Complete nucleotide sequences of \( \text{bla}_{\text{CTX-M}} \)-harboring IncF plasmids from community-associated \textit{Escherichia coli} strains in the United States

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Running title: Sequences of \( \text{bla}_{\text{CTX-M}} \)-carrying IncF plasmids in U.S.

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ABSTRACT

Community-associated infections due to *Escherichia coli* producing CTX-M-type extended-spectrum β-lactamases are increasingly recognized in the United States. The *bla*<sub>CTX-M</sub> genes are frequently carried on IncF-group plasmids. In this study, *bla*<sub>CTX-M-15</sub>-harboring plasmids pCA14 (ST131) and pCA28 (ST44), and a *bla*<sub>CTX-M-14</sub>-harboring plasmid pCA08 (ST131) were sequenced and characterized. The three plasmids were closely related to other IncFII plasmids from continents outside the U.S. in both the conserved backbone region and multiresistance regions (MRRs).

Both *bla*<sub>CTX-M-15</sub>-carrying plasmids pCA14 and pCA28 belonged to F31:A4:B1 and showed a high level of similarity (92% coverage of pCA14 and 99 to 100% nucleotide identity), suggesting a possible common origin. The *bla*<sub>CTX-M-14</sub>-carrying plasmid pCA08 belonged to F2:A2:B20 and was highly similar to pKF3-140 from China (88% coverage of pCA08 and 99 to 100% nucleotide identity). All three plasmids carried multiple antimicrobial resistance genes as well as modules associated with virulence and biochemical pathways, which likely confer selective advantages for their host strains. The *bla*<sub>CTX-M</sub>-carrying IncFII-IA-IB plasmids implicated in community-associated infections in the U.S. shared key structural features with those identified from other continents, underscoring the global nature of this plasmid epidemic.
INTRODUCTION

CTX-M-producing *Escherichia coli* has spread widely in both hospital and community settings in recent years (1-4). In particular, CTX-M-15, belonging to the CTX-M-1 group, appears to be the most common extended-spectrum-β-lactamase (ESBL) globally, followed by CTX-M-14, another common variant of CTX-M enzymes often reported from East Asia and parts of Europe (1, 2, 4, 5). The successful dissemination of *bla*<sub>CTX-M-15</sub>, the gene encoding CTX-M-15, has been mainly associated with the high-risk *E. coli* sequence type 131 (ST131) clonal group, as well as the epidemic multidrug resistance IncF plasmids (6). Moreover, the *ISecp1* element which could capture and mobilize *bla*<sub>CTX-M-15</sub> and *bla*<sub>CTX-M-14</sub> to IncF plasmids has greatly facilitated their spread (6). In our previous study that identified 107 community-associated ESBL-producing *E. coli* isolates from five states in the United States, 54% belonged to ST131, and 91% carried *bla*<sub>CTX-M</sub> (78 *bla*<sub>CTX-M-15</sub>, 12 *bla*<sub>CTX-M-14</sub>, and 5 other *bla*<sub>CTX-M</sub> variants) (2). In a follow-up study elucidating the molecular epidemiology of a subset of the isolates, 15 of the 24 *bla*<sub>CTX-M</sub>-carrying plasmids belonged to the IncF group by PCR-based replicon typing (PBRT), including IncFII-F1A-FIB, IncFII-F1A, IncFII, IncFIA-FIB, and IncFII-FIB (7). Many of these plasmids shared similar restriction profiles, and they occurred in both ST131 and various non-ST131 isolates, suggesting the role of plasmid-mediated dissemination of *bla*<sub>CTX-M</sub> across different sequence types of ESBL-producing *E. coli*. Whole plasmid sequencing and comparative analyses have facilitated our understanding of the process involved in the emergence and spread of these plasmids. To date, a handful of
IncF plasmids carrying bla_{CTX-M-15} in *E. coli* from different continents have been fully sequenced, which have demonstrated backbone region and multiresistance regions (MRRs) that are related to various degrees (8, 9). However, IncF plasmids in *E. coli* carrying bla_{CTX-M-15} from the U.S. have not been characterized. In addition, analysis of bla_{CTX-M-14}-carrying plasmids has been limited in spite of their global spread. In this study, we present the complete nucleotide sequences of three representative bla_{CTX-M}-carrying IncFII-IA-IB plasmids previously identified from *E. coli* causing community-associated infections in the U.S.

