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Mobile Genetic Elements Related to the Diffusion of Plasmid-Mediated AmpC β-Lactamases or Carbapenemases from *Enterobacteriaceae*: Findings from a Multicenter Study in Spain

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We examined the genetic context of 74 acquired *ampC* genes and 17 carbapenemase genes from 85 of 640 *Enterobacteriaceae* isolates collected in 2009. Using S1 pulsed-field gel electrophoresis and Southern hybridization, 37 of 74 bla_{AmpC} genes were located on large plasmids of different sizes belonging to six incompatibility groups. We used sequencing and PCR mapping to investigate the regions flanking the acquired *ampC* genes. The bla_{CMY-2}-like genes were associated with IS*Ecp1*; the surrounding bla_{DHA} genes were similar to *Klebsiella pneumoniae* plasmid pTN60013 associated with IS*26* and the *psp* and *sap* operons; and the bla_{ACC-1} genes were associated with IS*26* elements inserted into IS*Ecp1*. All of the carbapenemase genes (bla_{VIM-1}, bla_{IMP-22}, and bla_{IMP-28}) were located in class 1 integrons. Therefore, although plasmids are the main cause of the rapid dissemination of *ampC* genes among *Enterobacteriaceae*, we need to be aware that other mobile genetic elements, such as insertion sequences, transposons, or integrons, can be involved in the mobilization of these genes of chromosomal origin. Additionally, three new integrons (In846 to In848) are described in this study.

Beta-lactam resistance in *Enterobacteriaceae* due to acquired AmpC β-lactamases (pAmpC) or carbapenemases represents an emerging and increasing problem that limits therapeutic options. pAmpC confer resistance to most β-lactams, except cefepime and carbapenems, whereas carbapenemases, including classes A, B, and D, can confer resistance to most β-lactams, including carbapenems. The number of *Enterobacteriaceae* isolates carrying these enzymes is lower than that of extended-spectrum β-lactamase-producing isolates, but the number has increased over the last few years, particularly for pAmpC CMY-2 and DHA and for carbapenemase types NDM, VIM, IMP, and OXA-48 (1, 2).

Both families of enzymes (pAmpC and carbapenemases) are normally codified in plasmids, and their genes are associated with mobile genetic elements (MGE), such as insertion sequences, transposon-like elements, and class 1 integrons. All of these MGE can transfer these genes into mobilizable and conjugative plasmids and subsequently disseminate them into many bacterial species that naturally lack these genes (3–5).

As previously described (3), 100,132 *Enterobacteriaceae* isolates were collected from February to July 2009 from 35 Spanish hospitals. Among them, we found a total of 674 *Enterobacteriaceae* isolates with acquired *ampC* and/or carbapenemase genes. The following enzyme types were found: CMY-2-like (74.3%), DHA (17.8%), ACC (1.5%), FOX (0.6%), VIM (4.3%), and IMP (1.5%) (3). Although a great genetic diversity among pAmpC-producing strains was observed, some clonal relationships were established between these isolates, mainly in carbapenemase-producing strains (3).

This study aimed to describe the plasmid families and the surrounding regions involved in the dissemination of a great diversity of acquired *ampC* and metallo-β-lactamases genes in *Enterobacteriaceae* isolates lacking inducible chromosomal AmpC enzymes.

MATERIALS AND METHODS

Clinical isolates. To characterize the plasmids and flanking regions implicated in the expansion of these genes, we selected 85 strains from the collection cited above (3). The selection was made on the basis of prevalence, and strains that produced new enzymes were also included.

PCR-based replicon typing. PCR-based incompatibility (Inc)/replicon typing (PBRT) was used to identify the major Inc groups of the plasmids present (4, 6).

Plasmid profiles and Southern blot analysis. Plasmid analysis was carried out by DNA linearization with the S1 enzyme followed by pulsed-

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TABLE 1 Flanking regions and plasmid families associated with acquired AmpC and carbapenemases in *Enterobacteriaceae*

