DOI : 10.35124/bca.2020.20.1.1233

Biochem. Cell. Arch. Vol. 20, No. 1, pp. 1233-1239, 2020 | www.connectjournals.com/bca | *ISSN 0972-5075*

MOLECULAR DETECTION OF *BLA*OXA GENES IN *ACINETOBACTER BAUMANNII* COLLECTED FROM PATIENTS WITH VARIOUS INFECTIONS

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(Revised 25 April 2019, Revised 11 September 2019, Accepted 20 September 2019)

ABSTRACT : ***Acinetobacter baumannii*** **has registered recently as a highly disruptive pathogen worldwide. The present study aimed to set the antimicrobial susceptibility patterns and the prevalence of carbapenem-hydrolyzing oxacillinases among clinical isolates of *A. baumannii* isolated from Baghdad hospitals. A total of 165 samples with clinical cases of different ages included: wounds, urine, burns and sputum. All samples were cultured on some differential media and also on CHROMagar *Acinetobacter* selective medium. The isolates were identified according to microscopic examination and biochemical tests. The sensitivity of all isolates was examined *in vitro* against 10 different antibiotics using disc diffusion method on agar. They were subjected to DNA extraction, amplification of the target DNA by conventional PCR. The findings were clarified that all isolates were completely resistant to Aztreonam and Oxacillin, while most bacterial isolates were sensitive to both Colistin and Tigecycline. The study also accomplished that a big amount of *A. baumannii* clinical isolates had multidrug resistance against commercially used antibiotics even against carbapenems. The results related to Polymerase Chain Reaction cleared that the *bla*OXA51 gene was existed in all clinical isolates 100% (n = 54), which confirmed that this gene is important in the identification of *A. baumannii* and that it is intrinsic for this species. The results also manifested that the *bla*OXA23 gene was the predominant gene among *A.baumannii* isolates 90.74% (n=49), while the *bla*OXA24 gene had recorded 75.92% (n = 41), while the lowest rate was for the *bla*OXA58 gene 3.70% (n = 2). The study concluded that the isolation results of *A. baumannii* using CHROM agar was a perfect method for primary identification in addition to biochemical tests. Also, blaOXA-23-like genes recorded the highest percentage among carbapenem-hydrolyzing class D β-lactamases among *A. baumannii* isolates and blaOXA-58-like genes were the lowest.**

Key words : *Acinetobacter baumannii*, susceptibility patterns, *bla*OXA genes.

INTRODUCTION

Acinetobacter spp. consists of several species, but *Acinetobacter baumannii* is the most widespread member linked directly with hospital-acquired infections (Lin and Lan, 2014). *A. baumannii* is a gram-negative, strictly aerobic, glucose non-fermenting coccobacillus (Park *et al*, 2013). The bacterium *A. baumannii* is a hospital-acquired pathogen, usually affecting immunocompromised patients and patients with prolonged hospitalization, especially in Intensive Care Units (Doughari *et al*, 2011). It had been considered a low-level microbe, in spite of its capacity to cause vast and severe infections including: skin, bloodstream, secondary meningitis, urinary tract, and soft tissue infections (McConnell *et al*, 2013; Wsplinghoff and Seifert, 2014). A particular notoriety was gained for *A. baumannii* among soldiers injured during the Iraqi war though, it was given the name (*Iraqibacter*) after the pervasion of clones from Iraq to American military hospitals in Germany and US (Antunes *et al*, 2014). This bacterium has developed a global importance due to its potentiality to cause a wide number of infections and its possession to different mechanisms that gave it serious rates of resistant to all commercially available antibiotics coupled with the few number of new antimicrobial agents (Pendleton *et al*, 2013). *A. baumannii* is one of the six "ESKAPE" bacteria, which registered as common causes of life-threatening nosocomial infections amongst critically ill and immunocompromised individuals (WHO, 2017). In February 2017, *A. baumannii* has been recorded by the World Health Organization as the top seniority pathogenic bacterium in extreme need for new options of antimicrobials (Tiwari *et al*, 2012). Carbapenems were regarded as the most suitable choice for treating severe

