



RESEARCH ARTICLE

DETECTION OF METALLO β LACTAMASE IN LACTOSE NON-FERMENTING GRAM NEGATIVE ORGANISMS-COMPARISON OF DIFFERENT METHODS

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ABSTRACT

Background & Objectives: Increasing incidence of bacterial resistance to β lactam antibiotics is a potential healthcare hazard. In majority of the cases, this resistance is orchestrated through production of β lactamases. Among these, Carbapenemases, especially transferable metallo β lactamases (MBL), are the most important as they hydrolyze many antibiotics. MBL genes are often plasmid mediated and hence have potential for rapid dissemination. This study was conducted to phenotypically detect MBL in lactose non-fermenting Gram negative bacilli and to compare the different methods.

Methods: Strains of *Pseudomonas aeruginosa* and *Acinetobacter* spp. isolated from different clinical specimens were included in the study. MBL was detected using EDTA-Imipenem (EIC) & EDTA-Ceftazidime (ECC) combination assay and EDTA-Imipenem (EDTA-IPM) & EDTA-Ceftazidime (EDTA-CAZ) double disc synergy test (DDST). Carbapenemase was detected using Modified Hodge test (MHT).

Results: A total of 54 strains of *P.aeruginosa* and 55 strains of *Acinetobacter* spp. were studied. MBL was detected in most number of strains of *P.aeruginosa* using the EDTA-IPM-DDST (75%) method whereas the EIC and the ECC methods detected MBL in most number (53% each) of strains of *Acinetobacter* spp. MHT could detect Carbapenemase in 22% and 40% strains of *P.aeruginosa* and *Acinetobacter* spp. respectively.

Interpretation & Conclusions: Our results suggest that while EDTA-IPM-DDST is better method for MBL detection in *P.aeruginosa*, the EIC and ECC assays are equally good for MBL detection in *Acinetobacter* spp. MHT can only be used for screening of Carbapenemases in *Acinetobacter* spp.

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INTRODUCTION

Beta-lactam antibiotics, containing the beta lactam ring in their molecular structure, have a broad range of activity against both gram positive as well as gram negative bacteria. This class of antibiotics includes the Penicillins, Cephalosporins, Cephamycins, Carbapenems and Monobactams. While Penicillins, Cephalosporins and Cephamycins are prescribed more frequently, Carbapenems are usually used as antibiotics of the last resort. (Papp-Wallace *et al.*, 2011) As a result of evolutionary pressure, bacteria have developed various mechanisms to counter the lethal effect of these highly potent antibiotics. These mechanisms include enzymatic inactivation of antibiotics, chemical modification of the antibiotic, physical removal of the antibiotic from the cell by up regulation of efflux pumps, modification of the target site so that it is not

recognized by the antibiotic and/ or selective decreased permeability to the antibiotic due to mutation in porins and loss of certain outer membrane proteins. The production of β -lactamases is the main mechanism of bacterial resistance to β -lactam antibiotics in clinically important Gram-negative bacteria. (Livermore, 1995) Among the many β -lactamases discovered till date, Carbapenemases, especially transferable Metallo β -lactamases (MBLs) are the most dreaded since they are able to hydrolyze almost all the β -lactams except monobactams. (Noyal *et al.*, 2009; Drawz and Bonomo, 2010; Walsh, 2005) β -lactamases are classified into four classes A, B, C and D based on their molecular structures as per the Ambler classification. β -lactamases that are capable of hydrolyzing Carbapenems belong to classes A, B and D. (Queenan and Bush, 2007) Carbapenemases included in classes A and D require serine at the active site. Extended Spectrum β -lactamases (ESBLs) are Ambler Class A enzymes that hydrolyze extended spectrum cephalosporins with oxyiminoside chains. Those included in class B require Zinc

