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GES Extended-Spectrum β-Lactamases in *Acinetobacter baumannii* Isolates in Belgium▽

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During a PCR-based surveillance study of β-lactam resistance, 125 multidrug-resistant (MDR) *Acinetobacter baumannii* isolates were obtained from 18 hospitals in Belgium from January 2008 to December 2009. Nine GES-positive *A. baumannii* isolates were detected at 6 Belgian hospitals. DNA sequencing of the bla_{GES} genes identified GES-11, GES-12, and a novel variant GES-14, which differs from GES-11 by a single amino acid substitution (Gly170Ser). All index isolates were travel associated and originated from patients transferred from Turkey ($n = 2$), Egypt ($n = 2$), and Palestinian territories (Gaza) ($n = 2$). A nosocomial outbreak involving three additional patients occurred in a burn unit at a single hospital. No clonal relatedness could be established between the 6 index isolates by pulsed-field gel electrophoresis (PFGE) analysis. Three different alleles (the plasmid-located bla_{GES-11} and bla_{GES-12} and a likely chromosomally located novel variant bla_{GES-14}) were detected as part of a class 1 integron, also including the *aac6′Ib* and *dfrA7* genes. Restriction analysis of plasmids suggests a common origin for the plasmids bearing bla_{GES-11} and bla_{GES-12}. Cloning of the bla_{GES} genes in *Escherichia coli* identified GES-14 as hydrolyzing imipenem, while GES-12 showed the highest specific activity against ceftazidime. This report highlights the emergence of various $bla_{GES-like}$ genes, especially those conferring carbapenem resistance in *A. baumannii* and its importation in Western Europe from Middle Eastern countries.

Acinetobacter baumannii has been recognized over the last 2 decades as an emerging opportunistic pathogen that is associated mostly with life-threatening infections in highly debilitated patients and nosocomial outbreaks (5, 26, 29). In addition to their ability to survive for long-term periods in adverse environmental conditions, these organisms display numerous intrinsic and acquired antimicrobial mechanisms, such as changes in outer membrane proteins, overexpression of endogenous efflux pumps, chromosomal and plasmid-acquired β-lactamases, aminoglycoside-modifying enzymes, and fluoroquinolone resistance, among others (5). The genetic flexibility and high adaptability of this organism have resulted in the rapid and global emergence over the last few years of multidrug-resistant (MDR) *A. baumannii* strains resistant to most classes of antimicrobial drugs, including carbapenems (10). Carbapenem resistance in *A. baumannii* is most often mediated by acquired β-lactamases of class D (OXA-23-like, OXA-40-like, and OXA-58-like) and less frequently by metallo-β-lactamases (MBLs) (24). In addition, the low outer membrane permeability and/or the upregulation of intrinsic efflux systems are also contributory factors toward carbapenem resistance (24). Very recently the Ambler class A of the GES and KPC (carbapenemase) types have also been reported for *A. baumannii* (14, 25).

bla_{GES} genes are usually carried on integrons found in various species, predominantly *Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa*, and these resistance determinants have been reported in several countries in Europe, Asia, South America, and South Africa (18). Sixteen GES variants differing by one to three amino acid residues have been reported (18) (http://lahey.org/studies/other.asp). GES β-lactamases exhibit extended-spectrum properties, hydrolyzing oxyimino-cephalosporins. However, differences in the substrate spectra have been observed. Variants with either Gly to Asn (GES-2) or Gly to Ser (GES-4, -5, and -6) at Ambler position 170 are of special clinical importance since they exhibit increased carbapenemase activity compared to other GES enzymes (18). In addition GES-4, -5, and -6 also hydrolyze cefoxitin. Also Gly243Ser substitutions as observed with GES-9 expand the hydrolysis spectrum to aztreonam (21). Following the recent report of GES-11 β-lactamase in one *A. baumannii* isolate in France presenting a Gly243Ala substitution, we describe in this report the emergence of three different variants, including a novel GES variant, GES-14, that were identified during a screening for acquired carbapenemase genes in multidrug-resistant *A. baumannii* isolates collected from January 2008 to December 2009 from 18 Belgian hospitals.

