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Wide Dissemination of GES-Type Carbapenemases in *Acinetobacter baumannii* Isolates in Kuwait

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Acinetobacter baumannii **is an opportunistic pathogen that is an important source of nosocomial infections. Production of extended-spectrum β-lactamases (ESBLs) of the GES type in *A. baumannii* has been increasingly reported, and some of these GES-type enzymes possess some carbapenemase activity. Our aim was to analyze the resistance determinants and the clonal relationships of carbapenem-nonsusceptible *A. baumannii* clinical isolates recovered from hospitals in Kuwait. A total of 63 isolates were analyzed, and all were found to be positive for bla_{GES}-type genes. One isolate harbored the bla_{GES-14} gene encoding an ESBL with significant carbapenemase activity, whereas the other isolates harbored the bla_{GES-11} ESBL gene. Thirty-three isolates coharbored the bla_{OXA-23} and bla_{GES-11} genes. Analyses of the genetic locations indicated that the $bla_{GES-11/-14}$ genes were plasmid located. It is noteworthy that the bla_{OXA-23} and bla_{GES-11} genes were colocated onto a single plasmid. Nine different pulsotypes were observed among the 63 isolates. This study showed the emergence of GES-type ESBLs in *A. baumannii* in Kuwait, further suggesting that the Middle East region might be a reservoir for carbapenemase-producing *A. baumannii*.**

Acinetobacter baumannii is an opportunistic pathogen that is an important causative agent of nosocomial infections, such as pneumonia, septicemia, urinary tract infections, and wound infections (1). Multidrug-resistant (MDR) *A. baumannii* isolates are increasingly reported worldwide and are often a source of nosocomial infections. Treatment of infections due to this microorganism is becoming a serious clinical concern, since *A. baumannii* is very often resistant to multiple antibiotics (1). One of the main mechanisms of resistance to β-lactam molecules in this species is the production of β-lactamases (2). Resistance to carbapenems is mostly related to the production of carbapenem-hydrolyzing class D β-lactamases (CHDLs) and, to a lesser extent, of metallo-β-lactamases (MBLs). Among these CHDLs, OXA-23 is the most commonly identified CHDL worldwide (2), and its corresponding gene is located on either a plasmid or a chromosome and at the origin of its acquisition is associated with the insertion sequence IS*Aba1* or IS*Aba4* (3). Although resistance to carbapenems is mostly related to the production of CHDLs that do not include broad-spectrum cephalosporins in their hydrolytic spectrum, resistance to broad-spectrum cephalosporin molecules in *A. baumannii* usually results from overexpression of the natural AmpC-type enzyme but also from the acquisition of extended-spectrum β-lactamases (ESBLs) (2). Those ESBLs may correspond to TEM or SHV derivatives but mostly correspond to Ambler class A β-lactamases of the VEB, PER, and GES types (2). ESBLs of the GES type are being reported increasingly in Gram-negative rods, including *Pseudomonas aeruginosa*, *Enterobacter cloacae*, and *Klebsiella pneumoniae* (4) and were recently reported in *A. baumannii* (5–7). While the hydrolysis profile of GES-1 is similar to that of other ESBLs (8), including penicillins and broad-spectrum cephalosporins, GES-1 nonetheless does not hydrolyze monobactams, and some GES variants possess significant carbapenemase activity. A Gly170Ser substitution, located inside the omega loop of the catalytic site, was identified in GES-4, GES-5, and GES-6, enzymes that hydrolyze carbapenems and cephamycins (4). GES-11, identified in *A. baumannii* and differing from GES-1 by a single amino acid substitution (a Gly243Ala change), possesses increased activity against aztreonam (7). GES-14, also identified in *A. baumannii* and differing from GES-11 by a Gly170Ser amino acid substitution, possesses an extended spectrum of activity toward carbapenems and cephamycins, in addition to its ability to compromise monobactams, giving rise to a very broad-spectrum enzyme that is active against all β-lactams (6).

The aim of this study was to analyze the resistance determinants and the clonal relationships of a collection of carbapenem-nonsusceptible *A. baumannii* clinical isolates recovered from different hospitals in Kuwait.

MATERIALS AND METHODS

Bacterial isolates and susceptibility testing. Sixty-three nonduplicate and carbapenem-nonsusceptible *A. baumannii* clinical isolates were included in this study. These isolates were identified by using the API 20 NE system (bioMérieux, Marcy l'Etoile, France), 16S rRNA gene sequencing, and culture at 44°C, as described previously (9). The antibiotic susceptibilities of the isolates were determined by the disc diffusion technique on Mueller-Hinton agar. MICs were determined by using Etest strips (AB bioMérieux, La Balme-les-Grottes, France) and interpreted according to CLSI guidelines (10). The production of MBLs was evaluated using Etest strips as recommended by the manufacturer (AB bioMérieux) and by the combined-disc test as described previously (11).

