ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

New Delhi Metallo-β-Lactamase 1 (NDM-1) Producing *Acinetobacterbaumannii* in Egyptian Hospitals.

Reem R. Abd El-Glil

Microbiology & Immunology Department, Faculty of Medicine, Benha University, Egypt.

Manuscript Info

Manuscript History:

Received: 15 February 2015
Final Accepted: 22 March 2015
Published Online: April 2015

Key words:

Acinetobacterbaumanii,bla NDM-1,metallo-β-lactamases

Corresponding Author

Reem R. Abd El-Glil

Abstract

Resistance to carbapenems is developing around the world.*Acinetobacterbaumannii(A. baumannii*) resistance to carbapenems is associated with production of carbapenem-hydrolyzing class B metallo-β-lactamases (MBL).New Delhi metallo-β-lactamase 1 (NDM-1) is one of the most recently discovered MBL among *A. baumannii***.**

Aim: The present work was aimed to study the prevalence of bla NDM-1 gene amongcarbapenem-resistant*A. baumanii* isolates from two hospitals inBenha, Egypt.

Materials and Method:40 *A. baumanii* clinical isolates were investigated for different antibiotics and imipenem resistant using the standard disk diffusion method.Combination disc Test (CDT), Double Disc Synergy Test (DDST) and Epsilometer test(*E* test) were performed for phenotypic detection of MBLs and the molecular method polymerase chain reaction (PCR) was done for detection of NDM-1gene.

Results: Out of 40 imipenem resistant*A. baumanii* 62.5%,55% and65% of them had positive CDT,DDST and *E* test respectively. NDM-1 gene was detected in 5(19.2%) *A. baumanii* MBL producing isolates by molecular method.

Conclusion:bla NDM-1 production contributes to carbapenem resistance in *A. baumannii* in Egypt and highlights the need to screen and detect this enzyme to prevent and control their dissemination.

INTRODUCTION

Acinetobacterbaumannii (A.baumannii) is one of the most frequently isolated nosocomial pathogens in the hospital settings specially, intensive care units (**JAZANI et al., 2011**).Acinetobacter resistant to carbapenems, aminoglycosides and fluoroquinolones presents a challenge to the clinicians (**PELEG et al., 2005**). Increasing drug resistant rate among *A. baumannii* strains is a major concern worldwide. Carbapenem resistance caused by acquiring theMetallo-beta-lactamases (MBL) is considered to be more serious than other resistance mechanisms because MBLs can almost hydrolyse all beta-lactam antibiotics except monobactams (**Rahmati et al.,2013**).

Recently identified New Delhi MBL-1(NDM-1) is a new type of carbapenemasebelongs to the class B of Ambler *β*-lactamases produced by certain strains of bacteria, and is able to inactivate all β-lactams except aztreonam(**Walsh et al., 2005**). It was first reported in *K. pneumoniae* and *Escherichia coli* derived from a Swedish patient of Indian origin who was admitted to hospital in New Delhi, India in 2009 (**Yongeet al., 2009 b).**

bla NDM-1 -positive bacteria have been disseminated worldwide.The Indian subcontinent and China are the major reservoirs. Balkan state may be considered as a 'secondary' reservoir area, while the Middle East (Morocco, Algeria, Libya, Egypt, Iraq, Kuwait, Oman, Lebanon and Afghanistan), Southeast Asia may be additional reservoir areas (**Wailan and Paterson 2014**).
NDM-1 enzyme has been found clinically in *Enterobacteriaceae* and *A. baumanni* (**Karthikeyan et al.,*2010*).** The isolation of an NDM-1-producing *A. baumannii* in a Czech patient repatriated in 2011 from Egypt was described by **Hrabák et al., 2012.**

Most NDM-1-positive strains also express the CMY-4 and CTX-M-15 β-lactamases, which confer resistance to all β-lactams. Thus it provides resistance against all compounds that contain a beta-lactam ring such as penicillins, cephalosporins, and the carbapenems(**Karthikeyan*2010*).** Most of the NDM-1 producers remain susceptible only to two bactericidal antibiotics (colistin and fosfomycin) and a single bacteriostatic antibiotic (tigecycline) **(Falagas et al.,2011 , Rogers etal., 2013)**.Such diffusion pattern of multidrug resistance is unique for NDM producers and not observed currently for none of the producers of other types of carbapenemases (OXA-48, KPC, IMP…) ***(Lauren et al ., 2014***).
Lauren et al ., 2014 reported that among the most important features of NDM producers, one may retain that those NDM producers are not only nosocomial pathogens, but also community-acquired Gram-negative species, such as *A. baumanni* which may play a pivotal role for spreading blaNDM genes for its natural reservoir to Enterobacteriaceae.
Thus the need to screen and detect NDM-1 *A. baumanni* producers is necessary to aid in appropriate treatment and to prevent and control their dissemination.
The objective of this study is to determine the prevalence of carbapenemase encoding gene (blaNDM-1) among*A. baumanii* isolated from patients in the intensive care unit (ICU) from two hospitals in Benha, Egypt.

