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GES-2, a Class A β-Lactamase from *Pseudomonas aeruginosa* with Increased Hydrolysis of Imipenem

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***Pseudomonas aeruginosa* GW-1 was isolated in 2000 in South Africa from blood cultures of a 38-year-old female who developed nosocomial pneumonia. This isolate harbored a self-transferable ca. 100-kb plasmid that conferred an expanded-spectrum cephalosporin resistance profile associated with an intermediate susceptibility to imipenem. A β-lactamase gene, bla_{GES-2}, was cloned from whole-cell DNA of *P. aeruginosa* GW-1 and expressed in *Escherichia coli*. GES-2, with a pI value of 5.8, hydrolyzed expanded-spectrum cephalosporins, and its substrate profile was extended to include imipenem compared to that of GES-1, identified previously in *Klebsiella pneumoniae*. GES-2 activity was less inhibited by clavulanic acid, tazobactam and imipenem than GES-1. The GES-2 amino acid sequence differs from that of GES-1 by a glycine-to-asparagine substitution in position 170 located in the omega loop of Ambler class A enzymes. This amino acid change may explain the extension of the substrate profile of the plasmid-encoded β-lactamase GES-2.**

Clavulanic acid-inhibited extended-spectrum β-lactamases (ESBLs) conferring resistance to expanded-spectrum cephalosporins have been reported, first in *Enterobacteriaceae* and then in *Pseudomonas aeruginosa* (11, 23). Rare reports of TEM- and SHV-type ESBLs in *P. aeruginosa* are known (SHV-2a, TEM-4, TEM-24, and TEM-42 [15, 23, 31]), while they have been extensively described in *Enterobacteriaceae* (11). Three non-TEM-, non-SHV-type ESBLs have been reported in *P. aeruginosa*, i.e., PER-1, VEB-1, and OXA-18 β-lactamases (20, 25, 27, 38). The PER-1 β-lactamase gene is widespread in Turkey, although not reported as plasmid mediated in *P. aeruginosa* (39). VEB-1 β-lactamase, originally described in *Escherichia coli* and *Klebsiella pneumoniae* isolates in Vietnam, has been found in *P. aeruginosa* and enterobacterial isolates in Thailand (8, 29, 38).

We have recently identified another Ambler class A β-lactamase, GES-1, in a *K. pneumoniae* isolate in French Guiana (28). It was found to be remotely related to other ESBLs. This ESBL differs by two amino acid substitutions from IBC-1 β-lactamase recently found in an *Enterobacter cloacae* isolate in Greece (7). bla_{VEB-1}, bla_{GES-1}, and bla_{IBC-1} are plasmid located and are part of gene cassettes integrated into class 1 integrons (7, 28, 29).

Mobile cassettes contain genes most often mediating antibiotic resistance and a cassette recombination site, designated the 59-base element (59-be) (9, 10). The 59-be sites vary in length (57 to 141 bp) and structure, but they are all bounded by a core site (GTTRRRY) at the recombinant crossover point and an inverse core site (RYYYAAC) at the 3′ end of the inserted gene (4, 9). Integrons are genetic elements capable of integrating individual gene cassettes by a site-specific recombination mechanism that involves a DNA integrase, IntI; an integron-specific recombination site, *attI*; and 59-be (4, 9, 10). The 5′ conserved segment (5′-CS) of the integrons contains the integrase gene (*intI*) and the recombination site *attI1*. The 3′-CS of class 1 integrons carries the antisepsis resistance *qacEΔ1* gene; the *sul1* gene, which confers resistance to sulfonamides; and an open reading frame (ORF) of unknown function, ORF5 (26).

While analyzing carbapenem-resistant *P. aeruginosa* isolates from South Africa, we retained a *P. aeruginosa* isolate that was resistant to ceftazidime with an unusual substrate profile that included imipenem, according to preliminary analysis. Thus, the β-lactamase content of this strain was further characterized. A plasmid-mediated Ambler class A ESBL was identified with a substrate profile extended to imipenem but with hydrolysis rates lower than those of the chromosome-encoded carbapenem-hydrolyzing class A β-lactamases NmcA, SME-1/2, and IMI-1 identified in rare isolates of *E. cloacae* and *Serratia marcescens* (19, 21, 24, 33, 35) and of the recently reported plasmid-encoded β-lactamase KPC-1 from *K. pneumoniae* (40).

