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Molecular Detection of Multidrug Resistant *Acinetobacter baumannii* from Different Clinical Samples

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Abstract

Background *Acinetobacter baumannii* (*A. baumanii*) has recently emerged as a major pathogen causing nosocomial infections in patients admitted to intensive care units with a surprisingly rapid acquisition of antibiotic resistance.

Objective To study the rate of occurrence of *A. baumanii* isolated from different clinical specimens and to study the rate of occurrence of multidrug resistance especially NDM-1 and qnrA genes.

Methods A total of sixty-two (62) clinical isolates of *A. baumanii* were tested against 14 antibiotics by disc diffusion method. Minimum inhibitory concentration was determined by agar dilution method for resistant isolates. Polymerase chain reaction (PCR) was performed to detect bla NDM-1 and qnrA genes.

Results All Acinetobacter isolates were complete resistant to Colistin and Tigacycline (100%), while high rate of resistance was to Aztronem (93.54%), Cefotaxime, (91.93%), Ceftriaxone (88.70%) and Meropinem (80.64%). Moderate - to- low rate of resistance was to Ceftazidime (77.41%), Cefepim (75.80%), Peperacillin and Ciprofloxacin (74.19%), Gentamicin (69.35%), Levofloxacin (64.51%), Amikacin (61.29%) and Impenim (50%). The highest minimum inhibitory concentration value 128 µg/mL was to Cefotaxime, Tigacycline and Colistin. While the lowest value 8 µg/mL was to Gentamicin, Imipenem, Ciprofloxacin and Levofloxacin. The PCR results showed that 50% of Metallo beta lactamase producers *A. baumannii* was carried bla NDM-1 gene in chromsomal DNA and 24 (48%) of Flouroquinolone resistance *A. baumannii* harbored qnrA gene in chromsomal DNA while the prevalence of qnrA gene was (60.6%) in plasmid DNA.

Conclusion There is a high prevalence of multidrug resistant *A. baumannii* in different samples from Baghdad with the high prevalence of bla NDM-1 and qnrA genes among this bacterium.

Keywords *Acinetobacter baumannii*, bla NDM-1, qnrA- gene, MDR

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List of abbreviation: bla NDM-1 = New Delhi Metallo beta-lactamase, MDR: Multi-drug resistance, qnrA = Quinolone resistance

Introduction

Acinetobacter baumannii (*A. baumanii*) is a gram-negative, non-lactose fermenting organism that is increasingly recognized as a major opportunistic pathogen causing nosocomial infections [(1)]. The organism is characterized by its tendency to acquire resistance to multiple classes of antimicrobials; including carbapenems, aminoglycosides, and fluoroquinolones [(2)]. Identified New Delhi MBL-1 (NDM-1) is a new type of carbapenemase belongs to the class B of Ambler β-lactamases

[3]. It was first reported in *Klebsiella pneumoniae* and *Escherichia coli* derived from a Swedish patient of Indian origin who was admitted to hospital in New Delhi, India in 2009 [4]. Carbapenem resistance caused by acquiring the Metallo-beta-lactamases (MBL) is considered to be more serious than other resistance mechanisms because MBLs can almost hydrolyse all beta-lactam antibiotics except monobactams [5].

Plasmid-mediated quinolone resistance (PMQR) genes, such as qnr family, can be horizontally transferred and contribute to reduced susceptibility to fluoroquinolones [6,7]. The qnr genes appear to be acquired from chromosomal genes in aquatic bacteria [8]. The gene was cloned and was found to produce a 219-aa protein belonging to the pentapeptide repeat family [9], members of which are involved in protein-protein interactions. Purified qnr protein was shown to bind to and protect both DNA gyrase and topoisomerase IV from inhibition by ciprofloxacin [9,10].

This study aimed to investigate the rate of occurrence of *A. baumanii* isolated from different clinical specimens and their resistance to different classes of antibiotics including Carbapenemes and Fluoroquinolone with the detection of (NDM-1) and (qnr) genes in this bacterium.

Methods

Clinical isolates

This study was conducted during the period from the first of November 2015 to the end of April 2016. Two hundred (200) of different clinical specimens include blood, urine, wound swabs, cerebrospinal fluid and sputum were collected from patients in Al-Imamein Al-Kadhimein Medical City in Baghdad and Child Protection Hospital. One hundred and thirty-two (132) swabs were obtained from injured soldiers and diabetic foot. The isolates were identified by conventional and biochemical methods.

Antibiotic susceptibility testing

Sixty-two of *A. baumannii* isolates were tested for their susceptibility to fourteen antimicrobial agents including; Amickacin (30 µg), Cefotaxime (30 µg), Ceftriaxone (30 µg) Ceftazidime (30 µg), Aztreonam (30 µg), Ciprofloxacin (10 µg), Cefepime (30 µg), Colistin sulphate (25 µg), Imipenem (10 µg), Gentamicin (30 µg), Meropenem (10 µg), Piperacillin (100 µg), Tigacyclin (15 µg), and Levofloxacin (5 µg) in accordance to Clinical and Laboratory Standards Institute (CLSI) 2014 recommendations [11].

