AAC Journals.ASM.org

Complete Sequence of pSAM7, an IncX4 Plasmid Carrying a Novel $bla_{CTX-M-14b}$ Transposition Unit Isolated from *Escherichia coli* and *Enterobacter cloacae* from Cattle

M. O. Stokes,[a,b] M. AbuOun,[a] S. Umur,[c] G. Wu,[d] S. R. Partridge,[e] D. J. Mevius,[f,g] N. G. Coldham,[a] M. D. Fielder[b]

Department of Bacteriology, Animal Health and Veterinary Laboratories Agency (AHVLA), Addlestone, United Kingdom[a]; Faculty of Science, Engineering and Computing, Kingston University, Kingston, United Kingdom[b]; School of Biological Sciences, Royal Holloway University of London, Egham, United Kingdom[c]; Epidemiology, Surveillance and Risk Analysis Group, Animal Health and Veterinary Laboratories Agency, Addlestone, United Kingdom[d]; Centre for Infectious Diseases and Microbiology, University of Sydney, Westmead Hospital, Sydney, New South Wales, Australia[e]; Department of Bacteriology and TSEs, Central Veterinary Institute of Wageningen, Lelystad, The Netherlands[f]; Department of Infectious Diseases and Immunology, Faculty if Veterinary Medicine, Utrecht University, The Netherlands[g]

The same plasmid carrying $bla_{CTX-M-14b}$ was identified from an *Escherichia coli* isolate and an *Enterobacter cloacae* isolate collected from cattle in the United Kingdom by complete plasmid sequencing. This 35,341-bp plasmid, pSAM7, had an IncX4 backbone that is 99% identical to that of pJIE143 from a human isolate in Australia. PCR screening identified pSAM7-like plasmids in three other *E. coli* isolates of different multilocus sequence types isolated from cattle on different farms in the United Kingdom.

The dissemination of extended-spectrum-β-lactamase (ESBL) genes via plasmids is a worldwide problem, with genes of the bla_{CTX-M} group being the most widespread plasmid-mediated genes encoding ESBL (1, 2). bla_{CTX-M} genes have been found in various members of *Enterobacteriaceae* isolated from both humans and animals. The $bla_{CTX-M-14}$ gene is frequently found in human isolates in Asia (3, 4) and some parts of Europe (5), but in the United Kingdom it is more prevalent in animal isolates (6). $bla_{CTX-M-14}$ has been associated with pCT-like IncK plasmids in the United Kingdom and around the world, with apparent transmission of this plasmid between human and animal isolates (6, 7). Here, small (35-kb) plasmids carrying $bla_{CTX-M14b}$ from *Escherichia coli* and *Enterobacter cloacae* isolates collected from cattle in the United Kingdom were sequenced and annotated.

E. coli isolate SAM7 was obtained from cattle feces in 2008 and *Enterobacter cloacae* ECR528 from waste milk (milk from cows treated with antibiotics that was not suitable for human consumption) from a different farm in 2012, during routine screening for ESBL genes. The bacterial species were identified using matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) analysis (8). ESBL plasmids from both bacterial species were conjugated into *Salmonella enterica* serovar Typhimurium 26R, using a surface mating method with a 10:1 recipient-to-donor ratio, and transconjugants were selected on Rambach agar containing 100 μg/ml rifampin and 1 μg/ml cefotaxime. Transconjugants from both isolates were found to carry a 35-kb plasmid by S1 nuclease digestion and pulsed-field gel electrophoresis (PFGE) (9 and data not shown). They could not be typed by the original PCR-based replicon typing (PBRT) scheme available at the time (10), so both plasmids were sequenced for further characterization. Total plasmid DNA was extracted from each isolate using a Qiagen Hi Speed plasmid Midi kit following the manufacturer's protocol and electroporated into Electromax DH10B *E. coli* cells (Invitrogen), and transformants were selected with 4 μg/ml cefotaxime. Plasmid DNA was isolated from transformants using a Qiagen large construct kit. A DNA library prepared from 500 ng of DNA following Roche protocol 2.3 was sequenced on one-eighth of a plate using a Roche 454 GS-FLX system. Sequences were assembled using Newbler version 2.3 (Roche), and >240-fold coverage was achieved. Single contigs were closed by PCR (Table 1) and ABI sequencing.

