



ISSN NO. 2320-5407

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

INTERNATIONAL JOURNAL  
OF ADVANCED RESEARCH

## RESEARCH ARTICLE

**FIRST DETECTION OF THE *bla*NDM-1 and *bla*NDM-2 GENES IN A CLINICAL ISOLATES OF *Acinetobacter baumannii* IN HILLAH HOSPITALS-IRAQ**

Raad Abdulbass AL-Harmoosh<sup>1</sup> And Eman M. Jarallah<sup>2</sup>

1. University of Kufa / College of Science, Najaf, IRAQ.
2. University of Babylon / college of Science, Babylon, IRAQ

**Manuscript Info****Manuscript History:**

Received: 18 August 2015  
Final Accepted: 22 September 2015  
Published Online: October 2015

**Key words:**

NDM, *Acinetobacter*, Carbapenem, MDR

**\*Corresponding Author**

**Raad Abdulbass AL-Harmoosh**

**Abstract**

Carbapenemase producing *Acinetobacter baumannii* is frequently associated with nosocomial infections. Increasing resistance to carbapenems, may significantly reduce the choice of effective antibiotics. This study was conducted to determine the occurrence of carbapenemase producing *A. baumannii* isolates obtained from Hillah hospitals. Isolates were identified according to biochemical tests and more confirmed using API 20E system. Carbapenem susceptibility was assayed by using disks diffusion test. Phenotypic detection of carbapenemase was performed using the imipenem-EDTA disk and modified Hodg tests. Then isolates were subjected to monoplex PCR targeting *bla*NDM-1 and *bla*NDM-2 genes. 10 (0.76%) *A. baumannii* isolates were recovered from clinical samples. One (10%) of isolate was found to be imipenem and meropenem resistant (MIC > 512 µg/ml). 6(60%) gave positive result with the imipenem-EDTA disk and modified Hodg test. PCR experiments showed only two (20%) isolates were harbored *bla*NDM-1 gene and four (40%) *bla*NDM-2. Partial DNA sequencing of NDM genes revealed to there is some variation from NDM-1 and NDM-2 genes in two isolates. The present findings suggest that emergence of *bla*NDM carbapenemase producing *A. baumannii* clinical isolates in Hillah hospitals.

Copy Right, IJAR, 2015.. All rights reserved

**INTRODUCTION**

*Acinetobacter baumannii* have been implicated in recent years as important nosocomial pathogen especially in intensive care unit (9). *A. baumannii* is responsible for 3-5% of nosocomial pneumonia, and one of the most common presentations in mechanically ventilated patients in intensive care units (16). Carbapenems are the drugs of choice for this pathogen and carbapenem-resistant *A. baumannii* has been frequently hospital encountered (15). Meropenem and imipenem are carbapenems that remain active against organisms carrying most Ambler classes of β-lactamases which include many Gram-negative bacilli, including *Acinetobacter* spp. However, carbapenem resistance is increasingly encountered in *Acinetobacter* isolates worldwide (1). One of the major mechanisms of carbapenem resistance in this pathogen is the production of carbapenem hydrolyzing β-lactamases. These specific groups of β-lactamases are categorized into class B metallo β-lactamases (MBLs) including IMP and VIM, NDMs and class D (Oxacillinases) including OXA-23-like, OXA-24/40-like and OXA-58 (4). The new MBL, New Delhi metallo-β-lactamase 1 (NDM-1), initially reported in *Klebsiella pneumoniae* and *Escherichia coli* recovered from a Swedish patient who was previously hospitalized in India (51), has disseminated to several countries and other Enterobacteriaceae (38,39,44,50). Recently, cases of NDM-producing *Acinetobacter baumannii* have been described in India, Egypt, and China (13,27,28).

The bacteria with NDM-1 gene are known as superbugs and public health must pay more attention to them (10). In 2010, *bla*NDM-1 and *bla*NDM-2 were found in *A. baumannii* (20), and in 2011, *bla*NDM-5 was found in *E. coli* (24). More than six different *bla*NDM allotypes are known (21). NDM-1 is carried on plasmid or on chromosomes (41). The rapid emergence of *bla*NDM-1 has been related to a moveable plasmid which can be transferred from one bacteria to another, from man to man and even from country to country (21). In recent years, many Iraqi patients were travelled to India and to other countries for medical care purpose which may helped in acquiring this gene.

In Iraq, little attention has been paid to the carbapenemase producing isolates. However, in the Hillah city, no information are regarding the molecular studies of the occurrence of NDM *A. baumannii* producing clinical isolates. Hence, the proposed aim of this study is to identify and detect NDM producing *A. baumannii* isolates recovered from hospital settings in the Hillah city.

## MATERIAL AND METHODS

### *Isolation and Identification of Isolates:*

A total of 1300 clinical specimens (included 588 burn swabs, 136 wound swabs, 50 from throat, 204 urine, 110 stool, 20 sputum, 162 blood, 15 ears and 15 eyes) were collected from patients in Hillah hospitals over one year period starting from March, 2014 to March, 2015. Isolates were recovered from clinical samples after culturing on MacConkey agar and incubated for overnight at 37°C, non lactose fermenting bacteria were sub-cultured and incubated for additional overnights. Suspected bacterial isolates which their cells are Gram negative coccobacillary or diplobacillus and negative to oxidase which further identified using API20 E system.

### *Antimicrobial susceptibility testing*

Isolates were cultured on Mueller-Hinton agar and their susceptibilities to different antibiotics were tested by disk diffusion method according to the Clinical and Laboratory Standard Institute's guidelines (14).

### *MIC determination:*

Depending on manufacturer's instructions the antibiotic stripes were applied to the agar surface, the antibiotic instantaneously diffuses into the surrounding medium in high to low concentration from one end of the strip to the other. The gradient remains stable after diffusion, and the zone of inhibition created takes the form of ellipse (Liofilchem manufacture).