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ions at the active site. MBLs belong to Class B of the Ambler Classification of Carbapenemases and require presence of zinc ions at the active site for β -lactam hydrolysis. (Queenan and Bush, 2007) The genes encoding MBLs are frequently carried on plasmids or are associated with transposons and hence they have a high potential for dissemination across various genera and species. (Pitout *et al.*, 2007) Carbapenem resistance is mediated predominantly due to acquired MBLs. (Curcio, 2014) *Pseudomonas aeruginosa* and *Acinetobacter* spp. are innately resistant to a wide range of antibiotics and hence are important cause of nosocomial infections. Recent studies have shown a worldwide increase in prevalence of MBL producing strains of *Pseudomonas aeruginosa* and *Acinetobacter* spp. ranging as high as 75% in some cases. (Lucena *et al.*, 2014; Kabbaj *et al.*, 2013; Rit *et al.*, 2013; Kumar *et al.*, 2012) In India, prevalence of MBLs range from 7 –87.17% among the strains of *P.aeruginosa* (Navaneeth *et al.*, 2002; Jesudason *et al.*, 2005; Behera *et al.*, 2008; Kumar *et al.*, 2011; Rajput *et al.*, 2012; Ramakrishnan *et al.*, 2014) and 14%-41% among the strains of *Acinetobacter* spp. isolated from clinical specimens. (Rit *et al.*, 2013; Kumar *et al.*, 2012; De *et al.*, 2010) A study by Karthika *et al.* (2009) from Pondicherry Institute of Medical Sciences, Pondicherry University, has shown prevalence of MBL in *Acinetobacterbaumani* to be as high as 70.9%.

Detection of MBLs as described and advocated by different authors involves both phenotypic as well as genotypic methods. While genotypic detection is the gold standard, it is, however, performed only in reference laboratories and routine diagnostic centers still rely on culture based phenotypic detection methods. The phenotypic methods like EDTA based microbiological assays, EDTA – double disc synergy test and Combined disk test utilize chelating property of EDTA to detect MBLs. Modified Hodge test is a phenotypic test that utilizes a standard reference strain as an indicator for detection of Carbapenemase by the test strain. While the Clinical and Laboratories Standards Institute (CLSI) recommends this test for Carbapenemase detection in Enterobacteriaceae, it does not provide a standard guideline for MBL and Carbapenemase detection in lactose non fermenting bacteria. Thus, a simple, convenient, cost effective and sensitive method is required for Carbapenemase and MBL detection. Therefore, this study was undertaken to evaluate and compare three phenotypic methods of MBL detection in lactose non fermenting Gram negative bacilli isolated from different clinical specimens, to provide a simple and inexpensive method for MBL detection.

MATERIALS AND METHODS

The study was conducted over a period of one and a half years from October 2013 to April 2015 in the Microbiology Department of Sir HN Medical Research Society. Strains of *Pseudomonas aeruginosa* and *Acinetobacter* spp. isolated from different clinical specimens were identified based on colony characteristics and biochemical reactions. They were then subjected to EDTA based microbiological assay and EDTA Double Disc Synergy Test (DDST) for MBL detection and Modified Hodge Test for Carbapenemase detection. A total of 54 strains of *Pseudomonas aeruginosa* and 55 strains of *Acinetobacter* spp. were studied.

EDTA based Microbiological assay for detection of MBL/Carbapenemases

This microbiological assay allows one to detect MBL from cellular extracts of the test strain. The presence of Carbapenemases in these extracts can be readily detected and MBL can be distinguished from serine-Carbapenemases by evaluating the effect of EDTA on the growth of indicator strain of *E. coli* ATCC 25922 in the presence of a Carbapenem/Cephalosporin. The procedure consists of two steps, first step involves lysis of the bacterial cell to release the Carbapenemase and second step includes using this bacterial extract for an assay to detect Carbapenemase. The procedure was carried out as described by Marchiaro *et al.* 2005. The methodology in brief is as follows:

Preparation of crude enzyme extract

The test strain was inoculated on Mueller Hinton Agar (MHA) (Himedia Laboratories, Mumbai, India) and incubated overnight at 37°C. The overnight MHA growth was then transferred aseptically into a pre-weighed sterile microcentrifuge tube to obtain about 100mg bacterial wet weight. The cells were then suspended in 1 ml of 50 mM Tris-HCl (pH8) and pelleted by centrifuging at 5000 rpm for 10 mins. Cells were lysed by subjecting the pellet to repeated cycles of freezing and thawing at -20°C and 37°C respectively. After 10 cycles of freezing and thawing the pellet was spun for 10 min. at 10,000 rpm. Resulting supernatant containing crude enzyme extract was subjected to the EDTA- Imipenem and EDTA-Ceftazidime combination assay.

