$bla_{\text{CTX-M-1/9/1}}$ Hybrid Genes May Have Been Generated from $bla_{\text{CTX-M-15}}$ on an IncI2 Plasmid

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Three hybrid CTX-M β-lactamases, CTX-M-64, CTX-M-123, and CTX-M-132, with N and C termini matching CTX-M-1 group enzymes and centers matching CTX-M-9 group enzymes, have been identified. The hybrid gene sequences suggested recombination between $bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-14}}$, the two most common $bla_{\text{CTX-M}}$ variants worldwide. However, $bla_{\text{CTX-M-64}}$ and $bla_{\text{CTX-M-123}}$ are found in an IS$Ecp1$-$bla_{\text{CTX-M}}$ transposition unit with a 45-bp "spacer," rather than the 48 bp usually associated with $bla_{\text{CTX-M-15}}$, and 112 bp of IncA/C plasmid backbone. This is closer to the context of $bla_{\text{CTX-M-55}}$, which has one nucleotide difference from $bla_{\text{CTX-M-15}}$, on IncI2 plasmid pHN1122-1. Here, we characterized an IncI2 plasmid carrying $bla_{\text{CTX-M-15}}$ with a 45-bp spacer (pHNY2-1) by complete sequencing and also sequenced IncI2 plasmids carrying $bla_{\text{CTX-M-64}}$ (pHNAH46-1) or $bla_{\text{CTX-M-132}}$ (pHNLDH19) and an IncI1 plasmid carrying $bla_{\text{CTX-M-123}}$ (pHNAH4-1). pHNY2-1 has the same IS$Ecp1$-$bla_{\text{CTX-M}}$-IncA/C insertion as pHN1122-1, pHNAH46-1, and pHNLDH19, and all four plasmid backbones are almost identical. pHNAH4-1 (IncI1 sequence type 108 [ST108]) carries a transposition unit that includes a 2,720-bp fragment of the IncI2 backbone, suggesting IS$Ecp1$-mediated transfer of $bla_{\text{CTX-M}}$-IncA/C-IncI2 to an IncI1 plasmid. All three hybrid $bla_{\text{CTX-M}}$ genes may have resulted from recombination between $bla_{\text{CTX-M-14}}$ and $bla_{\text{CTX-M-15}}$ with a 45-bp spacer on an IncI2 plasmid. Five additional *Escherichia coli* isolates of different sequence types from different provinces, farms, and/or animals had $bla_{\text{CTX-M-64}}$ on a pHNAH46-1-like IncI2 plasmid and 9 had $bla_{\text{CTX-M-123}}$ on a pHNAH4-1-like IncI1 ST108 plasmid. Thus, epidemic IncI plasmids may be responsible for the spread of $bla_{\text{CTX-M-64}}$ and $bla_{\text{CTX-M-123}}$ between different animals and different locations in China.

CTX-M-type extended-spectrum β-lactamases (ESBLs) that exhibit potent activity against extended-spectrum cephalosporins are widespread, not only in human clinical settings but also in the community and in animals worldwide. The CTX-M family has been considered a model example of the evolution of drug resistance, and their worldwide spread represents a success story for antimicrobial resistance (1, 2). More than 160 CTX-M variants have been identified (http://www.lahey.org/Studies/other.asp#table1), and these can be divided into six groups (CTX-M-1, CTX-M-2, CTX-M-9, CTX-M-8, CTX-M-25, and KLUC) with an intergroup amino acid identity of ≤90% (2, 3). Evolution within different CTX-M groups is the result of gradual accrual of mutations under the selective pressure exerted by the presence of antibiotics (1, 2). However, recombination between genes from different CTX-M groups has also accelerated this evolution (1).

Four CTX-M enzymes (CTX-M-64, CTX-M-123, CTX-M-132, and CTX-M-137) that are hybrids of members of the CTX-M-1 and CTX-M-9 groups have emerged in recent years (4–6). CTX-M-137 is a simple hybrid, with the N terminus matching CTX-M-14 and the C terminus matching CTX-M-15 (6). The other three hybrids match CTX-M-1 group enzymes at the start and end but CTX-M-9 group enzymes in the middle, suggesting different double crossover events (4). Interestingly, these four CTX-M hybrids have all been found in China, where three of them were first reported, and have been detected in animals and in animal food (4, 6–11).