### *Imipenem-EDTA double disks method:*

Screening for metallo  $\beta$ -lactamases (MBL) was performed using disks containing 1900  $\mu$ g of EDTA plus 10  $\mu$ g of imipenem disk were placed on the inoculated plates containing Muller Hinton agar. An increase of  $\geq 17$  mm in zone diameter in the presence 1900  $\mu$ g of EDTA compared to imipenem alone indicated the presence of an MBL (30)

### *Modified Hodg test:*

Imipenem was used for carbapenemase detection as described by Lee and his colleagues (29). Positive test has a clover leaf-like indentation of *E. coli* Top-10 growing along the test organism growth streak within the imipenem disk diffusion zone.

### *PCR amplification:*

DNA was extracted from the isolates by using genomic extraction mini kit according to the manufacture instructions (Bioneer company, Korea). To amplify the genes encoding carbapenemases, a monoplex-PCR was run using the primers of NDM-1 (480bp: F/5'-ATG GAA TTG CCC AAT ATT ATG C-3' and R/5'-CGA AAG TCA GGC TGT GTT G-3') were designed in this study, and NDM-2 gene (500 bp: F/5'-CAC CTC ATG TTT GAA TTC GCC-3' and R/5'-CTC TGT CAC ATC GAA ATC GC-3') were described by Kaase and his colleagues (25).

Amplification was performed in a 20  $\mu$ l volume as recommended by Promega Master mix instruction. PCR amplifications were carried out on a thermal cycler (Prime, England). The cycling conditions for amplification were as follows: for *bla*NDM-1 gene, initial denaturation of 95°C for 2 min and 30 cycles of 30 sec at 95°C, 30 sec at 56°C, and 1 min at 72°C, followed by 5 min at 72°C, and for *bla*NDM-2 initial denaturation at 95°C for 2 min and 30 cycles of 30 sec at 95°C, 30 sec at 57°C, and 1 min at 72°C, followed by 5 min at 72°C. Amplified products were detected by agarose gel electrophoresis in 1% Tris-borate-EDTA (TBE) agarose (Promega, USA) and staining with ethidium bromide. The electrophoresis result was detected by using gel documentation system (Claver, England).

**DNA Sequencing:**

DNA sequencing method was performed for genotyping and phylogenetic analysis study of *Acinetobacter baumannii* isolates based NDM genes. The sequencing of the PCR product 480bp, and 500bp, for NDM-1, and NDM-2, gene respectively, where the PCR product was purified from agarose gel by using (EZ EZ-10 Spin Column DNA Gel Extraction Kit, Biobasic. Canada).

**RESULTS**

Based on the conventional biochemical tests and API20E system enabled to isolation and identification of 10 (0.76%) isolates as *A. baumannii* from the 1300 clinical samples (Table 1). As determined by disk-diffusion method, all *A. baumannii* isolates exhibited different pattern of resistance to different antibiotic agents (Fig.1), demonstrating highest resistance to penicillins (carbenicillin and ampicillin) with rate of resistance of 10(100%) isolates, whereas 3(30%) of isolates were resistance to piperacillin.

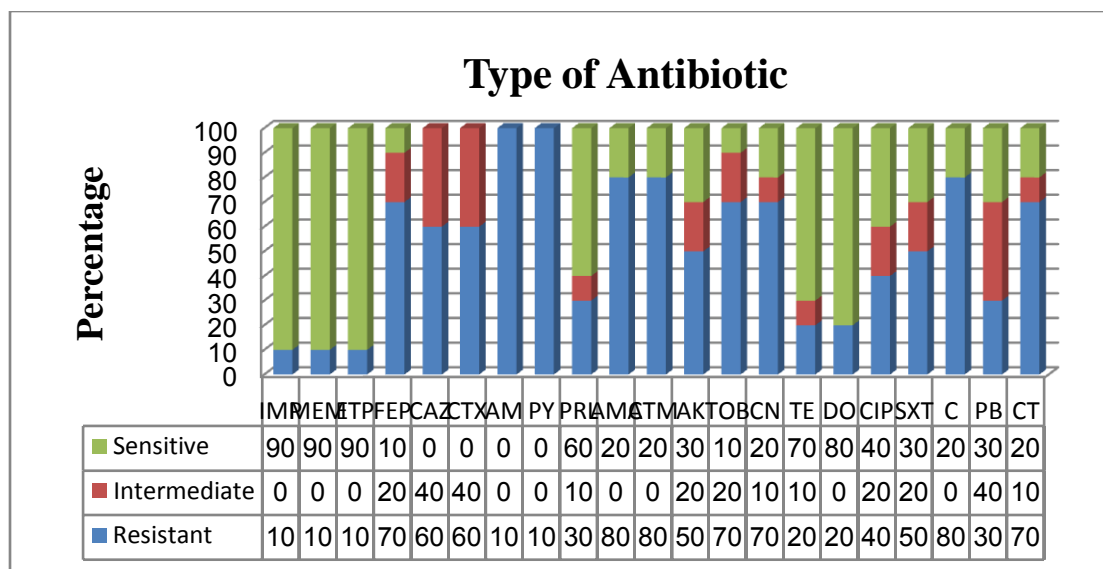
Resistance to other drug classes varied among the isolates. For cephalosporin antibiotics, a higher resistance was also detected with 6(60%) of isolates being resistant to ceftazidime and cefotaxime each other , and 7(70%) to cefepime. The results also revealed that were high resistant rates 8(80%) isolates for each amoxi-clav and aztreonam of monobactam's antibiotics.

For the carbapenem antibiotics, imipenem , meropenem and ertapenem displayed the lowest resistant rate 1(10%) isolates. Aminoglycosides resistance was variable , 7(70%) to each of tobramycine, andgentamicin and 5 (50 %) to amikacin.

