

# Novel Conjugative Plasmid from *Escherichia coli* of Swine Origin That Coharbors the Multiresistance Gene *cfr* and the Extended-Spectrum- $\beta$ -Lactamase Gene *bla*<sub>CTX-M-14b</sub>

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**Two porcine *Escherichia coli* isolates harbored the *cfr* gene on conjugative plasmids of 38,405 bp (pGXEC6) and 41,646 bp (pGXEC3). In these two plasmids, the *cfr* gene was located within a 4,612-bp region containing a *tnpA-IS26-cfr-IS26- $\Delta$ hyp* element. Plasmid pGXEC3 was almost identical to pGXEC6 except for a 3,235-bp *ISEcp1-bla*<sub>CTX-M-14b</sub> insertion. The colocation of the multiresistance *cfr* gene with an extended-spectrum- $\beta$ -lactamase gene on a conjugative plasmid may support the dissemination of these genes by coselection.**

During the past decade, the transferable multiresistance gene *cfr*, which confers resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A (1), has been identified mainly in Gram-positive bacteria, including *Staphylococcus* spp., *Bacillus* spp., *Enterococcus* spp., *Macrococcus caseolyticus*, *Jeotgalicoccus pinnipedialis*, and *Streptococcus suis* (2, 3). However, a small number of reports have described the presence of the gene *cfr* in Gram-negative bacteria, such as *Proteus vulgaris* (4) and *Escherichia coli* (5–7). The global spread of this gene in bacteria of human and animal origin has gained particular public health attention, since *cfr* confers resistance to linezolid, the last-resort antibiotic against vancomycin-resistant enterococci, methicillin-resistant *Staphylococcus aureus*, and penicillin-resistant pneumococci (2, 8). In *Enterobacteriaceae* from human and animal sources, extended-spectrum- $\beta$ -lactamase (ESBL) genes have emerged during recent years globally (9). Since the year 2000, ESBL genes of the type *bla*<sub>CTX-M</sub> have become the predominant ESBL genes (10, 11). Similar to the *cfr* gene (2), ESBL genes are often located on plasmids, including plasmids that carry additional resistance genes which may facilitate the dissemination and persistence of the ESBL genes (8, 11–13).

So far, a single conjugative IncA/C broad-host-range plasmid of *E. coli*, designated pSCEC2 (6), has been described, in which the *cfr* gene was collocated with the resistance genes *tet(A)* (tetracycline resistance), *strA-strB* (streptomycin resistance), and *sul2* (sulfonamide resistance). In the present study, two novel *cfr*-carrying plasmids, pGXEC6 and pGXEC3, were identified, one of which also harbored a *bla*<sub>CTX-M14b</sub> gene. These two plasmids were completely sequenced to gain insight into their genetic relatedness and understand how they may have developed.

Thirty-one pericardial fluid swab samples collected from diseased swine from June 2013 to January 2014 in four different commercial pig farms in the Guangxi Province, China, were inoculated on brain heart infusion agar plates supplemented with 10 mg/liter florfenicol. Two *cfr*-positive *E. coli* isolates, designated GXEC6 and GXEC3, were obtained from samples from two different pig farms, farm A (a breeding pig farm) and farm B (a fattening pig farm), respectively. The antimicrobial usage records for these two pig farms indicated that aside from ceftriaxone, which was used for prophylactic purposes at farm A, other anti-