MATERIALS AND METHODS

Bacterial strains. A phenotypic and genotypic surveillance study of β-lactam resistance was conducted in Belgium between January 2008 and December 2009. The participating hospitals ($n = 90$) were requested, on a voluntary basis, to send to the reference bacteriology laboratory of UCL Mont-Godinne University hospital all consecutive clinical isolates of multidrug-resistant *Acinetobacter* species (resistance to at least three classes of antibiotics among penicillins, carbapenems,

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aminoglycosides, and/or fluoroquinolones) with their corresponding susceptibility report and basic epidemiologic information. Identification of the isolates as *Acinetobacter baumannii* was initially determined by VITEK2 Gram-negative identification cards and confirmed by mass spectrometry using MALDI-TOF and MALDI Biotyper version 2.0 software (Bruker Daltonics) and the specific PCR targeting the bla_{OXA-51} gene (27).

K. pneumoniae ORI-1 (23), *Enterobacter cloacae* CHE-1 (22), and *P. aeruginosa* DEJ (21), producing GES-1, GES-5, and GES-9, respectively, were used in this study. Electro-competent *E. coli* TOP10 (Invitrogen, Eragny, France) and *A. baumannii* CIP7010 (Pasteur Institut, Paris, France) and N9040364 (wild-type clinical isolate) were used as recipients in electroporation experiments (16). Sodium azide *E. coli* J53Az^R and rifampin-resistant *A. baumannii* CIP7010 strains were used for conjugation experiments. *E. coli* 50192 was used as a reference strain for plasmid extraction (16). The PCR-Script Cam cloning vector was used for PCR cloning experiments (Stratagene, Massy, France).

Antimicrobial agents and MIC determinations. Antibiograms were determined by the disc diffusion method against a panel of 16 antimicrobial agents and MICs of aztreonam, ceftazidime, cefepime, piperacillin-tazobactam, imipenem, meropenem, doripenem, ciprofloxacin, amikacin, gentamicin, tobramycin, and colistin were determined by Etest (AB Biodisk, Solna, Sweden) on Mueller-Hinton agar (bioMérieux, Marcy-L'Etoile, France) and interpreted as recommended by the Clinical and Laboratory Standards Institute (CLSI) and by the European Committee for Antimicrobial Susceptibility Testing (EUCAST) (http://www.eucast.org/). All plates were incubated at 37°C for 18 h.

PCR detection of *bla* genes. To detect the presence of the most common carbapenemases, multiplex PCR was performed with specific primers targeting $bla_{OXA-51-like}$, $bla_{OXA-23-like}$, $bla_{OXA-40-like}$, and $bla_{OXA-58-like}$ carbapenemases and the MBLs bla_{IMP} and bla_{VIM} as previously described (2). The presence of the insertion element IS*Aba1* upstream of $bla_{OXA-51-like}$ and bla_{ADC} was investigated by PCR (1). PCR screening with family-specific β-lactamase primers for GES600FW (5′-CTG GCA GGG ATC GCT CAC TC-3′), GES600RV (5′-TTC CGA TCA GCC ACC TCT CA-3′), KPC, OXA-48, VEB, and PER was also performed as previously described (8, 17). The genetic context of GES was obtained with a primer located at the 5′ end inside the integrase I coding gene (primer intI, 5′-CAG TGG ACA TAA GCC TGT TC-3′) and with primer CSRV (12) combined with the primers GESFW600 and GESRV600. Selected PCR products were sequenced by external service (Macrogen Inc., Seoul, South Korea), and sequence alignment and analysis were done online by using the BLAST program at www.ncbi.nlm.nih.gov.

PFGE. Pulsed-field gel electrophoresis (PFGE) was performed on all *A. baumannii* isolates as previously described, except that DNA was digested with ApaI (Invitrogen, Merelbeke, Belgium) and PFGE separation conditions were 1 to 10 s for 13 h and then 10 to 15 s for 10 h (3). PFGE patterns were analyzed using BioNumerics software (Applied Maths, Kortrijk, Belgium), and clonal relatedness was determined following the classification criteria previously described (3). PFGE types included profiles showing 0 to 6 DNA fragment differences corresponding generally to a ≥80% level of Dice similarity and were designated by numerals.

