Mobilization of Tn*1721*-like transposons harboring *bla*$_{CTX-M-27}$ between P1-like bacteriophage in *Salmonella* and plasmids in *Escherichia coli*

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Sir,

ESBL CTX-M-27, differing by only one amino acid residue from the globally disseminated CTX-M-14, has recently been detected at increasing prevalence in *E. coli* human isolates from France, Japan and Korea.[1-3] The prevalence of CTX-M-27 in *E. coli* and *Salmonella* strains has also recently been described in food-producing animals in China.[4,5] IncF plasmids and IS*Ecp1*-associated transposons are frequently associated with CTX-M-27 transfer in *E. coli*.[1,6] However, the transmission mechanisms of CTX-M-27 amongst *Salmonella* from food animals remains to be explored. Our recent work revealed that the presence of CTX-M-27 among *Salmonella* isolated from pork was primarily mediated by a P1-like bacteriophage that integrated an 8.6-kb Tn*1721*-like structure harboring $bla_{\text{CTX-M-27}}$ (ΔIS*Ecp1B*-$bla_{\text{CTX-M-27}}$-IS*903D*-ΔTn*1721*).[7] This evidence for distinct transmission mechanisms of $bla_{\text{CTX-M-27}}$ in two common foodborne pathogens within the Enterobacteriaceae prompted us to determine whether the transmission pathways between *E. coli* and *Salmonella* were linked. In the present study, a total of 2509 isolates (2280 *E. coli* and 229 *Salmonella*) collected in our laboratory over the past decade were screened for the presence of $bla_{\text{CTX-M-27}}$ to explore the characteristics and genetic context of $bla_{\text{CTX-M-27}}$-bearing vectors within these bacteria.

The presence of $bla_{\text{CTX-M-27}}$ gene was confirmed in 18 (0.79%, 18/2280) *E. coli* isolates, of which 12 (66.7%, 12/18) were from ducks, 4 (22.2%, 4/18) from chickens and 2 (11.1%, 2/18) from pigs. The $bla_{\text{CTX-M-27}}$ gene was also detected in 34 (15.72%, 34/229) *Salmonella* isolates that were predominantly recovered from chickens (55.88%, 19/34),

followed by pigs (35.29%, 12/34) and ducks (8.82%, 3/34) (Table 1). The recovery rate of CTX-M-27-producing *E. coli* from food animals was generally low and remained constant (0.57-0.83%) during the past decade. In contrast, the prevalence of CTX-M-27-containing *Salmonella* was much higher and rose from 5.88% in 2009 to 19.25% in 2014.

Pulse field gel electrophoresis (PFGE) was used to compare relationships between all CTX-M-27 positive *E. coli* and *Salmonella*. In addition, *bla*$_{\text{CTX-M-27}}$-positive *Salmonella* were also serotyped using slide agglutination with hyperimmune sera (S and A Reagents, Bangkok, Thailand). The 18 *bla*$_{\text{CTX-M-27}}$-positive *E. coli* isolates were grouped into seven *XbaI*-PFGE clusters designated A-G and were 85% similar (Figure. S1). The clusters C and F contained isolates from different cities which suggested that both horizontal transmission and clonal dissemination contribute to the *bla*$_{\text{CTX-M-27}}$ distribution in *E. coli*. Clonal spread of ST131 *E. coli* harboring *bla*$_{\text{CTX-M-27}}$ is prevalent in humans in South Korea, Japan, China and Europe,[1-3,8] but sequence type ST131 was not detected in the *bla*$_{\text{CTX-M-27}}$-positive *E. coli* in the current study. The 34 *bla*$_{\text{CTX-M-27}}$-positive *Salmonella* isolates presented distinct PFGE profiles, suggesting that most of the strains were epidemiologically unrelated (data not shown). *S.* Indiana predominated (27/34), followed by *S.* Typhimurium (6/34) and *S.* Enteritidis (1/34). All the 52 isolates of both species were multi-drug resistant to various antimicrobials from a panel of 17 tested as previously described,[9] and the most frequent pattern of MDR observed was resistance to ampicillin, cefotaxime, ceftiofur, tetracycline, ciprofloxacin, and enrofloxacin (Table S1).

