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OXA-Carbapenemases Present in Clinical *Acinetobacter baumannii-calcoaceticus* Complex Isolates from Patients in Kurdistan Region, Iraq

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In addition to intrinsic resistance in *Acinetobacter baumannii*, many different types of acquired resistance mechanisms have been reported, including the presence of VIM and IMP metallo β-lactamases and also of $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-58-like}}$ enzymes. In the Kurdistan region of Iraq, the multiresistant *A. baumannii--calcoaceticus* complex is prevalent. We characterized the different mechanisms of resistance present in clinical isolates collected from different wards and different hospitals from the Kurdistan region. One hundred twenty clinical nonduplicate *A. baumannii-calcoaceticus* complex isolates were collected from four hospitals between January 2012 and October 2013. The identification of the isolates was confirmed by MALDI-TOF. The susceptibility to different antibiotics was determined by disk diffusion and analyzed in accordance to EUCAST guidelines. By PCR, the presence of $bla_{\text{OXA-51-like}}$, $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, and $bla_{\text{OXA-58-like}}$ genes was determined as well as the presence of the insertion element IS*Aba1*. Clonal diversity was analyzed by pulsed-field gel electrophoresis (PFGE) using the restriction enzyme *Apa*I and, in addition, multilocus sequence typing (MLST) was performed on a selected subset of 15 isolates. All 120 *A. baumannii* isolates harbored $bla_{\text{OXA-51-like}}$ genes. One hundred one out of 110 (92%) imipenem (IMP)-resistant *A. baumannii-calcoaceticus* complex isolates additionally carried the $bla_{\text{OXA-23-like}}$ gene and four isolates (3%) were positive for $bla_{\text{OXA-24-like}}$. All 101 $bla_{\text{OXA-23-like}}$-positive isolates had the IS*Aba1* insertion sequence, 1,600 bp upstream of the $bla_{\text{OXA-23-like}}$ gene. The $bla_{\text{OXA-58-like}}$ gene was not detected in any of the 110 IMP-resistant strains. Eight different PFGE clusters were identified and distributed over the different hospitals. MLST analysis performed on a subset of 15 representative isolates revealed the presence of the international clone ST2 (Pasteur). Besides ST2 (Pasteur), also many other STs (Pasteur) were encountered such as ST136, ST94, ST623, ST792, and ST793, all carrying the $bla_{\text{OXA-23}}$ gene. In clinical *A. baumannii-calcoaceticus* complex isolates from Kurdistan-Iraq, the $bla_{\text{OXA-23}}$ gene in combination with the upstream ISA*ba*1 insertion element is largely responsible for carbapenem resistance. Several small clusters of identical genotypes were found from patients admitted to the same ward and during overlapping time periods, suggesting transmission within the hospital. Identification of source(s) and limiting the transmission of these strains to patients needs to be prioritized.

Keywords: *A. Baumannii-calcoaceticus* complex, OXA-23, ISAba1, multi-resistance, carbapenemases, clonal relationship

Introduction

ACINETOBACTER BAUMANNII is an important opportunistic nosocomial pathogen.[1,2] Infections caused by *A. baumannii* are difficult to treat because of its intrinsic resistance to various classes of antibiotics and its ability to acquire additional resistance.[3,4] Due to these characteristics, *A. baumannii* is included in the so-called group of ESKAPE organisms.[5]

The carbapenem antibiotics play an important role in the treatment of *A. baumannii*[6] infections. However, *A.*

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baumannii can also become resistant to carbapenems through a number of mechanisms, including the presence of metallo-beta-lactamases (class B) or the presence and overexpression of OXA-type carbapenemases (class D).[7] Especially, $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, $bla_{\text{OXA-58-like}}$ β-lactamases are often demonstrated in addition to the intrinsic presence of $bla_{\text{OXA-51}}$ in carbapenem-resistant *A. baumannii*.[8,9] These additional OXA resistance genes are often accumulated in large resistance islands, located in a region prone to insertion and deletion within the bacterial chromosome.[10] The presence of the $bla_{\text{oxa-51-like}}$ gene does not usually result in increased MICs for carbapenems, this in contrast to, for instance, the $bla_{\text{OXA-23-like}}$ genes. The expression levels of these additional $bla_{\text{OXA-23-like}}$ genes are often regulated by an upstream insertion sequence (IS*Aba1*) resulting in increased MICs for carbapenems.[3,11]

These multidrug- and pandrug-resistant strains of *A. baumannii* are increasingly reported worldwide and pose a serious concern for healthcare,[12,13] In most Western European hospitals, the prevalence of multidrug-resistant (MDR) *A. baumannii* is limited and only found during local outbreaks. However, OXA-23 was recently shown to increase in frequency in Italy, Greece, and Turkey.[14–18] The increasing prevalence of *A. baumannii* is not only described in Europe but also OXA-23 has been reported in the United States as the most prevalent mechanism of resistance in *Acinetobacter baumannii-calcoaceticus* complex isolates.[19] Besides its increasing prevalence, these outbreaks are mainly linked to the worldwide spread of successful clones, such as international clones I and II,[20] CCI (comprising ST1, ST7, ST8, ST19, and ST20), CC2 (comprising ST2, ST45, and ST47, Pasteur typing scheme).[21] However, OXA-23 is also increasingly found in other multilocus sequence types such as ST15 and ST25.[20]

The report from Hujer *et al.* from 2006 suggested that MDR Acinetobacter sp. were present in Iraq, including isolates with OXA-23-like and OXA-58-like, as these were found among patients who were injured in Iraq or Afghanistan and then treated in the United States.[22] Currently, the wounds resulting from burn injuries are frequently colonized with *A. baumannii-calcoaceticus* complex, but also *A. baumannii-calcoaceticus* complex is cultured from other specimens from patients with different underlying diseases. As epidemiological surveillance is not generally practiced in Iraqi hospitals, we collected in the present study 120 MDR *A. baumannii-calcoaceticus* complex isolates deriving from different hospitals in the region of Kurdistan. The aim was to characterize the mechanism of carbapenem resistance and to determine the clonal relatedness among the different carbapenem-resistant *A. baumannii-calcoaceticus* complex isolates.

Materials and Methods

Bacterial isolates and identification

Isolates from clinical specimens, including urine, wounds, blood, sputum, and few other sources, were collected. The isolates were collected from January 2012 to October 2013 in the Rizgari teaching hospital, Rozhawa teaching hospital, and Hawler teaching hospital, all located in the city of Erbil, Iraq (Fig. 1). Additional specimens were collected from the Sulaymaniyah teaching hospital located at Slemani city. The isolates included in the present study were all derived from patients admitted >48 hrs to the hospital. The isolates were initially identified by VITEK 2 (bioMérieux). The primary obtained identification was confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) by making use of the Biotyper system (Bruker Daltonics) and analyzed by software version V4.0.0.1_4613-5627. The patient's social and demographic information, including age, gender, and hospital status, was recorded.

Antimicrobial susceptibility testing

Antimicrobial susceptibility was determined for imipenem, meropenem, ceftazidime, ciprofloxacin, and tobramycin by disk diffusion and was performed according to the EUCAST guidelines.[23]

DNA preparation from bacteria

Genomic DNA of all organisms was extracted by suspending colonies from a blood agar plate into DNA extraction reagent of the InstaGene™ matrix commercial reagent kit. The DNA extraction procedure was subsequently performed in accordance with the guidelines of the InstaGene Matrix (Biorad). The extracted DNA was kept at −20°C until amplification.