MATERIALS and METHOD

This study was carried out at Microbiology and Immunology Department,Benha Faculty of Medicine, Egypt, in the period between September 2014 and February 2015. Forty imipenem resistant*A. baumanii*clinical isolates were obtained from different sites of infection in subjects with different clinical presentations from Benha University Hospital and Benha Teaching Hospital. All isolates were from patients in ICUsof two hospitals. Specimens were blood (n=6), exudative discharge (pus and woundswabs)(n=13),sputum (n=11),Bronchoalveolar lavage (n=7) and urine (n=3). The organisms were identified up to species level using Micrbact24E (Microbact™, Oxoid, UK).*A. baumanii* ATTC 19606 was used as a control strain.

Antimicrobial Susceptibility Testing

Susceptibility to antibiotics was determined by Kirby-Bauer disk diffusion method on Mueller Hinton agar(Oxoid,UK) in accordance with Clinical and Laboratory Standards Institute(CLSI)guidelines(2011). The antibiotics discs(Oxoid, UK) tested were amikacin (30 µg),Gentamicin (10 µg) , Cefotaxime(30 µg), ceftazidime (30 µg),Cefepime(30 µg), ciprofloxacin (5 µg), piperacillin(100 µg), imipenem (10 µg), and meropenem (10 µg) and aztreonam (30 µg). The tested isolate was picked up with sterile loop and suspended in peptone water and incubated at 37°C for 3 hours. The turbidity of the suspension was adjusted to 0.5 McFarland's standard, and the suspension was then spread on the surface of a Muller Hinton agar plates using sterile cotton swab. The antimicrobial susceptibility test disc was placed on the agar. The plates were incubated at 37°C overnight.Interpretation of inhibition zone diameters were measured according the guidelines of CLSI.Piparcillins, susceptible (S) ≥21, Intermediate (I) 18-20,resistant (R) ≤17 ,CefepimeS≥18 ,I15-17 ,R≤14 ,CefotaximeS≥23 ,I 15-22 ,R ≤14 ,CeftazidimeS ≥18, I 15-17,R ≤14,Ciprofloxacin S≥21,I 16-20,R ≤15,Imipenem and meropenemS ≥16,I 14-15,R ≤13 ,Gentamicin S ≥15 , I 14-13, R≤12 ,Amikacin S ≥17, I 15-16,S ≤14 ,Aztreonam S ≥22 I16-21 ,R ≤15.MIC to imipenem was performed by E test (range: 4-256 µg/ml).The CLSI breakpoints for *A. baumannii* of imipenem was as follows: S ≤4 µg/ml; R ≥16µg/ml.

Phenotypic methods

Detection of MBL production was done by Imipenem (IMP) – EDTA combined disk test, IMP-EDTA double disc synergy test and *E* test.

1-**IMP - EDTA combined disctest (CDT)**: The IMP-EDTA combined disk test was performed as described by (**Yong et al., 2002a**), on the MullerHinton plates inoculated with 0.5 McFarlandIMP resistant *A.baumanii* using cotton swab . Two 10 µgimipenem discs (Oxoid.UK) were placed on the plate at a distance of 4-5 cm from each other., and appropriate amounts of 10 µL of 0.5 M EDTA (Gibco BRL, USA) solution were added to one of them to obtain the desired concentration (750 µg). The inhibition zones of IMP and IMP-EDTA discs were compared after

16 to 18 hours of incubation in air at 35°C. If the increase in inhibition zone with the IMP andEDTA disc was ≥ 7 mm than the IMP disc alone, it was considered as MBL positive **(Yong et al., 2002a)**, figure 1.

2-IMP-EDTA double disc synergytest(DDST): The IMP-EDTA double disc synergy test was performed as described by **(Lee et al., 2003)**. Test organisms were inoculated onto plates with Mueller Hinton agar as recommended by the CLSI**.(Behera et al., 2008).** An imipenem (10 µg) disc was placed 20 mm center to center from a blank disc containing 10 µL of 0.5 M EDTA (750 µg). Enhancement of the zone of inhibition in the area between imipenem and the EDTA disc in comparison with the zone of inhibition on the far side of the drug was interpreted as MBL positive result **(Lee et al., 2003)**.

