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GES-11, a Novel Integron-Associated GES Variant in *Acinetobacter baumannii*▽

Carole Moubareck,[1] Sylvie Brémont,[1] Marie-Christine Conroy,[2] Patrice Courvalin,[1]* and Thierry Lambert[1]

Institut Pasteur, Unité des Agents Antibactériens, Centre National de Référence de la Résistance aux Antibiotiques, 75724 Paris Cedex 15,[1] and Laboratoire de Bactériologie, CHU, 54035 Nancy Cedex,[2] France

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New extended-spectrum β-lactamase GES-11 was detected in *Acinetobacter baumannii* BM4674. The enzyme conferred resistance to β-lactams, including aztreonam, and reduced susceptibility to carbapenems. The structural gene was part of a class 1 integron borne by self-transferable plasmid pIP847. GES-type β-lactamases have not been reported previously in *A. baumannii*.

Acinetobacter baumannii is a predominant species associated with outbreaks of nosocomial infections, such as pneumonia, urinary tract infections, septicemia, and meningitis. Its clinical significance is due to its ability either to upregulate indigenous efflux pumps (5) or to acquire numerous resistance mechanisms (7) that lead to therapeutic failure. A rapid, global emergence of *A. baumannii* strains resistant to all β-lactams, including carbapenems, aminoglycosides, quinolones, tetracyclines-glycylcyclines, polymyxins, and trimethoprim-sulfamethoxazole, has been observed (1). *A. baumannii* clinical specimens resistant to all known antibiotics, including polymyxins, have been reported, illustrating the genetic flexibility of this pathogen (13).

Resistance to β-lactams in *A. baumannii* is due mainly to the production of β-lactamases but can also result from several other mechanisms, including changes in outer membrane proteins, overexpression of multidrug efflux pumps, and alterations in the affinity or production of penicillin-binding proteins (9). β-Lactamases with carbapenemase activity, i.e., class D carbapenem-hydrolyzing oxacillinases or, less frequently, class B metallo-β-lactamases, represent the major clinical concern. To the best of our knowledge, Ambler class A carbapenemases KPC, GES, SME, NMC, and IMI have not yet been reported in *A. baumannii* (18).

A. baumannii BM4674 was isolated from the tibia fracture of a patient hospitalized at the Centre Hospitalier Universitaire in Nancy, France, in September 2008. MICs of antimicrobial agents for this strain were determined by Etest (AB Biodisk, Combourg, France) on Mueller-Hinton agar (bioMerieux, Marcy l'Etoile, France), and the breakpoints delivered by the Comité de l'Antibiogramme de la Société Française de Microbiologie were used for interpretations of results (3). *A. baumannii* BM4674 was resistant to all β-lactams, with decreased susceptibility to carbapenems ($MIC_{imipenem}$ = 4 μg/ml; $MIC_{meropenem}$ = 8 μg/ml). It was also resistant to aminoglycosides, co-trimoxazole, quinolones, and chloramphenicol but remained susceptible to tetracyclines-glycylcyclines, colistin, and rifampin (rifampicin).

The transfer of β-lactam resistance from *A. baumannii* BM4674 to *A. baumannii* BM4652 was performed by conjugation on solid medium, as described previously (11). Transconjugants selected on agar containing apramycin (80 μg/ml) and ceftazidime (16 μg/ml) were obtained at a high frequency of ca. 1×10^{-3} per recipient cell. They exhibited a broad spectrum of resistance to β-lactams, including aztreonam, diminished susceptibility to imipenem (MIC = 0.75 μg/ml) and meropenem (MIC = 1.5 μg/ml) compared to that of the recipient ($MIC_{imipenem}$ = 0.125 μg/ml; $MIC_{meropenem}$ = 0.094 μg/ml), and synergism between cefotaxime and clavulanic acid, suggesting production of an extended-spectrum β-lactamase (ESBL). The transconjugants were also resistant to amikacin, tobramycin, trimethoprim, and sulfonamides. Plasmid DNA extracted from *A. baumannii* BM4674 was electrotransformed into *A. baumannii* BM4454 and *Acinetobacter radioresistens* CIP1281. Transformants selected on agar containing ticarcillin (20 μg/ml) had a resistance phenotype to β-lactams and to the other drugs similar to that of the transconjugants. Analysis of plasmid DNA (2) from *A. baumannii* BM4674 and *A. baumannii* transformants by agarose gel electrophoresis following digestion with EcoRI or XbaI revealed the presence of plasmid pIP847 of ca. 90 kb (data not shown).

