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Evaluation of Biofilm Formation and Carbapenemase-Encoding Genes in Clinical A.baumannii Isolates

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Abstract :

Background: Carbapenems, a class of beta-lactam antibiotics, are commonly used to treat multi-drug-resistant *Acinetobacter baumannii* isolates.

Objective: This study aimed to detecting Carbapenem-resistant gene and biofilm formation in *Acinetobacter baumannii*

Materials and Methods: From January - June 2024, 300 specimens were collected from Al-Hilla Teaching Hospital. These specimens were cultured on blood agar, MacConkey agar, and Hi-CHROM agar. Antibiotic susceptibility was assessed using the disc diffusion test and the minimum inhibitory concentration for Meropenem. Biofilm formation was evaluated using the tissue plate assay, and *bla-OXA* and metallo-beta-lactamase genes were identified via PCR. **Results:** Out of 300 isolates, 25 (8.3%) were studied. The isolates came from burns 75%, wounds 80%, blood 75%, and urine 70%. Susceptibility testing revealed 100% resistance to cefepime, ceftazidime, cefotaxime, ciprofloxacin, and levofloxacin; 70% to gentamicin; 60% to tobramycin; 84% to imipenem; 75% to polymyxin B; and 72% to meropenem. MIC assays confirmed meropenem resistance in all but seven isolates, with an MIC > 16 µg/ml according to CLSI 2024 guidelines. Biofilm formation tests indicated 60% of the isolates were strong biofilm formers and 40% were moderate. All CRAB isolates carried *bla-OXA-51*-like genes, 82.1% had *bla-OXA-23*-like, 68% had *bla-VIM*, fewer than 10% had *bla-IMP*, and no bla-NDM genes were detected.

Conclusion: Meropenem remains a critical option against multi-drug-resistant *A. baumannii.* Variations in biofilm formation correlate with infection severity. Oxacillinase genes are more prevalent than MBL genes.

Keywords:

Acinetobacter baumannii, Oxacillinase, Metallo-B-Lactamase.

Introduction

Acinetobacter baumannii is one of the ESKAPE organisms (*E. faecium, S. aureus, K. pneumoniae*, *A. baumannii*, *Pseudomonas aeruginosa, and Enterobacter* spp.), posing a global threat to human health and presenting a significant therapeutic challenge due to its emerging and rapidly increasing resistance[1]. *A. baumannii* is a highly successful pathogen responsible for difficult-to-treat nosocomial infections. Multi-drug resistant A. baumannii outbreaks are common worldwide, with limited treatment options available. In 2018, the WHO prioritized research and development for carbapenem-resistant *A. baumannii* (CRAB) due to its broad antibiotic resistance[2].Beta-lactamase enzymes are categorized into four classes based on sequence motifs and hydrolytic mechanisms. Carbapenems are hydrolyzed by Class A, B, and D enzymes, which are classified by their active sites into serine carbapenemases and metallo-beta-lactamases (MBLs). The primary mechanism of carbapenem resistance in Acinetobacter species is the production of enzymes that degrade β-lactams. However, multiple resistance mechanisms can coexist within the same organism[3]. *A. baumannii* rapidly mutates and acquires foreign resistance determinants through mobile genetic elements, broadening its resistance spectrum. The global spread of resistant strains has been facilitated by increased tourism and human migration[4]. The epidemiology and genetic evolution of *A. baumannii* is crucial for managing this pathogen. Carbapenem resistance serves as a marker because it often correlates with resistance to multiple antibiotic classes[5]. *A. baumannii* can enhance its intrinsic resistance mechanisms and acquire new resistance determinants through horizontal gene transfer. Clinical isolates frequently exhibit carbapenem resistance, posing a significant public health concern as they are resistant to last-line antibiotics. The pathogen forms biofilms on both biotic and abiotic surfaces, enabling survival on medical devices, hospital surfaces, and other harsh environments. Carbapenem resistance in *A. baumannii* primarily arises from Ambler class D (oxacillinases) and class B Metallo-beta-lactamases (MBLs)

carbapenemases, with class A being less common. Specifically, carbapenem-hydrolyzing class D β-lactamases (oxacillinases) are the main contributors to resistance [6].The genes b*laOXA-51* and *blaOXA-143*-like are central to this mechanism. Metallo-beta-lactamase such as IMP, NDM, and VIM also play a role, with the first potent carbapenemase enzyme reported by Panton et al. [7].

