CATRINA (2021), 21(1):75-80

Traditional and Molecular Gene Detection (blaIMP-1 and *blaIMP*) of Multi-drug Resistant *Acinetobacter baumannii*

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ABSTRACT

Acinetobacter bacteria are widely resistant to β-lactam antibiotics. The formation of carbapenemases such metallo-β lactamases (MBLs), which hydrolyze a variety of β-lactams including penicillin, cephalosporins, and carbapenems, is one of the primary causes of resistance in *Acinetobacter baumannii*. MBL-producing carbapenem-resistant strains have been detected all over the world in recent years, and at a rising pace. For this investigation, fifty-two *A. baumannii* isolates were chosen based on imipenem (IMP) resistance (MIC >16 g/ml). The Modified Hodge test (MHT) and the CDDT were used to detect MBL phenotypic expression (Combine Disk Diffusion Test). PCR was used to detect genotypic expressions of the blaIMP-1 and blaIMP genes in all metallo-lactamase-producing *A. baumannii* strains. According to the MHT test, 49 of 52 *A. baumannii* isolates (94.2%) produced carbapenemase, whereas the CDDT test revealed that 47 isolates (90.4%) produced MBL. Despite being negative for MBL-producer in the phenotypic technique used for control isolates, 39 (75%) of 52 putative MBL- producer isolates were positive for the blaIMP-1 gene by PCR, while fifteen *A. baumannii* isolates (28.8%) were positive for the blaIMP gene by PCR. In 23% (12/52) of instances, the blaIMP-1 and blaIMP genes were found together. The genotypic approach must be used to confirm isolates of *A. baumannii* that have been identified as MBL-producers using the MHT test and the Combine Disk Diffusion Test.
Keywords: *Acinetobacter baumannii*, Metallo β-lactamase (MBL) Multidrug resistant, Imipenem resistance

INTRODUCTION

Acinetobacter baumannii is a glucose-non-fermentative, Gram-negative coccobacillus that has emerged in recent years as a main cause of nosocomial infections associated with elevated morbidity and mortality (Zarrilli *et al.*, 2013). *A. baumannii* is an opportunistic infection with a high occurrence among immunocompromised people, especially those who spend a lot of time in hospitals. It has been identified as a red alert human pathogen in recent years, causing concern among medical professionals due to its wide range of antibiotic resistance (Howard *et al.*, 2012). The most common and serious multidrug resistant (MDR) pathogens have been encompassed within the acronym ESKAPE, standing for *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *A. baumannii*, *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Enterobacter spp*. According to Centre for Disease Control (CDC) the six ESKAPE bacteria cause two third of all hospital acquired infections (Howard *et al.*, 2012 and Ahir *et al.*, 2012).

It is generally known that MBLs are Ambler class ß metallo-enzymes that are resistant to clavulanic acid. They require zinc as a cofactor for enzymatic activity, and Ethylene Di-amine Tetra Acetic Acid (EDTA) and other metal ion chelating agents decrease their action. *Pseudomonas spp*. and *Acinetobacter* spp. are the most important nosocomial pathogens with multiple drug resistance (Corvec *et al.*, 2003)

Carbapenems are considered the last-line drugs for treatment of infections caused by multiresistant (MR) Gram-negative bacilli (Sacha *et al.*, 2007). Recently, the emergence of carbapenem-resistant organisms such as *P. aeruginosa* and *A. baumannii* has become a major therapeutic challenge. Carbapenem resistance due to acquired MBLs is more serious than other resistance mechanisms because MBLs can hydrolyze all β-lactam antibiotics except monobactams. In addition, MBL-encoding genes on integrons can easily be passed between strains (Yousefi *et al.*, 2010).

So far, world widely there are five main categories of MBLs have been described, IMP hydrolyzing β-lactamase, VIM-Verona integron-encoded metallo-β-lactamases, GIM-German Imipenemase, SPM-Sao Paulo metallo-β-lactamases, and SIM-Seoul imipenemase enzymes. Recently, A novel MBL has been designated in *P. aeruginosa* from Australia-*bla*AIM-1. There are no standard guidelines by Clinical Laboratory Standards Institute's (CLSI) for detection of these enzymes in various bacteria (Lee *et al.*, 2011).

The aim of current research was to investigate the presence of metallo-beta-lactamase production among beta-lactam resistant *A. baumannii* and to compare results gathered from phenotypic and genotypic methods. Further, for molecular analysis of target genes (*bla*IMP and *bla*IMP-1 genes) encoding for metallo-beta-lactamase with specific primers by polymerase chain reaction.

