Genetic and Phenotypic Characteristics of Acinetobacter baumannii Isolates from Sputum Specimens of ICU Patients at KHMC, Jordan

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ABSTRACT

Objective: To find out the antimicrobial resistance pattern in *A. baumannii* isolates from sputum samples of hospitalized patients in ICUs and determine the incidence rates of most common OXA-type carbapenemases, NDM-1, KPC and Class1 integrons among *A. baumannii* isolates.

Methods: This retrospective study included a total of 50 *A. baumannii* isolates recovered from sputum specimens, obtained from adult ICU patients at King Hussein Medical Center (KHMC, Amman, Jordan) over a period of 18-months from February 2017 to August 2018. All isolates were identified and tested for susceptibility against 13 antibiotics by *VITEK 2* Automated Microbiology System using gram negative ID card and "VITEK 2 AST–N233 and AST-XNO5 susceptibility cards according to CLSI guidelines (2018). Genomic and plasmid DNA were extracted. PCR tests were used to determine the presence of six types of class D oxacillinases, one type of class A carbapenemases, one type of class B metallo β lactamases, and Class 1 Integron among MDR isolates.

Results: All 50 isolates were MDR, including 100% resistance to cephalosporins, ciprofloxacin, aztreonem, piperacillin/tazobactum, 94%-98% to carbapenemes and 2% to colistin. All isolates carried *blaOXA-51* and 94% were positive for *blaOXA-23*, while *blaOXA-24*, *blaOXA-58*, *blaOXA-143* and *blaOXA-235* were positive in smaller percentages (4%-12%). The isolates also were positive for NDM-1, KPC and Class 1 Integron at rates of 26%, 22% and 86%, respectively.

Conclusions: This study concludes that respiratory tract colonization must be taken seriously as a source of bacteraemia with aggressive MDR *A. baumannii*. In addition, *A. baumannii* are able to acquire a lot of genetic resistance factors which will cause difficulties in treatment and rapid transmission in hospitals, Therefore, healthcare facilities should follow infection control measures to control and stop the transmission of MDR organisms.

Keywords: Acinetobacter baumannii, MDR, PCR, bla OXA, Class1 Integron.
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Introduction

Acinetobacter baumannii, commonly found in wet hospital environments, can colonize different body parts of patients due to its ability to resist dryness for a few weeks. In addition it cannot be easily eradicated with disinfectants and ultraviolet methods and can accommodate nutritional starvation in moist environments.(1-4)

Bacteriologically, *A.baumannii* belongs to the *Moraxellaceae* family, which includes gram-negative coccobacilli and has the following characteristics: strictly aerobic, nonmotile, glucose and lactose non-fermenting, catalase positive, oxidase negative and urease negative (1,5).

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The precise natural reservoir of this organism is still unidentified. It can stay alive for a long duration in soil, waste water and hospital water system and can hold up in temperatures between −20 to 44 °C and neutral pH for 5 months (6).

The *Acinetobacter* species is considered to be important as it is associated with frequent hospital outbreaks (7). It can be isolated and identified through culturing on blood and MacConkey agar for at least 24 h incubation at room temperature and up to 40 °C (7).

A.baumannii is a special opportunistic pathogenic organism due to its ability to develop rapid changes in genetic contents, leading to acquisition of multiple and extensive antimicrobial resistance genes (8-9).

Moreover, the genetic characteristics of *A. baumannii* strains are responsible for antibiotic resistance (10) and exhibit wide differences in biological characteristics related to biofilm formation, cell capsule development, adherence, invasion, iron uptake, or penicillin binding protein modifications (11). All of these characteristics enhance the spread of resistant isolates among the patients in clonally pattern (4).

A. baumannii strains cause a wide spectrum of infections including ventilator-associated pneumonia (VAP), blood stream infection, urinary tract infection, traumatic wound and burn wound infection, meningitis and endocarditis (12).

Most of these infections are developed after prolonged period of hospitalization or usage of mechanical ventilation and in patients had a previous history of antibiotics (1, 13).