Materials and Methods

Clinical specimens & Culture Characteristics of *A. baumannii*

During the investigation period from January 2024 to June 2024, a total of 25 (8.33%) *A. baumannii* isolates were recovered from 300 clinical specimens collected at Al-Hilla General Teaching Hospital. These specimens were obtained from patients with various infections, including wounds, burns, urine, and blood. The samples were cultured on general and selective media, including MacConkey agar, blood agar, and Hi-CHROM agar, and incubated aerobically at 37°C for 24 hours. After incubation, colonies displaying different morphologies were isolated.Bacilli-containing colonies were then subcultured on Hi-CHROM agar for an additional 24 hours at 37°C. The bacterial colonies appeared purple on Hi-CHROM agar, aiding in the confirmation of *A. baumannii* isolates. Confirmation of the isolates was performed using conventional PCR to detect the intrinsic *blaOXA-51*-like gene, employing specific primers as described in Table 1. Samples negative for *blaOXA-51* were excluded from the study. Further PCR tests were conducted to detect the presence of other oxacillinases and metallo-β-lactamase genes.

Antibiotic susceptibility test to *Acinetobacter baumannii*

The susceptibility of 25 isolates of *A. baumannii* towards 14 different antibiotics was evaluated by using Kirby –Bauer method, This study was taken phenotypically by performing susceptibility testing to antibiotics (cefepime, ceftazidime, cefotaxime, piperacillin/tazobactam, ciprofloxacin, levofloxacin, gentamycin,Tobramycin, meropenem, Doripenem, imipenem and polymyxin B. The breakpoints used were according to CLSI, 2024 recommendations.

The Minimum Inhibitory Concentration (MIC)assay:

Minimum Inhibitory Concentration (MIC) test for meropenem at a concentration between (0.5 -128) µg/ml. Agar dilution was performed to determine MICs for seven *A.baumannii* isolates (AB16-AB17, AB21-AB25). The results were read according to CLSI 2024.

Phenotypic Detection of Biofilm Formation in *A.baumannii*

The tissue culture plate method was employed to detect biofilms. Each well of a flat-bottom microtiter plate was initially inoculated with 20 ml of overnight bacterial culture in 180 ml of sterile Brain Heart Infusion broth supplemented with 2% sucrose (0.5 gm sucrose/25 ml of medium). Control wells contained only 200 µl of Brain Heart Infusion broth with 2% sucrose. The plate was covered and sealed with paraffin film, then incubated at 37°C for 24 hours.After the incubation period, the wells were washed three times with normal saline (pH =7.2) to remove unattached bacterial cells. The plate was air-dried at room temperature for 15 minutes. Subsequently, 200 µl of 0.1% crystal violet solution was added to each well and left for 15 minutes. After carefully removing the crystal violet solution, the wells were washed three times with distilled water to eliminate excess dye and allowed to air-dry at room temperature. Next, 200 µl of 95% ethanol was added to each well to extract the bound dye. The absorbance was measured at 630 nm using a microplate reader. The absorbance value of the control well was subtracted from each sample value. The resulting absorbance values were then categorized into three groups based on their absorbance readings: strong biofilm, moderate biofilm, and non-biofilm, as detailed in Table 5.

Amplification of Oxacillinase like gene

Bacterial DNA was extracted using the alkaline lysis method. Pairs of primers (Bioneer® Korea) for *OXA-51, OXA-23, and OXA-143* were employed for PCR amplification of the respective genes. The amplification procedure involved a 25 µl master mix containing 0.2 µl of Taq polymerase (5 U/µl), 2.5 µl of 10X PCR buffer with MgCl2, 1 µl of 10 pM forward and reverse primers each, 2.5 µl of dNTPs mix (2 mM), 3 µl of DNA template, and 14.8 µl of DNase-Free and RNase-Free Distilled Water. PCR amplification was conducted using a thermal cycler. The amplified DNA products were then subjected to agarose gel electrophoresis, using a 1% agarose gel with a 100 bp size marker, run for 10 minutes at 70 V. The gel was stained with ethidium

bromide to visualize and detect the bands. The PCR reactions included both positive and negative controls. Table 1 shows the primers and their sequences used in this study.