PCR amplification

The extracted DNA of the isolates was subjected to PCR to detect $bla_{\text{OXA-51-like}}$, $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, and $bla_{\text{OXA-58-like}}$ and IS*Aba1*. The upstream location of the IS*Aba*1 insertion element of the different bla_{OXA} genes was demonstrated by using the IS*Aba1* forward primer and the $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, and $bla_{\text{OXA-51-like}}$ reverse primers, respectively.[24]

The reaction mixtures (50 µl volumes) contained 75 mM Tris-HCl (pH 8.8 at 25°C), 20 mM $(NH_4)_2SO_4$, 0.01% (v/v) Tween 20, 100 nM dNTPs, 2.5 µM $MgCl_2$, 500 nM of each primer, 0.4 U Taq DNA polymerase (Thermo Scientific), and 5 µl DNA template. The PCR conditions (thermocycler; Biometra) were initial denaturation at 94°C for 5 min, followed by 30 cycles of 25 sec at 94°C, 40 sec at 57°C, 50 sec at 72°C, and a final extension cycle of 5 min at 72°C.[8,25] Positive controls of $bla_{\text{oxa-23-like}}$, $bla_{\text{oxa-24-like}}$, and $bla_{\text{OXA-58-like}}$ and a negative control were included in each PCR run.

Pulsed-field gel electrophoresis

Genetic diversity determinations of the *A. baumannii-calcoaceticus* complex isolates were carried out by using pulsed-field gel electrophoresis (PFGE). DNA fragments obtained after restriction enzyme digestion were separated by making use of the CHEF Mapper DRII apparatus (Bio-Rad Laboratories). Colonies picked from blood agar plates were embedded in 0.5% low melting point InCert agarose (FMC Bioproducts) buffered in 5 mM Tris-HCl pH 8.0, 50 mM Na_2EDTA, and 5 mM EGTA. The blocks were deproteinated by overnight incubation in the same buffer containing 1% sodium dodecyl sulfate and 1 mg/mL of proteinase K (Sigma Chemicals) at 37°C. After extensive washing, blocks were stored at 4°C. Approximately 3×5 mm portions of the blocks were incubated in the presence of 30 units of the restriction endonuclease *Apa*I (Boehringer Mannheim) in an appropriate buffer. Incubation at 37°C continued for 18 hrs,

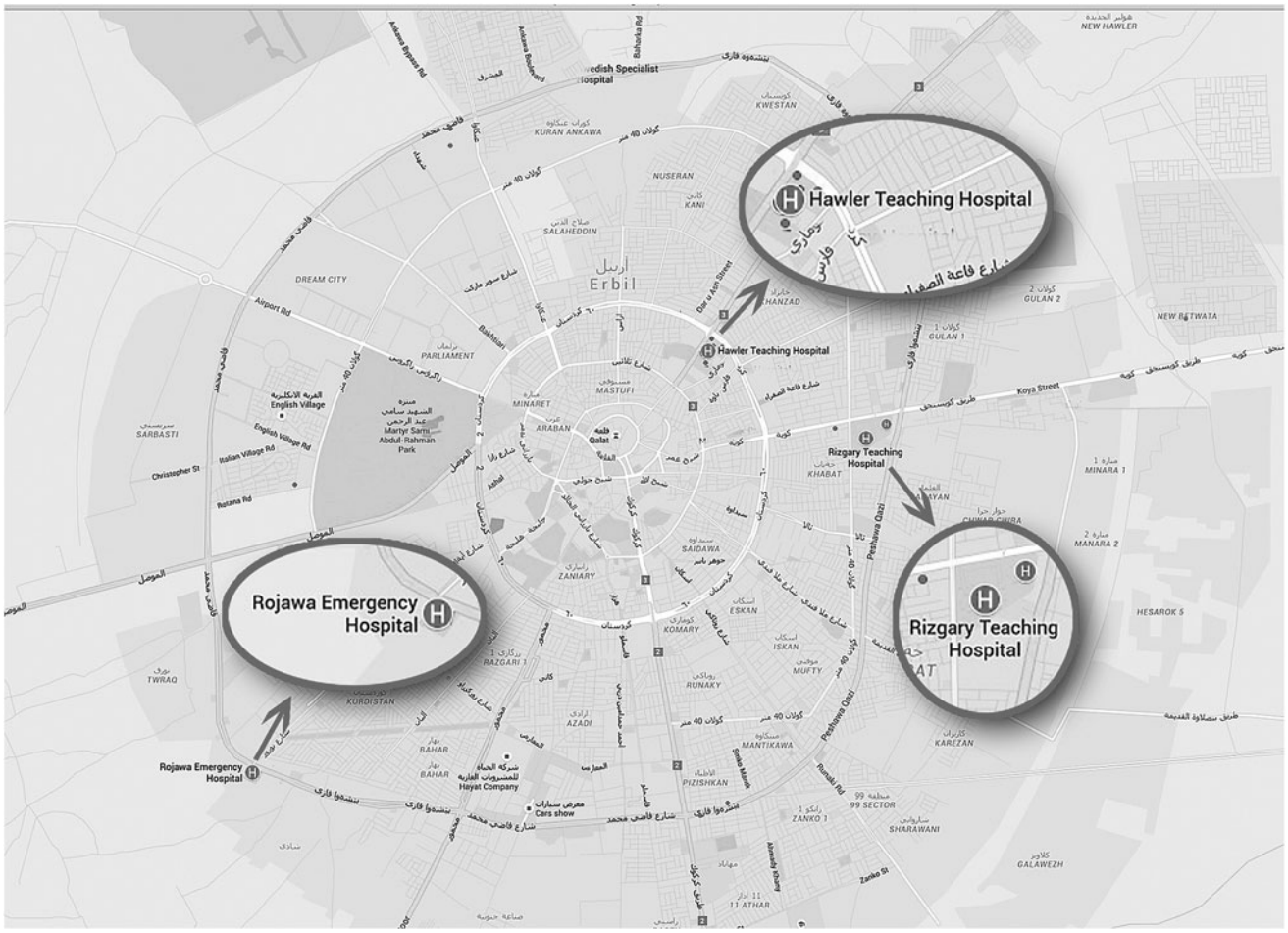

FIG. 1. Map of the city of Erbil with the respective hospitals, as indicated.

after which the blocks were incorporated in 1% Seakem GTG agarose slabgels (FMC BioProducts). The restriction fragments were separated at a field strength of 6 V/cm for 22 hrs at 14°C. The pulse time linearly increased from 5 to 35 sec during electrophoresis.[26] Concatemers of lambda DNA were used as molecular size markers (Bio-Rad). Gels were stained with ethidium bromide postelectrophoresis and photographed[27] (Mitsubishi Copy Processor, Progress Control, Waalwijk, The Netherlands).The PFGE banding patterns were subsequently analyzed by making use of BioNumerics software (version 7.1; Applied Maths). A dendrogram was produced using the Dice coefficient and an unweighted-pair group method using arithmetic averages (UPGMA). Band tolerance was set at 1.0%. Cluster designation was based on isolates showing approximately 85% or greater relatedness.

Whole genome sequencing and assembly

Genomic DNA of 15 isolates was extracted (DNeasy Blood and Tissue Kit; Qiagen) and fragment libraries constructed using the Nextera Kit (Illumina) followed by 251-bp paired-end sequencing (MiSeq; Illumina) according to the manufacturer's instructions.

The paired-end Illumina data were assembled using a pipeline based on SPAdes 3.5.0. (http://bioinf.spbau.ru/en/spades) with settings for Illumina 250 bp reads.

Multilocus sequence typing

Multilocus sequence typing (MLST) was performed from the whole genome sequences of a subset of 15 isolates using the MLST database (obtained on 2015-June-17), (https://github.com/tseemann/mlst). Two different MLST schemes were used: Oxford's MLST and Pasteur's MLST (http://pubmlst.org/abaumannii).