**MATERIALS AND METHODS**

**Study strains.** *E. coli* isolates CA08, CA14, and CA28 were selected from our previous study in order to represent different states of origin, bla_{CTX-M} types and ST131 status (Table 1) (7). Isolates CA14 and CA28 carried bla_{CTX-M-15}, while CA08 carried bla_{CTX-M-14}. Isolates CA08 and CA14 belonged to ST131, while CA28 belonged to a non-ST131 sequence type (ST44). All three bla_{CTX-M}-harboring plasmids belonged to IncFII-FIA-FIB by PBRT, the dominant replicon type among the bla_{CTX-M}-harboring plasmids in the U.S. (7). The antimicrobial susceptibility of these isolates was reported previously (7).

**Plasmid sequencing and bioinformatics.** Broth mating assay was performed using azide-resistant *E. coli* J53 as the recipient to test their self-transferability (10). The bla_{CTX-M}-harboring plasmids of CA08, CA14, and CA28 were extracted from the *E. coli* TOP10 transformants harboring them using the Qiagen Plasmid Maxi Kit (Qiagen,
Valencia, CA). Sequencing of the blaCTX-M-harboring plasmids was performed on a PacBio RSII single-molecule real-time (SMRT) sequencing instrument (Pacific Biosciences, Menlo Park, CA) at the Yale Center for Genome Analysis. The assembly process has been described previously (11). In brief, the first-pass reads were assembled de novo using the hierarchical genome assembly process (HGAP) with SMRT Analysis v2.1 (Pacific Biosciences) (12). The single contigs representing the plasmids were circularized and used as the references to reassemble the first-pass reads with Quiver v1 in SMRT Analysis v2.1. The plasmid sequences were initially annotated with RAST (http://rast.nmpdr.org) and the Prokaryotic Genome Annotation Pipeline (PGAP) available through the NCBI, and then curated manually using the BLASTn and BLASTp algorithms (http://blast.ncbi.nlm.nih.gov/Blast). Easyfig 2.0 was used to map the whole plasmids, and to conduct a limited comparison as well. The MRRs in each plasmid were mapped with reference to MRRs of pEC958 (HG941719), pIP1206 (AM886293), pKF3-140 (FJ876827), and pETN48 (FQ482074). A phylogenetic tree based on the alignment of the entire plasmids was constructed by the Neighbor Joining method and bootstrap analysis with 100 replicates using the CLC Genomic Workbench version 7 (Qiagen).

**Nucleotide sequence accession numbers.** The GeneBank accession numbers for the reported plasmids are CP009233 (pCA08), CP009231 (pCA14), and CP009232 (pCA28).

RESULTS AND DISCUSSION
In this study, we determined the complete nucleotide sequences of two plasmids (pCA14 and pCA28) harboring \( \text{bla}_{\text{CTX-M-15}} \) and a plasmid (pCA08) harboring \( \text{bla}_{\text{CTX-M-14}} \) from community-associated \( E. \text{coli} \) isolates in the U.S. Characteristics of the three plasmids are summarized in Table 1. Plasmids pCA08, pCA14, and pCA28 are circular molecules of 154,789 bp, 155,454 bp, and 172,280 bp in size, with average GC contents of 50.94%, 51.54%, and 51.55% in their conserved backbone regions, respectively. They harbor 185, 181, and 203 predicted genes, respectively. Detailed structural features and comparisons with related IncF plasmids including \( \text{bla}_{\text{CTX-M}} \)-carrying ones are shown in Figure 1. All three plasmids have a typical IncFII-IA-IB plasmid scaffold and can be divided into two regions: the IncFII-IA-IB backbone and variable MRRs. The backbone region includes conserved genes for plasmid maintenance and conjugation, relatively variable genes for plasmid replication, as well as highly variable modules associated with virulence and biochemical pathways. The MRRs include several genes conferring antimicrobial resistance and associated mobile elements, and are highly variable in both composition and arrangement.

