepime (77%), Gentamicin (73%). Low resistance was noted against Tetracycline (35%). All isolates were sensitive to Colistin. (Graph-4)

In the present study, we have also detected the Metallo-beta lactamase (MBL) producing strains by two different methods. The number of MBL producing strain by disc potentiation test was 42 and that of by E strip technique was 40.

Acinetobacter species are often present in the hospital environment and cause cross infection, which may result in life threatening disease conditions (Javed *et al.*, 2012). These species are notorious for their intrinsic resistance to multiple antibiotics and for their capacity to acquire genes encoding resistance determinants. Important mechanism of antimicrobial resistance in these organisms is production of beta-lactamases and aminoglycoside-modifying enzymes (Robert, 2006) Although, scarce knowledge has been obtained on tracing the development of resistance of antibiotics in *Acinetobacter* species. The aim of this study was to

characterize *Acinetobacter* species isolated from clinical specimens obtained from the infected patients and the antimicrobial susceptibility of these isolates to various antibiotics commonly used in clinical practice.

Out of total 4627 specimen, those were received in the Department of Microbiology during the study period, 101 were culture positive for *Acinetobacter* species. Therefore the prevalence rate of isolation of *Acinetobacter* at our rural tertiary care center was 2.18 %. Dash *et al.*, from Odisha reported the prevalence of 3%, while Rit *et al.*, reported it as 4.5% in a tertiary care hospital in West Bengal, India (Dash *et al.*, 2013; Rit, 2012) Higher prevalence rates of 14% and 9.6% were observed by Mostofi *et al.*, in Tehran, and Joshi *et al.*, in Pune respectively (Rit, 2012; Mostofi *et al.*, 2011; Joshi *et al.*, 2006).

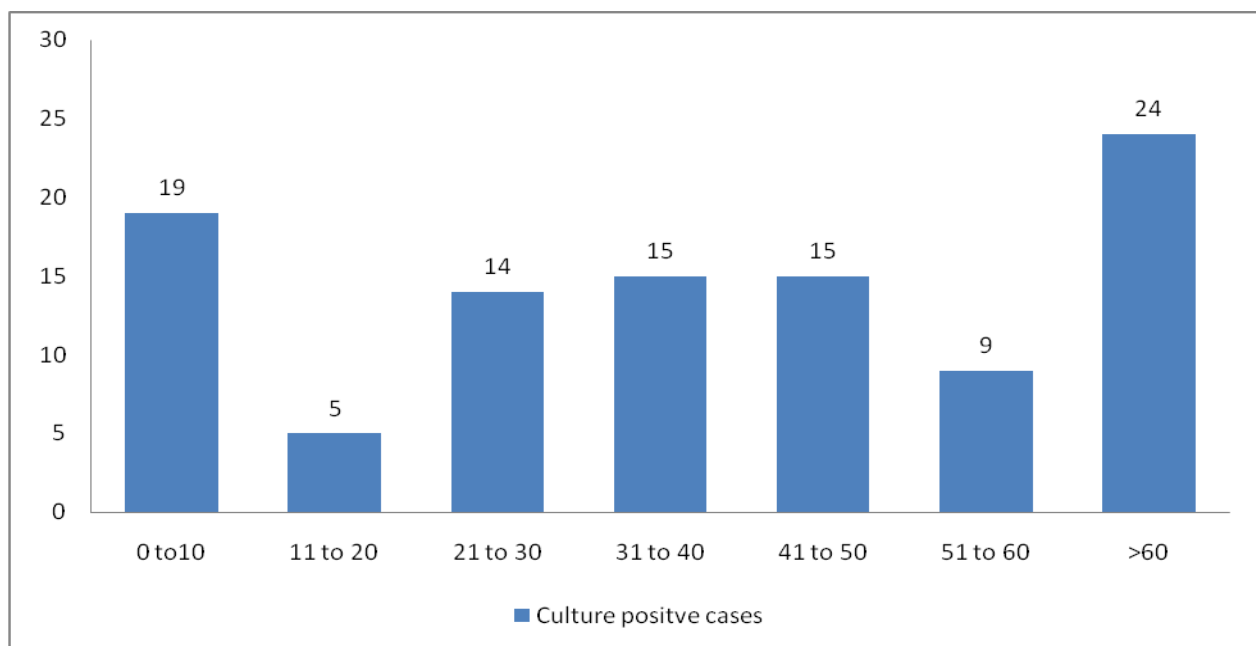
The isolation rate of the *Acinetobacter* species in our study was more common in males