A minimum of one isolate per PFGE cluster was selected for MLST analysis. In case of more than 10 isolates per PFGE cluster, a second or even a third isolate from the same PFGE cluster was included for MLST analysis.

Resistance gene finding

In silico resistance gene finding was done on the assembled genomic DNA using ResFinder-2.1 (https://cge.cbs.dtu.dk//services/ResFinder/) with 98% identity threshold and default for the remaining settings. ResFinder detects the presence of resistance genes, but the functional integrity and expression levels of resistance genes are not provided.

Results

Clinical bacterial strains

In the present study, 120 nonduplicate *A. baumannii-calcoaceticus* complex isolates were collected from four

hospitals in the Kurdistan region-Iraq: 34 isolates from Rizgari teaching hospital, 43 from Rozhawa teaching hospital, 8 from Hawler teaching hospital, and 35 from Sulaymaniyah teaching hospital. *A. baumannii-calcoaceticus* complex isolates were cultured from various clinical specimens, including urine $n=73$ (61%), burn wounds $n=33$ (27%), skin and soft tissue $n=8$ (7%), blood $n=2$ (2%), sputum $n=2$ (2%), and other specimens $n=2$ (2%). *A. baumannii-calcoaceticus* complex isolates were recovered from 37 men (31%) and 83 women (69%). The median age of the patients was 31 years and ranged from 2 to 78 years, see Table 1.

Antimicrobial resistance patterns

Of the 120 prospectively collected *A. baumannii-calcoaceticus* complex isolates, susceptibility determinations were performed by disk diffusion. One hundred ten isolates were determined as carbapenem resistant. In addition to the high prevalence of resistance to carbapenem antibiotics, most of the isolates also showed resistance to other antibiotics such as tobramycin (95%) and ciprofloxacin (89%).

Characterization mechanism(s) of carbapenem resistance

As the resistance to carbapenems in *A. baumannii* is often due to the presence of additional bla_{OXA} genes, besides the already present $bla_{\text{OXA-51-like}}$, the isolates were first screened by PCR for the most prevalent bla_{OXA} genes, that is, $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-24-like}}$, and $bla_{\text{OXA-58-like}}$. The majority ($n=101$) of the carbapenem-resistant isolates ($n=110$) harbored the $bla_{\text{OXA-23-like}}$ gene (84.1%). Of the remaining 9 carbapenem-resistant isolates that were $bla_{\text{OXA-23-like}}$ negative, four were positive for $bla_{\text{OXA-24-like}}$. $Bla_{\text{OXA-58-like}}$ could not be demonstrated in any of the isolates.

As the presence of an insertion sequence upstream of the bla_{OXA} gene can affect the expression of the additional bla_{OXA} gene, a PCR was performed for IS*Aba1*. The IS*Aba1* was detected in 116 (96.6%) isolates. All $bla_{\text{OXA-23-like}}$-positive isolates ($n=101$) showed a fragment of 1.6 kb using the IS*Aba1* forward and $bla_{\text{OXA 23-like}}$ reverse primer. Three of the four $bla_{\text{OXA-24-like}}$-positive isolates were also positive for IS*Aba1*, but we did not succeed in demonstrating its upstream localization of the $bla_{\text{OXA-24-like}}$ gene. The $bla_{\text{OXA-24-like}}$ isolate missing the insertion element had MICs for imipenem and meropenem of 8 and 16 μg/ml, respectively. The three isolates containing both the $bla_{\text{OXA-24-like}}$ and the IS*Aba1* had MICs of ≥32 μg/ml for both imipenem and meropenem.

For the five remaining isolates, negative for $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-24-like}}$, a PCR with the IS*Aba1* forward and $bla_{\text{OXA-51-like}}$ reverse primer was conducted and all five showed the presence of the IS*Aba1* 1,100 bp upstream of the $bla_{\text{OXA-51-like}}$ gene. The MICs for imipenem and meropenem were, respectively, 4–8 μg/ml for imipenem and 2–8 μg/ml for meropenem. The IS*Aba1* insertion element was also demonstrated in 7 of the 10 susceptible isolates.

PFGE analysis

As the majority of the isolates demonstrated the presence of the $bla_{\text{OXA-23-like}}$ gene in combination with the ISA*ba1* insertion element, a high similarity was expected between the different isolates. In the PFGE analysis (Fig. 2), clonal relatedness was based on a cutoff value of 85%. The number of isolates per cluster ranged from 3 to15. The rest, $n=39$, of the isolates were unique isolates. The isolates were distributed over eight clusters, represented by A to H. In Table 2, the epidemiological relatedness of patients (location and time) from whom isolates belonged to one of these eight PFGE clusters is shown per cluster. When combining the data from PFGE with the space-time information, cross-transmission has likely occurred in three hospitals (Rozhawa, Hawler, and Sulaymaniyah). In the Rizgari hospital, six isolates belonged to the PFGE cluster E and were from the Urology Department, but the correlation in time could not be made. The three largest clusters with likely cross-transmission were detected in a 3-month period in the Urology ward of the Sulaymaniyah hospital, with PFGE cluster D (15 isolates), cluster B (7 isolates), and cluster G (7 isolates) involved. In the Burn unit of the Rozhawa hospital, we could detect five smaller clusters with likely cross-transmission, with PFGE cluster C (3 isolates), cluster A (two events with two isolates), cluster B (two isolates), and cluster H (two isolates) involved. In cluster F, two isolates from the RCU were isolated at the same time, indicating possible transmission in the Hawler hospital. Interestingly, all clusters except cluster E harbored isolates from more than one hospital.

MLST

To be able to compare the typing data to other worldwide clones, we have performed MLST analysis on a subset of isolates ($n=15$). Using the Pasteur scheme, the 15 isolates belonged to six STs (ST2, ST94, ST136, ST623, ST792, and ST793). According to the Oxford MLST typing scheme, also six different ST types were determined (ST195, ST387, ST441, ST460, ST1092, and ST1093).

The two representative isolates belonging to PFGE cluster E and F belong to ST2 and in fact represent the European clone II and worldwide lineage. By using the Oxford MLST typing scheme, the two representative isolates of cluster A were divided into two ST clones 1092 and 460. The two isolates with ST1092 were collected at the same time from two patients admitted to the Burn unit of the Rozhawa hospital (isolate number 24 and 25; Table 2). Identical ST-136 (Pasteur) or ST 1092 (Oxford) type were also demonstrated in the Sulaymaniyah hospital. The same goes for ST-793 (Pasteur), which was demonstrated in isolates not only from Rozhawa but also from Sulaymaniyah hospital. Also, identical STs, that is, 623 (Pasteur) were found in the Rizgari and Sulaymaniyah hospital (Table 3).

Additional mechanisms of resistance

As we obtained the MLST results by whole genome sequencing, we were also able to detect additional mechanisms of resistance present in the selected 15 isolates (Table 3). Besides the $bla_{\text{OXA-23}}$ gene, already detected by PCR, additional variants of the $bla_{\text{OXA-51-like}}$ gene such as $bla_{\text{OXA-91}}$, $bla_{\text{OXA-66}}$, and $bla_{\text{OXA-69}}$, a variant of the chromosomal encoded *AmpC*-gene, that is, $bla_{\text{ADC-25-like}}$, and several aminoglycoside-modifying enzymes, as well as sulfamethoxazole and tetracycline resistance genes, were detected.