Plasmids harboring *bla*$_{\text{CTX-M-27}}$ were successfully transferred into streptomycin-resistant *E. coli* strain C600 by conjugation from each all the 18 *E. coli* isolates.[10] PCR-based Inc/rep typing (PBRT), S1-PFGE and Southern hybridization with specific probes confirmed that *bla*$_{\text{CTX-M-27}}$ gene was located on IncFII (12/18), IncN (4/18) and non-typeable plasmids (2/18) (Table 1). In 21 of the 34 *Salmonella* isolates (61.76%), *bla*$_{\text{CTX-M-27}}$ was detected on approximately 103 kb P1-like bacteriophages, which were identical to previously reported P1-like bacteriophage SJ46 (KU760857),[7] indicating that P1-like bacteriophage plays an essential role in the dissemination of *bla*$_{\text{CTX-M-27}}$ gene among *Salmonella* of food animal original. For the remaining 13 isolates of *Salmonella*, *bla*$_{\text{CTX-M-27}}$ could not be transferred into *E. coli* C600 by conjugation, but the gene was successfully transferred into *E. coli* DH5α by transformation. PBRT, S1-PFGE and Southern hybridization with specific probes confirmed that *bla*$_{\text{CTX-M-27}}$ gene was located on IncP (4/34), IncFIB (4/34), IncN (2/34), IncHI2 (2/34), and IncA/C (1/34) plasmids.

The *bla*$_{\text{CTX-M-27}}$-positive plasmids from the 18 *E. coli* isolates were typed into five patterns by *Eco*RI digestion. The most predominant plasmid pattern, pZ22 (derived from isolate Z22), was completely sequenced by Illumina HiSeq. pZ22 was an 80, 946 bp IncFII plasmid and contained two resistance genes *bla*$_{\text{CTX-M-27}}$ and *bla*$_{\text{TEM-1}}$. BLAST homology analysis demonstrated that the sequence of pZ22 showed high similarity to the *bla*$_{\text{CTX-M-27}}$-harbouring IncHI2 plasmid pA74 (MG014720) with 99% coverage and 99.95% identity. pA74 was found from an *E. coli* recovered from a duck in China. pZ22

was also highly homologous (74% coverage and 95.7% identity) to a *bla*$_{\text{CTX-M-27}}$-harbouring IncFII plasmid pGDD25-3 (MH316133) from a *Salmonella* Indiana isolate, again isolated from a duck in China (Figure S2).[11] The ~8.6 kb ΔIS*Ecp1B*-*bla*$_{\text{CTX-M-27}}$-IS*903D*-ΔTn*1721*-like structure was identified in pZ22, and this structure was bounded by IRR, with 5 bp DRs at both ends of the structure. The presence of DRs strongly indicated an insertion of the Tn*1721*-like structure. This Tn*1721*-like structure was also identified in an IncFIB plasmid, p11219-CTXM (MF133442) from *Klebsiella pneumoniae* in China (Figure S3), suggesting that the mobilization of *bla*$_{\text{CTX-M-27}}$ containing Tn*1721*-like structure amongst different plasmids from plasmid to P1 bacteriophage or *vice versa*.

The 8.6 kb Tn*1721*-like structure, found in each of the CTX-M-27-harboured plasmids in the remaining 15 *E. coli* strains, was located on IncFII (n=11) and IncN (n=4) plasmids determined by PCR mapping and sequencing (Table S2). In the *E. coli* strains carrying IncN plasmids, the Tn*1721*-like structure was inserted into a putative sodium: proton antiporter gene (Figure S4). NCBI BLASTn analysis revealed that a similar Tn*1721*-like structure has been found in the corresponding region containing a beta-lactamase gene *bla*$_{\text{Toho-1}}$ (MH430881) and *bla*$_{\text{CTX-M-24}}$ in *Salmonella* and *Klebsiella pneumoniae*, respectively.[12] Therefore, we speculate that Tn*1721* transposition serves as a common vehicle carrying *bla*$_{\text{CTX-M}}$ genes, representing an alternative mechanism mediating the mobilization of these genes in addition to the action of IS*Ecp1*.

In conclusion, we identified a high degree of genetic similarity of the Tn*1721*-like structure between P1-like bacteriophage from *Salmonella* and different *E. coli* plasmid replicon types from food-producing animals. These results suggested that $bla_{\text{CTX-M-27}}$ was able to transfer through mobilization of a Tn*1721*-like structure between plasmids of *Salmonella* and *E. coli*, using bacteriophages as vehicles. Therefore, more attention should be paid to the transmission mechanisms of $bla_{\text{CTX-M-27}}$ and drivers for selection of mobilization in the food chain.

Nucleotide sequence accession numbers

The complete nucleotide sequence of plasmids pZ22 and partial nucleotide sequence of plasmids pA61 have been deposited to the GenBank database and assigned accession numbers MT587865 and MN877942, respectively.

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Transparency declarations

None to declare.

Supplementary data

Table S1 to S2 and Figure S1 to S4 is available as Supplementary data at JAC Online..