microbial agents, such as ampicillin, florfenicol, sulfamethoxazole, amikacin, enrofloxacin, lincomycin, and tiamulin, were extensively used to prevent or treat bacterial infections at the two pig farms. The two *E. coli* isolates were typed by multilocus sequence typing (MLST) (see <http://mlst.warwick.ac.uk>) and subjected to pulsed-field gel electrophoresis (PFGE) (14). Conjugation experiments by filter mating and S1-PFGE combined with Southern blotting (15) were performed as described previously (15). PCR-based replicon typing (PBRT) followed previously published recommendations (16). The two *cfr*-positive original *E. coli* isolates and their transconjugants were tested for their susceptibility to amikacin, ampicillin, ceftazidime, chloramphenicol, enrofloxacin, florfenicol, gentamicin, sulfamethoxazole, and tetracycline by broth microdilution according to CLSI recommendations (17, 18). ESBL confirmatory tests also followed CLSI recommendations (18). The *cfr*-carrying plasmids of the transconjugants were sequenced using the 454 Life Sciences GS FLX system (Roche). The gaps between the contigs were closed by PCR, and the whole-plasmid sequences were draft assembled using the SeqMan software from DNASTar (Lasergene, Madison, WI, USA). The AatII, BstEII, ScaI, and SnaBI restriction profiles of *cfr*-carrying plasmids were determined and compared with the plasmid sequences to confirm the correct assembly of the contigs. The plasmid sequences were annotated using the Vector NTI program, and the putative coding sequences were identi-

Received 28 October 2014 Returned for modification 16 November 2014

Accepted 20 November 2014

Accepted manuscript posted online 24 November 2014

Citation Zhang W-J, Wang X-M, Dai L, Hua X, Dong Z, Schwarz S, Liu S. 2015. Novel conjugative plasmid from *Escherichia coli* of swine origin that coharbors the multiresistance gene *cfr* and the extended-spectrum- $\beta$ -lactamase gene *bla*<sub>CTX-M-14b</sub>. *Antimicrob Agents Chemother* 59:1337–1340. doi:10.1128/AAC.04631-14.

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doi:10.1128/AAC.04631-14

**TABLE 1** MICs for *E. coli* GXEC3 and GXEC6 and the corresponding *cfr*-positive *E. coli* J53 transconjugants

Strain	MIC (mg/liter) of <sup>a</sup> :								
	AMP	TAZ	GEN	AMK	CHL	FFC	ENO	TET	SMZ
GXEC3	≥256	64	64	16	256	128	≥32	128	32
<i>E. coli</i> J53 (pGXEC3) <sup>b</sup>	256	32	2	2	128	128	0.06	2	16
GXEC6	8	1	64	8	256	128	≥32	128	≥512
<i>E. coli</i> J53 (pGXEC6) <sup>c</sup>	4	0.06	2	2	128	128	0.06	2	16
<i>E. coli</i> J53	4	0.06	1	2	2	1	0.03	2	8

<sup>a</sup> AMP, ampicillin; TAZ, ceftazidime; GEN, gentamicin; AMK, amikacin; CHL, chloramphenicol; FFC, florfenicol; ENO, enrofloxacin; TET, tetracycline; SMZ, sulfamethoxazole.