Minimal inhibitory concentrations

The minimum inhibitory concentration was determined for *A. baumannii* by Vitik2-System and standard agar dilution method [12] according to the CLSI (2014) [11]. The antibiotic concentrations ranged from (0.125-128) µg/ml.

DNA Extraction

Chromosomal DNA were extracted using Genomic DNA extraction kit (Promega, USA), and analyzed on 1.5% agarose gel.

Plasmid Extraction

Rapid boiling method was used to extract plasmid DNA from clinical isolates according to Reischl et al. 2000 [13].

Molecular detection of bla NDM-1 gene and qnrA gene from *A. baumannii* isolates using PCR technique

Specific primer of NDM-1 gene was used as forward (5'-GGGCAGTCGCTTCCAACGGT) and reverse (5'-GTAGTGCTCAGTGTCGGCAT) [14]. While the qnrA specific primer forward was (5'-GATAAAGTTTTTCAGCAAGAGG) and reverse was (5'-ATCCAGATCGGCAAAGGTTA) [15] and Tri-phosphate isomerase genes were used as forward (5'-AAAGAAGCTACTAAGGGTACAAA) and reverse (5'-CATAATATTGGGTCTATTCCTAC) [16] that produce 475 bp, 593 bp and 230 bp respectively.

Twenty microliter of PCR mixture composed of 5 µl of PCR Master Mix (PCR Buffer, $MgCl_2$, dNTPs and Taq DNA polymerase) (Promiga,

USA), 2 µl of each 2 µM forward and reverse primer sequences (Alfa- DNA, Canada), 4 µl volume of chromosomal DNA sample, and nuclease free water to complete the volume to 20 µl. Internal control Triphosphate isomerase (tpi) 230 bp housekeeper gene was used as a positive control for compare with appositive results. The reaction was carried in Thermal cycler (Cleaver Scientific Thermal Cyclers-TC32/80, UK). PCR cycling program for detection of bla NDM, qnrA and tpi genes were showed in tables (1, 2 and 3) respectively. Of each amplified DNA, 10 µl were analyzed by electrophoresis in a 1.5% agarose gel at 70 V for 1 h in 1X Tris borate (TBE) containing 2.5% ethidium bromide. The samples were run alongside with 100 bp ladder (Promiga, USA) as molecular weight marker. The bands were visualized using UV Tran-illuminator (LKB, Sweden).

Table 1. PCR program for detection of bla NDM genes Amplification by thermal cycler

Steps	Temperature °C	Time	Cycles
Initial Denaturation	95 °C	5 minutes	1
Denaturation	95 °C	30 seconds	
Annealing	56 °C	30 seconds	40
Extension	72 °C	1 minute	
Final Extension	72 °C	15 minutes	1

Table 2. PCR program for detection of qnrA genes Amplification by thermal cycler

Steps	Temperature °C	Time	Cycles
Initial Denaturation	94 °C	10 minutes	1
Denaturation	94 °C	1 minute	
Annealing	57 °C	30 seconds	30
Extension	72 °C	1 minute	
Final Extension	72 °C	10 minutes	1

Table 3: PCR program for detection of tpi genes amplified by thermal cycler

Steps	Temperature °C	Time	Cycles
Initial Denaturation	95 °C	3 minutes	1
Denaturation	95 °C	30 seconds	
Annealing	60 °C	30 seconds	40
Extension	72 °C	30 seconds	
Final Extension	72 °C	10 minutes	1

Results

All Acinetobacter isolates were complete resistant to Colistin and Tigacycline 100%, while the high rate of resistance showed to Aztronem (93.54%), Cefotaxime (91.93%), Ceftriaxone (88.7%) and Meropinem (80.64%). Moderate-to-low rate of resistance to Ceftazidime (77.41%), Cefepim (75.80%), Peperacillin and Ciprofloxacin) 74.19%), Gentamicin (69.35%), Levofloxacin (64.51%), Amikacin (61.29%) and Impenim (50%) (Table 4).

The minimum inhibitory concentration (MIC) to detect the level of resistance was for Colitin and Tigacycline are ranged from (64-128 µg/ml), Cefotaxime (128 µg/ml), Gentamicin (8-

16 µg/ml), Ceftazidime (32-64 µg/ml), Ceftriaxone and Cefepim (16-64 µg/mL), Amikacin (62-125 µg/ml), Ciprofloxacin (2-8 µg/ml), Levofloxacin (4-8 µg/ml), Imipenem (8-16 µg/mL), Pipracillin (64- ≥128 µg/mL) and Meropenem (64-128 µg/ml).

By PCR essay, the amplified products of sequences of bla (NDM-1 gene) in chromosomal DNA, (qnrA gene) in both genomic and plasmid DNA and housekeeper gene (tpi) were of size 475 bp, 593bp and 230bp respectively (Figures 1, 2 and 3).

