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Carbapenem-hydrolyzing Oxacillinase Genes in Clinical Isolates of *Acinetobacter baumannii*

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Authors' contributions

This work was carried out in collaboration between all authors. Author MEM designed the study, performed the bacteriological diagnosis and statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors ATH and SMKA performed the clinical diagnosis of the cases and collect samples. All authors managed the literature searches. All authors read and approved e final manuscript.

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ABSTRACT

Aims: We aimed in this study to detect the prevalence of carbapenem-hydrolyzing oxacillinase genes among *Acinetobacter baumannii* clinical isolates recovered from Assiut University Hospitals, Egypt.
Methods: The antimicrobial susceptibilities of 23 non-repetitive *Acinetobacter baumannii* clinical isolates collected from patients with multiple types of infections were determined. Amplification of blaOXA-23, blaOXA-51, and blaOXA-58 genes was performed by PCR.
Results: *Acinetobacter baumannii* isolates showed high resistance to carbapenems and other antibiotics. Eleven (48%) isolates were extensively drug resistant and 12 (52%) isolates showed

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pandrug resistance. Among 23 *Acinetobacter baumannii* strains; oxacillinase genes were detected in 19 (83%) strains, none of the examined genes were found in 4 (17%) strains. Twelve (52%), 9 (39%), and 4 (17%) isolates harbored blaOXA-51, blaOXA-23, and blaOXA-58 genes, respectively, either in single form (12 isolates; 52%) or combined (7 isolates; 30%). blaOXA-producers associated with longer hospital stay and poor outcome. *A. baumannii* isolates expressed blaOXA-23 and blaOXA-58 genes, had higher MIC for carbapenems than blaOXA-51 gene.

Conclusion: We concluded that, the presence of oxacillinase genes, especially blaOXA-23 and blaOXA-58, may convey resistance to carbapenems in *Acinetobacter baumannii* isolates and are associated with high comorbidities and poor outcome in patients.

Keywords: Acinetobacter baumannii; oxacillinases; blaOXA-23; blaOXA-51; blaOXA-58.

ABBREVIATIONS

A. baumannii	:	*Acinetobacter baumannii*
ANOVA	:	*Analysis of variance*
CA	:	*community acquired*
CDC	:	*the Center of Disease Control and Prevention*
CLSI	:	*the Clinical and Laboratory Standards Institute*
COPD	:	*Chronic obstructive pulmonary disease*
DCP	:	*decompensated Core-pulmonale*
DM	:	*diabetes mellitus*
HAIs	:	*healthcare-associated infections*
HCV	:	*hepatitis C virus*
ICU	:	*intensive care units*
IHD	:	*ischemic heart disease*
IPF	:	*interstitial pulmonary fibrosis*
MDR	:	*Multidrug-resistant*
MV	:	*mechanical ventilation*
MIC	:	*minimum inhibitory concentration*
OXA	:	*carbapenem-hydrolyzing oxacillinases*
PDR	:	*pandrug-resistant*
R	:	*resistant*
RF	:	*Respiratory failure*
S	:	*susceptible*
SD	:	*Standard deviation*
SPSS	:	*Statistical package for social sciences*
VAP	:	*ventilator-associated pneumonia*
XDR	:	*extensively drug-resistant*

1. INTRODUCTION

Acinetobacter baumannii (*A. baumannii*) is ubiquitous opportunistic pathogen capable of causing both community-acquired and healthcare-associated infections (HAIs), although HAIs are the most common form [1], including bacteremia, ventilator-associated pneumonia (VAP), surgical wound infection, and urinary tract infections, particularly in patients admitted to intensive care units (ICU) [2]. Its great capacity to survive in low-moisture environments and its ability to develop resistance to antimicrobial agents afford *A. baumannii* the possibility of causing large HAI outbreaks. *A. baumannii* are usually resistant to multiple antimicrobial agents, because of its propensity to accumulate mechanisms of antimicrobial resistance that lead to pandrug resistance [1]. Of major concern, is the increased incidence of carbapenem resistance, which has risen dramatically over the last decade with limited therapeutic options [3]. The most common carbapenem resistance determinants in *Acinetobacter spp*. are the carbapenem-hydrolyzing oxacillinases (OXA). *A. baumannii* isolates harbor the intrinsic OXA-51, of which >80 variants have been identified so far, and five groups of acquired OXA genes (OXA-23, -40, -58, -143, and -235) [4,5]. Most *bla*OXA genes are often associated with insertion sequences (IS) that mediate their mobility and overexpression, thereby leading to carbapenem

resistance [6]. The identification of drug resistance mechanisms in *A. baumannii* will improve the outcome of infections caused by this organism. So, we aimed in this study was to determine the antimicrobial susceptibility patterns and the prevalence of carbapenem-hydrolyzing oxacillinase genes among *A. baumannii* strains isolated from clinical samples.