Table1: primers(Oxacillinase) and their sequences used in this study

NO	Primer name	Sequence(5'_3')	References
1.	*bla*$_{OXA-51}$	***F-TAATGCTTTGATCGGCCTTG*** ***R-TGGATTGCACTTCATCTTGG***	[9]
2.	*bla*$_{OXA-23}$	***F-GATCGGATTGGAGAACCAGA*** ***R-ATTTCTGACCGCATTTCCAT***	
3.	*bla*$_{OXA-143}$	***F-TTCTGTCAGTGCATGCTCATC*** ***R- CAGGCATTCCTTGCTTCATT***	

Amplification of metallo β-lactamase genes

Specific primers for VIM, IMP, and NDM were used for PCR amplification of the genes. The PCR amplification was conducted with a 25 µl master mix, which included 0.2 µl of Taq polymerase (5 U/µl), 2.5 µl of 10X PCR buffer with MgCl2, 1 µl of 10 pM forward and reverse primers, 2.5 µl of 2 mM dNTP mix, 3 µl of DNA template, and 14.8 µl of DNase-free and RNase-free distilled water. The PCR amplification was carried out in a thermal cycler.The amplified DNA products were then subjected to agarose gel electrophoresis using a 1% agarose gel with a 100-1500 bp size marker, run for 10 minutes at 70 V, and stained with ethidium bromide for visualization. Table 2 shows the primers and their sequences used in this study.

Table 2: The primers of MBL and their sequences used in this study

NO	Primers Names	Sequence(5'_3')	References
1-	***bla-VIM***	*F-GATGGTGTTTGGTCGCATA* *R- CGAATGCGCAGCACCAG*	[9]
2-	***bla-IMP***	*F-* *GGAATAGAGTGGCTTAAYTCTC* *R-CCAAACYACTASGTTATCT*	
3-	***bla-NDM***	*F-GGGCCGTATGAGTGATTG* *R- GCACACTTCCTATCTCGAC*	

Ethical approval

The necessary ethical approval from the health office directorate and the College of Medicine .University of Babylon and hospital ethics committee under reference No [IRB: 5-27, 1/12/2023]. was obtained. Moreover, all subjects involved in this work were informed and the agreement required for conducting the experiments and publication of this work was obtained from each one prior to the collection of samples.

Statistical analysis:

Statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS) version 25.0 (SPSS, IBM Company, Chicago, Illinois). Categorical variables were presented as frequencies and percentages.

Results

Distribution *of A. baumannii* in different specimens.

Of the total 25 isolates of *Acinetobacter baumannii*, 4.28% were isolated from blood, 2.6 % were from urine and 12.5 % were isolated from wound swabs, and 13.3 % were isolated from burn swabs as shown in Table 3.

Table 3: Distribution of *A.baumannii* isolates among clinical specimens (N=25)

Type of specimens	No. of specimens	No. of Isolates	%
Burn swab	75	10	13.3 %
Wound swab	80	10	12.5 %
Urine	75	2	2.6 %
Blood	70	3	4.28%
Total	300	25	8.3 %

Acinetobacter baumannii (n=25, 8.3%) was the major species isolated in this study and the remaining 175(33.3%) other bacterial species and 58.3% negative cultures.