55(54%) compared to those of females 46(46%). Similar observation was also made by other authors (Javed *et al.*, 2012; Parshant, 2006). Reason for this observation could not be known.

Maximum rate of isolation was seen in the age group of above 60 years old followed by those of below 10 years old. The old age of patients is recognized as an independent risk factor of the acquisition of *Acinetobacter* infection as reported by Uwingabiye *et al.*, (2016) as well as Dash *et al.*, (2013). This observation may be because of, the old age patients are definitely less immunocompetent than the younger ones.

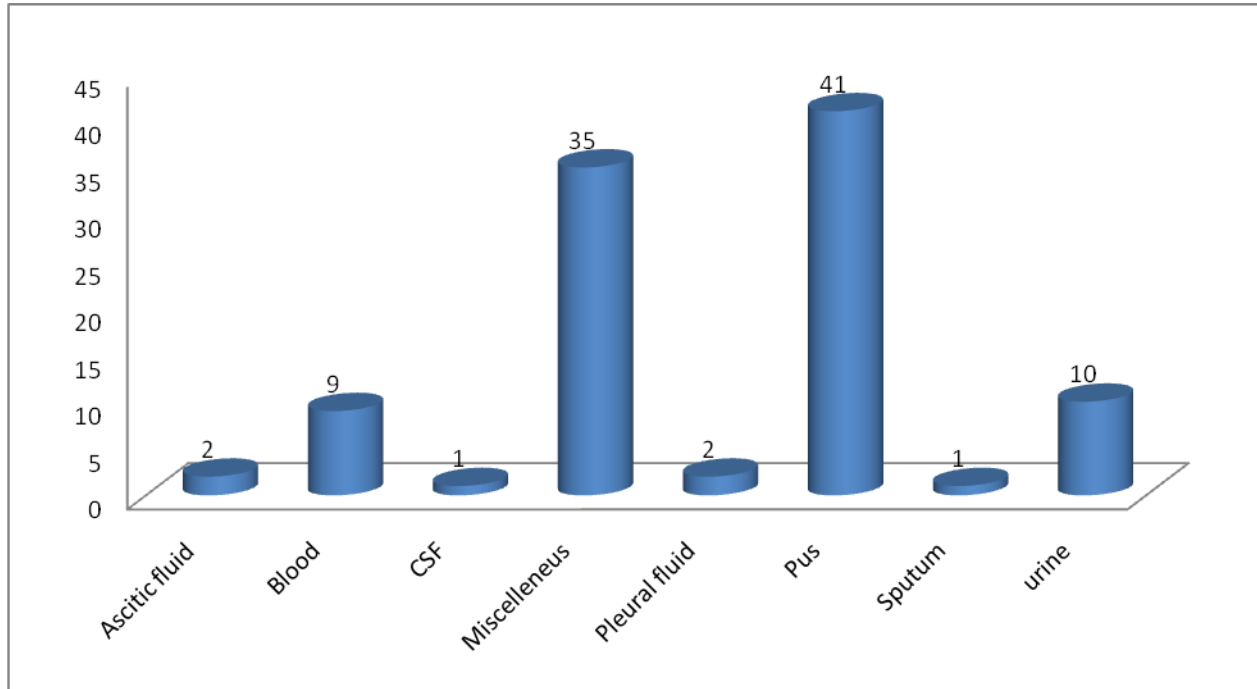
Maximum number of *Acinetobacter* species were isolated from pus sample (41) followed by miscellaneous samples (drainage tubes, catheter tips, endotracheal tubes) (35). Similar observation has been also made by Chakraborty *et al.*, in west Bengal and Dash *et al.*, in Odisha (Dash *et al.*, 2013; Chakraborty *et al.*, 2011).

