AAC Journals.ASM.org

A Plasmid-Encoded Class 1 Integron Contains GES-Type Extended-Spectrum β-Lactamases in *Enterobacteriaceae* Clinical Isolates in Mexico

Plasmid-located extended-spectrum-β-lactamase (ESBL) genes are mostly found in *Enterobacteriaceae* (6). A new class A ESBL was identified in *Klebsiella pneumoniae*. It was named GES-1, and it corresponds to the ceftazidime-hydrolyzing enzyme (8). GES-type ESBLs have emerged in a variety of countries, and there are 18 known variants (http://www.lahey.org/Studies/). In the present study, we investigated the prevalence of GES-type β-lactamases in ESBL-producing *Enterobacteriaceae* clinical isolates; two new alleles (GES-19 and GES-20) were identified in a plasmid-encoded class 1 integron (In724).

(This work was presented in part as an abstract at the 51st Interscience Conference on Antimicrobial Agents and Chemotherapy, abstract C1-1210, 2011.)

Between March 2005 and June 2009, 578 ESBL-producing *Enterobacteriaceae* clinical isolates were collected from 11 Mexican hospitals. All isolates were screened for the presence of β-lactamases from the GES family by means of PCR, using generic primers (14). Among the 578 ESBL-producing *Enterobacteriaceae* isolates studied, 8 (1.3%) contained the GES-type gene and were distributed as follows: 1/5 *K. oxytoca* isolates, 5/137 *K. pneumoniae* isolates, and 2/404 *Escherichia coli* isolates; no GES-positive (0/32) *E. cloacae* isolates were identified (Table 1). For GES-type-positive isolates, antibiotic susceptibility testing was carried out by broth microdilution, following CLSI recommendations (2). All isolates turned out to be resistant to ceftazidime, cefotaxime, piperacillin, and ciprofloxacin. Three isolates turned out to be resistant to gentamicin and two to imipenem and meropenem (Table 1). Genomic DNA was analyzed (4, 11), and it revealed a nongenetic relationship between the GES-positive isolates (data not shown).

GES-1 was identified in the *K. oxytoca* isolate (Kx09201). Whereas all *K. pneumoniae* and *E. coli* isolates contained ESBL GES-19 and carbapenemase GES-20, these proteins differed from GES-11 and GES-5 β-lactamases by the replacement of Ala by Gly at Ambler position 17 (leader peptide). The mating experiments (7, 9) showed that both GES-19 and GES-20 genes were transferred onto a 40-kb conjugative plasmid from *K. pneumoniae* and *E. coli* isolates and that GES-1 was transferred onto an 80-kb plasmid from *K. oxytoca* (Table 1). Plasmids were digested with XhoI and EcoRI restriction enzymes. The fingerprinting showed identical patterns among the 40-kb plasmids (data not shown). These data are in accordance with those corresponding to the FII_s incompatibility group identified in the plasmids. Moreover, members of incompatibility groups FII_y and FII_k and IncR were also identified in transconjugant TK06220, which contains an additional 50-kb plasmid (Table 1). The plasmid incompatibility groups were identified using recent PCR-based replicon typing (3, 13).

All GES-type alleles have been mainly described in class 1 integrons (15). The class 1 integron structure that encoded the GES-type alleles was determined using a PCR strategy with generic primers (1, 5, 14); in addition, GES-243F (5′-TGTGTTGTCGCCCATCTCCG-3′) and GES-104R (5′-ATGATCGTCGAATGGTCTCC-3′) were used to amplify the intergenic region between the two GES-type genes. All transconjugants harboring the 40-kb plasmid contained the class 1 integron with the following array: *aacA42*, bla_{GES-19}, and bla_{GES-20} (tandem duplication) and *aacA4′*, bla_{OXA-2}, *qacH4*, and *aadA1b* (named In724). The nucleotide analysis showed the following characteristics. The bla_{GES-19} gene is not followed by any *attC* recombination site; instead, there is a "TAAAACAAAGTTAG" fragment (2795 to 2908) that is a duplication at the end of the *attI1* (1141 to 1154) region. This In724 class 1 integron is very similar to the one located on the *Pseudomonas aeruginosa* chromosome (In647) previously described (12). Interestingly, the intergenic region between the GES-type tandem duplications in both class 1 integrons is the same fragment (with the exception of a deleted A) which separates the two bla_{GES} genes in In647; this situation supports the idea that the In724 integron derives from In647 by variations in the bla_{GES} alleles (GES-19 and GES-20). Most likely, this duplication occurred via an insertion sequence (IS)-mediated event (8). Therefore, the plasmid-located integron facilitates the dissemination of integrons of these classes. On the other hand, the GES-1 gene encoding the class 1 integron showed the following structure: *aacA4*, bla_{GES-1}, *qacF5*, *aacA4-18*, and Δ*aadA1*, corresponding to a new class 1 structure called In725.