According to the IncF replicon sequencing typing (RST) scheme which demonstrates a higher discriminatory power than PBRT (13), both \( \text{bla}_{\text{CTX-M-15}} \)-carrying plasmids pCA14 and pCA28 are assigned to formula F31:A4:B1, whereas the \( \text{bla}_{\text{CTX-M-14}} \)-carrying plasmid pCA08 is assigned to F2:A2:B20. These findings are consistent with the BLAST analysis, which indicates that pCA14 is closely related to pCA28, with 99 to 100% identity and 92% coverage, including both
the conserved backbone region and MRRs. pCA14 shares all backbone genes with pCA28, including genes for replication, plasmid maintenance and conjugal transfer, as well as modules associated with virulence and biochemical pathways. A total of 20 nucleotide differences are observed between their backbone regions. A one-nucleotide difference and a two-nucleotide difference are found in the genes traE and iucC, respectively, with the remainder observed in genes of hypothetical proteins or intergenic regions. In addition, pCA14 shares all resistance genes with pCA28 except for the truncated catA1 gene. In contrast, the blaCTX-M-14-carrying plasmid pCA08 only shares 99 to 100% identity and 63% coverage with pCA28. Notably, a second copy of FII allele is observed in both pCA14 and pCA28, which is composed of repA2, repA6 genes and a 3'-end truncated repA1 gene. This FII allele belongs to F36 according to the RST scheme, and is almost identical to the FII-a replicon in pIP1206, with only 3 nucleotide differences. The multiple replication regions in IncF plasmids could have provided them with a broader host range and promoted their spread.

F31:A4:B1 is a formula that includes a number of blaCTX-M-15-carrying plasmids, and has been reported in E. coli from humans in the U.K., Italy and Australia, as well as in those from cattle and pets in France (9, 13-15). These plasmids from cattle and pets were identified from E. coli in various non-ST131 sequence types, suggesting plasmid-mediated diffusion of blaCTX-M-15 gene across various E. coli lineages. F2:A2:B20 was also reported in E. coli ST131 from a pet cat from France, which harbored blaCTX-M-14 as in pCA08 (15).

pCA28 and pCA14 contain a complete transfer region consisting of 24 tra genes,
9 trb genes, artA, and finO. In contrast, trbG, and artA genes are absent in pCA08. However, all three plasmids are self-transmissible in spite of the incompleteness of the tra region in pCA08, which was supported by observation of their successful conjugal transfer to E. coli J53. Both pCA14 and pCA28 harbor several modules common across IncF plasmids that ensure stable plasmid inheritance and post-segregation killing: the sopABC operon for active plasmid partition, the post-segregation killing protein hok-mok system, the toxin-antitoxin systems pemKI and ccdAB, and the vagCD virulence-associated genes. pCA08 contains all these modules as well except for the vagCD virulence-associated genes. However, it possesses an additional restriction-modification (R-M) system (hsdMSR) that mediates plasmid maintenance. Upstream of the hsdMSR genes on pCA08, another type I R-M system encoded by doc-phd is located, which is also present in the genome of bacteriophage P1 and encodes an addiction system stabilizing the P1 prophage (16). The doc-phd and hsdMSR in pCA08 exhibit a high degree of identity with their counterparts on pIP1206 (99 to 100%), which originated from an E. coli clinical isolate in France and carried rmtB, qepA and an array of other antimicrobial resistance genes commonly found on IncF plasmids (16). Of note, the parB gene mediating plasmid partition is absent in all three plasmids in our study, which is located upstream of the psiAB genes and commonly observed in IncF plasmids.

