|| ISSN(online): 2589-8698 || ISSN(print): 2589-868X ||
International Journal of Medical and Biomedical Studies
Available Online at www.ijmbs.info
PubMed (National Library of Medicine ID: 101738825)
Index Copernicus Value 2018: 75.71
Original Research Article
Volume 3, Issue 8; August: 2019; Page No. 243-249

MOLECULAR EPIDEMIOLOGY OF METALLO BETA LACTAMASES IN *ACINETOBACTER BAUMANNII* AT A TERTIARY CARE HOSPITAL

Sulaiman A Mohammed[1] and Dr. Suman P Singh[2]

[1]PhD. Research Scholar, Medical Laboratory Technology at P.S. Medical College and Shree Krishna Hospital, Karamsad-388325.

[2]Professor & HOD of Microbiology, P.S. Medical College and Shree Krishna Hospital, Karamsad-388325.

Article Info: Received 10 July 2019; Accepted 30 August. 2019
DOI: https://doi.org/10.32553/ijmbs.v3i8.500
Corresponding author: Mr. Sulaiman A Mohammed
Conflict of interest: No conflict of interest.

Abstract

Background: Carbapenem resistance mediated by metallo beta lactamases (MBL) in *Acinetobacter baumannii* is a global challenge due to its rapid spread and limited therapeutic options.

Objective: To determine the prevalence of MBL in *A. baumannii* isolates in hospitalized patients by both phenotypic and genotypic methods.

Materials and Methods: The clinical samples were collected from inpatients and subcultured on routine culture media for growth. Identification of bacteria along with antimicrobial sensitivity testing was done by VITEK -2 Compact (bioMerieux). Antibiotics that were not tested by VITEK-2 were tested manually by Kirby-Bauer disk diffusion method according to CLSI 2017 and EUCAST 2016 guidelines. The isolates which were resistant to carbapenem (imipenem and/ or meropenem) were tested by phenotypic (imipenem-EDTA combined disk method) and genotypic method for presence of common metallo beta lactamases genes (*bla*IMP, *bla*NDM, *bla*GIM, *bla*VIM, *bla*SPM and *bla*SIM).

Results: 84 non duplicate *A.baumannii* were isolated out of 947 pathogenic gram negative isolates. Majority (47.6%) of isolates were obtained from tracheostomy/endotracheal/bronchoalveolar lavage (TT/ET/BAL) followed by sputum (21.4%). None of the isolates were found to be resistant to colistin and tigecycline. 73 (86.9%) isolates were found to be carbapenem resistant, among these 60 (82.2%) were found to be MBL positive by phenotypic and 32 (43.2%) by genotypic method. MBL genes detected were *bla*NDM (39.7%), *bla*GIM (2.7%) and *bla*VIM (1.4%). None of the isolates were positive for *bla*IMP, *bla*SPM and *bla*SIM.

Conclusion: The prevalence of MBL in carbapenem resistant isolates *of A.baumannii* was 87.7%. *bla*NDM was the most common gene detected. No significant difference was found in the ability of phenotypic and genotypic methods for MBL detection. The resistance rate of the *A.baumannii* is high for most antibiotics except for polymyxins (E&B) and tigecycline.

Key words: Metallo beta Lactamases, *Acinetobacter baumannii*, Carbapenem.

INTRODUCTION:

Acinetobacter baumannii is a nosocomial pathogen that is widely reported from hospitals, especially in ICU. The reports of multidrug-resistant isolates have increased during the last decade, probably due to increasing use of antimicrobial agents (1).

Carbapenems are used as the last resort antibiotics for treatment of these infections, however in the recent years many reports show increasing resistance to them as well (2). Resistance to carbapenems in *A. baumannii* may be due to various reasons like overexpression of an efflux pump, loss or reduced expression of the porin proteins and production of metallobeta lactamases that hydrolyse carbapenems (carbapenemases) (3).