These hybrid $bla_{\text{CTX-M}}$ genes have all been suggested to be the result of recombination between $bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-14}}$, the most dominant variants detected worldwide (2, 4–6). However, analysis of the genetic environment surrounding $bla_{\text{CTX-M-123}}$ and

$bla_{\text{CTX-M-64}}$ showed that IS$Ecp1$ lies 45 bp upstream of these genes, rather than the usual 48 bp seen with $bla_{\text{CTX-M-15}}$, and $orf477\Delta$ downstream of $bla_{\text{CTX-M}}$ is followed by a 112-bp fragment matching IncA/C plasmid backbones (4). This genetic environment resembles that of $bla_{\text{CTX-M-55}}$ in the IncI2 plasmid pHN1122-1, from an *Escherichia coli* isolate from a dog in Guangzhou (12), but $bla_{\text{CTX-M-55}}$ has a nucleotide substitution at position 239 (resulting in A77V) compared with $bla_{\text{CTX-M-15}}$ (4). One possibility was that the $bla_{\text{CTX-M-1/9/1}}$ hybrids resulted from recombination with $bla_{\text{CTX-M-15}}$ with a 45-bp spacer on an IncI2 plasmid (4), an arrangement which had only been reported in a single isolate from Russia in 2010 (13). Here we identified and characterized a plasmid with $bla_{\text{CTX-M-15}}$ and a 45-bp spacer by complete sequencing and also completely sequenced plasmids carrying $bla_{\text{CTX-M-64}}$, $bla_{\text{CTX-M-132}}$, or $bla_{\text{CTX-M-123}}$, for which the immediate contexts

TABLE 1 Isolates and plasmids carrying $bla_{CTX-M-15}$, $bla_{CTX-M-64}$, $bla_{CTX-M-123}$, or $bla_{CTX-M-132}$

Isolate[a]	bla_{CTX-M}	Date of isolation (mo/yr)	Source	Region/farm[b]	E. coli MLST	Plasmid marker[c]	IncI2/orf477[d]	IncI2/ISEcp1[e]	Plasmid size (kb)[f]
IncI2									
JC2[g]	15	11/2006	Chicken	SD17	224	**pHNY2-1**	P	P	65,358
AHC46[h]	64	6/2011	Chicken feces	AH14	1011	**pHNAH46-1**	P	P	62,194
LDH19[i]	132	1/2013	Human urine	GD-	4528	**pHNLDH19**	P	P	62,194
SG0514-2[c]	64	3/2012	Chicken heart	GD07	117	P	P	P	~62
ACH5[i]	64	6/2011	Chicken feces	AH03	162	P	P	P	~62
SG0532-2[i]	64	3/2012	Chicken heart	GD07	3851	P	P	P	~62
GDC16[i]	64	8/2010	Chicken feces	GD15	4474	P	P	P	~62
BSC1[i]	64	1/2010	Chicken feces	GD13	4346	P	P	P	~62
IncI1 ST108									
AHC4[i]	123	6/2011	Chicken feces	AH04	746	**pHNAH4-1**	P	NA	109,194
AHC13[h]	123	6/2011	Chicken feces	AH06	746	P	P	NA	~113
AHC14[i]	123	6/2011	Chicken feces	AH06	746	P (no MRR1)	P	NA	~113
AHC2[h]	123	6/2011	Chicken feces	AH03	746	P (no MRR1)	P	NA	~113
AHC54[i]	123	7/2011	Chicken feces	AH02	155	P	P	NA	~113
NKSC61[h]	123	1/2011	Chicken liver	GD07	155	P	P	NA	~109
AHC55[h]	123	7/2011	Chicken feces	AH02	162	P	P	NA	~113
FKP358[i]	123	11/2010	Pig feces	GD05	165	P	P	NA	~113
FKD567[h]	123	8/2012	Duck feces	GD01	1437	P	P	NA	~113
NKSC1[i]	123	1/2011	Chicken liver	GD07	2309	P	P	NA	~105
Inc group not determined									
FKP614F[j]	64	11/2011	Pig feces	GD16	746	ND	ND	ND	ND
TP36[g]	123	6/2012	Chicken heart	GD12	93	ND	ND	ND	ND
FKP587[j]	123	1/2011	Pig feces	GD09	156	ND	ND	ND	ND
FKD453[j]	123	1/2011	Duck feces	GD10	205	ND	ND	ND	ND
FKP97[j]	123	8/2010	Pig feces	GD11	1437	ND	ND	ND	ND
FKP745[j]	123	8/2012	Pig liver	GD08	1771	ND	ND	ND	ND

[a] Bold type indicates isolates from which plasmids were completely sequenced. Plasmid names are shown in the "Plasmid marker" column.

[b] AH, Anhui Province; GD, Guangdong Province; SD, Shandong Province. Farms are numbered. LDH19 is from a human clinical isolate.

[c] Transconjugants (Tc) or transformants (Tx) were screened for IncI2 or IncI1 (pHNAH4-1) markers, as appropriate, using primers in Table S1 in the supplemental material. P, positive; ND, not determined.

