Egyptian Journal of Medical Microbiology Volume 29 / No.2 / April 2020 107-115 Online ISSN: 2537-0979

ORIGINAL ARTICLE

Detection of Class-D OXA Carbapenemase Genes among Biofilm and Non-Biofilm Forming *Acinetobacter baumannii* Isolated from Suez Canal University Hospitals

Asmaa B. Hamady* and Yara E. Marei
Department of Medical Microbiology and Immunology, Faculty of Medicine, Suez Canal University, Ismailia, Egypt

Key words:
Acinetobacter baumannii-OXA Carbapenemase-biofilm- multidrug resistence

****Corresponding Author:***
Asmaa Bakeir Hamady
Department of Medical Microbiology and Immunology, Faculty of Medicine, Suez Canal University, Ismailia, Egypt
Tel.: 01004531482
asmaabakeir@yahoo.com

ABSTRACT

Background*: Acinetobacter baumannii is recognized as opportunistic pathogen of increasing relevance in healthcare-associated infections. The emergence of carbapenemases genes in MDR-AB forming biofilm complicated treatment of infected patients with this microorganism. This study aimed to assess the prevalence of blaOXA genes genes among biofilm and non-biofilm forming A. baumannii.* ***Methodology:*** *forty seven A. baumannii strains were isolated and identified by conventional microbiological tests and API 20NE assay. The presence of blaOXA genes were studied by polymerase chain reaction. Biofilm formation was detected by microtiter plate method.* ***Results****: The highest percentage of resistance was to imipenem (85.1 %). 80.9 % of the isolates were resistant to ceftazidime, cefotaxime and meropenem. Thirty five A. baumannii isolates (74.5 %) were found to be MDR. Thirteen A. baumannii isolates (27.6%) were strong biofilm producers, 10 strains were moderate biofilm producers and 24 strains were weak or non-biofilm producers. blaOXA-23 was detected in 16 isolates (34.04 %), blaOXA-51 was detected in 8 isolates (17.02 %) and blaOXA-58 in 18 isolates (38.3%). None of the study isolates harbored blaOXA-24.****Conclusion:*** *Coexistence of the blaOXA genes along with biofilm formation, in MDR-AB strains, complicated treatment of infected patients with these bacteria.*

INTRODUCTION

Acinetobacter species are strictly aerobic, nonfermenting Gram-negative coccobacilli that are common in water and soil. The *Acinetobacter calcoaceticus-Acinetobacter baumannii* (ACB) complex is composed of *A. baumannii*, *A. pittii*, *A. nosocomialis*, *A. lwoffii* and the environmental species *A. calcoaceticus* [1]. The species of ACB complex have the same appearance and biochemical reactions, and also cannot be distinguished by the traditional biochemical reactions or by automatic or semi-automatic identification machines, such as the Vitek 2 system. The species of ACB complex have been categorized as *A. baumannii* which is the most common genospecies of Acinetobacter. Precise molecular biological techniques can now be used to identify and distinguish these species accurately [2].

Acinetobacter species are recognized as opportunistic pathogens of increasing relevance in healthcare-associated infections [3], and are likely to cause opportunistic life threatening infections such as septicemia, meningitis, urinary tract, pneumonia and wound infections in intensive care units [4].

Acinetobacter baumannii possess a remarkable ability to acquire mobile genetic elements as plasmids, transposons and integrons that carry clusters of resistant genes, and this leads to multi drug resistance (MDR) [5]. This property, along with biofilm formation, contributed significantly to the infections caused by this microorganism [6].

Multiple previous studies recognized the relationship between biofilm and antibiotic resistance biofilm-based infections. Also, *A. baumannii* is among the leading nosocomial pathogens having the ability to produce severe biofilm related infections such as lower respiratory tract infections and colonization on venous catheters [6].

In recent years, the gradual increase of carbapenem-resistant forms of these species from hospital isolates has caused great concern. Carbapenem resistance in *A. baumannii* is mediated by one or several of these mechanisms: decreased outer membrane permeability, target-site modification, up-regulation of multidrug efflux pumps and carbapenemases production [7].

The carbapenemases in the AB complex include the molecular class D oxacillinases (OXAs type) and less common the molecular class B metallo β-lactamases as Verona imipenemase (VIM), imipenemase (IMP), Seoul imipenemase (SIM), Sao Paulo metallo-β-lactamase (SPM), German imipenemase (GIM), and New Delhi metallo beta-lactamase (NDM) [8]. Nowadays, OXA-type carbapenemases are divided into eight subgroups which four of them are identified in *A. baumannii*: OXA-23-

like consists of (OXA-23, OXA-27 and OXA-49); OXA-24-like (OXA-24, OXA-25, OXA-26, OXA40 and OXA-72); OXA-58; and OXA-51-like. The last group is a family of chromosomal enzymes found in *A. baumannii* [9].