Metallo beta lactamases (MBL), are Ambler class B enzyme and include imipenemase (IMP), Verona imipenemase (VIM), Seoul imipenemase (SIM) and New Delhi metallo beta-*lactamase* (NDM)-1. They have been identified in *A. baumannii* throughout the World (4, 5) . The MBL encoding genes are located on integrons that are capable of horizontal gene transfer (5). This makes it important to detect MBL in *A. baumannii*, which could help in control and prevent further dissemination of such mobile genes (6).

Various studies have been conducted across India with a prevalence of 9.3%-70.9% in different states (6-8).The common genotypes reported are *bla*IMP and *bla*VIM (7). In Gujarat, the reported prevalence of MBL in *A. baumannii* is 29.6% (9), but the

genotypes responsible for MBL resistance in Gujarat state of India, is still unexplored. This study was undertaken to detect the MBL encoding genes (*bla*IMP, *bla*NDM, *bla*GIM, *bla*VIM, *bla*SPM and *bla*SIM) in *A. baumannii* isolates in a tertiary care hospital located in rural part of Gujarat.

Materials and Methods:

A cross sectional study was conducted from 30 September 2017 to 28 February 2018.The study was approved by Human Research Ethics Committee (HREC) of H.M Patel Centre for Medical Care and Education. The clinical samples like pus, endotracheal aspirates, blood, sputum, body fluid etc were collected from inpatients and subcultured on routine culture media for growth. Identification of bacteria along with antimicrobial sensitivity testing was done by VITEK -2 Compact (bioMerieux). Antibiotics that were not tested by VITEK-2 were tested manually by Kirby-Bauer disk diffusion method according to CLSI 2017 and EUCAST 2016 guidelines (only for tigecycline) (10, 11). The antibiotics that were tested by VITEK-2 Compact by using GN280 card included cefepime, ceftriaxone, ciprofloxacin, colistin, gentamicin, imipenem, levofloxacin, meropenem, piperacillin-tazobactam, tigecycline, trimethoprim-sulfamethoxazole, while the rest of antibiotics such as amikacin (30µg), doripenem (10µg), ampicillin-sulbactam (10/10µg), doxycycline (10µg), minocycline (30µg), netilmicin (30µg), piperacillin (100µg), polymyxin B (MIC) and tobramycin (10µg) were procured commercially from Hi-media laboratories limited, India and tested by disk diffusion method**.** The isolates which were resistant to carbapenem (imipenem and/ or meropenem) were tested by phenotypic (imipenem-EDTA combined disk test) method and genotypic by uniplex PCR to detect MBL genes such as *bla*GIM and *bla*NDM and multiplex PCR to detect other MBL genes such as, *bla*IMP, *bla*VIM, *bla*SIM, and *bla*SPM.

Imipenem-EDTA combined disk test (IMP-EDTA CDT) (12):

The suspension was prepared from an overnight bacterial culture and inoculated on Mueller-Hinton agar. 10µg imipenem disc and an imipenem-EDTA (10/750µg) disc were placed on it. After overnight incubation at 35°C, the established zone diameter difference of ≥7 mm between imipenem disc and imipenem plus EDTA disk was interpreted as MBL positive.

PCR amplification protocols: DNA extraction was done by boiling lysis method. The cell suspension from an overnight culture was boiled in 500 µl saline at 100°C for 10 minutes, then it was placed at room temperature for five minutes. The suspension was centrifuged at 8,000 rpm for 5 minutes. The supernatant was used as a template for PCR amplification(13). The PCR reaction mixture consisted of 10 µl of PCR master mix (2x), 3 µl distilled water, 1.0 µl of each primer (forward and reverse) and 5 µl of DNA in a total volume of 20 µl.

The PCR thermal cycling conditions:

For *bla*VIM, *bla*SPM, *bla*SIM and *bla*IMP genes, thermal profile was set according to the previously reported work by Mohanam *et al* (14). Thermal conditions for two genes, *bla*NDM and *bla*GIM, were standardised using gradient PCR for their annealing temperature, the final conditions were set as shown in the table 1.

Table 1: Steps and conditions of thermal cycling for *bla*NDM and *bla*GIM primer genes in PCR.