Plasmids from *E. coli* and *Enterobacter cloacae* were found to be 100% identical, and the 35,341-bp plasmid was designated pSAM7. BLAST searches of GenBank indicated that the backbone of pSAM7 was closely related to the newly defined IncX4 group of plasmids (11), with the highest identity (99%) to pJIE143 (GenBank accession no. JN194214) harboring $bla_{CTX-M-15}$ from an *E. coli* isolated from a human in Australia in 2006 (12). pSAM7 was also related to pJEG012 (KC354802) (93%) from *Klebsiella pneumoniae*, also isolated in Australia and carrying the antimicrobial resistance genes *aacA4*, *aadA1*, and bla_{OXA-9} (13), and other IncX4 plasmids, pBS512_33 (CP001059) (93%) from *Shigella boydii*, pSHS696_34 (JX258654) (91%) from *Salmonella enterica* serovar Heidelberg, and pCROD2 (FN543504) (88%) from *Citrobacter rodentium* (14), none of which harbor any antimicrobial resistance genes. Annotation using RAST (15) and Artemis and comparisons with pJIE143 and pBS512_33 identified 51 open reading frames (ORFs), of which 20 were hypothetical. Alignments carried out using the WebACT comparison tool (16) revealed high levels of identity in large parts of the plasmid backbones (Fig. 1). As described for pJIE143, the initiation of replication in pSAM7 is likely to be mediated by the *pir*-encoded π replication initiation protein and several iterons (12). All iterons in pSAM7 were identical to those in pJIE143, apart from γ3, which differed by two nucleotides (bold) (AAACATGA**T**AA**C**TTCCTC GGTT in pSAM7 and AAACATGA**G**A**G**CTTCCTCGGTT in pJIE143).

Conjugation of IncX plasmids involves the products of the 11

Received 30 May 2013 Returned for modification 23 June 2013
Accepted 29 June 2013
Published ahead of print 8 July 2013

Address correspondence to M. O. Stokes, K0959262@kingston.ac.uk, or M. D. Fielder, M.fielder@kingston.ac.uk.

doi:10.1128/AAC.01157-13

TABLE 1 Primers used in this study to close pSAM7, genetic markers, and environment

Primer	Primer sequence (5′–3′)	Amplicon size (bp)	Position in pSAM7 (bp)
Rpir FW	CAGTGTGGATTTTGAGCAT	774	822–1595
Rpir RV	GCCCCTATTGTATAAAGATTCA		
Rpilx5 FW	CTTAGTTCATTTGTGAATGCC	1,060	20925–21984
Rpilx5 RV	GAAAGTGTTGATGCTGTGAT		
RhicA FW	CCAGTTTTCCATACAGGACA	350	28212–28561
RhicA RV	GTTGCATATCTATAGGGGATG		
Hyex FW	CAAAGGGAGGGTGTGAAT	841	10553–11393
Hyex RV	GGAATGGCGATACAAACA		
Smet FW	CGATGGCCTTAAGACCTT	471	31316–31786
Smet RV	CGGACACGGTATTTGTTG		
ISEC	GAAAAGCGTGGTAATGCT	739	29776–30514
CTXISEC	GCACCTGCGTATTATCTGC		
CTXSMETH[a]	GTCGTGGACTGTAGGTGATA	767	31020–31786
ISECHIC[a]	GCAAATTGGATATTGTAGCA	1,098	28212–29309
pSAM7 FW	GCACGCATTAAAAGCCTTAT	307	508–814
pSAM7 RW	GGCAGATTAACAACAGATTCAA		
pSAM7-2 FW	GAGTGGGGATCAAGTTTACG	327	1364–1690
pSAM7-2 RV	CTTCCGTATGTTTCATGATTTC		