MATERIALS AND METHODS

Isolate selection and Antibiotic Susceptibility

A total of 52 non-repetitive imipenem and meropenem resistant *A. baumannii* strains were isolated from clinical samples (endotracheal aspirates, sputum, and urine) of different patients. The isolates were identified by conventional methods (Schreckenberger *et al.*, 2003). Identification and antibiotic susceptibility analysis of the strains were performed by VITEK 2 system (bioMerieux, Marcy l'Etoile, France) according to criteria mentioned by bioMérieux (bioMérieux 2010). Strains were stored in 20% glycerol at -80°C.

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Phenotypic Detection of MBLs

Modified Hodge Test (MHT)

A 0.5 McFarland dilution of *Escherichia coli* ATCC 25922 was prepared. A 1:10 dilution inoculated onto a Mueller Hinton Agar (MHA) plate (Merck, Darmstadt, Germany) and a 10 µg IMP (Imipenem) disk was placed in the plate center, while strains of *A. baumannii* were streaked in a straight line from the edge of the disk to plate margins. After overnight incubation, if the inoculates had carbapenemases, the test showed a cloverleaf-like indentation of *E. coli* growing along the test bacterium growth streak within IMP disk diffusion zone (Lee *et al.*, 2001). Each run using MHT-Positive *Klebsiella pneumoniae* (ATCC BAA-1705) and MHT-Negative *Klebsiella pneumoniae* (ATCC BAA-1706).

Combined disc diffusion test (CDDT)

EDTA-IMP disks were prepared by adding EDTA solution (10µl of 0.1M EDTA) to 10µg-IMP (imipenem) disks. Bacterial isolates were adjusted according to McFarland 0.5 turbidity standard and were inoculated to Mueller Hinton agar. A 10-µg- imipenem disk and imipenem disc with EDTA were placed on Mueller Hinton agar. After overnight incubation, the established zone diameter difference of ≥ 4 mm between IMP disk and IMP + EDTA was referred to synergy positive effect of EDTA (Franklin *et al.*, 2006).

Molecular Detection of *bla*IMP-1 and *bla*IMP Genes

DNA was extracted from the bacterial isolates using QIAamp DNA Kits (Qiagen, Germany) according to manufactures instruction. PCR assay was run using the *bla*IMP-1 primers: *bla*IMP-1-F (5′CATGG TTTGG-TGGTTCTTGT-3′) and *bla*IMP-1 R(5′ATAATTTGG-CGGACTTTGGC-3′) as described by Yum *et al.* (2002); Lee *et al.*, (2005) and *bla*IMP primers: *bla*IMP-F(5'CGGCCGTCAGGAG ACG GTCTTT-3') *bla*IMP-R(5'-AACCAGTTTTGCCTTTACCTAT-3') as described by Aktas and Kayacan (2008). PCR was carried out within a 50 µl reaction mix containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100, 200 µM of each of the deoxynucleotide triphosphate, 0.4 µM of each primer, 1 U of *Thermus aquaticus* DNA polymerase (Pharmacia), and 5 µl of template DNA. All tubes were transferred into thermal cycler. The PCR was started as in the following program. The initial denaturation for 5 minutes at 94°C. Thirty five cycles of: A-denaturation (94°C for 30 secs), B-annealing (at 55°C - 30 secs) for *bla*IMP-1 and *bla*IMP genes, C- extension (72°C for 45 secs) and Final exten-sion (72°C for 7 minutes). Hold temperature (4°C for 10 minutes). The PCR product of 620 bp for *bla*IMP-1 and 587 bp for *bla*IMP was visualized by 2% agarose gel electrophoresis with Novel Juice (Novel Juice 2012).

RESULTS

Bacterial cultures, colony characterization, Gram staining, microscopic examination, and species identification by VITEK-2 were done. A total of 52 isolates were identified as *Acinetobacter baumannii*. Carbapenem resistance was observed in 52 *A. baumannii* clinical isolates by broth microdilution MIC (minimal inhibitory concentration) using the VITEK-2 automated system with VITEK card: AST-N204 (Garcia 2010).