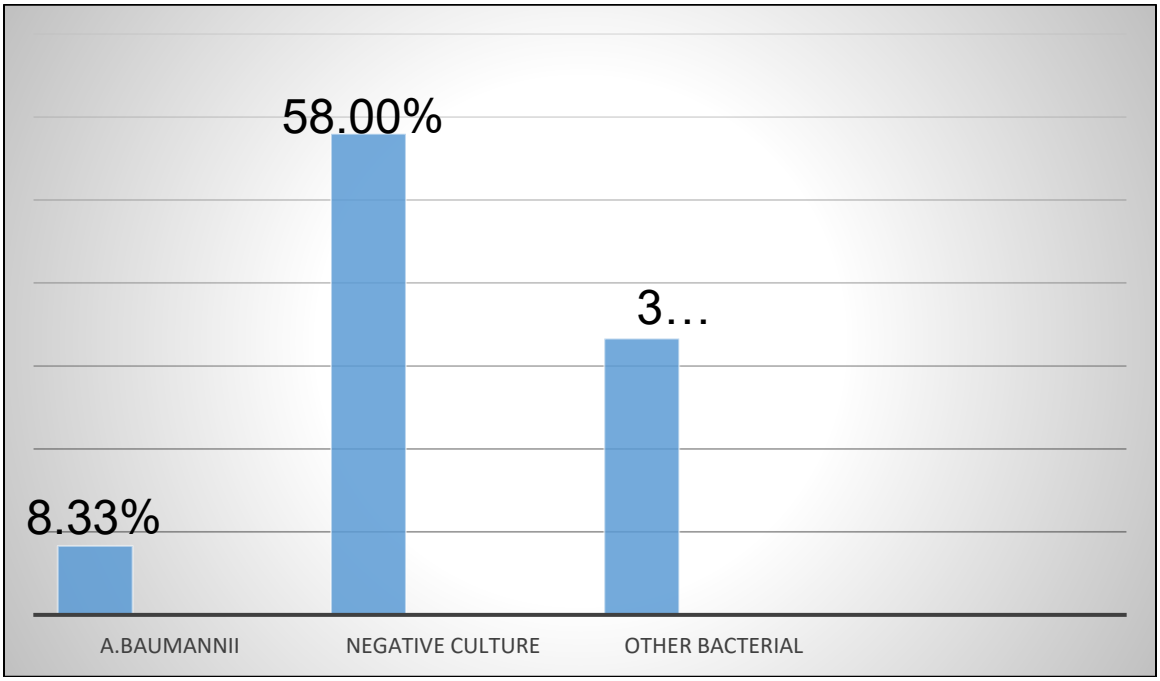

Figure 1. Distribution of *A. baummannii* among different clinical specimens (N=25).

Antibiotics susceptibility of *Acinetobacter baumannii* isolates by DDT.

Antibiotic susceptibility testing for all *Acinetobacter baumannii* isolates was conducted using the Disc Diffusion Method, with results interpreted according to the 2024 CLSI guidelines. The study revealed the following resistance patterns: 25 isolates (100%) exhibited the highest resistance to Cefepime, Ceftazidime, Cefotaxime, Piperacillin/Tazobactam, Ciprofloxacin, Levofloxacin, and Doripenem. 84% of the isolates were resistant to Imipenem.80% were resistant to trimethoprim/sulfamethoxazole.75% showed resistance to polymyxin B.72% were resistant to Meropenem. 70% showed resistance to Gentamycin.60% were resistant to Tobramycin.The antibiotic susceptibility results are presented in Table 4.

Table 4: Antibiotics Susceptibility Profile of *Acinetobacter baumannii* Isolates Detected by DDT(n=25).

Antibiotic categories	Antibiotic	Sensitive no. (%)	Resistant no. & (%)
B-lactam antibiotics	Piperacillin-tazobactam	0(0%)	25(100%)
	Cefepime	0(0%)	25(100%)
	Cefotaxime	0(0%)	25(100%)
	Ceftriaxone	0(0%)	25(100%)
	Imipenem	4(16%)	21 (84%)
	Meropenem	7 (28%)	18(72%)
	Doripenem	0(0%)	25(100%)
Aminoglycoside	Tobramycin	10(40%)	15 (60 %)
	Amikacin	10(40%)	15(60%)
	gentamycin	7(30%)	18(70%)
Fluoroquinolones	Ciprofloxacin	0(0%)	25 (100 %)
	Levofloxacin	0(0%)	25(100 %)
Sulfa drug	Trimethoprim - sulfamethoxazole	4 (20%)	20 (80%)

Lipopeptides	PolymyxinB	6(24%)	19(76%)

Detection of Meropenem susceptibility by (MIC)

To determine the lowest concentration of Meropenem that prevents the visible growth of a microorganism, an MIC assay was performed using the agar dilution method. All *A.baumannii* isolates were confirmed resistant to meropenem except 7 isolates (AB16,AB17, AB21-AB25) which resistant in MIC>16µg/mL) guidelineCLSI,2024.

Detection of Biofilm production by Tissue culture plate method.

In this study 60% of *A. baumannii* isolates capable to form a strong biofilm while 40% were moderate to formed biofilm as in Table 5 .

Table 5: Biofilm formation of (25) *A. baumanii* isolates

N0.of Isolates	O.D at 630 nm	Biofilm results	N0.of Isolates	O.D at 630nm	Biofilm results
AB1	0.287	+++	AB14	0.425	+++
AB2	0.144	+++	AB15	0.398	+++
AB3	0.158	+++	AB16	0.469	+++
AB4	0.479	+++	AB17	0.436	++
AB5	0.345	+++	AB18	0.149	++
AB6	0.250	+++	AB19	0.316	+++
AB7	0.383	+++	AB20	0.615	+++
AB8	0.501	+++	AB21	0.587	++
AB9	0.446	+++	AB22	0.261	++
AB10	0.476	++	AB23	0.259	++
AB11	0.145	+++	AB24	0.256	++
AB12	0.512	++	AB25	0.144	++
AB13	0.168	+++	Control	0.134	
p.value = 0.05, Strong +++,Moderate++,Weak+					

* represent the significant difference at p ≤0.05.(positive=+,Negative=-).