Plasmid content, mating out, and electroporation experiments. Direct transfer of resistance into azide-resistant *E. coli* J53 and rifampin-resistant *A. baumannii* CIP7010 was attempted as previously reported (9, 19). Plasmids were introduced by electroporation into *E. coli* TOP10 and *A. baumannii* CIP7010 and N9040364 (9) using a Gene Pulser II (Bio-Rad, Marnes-la-Coquette, France), as previously described. Electrotransformants were selected on brain heart infusion (BHI) agar medium containing ticarcillin at 100 μg/ml.

Natural plasmids were extracted using the Kieser extraction method (11) or with a Qiagen plasmid DNA maxi kit. Plasmid extracts were subsequently analyzed by electrophoresis on a 0.7% agarose gel. One microgram of natural plasmids was digested with 10 U of HindIII and 10 U of EcoRI (New England Biolabs, Hitchin, United Kingdom) at 37°C for 1 h. Restricted fragments were separated on a 0.8% agarose gel in 0.5× Tris-borate-EDTA (TBE) (44.5 mM Tris, 44.5 mM boric acid, 1 mM EDTA, pH 8.3) buffer containing 0.5 μg/ml of ethidium bromide for 1 h at 150 V and visualized under UV light.

Cloning experiments of GES β-lactamase genes. Whole-cell DNAs were extracted as described previously (15). The bla_{GES} genes from *K. pneumoniae* ORI-1, *E. cloacae* CHE-1, *P. aeruginosa* DEJ, *A. baumannii* 09027, *A. baumannii* 09135, and *A. baumannii* 09534, producing GES-1, GES-5, GES-9, GES-11, GES-12, and GES-14, respectively, were PCR amplified using the *Pfu* thermostable polymerase (Stratagene, Massy, France). The two primers used were GES-CasA, which starts at the beginning of the *ges* gene cassette, thus including the native consensus ribosomal binding site (RBS) 5 bp upstream of the ATG start codon (5′-TTAGACGGGCGTACAAAGAT-3′), and GES-CasB, which matched to the end of the bla_{GES} gene (5′-CACCTGAGTTAAGCCGCGGT-3′). These PCR fragments were then cloned into pPCRScriptCam, downstream of the pLac promoter, yielding plasmids pGES-1, -5, -9, -11, -12, and -14 that contained the respective alleles. The sequences of the cloned PCR-generated DNA fragments were confirmed by complete resequencing on both strands.

Recombinant plasmids were transformed by electroporation into *E. coli* Top10. Antibiotic-resistant colonies were selected onto Trypticase soy (TS) agar plates containing amoxicillin (50 μg/ml) and chloramphenicol (30 μg/ml). Recombinant plasmid DNAs were extracted with a Qiagen plasmid DNA maxi kit (Qiagen, Antwerp, Belgium) and analyzed by restriction endonuclease digestions (Amersham Biosciences, Orsay, France) and agarose gel electrophoresis (Invitrogen, Cergy Pontoise, France).

Specific activity. β-Lactamase extracts were obtained by sonication as described previously (20). The specific β-lactamase activity (nmol/min/mg) of the extracts was measured by UV spectrophotometry (ULTROSPEC 2000 spectrophotometer; Amersham Pharmacia Biotech, Orsay, France) as described previously (20). The specific β-lactamase activities were obtained with 100 μM aztreonam, ceftazidime, cefoxitin, and imipenem as substrates (21). The total protein content was measured with the Bio-Rad DC protein assay kit (Bio-Rad, Marnes-la-Coquette, France).

Nucleotide sequence accession numbers. The nucleotide sequences of bla_{GES-11}, bla_{GES-12}, and bla_{GES-14} have been assigned to the GenBank nucleotide database under the accession numbers HM622144 (GES-11), HM622145 (GES-12), and GU207844 (GES-14).

RESULTS

Origin of *A. baumannii* isolates and antimicrobial susceptibility. From a total of 125 multidrug-resistant *Acinetobacter* isolates originating from 18 Belgian hospitals (20% of the participating hospitals) between January 2008 to December 2009, 9 (7.2%) were identified as GES positive by PCR. These isolates were detected in 6 different hospitals (4 located in Brussels, 1 in Flanders, and 1 in the Wallonia region of Belgium).

The susceptibility tests showed that colistin had the lowest MICs of the tested antibiotics and was the only antimicrobial agent retaining *in vitro* activity against these strains (Table 1). High-level resistance to fluoroquinolones, aminoglycosides, and to the β-lactam antibiotics, including carbapenem, was observed.

