ORIGINAL RESEARCH PAPER | Medical Science | Volume : 6 | Issue : 7 | July 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

Antimicrobial Susceptibility Pattern and Detection of Metallobetalactamase Production in Acinetobacter Species Isolated From Clinical Samples

KEYWORDS: Acinetobacter, Antimicrobial susceptibility, MBL

Dr. Abhilasha Bansal	Dr. Rekha Bachhiwal	Dr. Rajni Sharma
Resident Doctor , S.M.S Medical College Jaipur	Professor, S.M.S Medical College Jaipur	Senior Professor, S.M.S Medical College Jaipur

Dr Anita Singhal	Dr. R.K. Maheshwari
Professor,, S.M.S Medical College Jaipur	Senior Professor, S.M.S Medical College Jaipur

ABSTRACT *Background: Acinetobacter species are common nosocomial pathogens and are resistant to several antibiotics in clinical practice. Metallo-beta-lactamase (MBL) mediated resistance to carbepenems is an emerging threat among isolates of Acinetobacter species*

Objectives: To study the antimicrobial susceptibility pattern of Acinetobacter isolates and detection of MBL production by phenotypic methods

Materials and Methods: 200 Acinetobacter isolates from various clinical samples were subjected to antimicrobial susceptibility testing. Imipenem resistant strains were further processed to detect MBL production.

Results: Acinetobacter baumannii was the most common species isolated (75.5%) from blood and respiratory samples from ICU patients. Maximum susceptibility was seen with Polymyxin B (91%) and colistin(87%). Among 106 Imipenem resistant isolates, 95 (89.62%) were found to be MBL producers using MBL E-test. Majority of MBL producing Acinetobacter were XDR (97.89%) followed by PDR (10.52%) and MDR. (2.1%).

Conclusion: High prevalence of MBL producing Acinetobacter spp among imepenem resistant strains demonstrated in our study calls for regular monitor of carpapenem resistance to control infections due to these bacteria.

Introduction

Infections with bacteria of genus Acinetobacter have become a significant problem worldwide. Acinetobacter baumannii is particularly formidable because of its propensity to acquire antibiotic resistance determinants.[1] Members of the genus *Acinetobacter* have been implicated in a wide spectrum of infectious diseases. Although this organism is associated primarily with nosocomial infections, it has also been involved in cases of community-acquired infections.[2] Outbreaks of infection caused by strains of *A baumannii* resistant to multiple antibiotic classes including carbepenems , are a serious concern in many specialized hospital units including Intensive care units (ICUs). Carbapenem resistance caused by acquiring the metallo-beta-lactamases (MBLs) is considered to be more serious than other resistance mechanisms because MBLs can almost hydrolyse all beta-lactam antibiotics except monobactams. Furthermore, the MBL-encoding genes located on integrons can be disseminated easily from one bacterium to another . Therefore rapid detection of metallo-β-lactamases production is necessary to modify therapy and to initiate effective infection control to prevent their dissemination. [4]

Material and Methods

Present study was conducted in the department of Microbiology, S.M.S. medical college and associated hospitals, Jaipur (Rajasthan) for a period of one year from May, 2014 to April, 2015.

A total 200 clinical isolates of *Acinetobacter* species isolated from various clinical samples received from various wards, intensive Care units (ICUs) and outpatient department (OPD) were included in the study. Samples included were mainly blood, respiratory samples (tracheal swab, throat swab, sputum, tracheal suction, tracheal aspirate, endotracheal aspirate, endotracheal tube tip), pus/wound swabs, CSF, urine and few other. Samples were processed according to standard procedures appropriate to the type of sample for isolation of bacteria and *Acinetobacter* species were identified as per conventional phenotypic methods.[5-8] Isolated *Acinetobacter* species were subjected to antimicrobial susceptibility testing against various classes of antimicrobials by Kirby Bauer disc diffusion method according to CLSI (clinical laboratory standard institute), 2013 guidelines. [9]

The *Acinetobacter* isolates showing reduced susceptibility (zone <16 mm.)/ resistant to imipenem were further processed for detection of metallo-β-lactamases (MBL) by phenotypic methods including Modified Hodge test (MHT)[10], Disc Potentiation test[11],Double Disc Synergy test (DDST) [12] and MBL E-test by E-test MBL strip(bioMerieux SA, France)[13].