Genes	Steps	Cycling conditions		
		Temperature	Time	
*bla*NDM	Initial Denaturation	95 °C	5 min	Repeated for 35 cycles
	Denaturation	95°C	0:15 sec	
	Annealing	**55°C**	0:20 sec	
	Extension	72°C	0:40 sec	
	Final Extension	72°C	5 min	
*bla*GIM	Initial Denaturation	95°C	5 min	Repeated for 35 cycles
	Denaturation	95°C	0:15 sec	
	Annealing	**57°C**	0:30 sec	
	Extension	72°C	0:45 sec	
	Final Extension	72°C	5 min	

The PCR was considered as the gold standard for MBL detection. On the basis of the phenotypic and/ or genotypic results of *A. baumannii* isolates were divided into two groups i.e. MBL-positive and MBL-negative strains. Statistical analysis was performed using SPSS software version 20.0. The results were presented as descriptive statistics in terms of relative frequency. Chi-square test was used to analyse the results wherever they needed. P value of < 0.05 was considered as statistically significant.

Table 2: Details of MBL genes specific primers used.

Name of primers	Primer sequence (5'-3')	Product length	Reference
*bla*NDM F	GGGCAGTCGCTTCCAACGGT	476 bp	Shenoy *et al*. (15)
*bla*NDM R	GTAGTGCTCAGTGTCGGCAT		
*bla*GIM F	AGAACCTTGACCGAACGCAG	749 bp	Castanheira *et al*. (16)
*bla*GIM R	ACTCATGACTCCTCACGAGG		
*bla*VIM F	GATGGTGTTTGGTCGCATA	390 bp	Mohanam *et al*. (14)
*bla*VIM R	CGAATGCGCAGCACCAG		
*bla*IMP F	GGAATAGAGTGGCTTAAYTCTC	232 bp	
*bla*IMP R	GGTTTAAYAAAAACAACCACC		
*bla*SIM F	TAC AAGGGATTCGGCATCG	570 bp	
*bla*SIM R	TAATGGCCTGTTCCCATGTG		
*bla*SPM F	AAAATCTGGGTACGCAAACG	271 bp	
*bla*SPM R	ACATTATCCGCTGGAACAGG		

Results:

During the study period 2843 non duplicate clinical specimens were processed for culture and sensitivity test that yielded 947 (33.3%) pathogenic organisms. 737(77.8%) out of 947 were found to be gram negative bacteria, of which 84 (11.4%) were *A. baumannii.*

Table 3, illustrate the sample-wise distribution of MBL-positive and MBL-negative *A. baumannii* isolates. The majority i.e. 40(47.6%) of samples source were tracheostomy/endotracheal/broncho alveolar lavage (TT/ET/BAL) followed by sputum, blood and pus/swab i.e. 18(21.4%), 12(14.3%) and 12(14.3%) respectively. For MBL positive isolates, also the majority i.e. 34 (85.0%) of samples source were (TT/ET/BAL) followed by blood and sputum i.e. 10 (83.3%, each).

Table 4, summarizes the result of an antibiotic susceptibility testing of MBL-positive and MBL-negative *A.baumannii* isolates. Resistance to all the antibiotics that were tested in the study, except piperacillin, colistin, polymyxin B and tigecycline, was significantly more in the MBL-positive strains, in comparison to the MBL-negative strains. The MBL positive isolates showed 100% resistance to piperacillin, ampicillin/sulbactam, piperacillin-tazobactam, ceftazidime, cefepime, cefotaxime, ceftriaxone, doripenem, ciprofloxacin and levofloxacin followed by 98.4% to imipenem, meropenem and doxycycline, while resistance to these antibiotics in the MBL negative isolates was in the range of 45-95%. No resistance was found to colistin and tigecycline in isolates of MBL positive and MBL negative *A. baumannii.*

Table 5, illustrate the distribution of carbapenem resistant isolates between phenotypic and genotypic detection methods. Out of 73 carbapenem resistant isolates only 28 isolates were positive by both phenotypic and genotypic method leading to a sensitivity and specificity of 87.5% & 21.95% by IMP-EDTA CDT, when compared to genotypic method as gold standard.