3-E Test MBL: MBL-Etest strips (AB Biodisk, Solna, Sweden): Each contains a double-sided dilution range of imipenem (4–256 mg/L) and imipenem (1–64mg/L) combined with (EDTA) (320mg/L).The E Test MBL was performed according to manufacture instructions briefly - :Individual colonies were picked from 18-h plates and suspended in0.85 %saline to a turbidity of a 0.5 McFarland standard . Cotton swabs were used to transfer the inoculum to the plates, whichwere thoroughly swabbed and dried before the E test MBL strips wereapplied.Plates were incubated for 16 to 20 h at 35°C .The MIC end points were read where the inhibition ellipsesintersected the strip .A reduction ofimpenem MICs by ≥8 dilutions in the presence ofEDTA was interpreted as being suggestive of MBLproduction .figure2.

Detection of NDM-1 gene

DNA was extracted from 40 isolatesby the boiling method (**Franco et al., 2010**).Specific primer that amplified 475 bp regionof*NDM-1 gene was used*;NDM1-Forward (5'-GGGCAGTCGCTTCCAACGGT) and NDM1-Reverse (5'-GTAGTGCTCAGTGTCGGCAT) **(Manchanda et al., 2011).**The PCR mixtures consisted of 25 µl of TaqPCR Master Mix(PCR Buffer, MgCl2 ,dNTPs and Taq DNA polymerase)(Fermentes, Germany),2µM primer (Fermentas, Germany), 5 µl of DNA sample, and nuclease free water to a final volume of 50 µL. The samples were overlaid with 100 µL of mineral oil.The reaction was carried in Thermal cycler(Biometra, Goettingen, Germany).Initial denaturation step at 95^{o}C for 5 minutes followed by 40 repeated cycles of: denaturation at 95^{o}C for 30 seconds, annealing at 56^{o}C for 30 seconds and extension at72^{o}C for 1 minute followed by final extension step at72^{o}C for 15 minutes. 10 µL of each amplified DNAwere analyzed by electrophoresis in a 1.5% agarose gel at 100 V for 1 h in 1XTris acetate EDTA (TAE) containing 0.3%ethidium bromide. The samples were run alongside a 100 bp ladder(Fermentans, Germany) that served as a molecular weight marker. The bands were visualized using UV transilluminator (254nm). figure 3.

Statistical analysis

The collected data were summarized in terms of frequency and percentage. The test of proportion (Z test) was used to compare the results of the IMP-EDTA combined disc test and IMP-EDTA double disc synergy test with the E test. The Fisher's Exact test was used to compare the proportions of different study groups. p-value <0.05 was considered statistically significant. The statistical analysis was conducted using STATA version 11 (STATA corporation, College Station, Texas).

RESULTS

The*A.baumanii*isolates were recovered from 40 (27 males and 13 females) patients in different age groups: 0–19 years (*n* = 6), 20–39 (*n* = 4), 40–59 years (*n* = 19) and ≥ 60 years (*n*= 11).

The resistance of *A. baumannii* isolates to different antibiotics was summarized in table (1).Out of 40 imipenem resistant *A. baumanii* strains ,IMP-EDTA -CDT, IMP-EDTA -DDSTand E test detected 25(62.5%) , 22(55%) , 26(65%) MBL producing isolates respectively,Table (2). The present study showedno significant differences in the proportion of positive cases using IMP-EDTA-CDT and IMP-EDTA -DDST compared with *E*-test.Among 26MBL producing isolates, resistant to amikan, gentamycin and aztreonamwere 92.3%, 88.5% and 84.6% respectively. All MBL producing isolates were 100% resistant to piperacillin ,cefotaxime, ceftazidime, cefepime and ciprofloxacin.

Highest percent of positive MBL producing isolates was from sputum samples (72.7%) followed by broncho-alveolar lavage samples (71.4%), Table3.

Five (12.5%) clinical isolatesof *A.baumanii* were positive for blaNDM-1 gene as detected by PCR.The 5 (19.2%) positive blaNDM-1 isolates were from MBL producers but, this finding was statistically insignificant, p=0.14, Table 4.

NDM-1 positive isolates were; 2 isolates from sputum specimens, 1 isolate from bronchoalveolar lavage and 2 isolates from exudative discharges. NDM-1 gene could not be detected in 35*A.baumanii* isolates. The study showed thatall specimens had non-significant differences in the proportions of NDM-1 gene detection between positive and negative MBL isolates, p=1, Table 5.