Emergence of acquired carbapenem hydrolyzing enzymes and their wide dissemination represents a global health threat which induces serious therapeutic and infection control problems as it is associated with elevated mortality rates and increase in hospital stay. For these reasons, clinicians became highly concerned about rapid identification of bacteria that carry these genes to limit their dissemination in hospitals and allow them to establish effective therapeutic regimens [10].

Due to the dramatic increase in carbapenem-resistant *A. baumannii* in Suez Canal University hospitals (SCUH) in Ismailia, Egypt, this study was conducted to assess the prevalence of molecular class-D OXA carbapenemase genes among biofilm and non-biofilm forming *A. baumannii* in order to apply a proper antibiotic policy to avoid emergence and spread of antibiotic resistant bacteria.

METHODOLOGY

Patients:

This cross-sectional study was done from February to December 2018 on different clinical specimens (sputum and endotracheal tube aspirates, pus, urine and blood). A total of 47 *A. baumannii* strains were isolated from 192 patients who were suffering from HAIs as urinary tract infections, respiratory tract infections, bed ulcers, burn infection, wound infections and bacteremia and admitted to different wards in SCUH in Ismailia. All age groups were included. Informed consent was taken from patients to use their data in the study. The ethics committee of Faculty of Medicine, Suez Canal University had approved the study.

Specimens were collected under aseptic conditions and transferred to the Medical Microbiology and Immunology department within 24 hours of the collection for processing.

Bacterial Identification:

Each clinical specimen was inoculated on blood agar and MacConkey's agar plates, incubated aerobically at 35˚C ± 2 for 24- hrs.

The individual colonies were initially identified by conventional microbiological tests as lack of lactose fermentation on MacConkey's agar, Gram staining as Gram negative coccobacillary rods, oxidase negative, catalase positive, non-motile and lack of glucose fermentation on Hugh and Liefson oxidative/ fermentative (OF) media covered with sterile mineral oil[11]. The identification of isolates was further confirmed by API 20NE assay (BioMerieux, France).

Antibiotic Susceptibility Testing

Antibiotic susceptibility tests of the AB isolates were performed by standard Kirby-Bauer disc diffusion method on Mueller- Hinton agar (Oxoid, UK) using the following antibiotic disks: piperacillin-tazobactam (100/10 µg), ceftazidime (30 µg), cefotaxime (30 µg), cefepime (30 µg), amikacin (30 µg), gentamicin (10 µg), tetracyclines (30 µg), meropenem (10 µg), imipenem (10 µg), ciprofloxacin (5 µg), levofloxacin (5 µg) and trimethoprim-sulfamethoxazole (1.25/23.75 µg) (Oxoid, Basingstoke, UK). Interpretations of the test results were done according to CLSI (2019) guidelines [12]. Multi-drug resistant *A. baumannii* (MDR-AB) was defined as the resistance of the isolate to at least three classes of antimicrobial agents including all penicillins and cephalosporins (including inhibitor combinations), fluoroquinolones and aminoglycosides [13].

Detection of biofilm formation by Tissue culture plate method:

All the AB isolates were tested for biofilm formation by the TCP method [14]. The procedure of the test was as follows:

- Isolates from fresh agar plates were inoculated in trypticase soy broth (TSB) (oxoid) containing 1% glucose and incubated for 24 hours at 37°C and diluted (1: 100) with fresh medium.
- Individual wells of sterile, flat-bottom; polystyrene tissue culture plates were filled with 200 µl of the diluted cultures. The plates were covered and incubated for 24 hours at 37°C.
- After incubation, the content of each well was removed by tapping the plates.
- The wells were washed 4 times with 200 µl of phosphate buffer saline (pH 7.2) to remove free-floating planktonic bacteria. 25 µl of 1% solution of crystal violet was added to each well plate. The plates were covered and incubated at room temperature for 15 minutes, rinse thoroughly and repeatedly with water.

Crystal violet-stained biofilm was solubilized in 200µl of 95 % ethanol (to extract the violet color), of which 125µl was transferred to a new polystyrene microtiter plate, which had been read. The optical density (OD) at 630nm was recorded and the results were interpreted according to table 1.

Table 1: Classification of bacterial adherence and biofilm formation by TCP method

Mean OD value at 630nm	Biofilm production
< 0.120	Non/weak
0.120 - 0.240	Moderate
> 0.240	Strong

Genomic DNA Isolation

Genomic DNA of all AB isolates was extracted by boiling method as follows: five to six colonies were suspended in 250 µl sterile water and boiled for 10 minutes. The samples were cooled on ice for 10 minutes and centrifuged at 14000 rpm for one minute at room temperature. The supernatant was then transferred to a new tube and kept at -20°c for further analysis [15]. DNA quantification was performed by spectrophotometer at 260 nm. The purity of DNA was evaluated by the ratio of the absorbance at 260 and 280 nm (A260/A28).

Detection of Carbapenemase (OXA) Genes by PCR:

The *bla*OXA genes in AB strains were amplified by PCR using a specific set of primers listed in table 2 according to the method described by [16].