TABLE 1. CHARACTERISTICS OF THE 120 *ACINETOBACTER BAUMANNII-CALCOACETICUS* COMPLEX ISOLATES

Number	*Hospitals*	*City*	*Specimen date*	*Gender*	*Age*	*Dept.*
1B	Rozhawa	Erbil	17-10-2012	Female	25 y	Burn unit
2	Rozhawa	Erbil	23-10-2012	Female	15 y	Burn unit
3A	Rozhawa	Erbil	18-12-2012	Female	14 y	ICU
4A	Rozhawa	Erbil	27-11-2012	Female	14 y	Burn unit
4B	Rozhawa	Erbil	27-11-2012	Female	24 y	Burn unit
6A	Rozhawa	Erbil	30-11-2012	Female	69 y	Burn unit
9A	Hawler	Erbil	25-11-2012	Male	17 y	Emergency
13A	Rozhawa	Erbil	22-11-2012	Female	28 y	Burn unit
15	Rozhawa	Erbil	1-8-2013	Male	18 y	Burn unit
16	Rozhawa	Erbil	1-8-2013	Female	5 y	Burn unit
17	Rozhawa	Erbil	1-11-2013	Female	20 y	Burn unit
20A	Rozhawa	Erbil	28-1-2013	Female	32 y	Burn unit
21	Rizgari	Erbil	15-9-2012	Female	48 y	Surgery
22	Rizgari	Erbil	11-12-2012	Female	20 y	Surgery
23	Rizgari	Erbil	26-2-2013	Female	31 y	Internal medicine
24	Rozhawa	Erbil	17-7-2012	Female	31 y	Burn unit
25	Rozhawa	Erbil	19-7-2012	Female	38 y	Burn unit
26	Hawler	Erbil	17-7-2012	Female	23 y	Burn unit
27	Rozhawa	Erbil	7-1-2012	Female	22 y	Surgery
31	Rozhawa	Erbil	22-7-2012	Female	24 y	Burn unit
32	Rozhawa	Erbil	8-12-2012	Female	25 y	Burn unit
33	Rozhawa	Erbil	8-12-2012	Female	16 y	ICU
35A	Rozhawa	Erbil	8-2-2012	Male	16 y	Neurology ICU
36A	Rozhawa	Erbil	8-2-2012	Male	19 y	Neurology ICU
38B	Rozhawa	Erbil	18-8-2012	Female	19 y	Burn unit
39	Rozhawa	Erbil	18-8-2012	Female	58 y	Burn unit
41	Rozhawa	Erbil	9-2-2012	Male	44 y	Burn unit
43	Rozhawa	Erbil	9-9-2012	Male	25 y	Internal medicine
46	Rozhawa	Erbil	28-9-2012	Male	31 y	?
48	Hawler	Erbil	9-9-2012	Female	15 y	Orthopedics
50	Rozhawa	Erbil	30-9-2012	Female	56 y	Burn unit
20PS	Rizgari	Erbil	30-1-2013	Male	77 y	Surgery
25PS	Rizgari	Erbil	2-3-2013	Female	17 y	RCU
33PS	Rozhawa	Erbil	2-3-2012	Female	8 y	ICU
41PS	Rozhawa	Erbil	31-1-2012	Male	2 y	ICU
44PS	Rozhawa	Erbil	2-11-2012	Male	11 y	Burn unit
57PS	Rozhawa	Erbil	3-9-2012	Male	22 y	Burn unit
63PS	Rozhawa	Erbil	3-10-2012	Female	42 y	Burn unit
79PS	Hawler	Erbil	21-4-2012	Male	16 y	Surgery
97PS	Hawler	Erbil	14-5-2012	Female	15 y	Ear Nose and Throat
100E-PS	Rozhawa	Erbil	5-12-2012	Female	16 y	Burn unit
101PS	Rozhawa	Erbil	16-5-2012	Female	78 y	Burn Unit
113PS	Hawler	Erbil	18-6-2012	Male	78 y	RCU
114PS	Hawler	Erbil	16-6-2012	Male	41 y	RCU
134PS	Rozhawa	Erbil	7-5-2012	Male	38 y	
140PS	Rozhawa	Erbil	29-7-2012	Female	33 y	Burn unit
148PS	Hawler	Erbil	23-8-2012	Male	48 y	
150PS	Rizgari	Erbil	?	Male	59 y	Urology
157PS	Rizgari	Erbil	18-8-2012	Male	20 y	Surgery
186PS	Rozhawa	Erbil	12-12-2012	Female	34 y	Burn unit
B1	Rozhawa	Erbil	9-1-2013	Female	16 y	Burn unit
B2	Rozhawa	Erbil	9-11-2013	Female	62 y	Burn unit
B3	Rizgari	Erbil	9-11-2013	Male	17 y	RCU
B4	Rozhawa	Erbil	9-11-2013	Female	36 y	Burn unit
B5-1	Rozhawa	Erbil	9-11-2013	Female	36 y	Burn unit
B5-2	Rozhawa	Erbil	9-11-2013	Female	11 y	Burn unit
B6	Rozhawa	Erbil	9-11-2013	Male	26 y	Burn unit
B7	Rozhawa	Erbil	10-2-2013	Female	25 y	Burn unit
B8	Rozhawa	Erbil	10-2-2013	Female	59 y	Burn unit
B9	Rizgari	Erbil	9-12-2013	Female	15 y	?
DL-1	Sulemani	Sulaimania	April 1st-June 30th 2012	Female	33 y	Urology
DL-2	Sulemani	Sulaimania	April 1st-June 30th 2012	Female	18 y	Urology

(*continued*)

Table 1. (Continued)