Total DNA of *A. baumannii* BM4674, selected transconjugants, and transformants was screened by PCR for the presence of bla_{VEB}, bla_{PER}, bla_{KPC}, bla_{GES}-type, bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-40}, and bla_{OXA-58} genes using laboratory-designed sets of primers (Table 1) and for the presence of bla_{TEM}, bla_{SHV}, $bla_{CTX-M-1}$-type, $bla_{CTX-M-2}$-type, $bla_{CTX-M-8}$-type, and $bla_{CTX-M-9}$-type genes, as described previously (10). Results (not shown) indicated that *A. baumannii* BM4674 harbored both bla_{OXA-58} and bla_{GES}-type genes, whereas the transconjugants and transformants carried only the bla_{GES}-type gene as part of plasmid pIP847.

The amplified bla_{GES}-type gene was cloned in pCR-Blunt vector (Invitrogen, Leek, The Netherlands) under the control of the *lac* promoter, generating the plasmid pAT517, which was electrotransformed in *Escherichia coli* Top10 and *E. coli* HB4 deficient in porins OmpF and OmpC (12). Transformants

* Corresponding author. Mailing address: Institut Pasteur, Unité des Agents Antibactériens, 25, rue du Docteur Roux, 75724 Paris Cedex 15, France. Phone: (33) (1) 45 68 83 20. Fax: (33) (1) 45 68 83 19. E-mail: patrice.courvalin@pasteur.fr.

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TABLE 1. Oligonucleotide primers used for PCR amplification of β-lactam resistance genes

bla gene	Primer (direction)[a]	Sequence (5′–3′)	Position[b]	Size (pb)	GenBank accession no.
PER	per (+)	CCTGACGATCTGGAACCTTT	157–176	715	EF535600.1
	per (−)	GCAACCTGCGCAAT(GA)ATAGC	872–853		
VEB	veb (+)	ATTTCCCGATGCAAAGCGT	188–206	542	AF010416
	veb (−)	TTATTCCGGAAGTCCCTGT	730–712		
GES	ges (+)	ATGCGCTTCATTCACGCAC	1–19	863	AF156486
	ges (−)	CTATTTGTCCGTGCTCAGGA	864–845		
KPC	kpc (+)	ATGTCACTGTATCGCCGTCT	1–20	881	AF297554
	kpc (−)	TTACTGCCCGTTGACGCCCA	882–863		
OXA-23	oxa23 (+)	ATGAATAAATATTTTACTTG	1–20	821	AJ132105
	oxa23 (−)	TTAAATAATATTCAGCTGTT	822–803		
OXA-24	oxa24 (+)	ATACTTCCTATATTCAGCAT	13–32	809	AJ239129
	oxa24 (−)	GATTCCAAGATTTCTAGCG	822–803		
OXA-40	oxa40 (+)	ATGAAAAAATTTATACTTCCTATA	1–24	819	AF509241
	oxa40 (−)	TTCCAAGATTTTCTAGCGAC	820–801		
OXA-58	oxa58 (+)	ATGAAATTATTAAAAATATTGAGT	143–166	840	AY570763
	oxa58 (−)	ATAAATAATGAAAAACACCCAA	983–962		

[a] +, primer forward; −, primer reverse.
[b] Refers to the first base of each β-lactamase gene.

E. coli Top10 (pAT517) and *E. coli* HB4 (pAT517) exhibited the ESBL phenotype and reduced susceptibility to carbapenems, as observed for transconjugants (Table 2). The MIC of imipenem for HB4 (pAT517) was 8 μg/ml, revealing that the expression of bla_{GES-11} in a porin-deficient *E. coli* strain could lead to imipenem resistance and likewise indicating the carbapenemase activity of GES-11.

Sequence determination of purified bla_{GES}-type amplicons revealed an 864-bp open reading frame. The deduced protein, designated GES-11, contained 287 amino acids and differed from GES-1 β-lactamase by a Gly-to-Ala substitution at Ambler position 243. A Gly-to-Ser change at this position has been previously reported in GES-9 (14). Substitution of the glycine at position 243 in GES-11 was associated with increased activity toward aztreonam, as had been observed for GES-9 (14). GES-11 did not have a substitution of the Gly170 residue that results in increased hydrolysis of imipenem as in GES-2, GES-4, GES-5, and GES-6 (17, 19, 20). The moles percent G+C content for bla_{GES-11} was 53.25%, a value higher than that of the genus *Acinetobacter*, which contains 38 to 39% G+C content (7). Using total DNA of *A. baumannii* BM4674 and of transformants BM4454 (pIP847) as a template and consensus primers for 5′-CS and 3′-CS ends of class 1 integrons (8), a 4,152-bp DNA fragment was obtained (Fig. 1). Sequence analysis revealed that the bla_{GES-11} gene was part of a class 1 integron containing, downstream, the *aac(6′)-Ib* gene encoding an aminoglycoside, 6′-*N*-acetyltransferase, type I, which modifies amikacin and tobramycin, and the *dfrA7* trimethoprim resistance gene. A deletion of 83 bp in the class 1 integrase gene revealed that this integron alone can no longer acquire or lose any resistance genes. The cassette organization differed from those of other GES-containing integrons (4, 6, 15, 21).

