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Prevalence of BlaOXA like Carbapenemase Genes in Multidrug Resistant Acinetobacter baumannii Isolated from burns and Wounds in Baghdad Hospitals.

Kais Kassim Ghaima[1]*, Shurook Mohammad K. Saadedin[1], and Kifah Ahmed Jassim[2].

[1]Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Baghdad, Iraq.
[2]Ministry of health, Central Public Health Laboratory, Baghdad, Iraq.

ABSTRACT

Multiple drug resistant strains of *Acinetobacter* have become common in hospitals. The problem becomes more acute with increasing resistance to carbapenems especially in hospitalized patients. *A. baumannii* has been increased during the last decade in many parts of the world. The present study was designed to determine the antimicrobial susceptibility patterns and prevalence of OXA-type carbapenemases genes among clinical isolates of *A. baumannii* in Baghdad hospitals, Iraq. 96 clinical isolates of *A. baumannii* were collected from burns and wounds infections. In vitro susceptibility of *A. baumannii* isolates to 16 antimicrobial agents was performed by the disk diffusion method. Also Minimum Inhibitory Concentration (MIC) was performed by the broth microdilution method. BlaOXA-23, blaOXA- 24, blaOXA-58, blaOXA-51 genes were detected by polymerase chain reaction and multiplex PCR. The results of antimicrobial susceptibility test of clinical isolates revealed that the rates of resistance to the majority of antibiotics tested varied between 52.1% and 87.5%, with the exception of Tigecycline and Colistin. High resistance and MICs levels were found of the local isolates for most antibiotics used especially for Amikacin, Imipenem, Meropenem, Cefotaxime, Ciprofloxacin, Ticarcillin - clavulanic acid, Oxaciillin and Piperacillin. Of 96 isolates tested 84 (87.5%) were multidrug resistant (MDR). The results of multiplex PCR confirmed that all strains carried a blaOXA-51 gene. BlaOXA-24 gene was the most prevalent among blaOXA-types (80.9%), 71.4% carried blaOXA-23-like and 7.1 % had blaOXA-58-like resistance genes. The results of this study demonstrated that blaOXA-51 gene was specific for detection this species also high prevalence of blaOXA-24-like and blaOXA-23-like resistance genes among MDR *A. baumannii* in Baghdad hospitals. Colistin and Tigecycline were the effective drugs. Continuous Surveillance for *A. baumannii* multidrug-resistant strains is necessary to prevent the further spread of resistant isolates.

Keywords: *Acinetobacter baumannii*, blaOXA genes, Antibiotic resistance.

Corresponding author

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INTRODUCTION

Acinetobacter baumannii is a gram-negative, strictly aerobic, nonfermenting coccobacillus belonging to the Moraxellaceae family; Species of this genus are opportunistic pathogens with increasing relevance in nosocomial infections, particularly among patients in intensive care units (ICUs) [1]. A. baumannii was responsible for between 3 and 20 percent of total ICU infections worldwide with increasing frequency from burn wound infections [2]. During the past decade, A. baumannii exhibited a remarkable ability to rapidly develop antibiotic resistance. Resistance to antimicrobial agents among A. baumannii clinical isolates is higher than community isolates. A. baumannii possesses mechanisms of resistance to most of antibiotic classes, as well as a great propensity for developing mechanisms of antibiotic resistance rapidly [3]. A global challenge in health-care facilities is A. baumannii, which possesses a high propensity to multi-drug resistance and epidemic spread [4].

Carbapenems are the first choice in the treatment of severe A. baumannii infections. Unfortunately, resistance to Carbapenems among A. baumannii clinical and environmental isolates is increasingly reported [5, 6] Increases in the prevalence of resistant strains have also been seen, which have compromised patient treatment with; aminoglycosides, penicillins, extended spectrum cephalosporins, and more recently, fluoroquinolones [7, 8]. The resistance to carbapenems is due to carbapenem- hydrolysing β-lactamase enzymes of Ambler molecular class B (metallo-β-lactamases) and D (oxacillinases). The OXA-type carbapenemases have emerged globally as the main mechanism responsible for this resistance. Currently, OXA-type carbapenemases are classified into eight subgroups and four of them were identified in A. baumannii: OXA-23, OXA-24, OXA-58, and OXA-51–like enzymes [9, 10]. The current study describes the prevalence of OXA-type carbapenemase genes among the local multiple drug resistant clinical isolates of A. baumannii isolated from burns and wounds patients hospitalized in Baghdad hospitals and develop a multiplex PCR assay for detecting alleles encoding these genes.