Several known virulence determinants, as well as several modules associated with biochemical pathways, are identified on these plasmids. The module that encodes iron permease and ATP binding proteins of the ABC transporter family is
present in all three plasmids. In addition, pCA28 contains two additional iron
acquisition systems IucABCD-IutA and SitABCD. This region from sitABCD to
iucABCD-iutA on pCA28 shares high-level identity (with 10 nucleotide differences)
with that on pSRB225, which originated from an unknown bacterium isolated from a
municipal sewage treatment plant in Germany (17). In contrast, pCA14 only contains
the IucABCD-IutA system. Although these two gene clusters are rather common in
IncF plasmids, they are rarely identified in bla_{CTX-M-15}-carrying IncF plasmids (8, 18,
19). pCA14 and pCA28 both have the arcACBD-argR gene cluster encoding proteins
involved in the arginine deiminase pathway, which was almost identical to that on
pIP1206, with only a two-nucleotide difference (16). pCA08 also carries an
S-methylmethionine metabolism operon composed of mmuP and mmuM, which
encode identical proteins as their counterparts on pIP1206 (16).

bla_{CTX-M-15} has always been found in association with ISEcp1, and is generally
located 48 bp beyond its right-hand inverted repeat (IR_R) (9). In our study, bla_{CTX-M-15}
is also located 48 bp beyond IR_R of ISEcp1 in both pCA14 and pCA28. However, the
ISEcp1 gene is truncated by IS26 at different locations, leaving ISEcp1 remnants of
different lengths in pCA14 (545 bp) and pCA28 (1,224 bp), which correspond to the
relatively common bla_{CTX-M-15} genetic environment 2b and 2c found in E. coli strains
isolated from travellers returning to the United Kingdom, respectively (20).
Truncations of ISEcp1 by IS26 have also been observed in other fully sequenced
bla_{CTX-M-15}-harboring plasmids such as pEK499, pEC958, and pJIE286, underscoring
the significant role of ISEcp1 in the initial spread of bla_{CTX-M-15} and the role of IS26 in
the subsequent rearrangement of MRRs (8, 9). In addition to \textit{bla}CTX-M-15, pCA14 and pCA28 also share other antimicrobial resistance genes, including \textit{tet}A(B)-\textit{tet}R (tetracyclines) in a truncated Tn10 derivative, \textit{sul}1 (sulfonamides)-\textit{qac}E\Delta (ammonium antiseptics)-\textit{aad}A5 (spectinomycin and streptomycin)-\textit{dfr}A17 (trimethoprim) cassette array carried by a class 1 integron, \textit{mph}(A)-\textit{mrx}-\textit{mph}R cluster (macrolides) following the class 1 integron, and the \textit{bla}_{OXA-1} (penicillins/oxacillin)-\textit{cat}B3 (chloramphenicol)-\textit{aac}(6')-\textit{ib}-\textit{cr} (aminoglycosides and fluoroquinolones) cassette array flanked by two copies of IS26 (Figure 2). The \textit{tet} cluster in pCA14 is truncated, probably as a result of insertion of an extra copy of IS1 followed by homologous recombination between directly oriented IS1 elements, deleting a circular molecule containing the intervenient region and one copy of IS1. The gentamicin resistance gene \textit{aac}(3)-\textit{Il}e is only present in pCA28 between directly oriented copies of IS26, and its absence in pCA14 could be readily explained by IS26-mediated deletion (9). The missing \textit{chr}A gene and truncated IS6100 in pCA28 is probably due to insertion of IS26 elements and IS26-mediated deletion between directly oriented copies of IS26 as well. Furthermore, it appears that IS4321 was inserted into the \textit{chr}A gene first, then was truncated by IS26. pCA08 also encodes a class 1 integron with \textit{sul}1-\textit{qac}E\Delta-\textit{aad}A5-\textit{dfr}A17 cluster and the \textit{chr}A-\textit{mph}(A) module. However, it carries \textit{tet}A(A) instead, and harbors additional common MRR components \textit{sul}2 (sulfonamides) and \textit{str}A/B (streptomycin) genes. The missing truncated Tn10 with \textit{tet}A(B) and truncation of the integron could have been caused by insertion of two copies of directly oriented IS26 and homologous recombination. The \textit{bla}_{TEM-1} gene,
which is commonly found on IncF plasmids, especially bla$_{CTX-M}$-carrying IncF plasmids, is noticeably absent in all three plasmids in our study. The compositions of MRRs on the three plasmids are in agreement with the susceptibility profiles of *E. coli* TOP10 transformants harboring them (7). These MRR components have been commonly found in IncF plasmids, but they are in different combinations and arrangement with certain common boundaries (the catA1-IR$_{mp21}$ boundary and the mp$_{21}$-IRi boundary) in the plasmids in our study, highlighting the modular and mosaic characteristics of MRRs (21). The abundance of mobile elements, especially IS26, could have greatly facilitated the process of gene rearrangement. However, the limited number of target duplication repeats observed also suggests that homologous recombination (HR) may have played a key role in the gene rearrangement process. Moreover, a recent study has reported that IS26 could mobilize adjacent DNA segment that carry genes encoding antimicrobial resistance or other functions via a translocatable unit that includes a single copy of IS26 and insert next to another IS26, with no additional IS26 or target duplications generated, which is quite similar to the process of HR between two copies of IS26 in direct orientation (9, 21, 22).