Three positive blaNDM-1 isolates were from 3 patients in ICU atBenha University Hospital, The other 2 positive blaNDM-1 isolates were from 2 patients in ICU atBenha Teaching Hospital.

In the 5 PCR-positive isolates CDT and *E* test were positive while DDST was positive for only four isolates.
Among 5 NDM-1 producing isolates resistant to amikan,gentamycin were 80%and 60 % respectively. All NDM-1 producing isolates were 100% resistant to piperacillin,cefotaxime, ceftazidime,cefepime ,ciprofloxacin and aztreonam.

Table (1): Resistantof *A.baumanii* to different antibiotics.

Antibiotics	**No.**	**%**
Piperacillin	40	100.0
Cefotaxime	40	100.0
Ceftazidime	40	100.0
Cefepime	40	100.0
Ciprofloxacin	40	100.0
Imipenem	40	100.0
Meropenem	40	100.0
Aztreonam	31	77.5
Gentamycin	35	87.5
Amikian	37	92.5

Table (2): MBL producing isolates according to phenotypic methods.

IMP-EDTA CDT **+/-; (%+)**	IMP-EDTA DDST +/-; (%+)	*E* test +/-; (%+)	Z1; P1	Z2; P2
25/15; (62.5)	22/18; (55.0)	26/14; (65.0)	0.23; 0.82	0.91; 0.36

No.=40; Z1; P1 for IMP-EDTA -CDT versus *E* test; Z2; P2 for IMP-EDTA-DDST

Table (3): MBL producing isolates in relation to clinical specimens.

Specimen	**Positive MBL isolates**		**Negative MBL isolates**		**P***
	No.	**%**	**No.**	**%**	
Sputum (No.=11)	8	72.7	3	27.3	**0.26**
Broncho-alveolar lavage (No.=7)	5	71.4	2	28.6	
Exudative (No.=13)	9	69.2	4	30.8	
Blood (No.=6)	4	66.7	2	33.3	
Urine (No.=3)	0	0.0	3	100.0	

*Obtained using the Fisher's Exact Test.There were no significant differences between the different specimens regarding the status of MBL isolates.

Table (4): NDM-1 gene among imipenem resistant *A. baumanii*

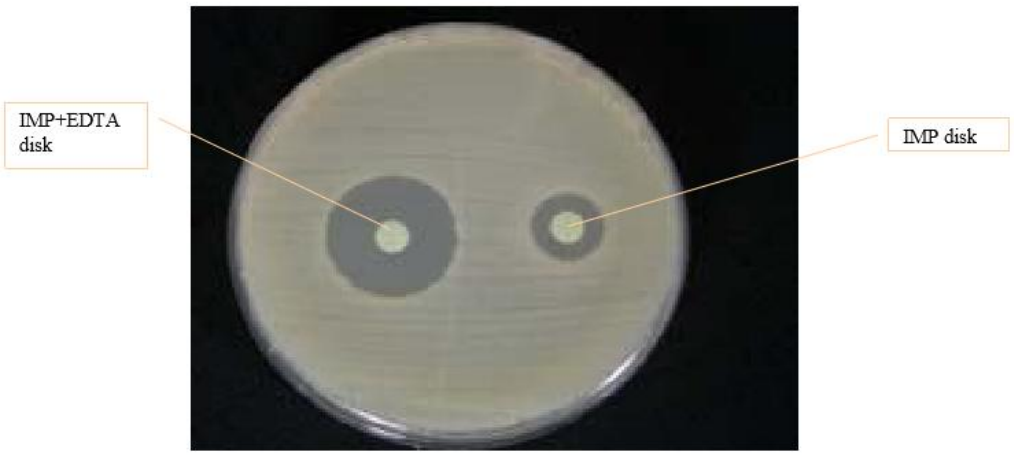
MBL isolates (No.=40)	**Positive NDM-1 gene**		**Negative NDM-1 gene**		**P***
	No.	**%**	**No.**	**%**	
Positive (No.=26)	5	19.2	21	80.8	**0.14**
Negative (No.=14)	0	0.0	14	100.0	

*Obtained using the Fisher's Exact Test.There were no significant differences between positive MBL isolates and negative MBL isolates regarding NDM-1 gene detection.

Table (5): NDM-1 gene among imipenem resistant *A. baumanii*in relation to clinical specimens.