EDTA-Imipenem/Ceftazidime combination assay

An overnight culture suspension of *E.coli* ATCC 25922 with the turbidity adjusted to 0.5 McFarland standard was inoculated on Mueller Hinton Agar plate. A 10 μ g Imipenem (IPM) (Himedia Laboratories, Mumbai, India) disc was placed at the center on the agar plate.

Four plain, sterile filter paper discs were placed at the periphery of the IPM disc at a distance within the expected zone of inhibition of the antibiotic as shown in the (Figure 1). One disc received 20 μ L of the crude enzyme extract. A second disc which has been previously supplemented with 0.1 mM ZnSO₄, received 20 μ L of extract. The third disc supplemented with 20 mM EDTA (pH 8), received 20 μ L of extract while the fourth disc was loaded with 20 μ L 50mM Tris HCl (pH 8). Similar procedure was carried out using Ceftazidime (30 μ g) disc (Himedia Laboratories, Mumbai, India) instead of Imipenem.

Plates were incubated overnight at 37°C. Growth of indicator *E.coli* around discs containing crude enzyme extract and ZnSO₄+ crude enzyme extract indicated the presence of Carbapenemase in the extract. Metallo β -lactamases were distinguished from other Carbapenemases by the growth inhibition of the indicator strain around the disc containing EDTA+ crude enzyme extract, while the disc containing only buffer acts as the negative control.

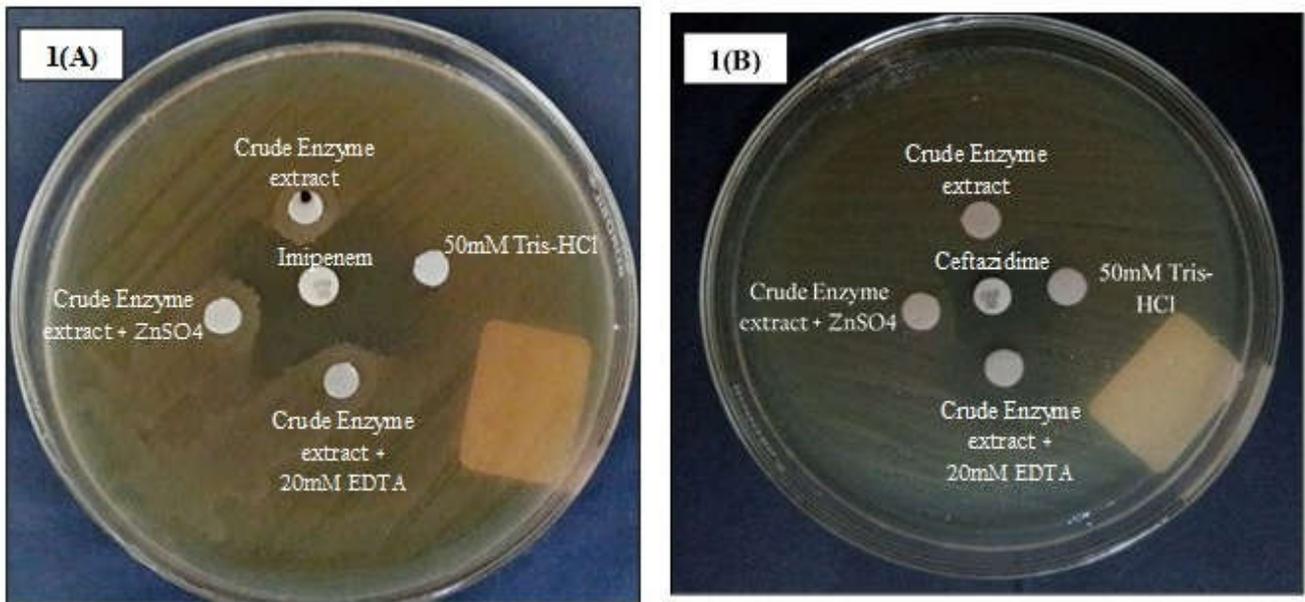


Fig.1. EDTA based microbiological assay

(A) Carbapenemase producing strain by EDTA Imipenem Combination (EIC) assay method.