The resistance to quinolones, (ciprofloxacin) was detected as 4(40%) isolates. Percentages of resistance of isolates to the remaining antibiotics were as follows : 2(20%) to each of tetracycline and doxycycline , trimethoprim-sulfamethoxazole 5(50%), chloramphenicol 8(80%) , Polymyxin B 5(50%) and Colistin sulphate 7(70%) . Results revealed that all tested isolates were resistant to at minimum of 3 classes of antibiotics, hence these isolates were considered to be multidrug resistant.

**Table (1): Distribution of bacterial isolates recovered from clinical samples among different hospitals in Hillah city.**

Hospital's name	No. of samples	No. (%) of <i>Acinetobacter baumannii</i> isolates	No. (%) of other bacterial spp. isolates	No. (%) of no growth cultures
Al- Hillah Teaching Hospital	885	7 (0.8%)	710 (80%)	168 (18%)
Babylon Teaching Hospital for Maternity and Pediatric	415	3 (0.7%)	235 (56.6%)	177 (42.6%)
Total	1300	10 (0.76%)	945 (72.69%)	345 (26.5%)



**Figure (1) : Antibiotics susceptibility profile of *A. baumannii* isolates by disk diffusion method (n=10)** (IMP,Imipenem ; MEM,Meropenem ; ETP,Ertapenem ; FEP,Cefepime ; CAZ,Ceftazidime ; CTX,Cefotaxime ; AM,Ampicillin ; PY,Carbenicillin ; PRL,Piperacillin ; AMC,Amoxi-clav; ATM,Aztreonam ; AK,Amikacin ; TOB,Tobramycine ; CN,Gantamicin ; TE,Tetracycline ; DO,Doxycycline ; CIP,Ciprofloxacin ; SXT,Trimethoprim-Sulfamethoxazole ; C,Chloramphenicol ; PB,Polymyxin B ; CT,Colistin sulphate).

#### **MIC determination:**

Minimum inhibitory concentrations of *A. baumannii* isolates against carbapenem antibiotics (imipenem, meropenem, ertapenem) were determined by using E-test and microbroth dilution methods according to the Clinical and Laboratory Standards Institute (CLSI, 2012) guidelines. Table (2) revealed that *A. baumannii* isolates were resistant to imipenem, meropenem, and ertapenem with concentrations beyond values: 0.032 µg/ml - >512 µg/ml.

**Table (2) : MIC of carbapenem antibiotics**

Isolates No.	MIC (µg/ml)		
	IMP	MER	ERT
1	0.75	0.032	0.032
2	0.25	0.38	2
3	1	0.047	0.023
4	0.19	0.38	1.5
5	0.38	1.5	6
6	> 512	> 512	> 512
7	0.19	0.75	3
8	1	0.5	2
9	1.9	0.047	0.032
10	1	0.064	0.047

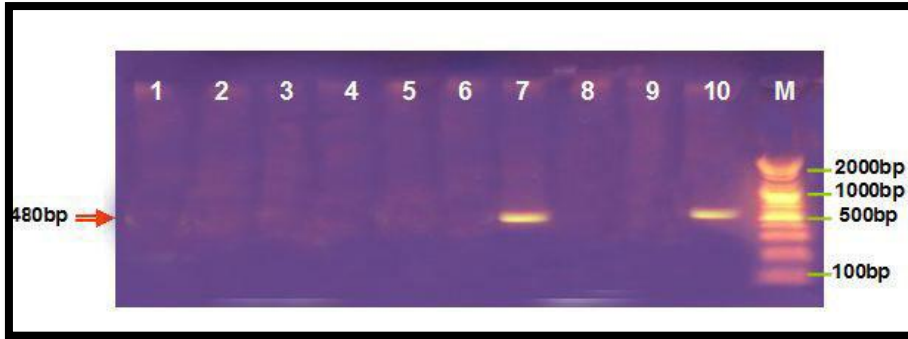
#### **Phenotypic detection of carbapenem production:**

1(10%) isolate demonstrated enhancement of inhibition zone, with the imipenem-EDTA test whereas six isolates showed positive results with modified Hodg test.

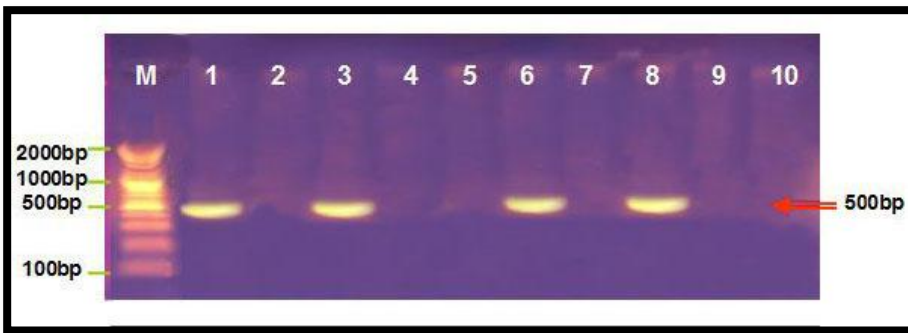
#### **Genotypic detection of NDM genes :**

*bla*NDM-1 genes were appeared in (20%) of *A. baumannii* isolates PCR products using specific primers gene. Moreover, the amplification of DNA of *A. baumannii* isolates with *bla*NDM-2 primers, observed presence of (40%) of *bla*NDM-2 gene positive isolates (Fig. 2 & Fig.3). Consequently, table (3) shows the isolates that harbored

*bla*NDM genes appeared as extensive drug resistant (XDR), which exhibited resistance to at least 5 classes of antibiotics were used in this study.