PCR amplification and sequencing. PCR experiments were performed using standard conditions to search for β-lactamase genes that have been identified previously in *A. baumannii*, i.e., narrow-spectrum β-lactamase genes bla_{SCO-1} and bla_{RTG-3}, ESBL genes bla_{PER}, bla_{GES}, and bla_{VEB}, MBL genes bla_{NDM}, bla_{VIM}, bla_{IMP}, and bla_{SIM}, acquired CHDL

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genes bla_{OXA-23}, bla_{OXA-40}, bla_{OXA-58}, and $bla_{OXA-143}$, and the intrinsic $bla_{OXA-51-like}$ CHDL gene (see Table S1 in the supplemental material). Detection of the IS*Aba1* element upstream of the bla_{ADC} and bla_{OXA-51} genes was also performed. The primers used in this study are listed in Table S1. Detection of the 16S RNA methylase genes was also performed as described previously (12). Amplified DNA fragments were purified with the QIAquick PCR purification kit (Qiagen, Courtaboeuf, France). Both strands of the amplification products obtained were sequenced with an ABI 3100 sequencer (Applied Biosystems, Foster City, CA). The nucleotide and deduced protein sequences were analyzed with software available over the Internet at the National Center for Biotechnology Information website (www.ncbi.nlm.nih.gov).

Acinetobacter PCR-based replicon typing (AB-PBRT) has been developed to type the plasmids circulating in *A. baumannii*. In total, 19 PCR amplifications were used as described previously (13).

Analysis of the genetic support of β-lactamase genes. In order to determine the genetic locations of carbapenemase and the ESBL genes, conjugation assays were performed using *A. baumannii* BM4547 (rifampin resistant) and azide-resistant *Escherichia coli* J53 (Invitrogen, Cergy-Pontoise, France) as recipient strains. Plasmid DNA was extracted by using the Kieser method (14), and electroporation was performed using *A. baumannii* CIP70.10 as the donor. Selection was based on ticarcillin (50 μg/ml) and sodium azide (100 μg/ml) or rifampin (100 μg/ml), depending on the recipient strain used.

DNA-DNA hybridization analyses were performed by using total plasmid DNA extracted as described above, separated by electrophoresis on 0.8% agarose gels, transferred onto Hybond-N+ membranes, and hybridized with enhanced chemiluminescence-labeled probes overnight at 42°C. Plasmid locations of the β-lactamase genes were assessed by the hybridization of plasmid DNA with DNA probes specific for the bla_{OXA-23} or bla_{GES} genes.

Molecular typing and clonal relationships. Isolates were typed by using ApaI macrorestriction analyses and pulsed-field gel electrophoresis (PFGE) according to the manufacturer's recommendations (Bio-Rad, Marnes-la-Coquette, France). Whole-cell DNA of *A. baumannii* isolates was digested with ApaI for 3 h at 37°C (Fermentas, St. Rémy-Les-Chevreuses, France) as described previously (3). Electrophoresis was performed on an agarose gel using a CHEF-DR II apparatus (Bio-Rad). Isolates were also typed by randomly amplified polymorphic DNA (RAPD) analyses using DAF4 and M13 primers as described previously (15).

The identification of PCR-based sequence groups was conducted by using 2 multiplex PCR assays designed to identify the worldwide clones as described previously (16). Clonal relationships were established by multilocus sequence typing (MLST) by using 7 standard housekeeping loci as described previously (17). Sequences of the 7 housekeeping genes were analyzed by using an *A. baumannii* database (www.pasteur.fr/recherche/genopole/PF8/mlst/Abaumannii.html).