Number	*Hospitals*	*City*	*Specimen date*	*Gender*	*Age*	*Dept.*
DL-3	Sulemani	Sulaimania	April 1st-June 30th 2012	Female	56 y	Urology
DL-4	Sulemani	Sulaimania	April 1st-June 30th 2012	Female	51 y	Urology
DL-5	Sulemani	Sulaimania	April 1st-June 30th 2012	Female	24 y	Urology
DL-6	Sulemani	Sulaimania	April 1st-June 30th 2012	Female	17 y	Urology
DL-7	Sulemani	Sulaimania	April 1st-June 30th 2012	Female	16 y	Urology
DL-8	Sulemani	Sulaimania	April 1st-June 30th 2012	Female	69 y	Urology
DL-10	Sulemani	Sulaimania	April 1st-June 30th 2012	Female	48 y	Urology
DL-11	Sulemani	Sulaimania	April 1st-June 30th 2012	Female	12 y	Urology
DL-12	Sulemani	Sulaimania	April 1st-June 30th 2012	Female	33 y	Urology
DL-14	Sulemani	Sulaimania	April 1st-June 30th 2012	Female	26 y	Urology
DL-17	Sulemani	Sulaimania	April 1st-June 30th 2012	Female	59 y	Urology
DL-18	Sulemani	Sulaimania	April 1st-June 30th 2012	Female	42 y	Urology
DL-19	Sulemani	Sulaimania	April 1st-June 30th 2012	Female	32 y	Urology
DL-20	Sulemani	Sulaimania	April 1st-June 30th 2012	Female	14 y	Urology
DL-21	Sulemani	Sulaimania	April 1st-June 30th 2012	Female	29 y	Urology
DL-22	Sulemani	Sulaimania	April 1st-June 30th 2012	Female	11 y	Urology
DL-23	Sulemani	Sulaimania	April 1st-June 30th 2012	Female	44 y	Urology
DL-24	Sulemani	Sulaimania	April 1st-June 30th 2012	Female	20 y	Urology
DL-25	Sulemani	Sulaimania	April 1st-June 30th 2012	Female	51 y	Urology
DL-26	Sulemani	Sulaimania	April 1st-June 30th 2012	Female	24 y	Urology
DL-30	Sulemani	Sulaimania	April 1st-June 30th 2012	Female	15 y	Urology
DL-31	Sulemani	Sulaimania	April 1st-June 30th 2012	Female	25 y	Urology
DL-32	Sulemani	Sulaimania	April 1st-June 30th 2012	Female	12 y	Urology
DL-33	Sulemani	Sulaimania	April 1st-June 30th 2012	Female	63 y	Urology
DL-36	Sulemani	Sulaimania	April 1st-June 30th 2012	Female	23 y	Urology
DL-37	Sulemani	Sulaimania	April 1st-June 30th 2012	Female	11 y	Urology
DL-38	Sulemani	Sulaimania	April 1st-June 30th 2012	Female	27 y	Urology
DL-39	Sulemani	Sulaimania	April 1st-June 30th 2012	Female	24 y	Urology
DL-40	Sulemani	Sulaimania	April 1st-June 30th 2012	Female	18 y	Urology
DL-41	Sulemani	Sulaimania	April 1st-June 30th 2012	Female	32 y	Urology
DL-42	Sulemani	Sulaimania	April 1st-June 30th 2012	Female	47 y	Urology
DL-43	Sulemani	Sulaimania	April 1st-June 30th 2012	Female	55 y	Urology
DL-134	Sulemani	Sulaimania	April 1st-June 30th 2012	Female	36 y	Urology
AB-1	Rizgari	Erbil	January 1st-April 30th 2013	Female	13 y	Urology
AB-2	Rizgari	Erbil	January 1st-April 30th 2013	Female	42 y	Urology
AB-3	Rizgari	Erbil	January 1st-April 30th 2013	Male	16 y	Urology
AB-4	Rizgari	Erbil	January 1st-April 30th 2013	Male	21 y	Urology
AB-5	Rizgari	Erbil	January 1st-April 30th 2013	Female	18 y	Urology
AB-6	Rizgari	Erbil	January 1st-April 30th 2013	Female	22 y	Urology
AB-7A	Rizgari	Erbil	January 1st-April 30th 2013	Female	32 y	Urology
AB-7B	Rizgari	Erbil	January 1st-April 30th 2013	Female	19 y	Urology
AB-8	Rizgari	Erbil	January 1st-April 30th 2013	Male	11 y	Urology
AB-10	Rizgari	Erbil	January 1st-April 30th 2013	Female	52 y	Urology
AB-11	Rizgari	Erbil	January 1st-April 30th 2013	Female	44 y	Urology
AB-12B	Rizgari	Erbil	January 1st-April 30th 2013	Male	10 y	Urology
AB-13	Rizgari	Erbil	January 1st-April 30th 2013	Female	22 y	Urology
AB-14B	Rizgari	Erbil	January 1st-April 30th 2013	Female	35 y	Urology
AB-15	Rizgari	Erbil	January 1st-April 30th 2013	Female	21 y	Urology
AB-16	Rizgari	Erbil	January 1st-April 30th 2013	Female	63 y	Urology
AB-17	Rizgari	Erbil	January 1st-April 30th 2013	Female	39 y	Urology
AB-18	Rizgari	Erbil	January 1st-April 30th 2013	Female	32 y	Urology
AB-19	Rizgari	Erbil	January 1st-April 30th 2013	Female	16 y	Urology
AB-20	Rizgari	Erbil	January 1st-April 30th 2013	Female	23 y	Urology
AB-21	Rizgari	Erbil	January 1st-April 30th 2013	Female	51 y	Urology
AB-22	Rizgari	Erbil	January 1st-April 30th 2013	Male	37 y	Urology
AB-23	Rizgari	Erbil	January 1st-April 30th 2013	Male	9 y	Urology
AB-24	Rizgari	Erbil	January 1st-April 30th 2013	Female	28 y	Urology
AB-26	Rizgari	Erbil	January 1st-April 30th 2013	Female	11 y	Urology

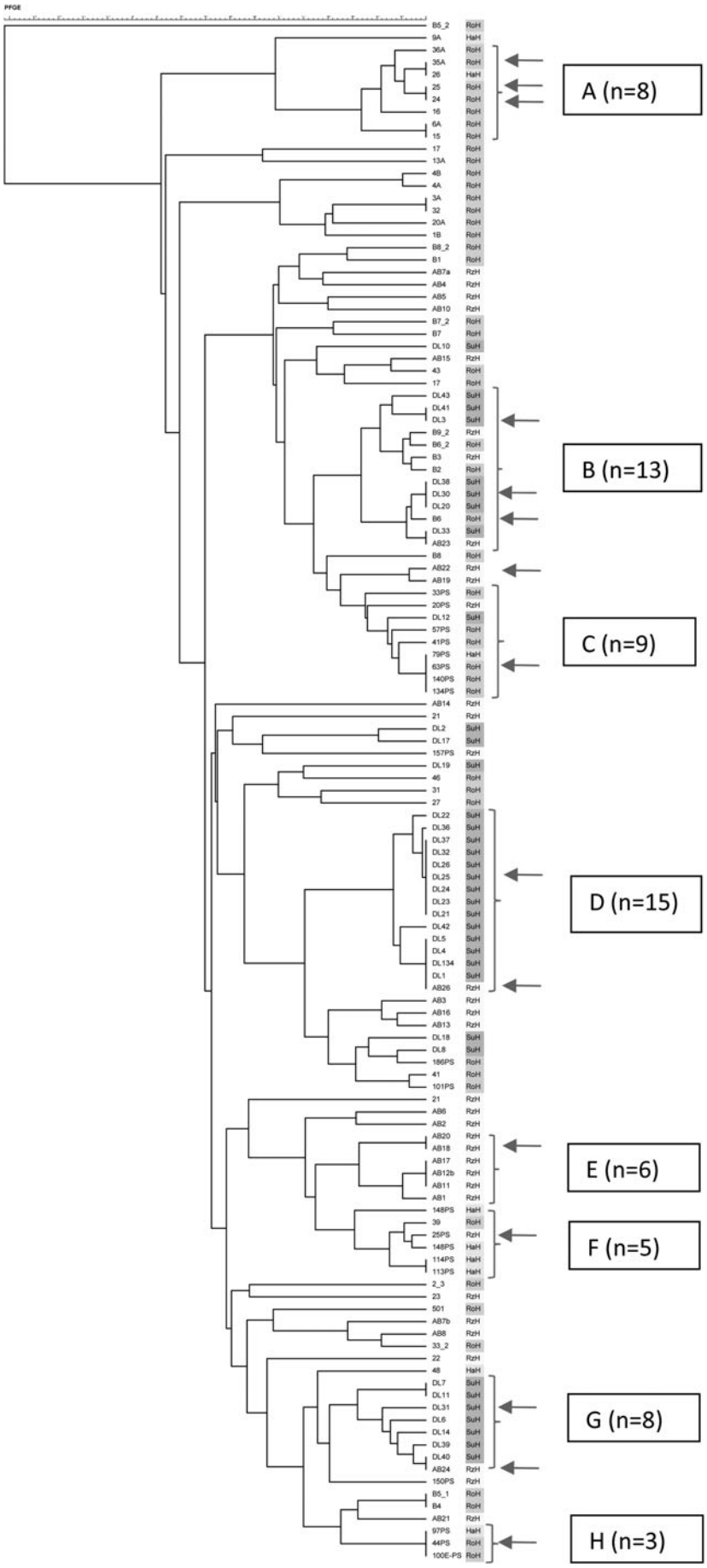

FIG. 2. PFGE dendrogram of *Apa*I digested genomic DNA from 120 *Acinetobacter baumannii-calcoaceticus* complex isolates. Cluster analysis of 120 *Acinetobacter baumannii-calcoaceticus* complex isolates by using the Dice coefficient and an unweighted-pair group method using arithmetic averages (UPGMA). All isolates depicted in the figure are unique isolates collected from patients admitted >48 hrs to one of the four teaching hospitals, Rizgari, Hawler, Rozhawa and Sulemani. The number of isolates per cluster are indicated in the *boxes*. The 15 isolates used for additional MLST typing are indicated by *arrows*. MLST, multilocus sequence typing; PFGE, pulsed-field gel electrophoresis.