MATERIALS AND METHODS

Bacterial isolates

A total of ninety six A. baumannii isolates were collected from 376 clinical specimens such as burns and wounds from patients at Baghdad Hospitals, Iraq during the February –July 2015 period. Bacterial isolation and identification were performed using standard laboratory methods [11]. The PCR of blaOXA-51-like genes was used as a final confirmation as to the presence of A. baumannii species [12].

Antibiotic resistance patterns and minimal inhibitory concentrations (MIC)

Antimicrobial susceptibility tests were performed by agar disk diffusion, according to manufacturer instructions and Clinical and Laboratory Standards Institute (CLSI) guidelines [13]. Broth microdilution method using Mueller-Hinton broth was used For determination Minimal Inhibitory Concentration (MIC) [14], according to the CLSI guidelines [15]. Pseudomonas aeruginosa ATCC- 27853 were used as quality reference strains.

Detection of bla OXA carbapenemase genes

Bla OXA-like genes including bla OXA-51, bla OXA-23, bla OXA-24, and bla OXA-58 were amplified as described previously (Woodford et al.,2006) [16]. Amplified DNA fragments were purified with Qiaquick PCR purification kits (Qiagen, USA). The PCR analysis was performed using the primers as in Table 1.

Table 1: PCR primers to detect bla OXA genes encoding carbapenemase (16)

Primers		Primer sequence	Product size (base pair)
BlaOXA 51	F	5- TAA TGC TTT GAT CGG CCT TG - 3	353bp
	R	5- TGG ATT GCA CTT CAT CTT GG - 3	
BlaOXA 23	F	5- GAT CGG ATT GGA GAA CCA GA - 3	501bp

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	R	5- ATT TCT GAC CGC ATT TCC AT - 3	
BlaOXA 24	F	5- GGT TAG TTG GCC CCC TTA AA - 3	246bp
	R	5- AGT TGA GCG AAA AGG GGA TT - 3	
BlaOXA 58	F	5- AAG TAT TGG GGC TTG TGC TG - 3	599bp
	R	5- CCC CTC TGC GCT CTA CAT AC - 3	

A multiplex polymerase chain reaction (PCR) assay was performed to detect the bla OXA genes in the A. baumannii isolates by The amplification conditions : initial denaturation at 94°C for 5 minutes, 30 cycles of 94°C for 45 seconds, 52°C for 40 seconds, 72°C for 45 seconds, and a final elongation at 72°C for 6 minutes.

The amplified DNA was detected by 2% agarose gel electrophoresis, stained with ethidium bromide and visualized under UV transillumination and photographed.

RESULTS AND DISCUSSION

96 isolates of A baumannii, were identified by conventional identification methods (CHROMagar Acinetobacter, API 20E and Vitek 2 system). 65 (20.6 %) isolates were obtained in burns infections and 31 (19.3 %) isolates in the wounds infections. The blaOXA-51-like gene was amplified from genomic DNA to detect A.baumannii. 96 isolates (100 %) that gave a band for bla OXA-51-like gene, were identified as A. baumannii as in Figure 1.