MATERIALS AND METHODS

Bacterial strains. *P. aeruginosa* clinical isolate GW-1 was identified with the API-20 NE system (bioMérieux, Marcy l'Etoile, France). *E. coli* DH10B was the host for cloning experiments, and in vitro-obtained rifampin-resistant *P. aeruginosa* PU21 was used as a recipient strain for conjugative transfer (30).

Susceptibility testing. Antibiotic-containing disks were used for routine antibiograms by the disk diffusion assay (Sanofi-Diagnostic Pasteur, Marnes-la-Coquette, France) as previously described (27). The double-disk synergy test was performed with disks containing ceftazidime and amoxicillin-clavulanic acid on Mueller-Hinton agar plates, and the results were interpreted as described previously (11). MICs were determined by an agar dilution technique with Mueller-Hinton agar (Sanofi-Diagnostic Pasteur) with an inoculum of 10^4 CFU, as described previously (27). All plates were incubated at 37°C for 18 h at ambient atmosphere. MICs of β-lactams were determined alone or in combination with

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a fixed concentration of clavulanic acid (2 μg/ml) and tazobactam (4 μg/ml). MIC results were interpreted according to the guidelines of the National Committee for Clinical Laboratory Standards (22).

PCR and hybridization experiments. Whole-cell DNA of *P. aeruginosa* GW-1 was extracted as described previously (27). This DNA was used as a template in standard PCR conditions (36) with a series of primers designed for the detection of class A β-lactamase genes and their extended-spectrum derivatives found in enterobacterial and *P. aeruginosa* isolates: bla_{TEM}, bla_{SHV}, $bla_{PER-1/-2}$, bla_{VEB-1}, $bla_{Toho-1/-2}$, bla_{SFO-1}, bla_{GES-1}, and $bla_{CTX-M-2}$ (8, 28). Similarly, a series of primers were designed for detection of genes coding for acquired carbapenem-hydrolyzing β-lactamases such as Van3 (5′-CCTGAGGGGATGACTAAA-3′) and Van6 (5′-GTTATGCACTAC GAAGGC-3′) for bla_{SME-1} (21); I4 (5′-CTA ATGAAATAG GAGTAC-3′) and I5 (5′-AACAGATTTCAATGGCAGG-3′) for bla_{NMC-A} (19), VIM-B and VIMF for $bla_{VIM-1/VIM-2}$ (30) and Imp-1 (5′-CT ACCGCAGCAGAGTCTTTGC-3′) and Imp-2 (5′-GAACAACCAGTTTTGC CTTAC C-3′) for bla_{IMP-1} (2). Since several β-lactamase genes are part of gene cassettes that are class 1 integron encoded, primers located in the 5′-CS (INT2F, 5′-TCTCGGGTAACATCAAGG-3′) and 3′-CS (5′-AAGCAGACTTGACCTG A-3′) regions were used for PCR amplifications (14). Southern hybridizations were performed as described by Sambrook et al. (36) using the ECL nonradioactive labeling and detection kit (Amersham Pharmacia Biotech, Orsay, France). Natural plasmid pGW-1 was hybridized with a PCR-generated probe consisting of the internal 860-bp PCR fragment for bla_{GES-1}, as described previously (28).

Cloning experiments, recombinant plasmid analysis, and DNA sequencing. The obtained PCR fragment (1.8 kb) with 5′-CS and 3′-CS integron primers was purified with a QIAquick column (Qiagen, Courtaboeuf, France) and cloned into the *Srf*I site of plasmid pPCR Cam SK(+) (Stratagene, Amsterdam, The Netherlands). Recombinant plasmids were selected onto Trypticase soy (TS) agar plates containing amoxicillin (100 μg/ml) and chloramphenicol (30 μg/ml). The cloned DNA fragment inserted into one of the recombinant plasmids (pLAP-1) was sequenced on both strands with an Applied Biosystems sequencer (ABI 377). The nucleotide and deduced amino acid sequences were analyzed and compared to sequences available over the Internet at the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov).