[d] PCR with primers CHP1-F and Orf477-R (see Table S1), linking the IncI2 backbone fragment to $orf477$.

[e] PCR with HP2-R and ISEcp1-F (see Table S1) linking the IncI2 backbone to IS$Ecp1$. NA, not applicable.

[f] Approximate plasmid sizes were estimated from S1 gels.

[g] Gave Tx carrying bla_{CTX-M} but more than one plasmid.

[h] Gave Tx carrying a single plasmid with bla_{CTX-M}.

[i] Gave Tc carrying a single plasmid with bla_{CTX-M}.

[j] No Tc/Tx carrying the relevant bla_{CTX-M} gene were obtained.

had already been identified ([12]), to try to define how these hybrid genes may have arisen.

MATERIALS AND METHODS

E. coli isolates. AHC4, carrying $bla_{CTX-M-123}$, and AHC46, carrying $bla_{CTX-M-64}$, were found in chicken samples submitted to a veterinary diagnostic center in Anhui Province, China, and the contexts of their bla_{CTX-M} genes had already been partially characterized ([4]). JC2, from a chicken sample from Shandong Province, was identified as carrying $bla_{CTX-M-15}$ in a previous study ([14]). LDH19 was from a urine sample collected in 2013 from a female patient at a community hospital in Guangzhou. PCR with published primers (see Table S1 in the supplemental material) was used to amplify $bla_{CTX-M-1}$ group and $bla_{CTX-M-1/9/1}$ hybrid genes in LDH19 and to determine the spacer length in both isolates. Five more E. coli isolates carrying $bla_{CTX-M-64}$ and 15 carrying $bla_{CTX-M-123}$ were available from a set of isolates collected from animals in China in 2010-2012 (Table 1) ([14]). Multilocus sequence typing (MLST) was performed according to http://mlst.warwick.ac.uk.

Conjugation experiments. Transconjugants of AHC4 carrying pHNAH4-1 ($bla_{CTX-M-123}$) and transformants of AHC46 carrying pHNAH46-1 ($bla_{CTX-M-64}$) were obtained previously ([4]). LDH19, JC2, and the 20 isolates carrying $bla_{CTX-M-123}$ or $bla_{CTX-M-64}$ were conjugated by filter mating with streptomycin-resistant E. coli C600 and selection on Luria-Bertani agar supplemented with 2 µg/ml cefotaxime and 2,000 µg/ml streptomycin. Where multiple plasmids were cotransferred by conjugation, transformation was performed to try to obtain a single plasmid carrying the relevant bla_{CTX-M} gene, as verified by S1 nuclease pulsed-field gel electrophoresis (PFGE) ([15]). The presence of bla_{CTX-M} genes in transconjugants/transformants was confirmed by PCR and sequencing using published primers ([7]).

Plasmid analysis. PCR-based replicon typing (PBRT) ([16]) was performed on all transconjugants/transformants carrying a single plasmid plus the transformant from JC2 carrying two plasmids. An IncI2-type replicon was screened for with published primers (see Table S1 in the supplemental material) ([12]). IncI1 plasmid multilocus sequence typing (pMLST) was performed as described previously ([17]), and alleles were

assigned by www.pubmlst.org/plasmid/. IncI2 plasmids carrying hybrid bla_{CTX-M} genes were compared by restriction digestion with ApaLI (TaKaRa Biotechnology, Dalian, China), according to the manufacturer's instructions. The sizes of the plasmids were estimated by S1 nuclease PFGE.

Plasmid sequencing. Plasmids pHNY2-1 and pHNY2-2 from the only isolate with $bla_{CTX-M-15}$ and a 45-bp spacer, the partially characterized pHNAH4-1 (IncI1, $bla_{CTX-M-123}$) and pHNAH46-1 (IncI2, $bla_{CTX-M-64}$), both from chickens in the same province, and pHNLDH19 (IncI2, $bla_{CTX-M-132}$), from the only isolate with $bla_{CTX-M-132}$, were selected for sequencing. Plasmid DNA purified from a transformant or transconjugant using a Qiagen plasmid midi kit (Qiagen, Hilden, Germany) was sequenced using the GS-FLX system (454 Life Sciences). Contigs were assembled with the 454 GS de novo assembler (Newbler) v2.8. Gaps between contigs (average coverage, ~100-fold) were closed by PCR and sequencing. PCR (see Table S1 in the supplemental material) and sequencing across the shufflon region gave mixed sequences for all plasmids, suggesting active shufflon rearrangement. As a contig covering the whole region was obtained during automated assembly of each IncI2 plasmid, these arrangements were used to assemble the final sequences. For the IncI1 plasmid pHNAH4-1, additional cloning of a PCR product in pMD19T (TaKaRa Biotechnology) and sequencing revealed that shufflon segment B, initially missing from the assembled sequence, was actually present in the plasmid population. In this plasmid, shufflon segments were assembled in the order of the cloned fragment to close the sequence. Gene prediction and annotation were performed using Glimmer 3.02 (http://ccb.jhu.edu/software/glimmer/index.shtml) and the BLASTp program (http://blast.ncbi.nlm.nih.gov/Blast.cgi). IncI1 plasmid R64 (GenBank accession no. AP005147) and IncI2 plasmid pHN1122-1 (JN797501) were used as reference plasmids for annotation.