pCA08 is highly similar to pKF3-140, described in a *Klebsiella pneumoniae* clinical strain from China (23). They share almost all genes for the plasmid scaffold and most resistance genes. A major difference between pCA08 and pKF3-140 lies in that, in the region located downstream of the pemKI genes, bla$_{CTX-M-14}$ is identified on pCA08, while aacC2 resides instead on pKF3-140. bla$_{CTX-M-14}$ and its environment (ISEcpI and IS903) together with the iroN gene located downstream of IS903
constitute a functional transposition unit typical for the \textit{bla}_{CTX-M-9}\text{-}group genes and have been reported mostly in \textit{bla}_{CTX-M-14}\text{-}carrying plasmids such as pETN48 (FQ482074.1) and pE66An (NC_020086.1), as well as plasmids harboring \textit{bla}_{CTX-M-17} (pIP843, AY033516.1), \textit{bla}_{CTX-M-19} (pILT-3, AF458080.1), and \textit{bla}_{CTX-M-24} (pKP96, EU195449.1) (24-28). This module is flanked by direct repeats TAAAA in pCA08. Interestingly, the \textit{K. pneumoniae} strain from which pKF3-140 originated also contained another plasmid pKF3-70 harboring \textit{bla}_{CTX-M-14} (23). Therefore, acquisition of the module containing \textit{bla}_{CTX-M-14} by pKF3-140 from pKF3-70 might have generated a pCA08\text{-}like plasmid.

The phylogenetic tree generated from the nucleotide alignment of the entire plasmids shows that pCA08 clusters with the plasmid pKF3-140, which coincides with the above BLAST findings and further suggests a possible common origin (Figure 3). pIP1206 and the \textit{bla}_{CTX-M-14}\text{-}harporing plasmid pETN48 both originating from France also cluster together. pCA14 and pCA28, which are closely related based on the BLAST search and FAB formula, does not form a single cluster. This distance is likely due to the large rearrangements present between the regions encoding ABC transporter and arginine deiminase pathway. pCA28 is also related to the above plasmids from France, suggestive of shared evolutionary relationship of these \textit{bla}_{CTX-M-15}\text{-}harporing plasmids.