PCR was carried out with 2 µL template DNA, 0.9 µL of each primer, 0.7 µM of each deoxyribonucleoside triphosphates, 1x PCR reaction buffer, 1.5 mM MgCl2 and 1U Taq DNA polymerase in a total volume of 25 µL.

The DNA was amplified in the thermal cycler (Eppendorf Co., Germany) using the following protocol: initial denaturation (94 ºC for 5 minutes), followed by 30 cycles of denaturation (94 ºC for 35 seconds), annealing (60 ºC for 35 seconds) and extension (72 ºC for 40 seconds), with a single final extension of 6 minutes at 72 ºC. Positive and negative contxrols were run along with the tested samples. The amplified products were visualized by electrophoresis on 1.5% agarose gels stained with ethidium bromide and then visualized under UV illumination. Amplicons obtained were compared to a molecular-weight DNA ladder with the size range 100-1200 bp (Fermentas, Germany).

Table 2: Primers used to detect *bla*OXA genes.

Gene	Sequence (5´→3´)	Size (bp)	References
OXA 23	F:GAT CGG ATT GGA GAACCAGA R:ATT TCT GAC CGC ATT TCC AT	501	[17]
OXA 24	F:GGT TAG TTG GCC CCC TTA AA R:AGTTGAGCGAAA AGGGGATT	249	[18]
OXA 58	F:AAG TAT TGG GGC TTG TGC TG R:CCCCTCTGCGCTCTACATAC	599	[19]
OXA 51	F: TAA TGC TTT GAT CGG CCT TG R: TGG ATT GCA CTTCATCTT GG	353	[20]

RESULTS

- **Identification of *A. baumannii* strains:**

A total of forty seven non-duplicated *A. baumannii* strains (24.5%) were isolated from 192 hospitalized patients with HAIs. The isolates were initially identified by conventional microbiological tests and further confirmed by API 20 NE assays as shown in figure 1.These isolates were mostly collected from sputum and endotracheal tube aspirates (46.8 %), followed by wound pus (27.7 %), urine (14.9 %) and then blood (10.6 %).

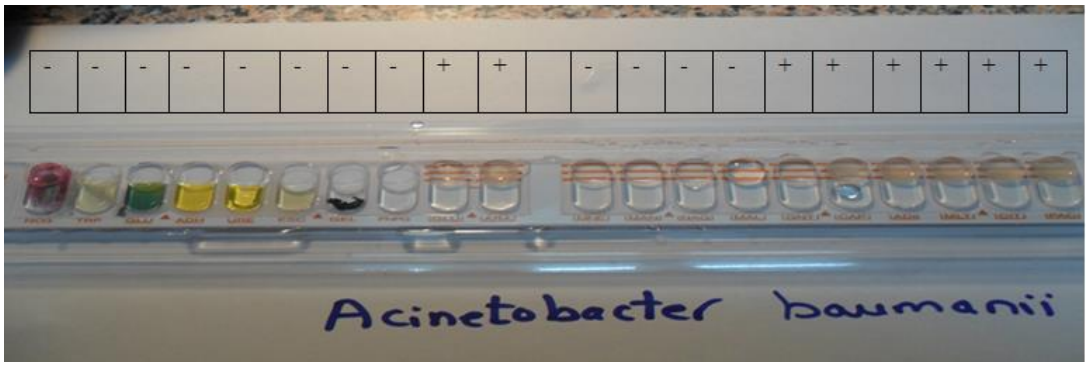

Fig. 1: API 20 NE of *A. baumannii*

- **Antibiotic susceptibility testing:**

The results of antibiotic susceptibility testing of the *A. baumannii* isolates to various antibiotics were demonstrated in figure 2. The highest percentage of resistance was to imipenem (85.1 %). 80.9 % of the isolates were found to be resistant to ceftazidime, cefotaxime and meropenem. Thirty five out of the 47 *A. baumannii* isolates (74.5 %) were found to be MDR.