bla gene(s)	Flanking region(s)[a]	Plasmid size(s) or localization (kb)[b]	Replicon	Microorganism(s) (no. of isolates)
bla_{ACC-1}	ACC-1(B)	64.5	N	*K. pneumoniae* (1); *E. coli* (1)
	ACC-1(B)	Chromosomal		*E. coli* (1); *Proteus mirabilis* (1)
	ACC-1(B)	80	Unidentified	*K. pneumoniae* (1)
	ACC-1(C)	32.5	Unidentified	*P. mirabilis* (1)
	ACC-1(D)	48.5	Unidentified	*K. pneumoniae* (1)
bla_{ACC-1} + bla_{FOX-3}	ACC-1(A) + FOX (A)	64.5 + 80.5	Unidentified + U	*E. coli* (1)
bla_{CMY-2}	CMY-2(A)	177.5 to 300.7	A/C	*K. pneumoniae* (2); *P. mirabilis* (1)
	CMY-2(A)	43.7 to 83.1	I1	*Citrobacter koseri* (1); *E. coli* (4)
	CMY-2(A)	69.3	I1 + FIB	*E. coli* (1)
	CMY-2(A)	48.5 to 83.1	K	*E. coli* (3); *K. pneumoniae* (1)
	CMY-2(A)	Chromosomal		*E. coli* (1); *Klebsiella oxytoca* (1)
	CMY-2(B)	55.4	K	*E. coli* (1)
	CMY-2(B)	76.2	I1	*E. coli* (1)
	CMY-2(C)	145.5	I1	*K. pneumoniae* (1)
	CMY-2(C)	Chromosomal		*P. mirabilis* (3); *K. pneumoniae* (1); *Proteus penneri* (1)
	CMY-2(D)	83.1 to 97.6	I1	*E. coli* (1); *K. pneumoniae* (1)
bla_{CMY-4}	CMY-2(A)	105.1	K + FIB	*E. coli* (1)
	CMY-2(A)	Chromosomal		*E. coli* (1)
	CMY-2(B)	80.8	I1	*E. coli* (1)
	CMY-2(C)	88.9	I1	*K. pneumoniae* (1)
bla_{CMY-7}	CMY-2(A)	80.8	I1	*E. coli* (1)
bla_{CMY-27}	CMY-2(A)	113.2	I1	*E. coli* (2)
bla_{CMY-43}	CMY-2(A)	Chromosomal		*E. coli* (1)
bla_{CMY-48}	CMY-2(B)	Chromosomal		*E. coli* (1)
bla_{CMY-54}	CMY-2(C)	105.1	K + FIB	*E. coli* (1)
bla_{CMY-55}	CMY-2(A)	282.9	A/C	*E. coli* (1)
bla_{CMY-56}	CMY-2(A)	299.1	A/C	*K. pneumoniae* (1)
bla_{CMY-57}	CMY-2(C)	97.0	I1	*E. coli* (1)
bla_{CMY-2} + bla_{DHA-1}	CMY-2(A) + DHA-1(G)	Chromosomal + 218	FIIA	*K. pneumoniae* (1)
bla_{CMY-2} + bla_{VIM-1}	CMY-2(C) + VIM-1(B)	Chromosomal + 48	Unidentified	*P. mirabilis* (1)
bla_{DHA-1}	DHA-1(A)	72.8 to 87.3	Unidentified	*C. koseri* (1); *E. coli* (1); *K. pneumoniae* (2)
	DHA-1(A)	203.7	A/C	*K. pneumoniae* (1)
	DHA-1(A)	Chromosomal		*C. koseri* (1); *Enterobacter cloacae* (1)
	DHA-1(B)	72.8	Unidentified	*K. oxytoca* (2)
	DHA-1(B)	77.6	I1	*E. coli* (1)
	DHA-1(C)	Chromosomal		*P. mirabilis* (3)
	DHA-1(D)	72.8	Unidentified	*K. oxytoca* (1)
	DHA-1(E)	72.8	Unidentified	*Salmonella* spp. (1)
	DHA-1(F)	87.3	Unidentified	*E. coli* (1)
	DHA-1(H)	Chromosomal		*E. coli* (1)
bla_{DHA-6}	DHA-1(G)	87.3	I1	*E. coli* (1)
bla_{DHA-1} + bla_{VIM-1}	DHA-1(B) + VIM-1 (D) (In846)	76.6 + chromosomal	FIIA	*K. pneumoniae* (1)
bla_{DHA-7} + bla_{VIM-1}	DHA-1(D) + VIM-1(E) (In847)	310.4 + 48.5	HI2 + unidentified	*E. cloacae* (2)
bla_{FOX-3}	FOX(A)	72.5	U	*E. coli* (1)
bla_{FOX-8}	FOX(A)	Chromosomal		*E. coli* (2)
bla_{IMP-22}	IMP-22(A)	485.0	Unidentified	*K. pneumoniae* (2)
bla_{IMP-28}	IMP-28(A) (In767)	Chromosomal		*K. oxytoca* (1)
bla_{VIM-1}	VIM-1(A) (In488)	48.0	Unidentified	*K. oxytoca* (1)
	VIM-1(B) (In624)	48.0 and 66.2	Unidentified	*E. cloacae* (2); *K. oxytoca* (2); *K. pneumoniae* (2)
	VIM-1(C) (In846)	48.0 and 72.5	U	*K. pneumoniae* (1); *E. cloacae* (1)
	VIM-1(D) (In848)	48.0	Unidentified	*E. coli* (1)

[a] The type of surrounding regions found for each *bla* gene is in parentheses (see Fig. 1 to 3 for more data).
[b] The plasmid size was determined after the hybridization procedure. Chromosomal means possible chromosomal localization of the gene.

field gel electrophoresis (PFGE), as previously described (7). Plasmid sizes were estimated using Fingerprinting II Informatix software (Bio-Rad) (7). A PCR Dig probe synthesis kit (Roche Diagnostics GmbH, Mannheim, Germany) was used to obtain bla_{AmpC} or Inc probes for hybridization of the S1-PFGE blots. These probes were labeled with the commercial kit (Dig high-prime DNA labeling and detection starter kit II; Roche Diagnostics GmbH).