Clinical data. The patient's pertinent clinical information is shown in Table 2. There were six male and three female patients, with an average age of 45 years (ranging from 5 to 79 years). Eight isolates were identified from patients in the intensive care units or in burn units (4 patients each), and one isolate was identified from a patient in a general surgical ward; isolates were either from wounds or abscesses (five isolates), endotracheal aspirates (two isolates), or pleural fluid or blood (one isolate each). The average hospital length of stay was 37 days (range, 9 to 78 days).

Six patients had skin and soft tissue infections with or without sepsis, two patients had ventilator-associated pneumonia (one with empyema), and one was colonized only (asymptomatic intestinal carriage). All patients had significant comorbidity conditions, such as chronic lung disease, cardiovascular disease, severe burns, or traumatic war injuries. Six patients had been transferred from hospitals in countries abroad (Turkey [$n = 2$], Egypt [$n = 2$], and Palestinian territories [Gaza] [$n = 2$]) (Table 2). At one center (CHA) a nosocomial outbreak occurred in a burn unit following the transfer of a patient with extensive burn wounds who had been transferred from a hospital in Cairo, Egypt. The crude mortality rate for the nine patients was 33%. All but one patient (the colonized patient F) had been treated with multiple antibiotic courses before their

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TABLE 1. MICs of selected antibiotics of GES-positive *A. baumannii* isolates (A to I) and recipient/electroporant *A. baumannii* strains

Patient[a]	MIC (μg/ml)[b]															
	TIC	PIP	TCC	TZP	ATM	CAZ	FEP	IPM	MEM	DORI	COL	CIP	GEN	TOB	AMI	SXT
A	>256	>256	>256	128	>256	>256	>256	32	32	6	0.5	>32	12	>32	>32	>128/8
B	>256	>256	>256	128	>256	>256	>256	8	12	4	0.5	>32	>32	>32	>32	>128/8
C	>256	>256	>256	256	>256	>256	>256	>32	>32	8	0.5	>32	>32	>32	>32	>128/8
D	>256	>256	>256	256	>256	>256	>256	>32	>32	32	1	>32	>32	>32	32	>128/8
E	>256	>256	>256	256	>256	>256	>256	>32	>32	32	0.5	>32	>32	>32	24	>128/8
F	>256	>256	>256	256	>256	>256	>256	>32	>32	32	0.5	>32	>32	>32	32	>128/8
G	>256	>256	>256	256	>256	>256	>256	>32	>32	12	0.5	>32	>32	>32	16	>128/8
H	>256	>256	>256	128	256	>256	128	>32	>32	6	0.5	>32	16	>32	>32	>128/8
I	>256	>256	>256	128	>256	>256	>256	12	16	3	0.5	>32	32	>32	>32	>128/8
N9040364 recipient	8	16	16	8	16	4	2	0.5	0.5	0.25	0.5	0.125	1	1	4	8/0.5
TrCGES-11	>256	128	>256	64	>256	>256	>256	4	8	2	0.5	0.125	4	16	>32	>128/8
TrDGES-12	>256	>256	256	128	>256	>256	>256	6	32	4	0.5	0.125	32	32	>32	>128/8

[a] N9040364, wild-type *A. baumannii* used as recipient strain for electroporation. TrC/DGES-11 and -12, electroporant obtained from plasmid preparation of isolates recovered from patients C and D.

[b] TIC, ticarcillin; PIP, piperacillin; TZP, piperacillin + tazobactam; ATM, aztreonam; CAZ, ceftazidime; TCC, ticarcillin + clavulanate; FEP, cefepime; IPM, imipenem; MEM, meropenem; DORI, doripenem; COL, colistin; CIPRO, ciprofloxacin; GEN, gentamicin; TOB, tobramycin; AMI, amikacin; SXT, trimethoprim-sulfamethoxazole.

transfer or during hospitalization (data not shown). Four patients were not treated, two patients were treated with colistin in association with a carbapenem (one of them also received amikacin), one patient was treated with an association of meropenem and ciprofloxacin, and the two remaining patients were treated with amoxicillin plus clavulanate.