nosocomial infections caused by *A. baumannii*, but the emergence of carbapenemase-producing *A. baumannii* isolates made these bacteria capable of evading the action of most traditional antibiotics including carbapenems (Santajit and Indrawattana, 2016). The current study aimed to isolate and identify *A. baumannii* isolates using conventional and molecular methods and to survey the existence of class D β-lactamases (*bla*OXA-23-Like, *bla*OXA-24-Like, *bla*OXA-51-Like and *bla*OXA-58-Like genes) among *A. baumannii* positive isolates.

MATERIALS AND METHODS

Bacterial isolation and identification

A total of fifty four *A. baumannii* isolates were collected from 165 non-repetitive clinical specimens comprising; wounds, urine, burns and sputum from patients at Baghdad hospitals from the beginning of October 2016 to the end of February 2017. Bacterial isolation and identification were performed using standard laboratory methods (Turton *et al*, 2010). Then, stock cultures were maintained in both agar slants and Trypticase Soy Broth (Oxoid, England) with 20% sterile buffered glycerol at –20^{0}C to be submitted for additional molecular identification tests. The PCR of *bla*OXA-51-like genes was used as a final confirmation to the presence of *A. baumannii* species (Braun and Vidotto, 2004).

Susceptibility testing

Nowadays controlling infections caused by gram negative pathogenic bacteria and the emergence of resistant isolates has become a clinical challenge (CLSI, 2006). Antimicrobial susceptibility tests were performed by disc diffusion method against 10 different antibiotics on Mueller Hinton agar plates according to manufacturer instructions and Clinical and Laboratory Standards Institute guidelines (Musleh, 2017). The applied antibiotics were as follows: Amikacin (10µg), Colistin (10µg), Gentamicin (10µg), Meropenem (10µg), Tigecycline (15µg), Trimethoprim / Sulphamethoxazole (1.25/23.75µg) (Bioanalyse, Turkey), Aztreonam (30µg), Cefotaxime (30ìg) (MAST/ UK), Imipenem (10µg) and Oxaciillin (5µg) (Himedia, India). After using an overnight culture of McFarland standard 0.5 and incubated at 37ºC for 24 hours. The results of inhibition zones developed around the discs were interpreted as susceptible, intermediate or resistant to a particular drug by comparison with standards inhibition zones according to Clinical Laboratories Standards Institute (2011).

Detection of Carbapenem resistance genes (*bla*OXA) by PCR

*Bla*OXA-like genes including *bla*OXA-51, *bla*OXA-23, *bla*OXA-24 and *bla*OXA-58 were amplified according to Woodford *et al* (2006). Amplified DNA fragments were purified with Presto™ Mini gDNA Bacteria Kit/ Geneaid, Taiwan according to the manufacturer's instructions. The PCR analysis was performed using the primers listed in Table 1.

The amplification conditions for this set of genes were as follows: initial denaturation at 94°C for 5 minutes and 30 cycles of 94°C for 45 seconds, 52°C for 40 seconds, 72°C for 45 seconds and a final extension at 72°C for 6 minutes (Mosafer, 2007). Amplified fragments were separated by electrophoresis in 2% agarose gel at 5 volt/ cm for 2hours. Finally, fragments were stained with ethidium bromide and detected under UV transilluminator documentation system (Al-Ouqaili, 2018).

The Statistical Analysis System- SAS (2012) program was used to explain the difference factors among the study percentages. Chi-square test (χ^2) was used for significant comparison among percentages listed in this study.

RESULTS AND DISCUSSION

Bacterial isolation and identification

In the present study, out of 165 clinical specimens were collected from wounds, urine, burns and sputum and identified by conventional identification methods (CHROMagar *Acinetobacter* medium and biochemical

Table 1 : PCR primers to detect *bla*OXA genes encoding carbapenemase (Hou and Yang, 2015).