The chromosomal location of *bla* genes was analyzed by digesting the

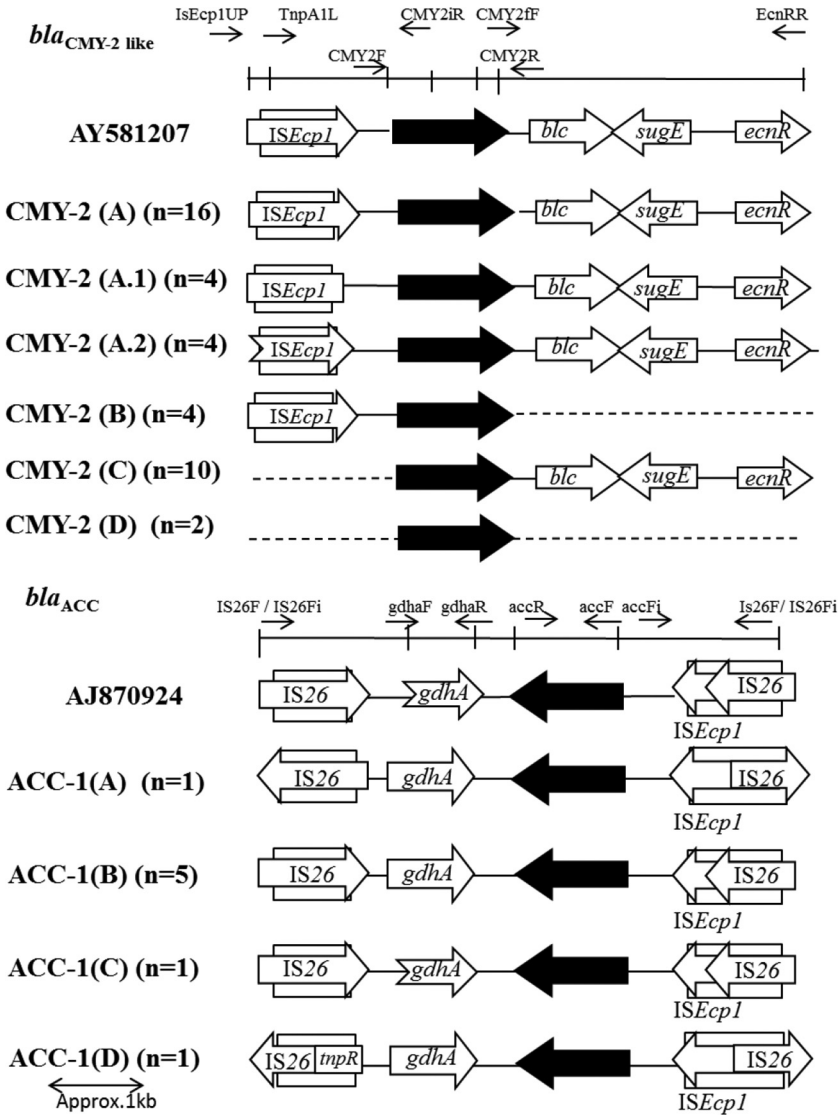

FIG 1 The genetic environment of bla_{CMY-2}-like and bla_{ACC} genes, which are indicated by solid black arrows; the surrounding genes are indicated by white arrows. The dashed lines indicate the areas that could not be amplified by PCR. Eight isolates contained truncated versions of IS*Ecp1* at the 3′ end (A.1) and the 5′ end (A.2). Truncated genes are indicated by disrupted arrows. One isolate (C) carrying the bla_{ACC} gene showed a structure identical to that of GenBank accession no. AJ870924. In one isolate (D), IS*26* was truncated by a *tnpR* gene of insertion Tn*5393*. The primers used for PCR amplification are also shown.

genomic DNA with the ICeuI enzyme, followed by PFGE and hybridization blotting as described above.

Genetic environment characterization of acquired *ampC* and carbapenemase genes. The genetic context was investigated by exploring the regions surrounding acquired bla_{AmpC} and carbapenemase genes frequently reported in the literature (8–14), employing PCR and sequencing with previously described primers. Additionally, primers designed in accordance with accessible DNA sequences in GenBank (accession numbers AY581207, AJ870924, Y11068, AJ971345, and EF577408) were used to ascertain the presence of genes linked to the acquired bla_{AmpC} and carbapenemase genes (see Table S1 in the supplemental material). Sequencing reactions were performed with the BigDye terminator kit (PE Applied Biosystems, Foster City, CA), and sequences were analyzed on an ABI Prism 3100 DNA sequencer (PE Applied Biosystems). The resulting sequences were then compared with those available in the GenBank database (www.ncbi.nih.gov/BLAST).

Nucleotide sequence accession numbers. Sequences with new integrons were submitted to GenBank under the accession numbers KC417378 (In846), KC417379 (In847), and KC417377 (In848).

RESULTS

Among the 85 selected strains (66 pAmpC-producing strains, 13 IMP/VIM-producing strains, 4 strains that produced both enzymes, and 2 strains that produced two pAmpC), we characterized the plasmids and flanking regions of 91 genes: 74 bla_{AmpC} genes, 3 bla_{IMP} genes, and 14 bla_{VIM} genes (Table 1).

The studied bla_{AmpC} genes included bla_{CMY-2}-like (40), bla_{DHA} (22), bla_{ACC} (8), and bla_{FOX} (4), while the metallo-β-lactamase genes included bla_{VIM-1} (14), bla_{IMP-22} (2), and bla_{IMP-28} (1).