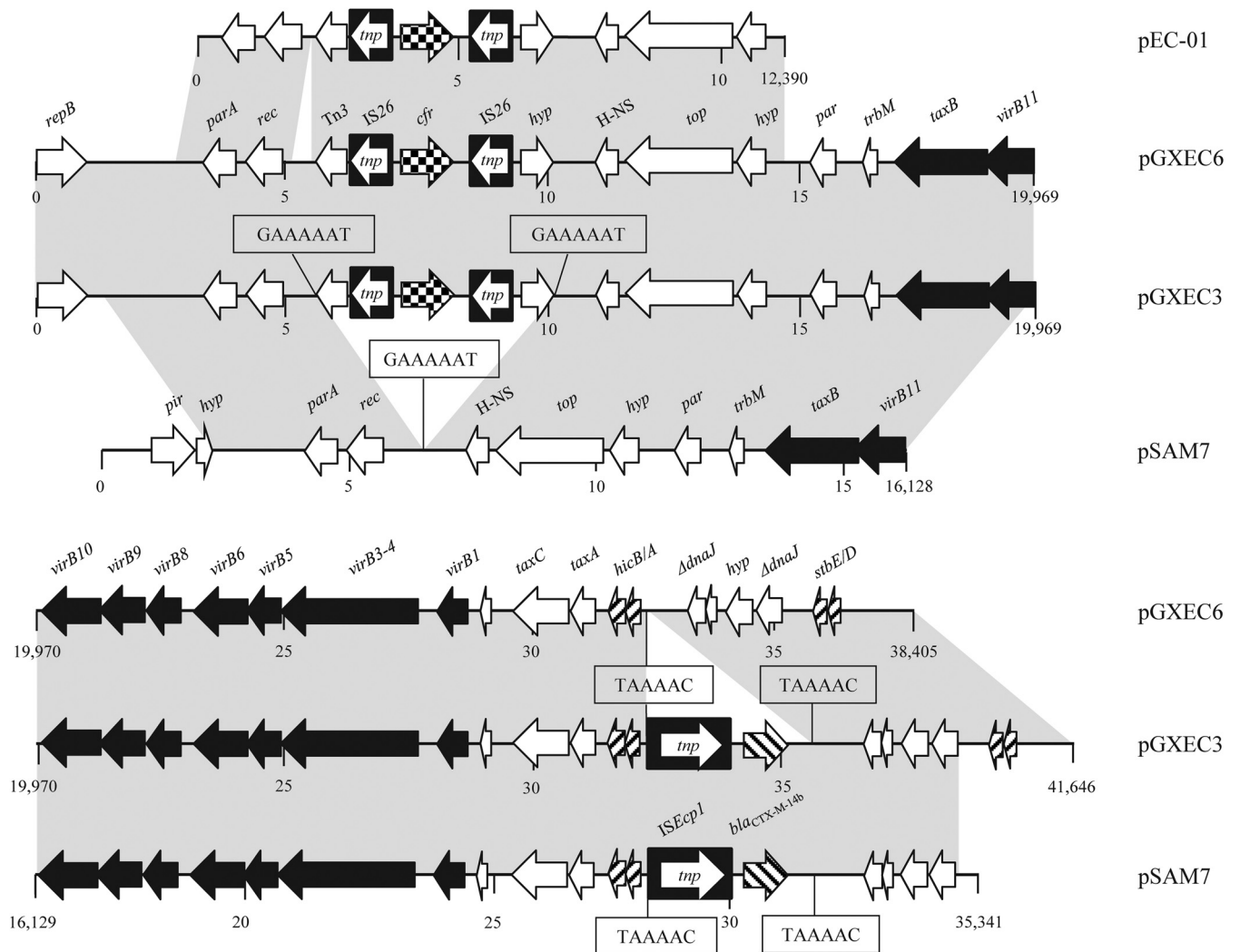
<sup>b</sup> *E. coli* J53 transconjugants of GXEC3.

<sup>c</sup> *E. coli* J53 transconjugants of GXEC6.

fied using the ORF Finder program (<http://www.ncbi.nlm.nih.gov/projects/gorf/>). Sequence comparisons were performed using BLASTN and BLASTP (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Although isolates GXEC6 and GXEC3 originated from different farms, PFGE analysis revealed two related XbaI patterns. This observation may be explained by the fact that fattening pig farm B, from

which isolate GXEC3 originated, imports weaned piglets from pig-breeding farm A, from which isolate GXEC6 was obtained. MLST identified each isolate as sequence type (ST) 746, and according to the MLST database, this ST was found in *E. coli* isolates of human and horse origins from Egypt and Germany, respectively. Moreover, a recent study reported an ST746 chicken *E. coli* isolate from China coharboring the *bla*<sub>CTX-M-55</sub> and *bla*<sub>CTX-M-123</sub> genes (19). The *cfr*



**FIG 1** Linear comparison of plasmids pGXEC6 and pGXEC3 with pEC-01 from *E. coli* (GenBank accession no. JN982327) and pSAM7 from *E. coli* (GenBank accession no. JX981514). The arrows indicate the positions and directions of the transcription of the genes. Gray shading indicates >99% nucleotide sequence identity. Direct repeats are shown in boxes. Δ indicates a truncated gene. A distance scale in kb is displayed below the plasmid sequences.