PCR screening for pHNAH4-1-like and pHNAH46-1-like plasmids. Published primers designed to amplify selected regions (see Table S1 in the supplemental material) of the IncI2 backbone (12) were used to characterize IncI2 plasmids. The insertion site of the IS$Ecp1$ transposition unit in IncI1 or IncI2 plasmids and junctions between the IncI1 backbone and the other two insertions found in pHNAH4-1 were also determined by PCR (see Table S1).

Nucleotide sequence accession numbers. The nucleotide sequences of pHNY2-1, pHNAH46-1, pHNAH4-1, and pHNLDH19 have been deposited in GenBank under accession numbers KF601686, KJ020576, KJ125070, and KM207012, respectively.

RESULTS AND DISCUSSION

IncI plasmids carrying hybrid bla_{CTX-M} genes. PCR and sequencing revealed that LDH19 carries $bla_{CTX-M-132}$ with a 45-bp spacer and that the same spacer separates $bla_{CTX-M-15}$ from IS$Ecp1$ in JC2. pHNAH46-1, carrying $bla_{CTX-M-64}$, had previously been identified as IncI2 and pHNAH4-1, carrying $bla_{CTX-M-123}$, as IncI1 sequence type 108 (ST108) (4). A single IncI2 plasmid (pHNLDH19) carrying $bla_{CTX-M-132}$ was transferred from LDH19 by conjugation. Transconjugants from JC2 carried two plasmids (~65 kb and ~55 kb, designated pHNY2-1 and pHNY2-2, respectively) and both an IncI2 and an IncN replicon. Transformants with a single plasmid carrying $bla_{CTX-M-15}$ could not be obtained, despite repeated attempts. The IncI2 replicons in pHNAH46-1, pHNLDH19, and the JC2 transconjugant were all 100% identical to that of pHN1122-1 carrying $bla_{CTX-M-55}$ (GenBank accession no. JN797501) (12). pHNAH46-1 and pHNLDH19 gave ApaLI patterns indistinguishable from those of pHN1122-1, while pHNY2-1 plus pHNY2-2 gave a similar pattern (see Fig. S1 in the supplemental material). Plasmids from all four isolates were completely sequenced.

IncI2 plasmids pHN1122-1, pHNY2-1, pHNAH46-1, and pHNLDH19 are almost identical. Sequencing and assembly re-

vealed that $bla_{CTX-M-15}$ is carried by the 65,358-bp plasmid pHNY2-1, which is IncI2. pHNY2-1, pHNAH46-1 ($bla_{CTX-M-64}$; 62,194 bp), and pHNLDH19 ($bla_{CTX-M-132}$; 62,194 bp) are almost identical to each other and to pHN1122-1($bla_{CTX-M-55}$; 62,196 bp) (12). These plasmids are also closely related to p1081-CTXM (GenBank accession no. KJ460501; $bla_{CTX-M-55}$; 62,194 bp) from a clinical *Shigella sonnei* isolate (18), pSTH21 (LN623683; $bla_{CTX-M-55}$; 62,139 bp) from a *Salmonella enterica* isolate from China, and pCTXM64_C0967 (KP091735; $bla_{CTX-M-64}$; 62,194 bp) and pCTXM132_P0421 (KP198615; $bla_{CTX-M-123}$; 63,124 bp) from chicken and swine *E. coli* isolates, respectively, from Hong Kong (11). They all have a typical IncI2 backbone, including a replicon region, plasmid stability functions, and genes encoding two types of pili (*tra* and *pil* operons), with only minor sequence differences (see Table S2 in the supplemental material).

Automated assembly suggested that, like pHN1122-1, the three plasmids sequenced here were missing segment C of the shufflon, which generates variation in the C terminus of the PilV tip adhesin compared with the archetypal IncI2 plasmid R721 (19) and had different arrangements of the remaining two shufflon segments. PCR across the shufflon gave a product of the size expected if segment C is missing (1,650 bp), but sequencing suggested multiple arrangements of shufflon segments in each sample. The sequences of the remaining A and B′/D′ segments are identical for all plasmids except for pCTXM132_P0421, which has differences in segment A.