Molecular Detection of Oxacillinase and metallo β-lactamase genes by PCR.

The intrinsic *BlaOXA-51* gene was detected in all 25 (100%) out of 300 isolates as in figure 2 confirming *A.baumannii*. *bla-OXA 23* gene was seen in 20 (80%)

isolates as in figure 3 , no isolates was observed carrying for $bla_{OXA-143}$−like genes as in figure 4 ,Table 6.

Table 6: Distribution of OXA genes in this study (N=25).

OXA genes	No of isolates	% of genes
BlaOXA-51	25	100%
BlaOXA-23	20	80%
BlaOXA-143 like	**0**	**0%**

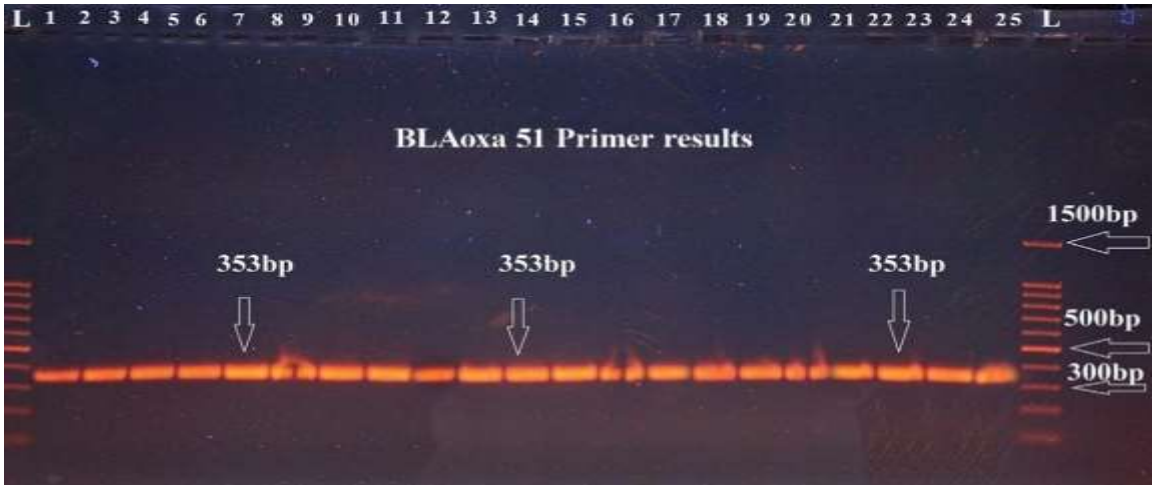

Fig 2: Gel electrophoresis of PCR product of ***bla$_{OXA-51}$***(353 bp) gene,L = ladder (100–1500), melting temperature (Tm) of 53°C, 1% agarose gel, starting at 100 volts for 10 minutes and then reduced to 70 volts for 60 minutes,the 1–25 represents of the positive results in samples for this gene, The electric current was allowed at 70 V for 1 h. PCR

Fig 3: Gel electrophoresis of PCR product of ***bla$_{OXA-23}$*** (501 bp) gene,L = ladder (100–1500), melting temperature (Tm) of 55°C, 1% agarose gel, starting at 100 volts for 10 minutes and then reduced to 70 volts for 60 minutes,the 1–20 represents of the positive results in samples for this gene, while lanes 21-25 and lane N represented negative results and the negative control, respectively. The electric current was allowed at 70 V for 1 h. PCR.