Fig.1 (B) MBL producing strain by EDTA Ceftazidime Combination (ECC) assay method. EDTA Imipenem /Ceftazidime Combination Assay

EDTA- Double Disc Synergy Test (EDTA-DDST)

This test exploits the inhibitory action of EDTA on MBL in order to detect MBL production by the test strain. The procedure was carried out as described by Lee K *et al*, 2001. An overnight liquid culture of the test strain, adjusted to a turbidity of a 0.5 McFarland standard was spread on a Mueller Hinton Agar (MHA) (Himedia Laboratories, Mumbai, India) plate. A 10 µg Imipenem (IPM) (Himedia Laboratories, Mumbai, India) disc was placed on the agar surface. A blank disc was kept on the inner surface of the lid of the MHA plate and 10 µL of sterile 0.5 mM EDTA was added to it to achieve a concentration of 750 µg. This EDTA disc was then placed on the MHA surface at a 10 mm edge-to-edge distance from the Imipenem disc. On the same plate a 30 µg Ceftazidime (CAZ) and an EDTA disc was placed in a similar fashion as described previously (Figure 2). The plate was then incubated overnight at 37°C. Presence of an expanded growth inhibition zone between the two discs was taken as a positive test for MBL production.

Modified Hodge Test for Carbapenemase detection

This test relies on Carbapenem inactivation by the test strain due to Carbapenemase production where an indicator strain like *E.coli* ATCC 25922 shows a distorted zone of inhibition. An overnight culture suspension of *E.coli* ATCC 25922 with turbidity adjusted to 0.5 McFarland standard is swabbed using a sterile cotton swab on a Mueller Hinton Agar plate. A 10 µg Imipenem disc is placed at the center of the plate and the test strain is streaked from the edge of the disc to the periphery of the plate in four different directions. The plate is then incubated at 37°C overnight. A 'cloverleaf' shaped zone of inhibition (Figure 3) due to carbapenemase production by the test strain is taken as a positive test.

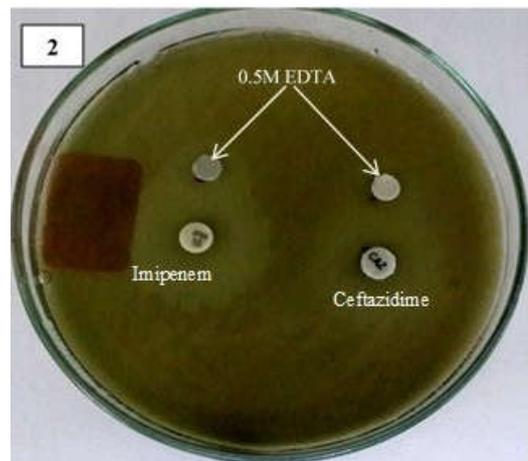


Fig. 2. EDTA-DDST. MBL producing *Pseudomonas aeruginosa* strain showing enhanced zone of inhibition with the EDTA disc

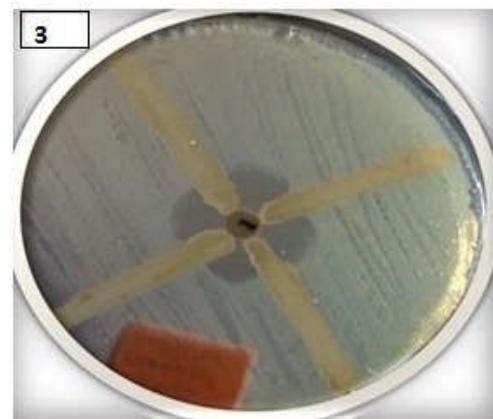


Fig. 3. Modified Hodge Test

Carbapenemase producing *Acinetobacter* strain showing cloverleaf shaped zone of inhibition.