Table 4. Numbers and percentages of susceptibility of different isolates of *A. baumannii*

AB (14)	Blood (30)		Urine (6)		CSF (7)		Wound (15)		Sputum (4)		Total (62)	%	
	No	%	No	%	No	%	No	%	No	%		R*	S*
IMP	11	36.6	3	50	7	100	9	60	1	25	31	50	50
CTX	25	83.3	6	100	7	100	15	100	4	100	57	91.93	8.06
CAZ	20	66.6	5	83.3	7	100	12	80	4	100	48	77.41	22.58
CIP	20	66.6	3	50	7	100	12	80	4	100	46	74.19	25.80
LEV	20	66.6	3	50	5	71.4	8	53.3	4	100	40	64.51	35.48
PRL	21	70	5	83.3	6	85.7	13	86.6	4	100	49	74.19	25.80
GEN	19	63.3	4	66,6	7	100	9	60	4	100	43	69.35	30.64
FEP	20	66.6	5	83.6	6	85.7	12	80	4	100	47	75.80	24.19
CO	30	100	6	100	7	100	15	100	4	100	62	100	0
TG	30	100	6	100	7	100	15	100	4	100	62	100	0
MEM	25	83.3	4	66.6	6	85.7	11	73.3	4	100	50	80.64	19.35
Ak	18	60	2	33.3	6	85.7	8	53.3	4	100	38	61.29	38.70
AT	26	86.6	6	100	7	100	15	100	4	100	58	93.54	6.45
CTR	23	76.6	6	100	7	100	15	100	4	100	55	88.70	11.29

AT, Aztronem; CTX, Cefotaxime; CRT, Ceftriaxone; CAZ, Ceftazidime; CIP, Ciprofloxacin; LEV, Levofloxacin; TG, Tigacycline; PRL, Peperacillin; CO, Colistin; GEN, Gentamicin; Ak, Amikacin; FEP, Cefepim; IMP, Imipenem; MEM, Meropinem, R*, resistance; S*, sensitive

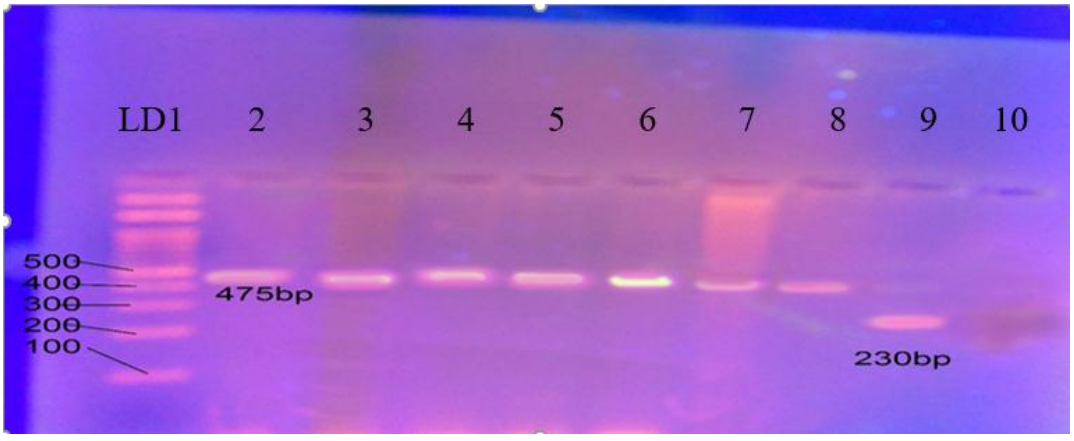

Figure 1. Gel electrophoresis of amplified PCR product (475 bp) for bla NDM-1 gene. Lane 1: 100 bp ladder. Lanes 2-8: Clinical isolates showing positive result. Lane 9: Internal control (tri–ph. iso.230 bp). Lane 10: Negative control. (1.5% agarose, 7 v/cm^2, 1.5 hrs)