RESULTS AND DISCUSSION

Antimicrobial susceptibilities. A total of 63 consecutive nonrepetitive carbapenem-nonsusceptible *A. baumannii* clinical isolates, including 59 invasive strains and 4 colonizers, were obtained from clinical specimens taken from patients on admission to one of six hospitals located in different parts of Kuwait between December 2007 and June 2008. Of these 63 isolates, 32 (50.8%) were from patients with proven lower respiratory tract infections, 11 (17.5%) from blood cultures, 7 (11.1%) from urine, 1 (1.6%) from body fluid, and 4 (6.4%) from other sites. Among these 63 isolates, all were resistant to broad-spectrum cephalosporins, and 54% (34/63) were resistant to carbapenems (Table 1). All isolates were resistant to ciprofloxacin, chloramphenicol, and sulfonamides, and 92% and 46% were resistant to amikacin and gentamicin, respectively. All the isolates remained susceptible to colistin and rifampin. Four isolates were resistant to tigecycline (MIC,

TABLE 1 β-Lactams for *A. baumannii* clinical isolates K22, K31, and K78, *A. baumannii* BM4547(pK22), BM4547(pK31), and BM4547(pK78) (transconjugant), and the *A. baumannii* BM4547 reference strain

β-Lactam(s)[a]	MIC (μg/ml) of:						
	A. baumannii isolate K22 (GES-11)	*A. baumannii* isolate K31 (GES-14)	*A. baumannii* isolate K78 (GES-11, OXA-23)	*A. baumannii* BM4547(pK22) (GES-11)	*A. baumannii* BM4547(pK31) (GES-14)	*A. baumannii* BM4547(pK78) (GES-11, OXA-23)	*A. baumannii* BM4547[b]
Ticarcillin	>256	>256	>256	>256	>256	>256	8
Ticarcillin + CLA	256	256	>256	256	128	>256	8
Piperacillin	>256	>256	>256	>256	>256	>256	4
Piperacillin + TZB	256	256	>256	256	128	>256	4
Cefoxitin	>256	>256	>256	>256	>256	>256	256
Cefotaxime	>64	>64	>64	>64	>64	>64	>32
Cefotaxime + CLA	>1	>1	>1	>1	>1	>1	ND
Ceftazidime	>64	>64	>64	>64	>64	>64	4
Ceftazidime + CLA	>1	>1	>1	>1	>1	>1	ND
Cefepime	>64	>64	>64	32	32	32	16
Aztreonam	>64	>64	>64	64	>64	>64	32
Meropenem	4	16	32	2	12	24	0.5
Doripenem	2	16	32	2	12	24	0.25
Imipenem	2	24	32	1	24	24	0.25

[a] CLA, clavulanic acid (4 μg/ml); TZB, tazobactam (4 μg/ml).
[b] ND, not determined.

1.5 to 3 μg/ml) according to guidelines described by Jones et al. (18), and eight isolates had reduced susceptibilities to tigecycline (MICs, 1 μg/ml).

Characterization of β-lactamase genes. Phenotypic assays showed that all the isolates remained negative for MBL production. PCR experiments for the detection of the bla_{NDM-1}, bla_{IMP}-type, bla_{VIM}-type, and bla_{SIM-1} genes gave negative results. Synergy tests with discs containing ceftazidime and ticarcillin-clavulanic acid, using cloxacillin-containing Mueller-Hinton agar plates as described previously (6), gave positive results. PCR followed by sequencing identified the bla_{GES} ESBL genes in all the isolates (Table 2). The bla_{VEB-1} and bla_{PER-1} genes were not identified. Additionally, PCR experiments followed by sequence analyses led to the identification of the bla_{OXA-23} gene in 33 isolates, in addition to a natural bla_{OXA-51}-like gene identified in all the isolates (Table 2). Sequencing of the bla_{OXA-51}-like gene revealed several variants corresponding to bla_{OXA-66}, bla_{OXA-64}, bla_{OXA-98}, and bla_{OXA-71} (19).

Genetic support of the bla_{GES-14} gene and transfer of β-lactam resistance. Plasmid DNA analyses showed that all *A. baumannii* clinical isolates harbored plasmids of ca. 90 to 100 kb in size (data not shown). Mating-out assays revealed *A. baumannii* BM4547 transconjugants harboring either the bla_{GES-14} or the bla_{GES-11} gene. All transconjugants obtained using the OXA-23-producing *A. baumannii* isolates as donors coharbored the bla_{OXA-23} gene. The bla_{GES-11}-carrying transconjugants exhibited an ESBL phenotype but also showed reduced susceptibilities to carbapenems, suggesting the presence of weak hydrolysis of carbapenems by GES-11 potentiated by the efflux overproduction in *A. baumannii* BM4547. The *A. baumannii* transconjugants harboring the bla_{GES-14}-positive plasmid showed resistance to carbapenems, confirming that the expression of GES-14 led to resistance to carbapenems, as reported previously (6). Finally, all *A. baumannii* transconjugants expressing both OXA-23 and GES-11 and actually harboring a single plasmid bearing both the bla_{GES-11} and bla_{OXA-23} genes were resistant to all β-lactams, including carbapenems. All transconjugants showed acquired resistance to chloramphenicol, tetracycline, and aminoglycosides. Southern hybridizations performed with bla_{GES}- and bla_{OXA-23}-specific probes confirmed that these two genes were colocated onto a ca. 95-kb plasmid (data not shown).