In summary, we described the complete nucleotide sequences of three \textit{bla}_{CTX-M}\text{-}carrying IncFII-IA-IB plasmids from community-associated \textit{E. coli} strains in the U.S. Both \textit{bla}_{CTX-M-15}\text{-}harporing plasmids pCA14 and pCA08 are closely related to
other IncFII plasmids from other continents in both the backbone region and MRRs, especially pETN48 and pIP1206 from France. They belong to F31:A4:B1 and shared high degree of identity, suggesting a possible common origin. The blaCTX-M-14-harboring plasmid pCA08 is very similar to pKF3-140 from China. All three plasmids carry multiple antimicrobial resistance genes as well as modules associated with virulence and biochemical pathways, which together likely confer significant selective advantages for their host strains.

Acknowledgment

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Transparency declaration

Y.D. has served on an advisor board for Shionogi Inc., consulted for Melinta Therapeutics, and received research funding from Merck & Co. for a study unrelated to this work. All other authors: No potential conflicts of interest.

References


sequence of pKP96, a 67,850 bp multiresistance plasmid encoding \( qnrA1, aac(6')-Ib-cr \) and \( bla_{CTX-M-24} \) from \( \textbf{Klebsiella pneumoniae} \). \textit{J Antimicrob Chemother} \textbf{62}:1252-1256.

Table 1. Characteristics of three IncFII-IA-IB \textit{bla}_{\text{CTX-M}}\text-harboring plasmids from community-associated \textit{E. coli} isolates in the United States

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Location</th>
<th>ST of host</th>
<th>Size (bp)</th>
<th>no. of genes</th>
<th>FAB formula</th>
<th>Resistance genes</th>
<th>Putative virulence factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>pCA14</td>
<td>Texas</td>
<td>131</td>
<td>155,454</td>
<td>181</td>
<td>F31:A4:B1</td>
<td>\textit{bla}<em>{\text{CTX-M}-15}, ΔcatB3-\textit{bla}</em>{\text{CTX-M}-15}-aac(6')-Ib-cr, tet(A)-tetR-tetC-tetD, mph(A)-mrx-mphR(A), dfrA17-aadA5-qacEΔ-sul1, ΔcatA1,</td>
<td></td>
</tr>
<tr>
<td>pCA28</td>
<td>Iowa</td>
<td>44</td>
<td>172,280</td>
<td>203</td>
<td>F31:A4:B1</td>
<td>\textit{bla}<em>{\text{CTX-M}-15}, ΔcatB3-\textit{bla}</em>{\text{CTX-M}-15}-aac(6')-Ib-cr, mph(A)-mrx-mphR(A), tet(A)-tetR-tetC-tetD, sitABCD, vagAB</td>
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<td></td>
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<td>ABC transporter system, \textit{iucABCD-istA}, vagAB</td>
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Figure 1

Major structural features of plasmids pCA08 (ST131, \(\text{bla}_{\text{CTX-M-14}}\)), pCA14 (ST131, \(\text{bla}_{\text{CTX-M-15}}\)), and pCA28 (ST44, \(\text{bla}_{\text{CTX-M-15}}\)) indicated with bold type, compared with IncF plasmids pIP1206 (AM886293), pRSB225 (JX127248), and pKF3-140 (FJ876827), and the IncF \(\text{bla}_{\text{CTX-M-15}}\)-harboring plasmid pEC958 (HG941719), and IncF \(\text{bla}_{\text{CTX-M-14}}\)-harboring plasmid pETN48 (FQ482074). Light blue shades mainly indicate shared backbone regions and virulence factors with a high degree of similarity (>90% identity of nucleotide sequence). ORFs are portrayed by arrows and colored according to their putative functions. Dark-blue arrows indicate replication associated genes. Genes associated with plasmid conjugal transfer are indicated by green arrows, and genes involved in plasmid stability are indicated by brown arrows. Red and yellow arrows indicate antimicrobial resistance genes and mobile element genes, respectively. Genes involved virulence and biochemical pathways are both indicated by dark-purple arrows. Grey arrows indicate genes for hypothetical proteins as well as proteins of unknown function. UK, United Kingdom; DE, Germany; FR, France; US, United States; CN, China.
Figure 2