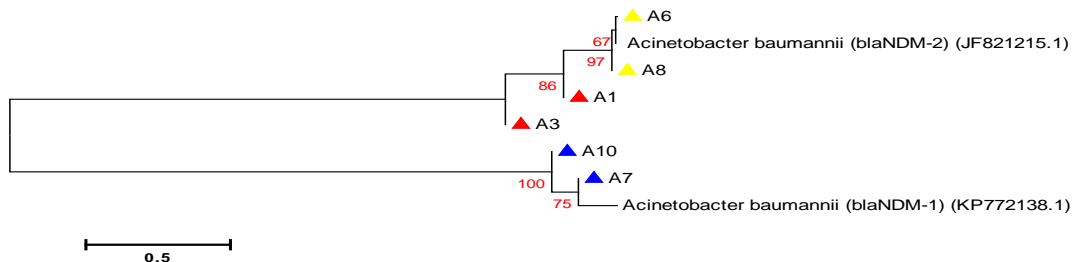
**Figure (2):** Agarose gel electrophoresis (1.5% agarose,70 volt for 1-2 hrs) for *bla*NDM-1 gene product (amplified size 480 bp) using DNA template of *Acinetobacter baumannii* isolates extracted by using genomic extracting kit. Lane (M), DNA molecular size marker (100- bp Ladder). Lanes (7 and 10) of *A. baumannii* isolates show positive results with *bla*NDM-1 gene . lanes (1, 2, 3, 4, 5, 6, 8 and 9) show negative results with *bla*NDM-1 gene.



**Figure (3):** Agarose gel electrophoresis (1.5% agarose,70 volt for 1-2 hrs) for *bla*NDM-2 gene product (amplified size 500 bp) using DNA template of *Acinetobacter baumannii* isolates extracted by using genomic extracting kit. Lane (M), DNA molecular size marker (100- bp Ladder). Lanes (1 , 3 , 6 and 8) of *A. baumannii* isolates show positive results with *bla*NDM-2 gene . lanes (2, 4, 5, 7, 9 and 10) show negative results with *bla*NDM-2 gene.

**Table (3) : Antibiotics resistant pattern of carbapenemase genes positive *A. baumannii* isolates.**

Type of resistance	No. (%) of <i>A. baumannii</i> isolates (N=10)	No. of antibiotics classes resisted
MDR	10 (100%)	3 or more
XDR	2 (20%)	9
	3 (30%)	7
	4 (40%)	6
	5 (50%)	5
PDR	1 (10%)	11



**Figure (4):** Phylogenetic tree analysis based on the partial NDM gene sequence that used for genotyping study.

The phylogenetic tree was constructed using the neighbor joining algorithm in (MEGA 6.0 version). The local *Acinetobacter baumannii* (Isolates No. A6 and A8) were show closed related to NCBI-Blast *Acinetobacter baumannii* NDM-2 (JF821215.1), and the local *Acinetobacter baumannii* (Isolates No. A7 and A10) were show closed related to NCBI-Blast *Acinetobacter baumannii* NDM-1 (KP772138.1), whereas, other local *Acinetobacter baumannii* (Isolates No. A1 and A3) were show some different than NCBI-Blast *Acinetobacter baumannii* NDM-1 and NDM-2 it may as a unique genotyping.

## DISCUSSION

The current report provided an evidence that the *A. baumannii* may be silently spread with low proportion 0.76% (Table 1) in a hospital settings and highlighted the threat of undetected reservoirs. However, The source of infection may include health care device or the environment can involve with transfer of microorganisms between staff and patients (Kollef and Schuster, 1994).

Results from Figure (1) revealed that higher resistant rate was found for ampicillin and carbenicillin were (100%) for each other, while for piperacillin was (30 %). This result in agreement with a pervious study in Hillah by Al-Warid (3), who found that all 11 (100%)  $\beta$ -lactam resistant *A. baumannii* isolates were resistant to ampicillin, carbencillin and piperacillin. Alsehlawi and his colleagues (2) stated that all *A. baumannii* isolates were resistant to ampicillin, carbenicillin (100%) for each other and (91.6%) to ticarcillin. High resistance to this class of antibiotics may be due to widespread use of these antibiotics in Hilla hospitals. The present study showed a high level of resistance to cephalosporins: ceftazidime and cefotaxime (60%) each other, and (70%) for cefepime. There is also a wide range of resistance to aztreonam (80%). Alsehlawi, and his colleagues (2) reported that resistance rate of *A. baumannii* isolates to ceftazidime, cefotaxime and cefepime were (100%). A report by Chaiwarith and his colleagues (11) documented that susceptibility to ceftazidim and cifirome were equal, (70%) in *A. baumannii* isolates collected from Thailand hospitals. High level of resistance to third generation cephalosporins could be attributed to the production of ESBLs, since it mediates resistance to broad spectrum cephalosporins (e.g. ,ceftazidime ,ceftriaxone and cefotaxime) and aztreonam (42). In the present study there was a high level of resistance (80%) to  $\beta$ -lactam /  $\beta$ -lactamase inhibitor combination (amoxicillin / clavulanic acid). This is likely due to the heavy selection pressure from overuse of this antibiotic and seem to be losing the battle. A similar result was recorded by Alsehlawi and his colleagues (2) who noted that (75%) of *A. baumannii* were resistant to amoxi-clav. In spite of the restricted use of cefotaxime in treatment of bacterial infection in Iraq, results of this study revealed a higher resistance (70%) to cefotaxime among clinical isolates of *A. baumannii*. This result is in accordance with the findings recorded by other researchers, Al-Warid,(100%) in Hillah(3) and AlSehlawi and his colleagues,(100%) in Najaf(2). The resistance to cephalosporins may be as a result of the development of porin-deficient mutants (32). In addition, increasing numbers of bacterial strains express different types of  $\beta$ -lactamases including inducible and / or plasmid mediated AmpC type of enzyme may also increase the chance for resistance to cephalosporins (43).