MBL E test was used to detect minimum inhibitory concentration (MIC) and to confirm MBL production. The E-test MBL strip (bioMerieux SA, France) containing a double sided seven-dilution range of Imipenem (IP) (4 to 256 µg/ml) and Imipenem (1 to 64 µg/ml) in combination with a fixed concentration of EDTA (IPI) was used for MBL detection. The test was done according to manufacturer's instructions (E-test technical manual, bioMerieux SA, France). MIC ratio of ≥8 for the 2 reagent sides or a phantom zone between IP/IPI and deformation of either ellipse was indicative MBL production.

Results

In the present study 200 isolates of Acinetobacter spp

Volume : 6 | Issue : 7 | July 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

from various samples were studied.

Among 200 isolates, the majority of isolates 195 (97.5%), were from IPD samples Out of which, 109 (55.9%) were from samples received from ward patients and 86 (44.1%) were from ICU patients samples.

Table 1Sample wise distribution of *Acinetobacter* species isolates (N=200)

Sample	Number	Percent
Blood	67	33.5%
Resp. samples	51	25.5%
Pus/wound swab	38	19%
CSF	23	11.5%
Urine	7	3.5%
Others	14	7%

(Resp.- Respiratory, CSF- cerebrospinal fluid)
Maximum Acinetobacter isolates were from blood (33.5%) and respiratory samples (25.5%) followed by CSF, urine and other samples.

Table 2 Comparison of sample wise distribution of *Acinetobacter* species isolates in ICU and wards (N=195)

Clinical sample	ICU (N=86)	Percentage	Wards(N=109)	Percentage	p value
Blood	37	43.02%	30	27.52%	0.02
Resp. sample	32	37.20%	18	16.51%	0.001
Pus/ Wound swab	3	5.81%	32	29.35%	<0.001
CSF	10	11.62%	13	11.92%	0.94
Urine	1	1.16%	6	5.50%	0.1
Others	3	5.81%	10	9.17%	0.11

(Resp.-Respiratory,CSF-cerebrospinal fluid)

The difference in isolation from blood and respiratory samples among ICU and ward was found to be statistically significant with p values 0.02 and 0.001 respectively. On the other hand isolation from pus was higher in ward samples (29.3%) than ICU (5.8%), which was found to be statistically significant (p value <0.001). There was no significant difference among ICU and ward isolation from CSF, urine and other samples.

Most common *Acinetbacter* species isolated was *A.baumannii,* 151 (75.5%) followed by *A.lwoffii*, 26 (13%), *A.calcoaceticus*, 21 (10.5%) and *A.haemolyticus*, 2 (1%). *Acinetobacter baumannii* was found to be the most common species both in ICUs (83.72%) and wards (69.72%).

Table 3 Antimicrobial susceptibility pattern of *Acinetobacter* species isolates (N=200)

Antibiotic	Penicillin	Piperacillin	Amp-sulb.	Pip-tazo.	Tic-dav.	Cefotaxime	Ceftazidime	Cefepime	Meropenem	Imipenem	Gentamicin	Tobramycin	Amikacin	Doxycycline	Ciprofloxacin	Levofloxacin	Cotrimoxazole	Polymyxin-B	Colistin
No	1	33	30	40	25	28	34	25	86	94	48	41	43	84	50	58	27	181	173
%	0.5	17	15	20	13	14	17	13	43	47	24	26	23	42	25	29	14	91	87

(Amp-sulb.- Ampicillin-Sulbactam, Pip-tazo- Piperacillin-tazobactam, Tic-clav- Ticarcillin-clavulanic acid)

Antimicrobial susceptibility of isolated *Acinetobacter* revealed least sensitivity against penicillin, (0.5%) and 3rd & 4th generation cephalosporins (13-17%). Maximum susceptibility observed was 91% with polymyxin-B and 87% with colistin.

Out of 200 *Acinetobacter* isolates 53% (106) were Imipenem resistant while 47 %(94) were susceptible to Imipenem.