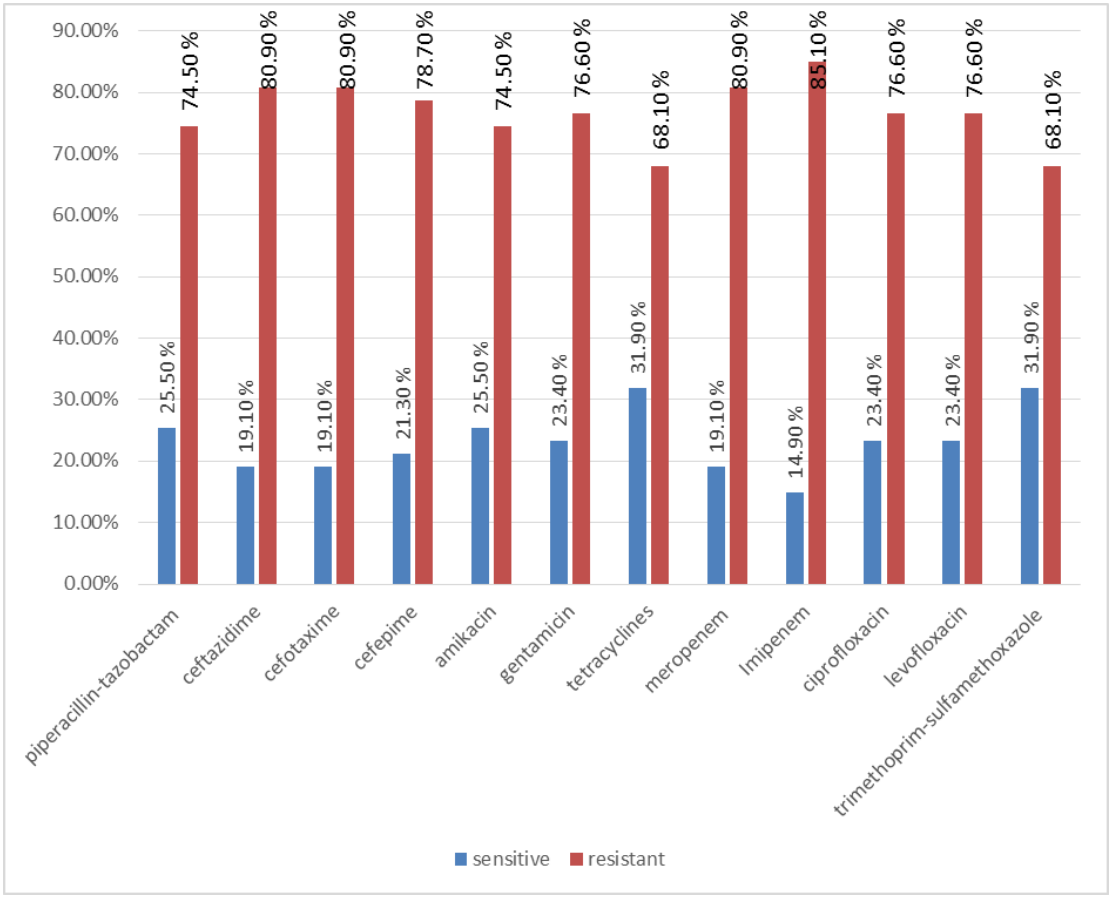

Fig. 2: Antibiotic susceptibility patterns of *A. baumannii* isolates using the disk diffusion method.

- **Biofilm detection by TCP method**

Detection of biofilm production was done by the TCP method (figure 3). Results showed that 13 out of 47 *A. baumannii* isolates (27.6%) were strong biofilm producers, 10 strains (21.3%) were moderate biofilm producers and 24 strains (51.1%) were weak or non-biofilm producers.

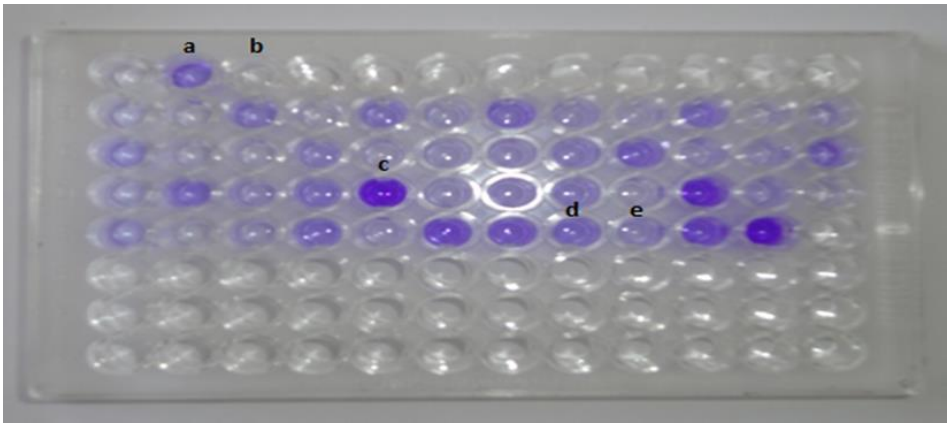

Fig. 3: Detection of biofilm production by tissue culture plate method.

a: positive control, b: negative control, c: strong biofilm producer,
d: moderate biofilm producer, e: non biofilm producer

- **Detection of Class-D OXA Carbapenemase genes by PCR:**
  The results of *bla*OXA genes detection among the isolates by PCR are presented in figures 4-6.