Gene name	Primer name	Sequence	Product size
*bla*OXA51	F	5'-TAATGCTTTGATCGGCCTTG-3'	353 bp
	R	5'-TGGATTGCACTTCATCTTGG-3'	
*bla*OXA23	F	5'-GATCGGATTGGAGAACCAGA-3'	501 bp
	R	5'-ATTTCTGACCGCATTTCCAT-3'	
*bla*OXA24	F	5'-GGTTAGTTGGCCCCCTTAAA-3'	246 bp
	R	5'-AGTTGAGCGAAAAGGGGATT-3'	
*bla*OXA58	F	5'-AAGTATTGGGGCTTGTGCTG-3'	599 bp
	R	5'-CCCCTCTGCGCTCTACATAC-3'	

tests). 54 isolates were identified as *A. baumannii*. These positive isolates were obtained in high percentage; 42.59% (n = 23) from wound specimens; while the percentage of urine specimens was 31.48% (n = 17), burn specimens constituted 20.37% (n = 11) and the low percentage was obtained from sputum specimens which achieved 5.55% (n = 3).

The susceptibility of studied isolates demonstrated a remarkable rise in the antimicrobial resistance not just only against carbapenems, but also against most classes of antibiotics, especially the antibiotics of choice for treating *A. baumannii* infections (Musleh *et al*, 2019). The present study showed different rates of resistance against 10 antibiotics, as shown in Table 2.

Based on the results of antibiotic susceptibility (Table 2), among 54 isolates of *A. baumannii*, most of them were highly resistant to more than 3 classes of selected antibiotics, in other words, they considered MDR *A. baumannii*, except for Colistin and Tigecycline which were effective against these bacterial isolates with moderate rates. A local study done by Mosafer,(2007) reported that *A. baumannii* clinical isolates showed 100% sensitivity to Meropenem. Additionally, Al-Mash'hadani (2010) found that *A. baumannii* clinical isolates developed 100% resistance to Cefotaxime, 95.45% to Aztreonam and 40.90% to Imipenem. Hussein (2013) found that the clinically identified *A. baumannii* isolates possessed a moderate resistant to Imipenem and Meropenem 58.26%, while they were 100% resistant to Cefotaxime; moreover, they found that the highest resistance represented against wide range of the used antibiotics especially cephalosporins, in addition to Colistin. In the study carried out by Al-Warid and Al-Thahab (2014), it was found that 63.63% of *A. baumannii* clinical isolates were resistant to Amikacin, 81.81% were resistant to Trimethoprim/ Sulfamethoxazole. Furthermore, Ghaima (2016) found that the clinical isolates of *A. baumannii* have shown a high-level resistance to Cefotaxime 87.5%, Trimethoprim/ Sulfamethoxazole 84.4%, Oxacillin 83.3%, Imipenem 81.3%, Amikacin 79.2%, Gentamicin 78.1% and 75% for Meropenem, while the low-level resistance was shown against Tigecycline 11.5% and Colistin 7.3%, respectively. In another Iraqi study, fifty strains of *A. baumannii* demonstrated high level resistance to most of the antibiotics under testing as follows; 92% for Cefotaxime, 86% for Amikacin, 84% for Meropenem and 66% for Trimethoprim/ Sulfamethoxazole (Al-Samaree and Al-Khafaji, 2016). The increasedantibiotic resistance of *A. baumannii* in an alarming rate leading to increased morbidity, mortality and treatment costs in Intensive Care Units settings as revealed by surveillance studies all over the world (Mosafer, 2007). The differences in the susceptibility patterns may due to the type of patients enlisted for the study, source of collected specimens, and the variation in geographical regions.