Results from (Fig.1) showed that resistance rates to imipenem, meropenem and ertapenem were (10%). This result is lower than that reported by other studies contacted in Turkey which reported that the resistance rate of *A. baumannii* isolates collected from clinical samples to imipenem was (46.7%) and to meropenem was (53.3%) (35). While in a local study in Najaf, AlSehlawi and his colleagues (2) found that only four isolates (41.6 %) of *A. baumannii* were resistant to imipenem and meropenem antibiotics. Leepethacharat and Oberdorfer demonstrated (65%) and (50%) resistance to imipenem and meropenem, respectively by *A. baumannii* in a surveillance study at Chiang Mai University Hospital in Thailand (31). While in USA Trottier and his colleagues who found that (87%) of *A. baumannii* resist to imepenem(48).

Meropenem is well tolerated and offers several potential advantages, including greater *in vitro* activity against Gram negative pathogens and the option of bolus administration (49). Beside these, problem of renal metabolism of imipenem, and risk of seizures (40), and availability of meropenem only in Hillah hospitals might be the reasons behind possible greater use of meropenem over imipenem and hence the high prevalence of resistance.

Ertapenem is the least active carbapenem against most strains producing carbapenemase and therefore the first marker that indicates the likelihood of carbapenemase occurrence (34, 45). Specificity is questioned because bacteria with ESBL and porin mutations are also resistant to ertapenem (23)

Reasons behind resistance may be due to inappropriate duration of antibiotic therapy and sub-therapeutic concentrations of the drug (6,37).

Results of the present study revealed that amikacin was more effective (80%) than other aminoglycosides, gentamicin and Tobramycine (60%) for each other. This result was parallel with other studies worldwide, as with Leepethacharat and Oberdorfer in Thailand (31) and Özdemir and his colleagues In Turkey(35). In another study in Najaf Alsehlawi and his colleagues who found that resistance against aminoglycosides were (58.3%) to amikacin, whereas gentamicin and Tobramycine (83.3%),(2).



High efficiency of amikacin may be due to its less vulnerability to bacterial enzymes than other aminoglycosides. In both *A. baumannii*, gentamicin and Tobramycin resistance is often due to the expression of a variety of modifying enzymes including aminoglycoside modifying enzymes (AME), acetylases, phosphorlyases and adenylases which can impair the effectiveness of antibiotics. Other resistance mechanisms include changes in bacterial membrane permeability and altered ribosomal proteins (8).

As shown in figure (1) resistance to quinolones antibiotics, (ciprofloxacin) was (40%). Quinolone resistance is typically encoded chromosomally. In this study, resistance against fluoroquinolones may reflect antibiotic pressure in Hilla hospitals. In China, Zhou and his colleagues reported high resistance to quinolones (> 95%) among clinical isolates of *A. baumannii* (52). In another study in Najaf Alsehlawi and his colleagues who found that resistance against ciprofloxacin was (91.6 %),(2).

Quinolone resistance results from mutations in the chromosomally encoded type II topoisomerases, and via the up regulation of efflux pumps, or point-related genes (17,47). The plasmid *qnr* genes play an emerging role in the dissemination of fluoroquinolone resistance (22).

Results of present study show highly resistant level to colistin sulphate (80%) with moderate resistance to Polymyxin B (50%). This result had been convergent with study at Chiang Mai University Hospital in Thailand (31).

Percentages of resistance of isolates to the remaining antibiotics were as follows: tetracycline (20%), doxycycline (20%), trimethoprim-sulfamethoxazole (50%) and chloramphenicol (80%). These results were parallel with previous studies in Brasil (46) and with Patwardhan, and his colleagues at India (36). This may be due to multiresistance plasmid harboring *A. baumannii* (36).

The high levels of resistance to antibiotics in the present study may be as a result of both intrinsic and acquired mechanisms. The resistance is widespread and constitutes serious clinical threat (33). In addition, the selection pressure of antibiotics in hospital environment lead to multiple resistance to these drugs. El-Astal, mentioned that inappropriate and incorrect administration of antimicrobial agents and lack of appropriate infection control strategies may be the possible reasons behind increasing resistant rate of *A. baumannii* to common used antimicrobial drugs (19).

All *A. baumannii* isolates were screened by two phenotypic tests for carbapenemase production. Figure (4-4) show that 6 (60%) of the isolates gave positive results by imipenem- EDTA disk test. Different studies which have used the IMP-EDTA to detect MBLs production in *A. baumannii* reported that (33%) of isolates have enhancement of inhibition zone, with the IMP-EDTA test (2). However, there are four isolates which gave negative results with EDTA disk synergy test.

The most easily performed test for Carbapenemase detection is the modified Hodge's test (Figure 4-5), which has been found to be 100% sensitive for the detection of the carbapenemase (29). Out of the 10 *A. baumannii* isolates which were enrolled in this study, 6 (60%) isolates were found to produce the carbapenemase enzyme by MHT and all the remaining isolates were found to be carbapenemase negative. In a previous local study, Alsehlawi and his colleagues reported that 4 (33.3%) of *A. baumannii* isolates recovered from Najaf hospitals were confirmed as carbapenemase producer using modified Hodge's test, whereas the same isolate gave negative result with imipenem-EDTA synergy test (2). Another study from Croatia the Hodge test showed that 74% (72/97) of the *A. baumannii* isolates were positive for carbapenemase production. (18), whereas in a study from Pakistan has shown that 17% of *A. baumannii* were positive for carbapenemase production by MHT (5).

In the present study, the overall prevalence of *bla*<sub>NDM-1</sub> and *bla*<sub>NDM-2</sub> possessing *A. baumannii* were found to be 2 (20%) and 4(40%) for *bla*<sub>NDM-1</sub> and *bla*<sub>NDM-2</sub> respectively. (Table 4-4 and Figures 4-13 & 4-14). This is the first report on prevalence of NDM-1 and NDM-2 genes in Hilla hospitals among *A. baumannii* isolates. Moreover, detection of NDM-2 is considered as a first report on prevalence of this gene in Iraq.