Specimen		Positive NDM-1 gene		Negative NDM-1 gene		P*
		No.	%	No.	%	
Sputum (11)	+MBL(8)	2	25.0	6	75.0	**1.00**
	-MBL(3)	0	0.0	3	100.0	
Broncho alveolar lavage (7)	+MBL(5)	1	20.0	4	80.0	**1.00**
	-MBL(2)	0	0.0	2	100.0	
Exudative (13)	+MBL(9)	2	22.2	7	77.8	**1.00**
	-MBL(4)	0	0.0	4	100.0	
Blood (6)	+MBL(4)	0	0.0	4	100.0	-
	-MBL(2)	0	0.0	2	100.0	
Urine (3)	+MBL(0)	0	0.0	0	0.0	-
	-MBL(3)	0	0.0	3	100.0	

*Obtained using the Fisher's Exact Test.All specimens showed non-significant differences in the proportions of NDM-1 gene detection between positive and negative MBL isolates.



IMP: Imipenem disk

Fig(1): IMP-EDTA -CDT: The zone of inhibition around IMP disc to the right of the figure is 13 mm and the zone of inhibition around IMP + EDTA disc to the left of the figure is 24 mm with a difference of 11 mm between them .

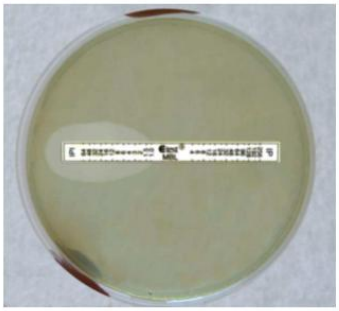


Fig (2): E test;Positive MBL strain.

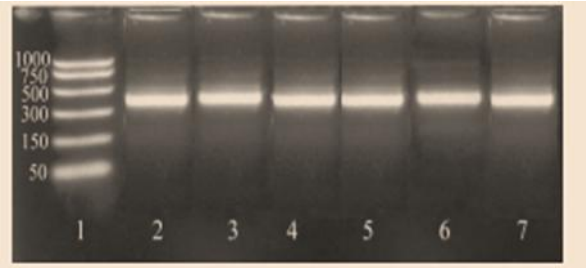


Figure (3): Lane 1: Molecular marker (100bp DNA ladder), Lane 2,3,4,5,6 and6: Positive NDM-1 (475 bp).

DISCUSSION

The emergence ofcarbapenemase-producing *A. baumannii* strains is increasing worldwide**(Shahcheraghi et al 2011)**. Infections caused by these bacteria have limited treatment options and have been associated with high mortality rates. The types of carbapenemase vary among countries, partially depending on the population exchange relationship between the regions and the possible reservoirs of each carbapenemase**(Djahmi et al., 2014**).

NDM-producing bacteria are commonly resistant to almost all groups of antibiotics, including fluoroquinolones, aminoglycosides, and *β*-lactams (especially carbapenems) but are susceptible to colistin and sometimes tigecycline. The bla NDM-1 gene has been detected on different large plasmids, which were readily transferable among bacteria, making NDM-1-producing bacteria a serious clinical and public health threat **(Chen et al., 2011). Hrabák et al., 2012** described the isolation of an NDM-1-producing *A. baumannii* in a Czech patient repatriated in 2011 from Egypt.

This study aimed todetermine the prevalence of NDM-1-gene among imipenem resistant *A. baumanii*isolates at two hospitals in Egypt.

The present study showed that all isolates were 100% resistant to piperacillin,cefotaxime ,ceftazidime,cefepime and ciprofloxacin with resistant percentage to toaztreonam (77.5%) gentamycin(87.5%) and amikacin (92.5). This finding goes in hand with **Nasr and Attala(2012)** who found that all isolates (n = 20) were 100% resistant to ampicillin–sulbactam, ceftazidime, ceftriaxone, ciprofloxacin, and piperacillin–tazobactam and high resistance rates were also observed to amikacin (90%), gentamicin (85%), and doxycycline (75%).

In the present work (82.5%) of *A. baumanii*isolates were resistant to imipenem (MIC >16 μg/ml) and (47.5 %)were resistance to imipenem, with MIC values ≥128 μg/ml. This results is consistent with **Al-Agamy et al., 2014**who reported that the majority of *A. baumanii*isolates (70%) were resistant to imipenem (MIC >8 μg/ml)and 50% of the isolates displayed unusually high levels of resistance to imipenem, with MIC values ≥128 μg/ml. They stated that resistance to carbapenems in clinical *A. baumannii* isolates has been notable recently in Egypt that reflects a problem that might be described as countrywide. Our results also in agreement with **Mohamed and Raafat, 2011** who found that all *A. baumanii* isolates in their study displayed elevated values MIC of imipenem (MIC_{50} and MIC_{90} were 64 and 512 μg mL^{-1}, respectively).