Molecular analysis of GES *A. baumannii* isolates. PFGE analysis revealed that the GES-positive *A. baumannii* isolates clustered into 6 different PFGE types (Table 2). All the *A. baumannii* isolates, except those outbreak related and recovered from the CHA center, displayed distinct PFGE types. Interestingly, the PFGE profile of the isolate recovered from patient A presented more than 80% similarity (but 9 DNA fragment differences) with the European clone I (4) (Fig. 1), and it displayed a PFGE type, 39, identical to that of the OXA-23-producing *A. baumannii* strains formerly recovered in a Belgian hospital in 2007 (1) (data not shown).

The PCR results obtained with family-specific ß-lactamase primers revealed that all isolates were positive for $bla_{\text{GES-like}}$ genes and for $bla_{\text{OXA-51-like}}$ groups (OXA-82 [four isolates], OXA-69 [two isolates], and OXA-51, OXA-66, OXA-94 [one isolate each]) but that they were negative for all acquired carbapenem-hydrolyzing bla_{OXA} genes (OXA-23, OXA-40, and OXA-58 groups) as well as for bla_{IMP} and bla_{VIM} genes. IS*Aba*1 was detected upstream of the bla_{ADC} gene in seven isolates but never upstream of the $bla_{\text{OXA-51-like}}$ genes (Table 2).

Sequencing of bla_{GES} genes and subsequent amino acid sequence deduction revealed the presence of novel GES variants. GES-11, described for one single isolate from France, was found in four isolates presenting a Gly243Ala substitution compared to GES-1 (14). GES-12, identified in all four *A. baumannii* isolates detected at CHA, presented two substitutions, Thr237Ala and Gly243Ala, compared to GES-1. Finally, GES-14 was identified in one *A. baumannii* isolate from patient H transferred from Turkey and presented two substitutions, Gly170Ser and Gly243Ala, compared to the GES-1 sequence.

Genetic environment of bla_{GES} genes. Sequence analysis of the close genetic context of bla_{GES} genes revealed that they were all part of a class 1 integron containing, downstream, the *aac(6′)*-Ib gene encoding an aminoglycoside, 6′-*N*-acetyltransferase, which modifies amikacin and tobramycin, and the *dfrA7* resistance gene as described by Moubareck et al. (14) and differing only by the point mutations encountered in the bla_{GES} alleles. Plasmid extraction experiments identified plasmids in $bla_{\text{GES-11}}$- and $bla_{\text{GES-12}}$-expressing *A. baumannii* (data not shown) but not in $bla_{\text{GES-14}}$ isolates despite several attempts using different extraction methods. In addition, total DNA extracts of GES-14-expressing *A. baumannii* were repeatedly unable to transform a competent wild-type *A. baumannii* clinical isolate (no. N9040364), further suggesting that the $bla_{\text{GES-14}}$ gene was chromosomally located. On the other hand, GES-11- and GES-12-expressing transformants could be obtained by electroporation, confirming the plasmid location of the $bla_{\text{GES-11}}$ and of the $bla_{\text{GES-12}}$ genes.

The GES-11 and GES-12 *A. baumannii* electroporants were highly resistant to all β-lactam antibiotics, including the carbapenems (with imipenem, meropenem, and doripenem MICs ranging between 4 and 32 μg/ml), to trimethoprim, and to aminoglycosides but not to ciprofloxacin. Double digestion of plasmid extract (about 75 kb) from these transformants with EcoRI and HindIII restriction enzymes revealed a very similar banding pattern for pGES-11 and pGES-12 (4 and 5 band differences), suggesting a common origin for these plasmids. As an example, restriction profiles of pGES-11 and pGES-12 (originating from isolates 9027 and 9035, respectively) are shown in Fig. 2.

Biochemical properties. The resistance profile and specific activity of each GES-type-expressing *E. coli* clone (Table 3) show variable activity against ceftazidime, aztreonam, cefoxitin, and imipenem. GES-11 and GES-12 variants shared with GES-9 a high hydrolytic activity against ceftazidime, the specific activity of GES-12 (85.2 nmol/min/mg) being over 10 times higher than that of GES-1 (6.5 nmol/min/mg), GES-5 (activity too low to be measurable), and GES-14 (2.0 nmol/

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min/mg). Further, GES-11 and -12 variants did also hydrolyze aztreonam but had very weak hydrolytic activity against imipenem and no measurable activity against cefoxitin.