This is the first report of an NDM-1 variant, although the ongoing spread of strains carrying the *bla*<sub>NDM-1</sub> gene will enhance the likelihood of variants emerging. This is an important consideration when designing genetic tools to target carbapenem resistance genes. Interestingly, we have evidence that NDM-encoding genes may be widespread in *A. baumannii*, and further molecular surveys will be necessary to evaluate their distribution in that species. We showed here the first identification of a *bla*<sub>NDM</sub> gene in a clinical isolate originating from Egypt, with no obvious link with the Indian subcontinent. This clinical case suggests 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.(38,39).

As in the present study, such isolates (*bla*<sub>NDM</sub> positive *A. baumannii*) exhibit resistance to most antimicrobials that recommended by CLSI (2012) and appeared to be extensive antibiotic resistance (XDR) (Table 3), this may creating a serious problem for choice of therapy, this results was more identical with the report of emergence XDR in *A. baumannii* isolates from patients in ICUs of Samsung Medical Center in Seoul, South Korea

(28). Hence, The occurrence of isolates contain blaNDM in Hillah hospitals may resulted from transfer of plasmid among resistant isolates rather than, several isolate may produce identical restriction pattern suggest the dissemination of blaNDM due to a clonal spread of resistant *A. baumannii* isolates (12).

In the present study partial NDM genes DNA sequencing had been done to investigate where any variation on NDM gene was occurs . As figure (4-28) shown NDM-1 genes were detected in two isolates (Isolates No.7 and No.10) , NDM-2 genes also were detected in two *A. baumannii* isolates (Isolates No. 6 and No. 8) . whereas in isolates No.1 and No.3 there are some different than NCBI-Blast *A. baumannii* NDM-1 and NDM-2 it may as a unique genotyping . So that in view of these requirements, the current widely used PCR sequencing method is not sufficient, because phenotypic testing (expression of recombinant protein) is needed to confirm whether a new variant identified by sequencing has an alteration in the function of its coded protein (7).

## CONCLUSION

Our study has shown the spreading of multidrug resistant and blaNDM harbored *A. baumannii* isolates among patients with different infections. Hence, it is suggested that, such isolates, which consequently poses an increased threat to hospitalized patients in Hillah hospitals and more importantly, avoiding misuse and overuse of antibiotics may reverse the undesired effects of multidrug resistant and NDM-producing bacteria.

## ACKNOWLEDGMENT

The authors are very grateful to department of biology in faculty of science. The authors thank the anonymous reviewers for their valuable comments and helpful revision suggestions.

## REFERENCES

- 1-Afzal-Shah, M., and Livermore, D.M.(1998). Worldwide emergence of carbapenem-resistant *Acinetobacter* spp. J. Antimicrob. Chemother.,41: 576–7.
- 2-Elsehlawi, Z. S. ; Alshara, J. M. ; Hadi, Z.J. ; Almohana, A. M. (2014). First report of the bla<sub>oxa</sub>-23 gene in a clinical isolates of *Acinetobacter baumannii* in Najaf hospitals-Iraq. Int. J. Recent Sci. Research. 5( 8) : 1407-1411.
- 3-Al-Warid, R.J.M. (2014). Immunological and Molecular Study on *Acinetobacter baumannii* Isolated from Clinical Samples. Ph.D. Thesis. University of Babylon .
- 4-Ambler, R.P., Coulson, A.F., Frère, J.M., Ghuysen, J.M., Joris, B., Forsman, M., et al. (1991). A standard numbering scheme for the class A  $\beta$ -lactamases. Biochem. J., 276:269-70.
- 5-Amjad, A. ; Mirza, I.A. ; Abbasi, S.A. ; Farwa, U. ; Malik, N. and Zia, F. (2011). Modified Hodge test: A simple and effective test for detection of carbapenemase production. Iran J. Microbiol. 3(4):189-193.
- 6-Baquero, F. ; Negri, M.C. ; Morosini, M.L. and Blazquez J.(1997). The antibiotic selective process: Concentration-specific amplification of low- level resistant populations. Ciba. Found. Symp.207:93-111.
- 7-Barczak, A.K. ; Gomez, J.E. ; Kaufmann, B.B. ; Hinson, E.R. ; Cosimi, L. ; Borowsky, M.L. ; Onderdonk, A.B. ; Stanley, S.A. ; Kaur, D. ; Bryant, K.F. ; Knipe, D.M. ; Sloutsky, A. and Hung, D.T.(2012). RNA signatures allow rapid identification of pathogens and antibiotic susceptibilities. Proc. Natl. Acad. Sci. U.S.A.109:6217-6222.
- 8-Barros , J.C.S. ; Bozza , M. ; Gueiros-Filho, F.J ; Bello, A.B. ; Lopes, U.G and Pereira , J.A.A. (1999). Evidences of gentamicin resistance amplification in *Klebsiella pneumoniae* isolated from faeces of hospitalized newborns. Mem. Inst. Oswaldo Cruz. Rio de Janeiro. 94(6): 795-802.
- 9-Bergogne-Berezin, E. and Towner, K .J. (1996). *Acinetobacter* spp. as nosocomial pathogens: microbiological , clinical and epidemiological features. Clin. Microbiol. Rev., 9(2):148- 165.
- 10-Bonomo, R.A. (2011). New Delhi-metallo- $\beta$ -lactamase and multidrug resistanc: a global SOS?.Clin.Infect.Dis.52(4) : 485-48.
- 11-Chaiwarith, R. ; Mahatthanaphak, S. ; Boonchoo, M. ; Supparatpinyo, K. and Sirisanthana, T. (2005). Pandrug Resistant *Acinetobacter baumannii* at Maharaj Nakorn Chiang Mai Hospital. J. Infect. Dis. Antimicrob. Agents.22 (1) : 1-8.
- 12-Chang, K., Lin, M., Lin, N., Wu, W., Kuo, H., Lin, T., Yang, T., Chen, Y. and Liou, M. (2012). Clonal spread of multidrug-resistant *Acinetobacter baumannii* in eastern Taiwan. J. Microbiology, Immunology and Infection.45: 37-42.
- 13-Chen, Y. ; Zhou, Z. ; Jiang, Y. and Yu Y. (2011). Emergence of NDM-1-producing *Acinetobacter baumannii* in China. J Antimicrob Chemother . 66: 1255-1259.