This work revealed that out of 40 imipenem resistant *A. baumanii* isolates,CDT, DDST and *E* test detect 62.5%,55% and 65% MBL producing isolates respectively.In astudy done by **Mohamed and Raafat, 2011**phenotypic detection of MBLs producer among *A. baumanii* isolates by CDT and Modified Three-Dimensional Test identified a proportion of 70% as MBL producers.

Safari et al., 2013 illustrated that results of *E*-test MBL 99% of all isolates *A. baumannii* were MBL producers. **Peymani et al. 2011** reported that among 63 carbapenem non-susceptible *A. baumannii* isolates, 49% were found to be MBL producers. These discrepancies in phenotypic methods make researchers confirm phenotypic results by using molecular methods.**Cuzon et al., 2011**, reported that phenotypic methods are growth dependent, turn around time is 18 - 24 h, not clinically useful and results are also subjective.

Our study revealed that MIC of imipenem among MBL producers ranged from16 μg/ml to ≥256 μg/ml. **Livermore1992**explained that discrepancy in the level of imipenem resistant for MBL producers may be due to the variation in the permeation of imipenem through bacterial outer membranes.

The present study demonstrated that 12.5% of imipenem resistant *A.baumanii* isolates had NDM-1 gene. This finding is in agreement with previous studies which reported that MBL NDM-1 and NDM-2 were first described in *A. baumannii* from Egypt,**Hrabák et al.,2012,'Kaase, et al.,2011.** and then spread in the Middle East**Higgins et al.,2010.**But, it is in contrast with **Al-Agamy et al., 2014** who demonstrated that none of the *A. baumannii* isolates in their study harboredbla NDM-1-MBL-encoding genes.

Kaase, et al.,2011 showed the first identification of a bla NDM gene in a clinical isolate originating from Egypt, with no obvious link with the Indian subcontinent. They stated that they have evidence that NDM-encoding genes may be widespread in *A. baumannii*, and recommended further molecular surveys will be necessary to evaluate their distribution in that species. They also suggested that NDM-producing isolates have already disseminated in the Middle East, after the recent identification of NDM producers in Iraq and the Sultanate of Oman (**Poirel et al., 2011 a,Poirel et al., 2011b)**

Ho et al.,2011 explained that high transmissibility of NDM-1 was due to the high rates of transfer of plasmids carrying thebla NDM-1 gene . This may assist acquisition of the newly emergent resistant gene via normal flora and other environmental microorganisms, resulting in worldwide spread (**Nordmann et al., 2011**).

The present study identified that *A.baumanii* isolates had NDM-1 geneexhibited high-level resistance to imipenem with MIC 128 ug/ml which may be pointed to the responsibility of bla NDM-1 for this high-level resistance.However, the presence of bla MBLs other than NDM-1gene may be the reason for high-level resistance to imipenem in*A.baumanii* isolates had negative NDM-1 gene.

Among the 40 study isolates, the MBL screen test was positive in 65 % (n = 26). PCR detected the bla NDM-1 genein only 19.2% (n =5). In 21 isolates that were MBL screen test positive, bla NDM-1 was not found, suggesting the presence of other MBL genes.

Recent reports also suggested that the Middle East might be an additional reservoir of NDM producers **(Sonnevendet al., 2013,Jamal et al.,2012 and Shibl et al.,2013).** This dissemination of NDM producers in the Middle East could mostly be linked to the population exchange between the Middle East and the Indian subcontinent**(Laurent et al., 2014**).

In conclusion, bla NDM-1 production contributes to carbapenem resistance in *A. baumannii* in Egypt. *A. baumannii* may become a reservoir for **NDM**-1-producing bacteria.It emerges therapeutic problem and highlights the need of infection control measures.

REFERENCES

Al-Agamy MH, Khalaf NG, Tawfick MM, Shibl AM, El Kholy A.(2014): Molecular characterization of carbapenem-insensitive *Acinetobacterbaumannii* in Egypt.Int J Infect Dis, May;22:49-54.

Behera B, Mathur P, Das A, Kapil A and Sharma V. (2008): An evaluation of four different phenotypic techniques for detection of metallo-beta- lactamases producing *Pseudomonas Aeruginosa*. Indian Journal of Medical Microbiology, 26 (3):233-237.