Graph.1 Age wise distribution of the cases



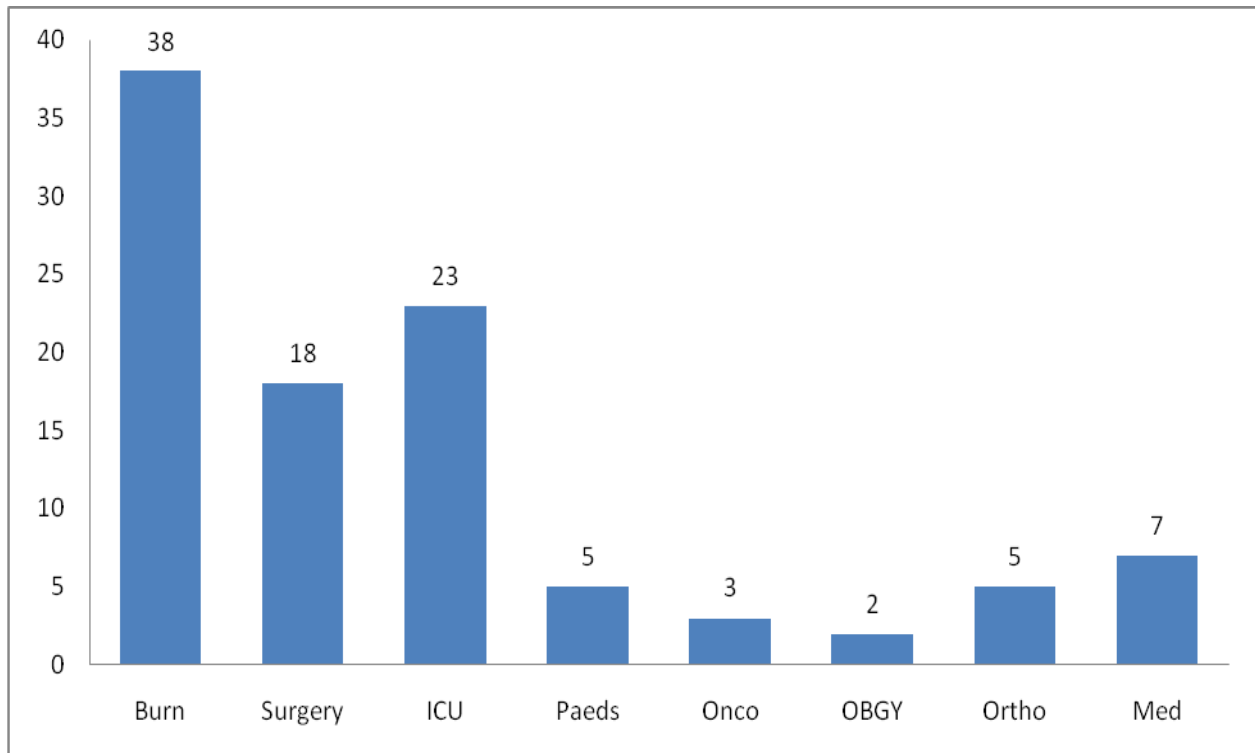
X axis- age group in years, Y axis- No of positive cases