Table (1): Antimicrobial sensitivity of MBL producing-*A. baumannii*

Antibiotic used	Antibacterial class	No of isolates and represented %	
		Resistant	Sensitive
Amoxicillin/Clavulanic	ß-lactam/inhibitor combination	51(98.1%)	1(1.9%)
Piperacillin/Tazobactam	Extended spectrum – β lactams	51(98.1%)	1(1.9%)
Cefotaxime	Third generation Cephalosporin	50(96.2%)	2(3.8%)
Ceftazidime	Third generation Cephalosporin	50(96.2%)	2(3.8%)
Imipenem	Carbapenems	52(100%)	0 (0.0%)
Meropenem	Carbapenems	52(100%)	0 (0.0%)
Amikacin	Aminoglycosides	45(86.5%)	7 (13.5%)
Gentamycin	Aminoglycosides	47(90.4%)	5(9.6%)
Ciprofloxacin	Fluoroquinolone	51(98.1%)	1(1.9%)
Ofloxacin	Fluoroquinolone	51(98.1%)	1(1.9%)
Colistin	polymyxin	0 (0.0%)	52(100%)

Using the VITEK-2 system method to test the susceptibility of *A. baumannii* isolates to different antibiotics, according to the Clinical Laboratory Standards Institute's (CLSI) guidelines (CLSI, 2017) we found that; the highest sensitivity of *A. baumannii* isolates was for Colistin (100% of isolates). Followed by Amikacin (13.5% of isolates) then Gentamycin by (9.6% of isolates). The lowest sensitivity of *A.baumannii* was for Amoxicillin/Clavulanic, Piperacillin/Tazobactam, Ciprofloxacin and Ofloxacin (1.9%) then Cefo-

taxime and Ceftazidime with 3.8% (Table 1). These carbapenem resistant *A.baumannii* isolates were tested Modified Hodge Test (MHT) for production of carbapenemase and 49 (94.2 %) were carbapenemase producers (Figure 1).

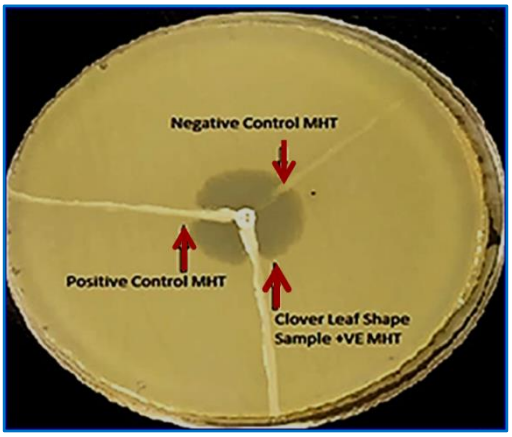

Figure (1): Modified Hodge Test (MHT) showing positive and negative test.

The carbapenem resistant isolates were also tested for MBL production and forty-seven (90.4%) of these isolates gave positive result by CDDT, (Figure 2, A). The difference in zone diameter between IMP disc and IMP + EDTA of 4 mm was evaluated as EDTA synergy positive (the presence of an enlarged zone of inhibition was interpreted as EDTA-synergy test positive). Five isolates were MBL negative in IPM-EDTA-disk synergy test (Figure 2, B). Thirty-nine (75%) of MBL producer isolates of *A. baumannii* (out of 52 isolates) were positive for blaIMP-1 by PCR, while fifteen (28.8%) isolates were positive for blaIMP gene only. The co-appearance of *bla*IMP-1 and *bla*IMP genes in 23% (12/52) of cases (Figures 3 and 4) were reported. No *bla*IMP genes were found in isolates negative by the phenotypic test (CDDT).

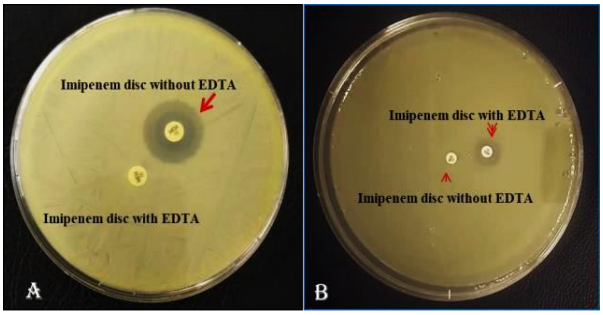

Figure (2): The combine disk diffusion test (CDDT) for metallo-β- lactamases (MBL) production. A, Positive CDDT with inhibition zone >4mm; B, negative CDDT.