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Table 1 Characteristics of CTX-M-27-carrying *Escherichia coli* and *Salmonella* isolates from food-producing animals in China

Isolate	Species	Serotype	Source	Date	Plasmid (kb)	Plasmid replicon	RFLP-*Eco*RI
Z22, Z39, Z40	*Escherichia*	N/D	Chicken	2003	80kb	IncFII	A1
A64,A87	*coli*		Duck	2007			
Z5			Chicken	2003	78kb		A2
A72			Duck	2007			
20,22			Duck	2009			
28,157,199			Duck	2009	90kb		A3
A97			Duck	2005	55kb	IncN	B
A61, A67,A66			Duck	2007			
229,230			Pig	2009	50kb	Non-typed	C
MM62	*Salmonella*	Enteritidis	Chicken	2014	80kb	IncP	D1
SP129		Typhimurium	Pig				
K46		Indiana	Chicken		100kb		D2
SP96		Indiana	Pig		150kb		D3
CL129, K21, SP123, SP125		Indiana	Chicken		150kb	IncFIB	E
XC48		Typhimurium	Chicken		150kb	IncN	F
SP132		Indiana	Pig				
SP108		Indiana	Pig		150kb	IncA/C	G
CL189, XC164		Typhimurium	Chicken		200kb	IncHI2	H
S31, S47, S56		Indiana	Duck	2009	100kb	P1-like	I
SP85,SP91,SP95,SP103,SP115,SP118,SP132			Pig	2014		bacteriophage	
CL108,CL135,CL140,CL146,HB137,SG119,K13,K14,K47			Chicken				
SP99		Typhimurium	Pig				
K17			Chicken				

Supplementary data

Table S1 Antimicrobial resistance of CTX-M-27-carrying *Escherichia coli* and *Salmonella enterica* isolates from food-producing animals in China.

Antimicrobial agents	Number (%) of resistant isolates (n = 52)
β-Lactams	
Ampicillin	51 (98.1)
Cefotaxime	52 (100)
Cefoxitin	45 (86.5)
Ceftiofur	47 (90.4)
Ceftazidime	20 (38.5)
Meropenem	0 (0)
Quinolones	
Ciprofloxacin	40 (76.9)
Enrofloxacin	39 (75)
Aminoglycosides	
Kanamycin	27 (51.9)
Gentamicin	27 (51.9)
Amikacin	10 (19.2)
Other Antibiotics	
Tetracycline	49 (94.2)
Tigecycline	0 (0)
Chloramphenicol	21 (40.4)
Florfenicol	23 (44.2)
Colistin	1 (1.9)

Table S2 Selected primers used in this study.

Primer	Nucleotide sequence (5′→3′)	Target DNA sequence	Reference/Source
U-IncN-F	GAGCGGGTCACCTTGGTC	Up of *bla*$_{CTX-M-27}$	This study
U-IncN-R	CTCTGCGTTCTGTTGCGG		
D-IncN-F	ACGCAGGTGCTTTATC	Down of *bla*$_{CTX-M-27}$	This study
D-IncN-R	CGCAAGTATGGTTTCC		
IS-fw	AGAATCATCGCCGAAGGGCTGT AACTGGTTTT	Insertion sequence on the P1-like bacteriophage	(1)
IS-rev	GCGAACATCATCCGTTGCACTC TCTTTGT		

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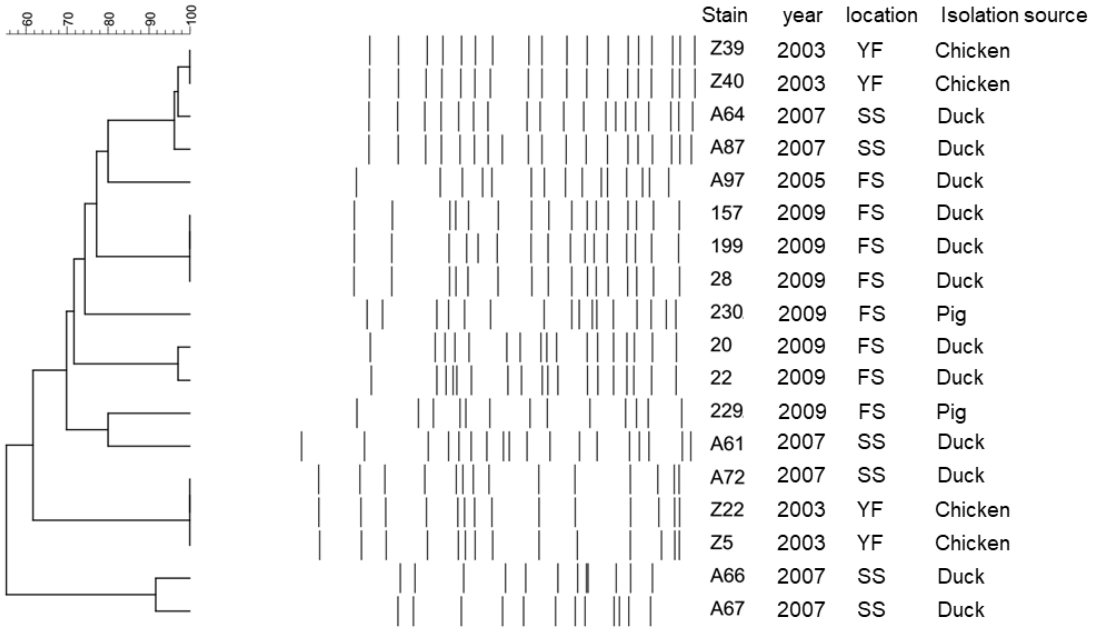