[a] CTXSMETH was used with Smet RV and ISECHIC was used with RhicA FW.

traX (*pilX*) genes, three *tax* genes (*taxA*, *taxB*, and *taxC*), and *oriT*α (17). Like other IncX4 plasmids, pSAM7 contains the *hicA-hicB* addiction system (18) and a partitioning gene, *parB*, adjacent to a resolvase. pSAM7 has two short insertions absent from pJIE143, the first between positions (bp) 6061 and 6377 in a conserved hypothetical gene in pJIE143 (positions 8971 to 9222) (12). This insertion is flanked by nearly perfect direct repeat sequences GGACA**G**AAT**C**ACCTGTATGTC (positions 6040 to 6060) and

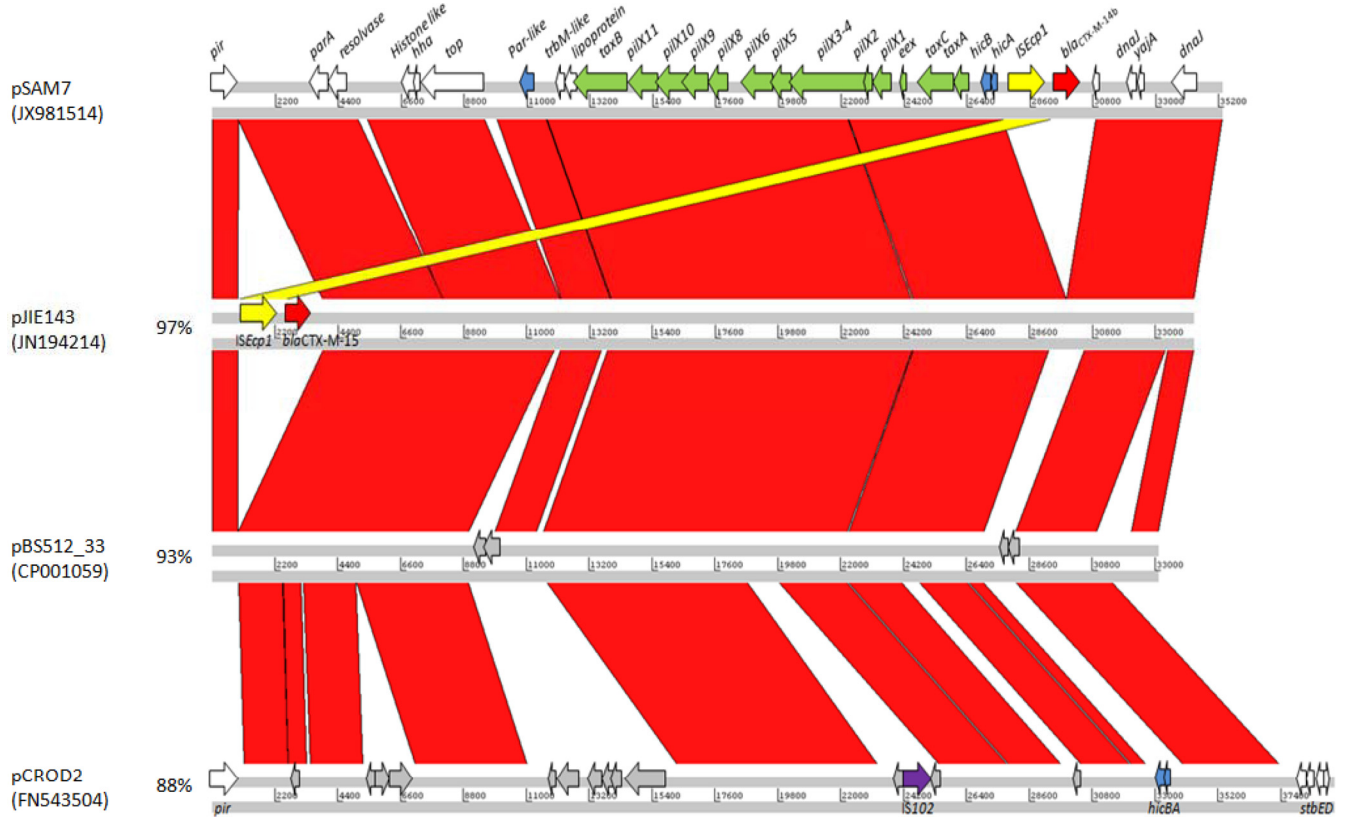

FIG 1 Artemis Comparison Tool (ACT) comparison of pSAM7 with related plasmids pJIE143, pBS512_33, and pCROD2 (which has an unrelated replication protein). Green arrows, genes relating to conjugation; blue arrows, stability genes; yellow arrows, IS*Ecp1*; red