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Comparative Analysis of IncHI2 Plasmids Carrying $bla_{CTX-M-2}$ or $bla_{CTX-M-9}$ from *Escherichia coli* and *Salmonella enterica* Strains Isolated from Poultry and Humans[▽]

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***Salmonella enterica* $bla_{CTX-M-2}$ and $bla_{CTX-M-9}$ plasmid backbones from isolates from Belgium and France were analyzed. The $bla_{CTX-M-2}$ plasmids from both human and poultry isolates were related to the IncHI2 pAPEC-O1-R plasmid, previously identified in the United States in avian *Escherichia coli* strains; the $bla_{CTX-M-9}$ plasmids were closely related to the IncHI2 R478 plasmid.**

The plasmid-encoded CTX-M family have recently become dominant extended-spectrum β-lactamases (ESBLs) in human isolates in Europe (13). Significantly, the CTX-M enzymes have emerged in isolates of *Escherichia coli* and *Salmonella* spp. from food, companion, and wild animals, raising a potential public health concern (1, 2, 4, 6, 12, 14–16).

In Belgium and France, *Salmonella enterica* serovar Virchow producing the CTX-M-2 ESBL was firstly identified in poultry flocks, then in poultry meat, and subsequently in humans over the period 2000 to 2003 (3). The chronology of isolation of these strains suggested that these bacteria were transmitted to humans via the food chain, specifically by poultry meat. In French Guiana in 2004, a *Salmonella enterica* serovar Typhimurium strain producing CTX-M-2 was isolated from a patient with gastroenteritis. In France in 2002, one CTX-M-9-producing *S. enterica* rough strain was isolated from a patient, and in 2003, *S.* enterica serovar Virchow strains were isolated from poultry (17).

The objective of this study was to characterize plasmids carrying $bla_{CTX-M-2}$ and $bla_{CTX-M-9}$ from poultry sources and to compare them with those circulating in humans. CTX-M-positive transconjugants were obtained in *E. coli* K-12 from CTX-M-2 or CTX-M-9 producers. The following transconjugates were selected for this study: six from the *S. enterica* serovar Virchow strain of animal origin, one from the rough isolate, and one from the *S. enterica* serovar Typhimurium strain (Table 1). The parental strains of these transconjugants were representative of CTX-M-2 or -9 producers for country of isolation, source, pulsed-field gel electrophoresis patterns, and resistance profiles, as previously determined (3, 17). The $bla_{CTX-M-9}$ integrons in these strains were previously characterized, and gene cassette arrays are reported in Table 1 (17).

To determine the structure of the $bla_{CTX-M-2}$-carrying integrons, the 8,690-bp and 9,017-bp fragments were amplified and fully sequenced from the plasmids of the *S. enterica* serovar Virchow p1639-SA-00-1 and *S. enterica* serovar Typhimurium p04-9275-1 strains, respectively. Sequencing results indicate that the integron in *S. enterica* serovar Virchow was 99% identical to the CTX-M-2 integron found in the *E. coli* strain of human origin identified in France in 2006 (7) carrying the *dfrA1* and *aadA1* gene cassettes (deposited in GenBank under accession no. EF592570). The integron from the *S. enterica* serovar Typhimurium strain showed a different variable region, including the *dfrA12*, *orfF*, and *aadA2* gene cassettes (Table 1 [deposited in GenBank under accession no. EF592571]).

Plasmids carrying $bla_{CTX-M-2}$ or $bla_{CTX-M-9}$ were analyzed by PCR-based replicon typing (PBRT) (5): PBRT indicates that all the strains were positive for the HI2 replicon, thus suggesting the presence of common IncHI2 plasmids in these strains. The HI2 replicon was previously detected on CTX-M-9-positive *Salmonella* and *E. coli* strains of human origin from Spain (8, 9). Three transconjugants obtained from representative strains of the Spanish collection (one from an *S. enterica* serovar Virchow strain and two from *E. coli*) were also included in this study for comparison (Table 1).

The HI2 amplicon was sequenced to confirm the PBRT result, and two types of DNA sequences were observed: the R478 type, showing 100% identity with the HI2 replicon of the reference plasmid R478 isolated from *Serratia marcescens* in the United States in 1969 (10); and the HI2-APEC-O1-R type, showing 100% homology with the HI2 replicon of the pAPEC-O1-R plasmid, recently described in avian pathogenic *E. coli* strains in the United States (11). HI2-R478 and HI2-pAPEC-O1-R differ by 29 nucleotide substitutions located within the *cis*-repeats (iterons) flanking the replicase (*repA*) gene (accession no. BX664015 and DQ517526, respectively).

Interestingly, the HI2-R478 replicon type was identified in plasmids carrying the $bla_{CTX-M-9}$ gene, while the HI2-pAPEC-O1-R replicon type was identified in plasmids carrying the $bla_{CTX-M-2}$ gene (Table 1).