VAP accounts for 86% of hospital-acquired infections in seriously ill patients. *Acinetobacter* species accounts for 8.4% of VAP and 2.2% of catheter-associated bloodstream infections in the USA (14, 15).

According to a recent study in the USA, most *Acinetobacter* isolates (57.6%) were obtained from the sputum, followed by blood (23.9%) and wounds (9.1%). In addition, carbapenem-resistant *A. baumannii* accounted for 65% of all pneumonias in USA and Europe (16) and had a mortality rate of 73% (17).

Almost all studies concluded that multidrug-resistant (MDR) *A. baumannii* infections are difficult to treat, spread rapidly among hospitalized patients, and have high morbidity and mortality due to blood sepsis or VAP (18, 1, 19-21).

The present study describes the genetic and phenotypic characteristics of antibiotic-resistant *A. baumannii* isolates obtained from ICU patients.

Materials and Methods

1. Subjects

This retrospective study included a total of 50 *A. baumannii* isolates that were recovered from sputum specimens from adult ICU patients admitted to King Hussein Medical Center (KHMC) in Amman, Jordan over a period of 18 months.

Approval was obtained from the Institution Ethical Review Board at KHMC/Royal Medical Services, Amman, Jordan.

2. Identification and antibiotic susceptibility tests of *A. baumannii* isolates

At the beginning, suspected pure growth of clinical specimens were identified by BioMerieux *VITEK 2 Automated Microbiology* System (France) using gram-negative ID card that can identify many different targets of gram negative. A total of five colonies were inoculated in brain-heart infusion agar plus 15% glycerol and kept frozen at -70 °C until used for further analysis.

All stored isolates were then sub-cultured on blood and MacConkey agar plates and incubated at 37 °C for 24 h. *A. baumannii* strains were confirmed according to the following characteristics: gram-negative coccobacilli, negative oxidase test, and non-lactose and non-glucose fermentation, positive

citrate and negative indol test. Later, they were confirmed by the presence of *blaOXA*-51 gene on using PCR**.**

A. baumannii strains were tested for susceptibility against 13 different antibiotics using VITEK 2 AST–N233 and AST-XNO5 susceptibility cards according to the manufacturer's instructions and recommendations (bioMerieux, France) and guidelines of the Clinical and Laboratory Standards Institute (CLSI) (2018).

Antimicrobial E-test strips (bioMérieux, France) were used to detect the minimum inhibitory concentration (MIC) values for three antibiotics (Amikacin, Colistin and Imipenem) as per the CLSI guidelines (CLSI, 2018) (22).

In addition, one antimicrobial disk (Mast group LTD, UK) was used to investigate the susceptibility of *Acinetobacter* isolates to amikacin (30 μg/L) by disc diffusion method according to the CLSI guidelines (CLSI, 2018).(22)

DNA and Plasmid extraction of *A. baumannii*

Genomic DNA was extracted during cell lysis, protein precipitation, DNA precipitation and rehydration at room temperature using Wizard Genomic DNA purification kit (Promega, USA). The bacterial plasmid was extracted using the Pure Yield Plasmid minipreps system (USA) according to manufacturer's instructions. The extracted DNA and plasmid are stored at -20 °C for further investigations.

PCR for detection of genes encoding *blaOXA* carbapenemases, metalo-beta-lactamases, and class-1 integron in *A. baumannii*

All primers of investigated genes and related information are listed in Table I (Alpha DNA, Montreal, Canada). The primers were dissolved in nuclease-free water (Promega, USA) to prepare them for investigation. The following control strains were used during PCR steps, which were kindly donated by Prof. Monzer Hamza, Laboratoire Microbiologie Sante et Environnement, Ecole Doctorale des Sciences et de Technologie, Faculte de Sante Publique, Universite Libanaise, Tripoli, Lebanon): *A. baumannii* (OXA-51 positive), *A. baumannii* (OXA-23 positive), *K. pneumonia* (blaKPC positive; ATCC BAA-1705), and *K. pneumonia* (*bla*NDM-1 positive; ATCC BAA-2146). DNA concentrations of each sample was evaluated using Nanodrop 2000c (Thermo scientific, USA).