Attempts to transfer these natural plasmids by conjugation and electroporation into *E. coli* TOP10 as a recipient strain failed. This result suggests that those plasmids possessed a replication module that does not allow replication in *E. coli*, as previously suggested (6). The typing of these plasmids by *A. baumannii* PCR-based replicon typing (AB-PBRT) indicated that all plasmids belonged to the plasmid group Gr6. This group of plasmids has been shown to be highly prevalent in *A. baumannii* and was previously found to be associated with the bla_{OXA-23}, bla_{OXA-40}, and bla_{OXA-58} CHDL genes (20).

Genetic environment of the bla_{GES} genes. PCR mapping was performed to identify the genetic structure bracketing the bla_{GES} genes. Downstream of the bla_{GES-14} gene, the *aacA4* gene cassette encoding the AAC(6′)-Ib aminoglycoside acetyltransferase was identified. It was followed by the *dfrA7* gene cassette, which encodes resistance to trimethoprim, and then the 3′ extremity of class 1 integrons as observed previously (6). Also as previously observed, the integrase gene upstream of the bla_{GES} genes was truncated in its 5′ extremity. The complete In*125* integron, composed of two class 1 integrons, was identified for only the isolate possessing the bla_{GES-14} gene. For the isolates carrying the bla_{GES-11} gene, the 5′ extremity of the In*125* integron was not identified by PCR mapping as observed previously (7).

Genetic environment of the bla_{OXA-23} gene. The insertion sequence IS*Aba1* was detected upstream of the bla_{OXA-23} gene in all positive isolates, but no IS*Aba1* was detected downstream of the bla_{OXA-23} gene, ruling out the hypothesis that this gene could be part of the composite transposon Tn*2006*. Sequence analysis of the region separating IS*Aba1* from the bla_{OXA-23} gene revealed a 7-bp deletion corresponding to the sequence previously identified in Tn*2008* carrying bla_{OXA-23} in *A. baumannii* isolates from the United States, Romania, and China (21–23).

Genotyping of clinical isolates. Genotypic comparisons were performed by using different techniques. RAPD analysis clustered the collection into four groups with a major clonal group (group 1) (Table 2). PFGE analysis showed that the 63 isolates were grouped into nine distinct clones named A to I (Table 2). Among these pulsotypes, pulsotype A, including 30 isolates, was the main group, pulsotype B included 15 isolates, and pulsotype F included 9 isolates. The other groups included either 1 or 2 isolates each. Pulsotypes A, C, F, G, H, and I corresponded to RAPD group 1, pulsotype B corresponded to RAPD groups 2 and 4, and pulsotype E corresponded to RAPD group 3. MLST analyses showed that pulsotype A belonged to ST158 (41-42-13-1-5-4-14), a recently identified sequence type (ST) (http://www.pasteur.fr/cgi-bin/genopole/PF8/mlstdbnet.pl?file=acin_isolates.xml). All isolates belonging to ST158 were isolated in the same hospital but different wards. Therefore, this clone appears to be endemic in the Al Jahrah hospital (Table 2). This clone coproduced the two carbapenemases GES-11 and OXA-23 and showed high levels of resistance to carbapenems (Table 1). Pulsotype B belonged to ST49 (3-3-6-2-3-1-5), which is an ST that has already been identified in the United States and the Netherlands (15, 21). This ST is actually a double-locus variant of ST3 (3-3-2-2-3-1-3), which has been widely identified throughout the world and corresponds to the worldwide clone III (24, 25). Isolates corresponding to pulsotypes G and H belonged to ST3, confirming that this ST is prevalent in Kuwait. The third main sequence type corresponded to ST113 (3-3-3-4-7-4-4), which was recently described in Saudi Arabia among GES-producing *A. baumannii* (26), or to ST178 (3-1-3-4-7-4-4), which is a single-locus variant of ST113. The remaining clones belonged to worldwide clone II derivatives, either ST2 (2-2-2-2-2-2-2) or ST104 (2-2-2-2-2-2-14), the latter of which is a single-locus variant of ST2. This clone has also been shown to be widely distributed throughout the world and is associated with the production of CHDLs (1, 17, 21, 24). Only two isolates belonging to clonal complex II were identified among our isolates. Overall, clonal lineages identified in Kuwait were significantly different from those reported in Europe, where ST1 and ST2 have been the major clones recovered (16, 17, 24). We identified only a few isolates belonging to ST2 and found none belonging to ST1. These results may be explained by the fact that our collection was recovered during the 2007-2008 time period.