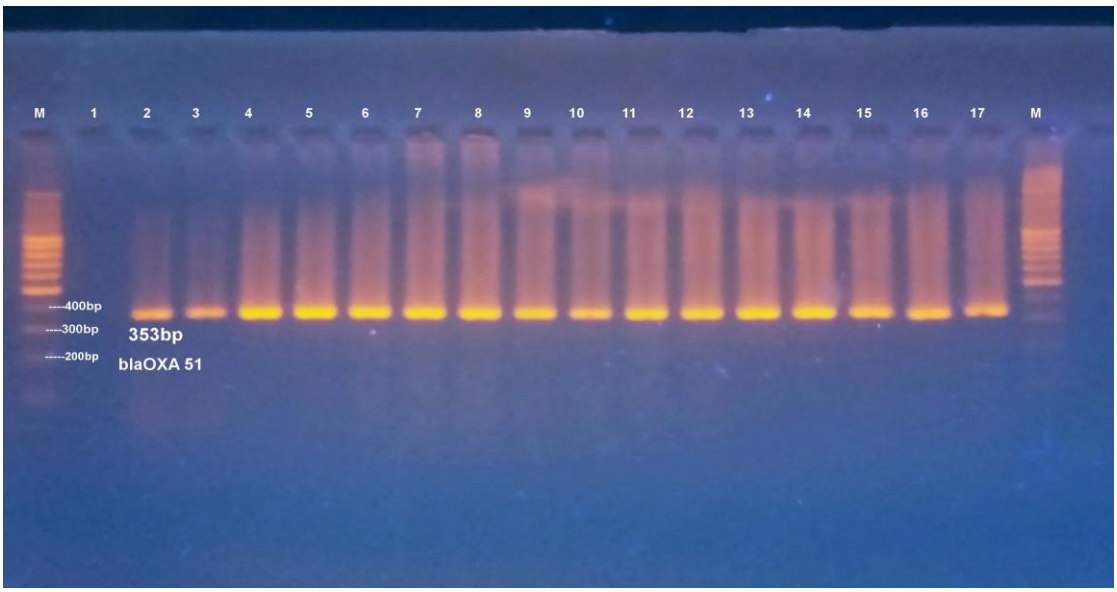

Figure 1: Ethedium bromide stained agarose gel (2%) electrophoresis of PCR products for the resistance genes *bla*OXA-51-like. Lane M, 100bp DNA ladder; lanes 2-17, *Acinetobacter baumannii* 1-16 isolates; lane 1, Negative control (had all PCR mixture including water instead of DNA template) (70V for 2hr).

Table 2: Minimum inhibitory concentrations (MICs) of different antibiotics and percentage of resistant isolates for 96 *A. baumannii* isolated from burns and wounds infections

Antibiotic*	MIC** range (µg/mL)	No. of resistant isolates (%)
AK	2 - 256	76 (79.2 %)
GM	0.5 - 64	75 (78.1 %)
IPM	1 - ≥ 265	78 (81.3 %)
MEM	2 - ≥ 265	72 (75 %)
CAZ	0.5 - 128	60 (62.5 %)
CTX	4 – ≥ 256	84 (87.5 %)

CIP	0.5 - 64	77 (80.2 %)
LVX	0.25 - 16	72 (75 %)
TE	1 – 128	50 (52.1 %)
TGC	0.25 - 32	11 (11.5 %)
ATM	16 – 128	74 (77.1 %)
TCC	4 – 256	67 (69.8 %)
OX	4 - ≥ 265	80 (83.3 %)
PI	2 - 256	78 (81.3 %)
TS	16 – 128	81 (84.4 %)
CT	0.25 - 16	7 (7.3 %)

*Amikacin (AK), Gentamicin (GM), Imipenem (IPM), Meropenem (MEM), Ceftazidime (CZ), Cefotaxime (CTX), Ciprofloxacin (CIP), Levofloxacin (LVX), Tetracycline (TE), Tigecyclin(TGC), Aztreonam (ATM), Ticarcillin - clavulanic acid (TCC), Oxaciillin(OX), Piperacillin(PI), Trimethoprim / Sulfamethoxazole (TS) and Colistin(CT) .
**MIC for each antibiotic was determined twice by the microdilution method in Mueller-Hinton broth, and CFU/mL was kept at 1.5×10^7.

The antimicrobial susceptibility of 96 A. baumannii isolates against 16 antimicrobial agents is shown in Table 2. The result of antimicrobial susceptibility test of clinical isolates by the disk diffusion method revealed that the majority of isolates were resistant to antibiotics used such as Cefotaxime (87.5 %), Imipenem (81.3%) and Meropenem (75%). The rates of resistance to the majority of antibiotics tested varied between 52.1% and 87.5%, with the exception of tigecycline (11.5%) and colistin (7.3%).