PCR was carried out in 25 μl reaction volumes with 2.5 μl of extracted DNA, 12.5 pmol of each primer in Table II (Alpha DNA, Montreal, Canada), and 12.5 μl of PCR green Go-taqR master mix (Promega, USA). The final volume was made up to 25 μl using nuclease-free water.

The amplification conditions of PCR were stabilized according to Woodford et al. (2006). The PCR assays for the target genes were performed by using programmable PCR system 9700 machine (Applied biosystem, US). Negative control tubes containing master mix but without template DNA were included in each run. At the end of each run, the tubes were held at 4 °C. The amplified products and the PCR DNA marker were separated via electrophoresis on 2% agarose gels containing 15% Red safe stain for 40-50 min at 120 volts and then visualized using Gel documentation system including UV camera, monitor and printer (UVP, USA).

Table I: Primers of blaOXA - carbapenemases, Metalo-beta-Lactamases ,Class-1 integrons.

Target genes	Primer name	Nucleotide sequence (5′ → 3′)	Product size (bp)	Annealing temperature (∘C)	References
Class D carbapenemases	*bla*OXA-23	F-ATCGGATTGGAGAACCAGA R-ATTTCTGACCGCATTTCCAT	501	58	(23)
Class D carbapenemases	*bla*OXA-24	F- GGTTAGTTGGCCCCCTTAAA R- GTTGAGCGAAAAGGGGATT	246	58	(23)
Class D carbapenemases	*bla*OXA-51	F- TAATGCTTTGATCGGCCTTG R- TGGATTGCACTTCATCTTGG	353	58	(23)
Class D carbapenemases	*bla*OXA-58	F- AAGTATTGGGGCTTGTGCTG R- CCCCTCTGCGCTCTACATAC	599	58	(23)
Class D carbapenemases	OXA-143	F-TGGCACTTTCAGCAGTTCCT	180	58	(23)

Class D carbapenemases	OXA-235	R-TAATCTTGAGGGGGCCAACC F-TTGTTGCCTTTACTTAGTTGC R-CAAAATTTTAAGACGGATCG	700	58	(23)
Class B carbapenemases	NDM-1	F- ATT AGC CGC TGC ATT GAT R- CAT GTC GAG ATA GGA AGT G	154	58	(24)
Class A carbapenemases	KPC	F-ATGTCACTGTA TCGCCGTCT R-TTTTCAGAGCCTTACTGCCC	246	52	25
Integrons	Integron-1	F- ATGTGATGGCGACGCACGA R- ATTTCTGTCCTGGCTGGCGA	600	55	Young, *et al*., 1999 (26)

Statistical analysis

We used mean and standard deviation to analyze continuous variables such as age in years. Other clinical features such as gender were analyzed using frequencies and percentages. Statistical analysis were performed using SPSS software 23 for Mac OS X (SPSS Inc., Chicago, IL, USA). We considered p value to be statistically significant at ≤ 0.05.

Results

Table II shows the demographic characteristics of ICU patients with positive sputum cultures for *A. baumannii* isolates. The majority of patients were adults aged over 50 years, and 24% were positive for blood culture at the same time or later on of sputum culture with negative history of central catheter at the time of positive blood culture. Most of the patients (64%) received medication prior to collection of specimens, and 76% patients were on mechanical ventilation.

Table II Major demographic characteristic of 50 ICUs patients .