Conclusion. This study revealed a high prevalence of GES-producing *A. baumannii* strains in Kuwait that were not linked to a single clone, even though one clone was more commonly identified (47%) within the 63 isolates studied. Recently, a study performed in Belgium also reported a series of GES-producing *A. baumannii* isolates for which a link with different geographical

TABLE 2 Clinical features, β-lactamase detection, and genotyping of *A. baumannii* clinical isolates

Isolate	Date of isolation (mo-day-yr)	Hospital	Hospital unit[a]	Specimen source[b]	Acquired carbapenemase	OXA-51-like	IS*Aba1-ampC*	ESBL	EC/ST[c]	RAPD pattern (group)	PFGE pattern (clone name)
K35	12-30-07	Al Jahra	ICU	Endotracheal tube	OXA-23	OXA-66	−	GES-11	nt/ST158	1	A
K36	12-30-07	Al Jahra	ICU	Endotracheal tube	OXA-23	OXA-66	−	GES-11	nt/ST158	1	A
K40	12-22-07	Al Jahra	ICU	Endotracheal tube	OXA-23	OXA-66	−	GES-11	nt/ST158	1	A
K45	12-30-07	Al Jahra	Medical	Urine culture	OXA-23	OXA-66	−	GES-11	nt/ST158	1	A
K46	12-24-07	Al Jahra	ICU	Endotracheal tube	OXA-23	OXA-66	−	GES-11	nt/ST158	1	A
K50	01-13-08	Al Jahra	ICU	Urine culture	OXA-23	OXA-66	−	GES-11	nt/ST158	1	A
K66	01-06-08	Al Jahra	ICU	Endotracheal tube	OXA-23	OXA-66	−	GES-11	nt/ST158	1	A
K68	01-06-08	Al Jahra	ICU	Endotracheal tube	OXA-23	OXA-66	−	GES-11	nt/ST158	1	A
K72	01-09-08	Al Jahra	ICU	Endotracheal tube	OXA-23	OXA-66	−	GES-11	nt/ST158	1	A
K73	01-09-08	Al Jahra	ICU	Endotracheal tube	OXA-23	OXA-66	−	GES-11	nt/ST158	1	A
K77	01-20-08	Al Jahra	ICU	Endotracheal tube	OXA-23	OXA-66	−	GES-11	nt/ST158	1	A
K78	01-22-08	Al Jahra	ICU	Endotracheal tube	OXA-23	OXA-66	−	GES-11	nt/ST158	1	A
K79	01-20-08	Al Jahra	ICU	Sputum	OXA-23	OXA-66	−	GES-11	nt/ST158	1	A
K80	01-22-08	Al Jahra	Surgical ward	Endotracheal tube	OXA-23	OXA-66	−	GES-11	nt/ST158	1	A
K81	02-07-08	Al Jahra	ICU	Leg	OXA-23	OXA-66	−	GES-11	nt/ST158	1	A
K82	02-18-08	Al Jahra	ICU	Bed swab	OXA-23	OXA-66	−	GES-11	nt/ST158	1	A
K86	01-14-08	Al Jahra	ICU	Endotracheal tube	OXA-23	OXA-66	−	GES-11	nt/ST158	1	A
K88	01-16-08	Al Jahra	ICU	Foot wound	OXA-23	OXA-66	−	GES-11	nt/ST158	1	A
K102	02-19-08	Al Jahra	ICU	Endotracheal tube	OXA-23	OXA-66	−	GES-11	nt/ST158	1	A
K103	02-19-08	Al Jahra	Medical ward	Endotracheal tube	OXA-23	OXA-66	−	GES-11	nt/ST158	1	A
K104	02-25-08	Al Jahra	ICU	Endotracheal tube	OXA-23	OXA-66	−	GES-11	nt/ST158	1	A
K105	02-24-08	Al Jahra	Medical ward	Endotracheal tube	OXA-23	OXA-66	−	GES-11	nt/ST158	1	A
K106	02-24-08	Al Jahra	Surgical ward	Endotracheal tube	OXA-23	OXA-66	−	GES-11	nt/ST158	1	A
K109	03-02-08	Al Jahra	Medical ward	Blood culture	OXA-23	OXA-66	−	GES-11	nt/ST158	1	A
K112	02-29-08	Al Jahra	ICU	Endotracheal tube	OXA-23	OXA-66	−	GES-11	nt/ST158	1	A
K118	02-07-08	Al Jahra	Medical ward	BAL	OXA-23	OXA-66	−	GES-11	nt/ST158	1	A
K144	03-23-08	Al Jahra	ICU	Blood culture	OXA-23	OXA-66	−	GES-11	nt/ST158	1	A
K162	04-26-08	Al Jahra	Medical ward	Sputum	OXA-23	OXA-66	−	GES-11	nt/ST158	1	A
K219	05-18-08	Al Jahra	Surgical ward	Urine culture	OXA-23	OXA-66	−	GES-11	nt/ST158	1	A
K221	05-07-08	Al Jahra	Medical ward	Urine culture	OXA-23	OXA-66	−	GES-11	nt/ST158	1	A
K5	12-04-07	Mubarak Al Kabeer	Medical ward	Blood culture	−	OXA-98	−	GES-11	nt/ST49	2	B1
K21	12-12-07	Al Adan	Neonatal ICU	Blood culture	−	OXA-98	−	GES-11	nt/ST49	2	B1
K22	12-12-07	Al Adan	Neonatal ICU	Blood culture	−	OXA-98	−	GES-11	nt/ST49	2	B1
K75	02-10-08	Mubarak Al Kabeer	ICU	Endotracheal secretions	−	OXA-98	−	GES-11	nt/ST49	2	B1
K107	03-02-08	Mubarak Al Kabeer	ICU	Blood culture	−	OXA-98	−	GES-11	nt/ST49	2	B1
K120	02-10-08	Al Adan	Neonatal ICU	Blood culture	−	OXA-98	−	GES-11	nt/ST49	2	B1
K130	03-19-08	Al Adan	Medical ward	Endotracheal tube	−	OXA-98	−	GES-11	nt/ST49	2	B1
K140	03-19-08	Mubarak Al Kabeer	Medical ward	Pus	−	OXA-98	−	GES-11	nt/ST49	2	B1
K147	04-02-08	Al Babtain	Medical ward	Urine culture	−	OXA-98	−	GES-11	nt/ST49	2	B1
K176	01-19-08	Al Adan	Neonatal ICU	Endotracheal sample	−	OXA-98	−	GES-11	nt/ST49	2	B1
K198	05-25-08	Mubarak Al Kabeer	Nephrology ward	Dialysis tip	−	OXA-98	−	GES-11	nt/ST49	4	B1
K202	02-22-08	Al Adan	Neonatal ICU	Endotracheal tube	−	OXA-98	−	GES-11	nt/ST49	4	B1
K228	05-27-08	Al Adan	Surgical ward	Urine culture	−	OXA-98	−	GES-11	nt/ST49	4	B1
K238	06-26-08	Al Adan	Neonatal ICU	Endotracheal tube	−	OXA-98	−	GES-11	nt/ST49	4	B1
K239	06-25-08	Al Adan	Neonatal ICU	Endotracheal tube	−	OXA-98	−	GES-11	nt/ST49	4	B1
K248	04-01-08	Al Adan	Neonatal ICU	Respiratory sample	−	OXA-98	−	GES-11	nt/ST49	4	B1
K63	01-08-08	Al Razi	Medical ward	Urine	−	OXA-98	−	GES-11	nt/ST49	2	B2
K65	11-23-08	Al Jahra	ICU	Tracheal sample	OXA-23	OXA-66	+	GES-11	II/ST104	2	D