Plasmid characterization. Analysis by S1-PFGE and Southern hybridization allowed us to determine the plasmid size for 74.7%

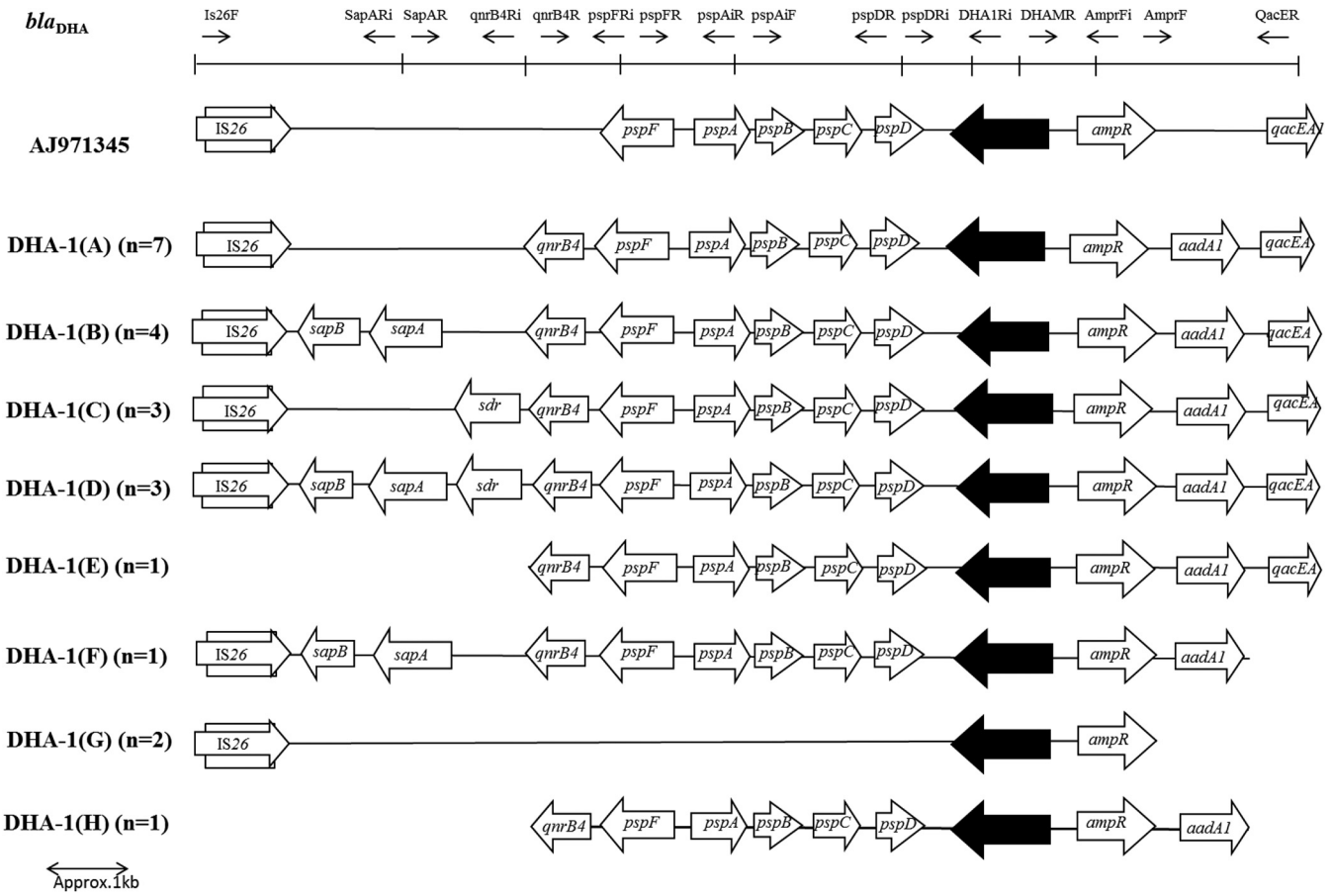

FIG 2 The genetic environment of the bla_{DHA} gene, which is indicated by solid black arrows; the surrounding genes are indicated by white arrows. Lines indicate an absence of the corresponding DNA fragments.

(68 of 91) of the studied genes, leaving 23 genes (25.3%) with a possible chromosomal location (positive hybridization in ICeuI-PFGE). Nevertheless, we were able to describe the incompatibility group by PCR-based replicon typing (PBRT) among these 68 plasmidic genes in only 41 cases (60.3%).

We found that 39 of 74 bla_{AmpC} genes (50%) were located in large plasmids of different sizes belonging to eight Inc groups, including A/C, FIB, FIIA, I1, K, HI2, N, and U. The most representative, present alone or together with other replicons, were I1 ($n = 18$; one also with FIB), K ($n = 7$; two also with FIB), A/C ($n = 6$), FIIA ($n = 2$), HI2 ($n = 2$), and N ($n = 2$) (Table 1). In 13 of 74 bla_{AmpC} genes (17.5%), the plasmid replicon was not identified. (In 10 cases, PBRT was positive for different replicons, but their hybridization bands did not match the bla_{AmpC} band, and PBRT was negative in 3 cases.) Finally, in 22 cases (29.7%), a possible chromosomal location of these genes was confirmed by ICeuI-PFGE.