Imipenem resistant isolates (106/200) were further subjected to MBL detection by different phenotypic methods. Highest positivity was seen by MBL E-test, 89.6% (95) followed by Modified Hodge test 86.8 %(92), Disc potentiation test 77.30 %,(82) and Double disc synergy test 76.40 %(81).

All Imipenem resistant isolates confirmed to be positive by MBL E- test (95/106, 89%) were considered as MBL producers.

Table4 Antimicrobial susceptibility pattern of MBL positive and MBL negative *Acinetobacter* species in imipenem resistant isolates

Antibiotic	MBLpositive(%) N=95	MBLnegative(%) N=11
Penicillin	0	0
Piperacillin	0	9.09
Ampicillin-sulbactam	0	9.09
Piperacillin-tazobactam	1.05	9.09
Ticarcillin-clavulanic acid	1.05	9.09
Cefotaxime	0	9.09
Ceftazidime	1.05	9.09
Cefepime	2.10	0
Polymyxin-B	89.47	100
Colistin	84.21	100
Gentamicin	6.31	18.18
Tobramycin	6.31	9.09
Amikacin	6.31	0
Doxycycline	22.10	54.54
Ciprofloxacin	7.36	18.18
Levofloxacin	12.63	27.27
Cotrimoxazole	2.10	18.18

MBL Positive *Acenatobacter spp*, isolates were less susceptible to all class of antimicrobials than MBL non **producer**. MBL producers (95/106) showed maximum sensitivity with polymixin B (89.47%) and colistin (84.21%) followed by doxycyclin and quinolones. A very low sensitivity was also seen with Aminoglycosides.. Penicillins , cephalosporins and β lactam –β lactamase inhibitor combination showed complete resistance or least sensitivity.

Among MBL producers, 97.89% (83) of the isolates were XDR (Extensively drug resistant) and 10.5% (10) were PDR (PAN drug resistant) strains while 2.1%(2) were MDR(Multi drug resistant)

Discussion

Acinetobacter species is the one of the most important nosocomial pathogens with multiple drug resistance, is of great concern because of its intrinsic and acquired resistance mechanisms, limiting the treatment options.

In the present study it was observed that isolation rate of *Acinetobacter* species was higher from samples received from IPD patients (97.5%) . Among *Acinetobacter* isolates from indoor patients, 44.1% belonged to ICU patients while 55.9% of them belonged to patients admitted in different wards. Our study correlates well with other studies conducted in various parts of country reporting an isolation rate of 38%- 45.2% in ICU. [14,15,] .Our findings are even in agreement with the other Indian studies reporting an isolation rate of 52.38%- 74% in ward patients. [16, 17, 18.] On the contrary some authors reported an isolation rate higher

Volume : 6 | Issue : 7 | July 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

in ICU (78.32%) samples than samples from ward patients (21.68%).[19]

Acinetobacter species were maximally isolated from blood samples (33.5%) followed by respiratory samples (25.5%), and least from urine. Kinikar AG [15] (48.86%) and Gupta Neetu et al [18]. (36.9%) also isolated *Acinetobacter* species maximally from blood sample while some authors reported highest isolation from respiratory samples. [14, 15-22].

It was further observed in our study that frequency of isolates from blood (p value = 0.02) and respiratory samples (p value =0.001) were significantly higher among the ICU patients than ward patients. *A. baumanni h*as been reported as the common cause of ICU acquired blood stream infections than of non ICU infection. [23]

The most common species identified from various samples was *Acinetbacter baumannii* (75.5%) followed by other species .Similar results have been reported in various other studies. [14, 18,19, 22.]

Alarmingly, in recent years an increasing number of *Acinetobacter spp* have become resistant to penicillins, cephalosporins and carbepenems. Acinetobacter isolated in our study were also found to be resistant to most of the commonly used antimicrobials.(Table3) Penicillins and 3rd and 4th generation cephalospostrins were found to be least susceptible (0.5%- 17%). .Aminoglycosides and quninolones revealed moderate susceptibility (23-29%). A high susceptibility was noted with polymixin B (91%) and colistin (85%). Variable results regarding antimicrobial susceptibility have been documented by various authors; however Polymyxin B and Colistin remained to be the most susceptible antibiotic to *Acinetobacter* isolates in various Indian as well as Asian countries. [3, 7, 24]

Although carbapenems are the drug of choice for treatment of multidrug resistant *Acinetobacter* species but these organisms have started developing resistance to carbapenems predominantly by producing metallo-β-lactamases. In the present study 53% (106/ 200) isolates were found to be imipenem resistant which is alarmingly high. Other Indian studies conducted by Singla P (70%) [25, kaur] A(40.3%)[22], S Aparna (50.59%) have also reported high resistance to Imepenem. Some Asian studies have also reported a high resistance to Imipenem ranging from 40.7% - 90 % [3, 27, 28]. Although a low resistance to Imipenem (20.5%) have also been reported by Kinikar A [15]in India.