MDR A. baumannii was defined as those resistant to 3 or more different classes of antibiotics, hence 84 of 96 isolates were MDR and used for detection blaOXA genes The MICs of imipenem and meropenem were higher than the CLSI susceptibility breakpoint, indicating that these antibiotics had a low antimicrobial activity against these isolates. The MICs of colistin and tigecycline indicating that A. baumannii possesses a low-level resistance against this antimicrobial agent.

A baumannii is one of the predominant MBL (Metallo beta lactamase) producers in burn patients; also it was found that extended-spectrum beta-lactamases in this bacterium is the main cause of resistance and it is one of the most important nosocomial pathogens especially in burn units [17]. The current study indicated that, 84 of 96 A. baumannii isolates (87. 5%) were MDR. These isolates were resistant to imipenem, meropenem, cefotaxim, ceftazidim, amikacin, ciprofloxine. Our results agreed with those of the study conducted in Tehran Hospitals, as high rates of resistance of A. baumannii to imipenem, meropenem and cefotaxime were observed [18]. Large increase in the rates of carbapenem-resistant A. baumannii from 8% in 2003 to 52% and 74% in 2005 and finally to 96% in 2007, indicated the spread resistance around the world [19, 20]. Also the previous results in Baghdad hospitals Among 128 A. baumannii isolates indicated that 67 isolates (58.26%) were resistant to imipenem and meropenem [21]. MDR Resistance rates can differ according to the country, hospital, and depend on methodical, biological, epidemiological factors [22]. Carbapenem antibiotics are considered the agents of choice to treat serious infections caused by A. baumannii, but progressive antimicrobial resistance has made treatment very difficult [23].

High resistance rates of the isolated A. baumannii from our clinical samples can be explained by the over consumption of antibiotics in our hospitals without documented proof of infection. The majority of the isolates in the current study were susceptible to colistin and tigecycline proved this result. Others also observed that; colistin and tigecycline were useful against carbapenem resistant strains [24]. Susceptibility to colistin was reported as 91.2 - 100% in various studies and it may be a good choice in the treatment of MDR A. baumannii, but adverse side effects, such as, renal toxicity has limited use of this agent [18, 25].

Results of PCR for detection of blaOXA genes revealed that all 84 strains carry a blaOXA-51-like gene, confirming the strain identification (Figure 1). The presence of blaOXA-24 was confirmed in 68 (80. 9%) (Figure 2), blaoxa-23 in 60 (71. 4%) (Figure 3), blaOXA- 58 in 6 (7.1%) strains (Figure 4). The co-existence of blaOXA-23-like and blaOXA- 24 in 43 (51.2%) strains. The frequency of blaOXA-types among A.baumannii isolates are summarized in Table 3.

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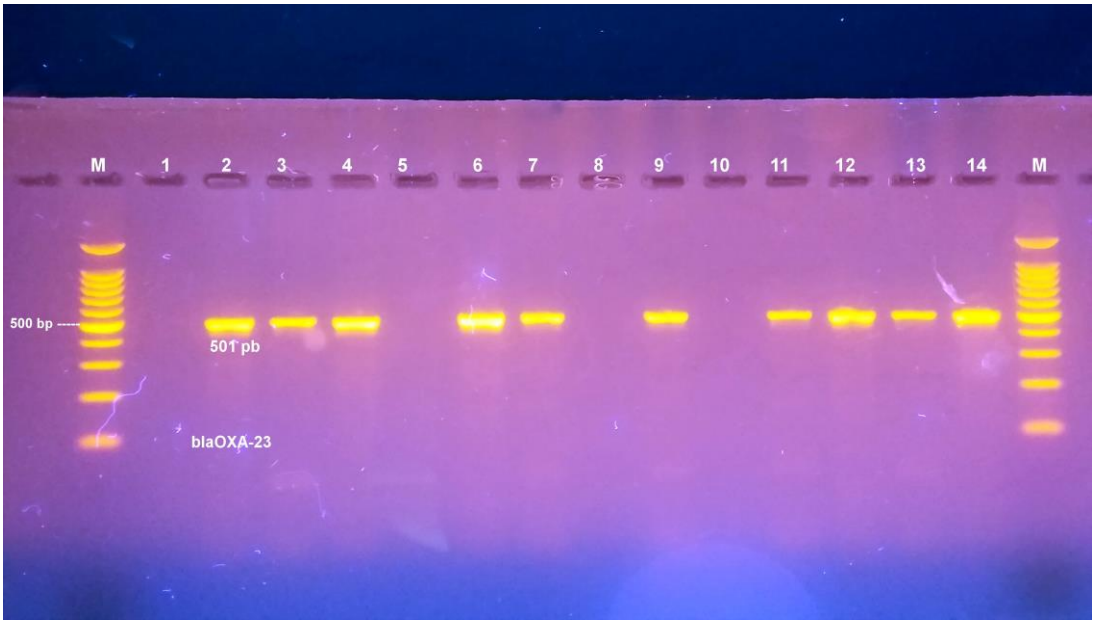