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TABLE 1. Plasmid backbone analysis of the IncHI2 plasmids in transconjugants obtained from *Salmonella* and *Escherichia coli* CTX-M-2 and CTX-M-9 producers

Plasmid (ESBL)	Parental strain (source)	Country (yr)	HI2 replicon type	PCR result for:												Reference
				smr10-11	*smr17-18*	*smr207-208*	*smr239-240*	O1R_160	*terF*	*arsB*	*smr201*	*smr92-93*	*smr136*	*tnsD*	Integron-borne gene cassettes	
p1639-SA-00-1 (CTX-M-2)	*S. enterica* serovar Virchow (poultry)	Belgium (2000)	HI2-pAPEC-O1-R	+	+	+	+	+	+	−	−	−	−	−	*dfrA1-aadA1*	This study
p142-SA-01-1 (CTX-M-2)	*S. enterica* serovar Virchow (poultry)	Belgium (2001)	HI2-pAPEC-O1-R	+	+	+	+	+	+	−	−	−	−	−	*dfrA1-aadA1*	This study
p03-1902-1 (CTX-M-2)	*S. enterica* serovar Virchow (poultry product)	Belgium (2003)	HI2-pAPEC-O1-R	+	+	+	+	+	+	−	−	−	−	−	*dfrA1-aadA1*	This study
p04-9275-1 (CTX-M-2)	*S. enterica* serovar Typhimurium (human feces)	French Guiana (2004)	HI2-pAPEC-O1-R	+	+	+	+	+	+	−	−	−	−	−	*drfA12-orfF-aadA2*	This study
pROU-1 (CTX-M-9)	*S. enterica* rough (human feces)	France (2002)	HI2-R478	+	+	+	+	+	+	+	+	+	−	−	*dfrA16-aadA2*	This study, 17
p2437-1 (CTX-M-9)	*S. enterica* serovar Virchow (chicken feces)	France (2003)	HI2-R478	+	+	+	+	+	+	+	+	+	−	−	*dfrA16-aadA2*	This study, 17
p2731-1 (CTX-M-9)	*S. enterica* serovar Virchow (retail chicken meat)	France (2003)	HI2-R478	+	+	+	+	+	+	+	+	+	−	−	*dfrA16-aadA2*	This study, 17
p3464b-1 (CTX-M-9)	*S. enterica* serovar Virchow (chicken environment)	France (2003)	HI2-R478	+	+	+	+	+	+	+	+	+	−	−	*dfrA16-aadA2*	This study, 17
112-D-T (CTX-M-9)	*S. enterica* serovar Virchow (human feces)	Spain (2000)	HI2-R478	+	+	+	+	+	+	+	+	+	−	−	*dfrA16-aadA2*	8, 9
1185-D-T (CTX-M-9)	*E. coli* (human feces)	Spain (1998)	HI2-R478	+	+	+	+	+	+	+	+	+	−	−	*dfrA16-aadA2*	8, 9
1406-D-T (CTX-M-9)	*E. coli* (human feces)	Spain (1999)	HI2-R478	+	+	+	+	+	+	+	+	+	−	−	*dfrA16-aadA2*	8, 9
R478[a]			HI2-R478	+	+	+	+	−	+	+	+	+	+	+	−	10
pAPEC-01-R[b]			HI2-pAPEC-O1-R	+	+	+	+	+	+	−	−	−	−	−	*aadA1*	11

[a] Expected PCR result based on the R478 sequence (EMBL accession no. BX664015).
[b] Expected PCR result based on the pAPEC-O1-R sequence (EMBL accession no. DQ517526).

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TABLE 2. Primers used in the plasmid backbone analysis[a]

Primer pair	Product of target gene in R478[b]	Primer sequence	PCR gene name (position of amplicon in R478[b] sequence)
10Fw 11Rv	HtdV, putative membrane protein HtdT, putative plasmid transfer protein	5′-AATCGCCTGAATCAGCTGG-3′ 5′-TTCTTTACTACACCAGAGCC-3′	*smr10-11* (6655–7713)
17Fw 18Rv	SMR0017, putative lipoprotein	5′-AACTCTTGAAAATCGTGG-3′ 5′-CTTCAGGCTATCGTTTCG-3′	*smr17-18* (18211–19101)
TerFw TerRv	Tellurium resistance protein	5′-ATGCAGGCTCAAGGAATCGC-3′ 5′-TTCATCGATCCACGGTCTG-3′	*terF* (80270–81163)
92Fw 93Rv	SMR0092, hypothetical protein SMR0093, putative inner membrane protein	5′-CTATGTAAGCAATGATCCTC-3′ 5′-TATAGAGAGCACCGAAGG-3′	*smr92-93* (88861–89862)
TnsDAFw TnsDARv	Tn*7*-like transposition protein	5′-AATCCTTGTTCAGCCGG-3′ 5′-CAAAAGCCAGCCATGCCC-3′	*tnsD* (119360–120825)
136Afw 136Arv	SMR0136, hypothetical protein	5′-TACGAAAATGAATTGTGGC-3′ 5′-AATTTACAATCTGCAGCCC-3′	*smr136* (120906–121768)
ArsBFw ArsBRv	Arsenical pump membrane protein	5′-AGTGAAAGACAGACGAAGCG-3′ 5′-GGCAGATAGTGTGGAATGCG-3′	*arsB* (159735–160870)
201Fw 201Rv	SMR0201 hypothetical protein	5′-TGTCAGGCTAAGTCACTGG-3′ 5′-ATTATACGGTAGATCC-3′	*smr201* (180398–181466)
207Fw 208Rv	SMR0207, conserved hypothetical protein SMR0208, hypothetical protein	5′-TTTCCCAAATAGGCGACGC-3′ 5′-ATGTGAAATTACTATACCGG-3′	*smr207-208* (190238–191131)
239Fw 240Rv	SMR0239, hypothetical protein SMR0240, hypothetical protein	5′-TGGAACGCGTGGTATGTGG-3′ 5′-ATACCTGCCGTTTACCC-3′	*smr239-240* (219372–220364)
O1R_160Fw O1R_160Rv	O1R_160, Tn*10* insertion site	5′-TTATGATGCTGGGCGTACC-3′ 5′-CACCATTACAATCACCTCC-3′	O1R_160 (202719–212075)[c]