The GES-positive isolates were also screened for plasmid-mediated quinolone resistance (PMQR), as well as for SHV, CTX-M, and TLA-1 ESBL genes by the use of the respective primers (10). Our study showed that 6/8 GES-positive isolates contained at least one PMQR gene (Table 1). The GES alleles coexist with *qnrA1*, *qnrB2*, *qnrS1*, and *aac(6′)-Ib-cr* determinants. On the other hand, the bla_{SHV-5}, bla_{SHV-12}, and $bla_{CTX-M-15}$ genes coexist with the *qnrB2*, *qnrS1*, and *aac(6′)-Ib-cr* determinants.

The transconjugants encoding the SHV- and CTX-M-type ESBLs (TK01256, TK06220, TE01298, and TKx09201) showed a high drug MIC value (≥256) with respect to ceftazidime (TK01256 and TE01298) and cefotaxime (TKx09201). In terms of imipenem, the transconjugants carrying the GES-20 allele displayed a 1-to->3-fold MIC increase with respect to *E. coli* J53-2. These multiple-ESBL-containing isolates could be playing an important role in terms of cephalosporin resistance, and they might limit the therapeutic options when combined with PMQR genes.

Nucleotide sequence accession numbers. The nucleotide sequence data reported in this paper appear in the GenBank/EMBL

Published ahead of print 23 April 2012

Address correspondence to Jesus Silva-Sanchez, jsilva@insp.mx.
Humberto Barrios and Ulises Garza-Ramos contributed equally to this article.

doi:10.1128/AAC.05980-11

nucleotide database under accession numbers JN596279 (In725) and JN596280 (In724).

ACKNOWLEDGMENTS

We are grateful to all the members of the Red-MEReBa (Red Mexicana para el Estudio de la Resistencia Bacteriana) Study Group: Instituto Nacional de Cardiología (INCard), MAOS, Distrito Federal (DF), Mexico (M. Rosario Velázquez and Veronica Rodríguez-Galicia); ISSSTESON (ISTESon), Hermosillo, Sonora, Mexico (Moises Navarro-Navarro); Hospital de Altas Especialidades (HAE), Monterrey, Nuevo León, Mexico; Hospital San Jose Tec (SJT), Monterrey, Nuevo León, Mexico (Jacobo Ayala and Claudia E. Guajardo-Lara); Sanatorio Durango (SD), DF, Mexico (Octavio Novoa-Farias and Ivan Sánchez-Castro); Centro de Especialidades Médicas del Edo. de Veracruz (CEMV) (Rafael Lucio and Jorge S. González-Hernandez); Hospital General de Acapulco (HGA), Acapulco, Guerrero, Mexico (Amparo Calderón-Navarro, Fausto Jaimes-Dominguez, and Bernardo González-Cervantes); and Hospital Central (HC), Ignacio Morones Prieto, San Luis Potosí (SLP), Mexico (Lilia E. Fragoso-Morales and Irma Y. Amaya-Larios). We thank A. Sanchez-Perez for his excellent laboratory assistance.

This work was supported by grants SALUD-2008-1-87334 and 136339 from CONACyT (Mexican Council for Science and Technology). L.E.O.-S. was a fellow from CONACyT.