Plasmid study. Conjugation experiments were performed with *P. aeruginosa* GW-1 and in vitro-obtained rifampin-resistant *P. aeruginosa* strain PU21 in solid and liquid media at 37°C (27). Transconjugants were selected on TS agar plates containing 150 μg of rifampin per ml and 5 μg of ceftazidime per ml. Plasmid DNAs of *P. aeruginosa* GW-1 and transconjugant *P. aeruginosa* PU21 were extracted with the Qiagen plasmid DNA maxi kit and analyzed by electrophoresis with a 0.8% agarose gel (Gibco BRL-Life Technologies, Cergy-Pontoise, France), as previously described (36). Plasmid DNAs extracted from *E. coli* NCTC 50192 were used as size standards (6).

β-Lactamase purification and isoelectric focusing (IEF) analysis. Cultures of *E. coli* DH10B(pLAP-1) were grown overnight at 37°C in 4 liters of TS broth containing amoxicillin (100 μg/ml). β-Lactamase was purified with exactly the same protocol as that described for GES-1 (28). Briefly, the β-lactamase extract was sonicated, cleared by ultracentrifugation, loaded on a Q-Sepharose column, and eluted with a linear NaCl gradient. The purity of the enzyme was estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis (36).

IEF analysis was performed with an ampholine polyacrylamide gel (pH 3.5 to 9.5), as described previously (27). Purified β-lactamase from a culture of *E. coli* DH10B(pLAP-1) and nonpurified extracts of 100-ml cultures of *P. aeruginosa* GW-1 and one of its *P. aeruginosa* PU21 transconjugants were submitted to IEF analysis. The focused β-lactamases were detected by overlaying the gel with 1 mM nitrocefin (Oxoid, Dardilly, France) in 100 mM phosphate buffer (pH 7.0). The pI values were determined and compared to those of known β-lactamases, including GES-1 β-lactamase.

Kinetic measurements. Purified β-lactamase was used for kinetic measurements performed at 30°C with 100 mM sodium phosphate (pH 7.0) with an ULTROSPEC 2000 UV spectrophotometer (Amersham Pharmacia Biotech) as previously described for the biochemical analysis of GES-1 β-lactamase (28).

Fifty percent inhibitory concentrations (IC_{50}s) were determined for clavulanic acid, tazobactam, sulbactam, and imipenem. Various concentrations of these inhibitors were preincubated with the purified enzyme for 3 min at 30°C to determine the concentrations that reduced the hydrolysis rate of 100 μM benzylpenicillin by 50%.

The specific activity of the purified β-lactamase from *E. coli* DH10B(pLAP-1) was obtained as described previously (29). One unit of enzyme activity was defined as the activity which hydrolyzed 1 μmol of benzylpenicillin per min per mg of protein. The total protein content was measured with the DC Protein assay kit (Bio-Rad, Ivry-sur-Seine, France). Specific activity was also determined with 100 μM ceftazidime as a substrate.

Nucleotide sequence accession number. The nucleotide sequence data reported in this paper will appear in the GenBank nucleotide database under accession no. AF326355.

RESULTS

Properties of *P. aeruginosa* isolate GW-1. GW-1 was isolated in May 2000 at the Pretoria Academic Hospital, Pretoria, Republic of South Africa, from a 38-year-old Zimbabwean refugee hospitalized for cerebral malaria. On the 12th day of her hospitalization in the intensive care unit, she developed nosocomial pneumonia, and blood cultures grew *P. aeruginosa* GW-1. Empirical treatment with a combination of imipenem and amikacin was unsuccessful. Imipenem was replaced after 3 days with aztreonam, and her clinical condition improved remarkably. She received no treatment prior to her hospitalization in South Africa.

P. aeruginosa GW-1 exhibited a broad spectrum of resistance to expanded-spectrum cephalosporins and an intermediate susceptibility to imipenem, according to antibiotic susceptibility testing by disk diffusion. Double-disk synergy testing remained negative with clavulanate- and ceftazidime-containing disks. *P. aeruginosa* GW-1 was also resistant to kanamycin, gentamicin, netilmicin, fluoroquinolones, sulfonamides, and tetracycline and susceptible to tobramycin. Preliminary experiments with crude extracts of a culture of *P. aeruginosa* GW-1 showed that imipenem hydrolysis was detectable (data not shown).