All of these plasmids have a 3,080-bp IS$Ecp1$-bla_{CTX-M} transposition unit containing 112 bp of IncA/C backbone inserted in the same position flanked by the same 5-bp direct repeats, differing only by the expected variations in the bla_{CTX-M} genes. pHNY2-1 only also has IS150 (inserted in the same position as in R721; GenBank accession no. AP002527) and IS$1294b$ (Fig. 1A) (19). This common context suggests that both $bla_{CTX-M-64}$ and $bla_{CTX-M-132}$ were generated in an IncI2 plasmid. IncI2 plasmids were not included in the original PBRT panel (16) and have not been well studied but have recently been found to harbor several other clinically important resistance genes, such as bla_{KPC} (20) and bla_{CMY-2} (21).

Transfer of IS$Ecp1$-bla_{CTX-M} from IncI2 to IncI1 plasmids. pHNAH4-1 (IncI1 ST108), carrying $bla_{CTX-M-123}$, is 109,194 bp and is made up of a typical IncI1 backbone with three insertions. As predicted from examination of a cloned fragment (4), an IS$Ecp1$ transposition unit containing $bla_{CTX-M-123}$, the 112-bp IncA/C backbone fragment, and a fragment of IncI2 backbone was identified. This 5,800-bp insertion includes 2,720 bp of the IncI2 backbone found adjacent to the IncA/C fragment in IncI2 plasmids and is flanked by a 5-bp duplication (TTATA) (Fig. 1B). This suggests IS$Ecp1$-mediated transposition of the IS$Ecp1$-bla_{CTX-M}-IncA/C region found in IncI2 plasmids plus the adjacent 2,720 bp of IncI2 backbone into an IncI1 plasmid backbone.

