AAC Journals.ASM.org

First Detection of bla_{IMI-2} Gene in a Clinical *Escherichia coli* Strain

The dissemination of carbapenem-resistant *Enterobacteriaceae* is increasing worldwide in the last decade, mainly due to the acquisition of beta-lactamase genes encoding carbapenemases. Among class A carbapenemases, KPC is widespread (14); however, IMI enzymes have been described so far only in *Enterobacter* genus (1, 10, 15). The detected bla_{IMI} genes are linked to a gene encoding a LysR-type transcriptional regulator, and as previously reported, the bla_{IMI-1} gene is located in the chromosome of *Enterobacter cloacae* (10), whereas bla_{IMI-2} is related to plasmids (1, 15). We report here the first description of a bla_{IMI-2}-positive *Escherichia coli* strain.

(Part of this study was presented at the 21st European Congress of Clinical Microbiology and Infectious Diseases Congress, Milan, Italy, 7 to 10 May 2011.)

A carbapenem-resistant *E. coli* strain, W635, was recovered from a blood sample of an elderly oncologic patient (without any history of travel) who was admitted with sepsis and treated with piperacillin-tazobactam at a Spanish hospital in 2010. *E. coli* W635 was ascribed to a new sequence type registered as ST1998 (http://mlst.ucc.ie/mlst/dbs/Ecoli) and showed resistance to imipenem (IPM), meropenem (MEM), ertapenem (ETP), doripenem, ampicillin (AMP), ticarcillin, amoxicillin-clavulanic acid (AMC), cephalothin, streptomycin, nalidixic acid, ciprofloxacin, norfloxacin, sulfonamides, and trimethoprim and intermediate resistance to aztreonam and chloramphenicol by the CLSI disk diffusion method (3). A class A carbapenemase phenotype was demonstrated in this strain by double-disk synergy test (5, 9).

After multiplex PCR (8) and subsequent sequencing, a partial sequence of the bla_{IMI-2} gene was detected in *E. coli* W635. To gain insight into the bla_{IMI-2} gene and its surrounding structure, the flanking regions were amplified by PCR using specific primers designed in this work (according to GenBank accession number AY780889). The LysR-type regulator gene (bla_{IMI-2R}) was found upstream of the bla_{IMI-2} gene, and their genetic environment was studied by inverse PCR using PvuII and BglII restriction enzymes. Sequence analysis revealed a total fragment of 6,184 bp that was deposited in GenBank database with the accession number JN412066. A new insertion sequence of 1,321 bp, designed IS*Ec36* by ISFinder (http://www-is.biotoul.fr/), was detected upstream of the bla_{IMI-2R} gene. This IS belongs to the IS*3* family and IS*2* group, and it exhibits an identity of 92% with respect to the IS*Ec27* sequence (GenBank accession number AY857617).

Testing for the presence of genes implicated in other antimicrobial resistances (beta-lactams, aminoglycosides, quinolones, trimethoprim, and sulfonamides) and the study of mutations in *gyrA* and *parC* genes, as well as the characterization of integrons and *sul2* gene environment, were performed by PCR and sequencing (4, 11, 13). Table 1 shows the MICs and genotypic results.

Two different transconjugants from W635 were obtained by mating experiments, using *E. coli* CSH26 as recipient and plates supplemented with either MEM (8 μg/ml) or AMP (50 μg/ml) and rifampin (100 μg/ml). Detection and typing of plasmids of *E. coli* W635 and transconjugant strains were carried out by PCR-based replicon typing (2, 6). Plasmids of strain W635 and its transconjugants belonging to incompatibility group I1 (IncI1) and F (IncF) were subtyped by plasmid multilocus sequence typing (7, 12). *E. coli* W635 contained the following typeable plasmids: IncI1 (ST26, CC-26), IncF (F43:A^-:B^-; Y2 variant:A^-:B^-), and $ColE_{TP}$. The location of bla_{IMI-2} in strain W635 and its transconjugants was studied by pulsed-field gel electrophoresis (PFGE)-S1 nuclease and PFGE-XbaI Southern blotting and hybridization (4) with bla_{IMI-2}, IncI1, IncF, and $ColE_{TP}$ probes. The bla_{IMI-2} gene in *E. coli* W635 was detected in an IncF plasmid of approximately 48.5 kb. The size of this plasmid is smaller than found in previously reported results that described the bla_{IMI-2} gene located on transferable plasmids with sizes of 66 kb or 80 kb (1, 15).

This is the first report of a carbapenem-resistant *E. coli* strain carrying the class A carbapenemase IMI-2. The bla_{IMI-2} gene located in a conjugative plasmid and linked to mobile elements might significantly spread between different members of the *Enterobacteriaceae*, and therefore this emerging resistance mechanism should be tracked in the future.

Nucleotide sequence accession number. The nucleotide sequence of the novel genetic environment of bla_{IMI-2} gene determined in this study was included in the GenBank database with the accession number JN412066.