MRRs of plasmids pCA14 (ST131, \textit{bla}_{CTX-M-15}), pCA28 (ST44, \textit{bla}_{CTX-M-15}), and pCA08 (ST131, \textit{bla}_{CTX-M-14}), compared with those of related IncF plasmids pIP1206 (AM886293) and pKF3-140 (FJ876827), the IncF \textit{bla}_{CTX-M-15}-harboring plasmid pEC958 (HG941719), and IncF \textit{bla}_{CTX-M-14}-harboring plasmid pETN48 (FQ482074).

Various transposons and other modules have different shading and are generally labeled once for each plasmid. # indicates that a transposon is incomplete. ISs are pointed boxes labeled with their number/name. Tall bars represent the 38-bp IR of transposons, as indicated. Positions/orientations of selected resistance and other genes are indicated by arrows, generally labeled once for each plasmid. Abbreviations: A, \textit{tnpA}; R, \textit{tnpR}; 30, \textit{bla}_{OXA-30}; cr, \textit{aac(6')-Ib-cr}; IIe, \textit{aac(3)-IIe}; 15, \textit{bla}_{CTX-M-15}; 1b, \textit{bla}_{TEM-1b}; 14, \textit{bla}_{CTX-M-14}; IIId, \textit{aac(3)-IIId}. Class 1 integron components are indicated as follows: 5', 5'-CS; 3', 3'-CS; small black boxes, \textit{attC} sites; narrow box 1, \textit{dfrA17-aadA5} cassette array; narrow box 2, \textit{aacA4-cmlA1} cassette array; IRi, 25-bp IR at \textit{intI1} end; IRt, 25-bp IR at \textit{tni} end. The \textit{chrA-mph(A)} module is usually located after the 3'-CS, which consists of part of a chromate resistance transposon (IR_{\textit{chr-chrA}}), the 123-bp IR end of \textit{tni}_{02}, \textit{IS6100}, and the \textit{mph(A)-mrx-mphR(A)} macrolide resistance gene cluster (9). Direct repeats (DRs) are paired filled circles, and paired squares represent flanking sequences that are reverse complementary to one another. An unpaired circle or square indicates the same boundary between regions present in another of the structure shown as well. Asterisks against plasmid names indicate that the structures shown have been rearranged to emphasize
relationship, usually by inverting regions flanked by IS26 (21). Vertical arrows indicate the positions of IS26 elements with DRs that have been removed for ease of comparison. Dashed lines represent the IncFII backbone. Structures named R1, R2 below CA14 and R below CA28 are hypothetical, and are intended to facilitate comparison between related MRRs. Dashed arrowed lines indicate where homologous recombination (HR) happens to invert or delete the regions between two IS26 elements. (c) pCA14 MRR. R1, two inversion events by homologous recombination between IS26 elements in opposite orientations could have occurred (shown by the dashed arrowed lines) in hypothetical structure R1 to yield pCA14 MRR; R2, the circular molecule above R2 could have been deleted in R2 by recombination between ISJ leading to the generation of R1. (d) pCA28 MRR. R, two inversion events by HR between IS26 elements in opposite orientations and a deletion event by recombination between IS26 in the same orientation (shown by the dashed arrowed lines) could have occurred and resulted in hypothetical structure R.