DISCUSSION

The multidrug resistance is now a worldwide problem with the increasing of antibiotic abuse that more and more selects for resistant strains. Carbapenem resistance in *Acinetobacter baumannii* strains has been on the rise for the past decade, and it has become a major public health concern (Falagas *et al.*, 2006 and Brusselaers *et al.*, 2011). *A. baumannii* is an opportunistic pathogen that is a leading cause of respiratory infections, particularly nosocomial and ventilator-acquired pneumonia (VAP). Recently there is a rise in community acquired infections induced by *A. baumannii*. The *A. baumannii* occurrence among hospitalized patients depends on the hospital populations, types of performed interventions and procedures done (Giamarellou *et al.*, 2008 and Howard *et al.*, 2012).

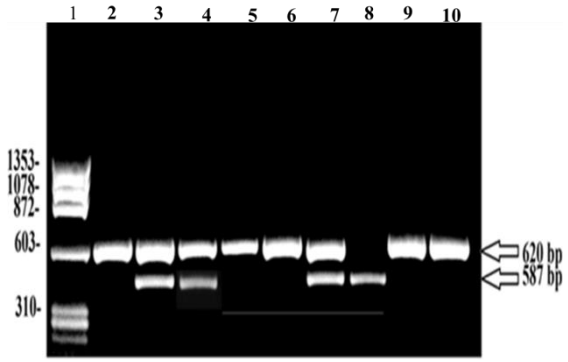

Figure (3): PCR detection of the *blaIMP*-1 and *blaIMP* genes. Lane 1: DNA ladder, lanes 2 to10: multiplex PCR products of Acinetobacter isolates. *blaIMP-1* was detected in all lanes except lane 8 was positive *bla IMP* - gene only. Lanes 3, 4 and 7 positive blaIMP-1 and *blaIMP* gens.

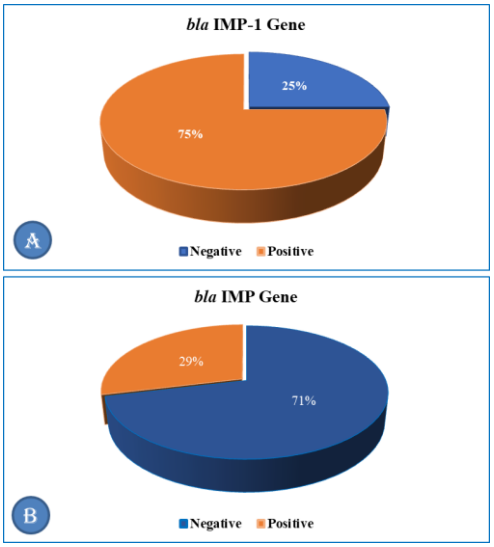

Figure (4): Distribution of *bla*IMP-1 and *bla*IMP genes in *A. baumannii* isolates

Detection of MBL production poses considerable technical difficulties, including differing results due to different MHA agar brands and lack of confirmatory criteria other than genetic analysis. Many phenotypic methodologies for detecting MBL-producing isolates are available; however the CLSI Institute has not endorsed a uniform procedure for MBL screening. Currently, the technique using a disc with IMP plus 750 µg of EDTA (combined disc method) is simple to perform and highly sensitive in differentiating MBL-producing isolates (Yousefi *et al.*, 2010 and Yong *et al.*, 2002). MHT was used to screen the Meropenem and Imipenem resistant strains for carbap-

enemase production, and 94.2 percent of them were found to be carbapenemase producers. According to Kumar *et al.* (2011), the MHT detected carbapenemase producers in 71% of the isolates. This was consistent with the findings of Lee *et al.* (2003) in Korea, who discovered carbapenemase positive isolates in 73 % of the isolates using the MHT.

The carbapenem resistant isolates were further screened for MBL production, 90.4% were positive by CDDT. Similar study conducted by Pandya *et al.* (2011) showed that 96.30% of strains were MBL positive by CDDT and 81.4% were positive by DDST. The findings of this study agree with those of Irfan *et al.* (2008), who found that 96.6 percent of carbapenem- resistant bacteria produced MBL when exposed to CDDT at Aga Khan University in Karachi. Similar findings were found with the study conducted by Noori *et al.* (2014), in which 86.8%of isolates were identified as MBL producers by CDDT.