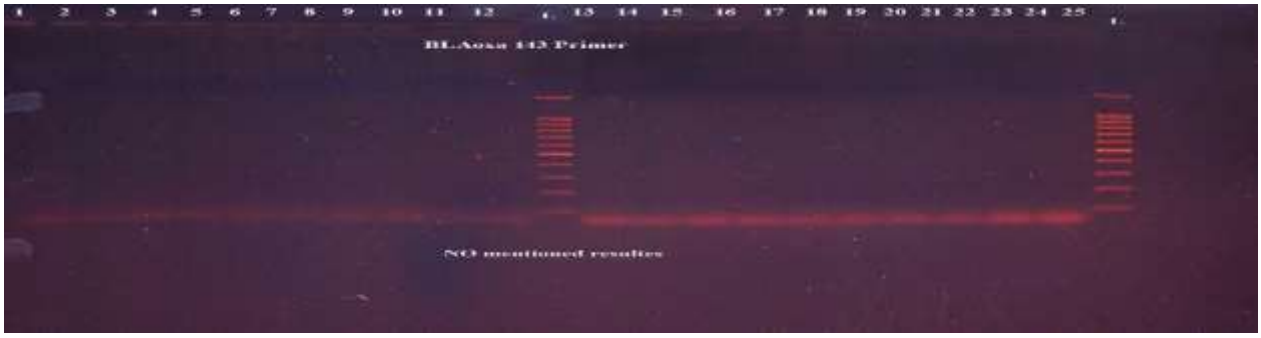

Fig 4: Gel electrophoresis of PCR product of ***blaOXA -143*** gene, L = ladder (100–1500), the 1–25 represents of the negative results in samples for this gene. The electric current was allowed at 70 V for 1 h. PCR

bla-VIM gene was detected in 10 isolates of Carbapenem resistant *A baumannii*. MBL gene was detected in 10 (68%) out of 300 isolates, figure 5, and follows: *bla-IMP* was seen in 8 (60%) isolates, Figure 6, followed by *Bla-VIM* gene, and no isolates were observed positive for ***bla***$_{OXA-NDM}$−like genes, Figure 7, Table 7.

Table 7 : Distribution MBL genes in this study(N=25).

MBL genes	NO.of isolates	% of Genes
blaVIM	10	68%
blaIMP	8	60%
blaNDM	0	0%

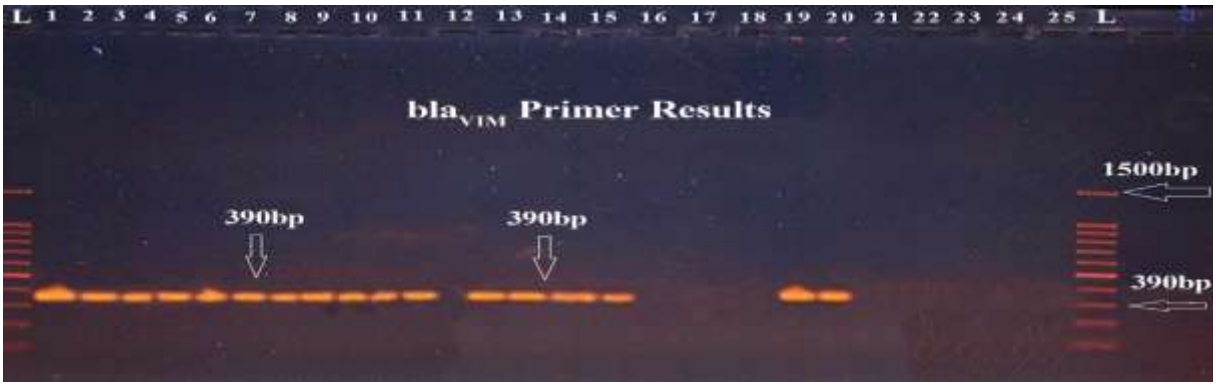

Fig 5:- Gel electrophoresis of PCR product of *bla-VIM* (390 bp) gene,L = ladder (100–1500), melting temperature (Tm) of 55°C, 1% agarose gel, starting at 100 volts for 10 minutes and then reduced to 70 volts for 60 minutes, Lanes 1-11, 13-16, and 19-20 showed positive results in samples for this gene, while lanes 12,14,15,17,18,21-25 and lane N represented negative results and the negative control, respectively.The electric current was allowed at 70 V for 1 h. PCR.

Fig 6 :-Gel electrophoresis was conducted for the PCR product using the bla-$_{IMP}$ primer, resulting in a band of 188 bp. The primer had a melting temperature (Tm) of 55°C. Electrophoresis was performed in a 1% agarose gel at 100 volts for 10 minutes, followed by lowering the voltage to 70 volts for 60 minutes. After staining with ethidium bromide, the gel was visualized under UV light. Lane L contained a DNA ladder (100-1500 bp). Lanes 1-9, 13-16, and 19-20 displayed positive results for the bacterial DNA isolates, while lanes 10-12, 17-18, and 21-25 represented negative results, along with the negative control lane (N).