arrows, bla_{CTX-M} genes. Other genes are shown in white, and conserved hypothetical genes are shown in gray. Red areas show nucleotide identity > 93%, the highlighted yellow area indicates IS*Ecp1*, and the percentages shown indicate the levels of identity of the complete plasmids to the pSAM7 backbone.

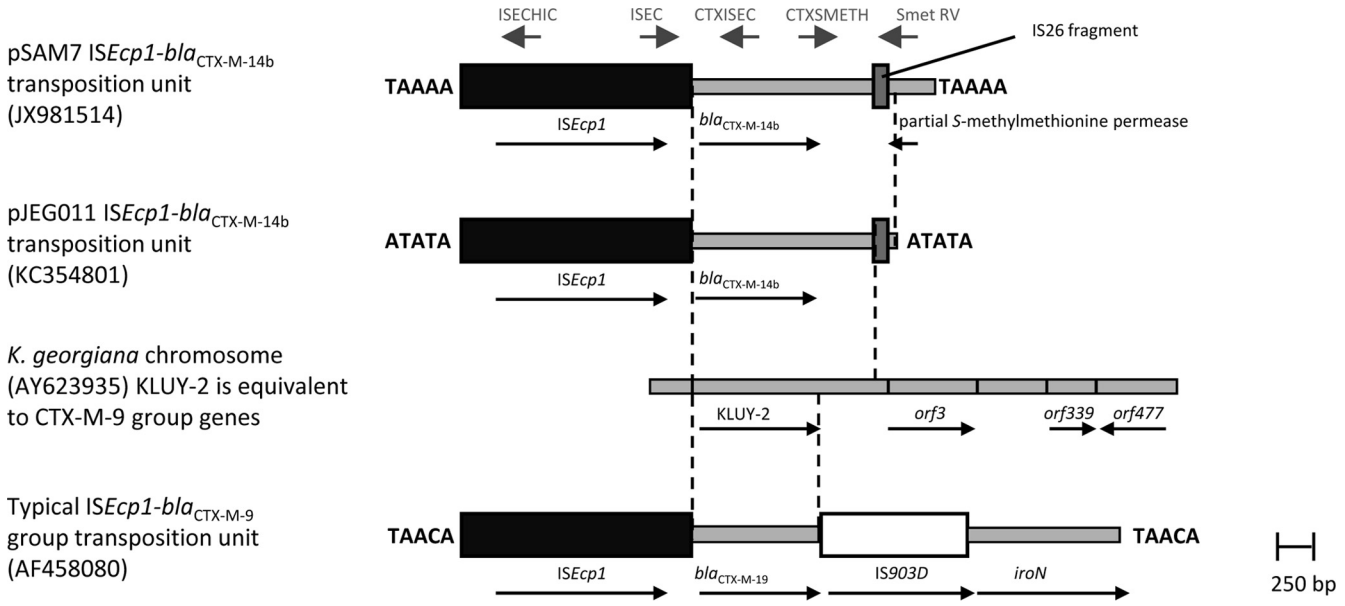

FIG 2 Comparison of the IS*Ecp1* transposition units in pSAM7 and pJEG011 with a typical IS*Ecp1*-bla$_{CTX-M-9}$ group transposition unit and with the chromosome of *Kluyvera georgiana*. Solid black boxes indicate transposons, black arrows indicate extents and directions of genes, and 5-bp direct repeats flanking insertions are indicated. Dashed lines indicated the boundaries of regions common to two or more sequences. Gray arrows indicate primers used for investigating the genetic environment of the bla$_{CTX-M-14b}$ gene in pSAM7.