- 14-Clinical and Laboratory Standards Institute. (2012). Performance standards for antimicrobial susceptibility testing: 22nd informational supplement. CLSI document M100-S22. CLSI, Wayne, PA.
- 15-Dalla-Costa L.M., Coelho J.M., Souza H.A., Castro M.E., Stier C.J., Bragagnolo K.L., Rea-Neto A., Pentead-Filho S.R., Livermore D.M. and Woodford, N.(2003). Outbreak of carbapenem-resistant *Acinetobacter baumannii* producing the OXA-23 enzyme in Curitiba, Brazil. J. Clin. Microbiol., 41: 3403-3406.
- 16-Dijkshoorn, L., Aucken, H. M., Gerner-Smidt, P., Kaufmann, M. E., Ursing, J. and Pitt. T. L.(1993). Correlation of typing methods for *Acinetobacter* isolates from hospital outbreaks. J. Clin. Microbiol., 31:702–705.
- 17-Drlica, K. and Zhao, X. (1997). DNA gyrase, topoisomerase IV, and the 4-quinolones. Microbiol .Mol. Biol .Rev. 61: 377-392.
- 18-Ehlers, M.M. ; Hughes, J.M. and Kock, M.M. (2012). Prevalence of Carbapenemases in *Acinetobacter baumannii*, Antibiotic Resistant Bacteria - A Continuous Challenge in the New Millennium, Dr. Pana, M. (Ed.). 213-246.
- 19-El-Astal, Z. (2005). Increasing ciprofloxacin resistance among prevalent urinary tract bacterial isolates in Gaza Strip, Palestine. J. Biomed Biotechnol. (3): 238-241.
- 20-Espinal, P., Fugazza G., López Y., Kasma M., Lerman Y., Malhotra-Kumar S., Goossens H., Carmeli Y., Vila J. (2011). Dissemination of an NDM-2-producing *Acinetobacter baumannii* clone in an Israeli rehabilitation center. Antimicrob Agents Chemother. 55:5396-5398.
- 21-Fallah, F. ; Taherpour, A. ; Vola, M.H. and Hashemi, A. (2011). Global spread of New Delhi metallo-beta-lactamase-1(NDM-1). Iran. J. Clin. Infect. Dis.6(4):171-176.
- 22-Firoozeh, F. ; Zibaei, M. and Soleimani-As, Y. (2014). Detection of plasmid-mediated *qnr* genes among the quinolone-resistant *Escherichia coli* isolates in Iran. J. Infect. Dev. Ctries. 8(7):818-822.
- 23-Flonta, M. ; Lupse, M. ; Craciunas, C. ; Almas, A. and Carstina, D. (2011). Ertapenem resistance among extended-spectrum-β- lactamase producing *Klebsiella pneumoniae* isolates. Therapeu. Pharmacol. Clin. Toxicol. 15(2): 121-125.
- 24-Hornsey, M. ; Phee, L. and Wareham, D.W. (2011). A novel variant NDM-5 of the New Delhi metallo-β-lactamase in a multidrug-resistant *Escherichia coli* ST648 isolate recovered from a patient in the United Kingdom. Antimicrob. Agents Chemother.5(11):5952-5954.
- 25-Kaase, M. ; Nordmann, P. ; Wichelhaus, T.A. ; Gatermann, S.G. ; Bonnin, R.A. and Poirer, L. (2011). NDM-2 carbapenemase in *Acinetobacter baumannii* from Egypt. J. Antimicrob. Chemother.66 :1260-1262.
- 26-Kai-Chih Chang, Ming-Feng Lin, Nien-Tsung Lin, Wen-Jui Wu, Han-YuehKuo, Teng-Yi Lin, Tsai-Lian Yang, Yu- Chuan Chen, Ming-Li Liou. (2012). Clonal spread of multidrug-resistant *Acinetobacter baumannii* in eastern Taiwan. Journal of Microbiology, Immunology and Infection. 45:37-42.
- 27-Karthikeyan, K. ; Thirunarayan, M.A. and Krishnan. P. (2010). Coexistence of blaOXA-23 with <sup>bla</sup>NDM-1 and armA in clinical isolates of *Acinetobacter baumannii* from India. J. Antimicrob. Chemother. 65:2253-2254.
- 28-Kim, K.M., Yoo, J.H., Choi, J.H., Park, E.S., Kim, K.S., Kim K.S.(2006). The nationwide surveillance results of nosocomial infections along with antimicrobial resistance in intensive care units of sixteen university hospitals in Korea. Kor., J. Noso. Infect. Control, 11:79–86.
- 29-Lee, K., Chong, Y., Shin, H. B., kim, Y.A ., Yong, D. and Yum, J. H. (2001). Modified Hodge and EDTA-disc synergy tests to screen metallo-β-lactamase-producing strains of *Pseudomonas* and *Acinetobacter* species .Clin. Microbiol .Infect.,7: 88-91.
- 30-Lee, K., Lim, Y.S., Yong, D., Yum, J. H. and Chong, Y. (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. J. Clin. Microbiol., 41(10): 4623-4629.
- 31-Leepethacharat, K. and Oberdorfer, P. (2007). *Acinetobacter baumannii* Infection and Colonization among Pediatric Patients at Chiang Mai University Hospital. J. Infect. Dis. Antimicrob. Agents.24 (2) : 63-73.
- 32-Manchanda, V. and Singh, N.P. (2003). Occurrence and detection of AmpC β -lactamases among Gram- negative clinical isolates using a modified three- dimensional test at Guru Tegh Bahadur Hospital, Delhi. India. J. Antimicrob. 51:415-418.
- 33-Mathur, P. ; Tatman, A. ; Das, B. and Dhawan, B. (2002). Prevalence of extended beta lactamase producing Gram negative bacteria in a tertiary care hospital. Indian J. Med. Res. 115 :153-7.
- 34-Overturf, G.D. (2010). Carbapenemases: a brief review for pediatric infectious disease specialists. Pediatr. Infect. Dis. J. 29: 68-70.
- 35-Özdemir1, H. ; Kendirli, T. ; Ergün, H. ; Çiftçi1, E. ; Tapısız1, A. ; Güriz, H. ; Aysev, D. ; İnce1, E. and Doğru, Ü.(2011). Nosocomial infections due to *Acinetobacter baumannii* in a pediatric intensive care unit in Turkey. The Turkish Journal of Pediatrics . 53: 255-260.