Figure 2: Ethedium bromide stained agarose gel (2%) electrophoresis of PCR products for the resistance genes blaOXA-23-like. Lane M, 100bp DNA ladder; lanes 2-14, *A. baumannii* 60 – 72 isolates; lane 1, Negative control (had all PCR mixture including water instead of DNA template) (70V for 2hr).

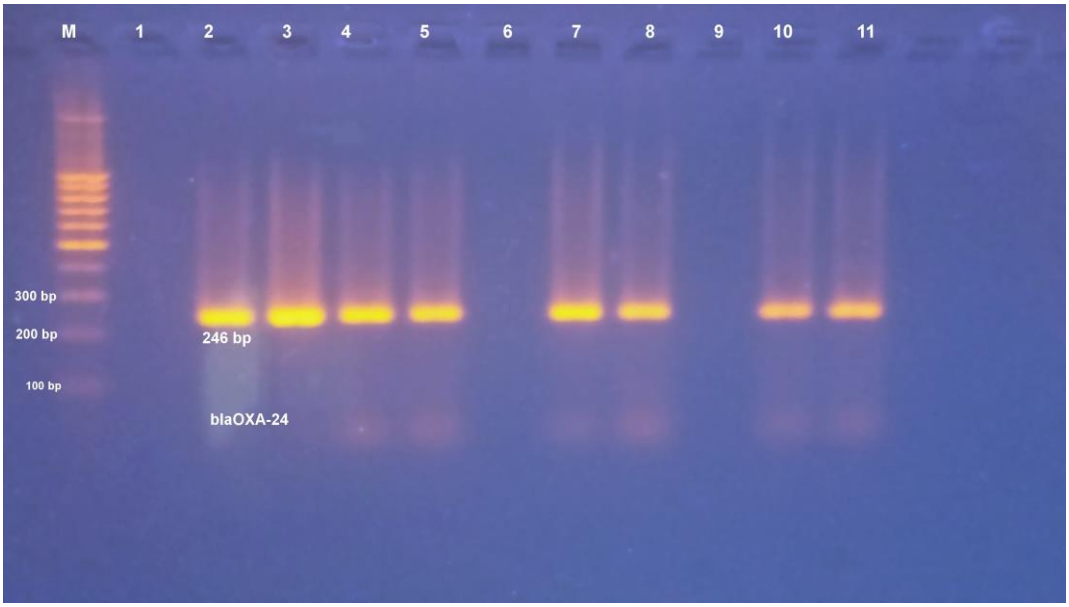

Figure 3: Ethedium bromide stained agarose gel (2%) electrophoresis of PCR products for the resistance genes blaOXA-24-like. Lane M, 100bp DNA ladder; lanes 1-32, *A. baumannii* 30-39 isolates; lane 1, Negative control (had all PCR mixture including water instead of DNA template) (70V for 2hr).