GGACA**A**AAT**G**ACCTGTATGTC (6377 to 6397). A similar insertion that includes these repeats is present in pCROD2 (91% identical) (14). The other insertion is between positions 10489 and 10925 of pSAM7, part of a hypothetical coding sequence (10268 to 10843). This region is also present in the IncX4 plasmids pSH696_34 and pBS512_33 and the IncX1 plasmid pE001 (19).

IS*Ecp1* transposition units carrying bla$_{CTX-M-9}$ group genes usually contain a 920-bp region corresponding to the *Kluyvera georgiana* chromosome with IS*903* 2 bp after the stop codon of bla$_{CTX-M}$ (Fig. 2) (20, 21). In pSAM7, a 1,256-bp region matching *K. georgiana* is followed by a 108-bp fragment of the IS*26* insertion sequence. Downstream of this is a region consisting of 177 bp corresponding to an internal part of a gene encoding an *S*-methylmethionine permease found in several *E. coli* chromosomes. This 3,246-bp transposition unit is flanked by 5-bp direct repeats (TA AAA) characteristic of IS*Ecp1* transposition. This combination of different components suggests that the transposition unit in pSAM7 may have been compiled from segments acquired in different IS*Ecp1* transposition events (22). A similar transposition unit with the IS*26* fragment but only 73 bp of the *S*-methylmethionine permease gene is also present, flanked by direct repeats of ATATA, in the IncL/M plasmid pJEG011 (KC354801) from the same *K. pneumoniae* isolate as pJEG012 (13). The transposition unit in pSAM7 is inserted 97 bp upstream of the *hicA* gene, while in pJIE143 the IS*Ecp1*-bla$_{CTX-M-15}$-*orf477*Δ transposition unit is inserted 22 bp downstream of the *pir* gene, flanked by direct repeats of GGATA (12), indicating different insertions of bla$_{CTX-M}$ genes in the same backbone. No other known antimicrobial resistance or virulence genes were identified in pSAM7.

Sequence comparisons were used to design primers to detect pSAM7-like plasmids (Table 1). The genetic markers selected were the *pir* (Rpir), *hicA* (RhicA), and *pilX5* (Rpilx5) regions, a hypothetical gene (Hyex), and the transposition unit (Smet). Sequence differences between *pir* genes would be expected to pre-

TABLE 2 Screening of ESBL isolates and transconjugants for pSAM7-like plasmids[a]

Isolate	Species[b]	Host	Origin	Yr	CTX-M	ST	pSAM7 markers[c]					
							Rpir	*Rpil*	*RhicA*	*Hyex*	*Smet*	bla$_{CTX-M-14}$ TU[d]
SAM7	*E. coli*	Cattle	United Kingdom	2008	14	10	Y	Y	Y	Y	Y	Y
ECR528	*E. cloacae*	Cattle	United Kingdom	2012	14		Y	Y	Y	Y	Y	Y
ESBL487	*E. coli*	Cattle	United Kingdom	2008	14	117	Y	Y	Y	Y	Y	Y
ESBL562	*E. coli*	Cattle	United Kingdom	2007	14	559	Y	Y	Y	Y	Y	Y
ESBL592	*E. coli*	Cattle	United Kingdom	2008	14	2177	Y	Y	Y	Y	Y	Y
ESBL219	*E. coli*	Human	Germany	Unknown	1	ND	Y	Y	Y	N	N	ND
ESBL220	*E. coli*	Human	Germany	Unknown	1	ND	Y	Y	Y	N	N	ND
ESBL484	*E. coli*	Cattle	United Kingdom	2007	1	ND	Y	Y	Y	N	N	ND