GES-14-expressing *E. coli* displayed a resistance profile (MIC values) similar to that of the GES-5-expressing clone (a carbapenem and cefoxitin hydrolyzing isoform) (28). It was able to hydrolyze imipenem and cefoxitin as efficiently as GES-5, while it displayed a very weak activity (like that of GES-5) against ceftazidime and aztreonam. Nevertheless, as observed for GES-5, *E. coli* expressing GES-14 remained susceptible to imipenem, suggesting the involvement of additional mechanisms in the carbapenem resistance observed in the GES-14-producing *A. baumannii* clinical isolate.

DISCUSSION

Whereas acquired resistance to carbapenem in *A. baumannii* was reported mainly as a consequence of acquired class D carbapenem hydrolyzing β-lactamases and to a lesser extent to class B MBLs (24), class A β-lactamase, such as KPC and GES, were until now essentially detected in *Enterobacteriaceae* and in *P. aeruginosa* (13). Very recently, a new GES-11 isoform was detected for the first time in one multidrug-resistant and carbapenem-resistant *A. baumannii* isolate recovered in France (14). Our data further point out the fact that several GES-expressing *A. baumannii* strains might now be emerging independently in different areas in the world and indicate that, along with KPC-type β-lactamases (that were also recently reported in an *Acinetobacter* isolate from Puerto Rico [25]), class A β-lactamases may now account as an additional mechanism of resistance to carbapenems in *A. baumannii*.

Three different GES isoforms were detected in our study: GES-11, -12, and -14, with GES-12 and GES-14 differing from GES-11 by a single amino acid substitution. The GES-11- and GES-12-associated plasmids, although displaying very similar banding patterns by restriction endonuclease analysis, were found in isolates with different PFGE types, suggesting horizontal plasmid transfer between different *A. baumannii* isolates. While GES-producing isolates were already discovered in *Enterobacteriaceae* isolates in Belgian hospitals (P. Bogaerts, unpublished data), their genetic contexts (*aacA4* $bla_{\text{GES-like}}$ *smr dhfrI aadA1*), as well as the GES isoforms of the *A. baumannii* isolates found here, were clearly different from those previously observed with *Enterobacteriaceae* isolates, thus ruling out the horizontal transfer of bla_{GES}-harboring plasmids from the *Enterobacteriaceae* to *A. baumannii* occurring in Belgian hospitals.

Moreover, the fact that all GES-producing *A. baumannii* isolates were recovered over a short period of time from patients transferred from different countries suggests that such isolates might be more widespread than it is currently appreciated.

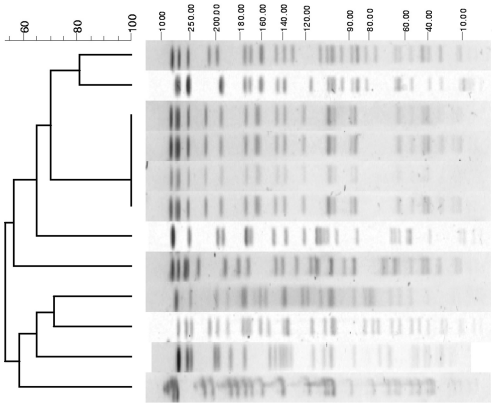
GES-11, GES-9, and GES-1 differ by one single amino acid substitution at Ambler position 243 (Gly243Ser for GES-9 or Gly243Ala in GES-11), a position known to be involved in the extension of the hydrolysis spectrum to aztreonam (21). GES-11 and GES-9 display a higher hydrolytic activity against ceftazidime and aztreonam than GES-1, but they do not hydrolyze cefoxitin or imipenem, confirming the involvement of Ser and Ala243 in the hydrolysis of aztreonam and ceftazidime.