The plasmids carrying bla_{CMY-2}-like genes belonged to the following Inc groups: I1 (16 of 40 [37.5%]; sizes ranged from 43.7 to 145.5 kb), K (7 of 40 [17.5%]; sizes ranged from 48.5 to 105.1 kb), and A/C (5 of 40 [12.5%]; sizes ranged from 177.5 to 300.7 kb). In three cases, the FIB plasmid was also found to be associated with one IncI1 or two IncK plasmids (Table 1).

Seven of 22 plasmids carrying bla_{DHA} genes were characterized, and the following Inc groups were found: I1 (9%; 77.6 and 87.3 kb), FIIA (9%; 76.6 and 218 kb), HI2 (9%; 291 kb), and A/C (4.5%; 203.7 kb). The remaining 15 cases were not resolved because no incompatibility group probes were hybridized ($n = 11$) or because of a negative PBRT ($n = 4$).

Only 2 of the 8 plasmids carrying bla_{ACC-1} were identified, and they belonged to the IncN group, with sizes ranging from 32.5 to 80 kb. The bla_{FOX-3} genes were found in plasmids of 72.5 and 80.5 kb, both in the IncU group, and the bla_{FOX-8} gene (15) was probably located on the chromosome.

Finally, the sizes of the 14 plasmids carrying the bla_{VIM-1} gene ranged from 48 to 72.5 kb, and one of them belonged to the IncU group; the bla_{IMP-22} genes were in plasmids of 485 kb with an unidentified Inc group. The bla_{IMP-28} gene was probably located on the chromosome, as previously described (16).

Detection of the flanking regions of acquired *ampC* and metallo-β-lactamase genes. The variable genetic environments detected for the most prevalent enzymes (CMY-2-like, DHA, ACC, and metallo-β-lactamase genes) are shown in Fig. 1, 2, and 3.

The analysis of the genetic environment revealed that bla_{CMY-2}-like genes (bla_{CMY-2}, bla_{CMY-4}, bla_{CMY-7}, bla_{CMY-27}, bla_{CMY-48}, bla_{CMY-54} and/or bla_{CMY-57}, bla_{CMY-59}, and bla_{CMY-60}) were associated with IS*Ecp1*, responsible for the transfer of the bla_{CMY-2}-like–*blc*–*sugE* region from the chromosome of *Citrobacter freundii* to plasmids (8). In our study, 16 strains contained IS*Ecp1* and *blc-sugE-ecnR* upstream and downstream of bla_{CMY-2}-like genes,

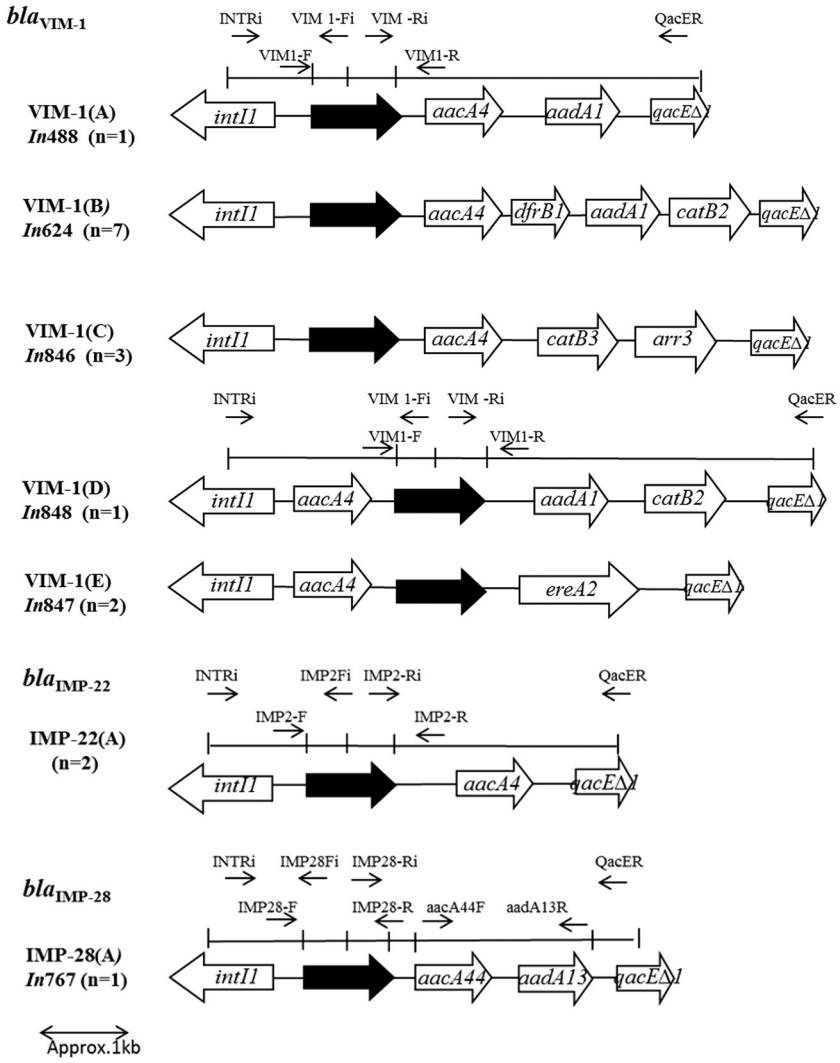

FIG 3 Structure of bla_{VIM-1}, bla_{IMP-22}, and bla_{IMP-28} genes carrying the integrons described in this work. Carbapenemase genes are indicated by solid black arrows, and the surrounding genes are indicated by white arrows. The locations of the primers used for PCR amplification are also shown.