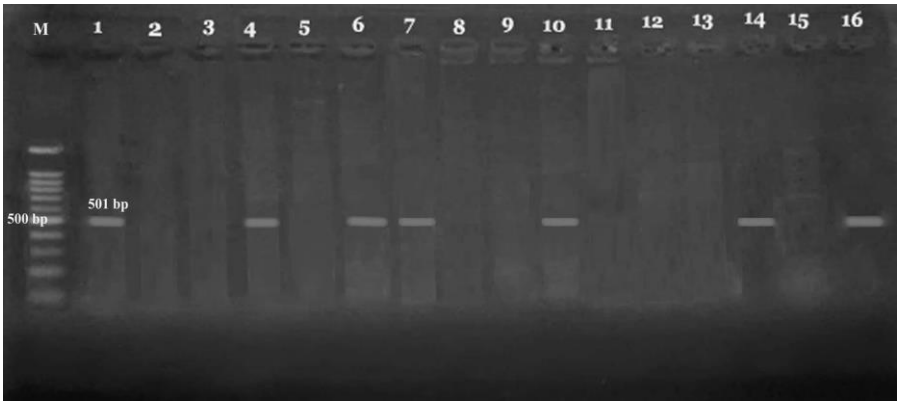

Fig. 4: Agarose gel electrophoresis of *bla*OXA-23 gene amplicons (501 bp). Lane M shows 100 bp molecular size standard DNA ladder. Lane 1 was positive control and lane 2 was negative control for the gene.

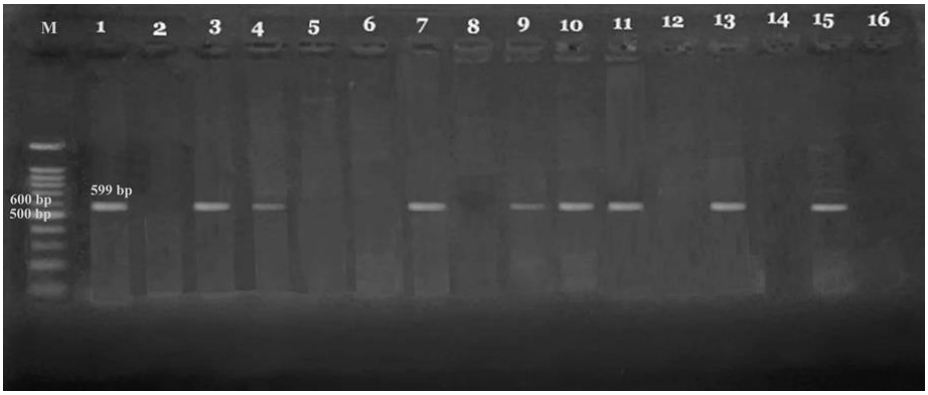

Fig. 5: Agarose gel electrophoresis of *bla*OXA-58 gene amplicons (599 bp). Lane M shows 100 bp molecular size standard DNA ladder. Lane 1 was positive control and lane 2 was negative control for the gene.

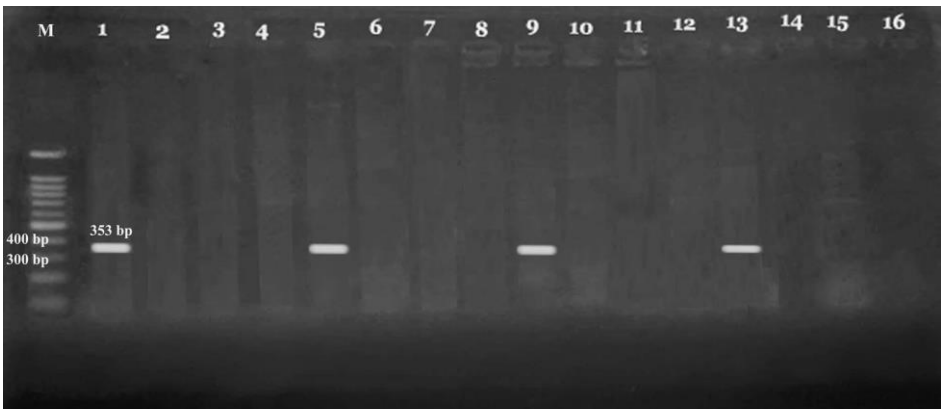

Fig. 6: Agarose gel electrophoresis of *bla*OXA-51 gene amplicons (353 bp). Lane M shows 100 bp molecular size standard DNA ladder. Lane 1 was positive control and lane 2 was negative control for the gene.

Eleven out of the 47 *A. baumannii* isolates (23.4 %) carried no *bla*OXA genes. *bla*OXA-23 was detected in 16 isolates (34.04%), *bla*OXA-51 was detected in 8 isolates (17.02%) and *bla*OXA-58 in 18 isolates (38.3%). None of the study isolates harbored *bla*OXA-24. Tables 3 and 4 illustrate the relation between phenotypic carbapenem resistance and the presence of *bla*OXA genes. The relation between biofilm production and the presence of *bla*OXA genes among *A. baumannii* isolates is illustrated in table 5.

Table 3: The relation between phenotypic imipenem resistance and the presence of *bla*OXA genes.