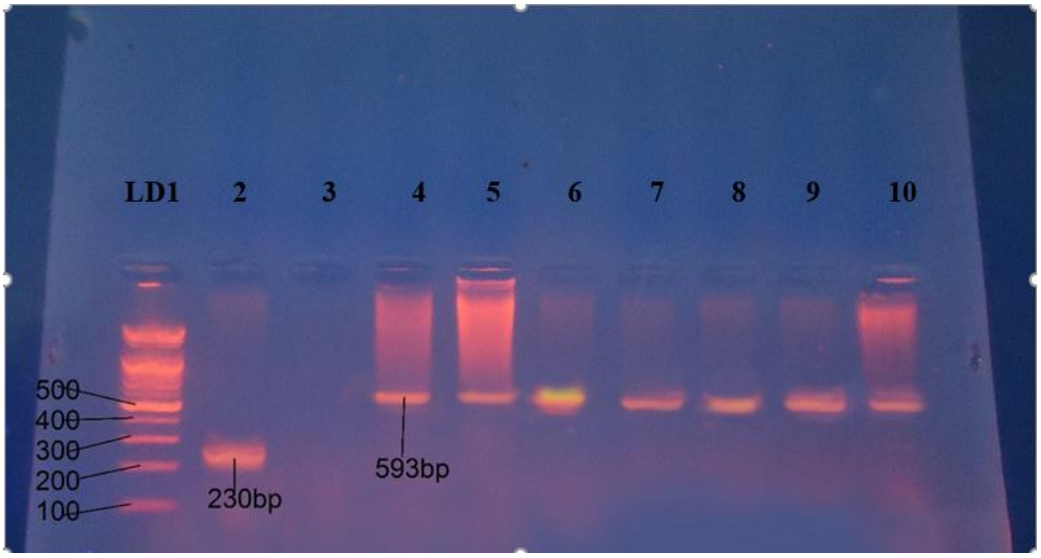

Figure 2. Gel electrophoresis of Amplified qnrA gene (593 bp) in genomic DNA using PCR with specific primers. Lane 1: 100 bp ladder. Lane 2: Positive control (tri-phosphotase 230 bp). Lane 3: Negative control. Lanes 4-10: Clinical isolates showing positive result. (1.5% agarose, 7 v/cm^2, 1.5 hrs)

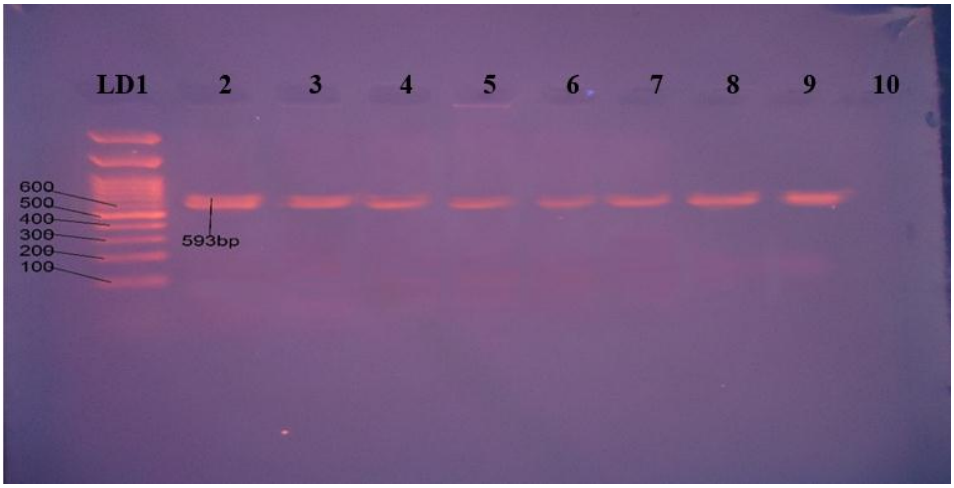

Figure 3: Gel electrophoresis of Amplified plasmid DNA for qnrA gene (593bp) using PCR with specific primers. Lane 1: 100 bp ladder. Lane 2-9: Clinical isolates showing positive result Lane 10: Negative control. (1% agarose, 7 v/cm^2, 1.5 hrs)

The present study also showed that the prevalence of bla NDM-1 was 25(50%) in chromosomal DNA, while the prevalence of qnrA gene was 24(48%) in chromosomal DNA and 20 (60.60%) in plasmid DNA (Table 5).

The presence of bla NDM-1 genes (48.0%) in *A. baumannii* was associated with high percentage of resistance to Carbapenems (Imipenim (53.6%) and Meropenim (60%). Also, the presence of qnrA gene (46.0%) in *A. baumannii* association with high percentage of resistance to Fluoroquinolones group (Ciprofloxacin (64.7%) and Levofloxacillin (60.7%) (Tables 6 and 7).

Table 5. The prevalence of bla (NDM-1 gene) and qnrA gene in Acinetobacter isolates

PCR-Results	NDM-1 gene (DNA)	qnrA gene (DNA)	qnrA gene (plasmid)
Positive No. (%)	25 (50.0%)	24 (48.0%)	20 (60.6%)
Negative No. (%)	25 (50.0%)	26 (52.0%)	13 (39.4%)
Total(No)	50	50	33

Table 6. Association between, Imipenim and Meropenim resistance and production of Metallo-β-lactamase (NDM-1gene) by Acinetobacter isolates

bla NDM-1 gene	Imipenem (10 µg)			Meropenem (10 µg)		
	R	S	Total	R	S	Total
Positive (No)%	15 (53.6%)	7 (36.8%)	22	24 (60.0%)	0 (0)	24
Negative (No)%	13 (46.4%)	12 (63.2%)	25	16 (40.0%)	10 (100%)	26
Total	28	19	47	40	10	50
P value		0.424			0.001*	

*Significant association (0.001)