REFERENCES

1. **Bogaerts P, et al.** 2010. GES extended-spectrum beta-lactamases in *Acinetobacterbaumannii* isolates in Belgium. Antimicrob. Agents Chemother. **54**:4872–4878.
2. **Clinical and Laboratory Standards Institute.** 2011. Performance standards for antimicrobial susceptibility testing. Document M100-S21. CLSI, Wayne, PA.
3. **GarcíA-Fernández A, Fortini D, Veldman K, Mevius D, Carattoli A.** 2009. Characterization of plasmids harbouring *qnrS1*, qnrB2 and *qnrB19* genes in Salmonella. J. Antimicrob. Chemother. **63**:274–281.
4. **Kaufmann ME.** 1998. Pulse-field gel electrophoresis. Methods Mol. Med. **15**:33–50.
5. **Lévesque C, Piche L, Larose C, Roy P.** 1995. PCR mapping of integrons reveals several novel combinations of resistance genes. Antimicrob. Agents Chemother. **39**:185–191.
6. **Medeiros AA.** 1997. Evolution and dissemination of beta-lactamases accelerated by generation of beta-lactam antibiotics. Clin. Infect. Dis. **24**(Suppl. 1):S19–S45.
7. **Miller J.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
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 beta-lactamase, and the class 1 integron In52 from *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. **44**:622–632.
9. **Silva-Sanchez J, et al.** 2011. Extended-spectrum β-lactamase-producing enterobacteriaceae causing nosocomial infections in Mexico. A retrospective and multicenter study. Arch. Med. Res. **42**:156–162.
10. **Silva-Sanchez J, et al.** 2011. Prevalence and characterization of plasmid-mediated quinolone resistance genes in extended-spectrum β-lactamase-producing Enterobacteriaceae isolates in Mexico. Microb. Drug Resist. **17**:497–505.
11. **Tenover FC, et al.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33**:2233–2239.
12. **Viedma E, et al.** 2009. Nosocomial spread of colistin-only-sensitive sequence type 235 *Pseudomonas aeruginosa* isolates producing the extended-spectrum beta-lactamases GES-1 and GES-5 in Spain. Antimicrob. Agents Chemother. **53**:4930–4933.
13. **Villa L, GarcíA-Fernández A, Fortini D, Carattoli A.** 2010. Replicon sequence typing of IncF plasmids carrying virulence and resistance determinants. J. Antimicrob. Chemother. **65**:2518–2529.
14. **Wachino J, et al.** 2004. Nosocomial spread of ceftazidime-resistant *Klebsiella pneumoniae* strains producing a novel class A beta-lactamase, GES-3, in a neonatal intensive care unit in Japan. Antimicrob. Agents Chemother. **48**:1960–1967.

TABLE 1 Molecular characteristics of GES-positive isolates and transconjugants[a]