Gene	Imipenem		Total	*P* value
	Sensitive No. (%)	Resistant No. (%)		
Oxa 23				0.167
Yes	4 (8.5%)	**12 (25.5%)**	16	
No	3 (6.4%)	28 (59.6%)	31	
Oxa 51				0.378
Yes	2 (4.3%)	**6 (12.8%)**	8	
No	5 (10.6%)	34 (72.3%)	39	
Oxa 58				0.450
Yes	2 (4.3%)	**16 (34%)**	18	
No	5 (10.6%)	24 (51%)	29	
Total	7	40	47	---------

Table 4: The relation between phenotypic meropenem resistance and the presence of blaOXA genes.

Gene	Meropenem		Total	*P* value
	Sensitive No. (%)	Resistant No. (%)		
Oxa 23				0.167
Yes	2 (4.3%)	**14 (29.8%)**	16	
No	7 (14.9%)	24 (51.1%)	31	
Oxa 51				0.378
Yes	0 (0%)	8 (17%)	8	
No	9 (19.1%)	30 (63.8%)	39	
Oxa 58				0.450
Yes	3 (6.4%)	**15 (31.9%)**	18	
No	6 (12.8%)	23 (48.9%)	29	
Total	9	38	47	---------

Table 5: The relation between biofilm production and the presence of *bla*OXA genes among *A. baumannii* isolates.

Genes	Biofilm producer n=23	Non biofilm producer n=24	*P. value*
***bla*OXA-23: positive No. (%)**	16 (34%)	0 (0.8%)	0.000
negative No. (%)	7 (14.8%)	24 (51%)	
***bla*OXA-51: positive No. (%)**	6 (12.7%)	2 (4.2%)	0.105
negative No. (%)	17 (36.2%)	22 (46.8%)	
***bla*OXA-58: positive No. (%)**	5 (10.6%)	13 (27.6%)	0.022
negative No. (%)	18 (38.3%)	11 (23.4%)	
Total No. (%)	23 (48.9%)	24 (51.1%)	

DISCUSSION

Acinetobacter baumannii is one of the most important MDR bacteria in hospitals worldwide. *A. baumannii* antimicrobial resistance has increased as the majority of strains became resistant to the commonly used antibiotics [21]. This study provides important results regarding the antimicrobial susceptibility, dissemination and diversity of carbapenem resistance encoding genes among MDR-AB in SCUH in Ismailia.

A total of 192 specimens were collected from patients with HAIs in SCUH, from which a 47 *A. baumannii* isolates were isolated (24.5%). The frequency of MDR among isolated strains was found to be 74.5 %. Lower isolation rate (2.9%) was reported by Nageeb *et al.* [22] in Egypt (the sample size was 350), and all the isolated strains were MDR. The percentage of *A. baumannii* is variable in different studies and this might be attributed to different sample sizes.

Another study was done by Gholami *et al.* [4] in Iran showed that the frequency of MDR among *A. baumannii* isolates was 100% (the sample size was 110). Carbapenems are successfully used for treatment of MDR-AB infections; however, in the recent years, the increase of carbapenem resistant isolates compromised their use and the emergence of carbapenem resistant *A. baumannii* is considered a global concern [23]. In this study, the rates of resistance to imipenem and meropenem by disk diffusion method were 85.1% and 80.9%, respectively. Nageeb *et al.* [22]in Egypt also reported high resistance to imipenem and meropenem (100% and 80% respectively). Similar results were reported by Gholami *et al.* [4].

Karmostaji *et al.* [9] found that 83 *A. baumannii* strains (67.4%) out of the 123 studied ones were resistant to imipenem, and 104 strains (84.5%) were resistant to meropenem.

In the current study, out of the 23 biofilm producing strains, sixteen strains carried *bla*OXA-23 gene, while only 6 strains carried *bla*OXA- 51 gene and 5 strains carried *bla*OXA-58 gene. Azizi *et al.* [6] found that *A. baumannii* strains containing both *bla*OXA-23 and *bla*OXA-24 like genes exhibited strong biofilm activity.

The OXA carbapenemase genes are well distributed among *Acinetobacter* spp. and also documented by multiple authors [24]. These genes have an important role in the antibiotic resistance among nosocomial bacteria and are reported on transposons, integrons or plasmid [25]. In the current study, 16 strains (34%) carried *bla*OXA-23 gene, 8 strains (17%) carried *bla*OXA-51 and 18 strains (38.3%) carried *bla*OXA-58, while *bla*OXA-24 was absent in all strains. The presence of the genes was higher among imipenem and meropenem resistant strains than sensitive ones (p value > 0.05). In a previous study that was done in Egypt, the *bla*OXA-23 gene was detected in 42.8% of isolates (the sample size was 14), while *bla*OXA-24 was detected among 7.1% of isolates[26]. Also, Hamam & Awad [27] in Egypt detected *bla*OXA-23 in 82.4 % of isolates and *bla*OXA-51 in 88.2 % of isolates (the sample size was 34); while, *bla*OXA-24 and *bla*OXA-58 were not detected.