The second insertion in pHNAH4-1 (MRR1, 8,587 bp) (Fig. 1D) is bounded by the ends of transposon Tn1721, including the 38-bp terminal inverted repeats, and is located in the *yafA* gene flanked by 5-bp direct repeats characteristic of this transposon. The central part of Tn1721 has been replaced by a region containing three copies of IS26, the *mph*(A)-*mrx*-*mphR*(A) macrolide resistance region and fragments of the class 1 integron 5′-conserved sequence (CS), 3′-CS, and IS6100. The third insertion in pHNAH4-1 is a 2,343-bp putative group II intron located upstream of the *ssb* gene.
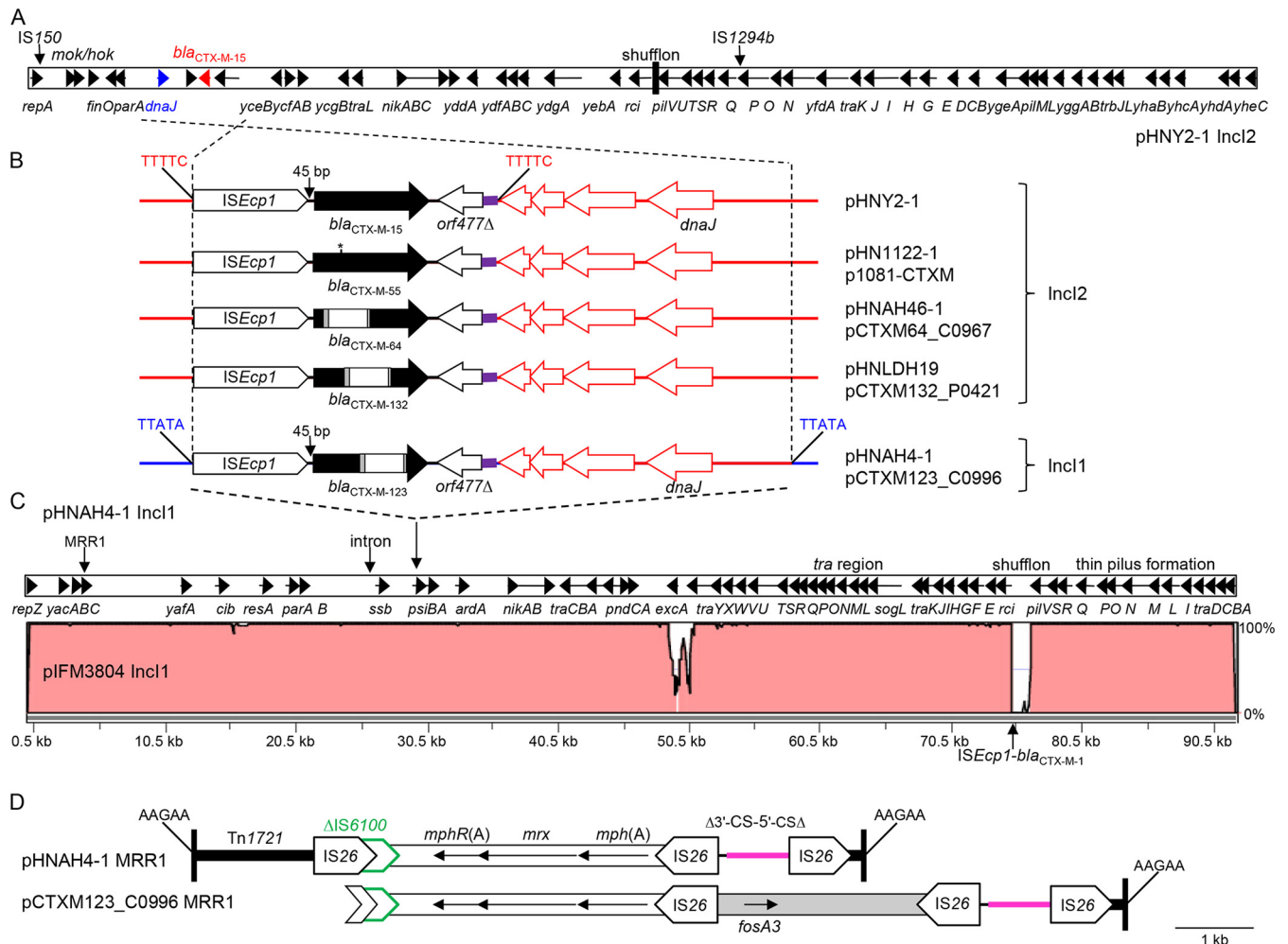
FIG 1 (A) Organization of IncI2 plasmids carrying $bla_{CTX-M-15}$, $bla_{CTX-M-55}$, or $bla_{CTX-M-1/9/1}$ hybrids, as represented by pHNY2-1 (GenBank accession no. KF601686) carrying $bla_{CTX-M-15}$. Arrows indicate the positions and directions of different genes, with $bla_{CTX-M-15}$ shown in red and the shufflon region shown as a black box. The positions of IS150 and IS1294b, found in pHNY2-1 only, are indicated by vertical arrows. (B) Comparison of the genetic environments of $bla_{CTX-M-15}$ (pHNY2-1), $bla_{CTX-M-55}$ (pHN1122-1, JN797501; p1081-CTXM, KJ460501; pSTH21, LN623683), and $bla_{CTX-M-1/9/1}$ hybrids $bla_{CTX-M-64}$ (pH-NAH46-1, KJ125070; pCTXM64_C0907, KP091735) and $bla_{CTX-M-132}$ (pHNLDH19, KM207012; pCTXM-132_P0421, KP198615) on IncI2 plasmids with the genetic environments of $bla_{CTX-M-123}$ on IncI1 plasmids (pHNAH4-1, KC160505; pCTXM123_C0996, KP198616). The IncI2 backbone is shown in red, the IncI1 backbone in blue, and the IncA/C backbone fragment in purple. The asterisk in $bla_{CTX-M-55}$ indicates the nucleotide that differs from $bla_{CTX-M-15}$. Within the bla_{CTX-M} genes, black corresponds to regions matching $bla_{CTX-M-15}$, white to regions matching $bla_{CTX-M-14}$, and gray to short sections matching both of these genes. The 5-bp direct repeats (DR) flanking the transposition unit in IncI2 plasmids are shown for pHNY2-1 only but are present in all IncI2 plasmids shown. The 5-bp direct repeats flanking the $bla_{CTX-M-123}$ transposition unit are shown. (C) Comparison of the pHNAH4-1 and pIFM3804 (CP006657) backbones, with insertions removed. The percent identity is displayed on the right of the diagram. The pCTXM123_C0996 backbone is almost identical to pHNAH4-1 except for missing shufflon segments and a deletion extending into the left end of MRR1, which may both be due to assembly issues, and this plasmid also has the intron insertion. (D) MRR1 in pHNAH4-1. The 38 bp of Tn1721 are shown by black bars and the sequences of the flanking 5-bp DR are given. The pink segment and adjacent black line represent the truncated class 1 integron 5′-CS and 3′-CS, respectively. IS6100 is truncated by IS26. pCTXM123_C0996 has an additional segment carrying fosA3.