Cloning and sequencing of the β-lactamase gene. Preliminary PCR detection of most of the class A ESBLs and carbapenem-hydrolyzing β-lactamase genes failed (data not shown). However, PCR amplification was positive with primers for bla_{GES-1}. With whole-cell DNA of *P. aeruginosa* GW-1 as a template and consensus primers for 5′-CS and 3′-CS ends of class 1 integrons, a 1.8-kb DNA fragment was obtained. It was cloned into the *Srf*I site of plasmid pPCR Cam SK(+), yielding recombinant plasmid pLAP-1. Sequence analysis of the cloned fragment revealed an 864-bp-long ORF encoding a 287-amino-acid preprotein. This protein was a β-lactamase with the STFK tetrad and structural elements characteristic of the active site of an Ambler class A β-lactamase (Fig. 1) (12). The β-lactamase, designated GES-2, had one amino acid change (glycine to asparagine in position 170) and three amino acid changes compared to GES-1 and IBC-1, respectively (Fig. 1). The G+C content of bla_{GES-2} was 51.5%, a value which is not within the range of G+C content of *P. aeruginosa* genes (60.1 to 69.5%).

As found for GES-1 and IBC-1, GES-2 was distantly related to the class A β-lactamases (7, 28). The highest percentage of amino acid identity was 36%, found with either carbenicillinase GN79 from *Proteus mirabilis*, a constitutive penicillinase from *Yersinia enterocolitica* YENT, or the L-2 chromosomally encoded extended-spectrum β-lactamase from *Stenotrophomonas maltophilia*.

Transfer of β-lactam resistance. Transconjugant *P. aeruginosa* PU21 strains were obtained with *P. aeruginosa* GW-1 as a donor. They showed a broad-spectrum β-lactam resistance phenotype, including an increased resistance against imipenem (Table 1). A slightly positive double-disk synergy test was done, with transconjugants indicating the presence of an ESBL (data not shown). Cotransferred antibiotic resistance markers were those carrying resistance for kanamycin, gentamicin, netilmi-

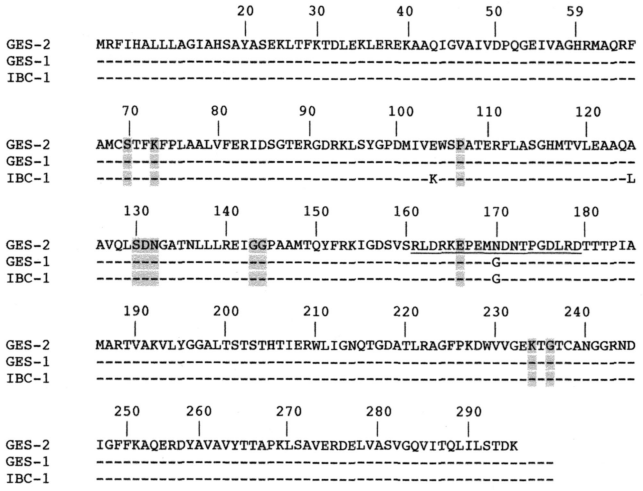

FIG. 1. Comparison of the amino acid sequence of GES-2 to those of GES-1 and IBC-1 β-lactamases (7, 28). The numbering is according to the Ambler designation (1). Highlighted amino acids are those strictly conserved in class A β-lactamases. The amino acids of the omega loop are underlined.

cin, and sulfonamides. The analysis of plasmid content of *P. aeruginosa* GW-1 and its transconjugant revealed a ca. 100-kb plasmid, designated pGW-1 (data not shown). Hybridization using an internal probe for $bla_{\text{GES-1}}$ confirmed the presence of a $bla_{\text{GES-1}}$-like gene on plasmid pGW-1 found in *P. aeruginosa* GW-1 and its transconjugant (data not shown).

β-Lactam susceptibility. MICs of β-lactams for *P. aeruginosa* GW-1 mirrored those for its transconjugant (Table 1). The GES-2-producing *P. aeruginosa* strains were characterized by resistance to carbenicillin, ureidopenicillins, cefotaxime, and ceftazidime and by an intermediate susceptibility to aztreonam (Table 1). The MIC of imipenem was eightfold higher for *P. aeruginosa* PU21(pGW-1) than that for *P. aeruginosa* PU21 (Table 1). A similar trend of resistance was noted once $bla_{\text{GES-2}}$ was expressed in *E. coli* DH10B. However, in this latter case, the recombinant strain was of intermediate susceptibility to extended-spectrum cephalosporins and susceptible to imipenem (Table 1). GES-2-producing *E. coli* DH10B(pLAP-1) was less resistant to cephalosporins than GES-1-producing *E. coli* DH10B(pC1) (Table 1). The imipenem MIC for *E. coli* DH10B(pLAP-1) was only slightly higher than that for *E. coli* DH10B (Table 1).