2. MATERIALS AND METHODS

2.1 Bacterial Isolates

This is a descriptive study that included 23 non-repetitive *A. baumannii* clinical isolates from Assiut University Hospitals, Egypt for the presence of carbapenem-hydrolyzing oxacillinase genes. The inclusion criteria included either community acquired (CA) or health care associated infections, multiple types of infections, mostly respiratory tract, urinary tract, wound infections, bacteremia, and sporadic infections. HAIs were diagnosed by the physicians according to the guidelines of the Center of Disease Control and Prevention (CDC), 2016 [7]. Samples were cultured on Herellea agar medium purchased from HIMEDIA. The colonies were identified by colony morphology (pale lavender colonies with yellow background), growth at 44° C, and the API 20 NE system (bioMérieux, France). An informed consent was obtained from all participants. The study was approved by the Ethical Committee of our university.

2.2 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility tests were performed by determining the minimum inhibitory concentration (MIC) values according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI), 2016 [8]. The MICs of the following antibiotics were determined; ciprofloxacin (5 µg), ofloxacin (30 µg), levofloxacin (30 µg), gentamicin (10 µg), amikacin (30 µg), tobramycin (10 µg), cefepime (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone, aztreonam (10 µg), meropenem (10 µg), and imipenem (10 µg). The breakpoints for imipenem and meropenem were susceptible (S) ≤ 2, intermediate 4 µg/ml, and resistant (R) ≥ 8 µg/ml [8]. Carbapenem-hydrolyzing enzyme activity was screened for by the CarbAcineto NP test as described by Dortet et al. [9]. Multidrug-resistant (MDR) bacteria was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, extensively drug-resistant (XDR) bacteria was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories, and pandrug-resistant (PDR) bacteria was defined as non-susceptibility to all agents in all antimicrobial categories [10].

2.3 PCR Amplification of Carbapenem-hydrolyzing Oxacillinase Genes

Genomic DNA was extracted by the boiling method [11]. We performed amplification reactions to target *bla*OXA-23, *bla*OXA-51, and *bla*OXA-58 oxacillinase genes in *A. baumannii* isolates using Master mix (supermix) and primers purchased from Invitrogen, United Kingdom. Primers were described previously: *bla*OXA-23 F-GATCGGATTGGAGAACCAGA, *bla*OXA-23 R-ATTTCTGACCGCATTTCCAT, *bla*OXA-51 F-TAATGCTTTGATCGGCC- TTG, *bla*OXA-51 R-TGGATTGCACTTCATCTTGG, *bla*OXA-58 F-TGGCACGCAT-TTAGACCG, and *bla*OXA-58 R-AAACCCACATACCAACCC, producing PCR products of 501, 353, and 507 bp, respectively [12,13]. PCR was performed for each gene in a 50 µl final volume containing 10x PCR buffer (5 µl), 2 mM deoxynucleoside triphosphates, 3.5 pmol of each primer, 2.5 mM MgCl (5 µl), 1 U Taq DNA polymerase and 2 µl of genomic DNA of the test strain, in a thermal cycler (Biorad, USA) using the following conditions: an initial denaturation step at 94°C for 5 min, followed by 30 cycles at 94°C for 30 s, 55°C for 30 s and 72°C for 90 s and a final extension step at 72ºC for 7 min. PCR products were separated by 1.3% agarose gel-electrophoresis, stained with ethidium bromide and visualized under UV light.

2.4 Statistical Analysis

Statistical analysis was performed using SPSS 20.0 program. Statistical significance was assessed via $\chi 2$ or Fisher's exact test for categorial variables and Student's *t*-test or ANOVA for continuous variables. *P* value <0.05 was considered statistically significant.

3. RESULTS

3.1 Antibiotic Susceptibilities and Characteristics of *A. baumannii* Infection Subjects

Bacterial strains were isolated from adults aged 27 to 73 years old. The average age of the