Graph.2 Sample wise distribution of the *Acinetobacter* isolates



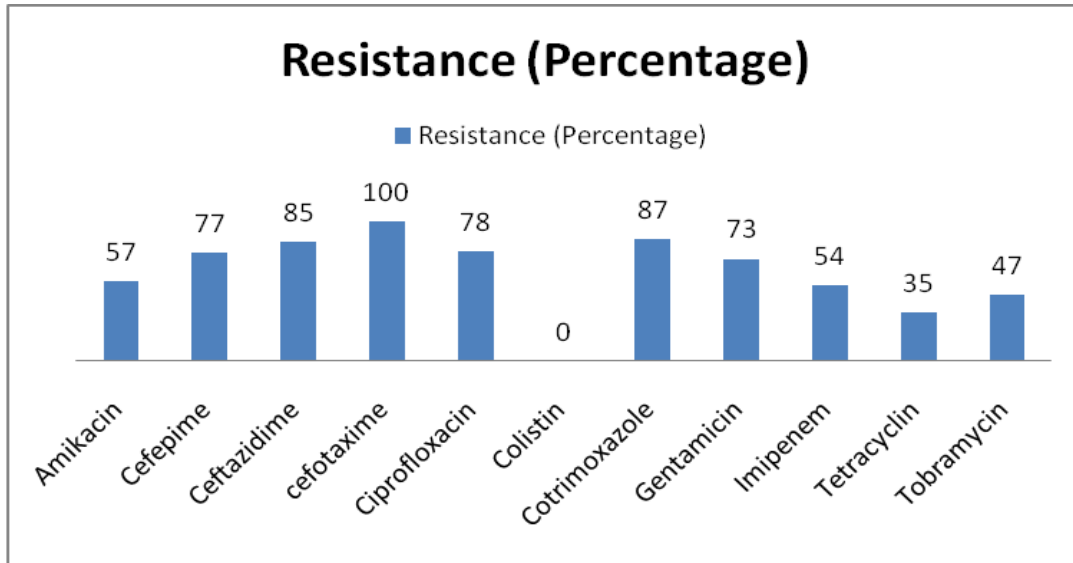
Miscellaneous-drainage tubes, catheter tips, endotracheal tubes. X axis- specimens, Y axis- No of positive cases.

Graph.3 Department- wise distribution of the *Acinetobacter* isolates



X axis- Departments, Y axis- No of positive cases

Graph.4 Antimicrobial resistance pattern of the *Acinetobacter* isolates



X axis- antibiotics, Y axis- Percentage of antibiotics resistance.

Table.1 Species wise distribution of *Acinetobacter* isolates

| Species | Number |
|----------------------------------|------------|
| <i>Acinetobacter baumannii</i> | 71 |
| <i>Acinetobacter lowfi</i> | 18 |
| <i>Acinetobater haemolyticus</i> | 12 |
| Total | 101 |

Table.2 Rate of MBL producing strains by two techniques

| Sr No | Technique | MBL producing <i>Acinetobacter</i> strains n=101 |
|-------|-----------------------------|--|
| 1 | Disc potentiation technique | 42 |
| 2 | E strip technique | 40 |

Many authors have reported the predominance of *Acinetobacter* strains in broncho-pulmonary samples (Lahsoune *et al.*, 2007; Jaggi *et al.*, 2012). *Acinetobacter* spp infections are often involved with organ systems with high fluid content (e.g. CSF, respiratory tract, peritoneal fluid, urinary tract). But, *Acinetobacter* infections also commonly affect patients with severe underlying disease conditions like burns, major surgery or trauma (Ghaima *et al.*, 2016).

Rate of isolation was maximum from the cases of burn department (38) followed by ICUs (23).

Acinetobacter is a major hospital pathogen and often affects immunocompromised patients and those who are in intensive care and burns units) (Murray, 2008).

Species wise distribution of the isolates shows that, *Acinetobacter baumannii* (71) was the commonest species isolated

amongst the total followed by *A. lowfii* (18) and *A. haemolyticus* (12). Chuang *et al.*, have also observed that among *Acinetobacter* species, *A. baumannii* is the main cause of *Acinetobacter* infections with very high antibiotic resistance rate which is also responsible for more serious infections than other *Acinetobacter* species (Chuang *et al.*, 2011).