Table 2. Analysis of the Epidemiological Relatedness of Patients (Location and Time) from Whom Isolates Belonged to One of the Eight PFGE Clusters, Shown per Cluster

PFGE cluster (no. of isolates)	*Hospital (no. of isolates)*	*Ward (no. of isolates)*	*No. of isolates recovered <3 months[a] (isolate numbers)*	*Month(s) with likely cross-transmission*
A (8)	RoH (7)	Burn unit (5)	2 (**24**[b], **25**)	July 2012
			2 (15, 16)	August 2013
		Neurology ICU (2)	2 (**35A**, 36A)	February 2012
	HaH (1)	Burn unit (1)	n.a.	
B (13)	SuH (7)	Urology (7)	7 (**DL-3**, DL-20, **DL-30**, DL-33, DL-38, DL-41, DL-43)	April–June 2012
	RzH (3)	RCU (1)	n.a.	
		Urology (1)	n.a.	
		Unknown (1)	n.a.	
	RoH (2)	Burn unit (2)	2 (B2, **B6**)	November 2013
C (9)	RoH (6)	Burn unit (3)	3 (57PS, **63PS**, 140PS)	July–October 2012
		ICU (2)	2 (33PS, 41PS)	January–March 2012
		Unknown (1)	n.a.	
	RzH (1)	Surgery (1)	n.a.	
	HaH (1)	Surgery (1)	n.a.	
	SuH (1)	Urology (1)	n.a.	
D (15)	SuH (14)	Urology (14)	14 (DL-1, DL-4, DL-5, DL-21, DL-22, DL-23, DL-24, **DL-25**, DL-26, DL-32, DL-36, DL-37, DL-42, DL-43)	April–June 2012
	RzH (1)	Urology (1)	n.a.	
E (6)	RzH (6)	Urology (6)	Unknown	
F (5)	HaH (3)	RCU (2)	2 (113PS, 114PS)	June 2012
		Unknown (1)	n.a.	
	RoH (1)	ICU (1)	n.a.	
	RzH (1)	RCU (1)	n.a.	
G (8)	SuH (7)	Urology (7)	7 (DL-6, DL-7, DL-11, DL-14, **DL-31**, DL-39, DL-40)	April–June 2012
	RzH (1)	Urology	n.a.	
H (3)	RoH (2)	Burn unit (2)	2 (**44PS**, 100E-PS)	November–December 2012
	HaH (1)	ENT (1)	n.a.	

[a]A period of 3 months is taken in accordance to the definitions of epidemiological relatedness as described by Voor in 't holt *et al.*[39]
[b]Isolate numbers in bold indicate that isolates were subjected to MLST analysis.
ENT, Ear, Nose, and Throat; HaH, Hawler teaching hospital; MLST, multilocus sequence typing; n.a., not applicable; no., number; RoH, Rozhawa teaching hospital; RzH, Rizgari teaching hospital; SuH, Sulaymaniyah teaching hospital; PFGE, pulsed-field gel electrophoresis.

Discussion

In this study, we collected *A. baumannii-calcoaceticus* complex isolates from four hospitals in Kurdistan region-Iraq and investigated the molecular mechanism of carbapenem resistance and the clonal relatedness. Although Hujer *et al.*[22] already reported in 2006 that various resistance mechanisms were present in Acinetobacter sp. isolates from military and civilian patients injured in Iraq and Afghanistan, and treated in a facility in Washington D.C., our report is the first on-site multicenter survey in this specific region of Iraq.

From a total of 120 isolates of *A. baumannii-calcoaceticus* complex, 110 (92%) were resistant to the last resort antibiotics imipenem and meropenem. These data are in accordance with reports from other countries in the Middle East, such as the report by Sharif *et al.*[28] that demonstrated resistance rates of 86% and 82% for imipenem and meropenem of *A. baumannii* isolates in Iran, respectively.[28] Similar observations have been made in other countries describing the dissemination of carbapenem-resistant *A. baumannii* isolates.[27,29]

Many mechanisms of resistance for imipenem in *A. baumannii* have been described as the overproduction of acquired bla_{OXA} genes such as $bla_{OXA\text{-}23\text{-}like}$, $bla_{OXA\text{-}24\text{-}like}$, $bla_{OXA\text{-}58\text{-}like}$[15] or the presence of VIM and NDM genes.[30]

The majority of the isolates in the present study carried a $bla_{OXA\text{-}23\text{-}like}$ gene. No isolate contained a $bla_{OXA\text{-}58\text{-}like}$ gene; only in four isolates the presence of $bla_{OXA\text{-}24\text{-}like}$ was demonstrated. These results are comparable with the results of Mendes *et al.* who demonstrated that $bla_{OXA\text{-}24/40}$ and $bla_{OXA\text{-}58}$ were less common in isolates recovered from six countries.[31]

In our study, 96.6% of *A. baumannii-calcoaceticus* complex isolates also carried the IS*Aba1* insertion element. In the $bla_{OXA\text{-}23\text{-}like}$-positive isolates, the IS*Aba1* was shown to be located 1,600 bp upstream of the $bla_{OXA23\text{-}like}$ gene located and thereby enhancing the upregulation of the $bla_{OXA\text{-}23\text{-}like}$ gene. In the 5 isolates negative for $bla_{OXA\text{-}23\text{-}like}$, we were able to demonstrate the localization of the IS*Aba1* element upstream of the $bla_{OXA\text{-}51\text{-}like}$ gene, indicating that the resistance for imipenem and meropenem could be due to the overproduction of $bla_{OXA\text{-}51\text{-}like}$. However, as we did not

Table 3. Isolate Characteristics of 15 Selected Multiresistant *A. baumannii-calcoaceticus* Complex Isolates

Isolate	*PFGE*	*Specimen*	*Date*	*MLST Pasteur*	*MLST Oxford*	*Hospital*	*Ward*	*Beta-lactamase gene*	*Genes encoding nonbeta-lactam resistance*
24	A	Wound	2012	136	1092	Rozhawa	Burn unit	*blaOXA-23, blaOXA-91-like, blaADC-25-like*	*aph(3')-VIa, strA, strB, sul2, tet*(39)
25	A	Wound	2012	136	1092	Rozhawa	Burn unit	*blaOXA-23, blaOXA-91-like, blaADC-25-like*	*aph(3')-VIa, strA, strB, sul2, tet*(39)
35A	A	Other	2012	136	460	Rozhawa	Neurology ICU	*blaOXA-23, blaOXA-91-like, blaADC-25-like*	*aph(3')-VIa, strA, strB, sul2, tet*(39)
B6	B	Wound	2013	793	1093	Rozhawa	Burn unit	*blaOXA-23, blaOXA-69, blaADC-25-like*	*sul2*
DL-30	B	Urine	2012	793	441	Sulemani	Urology	*blaOXA-23, blaOXA-69, blaADC-25-like*	*aph(3')-VIa, strA, strB, sul2*
DL-3	B	Urine	2012	793	441	Sulemani	Urology	*blaOXA-23, blaOXA-69, blaADC-25-like*	*aadB, aph(3')-VIa, strA, strB, sul2*
AB-22	—	Urine	2013	792	441	Rizgari	Urology	*blaOXA-23, blaOXA-69, blaADC-25-like*	*aph(3')-VIa, strA, strB, sul2*
63PS	C	Wound	2012	792	441	Rozhawa	Burn unit	*blaOXA-23, blaOXA-69, blaADC-25-like*	*strA, strB, sul2*
AB-26	D	Urine	2013	623	387	Rizgari	Urology	*blaOXA-23, blaOXA-69, blaADC-25-like*	*aph(3')-Vla, mph*(E), *msr*(E), *sul2*
DL-25	D	Urine	2012	136	1092	Sulemani	Urology	*blaOXA-23, blaOXA-91-like, blaADC-25-like*	*aph(3')-VIa, strA, strB, sul2*
AB-18	E	Urine	2013	2	195	Rizgari	Urology	*blaOXA-23, blaOXA-66, blaADC-25-like*	*armA, strA, strB, mph*(E), *msr*(E), *sul2*
25PS	F	Other	2013	2	195	Rizgari	RCU	*blaOXA-2, blaOXA-66, blaADC-25-like*	*armA, strA, strB, mph*(E), *msr*(E), *sul2*
AB-24	G	Urine	2013	623	387	Rizgari	Urology	*blaOXA-23, blaOXA-69, blaADC-25-like*	*aadB, aph(3')-VIa, mph*(E), *msr*(E), *sul2*
DL-31	G	Urine	2012	623	387	Sulemani	Urology	*blaOXA-23, blaOXA-69, blaADC-25-like*	*aadB, aph(3')-VIa, mph*(E), *msr*(E), *sul2*
44PS	H	Wound	2012	94	387	Rozhawa	Burn unit	*blaOXA-23, blaOXA-69, blaADC-25-like*	*aph(3')-Ic, aph(3')-VIa, mph*(E), *msr*(E), *sul2*