patients was 53.17 ± 10.4 years, and male–female ratio was 2.3:1. *A. baumannii* isolates were mainly distributed in ICU (7; 30%) and surgery department (4; 17%). Samples were mainly respiratory (7; 30%), pus (5; 22%), urine (3; 13%), and blood (3; 13%). *A. baumannii* associated with different types of infections including pneumonia (6 patients; 26%), wound infections (4 patients; 17%), urinary tract infection (3 patients; 13%), bacteremia (2 patients; 9%), and other infections. HAIs were found in 16 (70%) subjects, while, community-acquired infections were detected in 7 (30%) subjects. Patients have mean hospital stay of 8.7 ± 4.3 days (range 1-19 days). All patients received the inappropriate antibiotic therapy (as revealed from patients' files and data collected by their physicians) and 20 (87%) of them had associated comorbidities. Antibiotic susceptibility testing revealed that, almost all *A. baumannii* strains were resistant to the cephalosporin group, quinolones, and gentamicin. High resistance rates were detected against amikacin and tobramycin. Carbapenem-resistance was found in 15 (65%) and 16 (70%) isolates to imipenem and meropenem, respectively. Among 23 *A. baumannii* strains; 11 (48%) isolates were found to be extensively drug resistant (XDR), and 12 (52%) isolates showed pandrug resistance (PDR). Except for two isolates that wereo sensitive to aztreonam, carbapenem-resistant strains were resistant to all other antibiotics. Although did not reach a significant level ($\chi 2$; P= 0.069), 26% (6 isolates) of PDR strains were isolated from ICU. Other PDR strains were isolated from nephrology and gastroenterology units (2 isolates each; 17%), surgery and hematology unit (one isolate each; 9%). Respiratory tract infection was the most common type of infection caused by PDR isolates (Fisher's exact test; P= 0.024). For carbapenem-nonsusceptible strains; MIC to imipenem and meropenem ranged from ≥ 8 µg/ml to >256 µg/ml, and 6 (43%) out of the 14 carbapenem-resistant isolates had values ≥ 128 µg/ml. *A. baumannii* isolated from ICU showed significantly higher MIC for carbapenems than those isolated from many other wards (ANOVA; P values < 0.05). CarbAcineto NP test showed positive result in 16 (70%) of *A. baumannii* isolates.

3.2 Carbapenem-hydrolyzing Oxacillinase Genes and Univariate Analysis

PCR results showed that among 23 *A. baumannii* strains; oxacillinase genes were detected in 19 (83%) strains, we did not find any of the three genes tested in 4 (17%) strains. Twelve (52%), 9 (39%), and 4 (17%) isolates harbored *bla*OXA-51, *bla*OXA-23, and *blaOXA-58* genes, respectively, either in single form (12 isolates; 52%) or combined [7 isolates (30%); 5 (22%) isolates were positive for both OXA-51 and OXA-23 genes, and two (9%) isolates were positive for both OXA-51 and OXA-58 genes]. Univariate analysis showed that, there was highly significant association between the presence of *bla*OXA-genes and carbapenem-resistance in *A. baumannii* (Fisher's exact test; P values <0.001). Patients infected by OXA-producing *A. baumannii* strains exhibited longer (>7 days) hospital stay (Fisher's exact test; P=0.004) and significantly poor outcome (Fisher's exact test; P=0.021) than their counter partners, with even death in three (13%) patients. *A. baumannii* isolates expressed *bla*OXA-23 and *bla*OXA-58 genes, either alone or combined with *bla*OXA-51 gene, had significantly higher MIC for carbapenems than alone *bla*OXA-51 gene-producers (Student's *t*-test; P=0.029 and 0.033 for imipenem and meropenem, respectively).

4. DISCUSSION

In the hospital setting, carbapenems are reserved for treatment of the most severely ill patients. However, the emergence and spread of resistant *A. baumannii* strains, mostly due to the production of carbapenem-hydrolyzing enzymes, is often responsible for antibiotic treatment failure of those patients. The worldwide spread of carbapenem-resistant *A. baumannii* strains has become a challenge for both clinicians and microbiologists and represents a major public health problem [2]. Here, we reported the detection of carbapenem-hydrolyzing oxacillinase genes in *A. baumannii* isolated from clinical specimens. Our patients experienced multiple types of infections caused by *A. baumannii* including; pneumonia, wound infections, urinary tract infections, and bacteremia, and the isolates showed high resistance rates to cephalosporins, quinolones, aminoglycosides, and carbapenems [2]. Carbapenem-resistance among our isolates exceeded 50%, with many strains showed high MIC values which is in accordance with previous reports [13-18]. A considerable number of our patients had pneumonia. Pneumonia is the most common clinical presentation in *A. baumannii* infections. *A. baumannii* is among the most common pathogens to cause late-onset VAP and