K191	06-12-08	Al Sabah	Medical ward	Wound	OXA-23	OXA-66	–	GES-11	II/ST2	3	E
K55	01-20-08	Mubarak Al Kabeer	ICU	Endotracheal sample	–	OXA-64	–	GES-11	nt/ST113	1	C
K70	02-27-08	Mubarak Al Kabeer	ICU	Pus	OXA-23	OXA-64	–	GES-11	nt/ST113	1	C
K108	03-02-08	Mubarak Al Kabeer	CCU	Blood culture	–	OXA-64	–	GES-11	nt/ST113	1	F
K126	03-09-08	Mubarak Al Kabeer	CCU	Ventilator swab	–	OXA-64	–	GES-11	nt/ST113	1	F
K127	03-12-08	Mubarak Al Kabeer	ICU	Pus	–	OXA-64	+	GES-11	nt/ST113	1	F
K131	04-01-08	Al Razi	Medical ward	Urine culture	–	OXA-64	–	GES-11	nt/ST113	1	F
K138	02-22-08	Al Adan	Neonatal ICU	Endotracheal tube	–	OXA-64	–	GES-11	nt/ST113	1	F
K145	04-02-08	Mubarak Al Kabeer	ICU	Blood culture	–	OXA-64	–	GES-11	nt/ST113	1	F
K152	04-01-08	Mubarak Al Kabeer	ICU	Blood culture	–	OXA-64	–	GES-11	nt/ST113	1	F
K167	05-08-08	Mubarak Al Kabeer	ICU	Blood culture	–	OXA-64	–	GES-11	nt/ST113	1	F
K250	04-01-08	Mubarak Al Kabeer	ICU	Rectal swab	–	OXA-64	–	GES-11	nt/ST113	1	F
K89	02-10-08	Mubarak Al Kabeer	CCU	Endotracheal sample	–	OXA-64	–	GES-11	nt/ST178	1	G
K121	02-21-08	Al Adan	Medical ward	Tracheal sample	–	OXA-71	–	GES-11	III/ST3	1	H
K31	04-01-08	Al Adan	ICU	Bed rail	GES-14	OXA-71	+	GES-14	III/ST3	1	I