pHNAH4-1 has differences from another ST108 plasmid. pHNAH4-1 has a typical IncI1 backbone that includes the tra and trb gene clusters and the nikAB (DNA processing functions) and pil genes (type IV pilus) required for conjugation plus genes involved in plasmid partitioning and stability (parAB), plasmid addiction (pndCA), and inhibition of the bacterial SOS response (psiAB). Automated sequence assembly suggested that the shufflon was missing segments B and D compared with the archetypal IncI1 plasmid R64 (22). Additional PCRs across the shufflon region, cloning, and sequencing indicated that only segment D is missing and suggested multiple arrangements of the remaining three segments.

pHNAH4-1 is closely related to pCTXM123-C0996, which is also IncI1 ST108 and carries $bla_{CTX-M-123}$ (11) (Fig. 1), and its backbone is similar to that of IncI1 ST108 plasmid pIFM3804 (GenBank accession no. KF787110), which carries $bla_{CTX-M-1}$ and has disseminated across multiple genera at a United Kingdom pig farm (Fig. 1C) (23). Like pHNAH4-1, pIFM3804 is apparently missing shufflon segment D, but despite identity at the pMLST target sites, two other regions display low identity, suggesting variability within pMLST ST108, as previously observed for IncI1 ST2 plasmids (20). The main differences are in a region including the traY and excA genes, where pHNAH4-1 is 100% identical to the prototype IncI1 plasmid R64 (ST13; GenBank accession no.
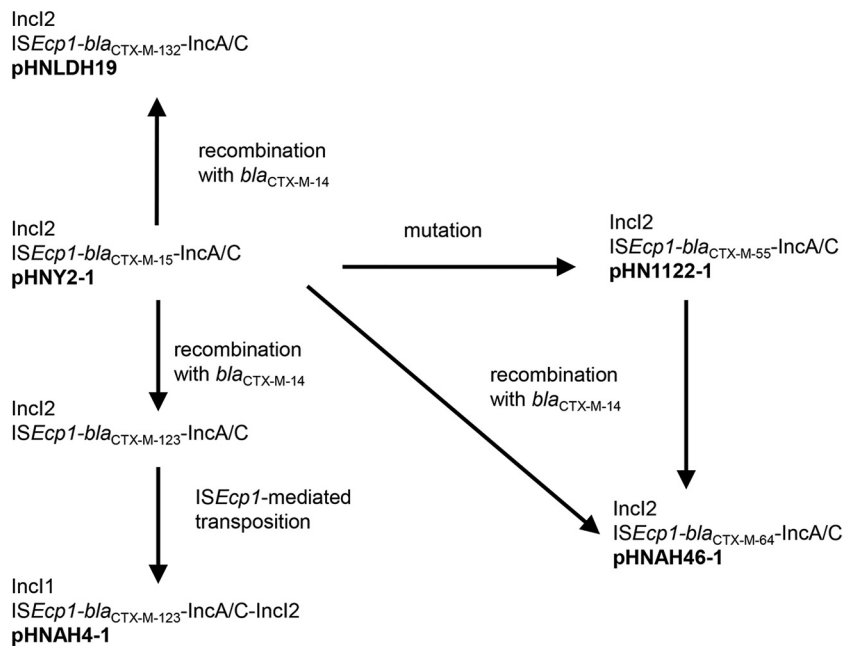
FIG 2 Suggested scheme for the formation of the observed hybrids and plasmids. $bla_{CTX-M-64}$ on an IncI2 plasmid could have been created by either of two different pathways, as the nucleotide that differs between $bla_{CTX-M-15}$ and $bla_{CTX-M-55}$ is not present in this hybrid gene.

AP005147, *Salmonella* Typhimurium, Japan), while pIFM3804 matches the IncI1 ST3 plasmid (e.g., pC49-108; KJ484638, *E. coli*, Switzerland). Interactions between the products of these genes influence entry exclusion of one plasmid by another (24).

Related plasmids are spreading between different *E. coli* sequence types and locations. Transconjugants or transformants carrying a single IncI2 plasmid were obtained from five of six other *E. coli* isolates carrying $bla_{CTX-M-64}$ from chicken samples (Table 1). These had all of the IncI2 backbone markers and the same IS*Ecp1*-$bla_{CTX-M-64}$-IncA/C transposition unit in the same position as the IncI2 plasmids sequenced here (Table 1). Four gave the same ApaLI pattern as these plasmids, while in pHNBSC1, from BSC1, a large ApaLI fragment seen in the other IncI2 plasmids seems to have been replaced by a slightly smaller band and an ~4.4-kb band (see Fig. S1 in the supplemental material), suggesting a rearrangement/and or insertion. Ho et al. (11) also identified an additional five related IncI2 plasmids carrying $bla_{CTX-M-64}$ from chicken isolates from Hong Kong.