Carbepenem resistance in Acinetobacter is growing concern now days. A major strategy employed by these pathogens is to use Zn (II)-dependent enzymes, the metallo-β-lactamases (MBLs), which hydrolyse the β-lactam ring. MBL production in imipenem resistant *Acinetobacter* species is an indicator of excessive use of carbepenems. Therefore all isolates showing reduced susceptibility or complete resistance to Imepenem were subjected to detection of MBL production by various phenotypic methods. MHT, DPT, DDST, and E-test MBL strip methods were used for detection of MBLs. Highest positivity was seen by E -test ,(89.6%) followed by Modified Hodge test (86.8%). Positivity by DPT and DDST was found to be 77.3% and 76.4% respectively. The MBL E-test (IMP-EDTA) have been evaluated in several studies and found to be the most sensitive method for detection of MBL production among phenotypic methods (Walsh 2002, Lee Kyngwon 2005).[13,29] This test found to have ability to detect MBL both chromosomally and plasmid mediated in aerobic and anaerobic bacteria.

Based on MBL E-test strip method 89.6 % (95/106) imepenem resistant isolates were found to be MBL producers in our study, which is alarmingly high. Our study correlate well with various other Indian studies from different regions which also reported a high rate of MBL production among imipenem resistant *Acinetobacter* isolates. (S. Aparna[26]-100%, Kaur A[22] -80.3%). A high prevalence of MBL production (74%-96%) among various Asian studies has also been documented. [3,20,27,28] However a low rate of MBL production (10.4%-38.5.%) have been reported by various other studies, [15,16,24,,30]

Variation in prevalence of MBL production among imipenem resistant *Acinetobacter spp* in different studies may be due to difference in type of phenotypic test used and variation in extent of use of carbepenems in different hospital settings.

In our study MBL producing *Acinetbacter* isolates were found to be less susceptible /resistant to most of the antimicrobials tested as compared to MBL non producers. Similar finding have been reported by others.[22, 24,25]

Majority of the MBL producing *Acinetobacter* species (95 positive by E-test), were XDR (97.89%), followed by PDR (10.52%) and MDR (2.10%).[31] MBL are encoded on transferable plasmids which also encodes for linked resistance to fluroquinolones , tetra cyclins and aminoglycosides[1] leading to strains to be MDR, XDR or PDR.

Park y et al (2009)[32] has also reported XDR and PDR among *Acinetobacter* isolates.

Conclusion

In the light of the above study the high prevalence of MBL producing *Acinetobacte*r should not go without our serious concern. Resistant strains are not only the major obstacle to treatment but once established, infections are likely to lead to the possibility of further transmission. Systematic surveillance to detect MBL producers is necessary. Judicious use of carbapenems is essential to prevent the spread of these organisms and to prevent potential risk of therapeutic failure.