Clavulanate and tazobactam partially restored the β-lactam activities against the GES-2-producing *E. coli* strain as previously found for the GES-1-producing *E. coli* strain (Table 1). Clavulanate did not lower significantly the β-lactam MICs for the GES-2-producing *P. aeruginosa* strains (Table 1). This result was likely due to a concomitant induction of the chromosomal cephalosporinase of *P. aeruginosa*.

IEF analysis and kinetic parameters. IEF analysis showed that *P. aeruginosa* GW-1, its transconjugant, and *E. coli* DH10B(pLAP-1) had β-lactamase activities with a pI value of 5.8, corresponding to that of GES-2 and identical to that of GES-1 (data not shown). β-Lactamase activities with a pI value of 8 to 8.5 were also detected for *P. aeruginosa* GW-1 and its transconjugant corresponding to the chromosomal cephalosporinase of *P. aeruginosa*. A nonidentified β-lactamase with a pI of 7.5 was found in GW-1 and in its transconjugant. Additional PCR experiments with whole-cell DNAs of *P. aeruginosa* GW-1 and its transconjugants as templates and primers for the

TABLE 1. MICs of β-lactams

β-Lactam(s)[b]	MIC (μg/ml)[a]					
	P. aeruginosa GW-1	*P. aeruginosa* PU21(pGW-1)	*P. aeruginosa* PU21	*E. coli* DH10B(pLAP-1)	*E. coli* DH10B(pC1)	*E. coli* DH10B
Amoxicillin	>512	>512	>512	>512	>512	4
Amoxicillin + CLA	>512	>512	>512	16	>128	4
Ticarcillin	>512	>512	8	256	>512	4
Ticarcillin + CLA	>512	256	4	4	64	4
Ticarcillin + TZB	>512	256	8	4	256	2
Piperacillin	128	64	8	8	64	1
Piperacillin + CLA	64	16	2	1	8	1
Piperacillin + TZB	128	16	16	8	8	1
Cephalothin	>512	>512	>512	32	256	2
Cefoxitin	>512	>512	>512	4	8	1
Ceftazidime	32	16	1	8	128	0.5
Ceftazidime + CLA	16	16	2	0.5	8	0.5
Ceftazidime + TZB	8	4	2	0.5	8	0.5
Cefotaxime	128	128	8	1	4	0.06
Cefotaxime + CLA	256	256	16	0.06	0.5	0.06
Cefotaxime + TZB	64	32	8	0.5	2	0.06
Cefepime	32	16	0.5	0.12	0.25	0.03
Cefsulodin	>512	512	2	32	32	1
Aztreonam	16	8	4	0.5	1	0.12
Imipenem	16	16	2	0.25	0.06	0.06
Meropenem	16	2	0.5	0.06	0.06	0.06
Moxalactam	16	8	8	0.12	0.12	0.12

[a] β-Lactamases: GES-2, AmpC type, pI 7.5, for *P. aeruginosa* GW-1; GES-2, AmpC type for *P. aeruginosa* PU21(pGW-1); AmpC type for *P. aeruginosa* PU21; GES-2 for *E. coli* DH10B(pLAP-1); GES-1 for *E. coli* DH10B(pC1).

[b] CLA, clavulanic acid at a fixed concentration of 2 μg/ml; TZB, tazobactam at a fixed concentration of 4 μg/ml.