The genes that were detected among IMP-EDTA CDT positive and negative isolates of *A. baumannii* included *bla*NDM in 29(39.7%), *bla*GIM in 2(2.7%) and *bla*VIM in one isolate (1.4%) (Table 6). No isolates were found positive for *bla*SIM, *bla*SPM and *bla*IMP genes. Four isolates with positive genes (three *bla*NDM & one *bla*VIM) were negative by phenotypic method.

Table 3: Distribution of clinical samples among MBL positive & MBL negative isolates of *A. baumannii* (n=84).

Specimen	MBL Positive n=64 (%)	MBL Negative n=20 (%)	Total n=84 (%)
Sputum	10 (55.6)	8 (44.4)	18 (100)
TT/ET/BAL	34 (85.0)	6 (15.0)	40 (100)
Pus/Swab	9(75.0)	3 (25.0)	12 (100)
Blood	10 (83.3)	2(16.7)	12 (100)
Body fluids	1 (50.0)	1 (50.0)	2 (100)

TT/ET/BAL: Tracheostomy/endotracheal/bronchoalveolar lavage

Table 4: Distribution of antimicrobial resistance among MBL positive & MBL negative isolates of *A. baumannii* (n=84).

Antibiotics	MBL Positive n=64 (%)	MBL Negative n=20 (%)	P value
Piperacillin	64 (100)	19 (95.0)	0.23*
Ampicillin-sulbactam	64 (100)	15 (75.0)	< 0.001
Piperacillin-tazobactam	64 (100)	16 (80.0)	0.002
Ticarcillin -clavulanate	62 (96.9)	12 (60)	< 0.001
Ceftazidime	64 (100)	15 (75.0)	< 0.001
Cefepime	64 (100)	15 (75.0)	< 0.001
Cefotaxime	64 (100)	15 (75.0)	< 0.001
Ceftriaxone	64 (100)	15 (75.0)	< 0.001
Doripenem	64 (100)	12 (60)	< 0.001
Imipenem	63 (98.4)	9 (45.0)	< 0.001
Meropenem	63 (98.4)	9 (45.0)	< 0.001
Colistin	0 (0.0)	0 (0.0)	Not applicable
Polymyxin B	2 (3.1)	0 (0.0)	0.97*
Gentamicin	57 (89.0)	5 (25.0)	< 0.001
Tobromycin	61 (95.3)	8 (40.0)	< 0.001
Amikacin	61(95.3)	9 (45.0)	< 0.001
Netilmicin	60 (93.8)	11 (55.0)	< 0.001
Ciprofloxacin	64 (100)	15 (75.0)	< 0.001
Levofloxacin	64 (100)	16 (80.0)	0.002
Doxycycline	63 (98.4)	13 (65.0)	< 0.001
Minocycline	62 (96.8)	14 (70.0)	0.001
Tetracycline	59 (92.1)	9 (45.0)	<0.001
Tigecycline	0 (0.0)	0 (0.0)	Not applicable
Trimethoprim-sulfamethoxazole	62 (96.8)	14 (70.0)	0.001

*indicate not significance at 5% level

Table 5: Comparison between phenotypic and genotypic MBL detection method among carbapenem resistant isolates of *A. baumannii* (n=73).

Phenotypic test	PCR+ve	PCR-ve	P value
IMP-EDTA +ve=60(%)	28(46.7)	32 (53.3)	0.295
IMP-EDTA-ve=13(%)	4(30.8)	9(69.2)	
Total= 73 (%)	32 (43.8)	41(56.2)	

Sensitivity=87.5%, specificity =21.95%, negative predictive value=69.23%, positive predictive value=46.67%.

Table 6: Expression of MBL genes among IMP-EDTA CDT positive and negative isolates of *A. baumannii*.