Detection of Carbapenem resistance genes (*bla*OXA) by PCR

In order to check the presence of carbapenems-resistant genes, uniplex PCR has been used for each DNA sample. The PCR reaction included 54 isolates, which processed to detect the set of *bla*OXA genes. Class D carbapenemases consists of OXA-type β-lactamases, which can hydrolyze carbapenem antibiotics and considered as the main factors that caused a multi-drug resistance of *A. baumannii* (Al-Samaree and Al-Khafaji, 2016). *Bla*OXA-51genes are factors of carbapenem-resistance in *Acinetobacter* which are inherently existed in all *A. baumannii* strains (Bonnin *et al*, 2012). The *bla*OXA-51-like gene was found in all 54 (100%) clinical isolatesof *A. baumannii* and according to these findings;

Table 2 : Number and percentage of antimicrobial susceptibility rates for 54 of *A. baumannii* isolates against 10 antibiotics.

Antibiotic	Resistant	Intermediate	Sensitive	Chi-square (÷²)
AK	43 (79.62%)	5 (9.25%)	6 (11.11%)	12.631 **
ATM	54 (100%)	0 (0%)	0 (0.00%)	15.00 **
CTX	53 (98.14%)	1 (1.85%)	0 (0.00%)	14.812 **
CO	7 (12.96%)	6 (11.11%)	41 (75.92%)	11.956 **
CN	44 (81.48%)	2 (3.70%)	8 (14.81%)	13.251 **
IPM	49 (90.74%)	1 (1.85%)	4 (7.40%)	14.024 **
MEM	52 (96.29%)	0 (0.00%)	2 (3.70%)	14.841 **
OX	54 (100%)	0 (0.00%)	0 (0.00%)	15.00 **
TGC	27 (50%)	4 (7.40%)	23 (42.59%)	9.623 **
SXT	47 (87.03%)	5 (9.25%)	2 (3.70%)	13.804 **
Chi-square (÷²)	10.471 **	4.816 *	11.326 **	—

*(P<0.05), ** (P<0.01).

*Amikacin (AK), Aztreonam (ATM), Cefotaxime (CTX), Colistin (CO), Gentamicin (CN), Imipenem (IPM), Meropenem (MEM), Oxacillin (OX), Tigecycline (TGC) and Trimethoprim /Sulfamethoxazole (SXT).

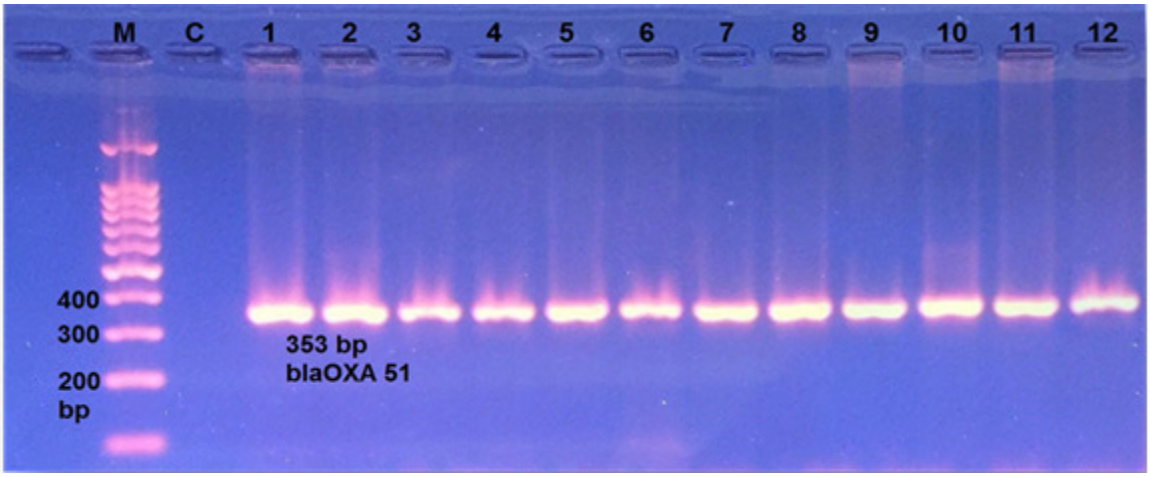

Fig. 1 : Electrophoresis of PCR products for the resistance genes *bla*OXA-51-like on agarose gel (2%) stained with ethidium bromide (5volt/cm for 2hr). Lane M: 100bp DNA ladder; lanes 1-12: *Acinetobacter baumannii* isolates; lane C: Negative control.