Figure 3

Phylogenetic tree of plasmids pCA08 (ST131, \(bla_{\text{CTX-M-14}}\)), pCA14 (ST131, \(bla_{\text{CTX-M-15}}\)), pCA28 (ST44, \(bla_{\text{CTX-M-15}}\)), and related IncF plasmids pIP1206 (AM886293), pRSB225 (JX127248), pKF3-140 (FJ876827), pEC958 (HG941719), and pETN48 (FQ482074).
Figure 1
Major structural features of plasmids pCA08 (ST131, blaCTX-M-14), pCA14 (ST131, blaCTX-M-14), and pCA28 (ST44, blaCTX-M-14) indicated with bold type, compared with IncF plasmids pIP1206 (AM866293), pRSB225 (JX127248), and pKF3-140 (FJ876827), and the IncF blaCTX-M-14-harborng plasmid pEC968 (HG941719), and IncF blaCTX-M-14-harborng plasmid pETN48 (FO482074). Light blue shades indicate shared backbone regions and virulence factors with a high degree of similarity. ORFs are portrayed by arrows and colored according to their putative functions. Dark blue arrows indicate replication associated genes. Genes associated with plasmid conjugal transfer are indicated by green arrows, and genes involved in plasmid stability are indicated by brown arrows. Red and yellow arrows indicate antimicrobial resistance genes and accessory genes of mobile elements, respectively. Genes involved virulence and biochemical pathways are both indicated by dark-purple arrows. Grey arrows indicate genes for hypothetical proteins as well as proteins of unknown function.
Figure 2
MRRs of plasmids pCA14 (ST131, bluCTX-M-15), pCA28 (ST44, bluCTX-M-15), and pCA08 (ST131, bluCTX-M-14), compared with those of related IncF plasmids pIP1206 (AM886293) and pIPF3-140 (FJ876827), the IncF bluCTX-M-14-harboring plasmid pEC958 (HG841719), and IncF bluCTX-M-15-harboring plasmid pETN48 (FQ482074). Various transposons and other modules have different shading and are generally labeled once for each plasmid. # indicates that a transposon is incomplete. ISs are pointed boxes labeled with their number/name. Tall bars represent the 38-bp IR of transposons, as indicated. Positions/orientations of selected resistance and other genes are indicated by arrows, generally labeled once for each plasmid. Abbreviations: A, trpA; R, trpR; 30, bluCTX-M-15 cr; aac(6’)-Ib-cr, tle, aac(3)-Ie-15; bluCTX-M-14-1b, bluCTX-M-14; bluCTX-M-14 cmlA cassette array; narrow box 1, dfrA17-aadA5 cassette array; narrow box 2, aacA4-cmlA1 cassette array; IR, 25-bp IR at intI1 end; IRl, 25-bp IR at trnI end. The chrA-mph(A) module is usually located after the 3’-CS, which consists of part of a chromate resistance transposon (IRchrA-chrA), the 123-bp IR of intA, IS6100, and the mph(A)-mnr-mph(A) macrolide resistance gene cluster. Direct repeats (DRs) are paired filled circles, and paired squares represent flanking sequences that are reverse complementary to one another. An unpaired circle or square indicates the same boundary between regions present in another of the structure shown as well. Asterisks against plasmid names indicate that the structures shown have been rearranged to emphasize relationships, usually by inverting regions flanked by IS26 (20). Vertical arrows indicate the positions of IS26 elements with DRs that have been removed for ease of comparison. Dashed lines represent the IncFII backbone. Structures named R1, R2 below CA14 and R below CA28 are hypothetical, and are intended to facilitate comparison between related MRRs. Dashed arrowed lines indicate where homologous recombination (HR) happens to invert or delete the regions between two IS26 elements. (c) pCA14 MRR: R1, two inversion events by homologous recombination between IS26 elements in opposite orientations could have occurred (shown by the dashed arrowed lines) in the hypothetical structure R1 to yield pCA14 MRR. R2, the circular molecule above R2 could have been deleted in R2 by recombination between IS1 leading to the generation of R1. (d) pCA28 MRR: R, two inversion events by HR between IS26 elements in opposite orientations and a deletion event by recombination between IS26 in the same orientation (shown by the dashed arrowed lines) could have occurred and resulted in hypothetical structure R.
Figure 3