Table 7. Association between, Ciprofloxacin and Levofloxacillin resistance with presence of qnrA-gene in Acinetobacter isolates

qnrA gene	Ciprofloxacin (5 µg)			Levofloxacin (5 µg)		
	R	S	Total	R	S	Total
Positive (No)%	22 (64.7%)	0 (0)	22	17 (60.7%)	0 (0%)	17
Negative (No)%	12 (35.3%)	13 (100%)	25	11 (39.3%)	15 (100%)	26
Total	34	13	47	28	15	43
P value		0.001*			0.001*	

*Significant association (0.001)

Discussion

The present study observed that 100% of the isolates of *A. baumannii* are multidrug resistant (MDR) when tested by standard disk diffusion method. The most prominent results of antibiotic sensitivity showed that this bacterium is completely resistant to Colistin and Tigacycline 100%. A study in Iraq done by Al-Samaree and Al-Khafaji in 2016 [17], they reported that, 30% of isolates resistant to Colistin. However, the current results are not in line with study in Thailand (2014), they found that *A. baumannii* isolates were susceptible to Colistin (97%) [18].

Modification of lipopolysaccharide outer membrane by addition of phosphor ethanol amine to the hepta-acylated lipid A structure has been suggested as a major mechanism of colistin resistance in *A. baumannii* [19-21]. While the resistance to Tigecycline due to over expression of a multi-drug efflux pump in *A. baumannii* [22]. In this study, a very high percentage resistance of *A. baumannii* to Aztronem. Also, similar findings showed by Al-Saleem 2013 [23] who found that *A. baumannii* clinical isolates developed 97.3% of resistance to Aztreonam and Ceftriaxone, 89.5% to Ceftazidime, 58.2% to Imipenem and Meropenem. Moreover, Al-Mashhadani in 2010 [24] studied the same bacterium revealed that, resistance percentage to Cefotaxime, Ceftazidime and Ceftriaxone were 100%. Carbapenem class represented by Imipenem and Meropenem. The current study was agreed with other study in Iraq; Al-Marjani in 2013 [25]

who found that a high level of resistance reached to 88.2% for Meropenem and 52.9% for Imipenem, with a resistance rate reached to 100% for Ceftriaxone, Cefepime and Azteronam, but not identical with the results obtained by Shareek et al. 2012 [26] whom found that (12.2%) of isolates were resistant to Cefotaxime.

Resistance to beta-lactams due to the presence of β-lactamases, which is the most prevailing mechanism of β-lactam resistance. These enzymes, at least partially, hydrolyze carbapenems along with other β -lactams [27]. Lately a new extended spectrum AmpC enzyme was identified *in A. baumannii*, which able to hydrolyze Ceftazidime, Cefepime and Aztroenam [28]. In regard to Fluoroquinolone resistance of *A. baumannii* is often caused by modifications in the structure of DNA gyrase secondary to mutations in the quinolone resistance-determining regions of the gyrA and parC genes [29,30]. These changes result in a lower affinity for the binding of the quinolone to the enzyme-DNA complex, a second mechanism of resistance to the quinolones is mediated by efflux systems that decrease intracellular drug accumulation [31]. In present study, the resistance of *A. baumannii* to Aminoglycoside is probably attributed to the fact that most clinical isolates of A. baumannii associated with Aminoglycoside-modifying enzymes or efflux pump mechanisms [32].

In the current study, these results are very close to study Done by Shali in 2012 [33] whom showed that MIC to Imipenem was ≥ 16, about 90% were resistant to Pipracillin/Tazobactam (MIC ≥ 128) whereas 85.71% of the strains showed resistance to Cefotaxim (MIC 16 - ≥ 64), Ceftazidime and Amikacin (MIC ≥ 64), but varied in Gentamicin (MIC ≥ 64) and Ciprofloxacin (64-320). However, this result disagreed with done by Al-Marjani in 2013 [25] whom found that *A. baumannii* isolates had MIC > 512 µg/ml for Cefotaxime. The current results showed that approximately 50% of *A. baumannii* have NDM-1 gene 50% in chromosomal DNA the percentage of NDM-1 gene in the current study was double of those in Egypt [34]. The present result was agreed with study done by Al-Harmoosh in 2015 whom found that (20%) of *A. baumannii* were harbored bla NDM-1 gene and (40%) bla NDM-2 [35].

A study on the dissemination of NDM-1 producing *A. baumannii* in Europe showed that the five isolates from Germany, France, Slovenia and Switzerland, which indicates a spread of NDM-producing clones in Europe [36]. Sixty percent (60%) Carbapenem-resistant *A. baumannii* strains isolated in 2012 in the Tripoli Government Hospital, Lebanon, from civilians wounded during the Syrian war (5.71%) were positive to NDM-1genes from Syrian isolates) and 1.42% (1 Lebanese isolate) [37]. However, two NDM-1-producing *Klebsiella pneumoniae* imported from Iraq were detected in 2010 [38].