PCR assay was carried out by utilizing previously published primers for amplification of genes encoding carbapenemases (*bla*IMP and *bla*IMP-1 genes). MBLs are less commonly identified in *A. baumannii* than the OXA-type carbapenemases but their carbapenem- hydrolyzing activities are 100–1000-fold more potent. Their presence in MDR *A. baumannii* isolates is in some instances difficult to detect, indicating that their contribution to the carbapenem resistance may be underestimated (Zarrilli *et al.*, 2013).

The high percentage for *bla*IMP-1 gene in present study (75%), confirmed by many studies in different percentages, in a surveillance study in 2003-2004, MBLs were detected in 135 of 545 (24.8%) IMP-resistant *A. baumannii* isolates, the proportion of *bla*IMP-1 was 61% (Lee *et al.*, 2011). In research published in 2006, *bla*IMP-1was found in 15 (48.4%) of 31 carbapenem-resistant *A. baumannii* isolates (Sung *et al.*, 2008). More than half of the isolates (55 percent) exhibited a positive *bla*IMP-1 in another investigation conducted by (Tognim *et al.*, 2006) at a Teaching Hospital in Brazil. The proportion of *bla*IMP-1-producing *A. baumannii* isolates among carbapenem-resistant strains grew from 0% in 1993-1997 to 29% in 1998 and 100% in 1999-2001, according to the same study.

The high percentage for *bla*IMP-1–producing strains of *A. baumannii* in present study (75%), indicating that this important mechanism of antimicrobial resistance was disseminated among distinct clones. A major contributing factor in the MDR emergence strains of *A. baumannii* is the acquisition and transfer of antibiotic resistance via plasmids and mobile genetic elements, including transposons and integrons (Sung *et al.*, 2008).

Fifteen (28.8%) *A. baumannii* out of 52 presumptive MBL producer isolates (with isolates were negative for MBL producer in control by phenotypic technique) were positive for *bla*IMP gene by PCR (Fig-4). No *bla*IMP genes were found in isolates negative by CDDT and MHT tests.

Previous research has reported that presence of *bla*IMP gene in the Acinetobacter species in low percentages (5.12%) as in the study of Hwa, (2008). The prevalence of the metallo-β-lactamase genes (*bla*IMP gene) is generally low within *A. baumannii* isolates as illustrated in a study by Mendes *et al.*, (2009) where the prevalence was 0.8% in Taiwan. Other research could not detected *bla*IMP genes (Aktas and Kayacan 2008; Mohamed and Raafat 2011; Ehlers *et al.*, 2012, and Purohit *et al.*, 2012).

The isolates which were positive MBL production by confirmatory test but negative for *bla*IMP amplification may have variant *bla*IMP or *bla*SIM genes (Uma *et al.*, 2009). This was established by the current study, by the presence of the *bla*IMP1 gene in proportion (75%). The IMP-resistant *Acinetobacter baumannii* strains in present study with no phenotypic or genotypic sign of MBL production may possess other enzymes mediating carbapenem resistance, such as OXA-type lactamases (class-D) or AmpC ß- Iactamases and other mechanisms such as outer- membrane permeability (OMP) and efflux mechanisms (Mohamed and Raafat 2011) .The mechanism of cleavage of ß-lactam ring is different for MBL's as compared to ß-lactamases; however, both gene products still share a unique αßßα fold in the active sites of the enzymes. The *bla*IMP gene is a foreign gene transferred from another bacterial species, and *A. baumannii* only retains it in situations where there is selective pressure in the form of IMP (Ehlers *et al.*, 2012). The coexistence of *bla*IMP-1 with *bla*IMP genes in current research 23% (12 out of 52) of cases exemplify the extraordinary ability presented by *A. baumannii* to acquire multiple resistance mechanisms.

CONCLUSION

According to the findings of this study, resistance to IMP was found to be a better indicator of MBL formation. The MHT and CDDT tests appeared to be useful in separating MBL from non-metalloenzyme producers. Most *A. baumannii* strains were found to produce metallo beta-lactamase using the IPMEDTA-disk synergy test and PCR for the *bla*IMP-1 gene (MBL). Finally, the widespread misuse, overuse, and exploitation of various antibiotics by healthcare professionals or patients may be to contribute for the growth in carbapenem resistance; hence, lowering antibiotic use aims to reduce costs and damage caused by *A. baumannii*.

REFERENCES

AHIR, H.R., P.H.PATEL, R.A. BERRY, R. PARMAR, S.T. SONI, P.K. SHAH, M.M. VEGAD, AND S. PATIL. 2012. Prevalence of Metallo-β-lactamases producing Pseudomonas and Acinet-obacter species in

tertiary care teaching hospital, Ggujarat. International Journal of Microbiology Research. 4(9):322-325.