check for additional carbapenemases such as KPC, NDM, or VIM, the increased MICs for imipenem and meropenem could also be due to the previously mentioned mechanisms. The same goes for the *bla*$_{\text{OXA-24-like}}$-positive isolates in which also a combination of the OXA enzyme with, for instance, reduced membrane permeability and/or efflux could explain the increased MICs for the carbapenem antibiotics.

Several other studies demonstrated the same findings on a worldwide scale.[32,33]

With PCR, only specific genes will be detected, whereas by WGS, all resistance genes can be demonstrated as long as the sequence is present in the database. As WGS was used to extract MLST data, we were also able to, at least for the 15 selected isolates, determine additional resistance genes.

ResFinder analysis of the WGS data identified and confirmed the presence of the bla$_{\text{OXA-23}}$ in all 15 isolates. Furthermore, variants of the bla$_{\text{oxa-51}}$ group were identified as bla$_{\text{OXA-66}}$, bla$_{\text{OXA-69}}$, and bla$_{\text{OXA-91-like}}$ (Table 3). To our knowledge, bla$_{\text{OXA-69}}$ and bla$_{\text{OXA-91}}$ are rare. Besides the OXA variants, also an AmpC variant described as bla$_{\text{ADC-25-like}}$ was demonstrated.[34] In addition to these β-lactamase variants, several aminoglycoside-modifying enzymes, tetracycline and sulfamethoxazole genes were demonstrated, providing evidence for the MDR character of these isolates.

Using PFGE typing, which is still considered a good typing method for local outbreaks, we showed multiple clusters of clonally related isolates from patients who were epidemiologically related. These results are indicative for cross-transmission. To prevent further transmission of these MDR *A. baumannii* strains, intensified infection control measures need to be taken, in addition to an antibiotic stewardship program.[35] Since identical clones were found in multiple hospitals, these measures should ideally be carried out in a program of regional collaboration.

With respect to the genetic relatedness, it has been demonstrated in several studies that the presence of *bla*$_{\text{OXA-23-like}}$ is often correlated with international clones I and II.[20] This suggests dissemination of one clone of *bla*$_{\text{OXA-23-like}}$ carbapenemase producers,[36] but besides the presence in international clones, numerous reports have also reported the presence of *bla*$_{\text{OXA-23-like}}$ in different ST types.[20]

In the present study, we were able to demonstrate the presence of the international clone ST 2 (Pasteur) in isolates obtained from the Rizgari hospital, but also different ST types were obtained. These findings confirm that OXA-23 is not restricted to one ST but that it is found in different ST types, which is not that surprising as OXA-23 is an acquired mechanism of resistance.

Our results are in accordance with the carbapenem-resistant *A. baumannii* epidemic clusters fueled by the spread of successful clones spreading among different cities and countries.[37,38]

In conclusion, our present results reveal that MDR *A. baumannii-calcoaceticus* complex isolates are spreading also in the Kurdistan region.

PFGE and MLST results demonstrate that identical clones are present in different hospitals. Surveillance cultures and prudent use of antibiotics accompanied with proper guidance and implementation of strict hygiene rules may reduce further spread of resistant bacteria.

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Author Disclosure Statement

All authors have no conflicts of interest.

References

1. Ben Othman, A., M. Zribi, A. Masmoudi, S. Abdellatif, S. Ben Lakhal, and C. Fendri. 2011. Multiresistance and endemic status of *Acinetobacter baumannii* associated with nosocomial infections in a tunisian hospital: a critical situation in the intensive care units. Braz. J. Microbiol. 42:415–422.
2. Deng, M., M.H. Zhu, J.J. Li, S. Bi, Z.K. Sheng, F.S. Hu, *et al*. 2014. Molecular epidemiology and mechanisms of tigecycline resistance in clinical isolates of *Acinetobacter baumannii* from a Chinese university hospital. Antimicrob. Agents Chemother. 58:297–303.
3. Poirel, L., T. Naas, and P. Nordmann. 2010. Diversity, epidemiology, and genetics of class D β-lactamases. Antimicrob. Agents Chemother. 54:24–38.
4. Poirel, L., R.A. Bonnin, and P. Nordmann. 2011. Genetic basis of antibiotic resistance in pathogenic Acinetobacter species. IUBMB Life 63:1061–1067.
5. Rice, L.B. 2008. Federal funding for the study of antimicrobial resistance in nosocomial pathogens: no ESKAPE. J. Infect. Dis. 197:1079–1081.
6. Liou, M.L., M.F. Lin, K.C. Chang, and H.Y. Kuo. 2010. Carbapenem-resistant *Acinetobacter baumannii* in Taiwan. J. Biomed. Lab. Sci. 22:81–87.
7. Opazo, A., M. Domínguez, H. Bello, S.G. Amyes, and G. González-Rocha. 2011. OXA-type carbapenemases in *Acinetobacter baumannii* in South America. J. Infect. Dev. Ctries. 6:311–316.
8. Brown, S., H. Young, and S. Amyes. 2005. Characterisation of OXA-51, a novel class D carbapenemase found in genetically unrelated clinical strains of *Acinetobacter baumannii* from Argentina. Clin. Microbiol. Infect. 11:15–23.
9. Higgins, P.G., L. Poirel, M. Lehmann, P. Nordmann, and H. Seifert. 2009. OXA-143, a novel carbapenem-hydrolyzing class D β-lactamase in *Acinetobacter baumannii*. Antimicrob. Agents Chemother 53:5035–5038.
10. Héritier, C., L. Poirel, P.E. Fournier, J.M. Claverie, D. Raoult, and P. Nordmann. 2005. Characterization of the naturally occurring oxacillinase of *Acinetobacter baumannii*. Antimicrob. Agents Chemother. 49:4174–4179.
11. Corvec, S., L. Poirel, T. Naas, H. Drugeon, and P. Nordmann. 2007. Genetics and expression of the carbapenem-hydrolyzing oxacillinase gene blaOXA-23 in *Acinetobacter baumannii*. Antimicrob. Agents Chemother. 51:1530–1533.
12. Bertini, A., L. Poirel, P.D. Mugnier, L. Villa, P. Nordmann, and A. Carattoli. 2010. Characterization and PCR-based replicon typing of resistance plasmids in *Acinetobacter baumannii*. Antimicrob. Agents Chemother. 54:4168–4177.
13. Howard, A., M. O'Donoghue, A. Feeney, and R.D. Sleator. 2012. *Acinetobacter baumannii*: an emerging opportunistic pathogen. Virulence 3:243–250.