A study done by Shali in 2012 [33] who detected that blaOXA-23-like in all of 21 strains of *A. baumannii* and resistant percentages for all isolates were recorded; highest resistant rate was against ampicillin (100%) while lowest rate was against imipenem (57.1%). The MICs of imipenem for the resistant isolates were ≥16. All isolates show multi drug resistance to different antibiotics. The bla NDM-1 positive bacteria have been disseminated worldwide [39]. This study noted, the presence of bla NDM-1 genes (48.0%) in *A. baumannii* was associated with high percentage of resistance to Carbapenems (Imipenim (53.6%) and Meropenim (60%)). The bla NDM-1 genes responsible for Carpapenems resistance by encoding to enzymes, these enzymes partially hydrolyze carbapenems along with other b-lactams [27].

Similar findings were reported in Brazil by Chagas et al. 2015 when they observed 37.5% of qnrA gene found in *A. baumannii* isolates [40]. Also, the presence of qnrA gene (46%) in *A. baumannii* association with high percentage of resistance to Fluoroquinolones group Ciprofloxacin (64.7%) and Levofloxacillin (60.7%). Members of which are involved in protein-protein interactions. Purified qnr

protein was shown to bind to and protect both DNA gyrase and topoisomerase IV from inhibition by Ciprofloxacin [9,10,41]. The qnr genes appear to be acquired from chromosomal genes in aquatic bacteria [8]. These results unlike some studies [42,43], that could not detect any qnrA in their clinical isolates of *A. baumannii*. None of the plasmid-mediated qnr determinants have been identified so far in non-enterobacterial Gram-negative species like *A. baumannii* in Iran [44]. On the other hand, these plasmid-mediated determinants have been identified in many Enterobacteriaceae species throughout the world [45]. The rapid emergence of multidrug-resistant (MDR) strains is generally because of their capacity to acquire and disseminate exogenous genes associated with mobile genetic elements such as plasmids, transposons, integrons, and genomic islands. Plasmid-mediated quinolone resistance (PMQR) genes, such as qnr family, can be horizontally transferred and contribute to reduced susceptibility to fluoroquinolones, these genes code for proteins of the pentapeptide repeat family that protects DNA gyrase and topoisomerase IV from quinolone inhibition [8].

This study concluded that *Acinetobacter baumannii* was the predominant species in patient with different infections in two hospitals in Baghdad. High frequency of *A. baumannii* infections was observed among neonates (<1 year). There was only a limited number of drugs sensitivity. Extended spectrum *B*-lactamase were increased in *A. Baumannii is* very alarming. Multidrug resistance *A. baumannii* to carbapenems (Impanel and Meropenim) and quinolones (Ciprofloxacin and Levofloxacin) was carried two genes have a role in the resistance. The bla NDM-1 genes is associated with the resistance to carbapenems specially IMP and MEM, and this gene is chromosomal- borne. The *qnrA*-gene is associated with the resistant to quinolones is chromosomal and plasmid-borne.

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Author contribution

Dr. Abdulrahman supervise this paper as part from a thesis. Mshachal and Dr. Khudair participated in sampling preparation. Mshachal, Dr. Abdulrahman and Dr. Hassan performed and did the tests. Dr. Abdulrahman interpret the results of the research

Conflict of interest

None.

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References

1. Peleg AY, Seifert H, Paterson DL. Acinetobacter baumannii: emergence of a successful pathogen. Clin Microbiol Rev. 2008; 21(3): 538-82. doi: 10.1128/CMR.00058-07.
2. Adams-Haduch JM, Paterson DL, Sidjabat HE, et al. Genetic basis of multidrug resistance in Acinetobacter baumannii clinical isolates at a tertiary medical center in Pennsylvania. Antimicrob Agents Chemother. 2008; 52(11): 3837-43. doi: 10.1128/AAC.00570-08.
3. Walsh TR. The emergence and implications of metallo-beta-lactamases in Gram-negative bacteria. Clin Microbiol Infect. 2005; 11 Suppl 6: 2-9. doi: 10.1111/j.1469-0691.2005.01264.x.
4. Yong D, Toleman MA, Giske CG, et al. Characterization of a new metallo-beta-lactamase gene, bla(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. 2009; 53(12): 5046-54. doi: 10.1128/AAC.00774-09.
5. Roodsari MR, Fallah F, Taherpour A, et al. Carbapenem-Resistant Bacteria and Laboratory Detection Methods. Arch Pediatr Infect Dis. 2014; 2(1): 188-91.
6. Luo Y, Yang J, Zhang Y, et al. Prevalence of β-lactamases and 16S rRNA methylase genes amongst clinical Klebsiella pneumoniae isolates carrying plasmid-mediated quinolone resistance determinants. Int J Antimicrob Agents. 2011; 37(4): 352-5. doi: 10.1016/j.ijantimicag.2010.12.018.
7. Zhang R, Ichijo T, Huang YL, et al. High prevalence of qnr and aac(6')-Ib-cr genes in both water-borne environmental bacteria and clinical isolates of

Citrobacter freundii in China. Microbes Environ. 2012; 27(2): 158-63.