AKTAS¸ Z., AND C.B. KAYACAN. 2008. Investigation of metallo-beta-lactamase producing strains of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* by E-test, disk synergy and PCR. Scandinavian Journal of Infectious Diseases. 40: 320-325.

BIOMÉRIEUX. 2010. VITEK® 2 Systems Product Information. bioMérieux, Inc. USA.

BRUSSELAERS, N., D. VOGELAERS, AND S. BLOT. 2011. The rising problem of antimicrobial resistance in the intensive care unit. Ann Intensive Care. 2011; 1:47.

CORVEC, S., N. CAROFF, E. ESPAZE, C. GIRAUDEAU, H. DRUGEON, AND A. REYNAUD. 2003. AmpC cephalosporinase hyperproduction in *Acinetobacter baumannii* clinical strains. Journal of Antimicrobial Chemotherapy. 52: 629-635.

EHLERS, M.M., J.M. HUGHES, AND M.M. KOCK. 2012. Prevalence of Carbapenemases in *Acinetobacter baumannii*. In: Antibiotic Resistant Bacteria-A Continuous Challenge in the New Millennium. By: Dr. Marina Pana. (Ed.). InTech.Rijeka, Croatia.

FALAGAS, M.E., P.K. KOLETSI, AND I.A. BLIZIOTIS. 2006. The diversity of definitions of multidrug resistant (MDR) and pandrug-resistant (PDR) *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. J Med Microbiol. Dec;55(Pt 12):1619- 29.

FRANKLIN, C., L. LIOLIOS, AND A.Y. PELEG. 2006. Phenotypic Detection of Carbapenem-Susceptible Metallo-ß-Lactamase-Producing Gram-Negative Bacilli in the Clinical Laboratory. J Clin Microbiol; 44:3139–44.

GARCIA, L.S. 2010. Staining Procedures. In: Clinical Microbiology Procedures Handbook (3rd ed.). American Society of Microbiology.

GIAMARELLOU, H., A. ANTONIADOU, AND K. KANELLAKOPOULOU. 2008. *Acinetobacter baumannii*: a universal threat to public health? Int J Antimicrob Agents. 32(2):106-19.

HOWARD, A., M. O'DONOGHUE, A. FEENEY, AND R.D. SLEATOR. 2012. *Acinetobacter baumannii* an emerging opportunistic pathogen. Virulence. 3(3): 243–250.

HWA, W.E. 2008. Mechanisms of resistance to carbapenems in Acinetobacter species. PhD thesis. Faculty of Medicine, University of Malaya, Malaysia.

IRFAN, S., A. ZAFAR, D. GUHAR, T. AHSAN, AND R. HASAN. 2008. Metallo β -lactamase-producing clinical isolates of *Acinetobacter* species and *Pseudomonas aeruginosa* from intensive care unit patients of a tertiary care hospital," Indian Journal of Medical Microbiology, 26, (3): 243–245.

KUMAR, A. V., V. S. PILLAI, K. DINESH, AND S. KARIM. 2011. The phenotypic detection of carbapenemase in meropenem resistant *Acinetobacter calcoaceticus-baumannii* complex in a tertiary care hospital in South India," Journal of Clinical and Diagnostic Research, vol. 5, no. 2, pp. 223–226, 2011.

LEE, K., D. YONG, S.H. JEONG, AND Y. CHONG. 2011. Multidrug-Resistant Acinetobacter spp.: Increasingly Problematic Nosocomial Pathogens. Yonsei Med J. 52(6):879-891.

LEE, K., J.H. YUM, D. YONG, H.M. LEE, H.D. KIM, J.D. DOCQUIER, G.M. ROSSOLINI, AND Y. CHONG. 2005. Novel acquired metallo-β-lactamase gene, blaSIM-1, in a class 1 integron from *Acinetobacter baumannii* clinical isolates from Korea. Antimicrob Agents Chemother. 49:4485–91.

LEE, K., Y. CHONG, H.B. SHIN, Y.A. KIM, D. YONG, J.H. YUM. 2001. Modified Hodge and EDTA-disk synergy tests to screen metallo-β-lactamase-producing strains of *Pseudomonas* and *Acinetobacter* species. Clin Microbiol Infect; 7:88- 91.