Transconjugants or transformants carrying a single IncI1 ST108 plasmid were identified in 9 of 14 additional isolates with $bla_{CTX-M-123}$ from chickens, a pig, and a duck (Table 1). All had both the intron and the IS*Ecp1* transposition unit containing the IncI2 fragment inserted in the same positions as in pHNAH4-1, but only seven had MRR1 (Table 1). All plasmids were similar in size to pHNAH4-1, although there was some variation (Table 1). Ho et al. (11) also identified an additional four IncI1 ST108 plasmids carrying $bla_{CTX-M-64}$ from chickens, a pig, and a dog in Hong Kong.

Sixteen different *E. coli* sequence types were identified among the 23 isolates (Table 1). Two isolates each of ST155 and ST1437 carried $bla_{CTX-M-123}$ and two ST162 isolates carried either $bla_{CTX-M-123}$ or $bla_{CTX-M-64}$. ST746 was the most prevalent type, corresponding to four isolates carrying $bla_{CTX-M-123}$ from three different chicken farms and one carrying bla_{CTX-64} on an untyped

plasmid from a pig. An *E. coli* ST746 strain carrying $bla_{CTX-M-132}$ on an untyped plasmid was also recovered from cattle in Hong Kong (11). It is also interesting that AHC46 and four isolates from Hong Kong (11) are all ST1011, and single isolates of ST93 and ST117 were identified in each location, with all nine carrying an IncI2/$bla_{CTX-M-64}$ plasmid and isolated from chickens. JC2 ($bla_{CTX-M-15}$ IncI2, human isolate) and a Hong Kong $bla_{CTX-M-64}$ IncI2 chicken isolate are also both ST224. Thus, although IncI2 and IncI1 plasmid transfer seems to play a major role in the epidemiology of $bla_{CTX-M-1/9/1}$ hybrid genes, some clonal dissemination of *E. coli* strains carrying these plasmids between locations and host species also appears to have occurred.

In conclusion, the almost identical sequences of IncI2 plasmids carrying $bla_{CTX-M-15}$, $bla_{CTX-M-55}$, $bla_{CTX-M-64}$, and $bla_{CTX-M-132}$, all with a 45-bp spacer, suggest that the hybrid genes could have been created on an IncI2 plasmid by a recombination with $bla_{CTX-M-14}$. In the case of $bla_{CTX-M-64}$, the nucleotide that differs between $bla_{CTX-M-15}$ and $bla_{CTX-M-55}$ is not present, due to the position of the first crossover with $bla_{CTX-M-14}$ (Fig. 1B), so either $bla_{CTX-M-15}$ or $bla_{CTX-M-55}$ could be the ancestor (Fig. 2). In the case of $bla_{CTX-M-132}$, the final sequence could result directly either from recombination of $bla_{CTX-M-14}$ with $bla_{CTX-M-15}$ or from recombination of $bla_{CTX-M-14}$ with $bla_{CTX-M-55}$ followed by reversion of the mutation leading to A77V. Although $bla_{CTX-M-15}$ with a 45-bp spacer sequence appears rare, reversion of the mutation in $bla_{CTX-M-55}$ seems less likely given the proposed evolutionary trajectory of these genes: $bla_{CTX-M-15}$ has a single nucleotide change from $bla_{CTX-M-3}$, the ancestral gene found on the *Kluyvera ascorbata* chromosome (25), and the resultant amino acid change (D240G) gives a smaller increase in ceftazidime hydrolysis (26). Another single nucleotide change gives $bla_{CTX-M-55}$ and the A77V mutation leads to a marked increase in ceftazidime hydrolysis (26). $bla_{CTX-M-123}$ has only been seen on Inc1 or untyped plasmids to date but could have been generated on an IncI2 plasmid and

then transferred to an IncI1 plasmid, along with 2,720 bp of IncI2 backbone, by IS*Ecp1*-mediated transposition, as suggested in Fig. 2.

All three of the $bla_{CTX-M-1/9/1}$ hybrid genes have been found in both mainland China, where two were first reported, and Hong Kong (11). It seems that these three hybrids might have been created in China, with $bla_{CTX-M-64}$ and $bla_{CTX-M-123}$ apparently spreading on epidemic plasmids (IncI2 and IncI1) between *E. coli* types, different animals, and different geographical regions in this country, while $bla_{CTX-M-132}$ has been identified more rarely. Only $bla_{CTX-M-64}$ has been reported in other countries: to date in Japan (5), the Netherlands (27), and the Lao People's Democratic Republic (28). Further studies are required to understand the distribution of these hybrid genes in other geographic regions and bacterial sources.

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