respectively. However, truncation of IS*Ecp1*(ΔIS*Ecp1*) was observed in 8 strains: in 4 at the 3′end and in 4 at the 5′end. In the 4 strains with truncation at the 3′ end, primers described to explore this region (IS*Ecp1* and CMY2Ri) (see Table S1 in the supplemental material) amplified a product of 1,560 bp instead of the expected 2,160 bp. In the 4 strains with truncation at the 5′ end, the amplicons were not obtained using IS*Ecp1* and CMY2Ri primers, and we required a new pair of primers (Tn*pA1L* and CMY2Ri). Finally, 12 strains did not contain IS*Ecp1* upstream, and the region downstream of the bla_{CMY-2}-like gene could not be amplified by PCR in 6 strains with the primers used. Only in 2 strains with complete bla_{CMY-2}-like genes was the genetic environment unknown (Fig. 1).

The surrounding regions of bla_{DHA} genes (bla_{DHA-1}, bla_{DHA-6}, and bla_{DHA-7}) were similar to those previously described in *Klebsiella pneumoniae* plasmid pTN60013 (GenBank accession number AJ971345) (5), although a certain variability was detected in accordance with the literature data (11, 17). This variability mainly concerned the absence or presence of *sapB*, *sapA*, and *sdr* genes (Fig. 2). The quinolone resistance determinant *qnrB4* and additional *aadA1* (streptomycin and spectinomycin resistance) genes were detected in most of the strains. This linkage between bla_{DHA-1} and *qnrB4* genes has been previously described in isolates of *K. pneumoniae* (11).

In the environment of the bla_{ACC} gene, IS*Ecp1* and the *gdhA* gene were detected upstream and downstream, respectively. In all cases, IS*Ecp1* was truncated in the 5′ end (13). Six of eight bla_{ACC} genes showed two IS*26* copies in the same orientation; one of these strains contained a truncated 5′ *gdhA*, and one contained a *tnpR* gene of Tn*5393* upstream of the *gdhA* gene.

Four bla_{FOX} genes, bla_{FOX-3} ($n = 2$) and bla_{FOX-8} ($n = 2$), were located in a class I integron, at the 5′ end of the integrase IN*I1*, and several attempts to identify the 3′ end by PCR were unsuccessful.

All the metallo-β-lactamase genes (14 bla_{VIM-1}, 2 bla_{IMP-22}, and 1 bla_{IMP-28}) were located in class 1 integrons (In). In this study, we detected 5 different structures harboring bla_{VIM} genes (Fig. 3); In846 (GenBank accession number KC417378), In847 (KC417379), and In848 (KC417377) were described for the first time.

DISCUSSION

We have characterized the genetic context of the largest available collection of acquired AmpC β-lactamases and metallo-β-lactamases in *Enterobacteriaceae* lacking inducible chromosomal AmpC enzymes recovered during 2009 from 35 Spanish hospitals (3).

Several authors have related the spread of different acquired *AmpC* genes with the expansion of plasmids of certain incompatibility groups (1, 2, 4, 5, 7, 18–21). In this context, the bla_{CMY-2} gene is associated with plasmids of I1, A/C, and K incompatibility groups (7, 18–21). Our results match these data, but differences were found in the percentage of each incompatibility group. In a previous study (7) carried out during 1999 to 2007, A/C was the most predominant incompatibility group found (33%) among plasmids carrying bla_{CMY-2}, followed by I1 (23%) and K (10%). In this study (with strains isolated in 2009), the most prevalent incompatibility group was I1 (40%), followed by K (17.5%) and A/C (12.5%). The fact that different Inc plasmids have been found to carry the same resistance gene is an indication of a successful and widespread distribution; moreover, this variability contributes to the genetic environment of these genes (20). The IncA/C and IncI1 are considered epidemic plasmids, because they are found in different countries and in bacteria of diverse origin, and because they carry a range of resistance mechanisms (4). IncA/C plasmids have been described carrying ESBLs that are TEM-type or VEB, as well as NDM-1 carbapenemase. On the other hand, IncI1 has an efficient conjugative system that could also contribute to the dissemination of different resistance mechanisms, such as ESBLs that are CTX-M-type and TEM-type (4).

The genetic environment of bla_{CMY-2} and its variants was highly conserved; 60% of isolates carried the transposon-like elements IS*Ecp1* (IS*Ecp1*/ΔIS*Ecp1*-bla$_{CMY}$-*blc-sugE*), as documented in previous reports (8, 10, 18). As these bla_{CMY-2}-derived bla_{CMY} genes differ from one other by only a few nucleotide substitutions, it is possible that these differences could have evolved within the same Inc plasmid (I1) (20). The genes bla_{CMY-55} and bla_{CMY-56} were found in the A/C plasmid, and the bla_{CMY-54} gene was found in a K plasmid, in this case cointegrated with FIB.