8. Jacoby GA, Strahilevitz J, Hooper DC. Plasmid-mediated quinolone resistance. Microbiol Spectr. 2014; 2(2). doi: 10.1128/microbiolspec.PLAS-0006-2013.

9. Tran JH, Jacoby GA. Mechanism of plasmid-mediated quinolone resistance. Proc Natl Acad Sci USA. 2002; 99(8): 5638-42. doi: 10.1073/pnas.082092899.

10. Tran J, Jacoby G, Hooper D. The plasmid-mediated protein Qnr protects topoisomerase IV from ciprofloxacin inhibition and interacts with the ParE subunit [abstract C1-604]. Program and abstracts of the 43rd Interscience Conference on Antimicrobial Agents and Chemotherapy (Chicago); Washington, DC: American Society for Microbiology; 2003. p. 71.

11. CLSI (Clinical and Laboratory Standards Institute). Performance standards for antimicrobial susceptibility testing; 16th informational supplement Wayne, PA.2014.

12. Wiegand I, Hilpert K, Hancock RE. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat Protoc. 2008; 3(2): 163-75. doi: 10.1038/nprot.2007.521.

13. Reischl U, Linde HJ, Metz M, et al. Rapid identification of methicillin-resistant Staphylococcus aureus and simultaneous species confirmation using real-time fluorescence PCR. J Clin Microbiol. 2000; 38(6): 2429-33.

14. Manchanda V, Sanchaita S, Singh N. Multidrug resistant acinetobacter. J Glob Infect Dis. 2010; 2(3): 291-304. doi:10.4103/0974-777X.68538.

15. Jacoby GA, Chow N, Waites KB. Prevalence of plasmid-mediated quinolone resistance. Antimicrob Agents Chemother. 2003; 47(2): 559-62.

16. Lemee L, Dhalluin A, Testelin S, et al. Multiplex PCR targeting tpi (triose phosphate isomerase), tcdA (Toxin A), and tcdB (Toxin B) genes for toxigenic culture of Clostridium difficile. J Clin Microbiol. 2004; 42(12): 5710-4. doi: 10.1128/JCM.42.12.5710-5714.2004.

17. Al-Samaree MY, Al-Khafaji ZM. Antibiogram of Acinetobacter baumannii isolated from Baghdad Hospitals. Int J Adv Res Biol Sci. 2016: 3(4): 238-42.

18. Tunyapanita W, Pruekpraserta P, Laoprasopwattanaa K, et al. Antimicrobial susceptibility of Acinetobacter baumannii isolated from hospital patients. Sci Asia 2014; 40: 28-34. doi: 10.2306/scienceasia1513-1874.2014.40.028.

19. Pelletier MR, Casella LG, Jones JW, et al. Unique structural modifications are present in the lipopolysaccharide from colistin-resistant strains of Acinetobacter baumannii. Antimicrob Agents Chemother. 2013; 57(10): 4831-40. doi: 10.1128/AAC.00865-13.

20. Arroyo LA, Herrera CM, Fernandez L, et al. The pmrCAB operon mediates polymyxin resistance in Acinetobacter baumannii ATCC 17978 and clinical isolates through phosphoethanolamine modification of lipid A. Antimicrob Agents Chemother. 2011; 55(8): 3743-51. doi: 10.1128/AAC.00256-11.

21. Beceiro A, Llobet E, Aranda J, et al. Phosphoethanolamine modification of lipid A in colistin-resistant variants of Acinetobacter baumannii mediated by the pmrAB two-component regulatory system. Antimicrob Agents Chemother. 2011; 55(7): 3370-9. doi: 10.1128/AAC.00079-11.

22. Maragakis LL, Perl TM. Acinetobacter baumannii: epidemiology, antimicrobial resistance, and treatment options. Clin Infect Dis. 2008; 46(8): 1254-63. doi: 10.1086/529198.

23. Al-Saleem NH. Genotyping relatedness of Acinetobacter baumannii isolated from medical City/Baghdad. PhD Thesis. College of Science. University of Baghdad; 2013.

24. Al-Mashhadani EIJA. Study the activity of Bacteriocin produced from Lactobacillus plantorum on Virulence factors of Acinetobacter baumannii. MSc Thesis. Al-Mustasiriyah University; 2010.

25. Al-Marjani MF, Ammar MHMA, kadhim EQ. Occurrence of ESBL and MBL genes in Pseudomonas aeruginosa and Acinetobacter baumannii isolated from Baghdad, Iraq. Int J Curr Res. 2013; 5(9): 2482-6.

26. Shareek P, Sureshkumar D, Ramgopalakrishnan, et al. Antibiotic sensitivity pattern of blood isolates of Acinetobacter species in a tertiary care hospital: A retrospective analysis. Am J Inf Dis. 2012; 8(1): 65-9.

27. Jain R, Danziger LH. Multidrug-resistant Acinetobacter infections: an emerging challenge to clinicians. Ann Pharmacother. 2004; 38(9): 1449-59. doi: 10.1345/aph.1D592.