[a] The DNA from the reference plasmid R478 of the incompatibility group IncHI2 was used as control for PCR assays. Amplification conditions were initial denaturation at 94°C for 5 min and 30 cycles at 94°C for 1 min, annealing at 60°C for 30 s, and elongation at 72°C for 1 min.
[b] EMBL accession no. BX664015.
[c] Positions 183121 to 182957 in EMBL accession no. DQ517526.

R478 and pAPEC-O1-R plasmids show many common regions, including the previously mentioned HI2 replicon, but also the Tra1 and Tra2 transfer regions and the genes coding for copper, silver, and tellurite resistance (10, 11). The two plasmids diverge in terms of insertion sequences, the presence or absence of a class 1 integron, the Tn*10* transposon, and arsenic and mercury resistance loci (11).

To further characterize the CTX-M-2 and CTX-M-9 IncHI2 plasmid backbones, we used a PCR-based scheme, previously applied to the CTX-M-9 producers isolated in Spain (Table 2) (9). This scheme recognizes five DNA targets unique for IncHI2 plasmids and common between R478 and pAPEC (*smr10-11*, *smr17-18*, *smr207-208*, *smr239-240* and *terF*) and five DNA targets that are specific for R478 but negative in pAPEC (*smr201*, *smr92-93*, *smr136*, *tnsD*, and *arsB*). An additional PCR is also included here to recognize the O1R_160 gene of pAPEC that is interrupted by a Tn*10* insertion in R478 (Table 2). Testing the transconjugants listed in Table 1, we observed that the O1R_160 gene was present in all of the strains, while the *smr136* and *tnsD* genes were absent: these results indicate that both the Tn*10* and Tn*7* transposons are missing in the CTX-M-2 and -9 plasmids analyzed in this study.

All of the plasmids were also positive for the five targets recognizing both R478 and pAPEC plasmids, while the $bla_{\text{CTX-M-2}}$-carrying plasmids were negative for the *arsB*, *smr92*, and *smr201* targets, which were positively detected in the $bla_{\text{CTX-M-9}}$-carrying plasmids (Table 1). These results provided evidence that the $bla_{\text{CTX-M-2}}$ and $bla_{\text{CTX-M-9}}$ plasmids show different plasmid scaffolds despite belonging to the same IncHI2 family: the CTX-M-2 producers from both human and poultry sources are associated with pAPEC-O1-R derivative plasmids, while the CTX-M-9 producers are associated with R478 derivative plasmids. The association between $bla_{\text{CTX-M-9}}$ and R478 was previously demonstrated for *E. coli* and *S. enterica* CTX-M-2 and -9 producers isolated in Spain (9). However, this is the first description of CTX-M-9-R478 derivative plasmids in animal sources.

This is also the first description of pAPEC-O1-R derivative plasmids carrying the $bla_{\text{CTX-M-2}}$ gene. It is of concern that the pAPEC-O1-R plasmid, largely prevalent in avian pathogenic and commensal *E. coli* strains in the United States (11), is detected with the $bla_{\text{CTX-M-2}}$ gene in *Salmonella* strains of human and animal origins in Europe.

Contamination with ESBL-producing strains of *S. enterica* has been extensively suggested to occur through the food chain, but very few *Salmonella* ESBL producers of animal origin have been reported, while these enzymes are frequently detected in human isolates. In this study, plasmid-mediated

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horizontal transfer of $bla_{CTX-M-2}$ and $bla_{CTX-M-9}$ genes has been demonstrated between poultry and human *S. enterica* and *E. coli* strains isolated in very different geographical regions. This result is of major concern because food animals may represent a large reservoir for a further dissemination of such genetic determinants to human pathogens.

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