MBL genes	Detected by IMP-EDTA CDT	Not detected by IMP-EDTA CDT	Total=32
*bla*NDM=29(%)	26(89.7)	3(10.3)	29(100)
*bla*GIM=2(%)	2(100)	0(0.0)	2(100)
*bla*VIM=1(%)	0(0.0)	1(100)	1(100)
Total=32(%)	28(87.5)	4(12.5)	32(100)

Discussion:

Infection by multidrug resistant (MRD) *A. baumannii* along with metallo beta lactamases activities represent the therapeutic trouble in hospital environment especially in ICU (5).

A.baumannii has been isolated from almost all samples as it has the potential to cause wide variety of clinical manifestations. Respiratory tract infections have been reported to be the commonest of these (5, 17). In the present study, this is supported as 69% isolates of *A. baumannii* were obtained from the respiratory specimen. This is also consistent with data presented by other investigators where more than 60% isolates were from respiratory specimens though blood has also been reported as the major clinical source of *A. baumannii* (5, 17, 18).

An alarming rise in antibiotic resistance rate of *A.baumannii* has now become a serious and an increasingly common public health concern (18). In the current study, antibiotic resistance rate in *A.baumannii* is high ranging from 73.8-98.8% for most antibiotics except for colistin, polymyxin B and tigecycline. This is similar to findings by Saranya A *et al*, where resistance to most of the antibiotics was in the range of 62.5 to 100% (19). The antibiotics resistance is higher among the MBL positive group as compared to the MBL negative group, which is in agreement with findings by Singla P *et al* and by Nandy S *et al* (8, 20).

Carbapenem resistance in *A.baumannii* has been reported in various studies, ranging from 40-98.7% (5, 21-24). We found 86.9% carbapenem resistance in *A. baumannii* which is similar to 85% resistance reported by Rynga D *et al* (2015) from Delhi(25). But previous studies conducted in our institute by Pandya *et al,* (2012) and Kalpesh *et al* (2016) have reported a comparatively higher resistance to carbapenems i.e. 98.8% and 88.5% emphasizing the need to keep a watch on current rates (9, 26).

In CLSI guidelines, no standard phenotypic method has been given for detection of MBL (8). The most commonly used method is IMP-EDTA CDT and has variable sensitivity & specificity when compared with genotypic methods as gold standard. In a study conducted by Kumar H *et al*, the sensitivity and specificity of IMP-EDTA CDT was 86.2% and 30.9% respectively, which is similar to our finding of 87.5% and 21.95% respectively (27). A very high sensitivity and specificity of IMP-EDTA CDT ranging from 96.11-100% has also been reported in the studies conducted by Sachdeva *et al* and Franklin C *et al* (28, 29) . We considered any isolates as MBL positive for MBL production if positive by either or both phenotypic and genotypic methods.

The rates of MBL positivity in *A.baumannii* is highly variable and ranges from 9.3-70.9% at different geographic locations in India (7-9, 30). In the present work, 87.7 % isolates of carbapenem resistant *A.baumannii* were positive for MBL production ,Similar higher rates have been reported from Iran (86.8%) and Egypt (97.5%) (5, 31).

The common gene reported from Asia include *bla*IMP and *bla*VIM. *Bla*IMP is found mainly in Japan, Korea, China, Taiwan, and Iran (32). The most recently described MBL, namely New Delhi Metallo beta-lactamase (NDM) has been reported from the Indian subcontinent, the Balkans regions, Southeast Asia and the Middle East (33).

In our study, the most predominant gene was blaNDM (39.7%) , similar to finding by Vijayakumar *et al* (2016) from Tamil Nadu and Solanki R *et al* (2014) from Hyderabad i.e. 19.2% and 36.4% respectively (34, 35). Some of the studies in India e.g. Nandy S *et al* (2015) and Goudarzi H *et al* (2015) have not yet detected NDM gene at their locations (20, 36).

We detected *bla*GIM gene in 2.7%, which is considerable lower than 24.0% reported by Rynga *Det al* (2015) from Delhi (25). A study from Egypt by Alkasaby NM *et al* (2017) has reported a much higher rate of 42.9 % for *bla*GIM but is still not detected in a study conducted by Kock MM *et al* (2013) from South Africa (5, 37).