In addition to the finding of a novel GES-type variant as part of a class 1 integron, this work reports the emergence of GES-type ESBLs in *A. baumannii*. The occurrence of GES enzymes in this species is probably underestimated, since synergism between extended-spectrum cephalosporins and clavulanic acid may be masked by the presence of the intrinsic AmpC cephalosporinase and OXA-51-like oxacillinase that are frequently associated with other β-lactamases such as OXA-58

TABLE 2. MICs of β-lactams for *A. baumannii* and *E. coli* strains

β-Lactam	MIC (μg/ml) for indicated strain (plasmid):						
	A. baumannii			*E. coli*			
	BM4674	BM4652	BM4652 (pIP847)	Top10	Top10 (pAT517)	HB4	HB4 (pAT517)
Cefoxitin	192	8	12	6	6	96	>256
Ceftazidime	>256	1.5	>256	0.75	>256	0.5	>256
Cefepime	>256	0.19	64	0.032	2	0.5	96
Cefotaxime	>256	1	>256	0.064	>256	0.5	>256
Aztreonam	>256	1	>256	0.125	24	0.75	48
Imipenem	4	0.125	0.75	0.19	0.25	0.125	8
Meropenem	6	0.064	1.5	0.016	0.047	0.25	4

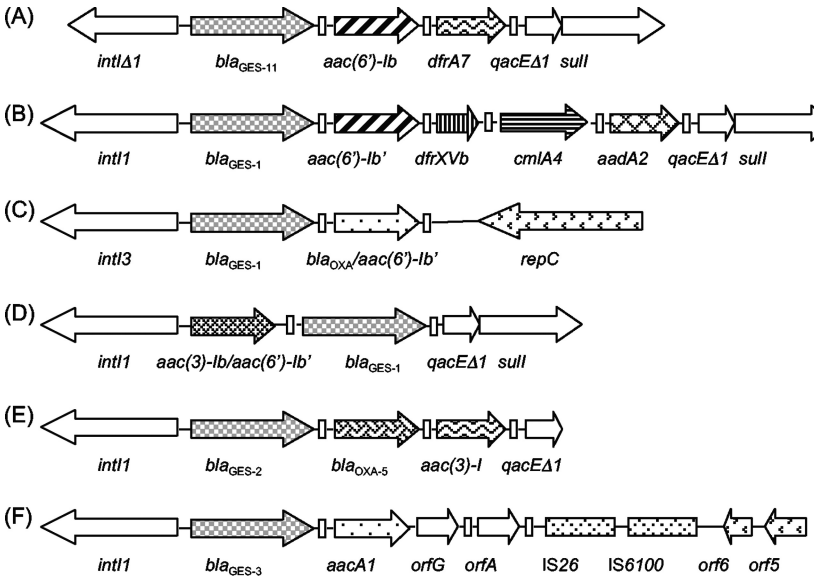

FIG. 1. Schematic representation of integrons containing bla_{GES}-like genes. Arrows represent coding sequences and indicate the direction of transcription. Rectangles represent *attC* sites. (A) bla_{GES-11} containing a class 1 integron of pIP847; (B) bla_{GES-1} containing a class 1 integron of pTK-1 (15); (C) bla_{GES-1} containing a class 3 integron of p22K9 (4); (D) bla_{GES-1} containing a class 1 integron of pC23 (6); (E) bla_{GES-2} containing a class 1 integron of p22K9 (GenBank accession no. AF326355); and (F) bla_{GES-3} containing a class 1 integron of pKGB525 (21).

(16). Genotypic screening is therefore required to assess the prevalence of GES-type enzymes in *A. baumannii*.

Nucleotide sequence accession number. The nucleotide sequence of the integron bla_{GES-11} is available in the GenBank nucleotide database under accession no. FJ854362.

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