[a] ICU, intensive care unit; CCU, coronary care unit.

[b] BAL, bronchoalveolar lavage.

[c] EC, multiplex PCR for determining the clonal complex (23); ST, multilocus sequence typing (9); nt, not typeable.

origins, including Middle East countries, Palestinian territories, Turkey, and Egypt, was evidenced (5). Overall, those results strongly suggest that the Middle East may be an important reservoir of GES-type carbapenemases. Our results have shown that the dissemination of those genes was not linked to clonal strain dissemination but rather to plasmid dissemination. Noticeably, the plasmid either was carrying the bla_{GES} carbapenemase gene alone or was coharboring the bla_{OXA-23} carbapenemase gene, making it able to more efficiently promote high-level resistance to carbapenems when acquired in *A. baumannii*.

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REFERENCES

1. **Peleg AY, Seifert H, Paterson DL.** 2008. *Acinetobacter baumannii*: emergence of a successful pathogen. Clin. Microbiol. Rev. **21**:538–582.
2. **Poirel L, Bonnin RA, Nordmann P.** 2011. Genetic basis of antibiotic resistance in pathogenic *Acinetobacter* species. IUBMB Life **63**:1061–1067.
3. **Mugnier PD, Poirel L, Naas T, Nordmann P.** 2010. Worldwide dissemination of the bla_{OXA-23} carbapenemase gene of *Acinetobacter baumannii*. Emerg. Infect. Dis. **16**:35–40.
4. **Poirel L, Bonnin RA, Nordmann P.** 2012. Genetic support and diversity of acquired extended-spectrum β-lactamases in Gram-negative rods. Infect. Genet. Evol. **12**:883–893.
5. **Bogaerts P, Naas T, El Garch F, Cuzon G, Deplano A, Delaire T, Huang TD, Lissoir B, Nordmann P, Glupczynski Y.** 2010. GES extended-spectrum β-lactamases in *Acinetobacter baumannii* isolates in Belgium. Antimicrob. Agents Chemother. **54**:4872–4878.
6. **Bonnin RA, Nordmann P, Potron A, Lecuyer H, Zahar JR, Poirel L.** 2011. Carbapenem-hydrolyzing GES-type extended-spectrum β-lactamase in *Acinetobacter baumannii*. Antimicrob. Agents Chemother. **55**:349–354.
7. **Moubareck C, Bremont S, Conroy MC, Courvalin P, Lambert T.** 2009. GES-11, a novel integron-associated GES variant in *Acinetobacter baumannii*. Antimicrob. Agents Chemother. **53**:3579–3581.
8. **Poirel L, Le Thomas I, Naas T, Karim A, Nordmann P.** 2000. Biochemical sequence analyses of GES-1, a novel class A extended-spectrum β-lactamase, and the class 1 integron In*52* from *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. **44**:622–632.
9. **Ibrahim A, Gerner-Smidt P, Liesack W.** 1997. Phylogenetic relationship of the twenty-one DNA groups of the genus *Acinetobacter* as revealed by 16S ribosomal DNA sequence analysis. Int. J. Syst. Bacteriol. **47**:837–841.
10. **Clinical and Laboratory Standards Institute.** 2012. Performance standards for antimicrobial susceptibility testing (M100-S22). Clinical and Laboratory Standards Institute, Wayne, PA.
11. **Bonnin RA, Naas T, Poirel L, Nordmann P.** 2012. Phenotypic, biochemical, and molecular techniques for detection of metallo-β-lactamase NDM in *Acinetobacter baumannii*. J. Clin. Microbiol. **50**:1419–1421.
12. **Bercot B, Poirel L, Nordmann P.** 2011. Updated multiplex polymerase chain reaction for detection of 16S rRNA methylases: high prevalence among NDM-1 producers. Diagn. Microbiol. Infect. Dis. **71**:442–445.
13. **Bertini A, Poirel L, Mugnier PD, Villa L, Nordmann P, Carattoli A.** 2010. Characterization and PCR-based replicon typing of resistance plasmids in *Acinetobacter baumannii*. Antimicrob. Agents Chemother. **54**:4168–4177.
14. **Kieser T.** 1984. Factors affecting the isolation of CCC DNA from *Streptomyces lividans* and *Escherichia coli*. Plasmid **12**:19–36.
15. **Grundmann HJ, Towner KJ, Dijkshoorn L, Gerner-Smidt P, Maher M, Seifert H, Vaneechoutte M.** 1997. Multicenter study using standardized protocols and reagents for evaluation of reproducibility of PCR-based fingerprinting of *Acinetobacter* spp. J. Clin. Microbiol. **35**:3071–3077.
16. **Turton JF, Gabriel SN, Valderrey C, Kaufmann ME, Pitt TL.** 2007. Use of sequence-based typing and multiplex PCR to identify clonal lineages of