References

1. Peterson DL and PelegAY (2015)Acinetobacter Infections in Kasper, and Fauci, (19 edition) Harrison's principles of internal medicines 1036-1038. USA
2. Joly-Guillou, m. L, and E. Bergogne-Berezein. Epidemiologie et resistance aux antibiotiques d'*Acinetobacter* en milieu hospitalier: bilan de 5 annees. *Presse Med*, 1990; 19:357-61.
3. Maryam Noori, Karimi Abdollah, Fallah Fatemeh, Hashemi Ali, Alimehr Shadi, Goudarzi Hossein, Aghamohammad Shadi. High Prevalance of Metallo-beta-lactamase Producing *Acinetobacter baumannii* Isolated From Two Hospitals of Tehran, Iran. *Arch Pediatr Infec Dis.,* 2014; 2(1):e15439.
4. Butt T, Usman M, Ahmed RN, Saif I. Emergence of metallo beta-lactamase producing *Pseudomonas aeruginosa. J Pak Med Assoc,* 2005; 55:302-4.
5. Smidt Peter Gerner, Tejernberg Ingella, Ursing Jan. Reliability of Phenotypic test for Identification of *Acinetobacter* Species. *Journal of Clinical Microbiology*,Feb. 1991; Vol. 29:277-82.
6. Tille Patricia M. Bailey & Scott's Diagnostic Microbiology. 13thEdition. Elsevier; 2014
7. Koneman Elmer, Jr., Winn Washington, Allen Stephen, Janda Williams, Procop Gary, Schreckenberger Paul, Woods Gail. Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 6th Edition. Philadelphia: Lippincott, Williams & Wilkins; 2006
8. Mackie & McCartney. Practical Medical Microbiology. 14th Edition; 2012

Volume : 6 | Issue : 7 | July 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