the second most common pathogen to cause bloodstream infections acquired in hospitals [19], that are most commonly associated with the presence of a central vascular catheter [20]. *A. baumannii* increased significantly in the last years as a cause of pneumonia in ICUs in many countries. In a previous survey, 36.8% of 427 *A. baumannii* isolates that caused VAP were resistant to carbapenems [20]. Apart from being associated with increased morbidity and mortality, suspected hospital-acquired pneumonia in the ICU can lead to the inappropriate use of antibiotic drugs, contributing to bacterial drug resistance and increases in toxic effects and health care costs [20]. In an earlier report, carbapenem-resistance reached up to 85% among *A. baumannii* isolates from ICU [21]. In accordance with previous findings [2,15,16,22-24], the main ward for drug-resistant *A. baumannii* isolates in our work was ICU and mainly in cases with respiratory tract infections. *A. baumannii* is adapted to survive and colonize in the hospital environment, especially in ICUs, and is responsible for serious outbreaks [19]. Most of our patients had potential risk factors for *A. baumannii* infection like; intravenous or urinary catheters, MV, prior surgery, use of broad-spectrum antibiotics as reported [1, 24-26]. Previous studies described an increase of MDR and carbapenem-resistant *A. baumannii* associated with the use of third-generation cephalosporins and carbapenems. Authors suggested that the use of one antimicrobial could improve resistance mechanisms to others [24]. Additionally, previous data have correlated invasive devices with carbapenem-resistant *A. baumannii* colonization, reinforcing the need for surveillance and control measures for these devices [27], mainly MV, as its habitat is a humid environment [1]. Moreover, patients who require invasive devices usually present with a more severe illness, demanding frequent medical interventions and have longer hospital stays, favoring colonization [24]. The death rate among our patients was 13%, which is relatively high. Previous case-control studies concluded that there is an association between infections by *A. baumannii* and mortality in hospitalized patients, even regardless of the resistance profile, especially in patients under inadequate antimicrobial therapy [28], which is the case in our patients. Another report described that patients infected by carbapenem-resistant *A. baumannii* displayed a 20% higher rate of hospital mortality when compared to those merely colonized [29]. As shown in our study, most *A. baumannii* isolates expressed *bla*OXA-genes. Moreover, seven isolates were positive for two genes. More than half of the isolates harbored *bla*OXA-51 gene. Our data support those of other studies that demonstrated OXA-51 may be used as a marker to identify *A. baumannii* [2,30]. *bla*OXA-51 gene appear to be naturally occurring in all *A. baumannii* isolates and has the ability to confer carbapenem resistance [12]. Carbapenem-hydrolyzing enzymes that belong to the OXA-51 group have been identified globally, due to their chromosomal location and the fact that every *A. baumannii* isolate carries an OXA-51-like gene [31]. Thirty nine of our isolates were positive for *bla*OXA-23 gene. This enzyme contributes to carbapenem resistance in *A. baumannii* globally [2,15,16,31,32]. Furthermore, OXA-23 has been documented in strains associated with outbreaks of carbapenem-resistant *A. baumannii* in Asia, Europe, and South America [33]. Previous studies reported that *bla*OXA-23 and *bla*OXA-51 are the most common detected genes in *A. baumannii* [13]. Four strains were OXA-58 positive. OXA-58 belongs to OXA-58 cluster, which has been reported in Kuwait, Saudi Arabia, Argentina, various European countries, USA, Oceania, and Asia [31,32,34-36]. Enzymes belonging to OXA-58-like subgroup can be located on plasmids, which may explain their wide distribution [31]. *A. baumannii* isolates harboring *bla*OXA-23 and *bla*OXA-51 genes are consistently resistant to imipenem and meropenem [12]. However, in the absence of additional carbapenemases, some isolates that harbor *bla*OXA-51 gene are carbapenem susceptible and others resistant, suggesting its controversial role in imipenem resistance [30]. This might be explained by its regulation by insertion sequences, which encode transposases (rendering them mobile) and have been found to affect the expression of neighboring genes [37]. The *bla*OXA genes have been related to a variety of insertion sequences, which have an important role in the expression of these genes in *A. baumannii* [36,37]. Insertion sequences may result in hybrid promoter sequences associated with increased expression rates, which represents a real mechanism of reduced susceptibility and resistance to carbapenems [36]. Antimicrobial susceptibilities of *A. baumannii* should be known especially in situation requiring empirical treatment. The easy spread of *bla*OXA genes among *A. baumannii* strains especially in hospital setting necessitates the implementation of rigorous control programs on infections caused by carbapenem-resistant isolates.

More studies are essential to explore the molecular mechanisms that confer carbapenem-resistant phenotypes for *A. baumannii* isolates and to investigate the genetic diversity of other OXA-genes, to prevent the spread of such genes and resistant clones. One restriction of our study is the small number of isolates. Resistance mechanism in *A. baumannii* might be affected by factors other than oxacillinase genes that detected in the present study. Future researches with larger sample size are needed to better clarify the role of oxacillinase genes in conveying resistance mechanism in *A. baumannii*.

5. CONCLUSION

The presence of oxacillinase genes, especially *bla*OXA-23 and *bla*OXA-58, may convey resistance to carbapenems in *Acinetobacter baumannii* isolates and are associated with high comorbidities and poor outcome in patients

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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