The bla_{DHA-1} genes were initially associated with IncFII plasmids (20), but recent studies link them with IncL/M plasmids and *qnrB* determinants (5, 7). Among our DHA-producing strains, 19 showed the *qnrB4* determinant (data not shown), and none was associated with the IncL/M plasmids. In fact, we were able to characterize the plasmid in only 7 cases (38.8%); I2, FIIA, and HI2 were the incompatibility groups found. The genetic organization of bla_{DHA} genes was more variable. Mobilization of this enzyme has been associated with IS*26* or class 1 integron-bearing IS*CR1* elements (11, 18). Among bla_{DHA}-carrying isolates, 86% were associated with IS*26*.

In the literature, characterization of plasmids carrying bla_{ACC-1} genes is scarce. In a previous study, a bla_{ACC-1} gene in an *Escherichia coli* strain was found in an IncI1 plasmid (7), but other authors could not type it (21). Regarding the genetic context of bla_{ACC-1} genes, an IS*Ecp1*element truncated at the 5′ end with an IS*26* insertion sequence was found in all of our bla_{ACC-1}-carrying isolates, as described in previous reports (12, 13).

bla_{FOX} and all carbapenemases detected in this study, including previously undescribed structures (in bla_{FOX}), were located in a class 1 integron and present in the most recent IncU plasmids.

There are few data on the types of plasmids involved in the spread of metallo-β-lactamases. In the literature, bla_{IMP} and bla_{VIM} genes are described in plasmids of incompatibility groups I1, N, W, and HI2 (4). In this study, the bla_{IMP-22} gene was located in a 485-kb plasmid of an uncharacterized incompatibility group, and two bla_{VIM-1} genes were found in IncU plasmids, curiously both isolated from different species but in the same hospital. Finally, the location of the bla_{IMP-28} gene seems to be chromosomal, as bla_{IMP-28}-positive hybridization was found in the ICeuI-PFGE membrane.

Accordingly, the high number of unidentified replicons could be associated with plasmids other than those tested; alternatively, they could be associated with one of the tested plasmids, albeit with some genetic variability, as has been described for the carbapenemase NDM in plasmids with a variant of the IncN or IncHI1 groups (4).

In conclusion, although plasmids have proven to be one of the main causes of the rapid dissemination of bla_{AmpC} and carbapenemase genes among bacteria, other MGE must play an important role in the increasing prevalence of these enzymes. Further studies, focused not only on plasmids but also on other MGE, such as insertion sequences, transposons, or integrative conjugative elements, are needed to gain a better understanding of the complex process involved in the dissemination of antibiotic resistance genes worldwide.

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REFERENCES

1. **Castanheira M, Farell SE, Krause KM, Jones RN, Sader HS.** 2014. Contemporary diversity of β-lactamases among *Enterobacteriaceae* in the nine U.S. census regions and ceftazidime-avibactam activity tested against isolates producing the most prevalent β-lactamase groups. Antimicrob Agents Chemother **58:**833–838. http://dx.doi.org/10.1128/AAC.01896-13.
2. **Lascols C, Peirano G, Hackel M, Laupland KB, Pitout JDD.** 2013. Surveillance and molecular epidemiology of *Klebsiella pneumoniae* isolates that produce carbapenemases: first report of OXA-48-like enzymes in North America. Antimicrob Agents Chemother **57:**130–136. http://dx.doi.org/10.1128/AAC.01686-12.
3. **Miró E, Agüero J, Larrosa MN, Fernández A, Conejo MC, Bou G, González-López JJ, Lara N, Martínez-Martínez L, Oliver A, Aracil B, Oteo J, Pascual A, Rodriguez-Baño J, Zamorano L, Navarro F.** 2013. Prevalence and molecular epidemiology of acquired AmpC β-lactamases and carbapenemases in *Enterobacteriaceae* isolates from 35 hospitals in Spain. Eur J Clin Microbiol Infect Dis **32:**253–259. http://dx.doi.org/10.1007/s10096-012-1737-0.
4. **Carattoli A.** 2013. Plasmids and the spread of resistance. Int J Med Microbiol **303:**298–304. http://dx.doi.org/10.1016/j.ijmm.2013.02.001.
5. **Mata C, Miró E, Mirelis B, Garcillán-Barcia MP, de la Cruz F, Coll P, Navarro F.** 2010. *In vivo* transmission of a plasmid coharbouring bla_{DHA-1} and *qnrB* genes between *Escherichia coli* and *Serratia marcescens*. FEMS Microbiol Lett **308:**24–28. http://dx.doi.org/10.1111/j.1574-6968.2010.01980.x.
6. **Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ.** 2005. Identification of plasmids by PCR-based replicon typing. J Microbiol Methods **63:**219–228. http://dx.doi.org/10.1016/j.mimet.2005.03.018.
7. **Mata C, Miró E, Alvarado A, Garcillán-Barcia MP, Toleman M, Walsh TR, de la Cruz F, Navarro F.** 2012. Plasmid typing and genetic context of AmpC β-lactamases in *Enterobacteriaceae* lacking inducible chromosomal *ampC* genes: findings from a Spanish hospital 1999 to 2007. J Antimicrob Chemother **67:**115–122, 2012. http://dx.doi.org/10.1093/jac/dkr412.