Azizi *et al.* [6] found that all of the isolates (the sample size was 65) carried *bla*OXA-23 and *bla*OXA-51 genes both in the imipenem resistant and imipenem sensitive strains, while *bla*OXA-24 was detected in 29 isolates. The *bla*OXA-58 gene was absent in the entire population. A previous study that was done by Shoja *et al.* [28]found that all the studied isolates (the sample size was 124) carried *bla*OXA-51 gene, while *bla*OXA-23 and *bla*OXA-24 genes were detected in 85.6% and 6.2% of carbapenem resistant isolates, respectively. No *bla*OXA-58 gene was detected in all studied isolates.

In another study done by Gholami *et al.* [4], the *bla*OXA-51 carbapenemase gene was detected in 100% of strains (the sample size was 110), while the percentage of *bla*OXA-23, *bla*OXA-24 and *bla*OXA-58 were 96.63%, 35.45% and 7.27%, respectively.

CONCLUSION

In conclusion, the experimental data obtained in this study revealed that many *A. baumannii* isolated from different wards in SCUH were resistant to imipenem and meropenem. The presence of *bla*OXA-51, *bla*OXA-58 and *bla*OXA-23 genes was higher among imipenem and meropenem resistant strains than susceptible strains. The *bla*OXA-24 carbapenemase gene was not detected in this study. This study was limited to OXA genes; however, future studies should be done on sequencing and molecular typing of the isolates. Also, it should be noted that, fight against MDR-AB and other organisms needs a strategy of health-care officials and decision makers, the challenge being to make hospitals as a safe place for all patients.

Conflicts of interest: The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.

- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

REFERENCES

1. TeoJWP, Kurup A, Lin RTP, Hsien KT. Emergence of clinical Klebsiella pneumoniae producing OXA-232 carbapenemase in Singapore. New Microbes and New Infections. 2013; 1(1), 13–15.

2. Wang TH, Leu YS, Wang NY, Liu CP, Yan TR. Prevalence of different carbapenemase genes among carbapenem-resistant *Acinetobacter baumannii* blood isolates in Taiwan. Antimicrobial Resistance & Infection Control. 2018; 7(1).
3. Esposito S and De Simone G. Update on the Main MDR Pathogens. Prevalence and Treatment Options. Infez Med. 2017; 25 (4), 301-310.
4. Gholami M, Moshiri M, Ahanjan M, Chirani A, Bibalan M, Asadi A, Eshaghi M, Pournajaf A, Abbasian S, Kouhsari E, Irajian G. The diversity of Class B and Class D Carbapenemases in Clinical *Acinetobacter Baumannii* Isolates. Le Infezioni in Medicina. 2018; 26 (4), 329-335.
5. Kamruzzaman M, Patterson JD, Shoma S, Ginn AN, Partridge SR, Iredell JR. Relative Strengths of Promoters Provided by Common Mobile Genetic Elements Associated with Resistance Gene Expression in Gram-Negative Bacteria. Antimicrobial Agents and Chemotherapy. 2015; 59(8), 5088–5091.
6. Azizi O, Shakibaie MR, Modarresi F, Shahcheraghi, F. Molecular Detection of Class-D OXA Carbapenemase Genes in Biofilm and Non-Biofilm Forming Clinical Isolates of *Acinetobacter baumannii*. Jundishapur Journal of Microbiology. 2015; 8(1).
7. Hsu LY, Apisarnthanarak A, Khan E, Suwantarat N, Ghafur A, Tambyah PA. Carbapenem-Resistant *Acinetobacter baumannii* and Enterobacteriaceae in South and Southeast Asia. Clinical Microbiology Reviews. 2016; 30(1), 1–22.
8. Cornaglia G, Giamarellou H, Rossolini GM. Metallo-β-lactamases: a last frontier for β-lactams? The Lancet Infectious Diseases. 2011; 11(5), 381–393.
9. Karmostaji A, Najar Peerayeh S, Hatef Salmanian A. Distribution of OXA-Type Class D β-Lactamase Genes Among Nosocomial Multi Drug Resistant *Acinetobacter baumannii* Isolated in Tehran Hospitals. Jundishapur Journal of Microbiology. 2013; 6(5).
10. Ramadan RA., Gebriel MG, Kadry HM, Mosallem A. Carbapenem-resistant *Acinetobacter baumannii* and Pseudomonas aeruginosa: characterization of carbapenemase genes and E-test evaluation of colistin-based combinations. Infection and Drug Resistance. 2018; 11, 1261–1269.
11. Bouvet PJ, Grimont PA. Identification and biotyping of clinical isolates of Acinetobacter. Annales de l'Institut Pasteur / Microbiologie. 1987; 138(5), 569–578.
12. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. 29th ed. CLSI Supplement M 100. USA: Wayne, PA. 2019.
13. Manchanda V, Sinha S, Singh N. Multidrug resistant Acinetobacter. Journal of Global Infectious Diseases. 2010; 2(3), 291.
14. Christensen G, Simpson W, Younger J, Baddour L, Barrett F, Melton D, Beachey EH. Adherence of Coagulase-Negative Staphylococci to Plastic Tissue Culture Plates: A Quantitative Model for the Adherence of Staphylococci to Medical Devices. J clinical microbial. 1985; 22 (6), 996-1006.
15. Andriamanantena TS, Ratsima E, Rakotonirina HC, Randrianirina F, Ramparany L, CarodN JF, Talarmin A. Dissemination of multidrug resistant *Acinetobacter baumannii* in various hospitals of Antananarivo Madagascar. Annals of Clinical Microbiology and Antimicrobials.2010; 9(1), 17.
16. Feizabadi M, Fathollahzadeh B, Taherikalani M, Rasoolinejad M, Sadeghifard N, Aligholi M., Soroush S, Mohammadi-Yegane S. Antimicrobial Susceptibility Isolated from Patients at Tehran Hospitals. Jpn J Infect Dis. 2008; 61(4), 274-278.
17. Liakopoulos A, Miriagou V, Katsifas E, Karagouni A, Daikos G, Tzouvelekis L, Petinaki E. Identification of OXA-23-Producing *Acinetobacter Baumannii* in Greece, 2010 to 2011. Euro Surveill. 2012; 17 (11), 20117.
18. Woodford N, Ellington M, Coelho J, Turton J, Ward M, Brown S, Livermore D. Multiplex PCR for genes encoding prevalent OXA carbapenemases in Acinetobacter spp. International Journal of Antimicrobial Agents. 2006; 27(4), 351–353.
19. Sohrabi N, Farajnia S, Akhi MT, Nahaei MR, Naghili B, Peymani A, Saeedi N. Prevalence of OXA-Type β-Lactamases Among *Acinetobacter baumannii* Isolates from Northwest of Iran. Microbial Drug Resistance. 2012; 18(4), 385–389.
20. Turton JF, Woodford N, Glover J, Yarde S, Kaufmann ME, Pitt TL. Identification of *Acinetobacter baumannii* by Detection of the blaOXA-51-like Carbapenemase Gene Intrinsic to This Species. Journal of Clinical Microbiology. 2006; 44(8), 2974–2976.
21. Gholami M, Chirani AS, Razavi S, Falak R, Irajian G. Immunogenicity of a fusion protein containing PilQ and disulphide turn region of PilA from Pseudomonas aeruginosa in mice. Letters in Applied Microbiology. 2017; 65(5), 439–445.
22. Nageeb W, Kamel M, Zakaria S, Metwally, L. Phenotypic characterization of *Acinetobacter baumannii* isolates from intensive care units at a tertiary-care hospital in Egypt. Eastern Mediterranean Health Journal. 2014; 20(3), 203–211.