[a] ST, serotype; ND, not determined.
[b] Confirmed by MALDI-TOF.
[c] pSAM7 transformed into DH10 was used as a positive control and *Salmonella* Typhimurium strain 26R was used as a negative control in PCR. Y, amplicon obtained; Y, amplicon also obtained from transconjugant; N, no amplicon.
[d] bla$_{CTX-M-14}$ TU, IS*Ecp1*-bla$_{CTX-M-14}$ transposition unit found in pSAM7.

vent binding of the Rpir primers to pCROD2 and pSH696_34, the RhicA region is absent from pBS512_32 and pJEG012, and Hyex is present only in pSAM7 and pSH696_34. The original isolates, transconjugants, and transformants carrying pSAM7 produced all five amplicons, while JIE143 carrying pJIE143 (12) produced Rpir, Rpilx5, and RhicA amplicons only, as expected.

The same primers were used to screen 42 *E. coli* isolates, collected from the United Kingdom, The Netherlands, or Germany between 2004 and 2009, which had been identified as having small plasmids carrying ESBL genes (unpublished data). Isolates carried $bla_{CTX-M-1}$ (human $n = 9$, cattle $n = 7$, poultry $n = 5$, pig $n = 4$), $bla_{CTX-M-14}$ (human $n = 2$, cattle $n = 5$), $bla_{CTX-M-15}$ (cattle $n = 2$), bla_{TEM-52} (poultry $n = 7$), or bla_{SHV-12} (human $n = 1$) as determined by PCR and sequencing. Six of the 42 isolates had at least three of the markers *pir* (Rpir), *hicA* (RhicA), and *pilX5* (Rpilx5) (Table 2). Transconjugants carrying ESBL genes from three isolates, collected from cattle at different farms in the United Kingdom, had an ~35-kb plasmid by S1/PFGE (data not shown) and all of the pSAM7 markers and carried $bla_{CTX-M-14}$ in the same genetic environment as in pSAM7 (Table 2). Comparison of these isolates by PFGE and multilocus sequence typing (MLST) (following the protocol available at http://mlst.ucc.ie/mlst/dbs/Ecoli) (23) showed that they were not related to each other (ST117, ST559, and ST2177) or to SAM7 (ST10). Thus, pSAM7-like plasmids are moving between *E. coli* sequence types.

The original PBRT scheme (10) included primers to detect IncX2 plasmids, typified by R6K, but not IncX1 plasmids, typified by R485 (24). Primers are now available to detect IncX1 and IncX2 plasmids and two additional groups, IncX3 and IncX4 (11). The IncX4 primers in the *taxC* gene are designed to detect all IncX4 plasmids (11), while the markers used here differentiated pSAM7 from other IncX4 plasmids. The similarity between pSAM7 and pJIE143 suggests that these plasmids share a ancestor which has been in the bacterial population for some time, as is evident from their identification from isolates with no epidemiological links on separate continents. To date, pSAM7-like plasmids have been found only in isolates from cattle in the United Kingdom, which may act as a reservoir, but the similarity to pJIE143 suggests that pSAM7 may be able to facilitate spread of $bla_{CTX-M-14b}$ from animal to human isolates. These plasmids are capable of conjugation, as is evident from the occurrence of pSAM7 in two different bacterial genera and its ability to transfer to *S. enterica* serovar Typhimurium from both original isolates and transformants. pSAM7 and pJIE143 and other IncX4 backbones may represent emerging vectors for bla_{CTX-M} genes, conferring resistance to extended-spectrum cephalosporins in *Enterobacteriaceae*. This work shows that IncX4 plasmids play a role in disseminating $bla_{CTX-M-14b}$ between different species of bacteria. Several other IncX plasmids bearing important β-lactamase genes, including IncX1 plasmids carrying bla_{TEM-52} in *E. coli* and *Salmonella* found in meat imported into Denmark from The Netherlands and Germany, have been reported recently (19). bla_{KPC} carbapenemase genes have also been identified on the IncX3 plasmid pKpS90 (bla_{KPC-2}) (25) and a novel IncX5 plasmid (bla_{KPC-5}) (26), both isolated from *K. pneumoniae*.