We had 1.4% isolates positive for *bla*VIM gene which is similar to finding by Ryoo NH *et al* (2010) from Korea(38) . Some Indian studies have reported rates of 4.5-16.2% for *bla*VIM gene (7, 25, 35) Some studies from India and Egypt have reported rates from 1-44.5% for *bla*IMP gene. (18, 20, 25). The gene was not detected in our study, which is similar to findings by some investigators from India and Africa. (7, 34, 37)**.**

*bla*SIM and *bla*SPM genes were also not detected by us and have not been detected in some studies from India, Iran and South Africa. (34, 37, 39). But studies conducted by Rynga D *et al* (2015 from India and Alkasaby NM *et al* (2017) from Egypt have reported presence of *bla*SIM gene from 8.0% to 47.1% (5, 25) *bla*SPM gene has also been reported in Iran by Vala MH *et al* (2014) and Shahcheraghi F *et al* (2011) in the range of 3.5 to 6.0% (40, 41).

Conclusion:

The prevalence of metallo beta lactamases is high (86.9%) in our setting and there is a difference in the ability of phenotypic (82.2%) and genotypic methods (43.8%) for MBL detection. *bla*NDM is the most common gene (39.9%) followed by *bla*GIM (2.7%) and *bla*VIM (1.4%). The clinical isolates of *A. baumannii* showed a high resistance rate to most of antibiotics except for polymyxins and tigecycline.

References:

1. Poirel L, Nordmann P. Carbapenem resistance in *Acinetobacter baumannii*: mechanisms and epidemiology. Clin Microbiol Infect. 2006;12(9):826-36.
2. Goudarzi H ME, Ghalavand Z, Vala MH, Mirjalali H, Hashemi A, et al. Molecular Detection of Metallo-Beta-Lactamase genes in Clinical Isolates of *Acinetobacter baumannii*. J Pure Appl Microbio. 2015;9:145-51.
3. Vila J, Martí S, Sanchez-Céspedes J. Porins, efflux pumps and multidrug resistance in *Acinetobacter baumannii*. J Antimicrob Chemother. 2007;59(6):1210-5.
4. Aksoy MD, Çavuşlu Ş, Tuğrul HM. Investigation of metallo beta lactamases and oxacilinases in carbapenem resistant *Acinetobacter baumannii* strains isolated from inpatients. Balkan Med J. 2015;32(1):79-83.
5. Alkasaby NM, El Sayed Zaki M. Molecular study of *Acinetobacter baumannii* isolates for Metallo-β-lactamases and extended-spectrum-β-lactamases genes in intensive care unit, Mansoura University Hospital, Egypt. Int J Microbiol. 2017;2017.
6. Karthika RU, Rao RS, Sahoo S, Shashikala P, Kanungo R, Jayachandran S, et al. Phenotypic and genotypic assays for detecting the prevalence of metallo-β-lactamases in clinical isolates of *Acinetobacter baumannii* from a South Indian tertiary care hospital. J Med Microbiol. 2009;58(4):430-5.
7. Purohit M, Mendiratta D, Deotale V, Madhan M, Manoharan A, Narang P. Detection of metallo-β-lactamases producing *Acinetobacter baumannii* using microbiological assay, disc synergy test and PCR. Indian J Med Microbio. 2012;30(4):456.
8. Singla P, Sikka R, Deep A, Chaudhary U. Phenotypic Detection And Prevalence Of Metallo B-Lactamases (Mbls) In Carbapenem Resistant Isolates Of Acinetobacter Species At A Tertiary Care Hospital In North India. Int J Pharm Med Bio Sci. 2013;2:85-91.
9. Pandya Y, Singh S, Badodariya D, Shethwala N. Metallo-B-Lactamase Producing Clinical Isolates Of *Acinetobacter baumannii* And *Pseudomonas aeruginosa* In A Teaching Hospital Of Rural Gujarat-India. Natl J Integr Res Med. 2016;7(6).
10. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. 2017;twenty-seven edition - M100-S26.
11. European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. 2016;Version 6.0.
12. Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, Chong Y. Imipenem-EDTA disk method for differentiation of metallo-β-lactamase-producing clinical isolates of *Pseudomonas spp*. and *Acinetobacter spp*. J Clin Microbiol. 2002;40(10):3798-801.
13. Manoharan A, Chatterjee S, Mathai D, Group SS. Detection and characterization of metallo beta lactamases producing *Pseudomonas aeruginosa*. Indian J Med Microbiol. 2010;28(3):241.
14. Mohanam L, Menon T. Coexistence of metallo-beta-lactamase-encoding genes in *Pseudomonas aeruginosa*. Indian J Med Res. 2017;146(Suppl 1):S46.
15. Shenoy KA, Jyothi E, Ravikumar R. Phenotypic identification & molecular detection of blaNDM-1 gene in multidrug resistant Gram-negative bacilli in a tertiary care centre. Indian J Med Res. 2014;139 (4):625.
16. Castanheira M, Toleman MA, Jones RN, Schmidt FJ, Walsh TR. Molecular characterization of a β-lactamase gene, blaGIM-1, encoding a new subclass of metallo-β-lactamase. Antimicrob Agents Chemother.. 2004;48(12):4654-61.
17. Khajuria A, Praharaj AK, Kumar M, Grover N. Molecular characterization of carbapenem resistant isolates of *Acinetobacter baumannii* in an intensive care unit of a tertiary care centre at central India. J Clin Diagn Res. 2014;8(5):DC38.
18. Gomaa FA-ZM, Tawakol WM, El-Azm A, Fatma I. Phenotypic and Genotypic Detection of Some