Published ahead of print 21 November 2011
Address correspondence to Yolanda Sáenz, ysaenz@riojasalud.es.

doi:10.1128/AAC.05478-11

TABLE 1 Resistance phenotype, genotype, and genetic elements of *E. coli* strain W635, transconjugants (TC), and recipient strain[a]

Strain	MIC (μg/ml) of[b]: AMP	ATM	IPM	MEM	STR	TMP	Resistance gene(s)				Amino acid change(s) detected: GyrA	ParC	Class 1 integron	Incompatibility group detected (size of plasmid)
W635 donor	>128	8	128	32	128	>128	bla_{TEM-1b}	bla_{IMI-2}	*strA-strB*[c]	*sul1 sul2*	S83L, D87N	S80I	*dfrA1 aadA1*	IncI1 (97 kb), IncF (48.5 kb), $ColE_{TP}$ (<10 kb)
TC1	>128	8	128	16	>128	>128	bla_{TEM-1b}	bla_{IMI-2}	*strA-strB*	*sul1 sul2*	ND	ND	*dfrA1 aadA1*	IncI1/IncF (160 kb),[d] $ColE_{TP}$ (<10 kb)
TC15	>128	0.125	0.25	0.032	>128	>128	bla_{TEM-1b}	–	*strA-strB*	*sul1 sul2*	ND	ND	*dfrA1 aadA1*	IncI1 (97 kb)
CSH26 recipient	16	0.125	0.25	0.032	4	<0.125	–	–	–	–	ND	ND	ND	ND

[a] ND, not determined; –, not detected.
[b] AMP, ampicillin; ATM, aztreonam; IPM, imipenem; MEM, meropenem; STR, streptomycin; TMP, trimethoprim.
[c] *strA-strB* genes were linked to the *sul2* gene (*repC-sul2-strA-strB*-IS*CR2*).
[d] IncI1 and IncF were detected by hybridization in a plasmid of approximately 160 kb in the bla_{IMI-2}-positive TC1 transconjugant.

ACKNOWLEDGMENTS

We thank Patricia Siguier for analyzing the new IS*Ec36* (http://www-is.biotoul.fr/).

The study did not receive financial support from third parties. M. López has a fellowship from the Gobierno de La Rioja.

REFERENCES

1. **Aubron C, Poirel L, Ash RJ, Nordmann P.** 2005. Carbapenemase-producing *Enterobacteriaceae*, U.S. rivers. Emerg. Infect. Dis. **11**:260–264.
2. **Carattoli A, et al.** 2005. Identification of plasmids by PCR-based replicon typing. J. Microbiol. Methods **63**:219–228.
3. **Clinical Laboratory Standards Institute.** 2011. Performance standards for antimicrobial susceptibility testing. Twenty-First International Supplement. M100–S21. Clinical and Laboratory Standards Institute, Wayne, PA.
4. **de Toro M, et al.** 2010. In vivo selection of *aac*(6′)-Ib-cr and mutations in the *gyrA* gene in a clinical *qnrS1*-positive *Salmonella enterica* serovar Typhimurium DT104B strain recovered after fluoroquinolone treatment. J. Antimicrob. Chemother. **65**:1945–1949.
5. **Doi Y, et al.** 2008. Simple disk-based method for detection of *Klebsiella pneumoniae* carbapenemase-type beta-lactamase by use of a boronic acid compound. J. Clin. Microbiol. **46**:4083–4086.
6. **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.
7. **García-Fernández A, et al.** 2008. Multilocus sequence typing of IncI1 plasmids carrying extended-spectrum beta-lactamases in *Escherichia coli* and *Salmonella* of human and animal origin. J. Antimicrob. Chemother. **61**:1229–1233.
8. **Hong SG, Hong SK, Huh JY, Kang MS.** 2010. Multiplex PCR for rapid detection of genes encoding class A carbapenemases. Clin. Microbiol. Infect. **16**(suppl. s2):S552. doi:10.1111/j.1469-0691.2010.03239.x.
9. **Nordmann P, Cuzon G, Naas T.** 2009. The real threat of *Klebsiella pneumoniae* carbapenemase-producing bacteria. Lancet Infect. Dis. **9**:228–236.
10. **Rasmussen BA, et al.** 1996. Characterization of IMI-1 beta-lactamase, a class A carbapenem-hydrolyzing enzyme from *Enterobacter cloacae*. Antimicrob. Agents Chemother. **40**:2080–2086.
11. **Sáenz Y, et al.** 2004. Mechanisms of resistance in multiple-antibiotic-resistant *Escherichia coli* strains of human, animal, and food origins. Antimicrob. Agents Chemother. **48**:3996–4001.
12. **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.
13. **Vinué L, et al.** 2010. Genetic environment of *sul* genes and characterisation of integrons in *Escherichia coli* isolates of blood origin in a Spanish hospital. Int. J. Antimicrob. Agents. **35**:492–496.
14. **Walther-Rasmussen J, Høiby N.** 2007. Class A carbapenemases. J. Antimicrob. Chemother. **60**:470–482.
15. **Yu YS, Du XX, Zhou ZH, Chen YG, Li LJ.** 2006. First isolation of bla_{IMI-2} in an *Enterobacter cloacae* clinical isolate from China. Antimicrob. Agents Chemother. **50**:1610–1611.

Beatriz Rojo-Bezares
Área de Microbiología Molecular
Centro de Investigación Biomédica de La Rioja (CIBIR)
Logroño, Spain

Carmen Martín
Laboratorio de Microbiología
Hospital San Pedro
Logroño, Spain

María López
Área de Bioquímica y Biología Molecular
Universidad de La Rioja
Logroño, Spain

Carmen Torres
Área de Bioquímica y Biología Molecular
Universidad de La Rioja
Logroño, Spain

Yolanda Sáenz
Área de Microbiología Molecular
Centro de Investigación Biomédica de La Rioja (CIBIR)
Logroño, Spain