Variables	**No. (%) of patients with positive blood culture N=12 (24%)**		**No. (%) of patients with negative blood cultures N= 38 (76%)**		**P-value**	**OR (CI 95%)**
Mean of age (17-87 years)	51.2 ± 22.5		58.1 ± 19.9		0.3151	
Gender						
Male	6	50%	25	66%	0.3259	0.520 (0.140, 1.937)
Female	6	50%	13	34%		
Mean length of stay in ICU						
Less than 7 days	1	8%	7	18%	0.7463	0.409 (0.008, 3.822)
More than 7 days	11	92%	31	82%		
Previous antibiotics treatments						
Yes	10	83%	22	58%	0.1095	3.55 (0.626, 37.75)
No	2	17%	16	42%		
Mechanical ventilation						
Yes	12	100%	27	71%	0.2046	undefined
No	0	0%	11	29%		

Figure 1 shows the results of antimicrobial susceptibility using VITEK 2 AST –N233 and AST-XNO5 susceptibility cards.
A. baumannii isolates were 100% resistant to piperacillin/tazobactam, aztreonam ceftazidime, cefepime and ciprofloxacin. The isolates were 94% resistant to imipenem, 72% resistant to amikacin, and 76% resistant to gentamycin.

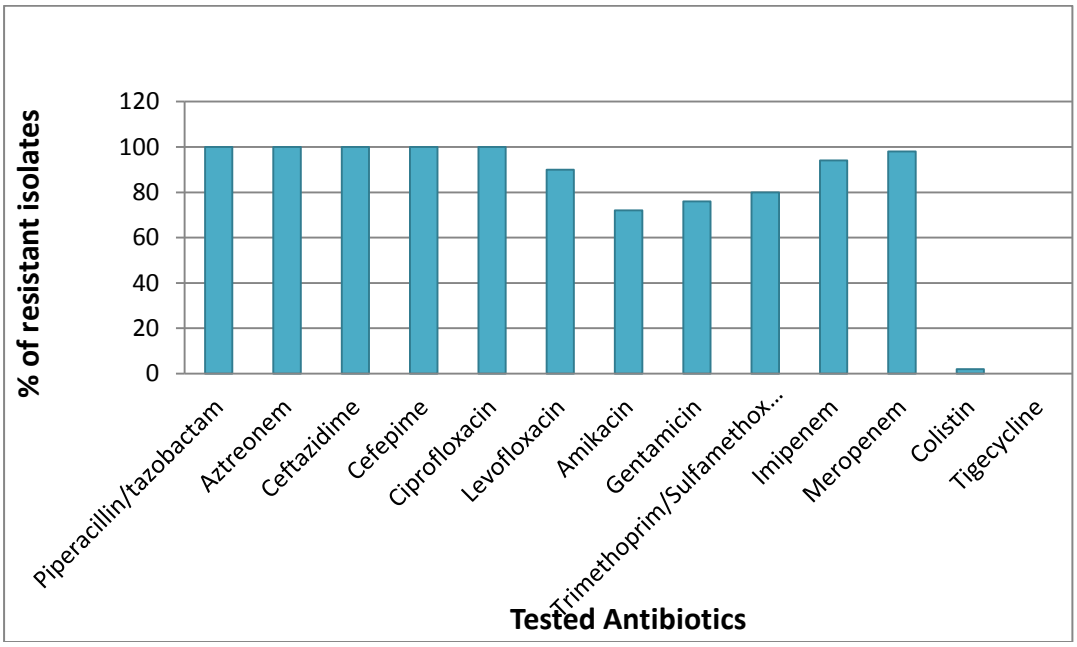

Figure 1: Antimicrobial resistance patterns of 50 *A. baumannii* isolates from ICU patients

Minimal inhibitory concentrations (MICs)

The results of MICs for the three commonly used drugs, i.e., amikacin, colistin and Imipenem, against *A. baumannii* isolates are shown in Table III.
It was found that 2% of isolates were resistant to colistin, whereas 94% and 72% of isolates were resistant to imipenem and amikacin, respectively**.**

Table III: MICs of 50 *A. baumannii* isolates to 3 most used antibiotics in treatment of ICU patients

Antimicrobial	No.(%) resistant isolates	MIC50 (mg/L)	MIC range (mg/L) ON THE STRIP
Colistin	1 (2)	0.37	**0.017-2**
Amikacin	36 (72)	80.6	**1-256**
Imipenem	47 (94)	25.5	**0.38-32**

PCR results

All 50 (100%) *A. baumannii* isolates were positive for *OXA-51*, and 47 (94 %) isolates were positive for *OXA-23* genes as shown in Figure 2.