Nucleotide sequence accession number. The sequence of pSAM7 from *E. coli* SAM7 has been submitted to GenBank under accession number JX981514.

ACKNOWLEDGMENTS

We thank Richard Ellis for sequencing of the plasmids and Onyi Diribe, Matthew Hayward, Hannah Preedy, Roberto La Ragione, Luke Randall, Robert Horton, Chris Teale, Jonathan Iredell, and Alison Kelly for technical help and useful discussions. We also acknowledge Beatriz Guerra, Reiner Helmuth, and Irene Rodriguez, who work on the ESBL-Safefoodera Consortium and kindly supplied isolates.

This work was funded by the Department for Environment, Food and Rural Affairs (project grant OD2028) and by Kingston University BPSRG funding.

REFERENCES

1. **Bonnet R.** 2004. Growing group of extended-spectrum β-lactamases: the CTX-M enzymes. Antimicrob. Agents Chemother. **48:**1–14.
2. **Cantón R, Coque TM.** 2006. The CTX-M β-lactamase pandemic. Curr. Opin. Microbiol. **9:**466–475.
3. **Chanawong A, M'Zali FH, Heritage J, Xiong JH, Hawkey PM.** 2002. Three cefotaximases, CTX-M-9, CTX-M-13 and CTX-M-14, among *Enterobacteriaceae* in the People's Republic of China. Antimicrob. Agents Chemother. **46:**630–637.
4. **Kim J, Bae IK, Jeong SH, Chang CL, Lee CH, Lee K.** 2011. Characterization of IncF plasmids carrying the $bla_{CTX-M-14}$ gene in clinical isolates of *Escherichia coli* from Korea. J. Antimicrob. Chemother. **66:**1263–1268.
5. **Weill FX, Lailler R, Praud K, Kerouanton A, Fabre L, Brisabois A, Grimont PAD, Cloeckaert A.** 2004. Emergence of extended-spectrum-β-lactamase (CTX-M-9)-producing multiresistant strains of *Salmonella enterica* serotype Virchow in poultry and humans in France. J. Clin. Microbiol. **42:**5767–5773.
6. **Stokes MO, Cottell JL, Piddock LVJ, Wu G, Wootton M, Mevius DJ, Randall LP, Teale CJ, Fielder MD, Coldham NG.** 2012. Detection and characterization of pCT-like plasmid vectors for $bla_{CTX-M-14}$ in *Escherichia coli* isolates from humans, turkeys and cattle in England and Wales. J. Antimicrob. Chemother. **67:**1639–1644.
7. **Cottell JL, Webber MA, Coldham NG, Taylor DL, Cerdeno-Tarraga AM, Hauser H, Thomson NR, Woodward MJ, Piddock LJV.** 2011. Complete sequence and molecular epidemiology of IncK epidemic plasmid encoding $bla_{CTX-M-14}$. Emerg. Infect. Dis. **17:**645–652.
8. **Eigner U, Holfelder M, Oberdorfer K, Betz-Wild U, Bertsch D, Fahr A.** 2009. Performance of a matrix-assisted laser desorption ionization-time-of-flight mass spectrometry system for the identification of bacterial isolates in the clinical routine laboratory. Clin. Lab. **55:**289–296.
9. **Barton BM, Harding GP, Zuccarelli AJ.** 1995. A general method for detecting and sizing large plasmids. Anal. Biochem. **226:**235–240.
10. **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.
11. **Johnson TJ, Bielak EM, Fortini D, Hansen LH, Hasman H, Debroy C, Nolan LK, Carattoli A.** 2012. Expansion of the IncX plasmid family for improved identification and typing of novel plasmids in drug-resistant *Enterobacteriaceae*. Plasmid **68:**43–50.
12. **Partridge SR, Ellem JA, Tetu SG, Zong Z, Paulsen IT, Iredell JR.** 2011. Complete sequence of pJIE143, a *pir*-type plasmid carrying IS*Ecp1*-$bla_{CTX-M-15}$ from an *Escherichia coli* ST131 isolate. Antimicrob. Agents Chemother. **55:**5933–5935.
13. **Espedido BA, Steen JA, Ziochos H, Grimmond SM, Cooper MA, Gosbell IB, van Hal SJ, Jensen SO.** 2013. Whole genome sequence analysis of the first Australian OXA-48-producing outbreak-associated *Klebsiella pneumoniae* isolates: the resistome and *in vivo* evolution. PLoS One **8:**e59920. doi:10.1371/journal.pone.0059920.
14. **Petty NK, Bulgin R, Crepin VF, Cerdeno-Tarraga AM, Schroeder GN, Quail MA, Lennard N, Corton C, Barron A, Clark L, Toribio AL, Parkhill J, Dougan G, Frankel G, Thomson NR.** 2010. The *Citrobacter rodentium* genome sequence reveals convergent evolution with human pathogenic *Escherichia coli*. J. Bacteriol. **192:**525–538.
15. **Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O.** 2008. The RAST server: rapid annotations using subsystems technology. BMC Genomics **9:**75. doi:10.1186/1471-2164-9-75.
16. **Abbott JC, Aanensen DM, Rutherford K, Butcher S, Spratt BG.** 2005.