Table 1. Results of the phenotypic tests for MBL/Carbapenemase production

Organism	EDTA Imipenem Combination (EIC) assay	EDTA Ceftazidime Combination (ECC) assay	Modified Hodge Test	EDTA Imipenem DDST	EDTA Ceftazidime DDST
<i>Pseudomonas aeruginosa</i> (n=54)	18 (33%)	21 (38%)	12 (22%)	41 (76%)	35 (65%)
<i>Acinetobacter</i> spp. (n=55)	29 (53%)	29 (53%)	22 (40%)	21(38%)	11(20%)

RESULTS

The study was carried out on Carbapenem resistant, strains of *Pseudomonas aeruginosa* (n=54) and *Acinetobacter* spp (n=55) isolated from different clinical specimens like blood, urine, wound swab etc. A few isolates of *Pseudomonas aeruginosa* (n=4) and *Acinetobacter* spp. (n=8) were found to be negative for both MBL and Carbapenemase production by all of the phenotypic tests employed in the study. The number of strains which tested positive by each of the methods employed is detailed in the Table-1. The results of EDTA-Imipenem Combination assay (EIC) and EDTA- Ceftazidime Combination (ECC) assay were taken into consideration for positivity of MBL. The results obtained are as presented in Table-1. We observed that some pyocyanin producing strains of *Pseudomonas aeruginosa* had an inhibitory effect on the *E.coli* ATCC 25922 indicator strain while performing the Modified Hodge test. Such results were noted as negative for Carbapenemase production. The EIC could detect MBL in 53% (n=29) strains of *Acinetobacter*spp. and in 33% (n=18) strains of *P. aeruginosa*. The ECC assay showed an overall better positivity rate of 38% (n=21) in strains of *P. aeruginosa* while in *Acinetobacter* spp. (53% (n=29)) it was as good as the EIC. Among strains of *P. aeruginosa*, the EDTA-IPM DDST was able to detect MBL in 76% (n=41) strains which was the highest among all the methods and EDTA-CAZ DDST was able to detect MBL in a comparably low 65% (n=35) strains. On the other hand, EDTA-IPM DDST and EDTA-CAZ DDST were able to detect MBL in only 38% (n=21) and 20% (n=11) strains of *Acinetobacter* spp. respectively. Rate of Carbapenemase detection by the Modified Hodge test was comparatively low in *P. aeruginosa* (22%, n=12) while in *Acinetobacter* spp. it was slightly better (40%, n=22).

DISCUSSION

Emergence of bacterial resistance to antibiotics is currently one of the most worrying developments in the field of antimicrobial therapeutics. Bacterial resistance due to production of Carbapenemases and Metallo β Lactamases (MBLs) is of great concern since the genes for these hydrolytic enzymes are carried chromosomally as well as on mobile genetic elements (transposons, plasmids etc.) thereby facilitating their rapid dissemination. Furthermore, Carbapenemase and MBL producing bacteria pose a therapeutic challenge as they are frequently known to be resistant to other β lactam antibiotics, aminoglycosides, macrolides (Strateva and Yordanov, 2009; Lee *et al.*, 2011) thereby further limiting treatment options. MBL producing bacteria are susceptible only to monobactams like aztreonam while Carbapenemase producing bacteria are susceptible to polymixin B and Colistin. (Urban *et al.*, 2010; Current Concepts in Antimicrobial Therapy against Resistant Gram-Negative Organisms, 2011) In this scenario, the early