14. Castanheira, M., S.E. Costello, L.N. Woosley, L.M. Deshpande, T.A. Davies, and R. Jones. 2014. Evaluation of clonality and carbapenem resistance mechanisms among *Acinetobacter baumannii-Acinetobacter calcoaceticus* complex and Enterobacteriaceae isolates collected in European and mediterranean countries and detection of two novel β-lactamases, GES-22 and VIM-35. 2014. Antimicrob. Agents Chemother. 58:7358–7366.
15. D'Arezzo, S., L. Principe, A. Capone, N. Petrosillo, A. Petrucca, and P. Visca. 2011. Changing carbapenemase gene pattern in an epidemic multidrug-resistant *Acinetobacter baumannii* lineage causing multiple outbreaks in central Italy. J. Antimicrob. Chemother. 66:54–61.
16. Liakopoulos, A., V. Miriagou, E.A. Katsifas, A.D. Karagouni, G.L. Daikos, *et al.* 2012. Identification of OXA-23-producing *Acinetobacter baumannii* in Greece, 2010 to 2011. Euro Surveill 17: pii:20117.
17. Mezzatesta, M., M. D'Andrea, R. Migliavacca, T. Giani, F. Gona, E. Nucleo, G. Fugazza, L. Pagani, G. Rossolini, and S. Stefani. 2012. Epidemiological characterization and distribution of carbapenem-resistant *Acinetobacter baumannii* clinical isolates in Italy. Clin. Microbiol. Infect. 18: 160–166.
18. Minandri, F., S. D'Arezzo, L.C. Antunes, C. Pourcel, L. Principe, *et al.* 2012. Evidence of diversity among epidemiologically related carbapenemase-producing *Acinetobacter baumannii* strains belonging to international clonal lineage II. J. Clin. Microbiol. 50:590–597.
19. Adams-Haduch, J.M., E.O. Onuoha, T. Bogdanovich, G.B. Tian, J. Marschall, *et al.* 2011. Molecular epidemiology of carbapenem-nonsusceptible *Acinetobacter baumannii* in the United States. J. Clin. Microbiol. 49:3849–3854.
20. Diancourt, L., V. Passet, A. Nemec, L. Dijkshoorn, and S. Brisse. 2010. The population structure of *Acinetobacter baumannii*: expanding multiresistant clones from an ancestral susceptible genetic pool. Plos One 5:e10034.
21. Woodford, N., J.F. Turton, and D.M. Livermore. 2011. Multiresistant Gram-negative bacteria: the role of high-risk clones in the dissemination of antibiotic resistance. FEMS Microbiol. Rev. 35:736–755.
22. Hujer, K.M., A.M. Hujer, E.A. Hulten, S. Bajaksouzian, J.M. Adams, C.J. Donskey, D.J. Ecker, C. Massire, M.W. Eshoo, and R. Sampath. 2006. Analysis of antibiotic resistance genes in multidrug-resistant Acinetobacter sp. isolates from military and civilian patients treated at the Walter Reed Army Medical Center. Antimicrob. Agents Chemother. 50:4114–4123.
23. The European Committee on Antimicrobial Susceptibility Testing. 2014. EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Available at www.eucast.org.
24. Woodford, N., M.J. Ellington, J.M. Coelho, J.F. Turton, M.E. Ward, S. Brown, S.G. Amyes, and D.M. Livermore. 2006. Multiplex PCR for genes encoding prevalent OXA-carbapenemases in Acinetobacter spp. Int. J. Antimicrob. Agents. 27:351–353.
25. Segal, H., S. Garny, and B.G. Elisha. 2005. Is IS(*ABA1*) customized for Acinetobacter? FEMS Microbiol. Lett. 243: 425–429.
26. Shi, Z.-Y., P. Liu, Y.J. Lau, Y.H. Lin, and B.S. Hu. 1996. Epidemiological typing of isolates from an outbreak of infection with multidrug-resistant *Enterobacter cloacae* by repetitive extragenic palindromic unit b1-primed PCR and pulsed-field gel electrophoresis. J. Clin. Microbiol. 34:2784–2790.
27. Karmostaji, A., S.N. Peerayeh, and A.H. Salmanian. 2013. Distribution of OXA-Type Class D β-Lactamase genes among nosocomial multi drug resistant *Acinetobacter baumannii* isolated in Tehran Hospitals. Jundishapur J. Microbiol. 6:1–5.
28. Sharif, M., R. Mirnejad, and N. Amirmozafari. 2014. Molecular identification of TEM and SHV extended spectrum beta-lactamase in clinical isolates of *Acinetobacter baumannii* from Tehran hospitals. JGMI 2014:1–9.
29. Kaliterna, V., and I. Goic-Barisic. 2013. The ability of biofilm formation in clinical isolates of *Acinetobacter baumannii* belonging to two different European clones causing outbreaks in the Split University Hospital, Croatia. J. Chemother. 25:60–62.
30. Poirel, L., and P. Nordmann. 2006. Carbapenem resistance in *Acinetobacter baumannii*: mechanisms and epidemiology. Clin. Microbiol. Infect. 12:826–836.
31. Mendes, R.E., J.M. Bell, J.D. Turnidge, M. Castanheira, and R.N. Jones. 2009. Emergence and widespread dissemination of OXA-23,-24/40 and-58 carbapenemases among Acinetobacter spp. in Asia-Pacific nations: report from the SENTRY Surveillance Program. J. Antimicrob. Chemother. 63:55–59.
32. Evans, B., A. Hamouda, and S. Amyes. 2013. The rise of carbapenem-resistant *Acinetobacter baumannii*. Curr. Pharm. Des. 19:223–238.
33. Valenzuela, J.K., L. Thomas, S.R. Partridge, T. van der Reijden, L. Dijkshoorn, and J. Iredell. 2007. Horizontal gene transfer in a polyclonal outbreak of carbapenem-resistant *Acinetobacter baumannii*. J. Clin. Microbiol. 45:453–460.
34. Beceiro, A., A. Peŕez, F. Fernańdez-Cuenca, *et al.* 2009. Genetic variability among ampC genes from Acinetobacter genomic species 3. Antimicrob. Agents Chemother. 53:1177–1184.
35. Apisarnthanarak, A., U. Pinitchai, B. Warachan, D.K. Warren, T. Khawcharoenporn, and M.K. Hayden. 2014. Effectiveness of infection prevention measures featuring advanced source control and environmental cleaning to limit transmission of extremely-drug resistant *Acinetobacter baumannii* in a Thai intensive care unit: An analysis before and after extensive flooding. Am. J. Infect. Control. 42:116–121.
36. Lee, K., D. Yong, S.H. Jeong, and Y. Chong. 2011. Multidrug-resistant Acinetobacter spp.: increasingly problematic nosocomial pathogens. Yonsei Med. J. 52:879–891.
37. Prashanth, K., and S. Badrinath. 2005. Epidemiological investigation of nosocomial Acinetobacter infections using arbitrarily primed PCR & pulse field gel electrophoresis. Indian J. Med. Res. 122:408–418.
38. Villalón, P., S. Valdezate, M.J. Medina-Pascual, V. Rubio, A. Vindel, and J.A. Saez-Nieto. 2011. Clonal diversity of nosocomial epidemic *Acinetobacter baumannii* strains isolated in Spain. J. Clin. Microbiol. 49:875–882.
39. Voor in 't Holt, A.F., J.A. Severin, W.H. Goessens, R. te Witt, and M.C. Vos. 2015. Instant typing is essential to detect transmission of Extended-Spectrum Beta-lactamase-produing Klebsiella species. Plos One. 10:e0136135.

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