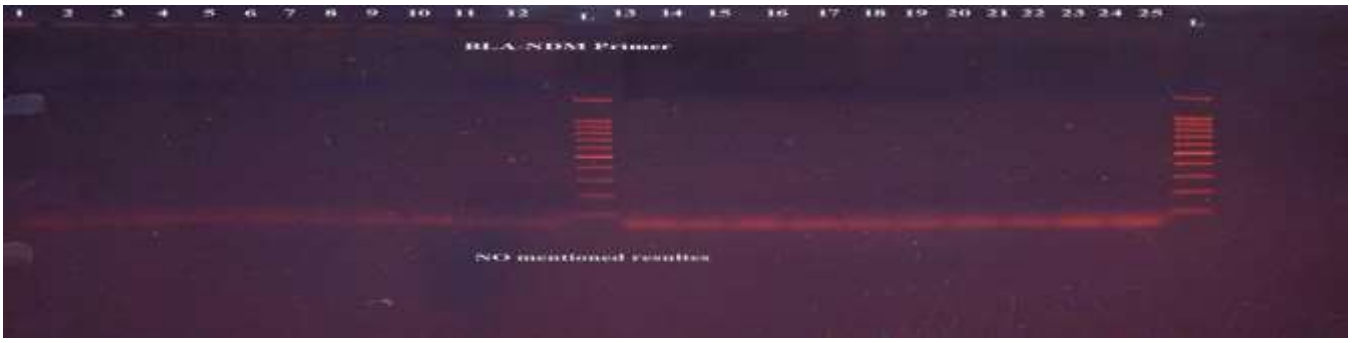

Fig 7 :- Gel electrophoresis of PCR product of *bla-$_{NDM}$* gene,L = ladder (100–1500), the 1–25 represents of the negative results in samples for this gene, The electric current was allowed at 70 V for 1 h. PCR

Discussion

The results of the current study revealed that out of 300 specimens, 25(8.33%) isolates belonged to *A. baumannii* collected from various clinical specimens. The current study reported that the isolation rate of 8.33% was different from the study by Zehra et al. [10] who found that the isolation rate was 74.3% while the studies conducted by Hasani et al.[11] and Mekkey et al. reported that the isolation rate did not exceeded 10%. The study by Wong *et al.* [12] established that the isolation rate of *A. baumannii* was 3.09%.

In the present study, the specimens distributed in burn swabs 13.3%, wound swabs 12.5%, urine 2.6% , blood 4.28%. AL-Baroody and Al-Ghanimi [13] found that the *A.baumannii* distribution percentage rate in clinical samples from patients urine 9%, blood 8%, swabs of wounds 10% and burns 12% And study of Al-Hasnawy et al. [14] who revealed that the isolation rates as; burn 3.57%, blood 4.44%, wound 2.38 % and urine 4.61%. This suggests that the percentage of isolation from wounds and burns is higher by isolation, which is also what the current study found.

Antibiotic susceptibility by disk diffusion test: All isolates had the highest resistance level of 25 (100%) to cefepime, ceftazidime, cefotaxime, piperacillin/tazobactam, ciprofloxacin, and levofloxacin was similar to the local study in Babylon province by Khulaif et al. [15] who recorded high resistance to all antibiotics and other study by Alzaidi et al. [16] who reached 100% resistance to cefepime, ceftazidime, cefotaxime, piperacillin/tazobactam, ciprofloxacin, and levofloxacin the results similar to the current study. The present study revealed that one of carbapenem class (Imipenem) conducted a resistance rate of 84% while meropenem resistant rate of 72% and a study of AL-Warid *et al*. [17] found that *A.baumannii* isolates were resistant 100% for Imipenem and meropenem, and another study conducted by Thirapanmethee et al. [18] showed resistance rate 50% for imipenem and 40% meropenem. The antibiotic susceptibility patterns in this study were similar to other studies in Jordan conducted by

Mshachal et al. [19] which showed the lowest level of resistance rate to meropenem 72%. Detection of Meropenem susceptibility by (MIC): minimal inhibitory concentration (MIC) assay for resistance against meropenem to 7 *Acinetobacter baumannii* isolates, the concentration used between (0.5 µg/ml -128 µg/ml), [20].