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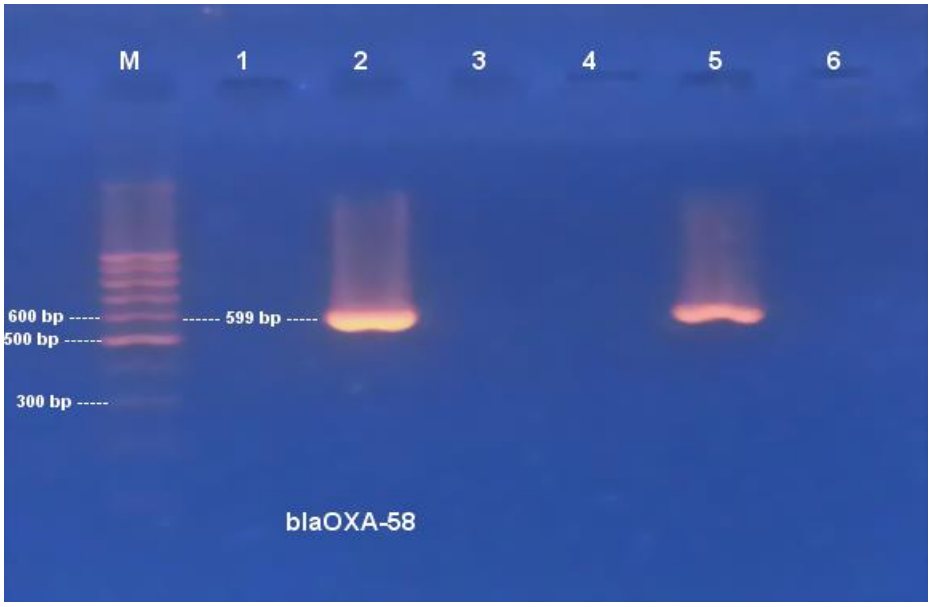

Figure 4: Ethedium bromide stained agarose gel (2%) electrophoresis of PCR products for the resistance genes blaOXA-58-like. Lane M, 100bp DNA ladder; lanes 2-6, *A. baumannii* 50-54 isolates; lane 1, Negative control (had all PCR mixture including water instead of DNA template) (70V for 2hr).

Table 3: The distribution of blaOXA-types among multidrug resistant A. *baumannii* isolates

blaOXA-type	MDR *A. baumannii* isolates	
	No.(%) of positive isolates	No.(%) of negative isolates
blaOXA-51-like	84 (100)	0 (0)
blaOXA-23-like	60 (71. 4)	24 (28.6)
blaOXA -24-like	68 (80. 9)	16(19.1)
blaOXA -58-like	6 (7.1)	78 (92. 9)
blaOXA -23/ blaOXA -24-like	43 (51.2)	41 (48. 8)
blaOXA -58/ blaOXA -23-like	6 (7.1)	78 (92. 9)
blaOXA -58/ blaOXA -24-like	5 (6)	79 (94)

The class D carbapenemases consist of OXA-type β-lactamases which can hydrolyze carbopenems antibiotics, and are regarded the main factors that caused the multi-drug resistance of A. baumannii , Oxacillinase type carbapenemases (OXA) have been reported in Acinetobacter species worldwide, particularly in hospital environment [26, 27].

Amplification Results for detection of blaOXA-51-like showed that all A. baumannii isolates had blaOXA-51 gene. This finding confirmed the proposal that blaOXA-51-like gene is intrinsic in A. baumannii [28]. As Table 3 shows, 51.2% of isolates which carried more than two oxacillinase genes. In this study blaOXA-40-like gene (blaOXA-24-like gene) was detected in 68 isolate (80. 9%). High prevalence of blaOXA-40-like gene in contrast with most previous studies revealed the role of this gene in the resistance in local A. baumannii isolates and was much higher than the rate of this gene reported from Tehran , Iran [18,29], and in Baghdad hospitals in 2013 [21]. The highest prevalence of blaOXA-40-like gene have been reported from European countries such as Spain and Portugal [30, 31]. The varied prevalence results are due to OXA-24 being an acquired gene within A. baumannii and thus not all isolates will contain the gene [32]. Isolates that carry an OXA-40-like beta-lactamase are typically resistant to the carbapenems. In a study where the bla OXA-40 gene was insertionally inactivated, the bacterium became more sensitive to the carbapenems, penicillins, and

cephalosporins, demonstrating the role that this enzyme plays in increasing resistance to the beta-lactams in general [33].