TABLE 2. Steady-state kinetic parameters of GES-2[a]

Substrate	GES-2			GES-1		
	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m ($mM^{-1} \cdot s^{-1}$)	k_{cat}(s^{-1})	K_m (μM)	k_{cat}/K_m ($mM^{-1} \cdot s^{-1}$)
Benzylpenicillin	0.4	4	96	2.8	40	70
Amoxicillin	0.7	25.8	26	13	200	65
Ticarcillin	0.06	13.3	4.5	0.3	400	0.7
Piperacillin	0.3	22.8	23	8	900	13
Cephalothin	0.3	3	112	179	3,400	52
Cephaloridine	0.5	7.7	65	53	2,000	26
Cefoxitin	—[b]	—	—	0.9	30	33
Ceftazidime	ND[c]	>3,000	ND	380	2,000	188
Cefepime	1.1	1,900	0.6	2.8	1,800	1.6
Cefotaxime	2.2	890	2.5	68	4,600	15
Imipenem	0.004	0.45	9	0.003	45	0.07
Meropenem	—	—	—	—	—	—
Aztreonam	—	—	—	—	—	—

[a] Parameters of GES-2 are compared to those of GES-1 β-lactamase, as previously published (28). Values are expressed as the mean of three independent measures (standard deviations of the values were within 15%).
[b] —, not hydrolyzed (the initial rate of hydrolysis was lower than 0.001 $\mu M^{-1} \cdot s^{-1}$).
[c] ND, not determinable due to very high K_m values.

detection of OXA-1, OXA-2, OXA-3, OXA-10, OXA-18, and OXA-20 genes failed (data not shown).

The specific activity of the purified β-lactamase GES-2 was 720 mU · mg of $protein^{-1}$, determined with 100 μM benzylpenicillin as a substrate. Its overall recovery was 70% with a 20-fold purification. The purity of the enzyme was estimated to be 90% according to sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis. Kinetic parameters of GES-2 showed its broad-spectrum activity against most β-lactams, except aztreonam and meropenem (Table 2). The hydrolysis rates of GES-2 for penicillins were similar to those of GES-1 because the K_m values were 10-fold lower. However, the hydrolysis efficiency of GES-2 for extended-spectrum cephalosporins was slightly lower than that of GES-1 β-lactamase. Although ceftazidime was hydrolyzed, its kinetic parameters could not be determined precisely. This was due to a very high K_m value (>3,000 μM), reflecting a very low affinity of GES-2 for ceftazidime. Nevertheless, its V_{max} value was high (data not shown), and the specific activity of GES-2 was 57 mU · mg^{-1} with 100 μM ceftazidime as a substrate. As reported for GES-1 (28), high k_{cat}/K_m values were obtained for most cephalosporins for GES-2 when expressed in millimolar units and not, as usual for an ESBL activity of a class A enzyme, in micromolar units.

GES-2, unlike GES-1, measurably hydrolyzed imipenem (Table 2). Meropenem hydrolysis by GES-2 was not detected. The hydrolysis efficiency of GES-2 against imipenem was 100-fold higher than that of GES-1 due to a 100-fold-lower K_m value (Table 2) but still remained marginal. GES-2 was probably saturated by imipenem, while GES-1 was not. Inhibition studies as measured by IC_{50}s with benzylpenicillin as a substrate showed that GES-2 activity was inhibited by clavulanic acid and tazobactam more than GES-1 is, while GES-2 was less inhibited by imipenem (Table 3).

Genetic environment of bla_{GES-2}. Upstream of bla_{GES-2}, two putative promoter sequences named P_1 and P_2 were located in the structural integrase gene (data not shown). Comparison of P_1 with known promoters for which expression studies were performed (5, 13) identified P_1 as a weak promoter. As described, the insertion of three guanosine molecules 119 bases downstream of the promoter P_1 creates a secondary promoter (P_2) for bla_{GES-1} expression. This triple nucleotide insertion brings the spacing between the −35 and −10 regions of P_2 to 17 bp. Therefore, P_2 expression may be responsible for 90% of bla_{GES-2} transcription, as shown for other genes (5, 13). The bla_{GES-2} gene cassette which was inserted at the *att*I recombination site has a core site (GTTAGAC) and an inverse core site (GTCTAAC). Downstream of this gene, the 59-be sequence was made up of 110 bp. It was exactly identical to that found downstream of bla_{IBC-1} and shared 70% nucleotide identity within the 19-bp-long and truncated 59-be of bla_{GES-1} (data not shown).

DISCUSSION

This report characterized another non-SHV-, non-TEM-type ESBL, showing that class A ESBLs are not limited to SHV and TEM derivatives. GES-2 β-lactamase is the fourth example of a non-TEM-, non-SHV-type ESBL in *P. aeruginosa* after PER-1, VEB-1, and OXA-18. A study of the GES-2-producing *P. aeruginosa* GW-1 isolate further indicated that ESBL genes in *P. aeruginosa* are difficult to detect by double-disk synergy tests (23) and may therefore be clinically underestimated.