LEE, K., Y.S. LIM, D. YONG, J.H. YUM, AND Y. CHONG. 2003. Evaluation of the Hodge test and the imipenem-EDTA double-disk synergy test for differentiating metallo β -lactamase-producing isolates of *Pseudomonas* spp. and *Acinetobacter* spp.," Journal of Clinical Microbiology, 41(10): 4623–4629.

MENDES, R.E., J.M. BELL, J.D. TURNIDGE, M. CASTANHEIRA, AND R.N. JONES. 2009. Emergence and widespread dissemination of OXA-23, -24/40 and -58 carbapenemases among *Acinetobacter* spp. in Asia-Pacific nations: report from the SENTRY Surveillance Program. Journal of Antimicrobial Chemotherapy.63: 55-59.

MOHAMED, N. AND D. RAAFAT. 2011. Phenotypic and Genotypic Detection of Metallo-beta-Iactamases in Imipenem-resistant *Acinetobacter baumannii* Isolated from a Tertiary Hospital in Alexandria, Egypt. Research Journal of Microbiology. 6 (10): 750-760.

NOORI, M., A. KARIMI, F. FALLAH, A. HASHEMI, S. ALIMEHR, H. GOUDARZI, AND S. AGHAMOHAMMAD. 2014. High prevalence of metallo- beta-lactamase producing *Acinetobacter baumannii* isolated from two hospitals of Tehran, Iran," Archives of Pediatric Infectious Diseases, 2(3): e15439.

NOVEL JUICE. 2012. www.genedirex.com.

PAN-DYA, P. N., B.S. PRAJAPATI, J.S. MEHTA, M.K. KIKANI, AND J. P. JOSHI. 2011. Evaluation of various methods for detection of MBL (MBL) production in Gram negative bacilli," International Journal of Biological and Medical Research, 2(3): 775–777.

PUROHIT, M., D.K. MENDIRATTA,V.S. EOTALE, M. MADHAN, A. MANOHARAN, AND P. NARANG. 2012. Detection of metallo-β- lactamases producing *Acinetobacter baumannii* using microbiological assay, disc synergy test and PCR. Indian Journal of Medical Microbiology. 30(4): 456-461.

SACHA, P., M. ZÓRAWSKI, T. HAUSCHILD, P. WIECZOREK, J. JAWOROWSK P JAKONIUK,

AND E. TRYNISZEWSKA. 2007. The presence of *bla*IMP genes on plasmids DNA isolated from multidrug-resistant Pseudomonas aeruginosa strains at University Hospital in Bialystok (Poland)—first report. Folia Histochem Cytobiol.45:405–408.

SCHRECKENBERGER, P. C., M. I. DANESHVAR, R. S. WEYANT, AND D. G. HOLLIS. 2003. *Acinetobacter*, *Chryseobacterium*, *Moraxella*, and other non-fermentative Gram-negative rods, p. 749–779. In P. R. Murray, E. J. Baron, J. H. Jorgensen, M. A. Pfaller, and R. H. Yolken (ed.), Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.

SUNG, J.Y., K.C. KWON, J.W. PARK, Y.S. KIM, J.M. KIM, K.S. SHIN, J.W. KIM, C.S. KO, S.Y. SHIN, J.H. SONG, AND S.H. KOO. 2008. Dissemination of IMP-1 and OXA type β-lactamase in carbapenem-resistant Acinetobacter baumannii. Korean J Lab Med. 28:16-23.

TOGNIM, M.C.B., A.C. GALES, A.P. PENTEADO, S. SUZANE, AND H.S. SADER. 2006. Dissemination of IMP-1 Metallo-b-Lactamase–Producing Acinetobacter species in a Brazilian Teaching Hospital. Infect Control Hosp Epidemiol. 27(7):742-747.

UMA KARTHIKA, R., R. SRINIVASA RAO, S. SAHOO, P. SHASHIKALA, R. KANUNGO, S. JAYACH-ANDRAN, AND K. PRASHAN-TH.2009. Phenotypic and genotypic assays for detecting the prevalence of metallo-ß-lactamases in clinical isolates of *Acinetobacter baumannii* from a South Indian tertiary care hospital. Journal of Medi-. Cal Microbiol. 58: 430-435.