detection of Carbapenemases and MBLs becomes important for the control of infection and prevention of spread of resistant organisms. While the CLSI has recommended standard tests for detection of β - lactamases and Carbapenemases in *Enterobacteriaceae*, it has not yet advocated any standard test for detection of MBLs and Carbapenemases in lactose non fermenting Gram negative bacilli. Some studies have advocated the use of molecular methods like PCR and multiplex PCR for detection of Carbapenemases and MBLs, but the sheer number of types of Carbapenemases and MBLs and the costs and skill involved make it infeasible for routine use. This study aimed at evaluating three phenotypic methods for detection of MBLs and Carbapenemases in Gram negative lactose non-fermenting bacilli i.e. *P.aeruginosa* and *Acinetobacter* spp. We employed EDTA based microbiological assay using Imipenem and Ceftazidime to detect MBLs from bacterial extract, EDTA DDST using Imipenem and Ceftazidime to detect MBLs from bacterial suspension and Modified Hodge test for Carbapenemase detection. The materials and skill required for performing these tests are those that are routinely available in any microbiological laboratory and no specialized equipment is required. Our results suggest that amongst the *P. aeruginosa* strains, the EDTA IPM DDST was able to detect MBL in most number of isolates (76%), which was the highest among all the methods used. EDTA CAZ DDST detected MBL in a comparably low 65% strains. MBL detection by EIC and ECC assays was comparatively low in *P. aeruginosa* isolates. The MHT could detect Carbapenemase in only 22% of *P. aeruginosa* isolates, which was the lowest among all three phenotypic methods. One of the reasons for this could be due to suspected false negatives of some of the pigment producing strains of *P. aeruginosa*. We noticed that some of these pigments were having an antibacterial effect on the indicator *E.coli* ATCC 25922 due to which the cloverleaf shaped zone of inhibition does not manifest. Some other studies have also reported similar observation and a few studies have reported that changing the indicator strain improves MHT for *P.aeruginosa*. (Pasteran *et al.*, 2011; Jeremiah *et al.*, 2014) We hypothesize that some compound of the pigment might interfere with the interaction between the Carbapenemase and Imipenem. Among *Acinetobacter* spp. isolates, ECC assay detected MBL in most number of strains (49%) followed closely by EIC assay (47%). The EIC and ECC assays showed a better concurrence with 19 isolates being positive by both assays. However, MBL positivity was low by both EDTA IPM DDST and EDTA CAZ DDST, with EDTA CAZ DDST detecting MBL in only 15% of strains, which was the lowest detection rate amongst both organisms. Carbapenemase detection using MHT was only slightly better as compared to *P.aeruginosa* strains.

While our study has found that the EDTA IPM DDST was the most effective method for detection of MBL in *Pseudomonas aeruginosa*, there are other studies that have reported the

EDTA combined disc test to be the best method. (Kumar *et al.*, 2011; Sharma *et al.*, 2015) This may be due to the prevalence of different strains in different geographical areas and also due to difference in interpretation of results, since the interpretation of DDST is more subjective, an opinion echoed by other authors as well. (Picao *et al.*, 2008; Pandya *et al.*, 2011) However, the DDST has been observed to be more specific than the combined disc test. (Khosravi *et al.*, 2012) Some authors like Singh *et al.* (2009), have found EDTA-IPM-DDST on par with other phenotypic methods like the combined disk test for MBL detection in *P. aeruginosa*. We found only one other study from India by Purohit *et al.* (2012) which has employed the EIC for MBL detection in *Acinetobacter* spp. They were able to detect MBL using EIC assay in 9.3% isolates. Our study is the first one to employ Ceftazidime in an EDTA based microbiological assay. Our study reports very low MBL detection rates using the EDTA-DDST, especially EDTA-CAZ-DDST in *Acinetobacter* spp. which is in contrast to that of the other studies. This may be attributed to the variation of strains in different institutions, type of strains and their response to the antibiotics and inhibitors. Thus, according to our results, the EDTA-IPM-DDST seems to be a better method for MBL detection in *P.aeruginosa*, whereas both EIC and ECC assays are equally good for MBL detection in *Acinetobacter* spp. MHT can be used strictly for screening of Carbapenemases in *Acinetobacter* spp. only while in *P.aeruginosa* it seems to be highly fallible and hence unsuitable.