- 36-Patwardhan, R.B.1. ; Dhakephalkar, P.K. ; Niphadkar, K.B. and Chopade, B.A.(2008). A study on nosocomial pathogens in ICU with special reference to multi-resistant *Acinetobacter baumannii* harbouring multiple plasmids. Indian J. Med. Res. 128(2):178-187.
- 37-Philippe, E. ; Weiss, M. ; Shultz, J.M. ; Yeomans, F. and Ehrenkranz, N.J.(1999). Emergence of highly antibiotic-resistant *Pseudomonas aeruginosa* in relation to duration of empirical anti-pseudomonal antibiotic treatment. Clin. Perform. Qual. Health Care. 7: 83-7.
- 38-Poirel, L. ; Fortineau, N. and Nordmann, P.(2011b). International transfer of NDM-1-producing *Klebsiella pneumoniae* from Iraq to France. Antimicrob Agents Chemother:55(4) : 1821-1822.
- 39-Poirel, L. ; Al Maskari, Z.; Al Rashdi, F. ; Bernabeu, S. and Nordmann, P. (2011a) . NDM-1-producing *Klebsiella pneumoniae* isolated in the Sultanate of Oman. J. Antimicrob. Chemother. 66:304–306.
- 40-Prakash, S. (2006). Carbapenem sensitivity profile amongst bacterial isolates from clinical specimens in Kanpur city. 10 (4) : 250-253.
- 41-Revathi, G. ; Siu, K.L. ; Lu, P-L. and Huang L-Y. (2013). First report of NDM-1-producing *Acinetobacter baumannii* in East Africa. International J. Infectious Dis. 17 : e1255-e1258.
- 42-Shaikh, S. Fatima, J. and Kamal, M. A.(2015). Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. Saudi J. Biol. Sci. 22(1) : 90-91.
- 43-Siu, L.K. ; Lu, P.L. ; Chen, J-Y. ; Lin, F.M. and Chang, S-C. (2003). High level expression of AmpC  $\beta$ -lactamase due to insertion of nucleotides between -10 and -35 promoter sequences in *Escherichia coli* clinical isolates: cases not responsive to extended-spectrum cephalosporin treatment. Antimicrob. Agents Chemother. 47: 2138-2144.
- 44-Struelens, M. J. ; Monnet, D. L. ; Magiorakos, A.P. ; Santos, F. ; O'Connor, and Giesecke, J. (2010). New Delhi metallo-beta-lactamase 1-producing Enterobacteriaceae: emergence and response in Europe. Euro Surveill. 15(46): 1-8.
- 45-Thomson, K. S. (2010). Extended- spectrum  $\beta$ - lactamase, AmpC, and carbapenemase issues. J. Clin. Microbiol. 48(4): 1019-1025.
- 46-Tognim , M. C. B. ; Gaziri, L. C.J. ; Vidotto, M. C. And Perugini. M.R. (1999). Association of Plasmid Typing to Biotyping and Antibiotyping in the Characterization of Outbreaks by *Acinetobacter baumannii*. Braz. Arch. Biol. Technol. 42 (1) :1-8.
- 47-Tran, J. H. ; Jacoby, G. A. and Hooper, D.C. (2005). Interaction of the plasmid encoded quinolone resistance protein Qnr with *Escherichia coli* DNA gyrase. Antimicrob.Agents Chemother. 49: 118-125.
- 48-Trottier ,V. ; Segura, P. G. ; Namias, N. ; King, D. ; Pizano, L. R. and Schulman, C. I.(2007). Outcomes of *Acinetobacter baumannii* Infection in Critically Ill Burned Patients. J. Burn Care & Research. 28(2) : 248-254.
- 49-Verwaest, C. and Belgian Multicenter Study Group. (2000). Meropenem versus imipenem / cilastatin as empirical mono-therapy for serious bacterial infections in the intensive care unit. Clin .Microbiol. Infect. 6: 294-302.
- 50-Wu, H.S. ; Chen, T.L. ; Chen I.C. ; Huang, M.S. ; Wang, F.D. ; Fung C.P. and Lee, S.D. (2010). First identification of a patient colonized with *Klebsiella pneumoniae* carrying blaNDM-1 in Taiwan. J. Chin. Med. Assoc. 73(11) :596-598.
- 51-Yong, D., Toleman, M.A. ; Giske, C.G. ; Cho, H.S. ; Sundman, K. ; Lee, K. and Walsh, T.R. (2009). Characterization of a new metallo-beta-lactamase gene, <sup>bla</sup>NDM-1, and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. Antimicrob. Agents Chemother. 53(12):5046–5054.
- 52-Zhou, H. ; Pi, B-R. ; Yang, Q. ; Yu, Y-S. ; Chen, Y-G. ; Li, L-J. and Zheng, S-S. (2007). Dissemination of imipenem-resistant *Acinetobacter baumannii* strains carrying the ISAbal–blaOXA-23 genes in a Chinese hospital. J. Medic. Microbiol. 56: 1076-1080.