YONG, D., K. LEE, J.H. YUM, H.B. SHIN, G.M. OSSOLINI, AND Y. CHONG. 2002. Imipenem-EDTA Disk Method for Differentiation of Metallo β-Lactamase Producing Clinical Isolates of Pseudomonas spp. and Acinetobacter spp. journal of clinical microbiology. 40(10): 3798-3801.

YOUSEFI, S., S. FARAJNIA, M.R. NAHAEI, M.T. AKHI, R. GHOTASLOU, M.H. SOROUSH, B. NAGHILI, AND N.H. JAZANI. 2010. Detection of metallo-β-lactamase–encoding genes among clinical isolates of *Pseudomonas aeruginosa* in northwest of Iran. Diagnostic Microbiology and Infectious Disease. 68:322–325.

YUM, J.H., K. YI, H. LEE, D. YONG, K. LEE, J.M. KIM, G.M. ROSSOLINI, AND Y. CHONG. 2002. Molecular characterization of metallo- β - lactamase-producing *Acinetobacter baumannii* and *Acinetobacter* genomo species 3 from Korea: identification of two new integrons carrying the blaVIM-2 gene cassettes. Journal of Antimicrobial Chemotherapy. 49: 837–840.

ZARRILLI, R., S. POURNARAS, M. GIANNOULI, AND A. TSAKRIS. 2013. Global evolution of multidrug-resistant *Acinetobacter baumannii* clonal lineages. International Journal of Antimicrobial Agents. 41:11-19.

استخدام الطرق التقليدية والجزيئية للكشف عن الجينات *bla*IMP-1 و *bla*IMP فى *Acinetobacter baumannii* المقاومه لعديد من المضادات الحيوية

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الملخص العربـــى

تم اكتشاف سلالات من *Acinetobacter baumannii* فى مقاومة للكاربابينيم ومنتجة للميتالوبيتالاكتاميز في جميع أنحاء العالم في السنوات الأخيرة وبوتيرة متزايدة. يعتبر تكوين الكاربامينيز مثل الميتالوبيتالاكتاميز أحد الأسباب الرئيسية لمقاومة الاسينيتوبكتر بيومينای للمضادات الحيوية على نطاق واسع والتي تحلل مجموعة متنوعة من البيتا لاكتم بما في ذلك البنسلين، السيفالوسبورين، والكاربابينيمات. والهدف من هذه الدراسة هو مقارنة الطرق المظهرية والجينية لتحديد منتجي الميتالوبيتالاكتاميز في الاسينيتوبكتر بيومينای المقاوم للكاربابينيم باستخدام تفاعل البلمرة المتسلسل كطريقه سريعه ودقيقة. تم اختيار 52 عزلة لهذه الدراسة من الاسينيتوبكتر بيومينای وفقًا لمقاومة هذه السلالات للمضاد الحيوى التينام. تم الكشف عن التعبير المظهري للميتالوبيتالاكتاميز باستخدام اختبار الهودج المعدل واختبار انتشار القرص المشترك. تم الكشف عن تعبيرات النمط الجيني لجينات *bla*IMP-1 و *bla*IMP بواسطة تفاعل البلمرة المتسلسل لجميع سلالات الاسينيتوبكتر بيومينای المنتجة للميتالوبيتالاكتاميز. وجد ان تسعة وأربعون عزلة من أصل 52 (94.2٪) من الاسينيتوبكتر بيومينای كانت منتجة للكاربامينيز بواسطة اختبار الهودج المعدل بينما سبعة وأربعون (90.4٪) من العزلات كانت منتجة للميتالوبيتالاكتاميز بواسطة اختبار انتشار القرص المشترك بينما كانت 39 عزلة (75٪)من الاسينيتوبكتر بيومينای (من 52 عزلة مفترض انها منتجه للميتالوبيتالاكتاميز موجبة لجين *bla* IMP-1 بواسطة تفاعل البلمرة المتسلسل ، بينما كانت خمسة عشر (28.8٪) من الاسينيتوبكتر بيومينای موجبة لجين *bla*IMP بواسطة تفاعل البلمرة المتسلسل .التواجد المشترك لجينات *bla*IMP-1 و *bla*IMP في 23٪ (52/12) من الحالات. ونستنتج من ذالك ان عزلات الاسينيتوبكتر بيومينای التي يتم اكتشافها كمنتجه للميتالوبيتالاكتاميز باستخدام الطرق المظهريه اختبار الهودج المعدل واختبار انتشار القرص المشترك، يجب عمل اختبار تاكيدى لها من خلال تطبيق طريقة النمط الجيني.