Other *OXA* genes were found in less percentage (4-12%), while *NDM-1*, *KPC* and *Integron-1* genes were detected in 26%, 22% and 86% isolates, respectively.

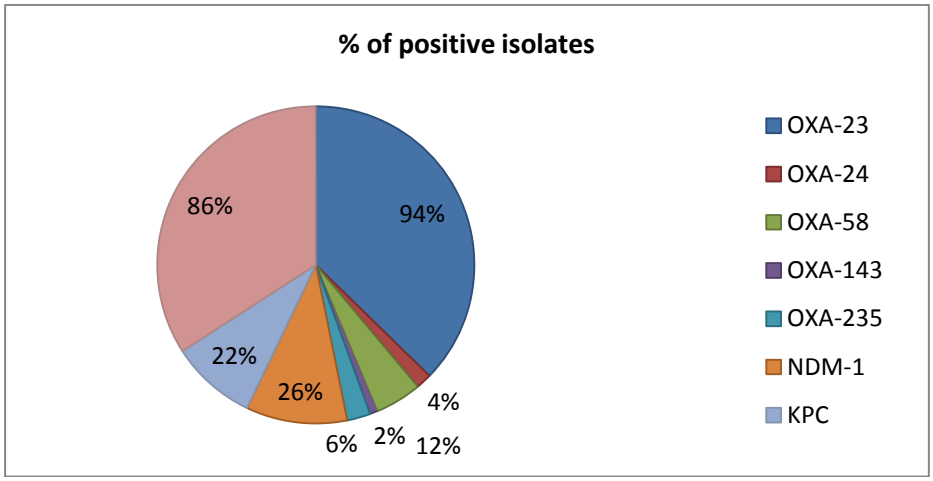

Figure 2: Prevalence of blaOXA-carbapenemases, MBLs and Integron-1 genes among 50 isolates of A.baumannii.

Table (IV): Distribution of blaOXA-carbapenemases, NDM-1, KPC and Integron-1 among ICU patients.

Gene name	No. (%) of patients with positive blood culture N=12 (24%)		No. (%) of patients with negative blood cultures N= 38 (76%)	
OXA-51	12	100%	38	100%
OXA-23	11	92%	36	95%
OXA-24	1	8%	1	3%
OXA-58	2	17%	4	11%
OXA-143	1	8%	0	0%
OXA-235	0	0%	3	8%
NDM-1	5	42%	8	21%
KPC	3	25%	8	21%
Integron-1	11	92%	32	84%

Discussion

This retrospective study was designed to identify the phenotypic characteristics of *A. baumannii* isolates, mainly the antibiogram, to illustrate the resistance pattern and identify the genetic composition of these isolates. The genetic compositions included extended-spectrum β-lactamases: blaOXA carbapenemases, Metallo-β-lactamases and class-1 integrons. Also, the study intended to detect the difference between the genetic contents of *A. baumannii* isolates that are associated with invasive infections represented by positive blood culture.

All of the patients in our study are adults and their ages ranged from 17 to 87 years. Most of these patients were aged more than 50 years. No significant differences were observed in general demographic characteristics due to the small sample size.

Many studies worldwide have reported that *A. baumannii* is the most frequently found species among *Acinetobacter* group causing healthcare-associated infections. This MDR organism is often associated with high mortality (18,27,28,8). Also, *A. baumannii* is frequently found to colonize many body sites of hospitalized patients, including respiratory tracts of those in ICUs. Invasive infections, especially bacteremia with *A. baumannii*, in hospitalized patients are frequently caused in those patients who carry asymptomatically the organism in their respiratory tract (29,30,8). The current study demonstrated that respiratory tracts of ICU patients are frequently colonized with *A. baumannii*, especially in those patients staying in ICU for a long duration and with a previous history of antibiotic admintration. Invasive blood infection was detected among 24% of ICU patients during the study period (Table 2). The rest of the isolates (76%) can be considered colonizers and might be converted into potential pathogens for patients under certain circumstances, especially in those who underwent invasive procedures and critically ill ICU patients (31,32).