Antimicrobial Resistance Mechanisms among Multidrug-Resistant Acinetobacter Baumannii Isolated from Immunocompromised Patients in Egypt. Egypt J Med Microbiol. 2014;38(3276):1-14.

19. Saranya A SM, Aboody M. Prevalence Of Metallo-B-Lactamases In Carbapenem Resistant *Acinetobacter baumannii* Isolated From Tracheal Secretions. Int J Recent Sci Res. 2017;8(1):15197-202,.
20. Nandy S, Asthana AK, Madan M. Molecular Characterization of Metallo Beta Lactamase Producing Clinical Isolates of Acinetobacter. IJCMR. 2015:2(4).
21. Anwar M, Ejaz H, Zafar A, Hamid H. Phenotypic detection of metallo-beta-lactamases in carbapenem resistant *Acinetobacter baumannii* isolated from pediatric patients in Pakistan J Pathog. 2016;2016.
22. babariya Resident M, Mehta T, Pethani J, Shah P, Chauhan B, Rathod S. Bacteriological Profile And Drug Resistance Pattern Of Nonfermenting Gram Negative Bacilli Infection In Tertiary Care Hospital In Ahmedabad, Gujarat. Int. J Sci Res. 2015;4(2).
23. Kaur A, Gupta V, Chhina D. Prevalence of metalo-β-lactamase-producing (MBL) Acinetobacter species in a tertiary care hospital. Iran J Microbiol. 2014;6(1):22.
24. Jiang M, Liu L, Ma Y, Zhang Z, Li N, Zhang F, et al. Molecular epidemiology of multi-drug resistant *Acinetobacter baumannii* isolated in Shandong, China. Front Microbiol. 2016;7:1687.
25. Rynga D, Shariff M, Deb M. Phenotypic and molecular characterization of clinical isolates of *Acinetobacter baumannii* isolated from Delhi, India. Ann Clin Microbiol Antimicrob.. 2015;14(1):40.
26. Kalpesh S MP, Suman S,, al e. Prevalence and Antimicrobial Drug Resistance of *Acinetobacter baumannii* Infection in a Tertiary Care Teaching Hospital of Rural Gujarat, India. Int J Curr Microbiol Appl Sci. 2017;6:769-1774.
27. Kumar H AD, Mishra B, Dogra V. Evaluation of phenotypic and genotypic detection method of metallo beta lactanase producing *Pseudomonas aeruginosa*. IJBAMR. 2018;2:647-59.
28. Sachdeva R, Sharma B, Sharma R. Evaluation of different phenotypic tests for detection of metallo-β-lactamases in imipenem-resistant *Pseudomonas aeruginosa*. J Lab Physicians. 2017;9(4):249.
29. Franklin C, Liolios L, Peleg AY. Phenotypic detection of carbapenem-susceptible metallo-β-lactamase-producing gram-negative bacilli in the clinical laboratory. J Clin Microbio. 2006;44(9):3139-44.
30. Mahajan G, Sheemar S, Chopra S, Kaur J, Chowdhary D, Makhija S. Carbapenem resistance and phenotypic detection of carbapenemases in clinical isolates of *Acinetobacter baumannii.* Indian J Med Res. 2011;65(1).