REFERENCES

- Behera B, Mathur P, Das A, Kapil A, Sharma V. An evaluation of four different phenotypic techniques for detection of metallo-beta-lactamase producing *Pseudomonas aeruginosa*. *Indian J Med Microbiol.*, 2008 Jul-Sep;26(3):233-7.
- Curcio D. Multidrug-resistant Gram-negative bacterial infections: are you ready for the challenge? *CurrClinPharmacol.*, 2014 Feb;9(1):27-38.
- Current Concepts in Antimicrobial Therapy against Resistant Gram-Negative Organisms: Extended-Spectrum β -Lactamase-Producing Enterobacteriaceae, Carbapenem-Resistant Enterobacteriaceae, and Multidrug-Resistant *Pseudomonas aeruginosa*. Kanj SS, Kanafani ZA. *Mayo Clin Proc.*, 2011 Mar;86(3):250-9.
- De AS, Kumar SH, Baveja SM. Prevalence of metallo- β -lactamase producing *Pseudomonas aeruginosa* and *Acinetobacter* species in intensive care areas in a tertiary care hospital. *IndianJCritCareMed.*, 2010 Oct;14(4):217-9.
- Drawz SM, Bonomo RA. Three decades of β -lactamase inhibitors. *ClinMicrobiol Rev.*, 2010; 23: 160–201.
- Jeremiah SS, Balaji V, Anandan S, Sahani RD. A possible alternative to the error prone modified Hodge test to correctly identify the carbapenemase producing gram negative bacteria. *Indian J Med Microbiol.*, 2014 Oct-Dec;32(4):414-8.
- Jesudason MV, Kandathil AJ, Balaji V. Comparison of two methods to detect carbapenemase & metallo-beta-lactamase production in clinical isolates. *Indian J Med Res.*, 2005 Jun; 121(6):780-3.
- Kabbaj, H., Seffar, M., Belefquih, B., Akka, D., Handor, N., Amor, M., Alaoui, A. E. Prevalence of metallo- β -lactamases producing *Acinetobacterbaumannii* in a Moroccan Hospital. *ISRN Infect. Dis*, 2013, pp:1-3.
- Karthika UR, Rao SR, Sahoo S, Shashikala P, Kanungo R, Jayachandran S, Prashanth K. Phenotypic and genotypic assays for detecting the prevalence of metallo-beta-lactamases in clinical isolates of *Acinetobacterbaumanii* from a South Indian tertiary care hospital. *J Med Microbiol.*, 2009;54:430–5.
- Khosravi Y, Loke MF, Chu EG, Tay ST, Vadivelu J. Phenotypic Detection of Metallo β -lactamase in Imipenem – Resistant *Pseudomonas aeruginosa*, *Scientific World, Journal* 2012;2012:654939.
- Kumar SH, De AS, Baveja SM, Gore MA. Prevalence and risk factors of Metallo β -lactamase producing *Pseudomonas aeruginosa* and *Acinetobacter* species in burns and surgical wards in a tertiary care hospital. *J Lab Physicians*, 2012 Jan;4(1):39-42.
- Kumar V, Sen MR, Anupurba S, Prakash P, Gupta R. An observational study of metallo beta lactamase production in clinical isolates of *Pseudomonas aeruginosa*: An experience at a Tertiary care Hospital in North India. *Indian J. Prev. Soc. Med.*, 2011. 42 (2): 173-176.
- Lee K, Lim YS, Yong D, Yum JH, Chong Y. Evaluation of the Hodge test and the imipenem-EDTA double-disk synergy test for differentiating metallo-beta-lactamase-producing isolates of *Pseudomonas* spp. and *Acinetobacter* spp. *J ClinMicrobiol.*, 2003 Oct;41(10):4623-9.
- Lee K, Yong D, Jeong SH, Chong Y. Multidrug-resistant *Acinetobacter* spp.: increasingly problematic nosocomial pathogens. *Yonsei Med J.*, 2011 Nov;52(6):879-91.
- Livermore DM. beta-Lactamases in laboratory and clinical resistance. *ClinMicrobiol Rev.* 1995 Oct;8(4):557-84.
- Lucena A, Dalla Costa LM, Nogueira KS, Matos AP, Gales AC, Paganini MC, Castro ME, Raboni SM. Nosocomial infections with metallo-beta-lactamase-producing *Pseudomonas aeruginosa*: molecular epidemiology, risk factors, clinical features and outcomes. *J Hosp Infect.*, 2014 Aug;87(4):234-40.
- Marchiaro P, Mussi MA, Ballerini V, Pasteran F, Viale AM, Vila AJ, Limansky AS. Sensitive EDTA-based microbiological assays for detection of metallo- β -lactamases in nonfermentative gram-negative bacteria. *J ClinMicrobiol.*, 2005 Nov;43(11):5648-52.
- Navaneeth BV, Sridaran D, Sahay D, Belwadi MR. A preliminary study on metallo-beta-lactamase producing *Pseudomonas aeruginosa* in hospitalized patients. *Indian J Med Res.*, 2002 Dec; 116:264-267.
- Noyal MJ, Menezes GA, Harish BN, Sujatha S, Parija SC. Simple screening tests for detection of carbapenemases in clinical isolates of nonfermentative Gram-negative bacteria. *Indian J Med Res.*, 2009 Jun;129(6):707-12.
- Pandya NP, Prajapati SB, Mehta SJ, Kikani KM, Joshi PJ. Evaluation of various methods for detection of Metallo β -lactamase (MBL) production in gram negative bacilli. *Int.J. Biol. Med. Res.*, 2011.2(3):775-777.
- Papp-Wallace KM, Endimiani A, Taracila MA, Bonomo RA. Carbapenems: past, present, and future. *Antimicrob Agents Chemother.*, 2011 Nov; 55(11):4943-60.