Chen Y, Zhou Z, Jiang Y, Yu Y.(2011): Emergence of NDM-1-producing *Acinetobacterbaumannii* in China.*Journal of Antimicrobial Chemotherapy*, 66(6):1255–1259.

Clinical and Laboratory Standards Institute (2011): Performance standards for antimicrobial susceptibility testing: 19th informational supplement. CLSI document M100–S21 Clinical and Laboratory Standards Institute, Wayne, PA.

Cuzon G, Ouanich J, Gondret R, Naas T, Nordmann P. (2011): Outbreak of OXA-48-positive carbapenem-resistant *Klebsiellapneumoniae* isolates in France. Antimicrob Agents Chemother,55:2420–2423.

Djahmi N, Dunyach-Remy C, Pantel A, Dekhil M, Sotto A, Lavigne JP. (2014): Epidemiology of carbapenemase-producing *Enterobacteriaceae* and *Acinetobacterbaumannii*in Mediterranean countries.Biomed Res Int, 305784.

Falagas ME, Karageorgopoulos DE, Nordmann P. (2011): Therapeutic options for infections with *Enterobacteriaceae* producing carbapenem-hydrolyzing enzymes. Future Microbiology,6(6):653–666.

Franco MR, Caiaffa-Filho HH, Burattini MN, Rossi F. (2010):Metallo-beta-lactamases among imipenem-resistant *Pseudomonas aeruginosa* in a Brazilian university hospital.Clinics (Sao Paulo),65(9):825-829.

Higgins, P.G., Dammhayn, C., Hackel, M., and Seifert, H. (2010):**Global spread of carbapenem-resistant** *Acinetobacterbaumannii***.** *J AntimicrobChemother*,65: 233–238.

Ho PL, Lo WU, Yeung MK, Lin CH, Chow KH, Ang I, Tong AH, Bao JY, Lok S, Lo JY. (2011):Complete sequencing of pNDM-HK encoding NDM-1carbapenemase from a multidrug-resistant *Escherichia coli* strain isolated in Hong Kong. PLoS One,Mar 21;6(3):e17989.

Hrabák J, Stolbová M, Studentová V, Fridrichová M, Chudáčková E, Zemlickova H. (2012): NDM-1 producing *Acinetobacterbaumannii* isolated from a patient repatriated to the Czech Republic from Egypt.*Eurosurveillance*, 17(7).pii: 20085.

Jamal W, Rotimi VO, Albert MJ, Khodakhast F, Udo EE, Poirel L. (2012):Emergence of nosocomial New Delhi metallo-β-lactamase-1 (NDM-1)-producing *Klebsiellapneumoniae* in patients admitted to a tertiary care hospital in Kuwait. International Journal of Antimicrobial Agents,39(2):183–184.

Jazani NH, Zartoshti M, Babazadeh H, Ali-Daiee N. (2011):Antibacterial effects of *Artemisia dracunculus* essential oil on multidrug resistant isolates of *Acinetobacterbaumannii*. Bacteriology, 1: 31-36.
Kaase, M., Nordmann, P., Wichelhaus, T.A., Gatermann, S.G., Bonnin, R.A., and Poirel, L. (2011): **NDM-2 carbapenemase in** *Acinetobacterbaumannii* **from Egypt.** *J AntimicrobChemother*,66: 1260–1262.
Karthikeyan K, Thirunarayan MA, Krishnan P. (2010): Coexistence of bla_{OXA-2} with bla_{NDM-1} and armA in clinical isolates of *Acinetobacterbaumannii* from India. J AntimicrobChemother,65:2253–2254.

Laurent Dortet, Laurent Poirel , Patrice Nordmann .(2014): Worldwide Dissemination of the NDM-Type Carbapenemases in Gram-Negative Bacteria. Biomed Res Int, 249856.
Lee K, Lee W G, Uh Y, Ha G Y, Cho J and Chong Y (2003): VIM- and IMP-type metallo-ß-lactamase-producing *Pseudomonas spp.* and *Acinetobacter spp*. in Korean hospitals. Emerg Infect Dis, 9:868-871.
Livermore DM. (1992): Interplay of impermeability and chromosomal beta-lactamase activity in imipenem-resistant P*seudomonas aeruginosa.*Antimicrob Agents Chemother, Sep;36(9):2046-2048.