Resistance to multiple antibiotics is an important aspect associated with the clinical outcomes of patients infected with *A. baumannii* in ICUs, especially carbapenem resistance that causes difficulties in treating patients using other antibiotics (33,34). In the present study, the majority of *A. baumannii* isolates were resistant to 11 commonly used antimicrobial agents (meropenem, piperacillin/tazobactam, aztreonam and ceftazidime, cefepime, imipenem, ciprofloxacin, levofloxacin, amikacin, and gentamycin, trimethoprim/sulfamethoxazole) in the range of 72% to 100%**.** Also, our isolates carried resistant genes to colistin in a small percentage (2%) and have been considered as the last treatment option for *A. baumannii* infections. The genetic section of this study demonstrates that all 50 MDR *A. baumannii* isolates were 100% positive for intrinsic genes of *blaOXA*-51, while 94% carried *blaOXA*-23 genes. A smaller percentage of isolates carried other less common genes: *blaOXA*-24 (4%), *blaOXA*-58 (12%), *blaOXA*-235 (6%) and *blaOXA*-143 (2%). A recent Jordanian study at King Hussein Cancer Center showed that all examined MDR *A. baumannii* isolates from patients also harbored 100% genes of *blaOXA*-51 and *blaOXA*-23, while they_lacked *blaOXA*-58 or *blaOXA*-24 genes (8). Recent results from a Palestinian study showed that 100% of *A. baumannii* isolates were positive for *blaOXA*-51 and 82.6% were positive for *bla*OXA-23. In comparison, their isolates carried *bla*OXA-24 (14.5%) and *bla*OXA-58 (3%), and none of their *A. baumannii* isolates were positive for *bla*OXA-143 and *bla*OXA-235 (35). In addition, ambler class A carbapenemase (KPC) has been detected during the last few years in *A. baumannii* clinical isolates in many regions of the world (36,37). Our study revealed that 22% of *A. baumannii* isolates carried KPC genes. This finding is in agreement with the findings of a previous Saudi study that had a prevalence rate of 34.5% among their isolates(38).

The present study also found that 86% of MDR *A. baumannii* isolates harbored *integrase* gene (Figure 2). This *integrase* gene is a member of mobile genetic elements, associated with both plasmids and transposons. The gene enhances circulation of antimicrobial resistance genes in *A. baumannii* and other gram-ND studies from Jordan and our region have confirmed that Class 1-integrons are commonly present in clinical and environmental isolates of gram-negative bacteria species such as *A. baumannii* and *E. coli* and are often associated with MDR of these bacteria species, one of these studies at KHCC,Amman, Jodan and the percent of harboring this gene is near our result (8,39-40).

Conclusion

A. baumannii infection is a serious infection in the immunocompromised patients, especially in ICUs. This study also demonstrated that respiratory tract colonization is a source for blood stream infection with a aggressive MDR *A. baumannii.*

In addition, the existence of the integrase gene in more than two third of our *A. baumannii* isolates in association with other resistance genes provided a powerful evidence that these isolates have the potential for gaining more antimicrobial resistance genes in future that may cause difficulty in the treatment of its infections and result in rapid transmission in a hospital setting in clonal pattern. Also, we conclude the *Acinetobacter baumannii* isolates from ICU patients were more aggressive than isolates from any other site of immuncopromised patients as cancer patients;the our isolates contain more resistant genes.

Thus, health care personnel and staff should follow infection control measures and guidelines, such as active surveillance, hand hygiene, and contact precautions, to control the transmission of MDR organisms.

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