gene was transferable by conjugation from these two original *E. coli* isolates to the recipient *E. coli* strain J53. Transconjugants were obtained with a high frequency ( $\sim 2 \times 10^{-3}$  to  $3 \times 10^{-3}$  per donor cell). S1-PFGE of the transconjugants revealed approximate sizes of the cfr-carrying plasmids of 38 kb (pGXEC6) and 41 kb (pGXEC3). None of the two cfr-carrying plasmids produced amplicons by PBRT using the 18 primer pairs described previously (16). While the pGXEC6-carrying transconjugant conferred resistance to only chloramphenicol and florfenicol, the pGXEC3-transconjugant was also resistant to ampicillin and ceftazidime and exhibited an ESBL phenotype (Table 1).

Plasmid pGXEC3 had a size of 41,646 bp, while that of pGXEC6 was 38,405 bp. Comparative sequence analysis of the two plasmids revealed that a 3,241-bp fragment was inserted upstream of the *hica* gene in pGXEC3 (Fig. 1), whereas the remaining parts of the two plasmids were virtually identical except for several nucleotide substitutions. The insertion included an intact 3,235-bp ISEcp1-*bla*<sub>CTX-M-14b</sub> transposition unit and one 6-bp direct repeat (Fig. 1). The detection of a functionally intact *bla*<sub>CTX-M-14b</sub> gene explained the observed ESBL phenotype. In these two plasmids, the cfr gene was located in a 4,612-bp segment, which included a 1,545-bp cfr-carrying central region that was identical to the corresponding sequence of plasmid pEC-01 from porcine *E. coli* (5) (Fig. 1). Moreover, comparative analysis of the 13,122-bp cfr-carrying fragment of pGXEC6/pGXEC3 and the 12,390-bp cfr-carrying fragment of pEC-01 revealed >99% nucleotide sequence identity, except for a 732-bp insertion in pGXEC6/pGXEC3 at positions 5041 to 5772. The cfr gene was flanked by two identical copies of the insertion sequence IS26 located in the same orientation. Further database searches revealed that pGXEC3 and pGXEC6 were related to the recently described IncX4 *bla*<sub>CTX-M-14b</sub>-carrying plasmid pSAM7 from bovine *E. coli*, isolated in 2008 in the United Kingdom (20), except for the cfr-carrying segment, the *repB* plasmid replication gene region, and a segment containing the *stbDE* toxin/antitoxin genes (Fig. 1). It is tempting to assume that the 4,612-bp region consisting of *tnpA*-IS26-*cfr*-IS26- $\Delta$ *hyp* may represent a transposition unit which has integrated into a pSAM7-like backbone to give rise to pGXEC3. Since ISEcp1 is able to mobilize itself and adjacent resistance genes, such as *bla*<sub>CTX-M</sub>-type genes (21–23), it is possible that plasmids pGXEC3 and pGXEC6 have developed from one another by either a gain or loss of the ISEcp1-*bla*<sub>CTX-M-14b</sub> segment.

To the best of our knowledge, this is the first report of the cfr gene and an ESBL gene (*bla*<sub>CTX-M-14b</sub>) being collocated on a conjugative plasmid in *E. coli*. Given the widespread use of penicillins in veterinary medicine (24), the occurrence and dissemination of plasmids carrying cfr and ESBL genes among Gram-negative bacteria need further surveillance.

**Nucleotide sequence accession numbers.** The sequences of plasmids pGXEC3 and pGXEC6 have been deposited in GenBank under accession no. KM580532 and KM580533, respectively.

## ACKNOWLEDGMENTS

This work was funded by the national 973 program (grant 2012CB518801), the National Natural Science Foundation of China (grant 31201862), and the Central Public-Interest Scientific Institution Basal Research Fund (grant 0302014016). The contribution of S.S. was funded by the German Federal Ministry of Education and Research (BMBF) through the German Aerospace Center (DLR), grant 01KI1313D (RESET II) and grant 01KI1301D (MedVet-Staph II).

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