In the present study, it was observed that the isolates were highly resistant to cefotaxime (100%), Co-trimoxazole (87%), Ceftazidime (85%), Ciprofloxacin (78%), Cefepime (77%), Gentamicin (73%), Imipenem (54%). All isolates were sensitive to Colistin. Uwingabiye *et al.*, reported the antimicrobial resistance to ciprofloxacin, ceftazidime, piperacillin / tazobactam, imipenem, amikacin, tobramycin, netilmicin, rifampicin and colistin respectively as 87%, 86%, 79%, 76%; 52%, 43%, 33% 32% and 1.7% (Uwingabiye *et al.*, 2016). The low resistance of *Acinetobacter* spp. to colistin, may be because of their recent introduction in the healthcare sector. Higher cost of these drugs is also responsible for their restricted use. Although, antibiotic resistance is a worldwide problem, it is the first and foremost a local problem. Selection for and amplification of resistant species which are present in each hospitals and communities, which then helps to spread it worldwide.

In our study, the Imepenem resistant strains were subjected to MBL detection by Disk Potentiation test and Epsilometer test. We observed that the resistance rate to Imipenem was 54 (54%) (Table 2). Out of that rate of MBL producing strain by disc potentiation test was 77.8% and that of by E strip technique was 74.07%. Irfan *et al.*, have reported 97% of the MBL producing *Acinetobacter* species by Disc potentiation technique (Irfan *et al.*, 2008). In one of the

Indian study on the *Acinetobacter baumannii* species stated that 70.9% of these isolates produced metallo beta lactamase by Disc potentiation technique, while another study reported from Kerala, India, states that 21% of the *Acinetobacter baumannii* isolates were found to be metallo- β -lactamase producers (Uma *et al.*, 2009; Anil *et al.*, 2011).

Yong *et al.*, of the study sensitivity with the imipenem-EDTA disk method was 95.7% for *Acinetobacter* spp, while Walsh *et al.*, stated that the sensitivity and specificity for E strip technique was 94 % and 95% respectively. However false negative results have been obtained by E strip method for the isolates who have MICs less than 4 $\mu\text{g/ml}$ (Yong *et al.*, 2002; Walsh *et al.*, 2002; Yan *et al.*, 2004).

Class B Metallo-beta-lactamases is characterized by the ability to hydrolyze carbapenems and by its resistance to the commercially available beta-lactamase inhibitors, but susceptibility to inhibition by metal ion chelators. The substrate spectrum is quite broad; in addition to the carbapenems, most of these enzymes hydrolyze cephalosporins and penicillins but lack the ability to hydrolyze aztreonam.

In the present study, we came to the conclusion that, in our hospital, the resistance to commonly used antibiotics against *Acinetobacter* infection is high and could pose a real problem in a patient care and management. Emergence of MBLs producing strains of *Acinetobacter* in our hospital is alarming and reflect excessive use of carbapenems. Therefore early detection and prompt infection control measures is important to prevent further spread of MBLs to other Gram negative bacilli. The Disc potentiation technique can be used for screening of MBL producing

strain. A strict control of the hospital infection by following infection control measures, sticking to antibiotic policies to avoid excessive use of carbapenems and other broad spectrum antibiotics is recommended.

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How to cite this article:

Kulkarni Vishal, Kinikar Anagha, Bhalerao Deepika, Deorukhkar Sachin and Roushani Shahriar. 2017. Study of *Acinetobacter* Species with Special Reference to Species Differentiation and Metallo-Beta Lactamase Production in a Rural Tertiary Care Hospital. *Int.J.Curr.Microbiol.App.Sci.* 6(9): 1103-1111. doi: <https://doi.org/10.20546/ijcmas.2017.609.132>