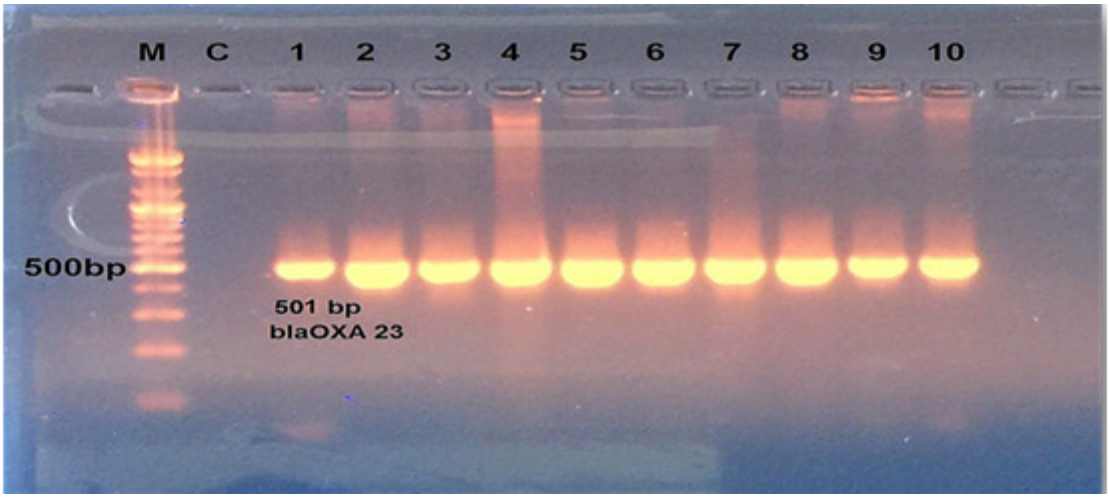

Fig. 2 : Electrophoresis of PCR products for the resistance genes *bla*OXA-23-like on agarose gel (2%) stained with ethidium bromide (5volt/cm for 2hr). Lane M: 100bp DNA ladder; lanes 1-10: *Acinetobacter baumannii* isolates; lane C: Negative control.

all the previous isolates were identified as *A. baumannii.* The current results demonstrated high specificity of this primer for *A. baumannii*; therefore, it was used for the identification of *A. baumannii* at the species level by PCR (Shali, 2012) (Fig. 1).

The current results were in agreement with many studies which recorded the presence of *bla*OXA-51-like gene in all clinical isolates of *A. baumannii* and not detected in the other species of *Acinetobacter* spp. (Hamouda *et al*, 2010). The presence of *bla*OXA-51-like gene is not related to the level of carbapenem resistance in *A. baumannii* isolates, because it controlled by the insertion sequence IS*Aba1* upstream of this gene which provides a promoter for the expression of *bla*OXA-51-like gene (Mosafer, 2007). The high rates of *bla*OXA-51-like gene are back to the fact that this gene is a ubiquitous, an intrinsic and chromosomally located gene in *A. baumannii*. Furthermore, the G+C content of *bla*OXA-51 is compatible with the genomic content of *A. baumannii*, that's why it's completely intrinsic to this bacterium (Mugnier *et al*, 2010).

The results of detecting *bla*OXA-23-like gene by PCR showed that this gene was existed in 49 (90.74%) of *A. baumannii* isolates (Fig. 2).