31. Noori M, Karimi A, Fallah F, Hashemi A, Alimehr S, Goudarzi H, et al. High prevalence of metallo-beta-lactamase producing *Acinetobacter baumannii* isolated from two hospitals of Tehran, Iran. Arch Pediatr Infect Dis. 2014;2(3).
32. Amudhan MS, Sekar U, Kamalanathan A, Balaraman S. blaIMP and blaVIM mediated carbapenem resistance in Pseudomonas and Acinetobacter species in India. J Infect Dev Ctries.. 2012;6(11):757-62.
33. Zmarlicka MT, Nailor MD, Nicolau DP. Impact of the New Delhi metallo-beta-lactamase on beta-lactam antibiotics. Infect Drug Resist. 2015;8:297.
34. Vijayakumar S, Gopi R, Gunasekaran P, Bharathy M, Walia K, Anandan S, et al. Molecular characterization of invasive carbapenem-resistant *Acinetobacter baumannii* from a tertiary care hospital in South India. Infect Dis Ther. 2016;5(3):379-87.
35. Solanki R, Vanjari L, Sreevidya Subramanian AB, Nagapriyanka E, Lakshmi V. Comparative evaluation of multiplex PCR and routine laboratory phenotypic methods for detection of carbapenemases among Gram negative bacilli. J Clin Diagn Res.. 2014;8(12):DC23.
36. Goudarzi H, Mirsamadi ES, Ghalavand Z, Vala MH, Mirjalali H, Hashemi A, et al. Molecular Detection of Metallo-Beta-Lactamase genes in Clinical Isolates of *Acinetobacter baumannii.* J Pure Appl Microbio. 2015;9:145-51.
37. Kock MM, Bellomo AN, Storm N, Ehlers MM. Prevalence of carbapenem resistance genes in *Acinetobacter baumannii* isolated from clinical specimens obtained from an academic hospital in South Africa. South Afr J Epidemiol Infect. 2013;28(1):28-32.
38. Ryoo NH, Ha JS, Jeon DS, Kim JR. Prevalence of Metallo-β-lactamases in Imipenem-non-susceptible *Pseudomonas aeruginosa* and *Acinetobacter baumannii.* Korean J Microbiol. 2010;13(4):169-72.
39. Safari M, Nejad ASM, Bahador A, Jafari R, Alikhani MY. Prevalence of ESBL and MBL encoding genes in *Acinetobacter baumannii* strains isolated from patients of intensive care units (ICU). Saudi J Biol Sci. 2015;22(4):424-9.
40. Vala MH, Hallajzadeh M, Hashemi A, Goudarzi H, Tarhani M, Tabrizi MS, et al. Detection of Ambler class A, B and D ß-lactamases among *Pseudomonas aeruginosa* and *Acinetobacter baumannii* clinical isolates from burn patients. Ann Burns Fire Disasters. 2014;27(1):8.
41. Shahcheraghi F, Abbasalipour M, Feizabadi M, Ebrahimipour G, Akbari N. Isolation and genetic characterization of metallo-β-lactamase and carbapenamase producing strains of *Acinetobacter baumannii* from patients at Tehran hospitals. Iran J Microbio . 2011;3(2):68.