In present study, the resistant for Carbapenems group (meropenem) with an all isolates except for 7 isolates (AB16,AB17,AB21,AB22,AB23,AB24,AB25) showed resistance in MIC>16 µg/ml was similar study by Zalacain *et a*l. [21] found resistant for meropenem MIC>16 µg/ml, while other study by Nelson *et al*.,(2020) collected 275 clinical isolates of *Acinetobacter baumannii* CRAB, were highly resistant to meropenem (MIC for meropenem, >64 µg/ml). While another study by Berneking *et al*. [22] recorded results of meropenem resistance in MIC value of 8 µg ml). A study of Sarshar *et al*. [23] conducted the MIC for resistance against meropenem at 16 µg / ml, other study by Shali *et al*. [24] used 108 isolates of *A. baumannii* who showed MIC rate of resistance to meropenem MIC > 8 g/L). Biofilm formation in *A.baumannii* isolates in present study, showed results of 60% strong biofilm producers and 40% moderate. The current result is consistent with another study conducted in Iran, reported by Mahdi et al. [25] who found 23% of *A. baumannii* showed strong biofilm activity. while this result disagreement with a local study by Khan *et al*. [26] and a study by Vahhabi et al. [27] with percentages of 100%, and 80% respectively of a strong form of biofilm. In the results for the detection of Oxacillinase genes by molecular methods (*blaOXA- 51, blaOXA- 23, blaOXA- 143* were used for PCR detection of these genes .The results showed that the *blaOX*A-51 gene was present in all 25 (100%) *A. baumannii* isolates. This is consistent with a study conducted by Su et al. [28] which reported *blaOXA-51* in 72 (100%) of their carbapenem-resistant *A. baumannii* isolates, and another study by Al-Rashed *et al.* [29] which found *blaOXA-51* in 121 (100%) *A. baumannii* isolates. Regarding the *blaOXA-23* gene, it was detected in 20 (80%) of the 25 *A. baumannii* isolates in this study. In comparison, a study by Alkaabneh *et al.* [30] reported bla_{OXA-23} in 4.5% of 154 carbapenem-resistant *A. baumannii* isolates, while Bolori *et al.* [31] found bla_{OXA-23} in 41 (82%) out of 50 *A. baumanni*i isolates. For the *blaOXA-143* gene, no isolates tested positive among the 25 *A. baumannii* isolates in this study. This aligns with a study by Mottaghiyan *et al*. [33] which also found no *blaOXA-143*-positive isolates. However, a study by Sales *et al*. [32] detected *blaOXA-143* in 2% of 50 carbapenem-resistant *A. baumannii* isolates.

In the detection of metallo-β-lactamase genes (*blaVIM, blaIMP, blaNDM*), PCR was used to identify these genes as detailed in Table 7. The blaVIM gene was found in 10 (68%) of the 25 A. *baumannii* isolates. This differs from a study by Abordo *et al*. [34] which found *blaVIM* in 21 (16.66%) of 129 carbapenem-resistant *A. baumannii* isolates, and another study by Al-Rashed *et al*. [29]which found *blaVIM* in 1 (2%) out of 50 isolates. The *blaIMP* gene was detected in 8 (60%) of the 25 *A. baumannii* isolates in this study. A study by Hajikhan *et al*. [35] reported *blaIM*P in 20 (20%) of 100 carbapenem-resistant *A. baumannii* isolates, while Abordo *et al*. [34] found *blaIMP* in 20 (4%) out of 500 isolates. For the *blaNDM* gene, no isolates tested positive among the 25 *A. baumannii* isolates in this study. This contrasts with a study by Hajikhan *et al*.[35] which detected *blaNDM* in 67 (100%) of 67 carbapenem-resistant *A. baumannii* isolates. Radhi and Al-Charrakh [36] detected the metallo β-lactamases in local *A.baumannii* isolates in Iraq using MBL E-test (Imipenem/Imipenem+ EDTA) and they found that 22 isolates out of 30 (73.3%) were positive for this test.

Conclusion

Class D carbapenems were more prevalent in our CRAB isolate collection. The coexistence of multiple oxacillinases and MBL along with biofilm formation may also lead to treatment failure and thus the spread of isolates PDR-*A.baumannii* that are resistant to all antibiotics.

Acknowledgement

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Nil.

Conflict of interests

There are no conflicts of interest.

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