TABLE 2. Case history and characteristics of *A. baumannii* clinical isolates

Patient	Strain	Country of origin	Center	Status	Date of hospitalization (mo/day/yr)	Date of isolation (mo/day/yr)	Length of stay before isolation (days)	Age (yr)	Sex[a]	Ward[b]	Underlying disease	Site of isolation[c]	ESBL	OXA-51 like	ADC	PFGE type
A	8265	Turkey	BRU-1	Infected	03/31/2008	03/31/2008	0	64	M	ICU	Prosthesis infection	Prosthesis biopsy	GES-11	OXA-69	ISAba1-ADC-11	39
B	10117	Palestinian territories	BRU-2	Infected	01/14/2009	01/14/2009	0	5	F	Pediatry	War injury	Wound	GES-11	OXA-51 like	ADC-2	13
C	9027	Palestinian territories	BRU-3	Infected	01/14/2009	01/14/2009	0	14	M	ICU	War injury	Wound	GES-11	OXA-69	ISAba1-ADC-11	7
D	9135	Egypt	CHA	Infected	03/26/2009	03/30/2009	4	32	M	Burn unit	Highly burned	Wound	GES-12	OXA-82	ISAba1-ADC7	49
E	9252	France	CHA	Infected	05/20/2009	05/22/2009	2	62	M	Burn unit	Highly burned	ETA	GES-12	OXA-82	ISAba1-ADC7	49
F	9463	Belgium	CHA	Colonized	06/01/2009	06/08/2009	7	52	M	Burn unit	Burned	Rectal swab/ETA	GES-12	OXA-82	ISAba1-ADC7	49
G	9465	Belgium	CHA	Infected	06/02/2009	06/18/2009	16	79	F	Burn unit	Burned	Rectal swab/Blood	GES-12	OXA-82	ISAba1-ADC7	49
H	9534	Turkey	BRU-4	Infected	08/30/2009	09/08/2009	9	53	M	ICU	Empyema	Pleural liquid	GES-14	OXA-66	ISAba1-ADC-30	18
I	9536	Egypt	HAS	Infected	09/20/2009	09/20/2009	0	35	F	General ward	Hernia	Post operative wound abscesses	GES-11	OXA-94	ADC-54	3

[a] M, male; F, female.
[b] ICU, intensive care unit.
[c] ETA, endotracheal aspirates.

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Strain	Origin	GES	PFGE type
08265	Turkey	11	39
RUH0875	Europe		4
09135	Egypt	12	49
09252	France	12	49
09463	Belgium	12	49
09465	Belgium	12	49
LUH05875	Europe		8
09027	Gaza	11	7
09534	Turkey	14	18
RUH0134	Europe		16
10117	Gaza	11	13
09536	Egypt	11	3

FIG. 1. Dendrogram of PFGE patterns of *A. baumannii* clinical isolates and reference strains of European clones I, II, and III. RUH0875, RUH0134, and LUH05875 correspond to European clones I, II, and III, respectively (4).

GES-12 differs from GES-11 by a single Thr237Ala substitution, increasing significantly the levels of hydrolysis of ceftazidime and of aztreonam and hence rendering GES-12 a very potent ceftazidimase. This mutation was observed for the first time with the GES-type enzyme. Nevertheless in another class A β-lactamase type (CTX-M-4), the position 237 was described as modifying the hydrolysis spectrum of the enzyme (7) and hence is suspected to participate in the shape of the catalytic pocket of the class A enzyme. Although both GES-11 and GES-12 possess only a very weak hydrolytic activity against imipenem (with a level of hydrolysis comparable to that of GES-1 and of GES-9), it seems sufficient to alter the carbapenem susceptibility in *A. baumannii* as observed with the transformants, and it may represent an additional mechanism of resistance in this organism.

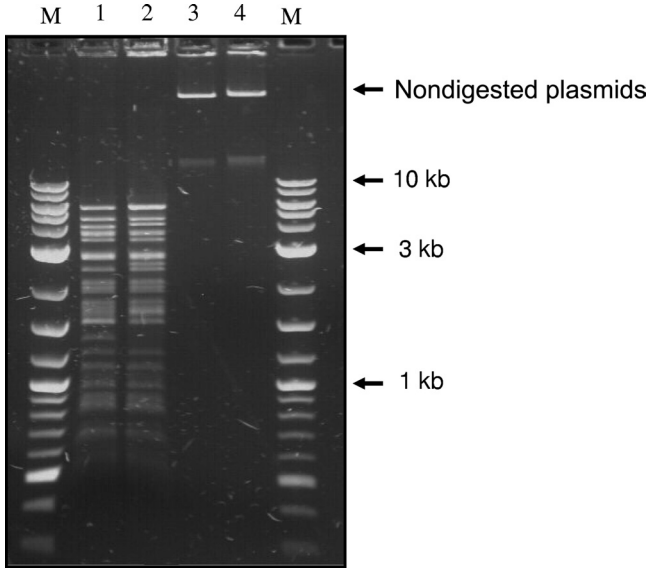


FIG. 2. Crude plasmid extracts and banding patterns of plasmids pGES-11 and pGES-12 corestricted with HindIII and EcoRI. Lane M, 2 log DNA ladder (0.1 to 10.0 kb); lane 1, pGES-11 (from isolate 9027) extract digested with EcoRI and HindIII; lane 2, pGES-12 (from isolate 9135) extract digested with EcoRI and HindIII; lane 3, undigested pGES-11 crude extract; lane 4, undigested pGES-12 crude extract.