23. Manageiro V, Jones-Dias D, Ferreira E, Louro D, Caniça M. Genetic diversity and clonal evolution of carbapenem-resistant *Acinetobacter baumannii* isolates from Portugal and the dissemination of ST118. International Journal of Antimicrobial Agents. 2012; 40(5), 398–403.
24. Grosso F,Quinteira S, Poirel L, Novais Â, Peixe L. Role of CommonblaOXA-24/OXA-40-Carrying Platforms and Plasmids in the Spread of OXA-24/OXA-40 among Acinetobacter Species Clinical Isolates. Antimicrobial Agents and Chemotherapy. 20121; 56(7), 3969–3972.
25. Shacheraghi F, Shakibaie M, Noveiri H. Molecular Identification of ESBL Genes blaGES-1, blaVEB-1, blaCTX-M blaOXA-1, blaOXA-4, blaOXA-10 and blaPER-1 in Pseudomonas Aeruginosa Strains Isolated from Burn Patients by PCR, RFLP and Sequencing Techniques. Int J Biol life Sci. 2010; 3(6), 138–42.27.
26. El-Masry EA, El-Masry HA. Characterization of Carbapenem-resistant *Acinetobacter baumannii* Isolated from Intensive Care Unit, Egypt. *Egyptian* Journal of Medical Microbiology. 2018; 27(3), 85-91
27. Hamam SS, Awad SM. Characterization of Antimicrobial Resistance and Prevalence of OXA Genes in the Emerging Threat *Acinetobacter baumannii* Causing Blood Stream Infection in ICU Patients. Egyptian Journal of Medical Microbiology. 2018; 27(4), 141-148.
28. Shoja S, Moosavian M, Rostami S, Abbasi F, Tabatabaiefar MA, Peymani A. Characterization of Oxacillinase and Metallo-β-Lactamas Genes and Molecular Typing of Clinical Isolates of *Acinetobacter baumannii* in Ahvaz, South-West of Iran. Jundishapur Journal of Microbiology. 2016; 9(5).