Strain[b]	Isolation date (mo/day/yr)	Hospital[c]	Ward	Origin	Plasmid size(s) (kb)	Incompatibility group	PMQR gene	ESBL gene type(s)		MIC (mg/ml)								
								Non-GES	GES	CAZ	CAZ/CLA	CTX	PIP	PIP/TAZ	CPO	IMP	IMP/CLA	MER	Gm
K01201	11/27/2006	1	Hematology	Blood	220, 140, 90, 40	NA	*qnrA1/qnrS1/aac (6′)-Ib-cr*	SHV-11	19, 20	256	128	>256	128	128	>32	1	0.25	0.125	8
TK01201	Not applicable	Not applicable	Not applicable	Not applicable	40	FII_s	Neg	19, 20		256	4	64	64	16	<0.002	1	0.25	0.125	2
K01239	10/10/2008	1	ICU	Wound	190, 160, 40	NA	*qnrA1/aac(6′)-Ib-cr*	SHV-11	19, 20	>256	>256	128	128	128	>32	32	16	8	16
TK01239	Not applicable	Not applicable	Not applicable	Not applicable	40	FII_s	Neg	19, 20		256	4	64	256	16	<0.002	0.5	0.125	0.03	2
K01256	09/22/2006	1	OR	Wound	190, 120, 110, 40	NA	Neg	SHV-26	19, 20	>256	>256	256	512	512	32	16	16	32	4
TK01256	Not applicable	Not applicable	Not applicable	Not applicable	40	FII_s	Neg	SHV-26	19, 20	>256	16	64	128	16	<0.002	0.5	0.125	0.03	2
K01295	04/01/2009	1	OR	Catheter	200, 100, 40	NA	*qnrA1*	SHV-1	19, 20	256	8	32	128	256	>32	1	0.25	0.125	8
TK01295	Not applicable	Not applicable	Not applicable	Not applicable	40	FIIs	*qnrA1*	Neg	19, 20	256	8	64	256	128	<0.002	0.5	0.125	0.03	2
K06220	09/14/10	2	ICU	Urine	180, 100, 50, 40	NA	*qnrS1/aac(6′)-Ib-cr*	SHV-5	19, 20	64	16	64	512	256	>32	1	0.5	0.5	>64
TK06220	Not applicable	Not applicable	Not applicable	Not applicable	50, 40	FII_s[d]	*aac(6′)-Ib-cr*	SHV-5	19, 20	64	4	32	128	8	<0.002	1	0.25	0.03	2
E01298	06/20/2009	1	Nephrology	Urine	110, 40	NA	*aac(6′)-Ib-cr*	CTX-M-15	19, 20	>256	8	>256	>512	64	>32	0.5	0.25	0.03	4
TE01298	Not applicable	Not applicable	Not applicable	Not applicable	40	FII_s	*aac(6′)-Ib-cr*	CTXM-15	19, 20	256	8	64	64	4	<0.002	0.5	0.125	0.03	2
E09280	03/10/2009	3	Hematology	Blood	190, 40	NA	*aac(6′)-Ib-cr*	Neg	19, 20	>256	16	128	32	8	>32	1	0.125	0.125	2
TE09280	Not applicable	Not applicable	Not applicable	Not applicable	40	FII_s	*aac(6′)-Ib-cr*	Neg	19, 20	256	8	64	32	8	<0.002	1	0.125	0.125	2
Kx09201	09/26/2007	3	Hematology	Blood	220, 190, 100, 80	NA	*qnrB2*	SHV-12/CTX-M-15	1	64	0.5	>256	>512	8	32	0.25	0.0625	0.015	64
TKx09201	Not applicable	Not applicable	Not applicable	Not applicable	80	FII_s	*qnrB2*	SHV-12/ CTXM-15	1	32	1	>256	>512	8	<0.002	0.25	0.0625	0.015	32
E. coli J53-2	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	NA	Not applicable	Not applicable	Not applicable	0.5	0.25	0.0625	8	8	<0.002	0.125	0.0625	0.015	0.5

[a] CAZ, ceftazidime; CLA, clavulanic acid; CTX, cefotaxime; PIP, piperacillin; TAZ, tazobactam; CPO, ciprofloxacin; IMP, imipenem; MER, meropenem; Gm, gentamicin; ICU, intensive care ward; NA, not analyzed; Neg, negative; OR, operating room.
[b] K, *K. pneumoniae*; E, *E. coli*; Kx, *K. oxytoca*; T, transconjugant.
[c] Hospitals: 1, Hospital Civil de Guadalajara (HCG); 2, Hospital Universitario (CRCEI); 3, Instituto Nacional de Cancerología (INCan).
[d] Additional incompatibility group identified in transconjugants FII_s, FII_k, and IncR (see text).

15. **Walther-Rasmussen J, Høiby N.** 2007. Class A carbapenemases. J. Antimicrob. Chemother. **60**:470–482.

Humberto Barrios
Ulises Garza-Ramos
Luz Edith Ochoa-Sanchez
Fernando Reyna-Flores
Teresa Rojas-Moreno
Instituto Nacional de Salud Pública (INSP)
Centro de Investigaciones Sobre Enfermedades Infecciosas (CISEI), Cuernavaca
Morelos, Mexico

Rayo Morfin-Otero
Eduardo Rodriguez-Noriega
Hospital Civil de Guadalajara (HCG-J) Fray Antonio Alcalde
Instituto de Patología, CUCS
UDG Guadalajara
Jalisco, Mexico

Elvira Garza-Gonzalez
Gloria Gonzalez
Hospital Universitario (CRCEI-NL) Dr. José Eleuterio González
Departamento de Microbiología
Facultad de Medicina
Universidad Autónoma de Nuevo León, Monterrey
Nuevo León, Mexico

Patricia Volkow
Patricia Cornejo-Juarez
Instituto Nacional de Cancerología (INCan)
Ciudad de México, Mexico

The Red-MEReBa Study Group

Jesus Silva-Sanchez
Instituto Nacional de Salud Pública (INSP)
Centro de Investigaciones Sobre Enfermedades Infecciosas (CISEI)
Cuernavaca, Morelos, Mexico