outbreak strains of *Acinetobacter baumannii.* Clin. Microbiol. Infect. **13**: 807–815.

17. **Diancourt L, Passet V, Nemec A, Dijkshoorn L, Brisse S.** 2010. The population structure of *Acinetobacter baumannii*: expanding multiresistant clones from an ancestral susceptible genetic pool. PLoS One **5**:e10034. doi:10.1371/journal.pone.0010034.
18. **Jones RN, Ferraro MJ, Reller LB, Schreckenberger PC, Swenson JM, Sader HS.** 2007. Multicenter studies of tigecycline disk diffusion susceptibility results for *Acinetobacter* spp. J. Clin. Microbiol. **45**:227–230.
19. **Zander E, Nemec A, Seifert H, Higgins PG.** 2012. Association between β-lactamase-encoding bla_{OXA-51} variants and DiversiLab rep-PCR-based typing of *Acinetobacter baumannii* isolates. J. Clin. Microbiol. **50**:1900–1904.
20. **Towner KJ, Evans B, Villa L, Levi K, Hamouda A, Amyes SG, Carattoli A.** 2011. Distribution of intrinsic plasmid replicase genes and their association with carbapenem-hydrolyzing class D β-lactamase genes in European clinical isolates of *Acinetobacter baumannii.* Antimicrob. Agents Chemother. **55**:2154–2159.
21. **Adams-Haduch JM, Paterson DL, Sidjabat HE, Pasculle AW, Potoski BA, Muto CA, Harrison LH, Doi Y.** 2008. Genetic basis of multidrug resistance in *Acinetobacter baumannii* clinical isolates at a tertiary medical center in Pennsylvania. Antimicrob. Agents Chemother. **52**:3837–3843.
22. **Bonnin RA, Poirel L, Licker M, Nordmann P.** 2011. Genetic diversity of carbapenem-hydrolysing β-lactamases in *Acinetobacter baumannii* from Romanian hospitals. Clin. Microbiol. Infect. **17**:1524–1528.
23. **Wang X, Zong Z, Lu X.** 2011. Tn*2008* is a major vehicle carrying bla_{OXA-23} in *Acinetobacter baumannii* from China. Diagn. Microbiol. Infect. Dis. **69**:218–222.
24. **Higgins PG, Dammhayn C, Hackel M, Seifert H.** 2010. Global spread of carbapenem-resistant *Acinetobacter baumannii.* J. Antimicrob. Chemother. **65**:233–238.
25. **van Dessel H, Dijkshoorn L, van der Reijden T, Bakker N, Paauw A, van den Broek P, Verhoef J, Brisse S.** 2004. Identification of a new geographically widespread multiresistant *Acinetobacter baumannii* clone from European hospitals. Res. Microbiol. **155**:105–112.
26. **Ribeiro A, Al-Agamy MH, Shibl AM, Tawfik AF, Courvalin P, Jeannot K.** 2012. Molecular epidemiology and mechanisms of carbapenem-resistant *Acinetobacter baumannii* in a Saudi Arabia hospital (P1256). Clin. Microbiol. Infect. **18**:318.