- Pasteran F, Veliz O, Rapoport M, Guerriero L, Corso A. Sensitive and specific modified Hodge test for KPC and metallo-beta-lactamase detection in *Pseudomonas aeruginosa* by use as a novel indicator strain, *Klebsiella pneumoniae* ATCC 700603. *J Clin Microbiol.*, 2011 Dec;49(12):4301-3.
- Picao RC, Andrade SS, Nicoletti AG, Campana EH, Moraes GC, Mendes RE, Gales AC. Metallo β -lactamase detection: comparative evaluation of double-disk synergy versus combined disk tests for IMP-,GIM-,SIM-,SPM-,or VIM-producing isolates. *J Clin Microbiol.*, 2008 Jun;46(6): 2028-37.
- Pitout JD, Chow BL, Gregson DB, Laupland KB, Elsayed S, Church DL. Molecular epidemiology of metallo- β -lactamase-producing *Pseudomonas aeruginosa* in the Calgary Health Region: emergence of VIM-2-producing isolates. *J Clin Microbiol.*, 2007;45(2):294-8.
- Purohit M, Mendiratta DK, Deotale VS, 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 Microbiol.*, 2012 Oct-Dec; 30(4):456-61.
- Queenan AM, Bush K. Carbapenemases: the versatile betalactamases. *Clin Microbiol Rev.*, 2007; 20 : 440-58.
- Rajput A, Prajapati B, Chauhan B, Shah A, Trivedi T, Kadam M. Prevalence of Metallo-beta-lactamases (MBL) producing *Pseudomonas aeruginosa* in a Tertiary care Hospital. *Indian. J. Basic. Appl. Med. Res.*, 2012. 1(4): 304-308.
- Ramakrishnan K, Rajagopalan S, Nair S, Kenchappa P, Chandrakesan SD. Molecular characterization of metallo β -lactamase producing multidrug resistant *Pseudomonas aeruginosa* from various clinical samples. *Indian J Pathol Microbiol.*, 2014; 57:579-582.
- Rit K, Chakraborty B, Dey R, Chakraborty P, Naha A, Saha R. Prevalence of *Pseudomonas aeruginosa* and *Acinetobacter* spp producing metallo-c in a tertiary care hospital. *J NTR Univ Health Sci.*, 2013;2 (1):18-21
- Sharma S, Sikka R, Deep A, Mittal S, Sharma A, Chaudhary U. Comparative study of three phenotypic methods for detection of Metallo β -lactamase in clinical isolates of *Pseudomonas aeruginosa*. *Int. J. Curr. Microbiol. App. Sci.*, 2015.4(4):366-370.
- Singh SP, Shariff M, Barua T, Thukral, SS. Comparative evaluation of phenotypic tests for identification of Metallo β -lactamase producing clinical isolates of *c. Indian Journal of Medical Research*, 2009;129(6):713-5
- Strateva T, Yordanov D. *Pseudomonas aeruginosa*- a phenomenon of bacterial resistance. *J Med Microbiol.*, 2009 Sep; 58(Pt9):1133-48.
- Urban C, Mariano N, Rahal JJ. In vitro double and triple bactericidal activities of doripenem, polymyxin B, and rifampin against multidrug-resistant *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherichia coli*. *Antimicrob Agents Chemother.* 2010;54:2732-2734.
- Walsh TR. The emergence and implications of metallo- β -lactamases in Gram-negative bacteria. *Clin Microbiol Infect*, 2005; 11 (Suppl 6) : S2-9.