WebACT—an online companion for the Artemis Comparison Tool. Bioinformatics **21:**3665–3666.
17. **Núñez B, Avila P, de la Cruz F.** 1997. Genes involved in conjugative DNA processing of plasmid R6K. Mol. Microbiol. **24:**1157–1168.
18. **Jørgensen MG, Pandey DP, Jaskolska M, Gerdes K.** 2009. HicA of *Escherichia coli* defines a novel family of translation-independent mRNA interferases in bacteria and archaea. J. Bacteriol. **191:**1191–1199.
19. **Bielak E, Bergenholtz RD, Jorgensen MS, Sorensen SJ, Hansen LH, Hasman H.** 2011. Investigation of diversity of plasmids carrying the bla_{TEM-52} gene. J. Antimicrob. Chemother. **66:**2465–2474.
20. **Olson AB, Silverman M, Boyd DA, McGeer A, Willey BM, Pong-Porter V, Daneman N, Mulvey MR.** 2005. Identification of a progenitor of the CTX-M-9 group of extended-spectrum β-lactamases from *Kluyvera georgiana* isolated in Guyana. Antimicrob. Agents Chemother. **49:**2112–2115.
21. **Poirel L, Decousser JW, Nordmann P.** 2003. Insertion sequence IS*Ecp1B* is involved in expression and mobilization of a bla_{CTX-M} β-lactamase gene. Antimicrob. Agents Chemother. **47:**2938–2945.
22. **Partridge SR.** 2011. Analysis of antibiotic resistance regions in Gram-negative bacteria. FEMS Microbiol. Rev. **35:**820–855.
23. **Wirth T, Falush D, Lan R, Colles F, Mensa P, Wieler LH, Karch H, Reeves PR, Maiden MC, Ochman H, Achtman M.** 2006. Sex and virulence in *Escherichia coli*: an evolutionary perspective. Mol. Microbiol. **60:**1136–1151.
24. **Jones CS, Osborne DJ, Stanley J.** 1993. Molecular comparison of the IncX plasmids allows division into IncX1 and IncX2 subgroups. J. Gen. Microbiol. **139:**735–741.
25. **Kassis-Chikhani N, Frangeul L, Drieux L, Sengelin C, Jarlier V, Brisse S, Arlet G, Decre D.** 2013. Complete nucleotide sequence of the first KPC-2- and SHV-12-encoding IncX plasmid, pKpS90, from *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. **57:**618–620.
26. **Chen L, Chavda KD, Fraimow HS, Mediavilla JR, Melano RG, Jacobs MR, Bonomo RA, Kreiswirth BN.** 2013. Complete nucleotide sequences of bla_{KPC-4}- and bla_{KPC-5}-harboring IncN and IncX plasmids from *Klebsiella pneumoniae* strains isolated in New Jersey. Antimicrob. Agents Chemother. **57:**269–276.