9. Clinical and Laboratory Institute CLSI) Guidelines January, 2013. M 100-S23. Performance Standard for Antimicrobial Susceptibility Testing. 23rd Informational Supplement; Volume 33.
10. Lee K, Chong Y, Shin HB, KimYM, YamJH. Modified Hodge and EDTA-disk synergy test to screen metallobetalactamase producing strains of *Pseudomonas* and *Acinetobacter* species. *Clin Microbiol Infect,* 2001; 7:88-91.
11. Bhalerao DS, Roushani S, Kinikar AG, Akhter I. Study of Metallo-beta lactamase producing *Pseudomonas aeruginosa* in Pravara Rural Hospital. Med Rev 2010; 5(3).
12. Lee K, Lim YS, Yong D, Yum JH, Chong Y. Evaluation of the Hodge test and the imipenem-EDTA double disk synergy test for differentiation of metallo- β-lactamases producing clinical isolates of *Pseudomonas* spp and *Acinetobacter* spp. J Clin Microbiol 2003;41:4623-9.
13. Walsh Timothy R., Blomstrom Anne, Qwarnstrom Anette and Gales Ana. Evaluattion of a New Etest for Detecting Metallo-β-lactamases in routine Clinical Testing. *Journal of Clinical Microbiology,* 2002; Vol. 40 (8):2755-59.
14. Rit Kalidas, Saha Rajdeep. Multidrug-resistant *Acinetobacter* infection and their susceptibility patterns in a tertiary care hospital. *Nigerian Medical Journal,* 2012; Vol.53 (3):126-28.
15. Kinikar Anagha G, Bhalerao Deepika S, Roushani S. B., Study of *Acinetobacter* species with special reference to Carbapenem Resistance. *Indian* Medical Gazette, 2013:420-23.
16. Mahajan Gomty, Sheemar Sheevani, Chopra Shashi, Kaur Jaspal, Chowdhary Deeksha, Makhija S. K. Carbapenem resistance and phenotypic detection of carbapenemases in clinical isolates of *Acinetobacter baumannii. Indian Journal of Medical Sciences*, 2011; Vol. 65(1):9-25.
17. Dash Muktikesh, Padhi Sanghmitra, Pattnaik Swetlana, Mohanty Indrani, Misra Pooja. Frequency, risk factors, and antibiogram of *Acinetobacter* species isolated from various clinical samples in a tertiary care hospital in Odisha, India. *Avicenna Journal of Medicine,* 2013; Vol. 3 (4):97-102.
18. Gupta Neetu, Gandham Nageswari, Jadhav Savita, Mishra Ravindra Nath. Isolation and Identification of *Acinetobacter* species with special reference to antibiotic resistance. *Journal of Natural Science, Biology and Medicine*, 2015; Vol. 6 (1):159-162.
19. Nagmoti M. B., Nagmoti Jyoti. M. and Budhagaonkar Sonali. *Acinetobacter* species as pathogen in tertiary care hospital – A reteospective study. *Journal of Experimental Sciences*, 2011; Vol.2 (2):52-
20. Kaleem Fatima, Usman Javaid, Afreenish Hassan, Khan Aslam. Frequency and susceptibility pattern of metallo-beta-lactamase producers in a hospital in Pakistan. *J Inf Dev Ctries.,* 2010; 4(12):810-13.
21. Muthusamy Dheepa, Boppe Appalaraju. Phenotypic Methods for the Detection of Various Betalactamases in Carbapenem Resistant isolates of *Acinetobacter baumannii* at a Tertiary Care Hospital in South India. *Journal of Clinical and Diagnostic Research,* 2012; Vol. 2(2):970-73.
22. Kaur Amarjeet, Gupta Veenu, Chhina Deepinder. Prevalance of metallo-β-lactamase producing *Acinetobacter* species in a tertiary care hospital. *Iranian Journal of Microbiology,* 2014; Vol.6 (1):22-25.
23. Anton Y. Peleg, Harald Seifert and David L. Paterson. *Acinetobacter baumannii* : Emergence of Successful Pathogen. *Clin. Microbiol. Rew.*, 2008; 21(3):538-82.
24. Rit Kalidas, Bipasa Chakeraborty, Dey Rupali, Chakeraborty Parthsarthi, Naha Amrita, Saha Rajdeep. Prevalance of *Pseudomonas aeruginosa* and *Acinetobacter* spp. producing metallo-β-lactamase in a tertiary care hospital. *Journal of Dr. NTR University of Health Sciences,* 2013; 2 (1):18-21
25. Singla Pooja, Sikka Rama, Deep Antariksh and Uma Chaudhary. Phenotypic detection and Prevalance of Metallo-β-lactamases in Carbapenem Resistant Isolates of *Acinetobacter* species at a Tertiary care hospital in North India. *Int. J. Pharma. Med. & Bio. Sc.,* 2013; ISSN 2278-5221, Vol. 2 (1):85-91.
26. Shivaprasad Aparna, Antony Beena, Shenoy Poornima. Comparative Evaluation of Four Phenotypic Test for Detection of Metallo-β-Lactamase and *Carbapenemase* Production in *Acinetobacter baumannii. Journal of Clinical and Diagnostic Research,* 2014; Vol.8 (5):DC05-DC08.
27. Irfan S, Zafar A, Guhar D, Ahsan T, Hasan R. Metallo-β-lactamase Producing Clinical Isolates of *Acinetobacter* species and *Pseudomonas areuginosa* from intensive care unit of a tertiary care hospital. *Indian Journal of Medical Microbiology,* 2008; 26 (3):243-45.
28. Kabbaj Hakima, Seffar Myriam, Belefquih Bouchra, Akka Dalal, Handor Najat, Amor Morad, and Alaoui Ahmed Essaid. Prevalance of Metallo-β-lactamases Producing *Acinetobacter baumannii* in a Moroccan Hospital. *ISRN Infectious Diseases,* 2013; Article ID 154921
29. K Lee Yong Dongeun, HY Jong , SL Yong , Blomstrom Anne, Qwarmstrom Anette, Karlsson A and Chong Y. Evaluation of E test for detection of bla_{IMP-1} and bla_{VIM-2}Allele- Positive Clinical isolates of *Pseudomonas* spp. and *Acinetobacter* spp., J C Microbiol. 2005; 43(2): 942–944.
30. Ahir H.R., Patel P.H., Berry R.A., Parmar R., Soni S.T., Shah P.K., Vegad M.M., Patil S. Prevalance of Metallo-β-lactamase Producing *Pseudomonas* and *Acinetobacter* species in Tertiary care hospital, Gujrat. *International Journal of Microbiological research*, 2012; ISSN: 0975-5276 & E-ISSN:0975-9174, Volume 4 (9):322-25.
31. Magiorakos A. P., Srinivasan A., Carey R. B., Carmeli Y., Falagas M.Et al. Multidrug- resistant, extensively drug resistant and pan drug-resistant bacteria: an international expert proposal for inerim standard definations for acquired resistance. *Clinical Microbiology and Infection,* 2012 Mar;18(3):268-81. .
32. Park Young Kyoung, Pick Ran Kyong, Cheong Hae Suk, Chung Doo Ryeon, Song Jae Hoon, Ko Kwan soo. Extreme drug resistance in *Acinetobacter baumannii* infection in intensive care unit, South Korea. *Emer Inf Dis,* 2009; 15(8):1325-2.