28. Rodriguez-Martinez JM, Nordmann P, Ronco E, et al. Extended-spectrum cephalosporinase in Acinetobacter baumannii. Antimicrob Agents Chemother. 2010; 54(8): 3484-8. doi: 10.1128/AAC.00050-10.

29. Seward RJ, Towner KJ. Molecular epidemiology of quinolone resistance in Acinetobacter spp. Clin Microbiol Infect. 1998; 4(5): 248-54.

30. Vila J, Ruiz J, Goni P, et al. Quinolone-resistance mutations in the topoisomerase IV parC gene of Acinetobacter baumannii. J Antimicrob Chemother. 1997; 39(6): 757-62.

31. Heinemann B, Wisplinghoff H, Edmond M, et al. Comparative activities of ciprofloxacin, clinafloxacin, gatifloxacin, gemifloxacin, levofloxacin, moxifloxacin, and trovafloxacin against epidemiologically defined Acinetobacter baumannii strains. Antimicrob Agents Chemother. 2000; 44(8): 2211-3.

32. Ahmed AM, Hussein AI, Shimamoto T. Proteus mirabilis clinical isolate harbouring a new variant of Salmonella genomic island 1 containing the multiple antibiotic resistance region. J Antimicrob Chemother. 2007 Feb;59(2):184-90. doi: 10.1093/jac/dkl471.

33. Shali AAK. Identification of Multidrug-Resistant Genes in Acinetobacter baumannii in Sulaimani City-Kurdistan Regional Government of Iraq. Asian J Med Sci. 2012; 4(5): 179-83.

34. El-Glil RRA. New Delhi Metallo-beta-Lactamase 1 (NDM-1) Producing Acinetobacterbaumannii in Egyptian Hospitals. Int J Adv Res (IJAR). 2015; 4: 470-8.

35. Al-Harmoosh RA, Jarallah EM. First detection of the blandm-1 and blandm-2 genes in a clinical isolate of acinetobacter baumannii in Hillah Hospitals-Iraq. Int J Adv Res (IJAR). 2015; 3(10): 1407-16.

36. Bonnin RA, Poirel L, Naas T, et al. Dissemination of New Delhi metallo-β-lactamase-1-producing Acinetobacter baumannii in Europe. Clin Microbiol Infect. 2012; 18(9): E362-5. doi: 10.1111/j.1469-0691.2012.03928.x.

37. Rafei R, Dabboussi F, Hamze M, et al. First report of blaNDM-1-producing Acinetobacter baumannii isolated in Lebanon from civilians wounded during the Syrian war. Int J Infect Dis. 2014; 21: 21-3. doi: 10.1016/j.ijid.2014.01.004.

38. El-Herte RI, Araj GF, Matar GM, et al. Detection of carbapenem-resistant Escherichia coli and Klebsiella pneumoniae producing NDM-1 in Lebanon. J Infect Dev Ctries. 2012; 6(5): 457-61.

39. Wailan AM, Paterson DL. The spread and acquisition of NDM-1: a multifactorial problem. Expert Rev Anti Infect Ther. 2014; 12(1): 91-115. doi: 10.1586/14787210.2014.856756.

40. Chagas TPG, Oliveira TRT, Ribeiro SS, et al. Detection of plasmid-mediated quinolone resistance genes (qnr) among acinetobacter baumannii isolates from Brazil. Conference: XXVIII Congresso Brasileiro Microbiologia; 2016.

41. Tran JH, Jacoby GA, Hooper DC. Interaction of the plasmid-encoded quinolone resistance protein Qnr with Escherichia coli DNA gyrase. Antimicrob Agents Chemother. 2005; 49(1): 118-25. doi: 10.1128/AAC.49.1.118-125.2005.

42. Vila J, Marti S, Sanchez-Cespedes J. Porins, efflux pumps and multidrug resistance in Acinetobacter baumannii. J Antimicrob Chemother. 2007; 59(6): 1210-5. doi: 10.1093/jac/dkl509.

43. Pannek S, Higgins PG, Steinke P, et al. Multidrug efflux inhibition in Acinetobacter baumannii: comparison between 1-(1-naphthylmethyl)-piperazine and phenyl-arginine-beta-naphthylamide. J Antimicrob Chemother. 2006; 57(5): 970-4. doi: 10.1093/jac/dkl081.

44. Maleki MH, Jalilian FA, Khayat H, et al. Detection of highly ciprofloxacin resistance acinetobacter baumannii isolated from patients with burn wound infections in presence and absence of efflux pump inhibitor. Maedica (Buchar). 2014; 9(2):162-7.

45. Touati A, Brasme L, Benallaoua S, et al. First report of qnrB-producing Enterobacter cloacae and qnrA-producing Acinetobacter baumannii recovered from Algerian hospitals. Diagn Microbiol Infect Dis. 2008; 60(3): 287-90. doi: 10.1016/j.diagmicrobio.2007.10.002.

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