Finally, GES-14 also differs from GES-11 by a single mutation, Gly170Ser, which results in a marked (almost 10-fold) increase in the level of hydrolysis of imipenem and of cefoxitin, making GES-14 the first true GES carbapenemase recovered in *A. baumannii*. A single Gly170Ser substitution was previously shown to be responsible for the expansion of the breadth of hydrolytic activity to carbapenem and to cefoxitin in GES-5 (28). It was recently demonstrated by Frase et al. that this Gly170 substitution in GES enzymes had a major impact on the turnover of imipenem during the catalytic cycle through a more than 5,000-fold enhancement in the rate of acetylation (6).

The expected increase of aztreonam hydrolysis consecutively to mutation Gly243Ala at position 243 is not observed in GES-14 possibly because of the counter effect of Ser170, which also diminishes the hydrolysis efficiency of GES-5 and GES-14 against ceftazidime.

The analysis of hydrolysis activity of the GES-expressing *E. coli* extracts confirmed that only GES-14 harbored a carbapenemase activity. For clinical isolates expressing GES-11 and GES-12, the observed resistance to carbapenem is probably due to additional mechanisms, such as impermeability (5) combined with ADC overexpression due to IS*Aba*1. This hypothesis is further suggested by the observation that the GES-11 expressing isolates harboring the insertion sequence IS*Aba*1 upstream of bla_{ADC} (isolates from patients A and C) present a higher level of resistance to carbapenems than those which do not overexpress ADC (isolates from patients B and I). The involvement of OXA-51-like enzymes in carbapenem-resistance could be ruled out since IS*Aba*1 was not detected upstream to these genes.

In conclusion, our results suggest that GES β-lactamases might now be emerging in *A. baumannii*. The emergence of carbapenem-hydrolyzing enzymes, such as GES-14, highlights the propensity of *A. baumannii* to acquire new resistance mechanisms, and it further underlines the absolute necessity to control inpatients transferred from hospitals in foreign coun-

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TABLE 3. Resistance profile and specific activity of cloned GES alleles expressed in *E. coli* TOP10[a]

Antibiotic	Parameter	GES-1	GES-5	GES-9	GES-11	GES-12	GES-14	*E. coli* Top10
IPM	Diam (mm)	36	26	36	34	35	30	36
	Resistance	S	S	S	S	S	S	S
	MIC (μg/ml)	0.25	1.5	0.19	0.38	0.25	0.5	0.12
	Sp act	3.7	20.9	2.5	1.8	2.4	16.7	ND
CAZ	Diam (mm)	13	27	11	9	6	16	27
	Resistance	R	S	R	R	R	R	S
	MIC (μg/ml)	32	2	>256	64	>256	16	0.25
	Sp act	6.5	ND	25.2	41.0	85.2	2.0	ND
ATM	Diam (mm)	31	38	28	27	25	33	38
	Resistance	S	S	S	S	I	S	S
	MIC (μg/ml)	0.25	0.016	1	1	2	0.125	0.016
	Sp act	11.4	3.8	15.9	17.9	38.5	4.4	ND
FOX	Diam (mm)	30	16	34	32	32	20	34
	Resistance	S	I	S	S	S	I	S
	MIC (μg/ml)	2	32	1	1	1	12	1
	Sp act	ND	10.9	ND	ND	ND	12.2	12.2

[a] Sp act, specific activity (nmol/min/mg) of a crude extract of each GES-expressing *E. coli* clone and recipient *E. coli* TOP10. The measurements were performed on three independent extracts, and the differences in the activities were below 10%. ND, not detectable; IPM, imipenem; CAZ, ceftazidime; ATM, aztreonam; FOX, cefoxitin; R, resistant; S, susceptible; I, intermediate.

tries known to have a high prevalence of resistance mechanisms.

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