GES-2 β-lactamase is also the third example of a GES-type ESBL, in addition to GES-1 from *K. pneumoniae* ORI-1 (28)

TABLE 3. Inhibition profile of GES-2 compared with those of other β-lactamases

β-Lactamase	IC_{50} (μM)[a]		
	Clavulanic acid	Tazobactam	Imipenem
GES-2	1 ± 0.5	0.5 ± 0.2	8 ± 2
GES-1	5	2.5	0.1
IBC-1	1	0.12	0.06
SME-1	0.28	0.16	—[b]
TEM-1	0.08	0.05	11.8

[a] Data were adapted from references 7, 28, 33, and 37.
[b] —, not done.

and IBC-1 from *E. cloacae* HT9 (7). The natural producer of the GES-type enzymes remains to be determined. Indeed, isolation of a GES-2 gene in *P. aeruginosa* with a G+C content of non-*P. aeruginosa* origin indicated a horizontal transfer of bla_{GES} genes in gram-negative species.

One interesting aspect of this study is the comparison of the substrate profiles of GES-1 and GES-2. A glycine-to-asparagine substitution in GES-2 extended its activity to imipenem. This substitution occurred in an amino acid position of the omega loop of class A β-lactamases that is of primary importance in the catalytic activity of these enzymes (3, 16). This substitution may enlarge the pocket that houses the hydroxyethyl moiety of imipenem on the alpha face of the acyl enzyme for GES-2 (17, 18).

GES-2, like GES-1 and IBC-1 β-lactamases and also like the class A enzymes with significant catalytic efficiency against imipenem (i.e., the chromosomally encoded β-lactamases SME-1/2, IMI-1, and Nmc-A and the plamid-encoded KPC-1 enzyme [40]), contains two cysteine residues in positions 69 and 238 that may form a disulfide bridge. The catalytic activity (k_{cal}/K_m, expressed in millimoles per second) of GES-2 versus that of imipenems (9 and 520, respectively) remained much lower than those of the carbapenem-hydrolyzing β-lactamases such as SME-1 (33). Thus, as reported (34), this cysteine bridge may enable the catalytic site to bind imipenem, but other amino acid residues are likely to be involved in the significant catalytic efficiency of the carbapenem-hydrolyzing enzymes against imipenem.

The IC_{50} of imipenem for GES-2 was similar to the values of other class A β-lactamases like TEM-1 (Table 3). Inhibitory activity profiles of GES-1, IBC-1, and GES-2 showed that clavulanic acid and tazobactam are similarly active against GES-2 and IBC-1 but less so against GES-1.

Comparison of the surrounding sequences of bla_{GES-2} to those of bla_{GES-1} and bla_{IBC-1} showed that bla_{GES} genes are part of gene cassettes. Identification of an identical 59-be consisting of 110 bp for bla_{GES-2} and bla_{IBC-1} indicated the spread of an identical gene cassette among *P. aeruginosa* and enterobacterial isolates. Additionally, it may confirm the hypothesis that a deletion occurs in the 59-be of the bla_{GES-1} cassette (28).

A striking similarity may be drawn between VEB and GES β-lactamase genes. They encode class A ESBLs, are located on broad-host-range conjugative plasmids, are part of gene cassettes in class 1 integrons, and are found in gram-negative isolates, and their origin (natural producer) remains unknown. Additionally, they may have spread worldwide, since VEB β-lactamases have been isolated in southeastern Asia and recently in Kuwait (8, 20, 29, 32, 38); GES β-lactamases are found in French Guiana, Greece, and now South Africa (7, 28).

This work shows that plasmid- and integron-mediated genes encoding ESBLs with some carbapenem hydrolytic activity are not limited to the Ambler class B β-lactamase genes. GES-2 β-lactamase may contribute in part to the decreased susceptibility of *P. aeruginosa* to imipenem. However, once expressed from a multicopy vector in *E. coli*, bla_{GES-2} expression did not significantly increase the imipenem MIC, thus making its clinical detection in enterobacterial isolates by a simple susceptibility study unlikely.

Finally, this report indicates that a class A β-lactamase with a substrate profile extended to imipenem may be selected in vivo through a single amino acid substitution in an ESBL sequence.

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