Upon the current results, the production of *bla*OXA-23-like gene is the dominant carbapenems resistance gene in the local isolates of *A. baumannii* isolates. The wide spread of *bla*OXA-23-like gene may be attributed to the ability of these bacteria to acquire resistance against many antibiotics which contributes in the distribution of this gene in hospitals all over the world. In comparison with the local studies, the present results were in agreement with the results of Hussein (2013) and Ghaima (2016), who established very close results. Hussein (2013) found that the *bla*OXA-23-like gene was the dominant among carbapenem resistance genes that existed in *A. baumannii* isolates collected from Medical City hospitals in Baghdad and achieved 71 (91.03%) out of 78 Imipenem

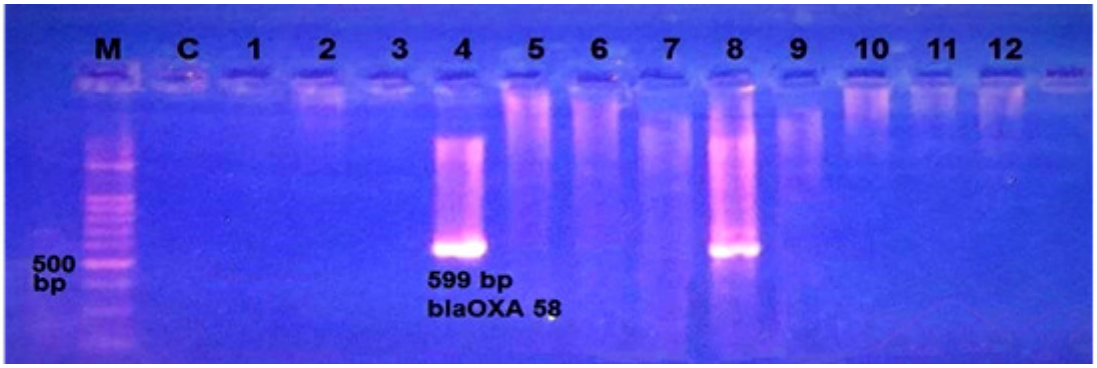

Fig. 3 : Electrophoresis of PCR products for the resistance genes *bla*OXA-24-likeon agarose gel (2%) stained with ethidium bromide (5volt/cm for 2hr). Lane M: 100bp DNA ladder; lanes 1-12: *Acinetobacter baumannii* isolates; lane C: Negative control.

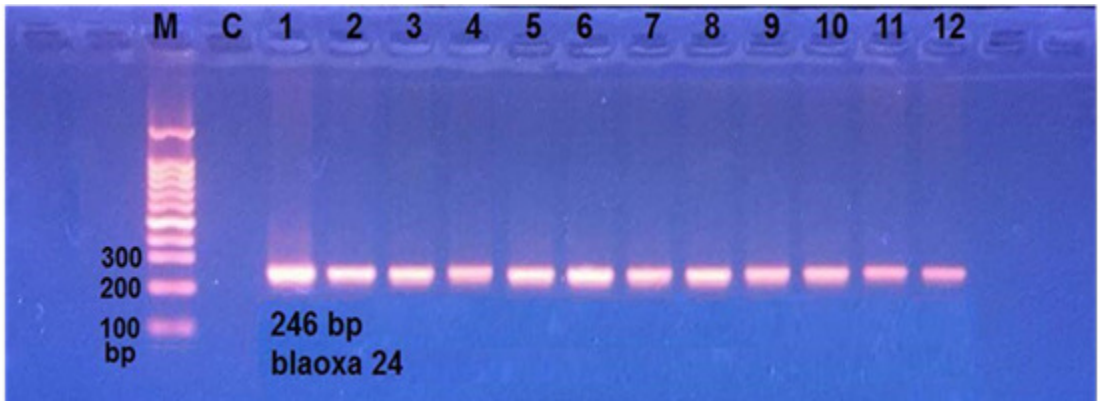

Fig. 4 : Electrophoresis of PCR products for the resistance genes *bla*OXA-58-like on agarose gel (2%) stained with ethidium bromide (5volt/cm for 2hr). Lane M: 100bp DNA ladder; lanes 1-12: *Acinetobacter baumannii* isolates; lane C: Negative control.