The bla OXA-23-like genes present in 71.4% of MDR A. baumannii. This is significantly higher than the rate of bla OXA-23-like gene reported from central part of Iran (25%) [18,29]. BlaOXA-23-like genes are among the most prevalent acquired carbapenemase-encoding genes worldwide, which can be on the chromosome or plasmids in different genetic structures [34]. Similar findings of prevalence carbapenemase encoding genes demonstrated that blaOXA-23-like genes in MDR-AB isolates were the most common genes encoding carbapenemase in patients with burns, suggesting that to prevent the spread of blaOXA-23-like genes in A. baumannii will be the main concern for both local communities and clinicians [35]. In our study, blaOXA-58 were detected in 6 (7. 1%) of isolates, that agree with the recent study in China which revealed that 18 (5.31%) were blaOXA-58 [36]. Previous reports about the percentage of blaOXA-58 in Asia and middleast region showed low levels [37-39].

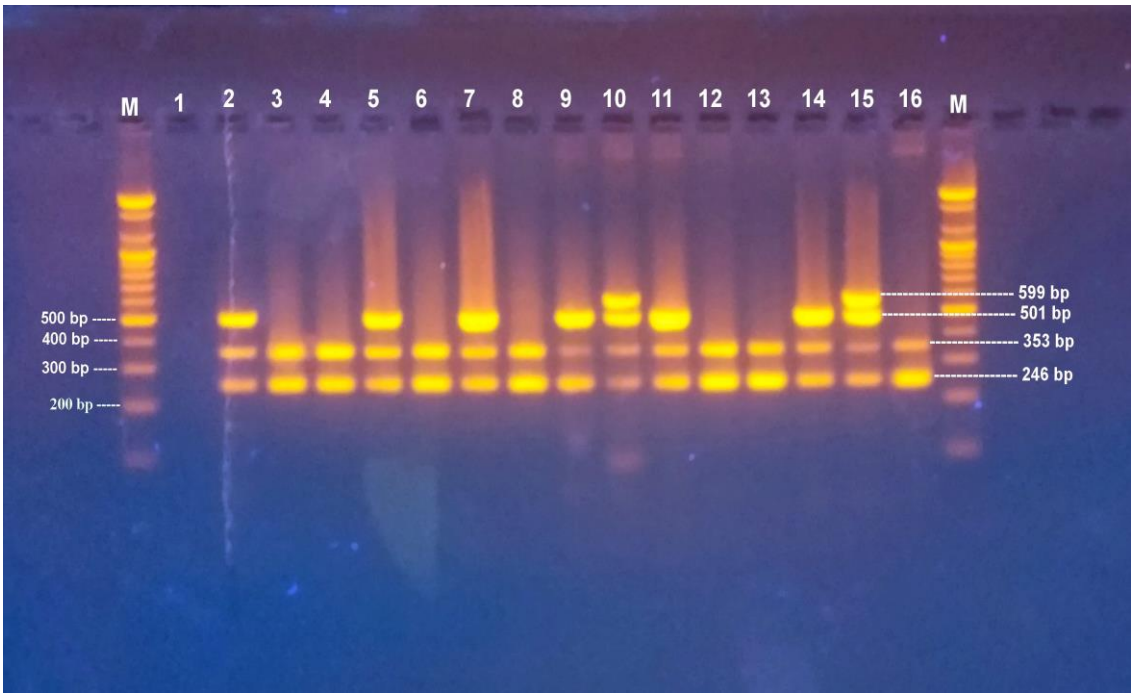

Figure 5: Detection of resistance genes blaOXA-51-like, blaOXA-23-like, blaOXA-24-like and blaOXA-58-like by multiplex PCR. Lane M, 100bp DNA ladder; lanes 2-16, *A baumannii* 40-54 isolates; lane 1, Negative control (had all PCR mixture including water instead of DNA template) (70V for 2hr).

The present study was developed a multiplex PCR assay for detecting alleles encoding oxacillinases to evaluate the presence of carbapenemase genes among MDR *A. baumannii* (figure 5). The combination of markers detected in the multiplex PCR provides powerful information for identification *A. baumannii* and probable outbreak. Results from the multiplex can be obtained rapidly and should prove highly useful to clinicians and infection control staff [13].

CONCLUSION

The resistance among A. baumannii strains is continuously rising; the treatment of nosocomial infections caused by A. baumannii strains is dependent upon the geographic region, the type of resistance genes observed, using antibiogram tests. BlaOXA-24 gene was the most prevalent among blaOXA-types in local isolates in Baghdad hospitals. The information proceeded will help us to create a baseline data for early management of the patients and to control the spread of these bacteria in our hospitals.

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