8. **Verdet C, Gautier V, Chachaty E, Ronco E, Hidri N, Decré D, Arlet G.** 2009. Genetic context of plasmid-carried bla_{CMY-2}-like genes in *Enterobacteriaceae*. Antimicrob Agents Chemother **53:**4002–4006. http://dx.doi.org/10.1128/AAC.00753-08.
9. **Wei ZQ, Chen YG, Yu YS, Lu WX, Li LJ.** 2005. Nosocomial spread of multiresistant *Klebsiella pneumoniae* containing a plasmid encoding multiple β-lactamases. J Med Microbiol **54:**885–888. http://dx.doi.org/10.1099/jmm.0.46151-0.
10. **Kang MS, Besser TE, Call DR.** 2006. Variability in the region downstream of the bla_{CMY-2} β-lactamase gene in *Escherichia coli* and *Salmonella enterica* plasmids. Antimicrob Agents Chemother **50:**1590–1593. http://dx.doi.org/10.1128/AAC.50.4.1590-1593.2006.
11. **Verdet C, Benzerara Y, Gautier V, Adam O, Ould-Hocine Z, Arlet G.** 2006. Emergence of DHA-1-producing *Klebsiella* spp. in the Parisian region: genetic organization of the *ampC* and *ampR* genes originating from *Morganella morganii*. Antimicrob Agents Chemother **50:**607–617. http://dx.doi.org/10.1128/AAC.50.2.607-617.2006.
12. **Partridge SR.** 2007. Genetic environment of IS*Ecp1* and bla_{ACC-1}. Antimicrob Agents Chemother **51:**2658–2659. http://dx.doi.org/10.1128/AAC.00364-07.
13. **Doloy A, Verdet C, Gautier V, Decré D, Ronco E, Hammami A, Philippon A, Arlet G.** 2006. Genetic environment of acquired bla_{ACC-1} β-lactamase gene in *Enterobacteriaceae* isolates. Antimicrob Agents Chemother **50:**4177–4181. http://dx.doi.org/10.1128/AAC.00619-06.
14. **Gutiérrez O, Juan C, Cercenado E, Navarro F, Bouza E, Coll P, Pérez JL, Oliver A.** 2007. Molecular epidemiology and mechanisms of carbapenem resistance in *Pseudomonas aeruginosa* isolates from Spanish hospitals. Antimicrob Agents Chemother **51:**4329–4335. http://dx.doi.org/10.1128/AAC.00810-07.
15. **Pérez-Llarena FJ, Kerff F, Zamorano L, Fernández MC, Nuñez ML, Miró E, Oliver A, Navarro F, Bou G.** 2013. Characterization of the new AmpC β-lactamase FOX-8 reveals a single mutation, Phe313Leu, located in the R2 loop that affects ceftazidime hydrolysis. Antimicrobial Agents Chemother **57:**5158–5161. http://dx.doi.org/10.1128/AAC.00818-13.
16. **Pérez-Llarena FJ, Fernández A, Zamorano L, Kerff F, Beceiro A, Aracil B, Cercenado E, Miro E, Oliver A, Oteo J, Navarro F, Bou G.** 2012. Characterization of a novel IMP-28 metallo-β-lactamase from a Spanish *Klebsiella oxytoca* clinical isolate. Antimicrob Agents Chemother **56:**4540–4543. http://dx.doi.org/10.1128/AAC.00776-12.
17. **Jiang Y, Yu D, Wei Z, Shen P, Zhou Z, Yu Y.** 2010. Complete nucleotide sequence of *Klebsiella pneumoniae* multidrug resistance plasmid pKP048, carrying bla_{KPC-2}, bla_{DHA-1}, *qnrB4*, and *armA*. Antimicrob Agents Chemother **54:**3967–3969. http://dx.doi.org/10.1128/AAC.00137-10.
18. **Naseer U, Haldorsen B, Simonsen GS, Sundsfjord A.** 2010. Sporadic occurrence of CMY-2-producing multidrug-resistant *Escherichia coli* of ST-complexes 38 and 448, and ST131 in Norway. Clin Microbiol Infect **16:**171–178. http://dx.doi.org/10.1111/j.1469-0691.2009.02861.x.
19. **Folster JP, Pecic G, Singh A, Duval B, Rickert R, Ayers S, Abbott J, McGlinchey B, Bauer-Turpin J, Haro J, Hise K, Zhao S, Fedorka-Cray PJ, Whichard J, McDermott PF.** 2012. Characterization of extended-spectrum cephalosporin-resistant *Salmonella enterica* serovar Heidelberg isolated from food animals, retail meat, and humans in the United States 2009. Foodborne Pathog Dis **9:**638–645. http://dx.doi.org/10.1089/fpd.2012.1130.
20. **Hopkins KL, Liebana E, Villa L, Batchelor M, Threlfall EJ, Carattoli A.** 2006. Replicon typing of plasmids carrying CTX-M or CMY β-lactamases circulating among *Salmonella* and *Escherichia coli* isolates. Antimicrob Agents Chemother **50:**3203–3206. http://dx.doi.org/10.1128/AAC.00149-06.
21. **Dierikx C, van Essen-Zandbergen A, Veldman K, Smith H, Mevius D.** 2010. Increased detection of extended spectrum β-lactamase producing *Salmonella enterica* and *Escherichia coli* isolates from poultry. Vet Microbiol **145:**273–278. http://dx.doi.org/10.1016/j.vetmic.2010.03.019.