resistant *A. baumannii* (IRAB) isolates while Ghaima (2016) determined 60 (71.4%)of 84 multidrug resistant *A. baumannii* (MDRAB) isolates (Al-Warid and Al-Thahab, 2014). Relative findings of *bla*OXA23 prevalence were recorded inother parts of the Middle East such as Bahrain, United Arab Emirates and Saudi Arabia (Alsultan, 2015). The prevalence of OXA-23-producing *A. baumannii* isolates was reported in many countries, such as Korea, Singapore, Thailand (Azimi *et al*, 2015). Bulgaria, China, Brazil and Afghanistan (Niumsup *et al*, 2009). Additionally, high percentage of *bla*OXA-23-like gene (83%) was found in burn patients in Tehran hospitals (Özcan *et al*, 2015). Moreover, the *bla*OXA 23-like was a common gene in MDR *A. baumannii* isolates in southeast of Turkey and the presence of this gene was found in 48 (60%) isolates (Jal'oot *et al*, 2016).

The results presented in Fig. 3 showed the presence of *bla*OXA-24-like genes in 41 (75.92%) of*A. baumannii* clinical isolates.

The *bla*OXA-58-like gene recorded different results compared with other OXA genes. This study results revealed that the presence of *bla*OXA58 gene was found in 2 (3.70%) of all *A. baumannii* isolates (Fig. 4).

The low prevalence of *bla*OXA-58-like gene among *A. baumannii* isolates may attributed to the number of collected samples or because this gene is most frequently reported in Europe, South and North America, Asia and Australia (Mendes *et al*, 2009). The results of this study were consistent with most of the findings of the Middle East and around the world which stated that the frequency of *bla*OXA58 gene was the lowest compared to other genes that responsible for the resistance against carbapenems. In comparison with local studies, the results represented in the current study were in agreement with the studies of Hussein (2013) and Ghaima (2016) in Baghdad. Hussein (2013) found that out of 71 (IRAB) isolates, 10 (12.82%) isolates have *bla*OXA-23-like genes, *bla*OXA-58-like genes and *bla*OXA-51-like genes. Ghaima (2016) demonstrated the presence of *bla*OXA-58-like gene in 6 (7.1%) of multidrug resistant isolates of *A. baumannii* (Hou and Yang, 2015*)*. Further studies showed that carbapenemase OXA-58 was very wide spread and may be the main cause of carbapenem resistance in *A. baumannii*, since it has been detected in *A. baumannii* isolates recovered from different countries like France, Argentina, Kuwait and United Kingdom (Ghaima, 2016). A total of 196 isolates of *A. baumannii* were collected from the Central Hospital of Zhumadian,

China between 2010 and 2014, the results of this study revealed the presence of *bla*OXA58 gene in 5.31% of multidrug-resistant isolates (Park *et al*, 2013). Another study performed in Saudi Arabia hospitals, Riyadh city indicated that no isolate had a positive PCR result for *bla*OXA58 in multi-drug resistant *A. baumannii* isolates (Bonnin *et al*, 2012).

CONCLUSION

The resistance among *A. baumannii* isolates is increasingly rising all over the world. Treating nosocomial infections caused by *A. baumannii* strains varies according to the geographic region, type of possessed resistance genes, performing sensitivity tests. The results established that a large number of *A. baumannii* clinical isolates had multidrug resistance to almost all classes of antibiotics, especially carbapenems, which represented a serious therapeutic challenge in hospitalized patients. Moreover, every strain of *A. baumannii* has its own chromosomally encoded OXA β-lactamase (OXA-51-like), and they also had (OXA-23-like, OXA-24-like and OXA-58-like) that could be acquired from the environment, which explains the global outbreak of these bacteria. The current results also manifested that among *bla*OXA genes, *bla*OXA-23 gene had the highest rate while *bla*OXA-58 gene was the lowest in *A. baumannii* clinical isolates.

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