Manchanda V, Rai S, Gupta S, Rautela RS, Chopra R, Rawat DS, et al. (2011):Development of TaqMan real-time polymerase chain reaction for the detection of the newly emerging form of carbapenem resistance gene in clinical isolates of *Escherichia coli*, *Klebsiellapneumoniae*, and *Acinetobacterbaumannii*. Indian J Med Microbiol,29:249–253.
Mohamed, N.M. and Raafat, D. (2011)**Phenotypic and genotypic detection of metallo-beta-lactamases in imipenem-resistant** *Acinetobacterbaumannii* **isolated from a tertiary hospital in Alexandria, Egypt.** *Res J Microbiol*,6: 750–760.
Nasr, R.A. and Attalah, M.F. (2012):**Molecular epidemiology of nosocomial** *Acinetobacterbaumannii* **isolates.** *Nature and Science*,10: 76–82.
Nordmann P, Poirel L, Toleman MA, Walsh TR. (2011): Does broad-spectrum beta-lactam resistance due to NDM-1 herald the end of the antibiotic era for treatment of infections caused by Gram-negative bacteria? J. Antimicrob. Chemother, 66:689–692.

Peleg AY, Franklin C, Bell JM, Spelman DW. (2005): Dissemination of the metallo-β-lactamase gene blaIMP-4 among Gram-negative pathogens in a clinical setting in Australia. Clin Infect Dis,41: 1549-1556.
Peymani A, Nahaei M, Farajnia S, et al. (2011): High prevalence of metallo-β-lactamase-producing *Acinetobacterbaumannii* in a teaching hospital in Tabriz, Iran. Japanese Journal of Infectious Diseases, 64(1):69–71.

Poirel L, Al Maskari Z, Al Rashdi F, et al. (2011) a: NDM-1-producing Klebsiellapneumoniae isolated in the Sultanate of Oman. J AntimicrobChemother, 66:304-306.

Poirel L, Fortineau N, Nordmann P. (2011) b: International transfer of NDM-1-producing *Klebsiellapneumoniae*from Iraq to France. Antimicrob.AgentsChemother,Apr;55(4):1821-1822.

RahmatiRoodsari M, Fallah F, Taherpour A, HakemiVala M, Hashemi A.(2013):Carbapenem-resistant bacteria and laboratory detection methods. *Archives of Pediatric Infectious Diseases*,1(4):188–191.
Rogers BA, Sidjabat HE, Silvey A, et al. (2013): Treatment options for New Delhi metallo-*β*-lactamase-harboring *Enterobacteriaceae*. Microbial Drug Resistance, 19(2):100–103.

Safari M, Saidijam M, Bahador A, Jafari R, Alikhani MY. (2013): High prevalence of multidrug resistance and metallo-beta-lactamase (MbetaL) producing *Acinetobacterbaumannii* isolated from patients in ICU wards, Hamadan, Iran. Journal of Research in Health Sciences,13(2):162–167.
Shahcheraghi F, Abbasalipour M, Feizabadi MM, Ebrahimipour GH, Akbari N. (2011): Isolation and genetic characterization of metallo-beta-lactamase and carbapenamase producing strains of *acinetobacterbaumannii*from patients at tehran hospitals. *Iranian Journal of Microbiology*,3(2):68–74.

Shibl A, Al-Agamy M, Memish Z, Senok A, Khader SA, Assiri A. (2013):The emergence of OXA-48- and NDM-1-positive *Klebsiellapneumoniae* in Riyadh, Saudi Arabia. International Journal of Infectious Diseases, 17(12):e1130–e1133.

Sonnevend A, Al Baloushi A, Ghazawi A, et al. (2013): Emergence and spread of NDM-1 producer *Enterobacteriaceae* with contribution of IncX3 plasmids in the United Arab Emirates. Journal of Medical Microbiology, 62, part 7:1044–1050.

Wailan AM, Paterson DL. (2014):The spread and acquisition of NDM-1: a multifactorial problem. Expert Rev Anti Infect Ther, 12:91–115.

Walsh TR, Toleman MA, Poirel L, Nordmann P. (2005):Metallo-β-lactamases: the quiet before the storm? ClinMicrobiol Rev, 18:306–325.

Yong D, Lee K, Yum JH, Shin HB, Rossolini GM and Chong Y.(2002)a :Imipenem-EDTA disk method for differentiation of metallo-Blactamases producing clinical isolates of *Pseudomonas spp and Acinetobacterspp*.JClin Microbial,40:3798-3801.

Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, et al. (2009)b: Characterization of a new metallo-beta-lactamase gene, bla(NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiellapneumoniae* sequence type 14 from India. Antimicrob Agents Chemother, 53:5046–5054.