Figure S1 Pulsed-field gel electrophoresis fingerprinting patterns of Xba I-digested total DNA preparations from *Escherichia coli* isolates harboring CTX-M-27-encoding genes

YF: Yunfu in Guangdong province; SS: Sanshui in Guangdong province; FS: Foshan in Guangdong province.

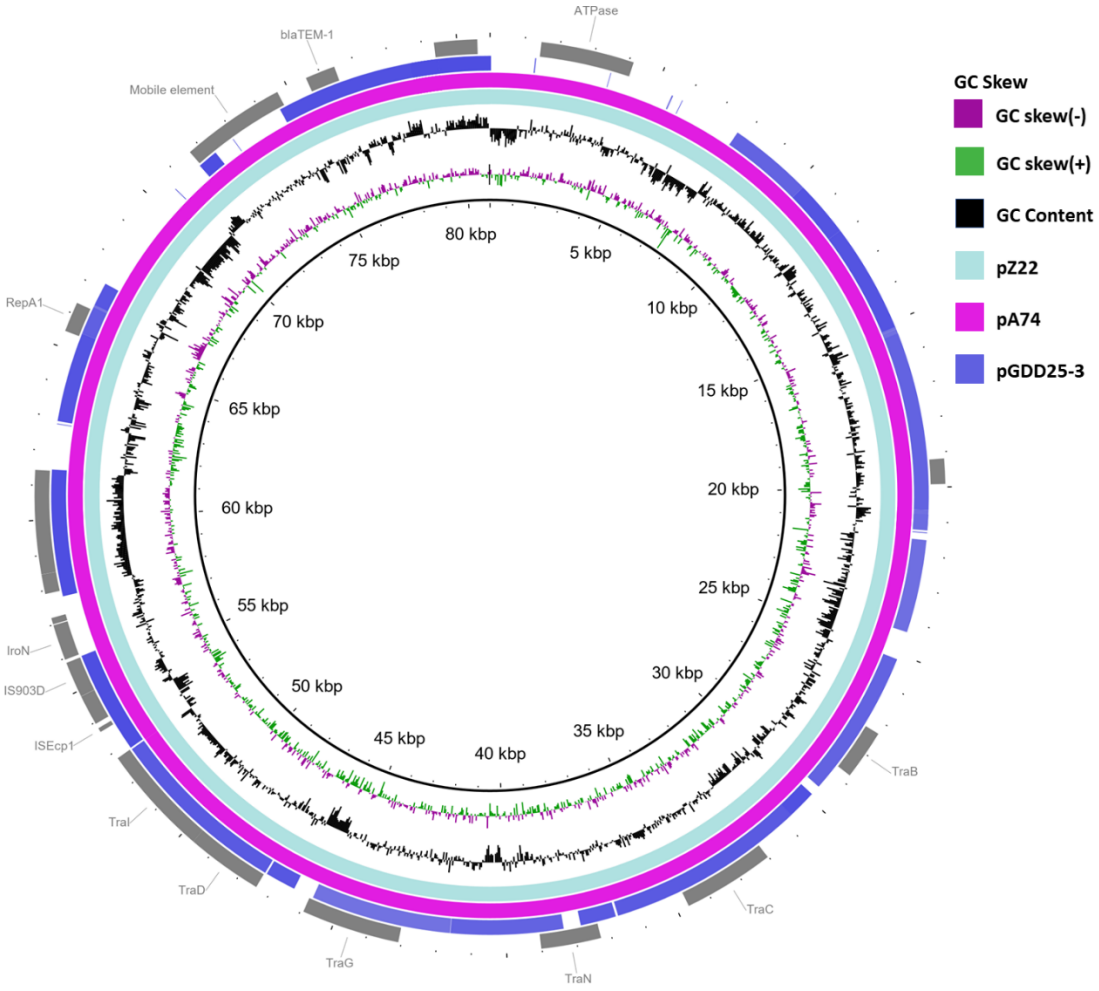

Figure S2 The comparison of pZ22, pA74 and pGDD25-3.

Comparison of IncFII plasmid pZ22, IncHI2 plasmid pA74 and IncFII plasmid pGDD25-3 . The comparison is a pairwise BLASTn alignment performed using BRIG.[1]

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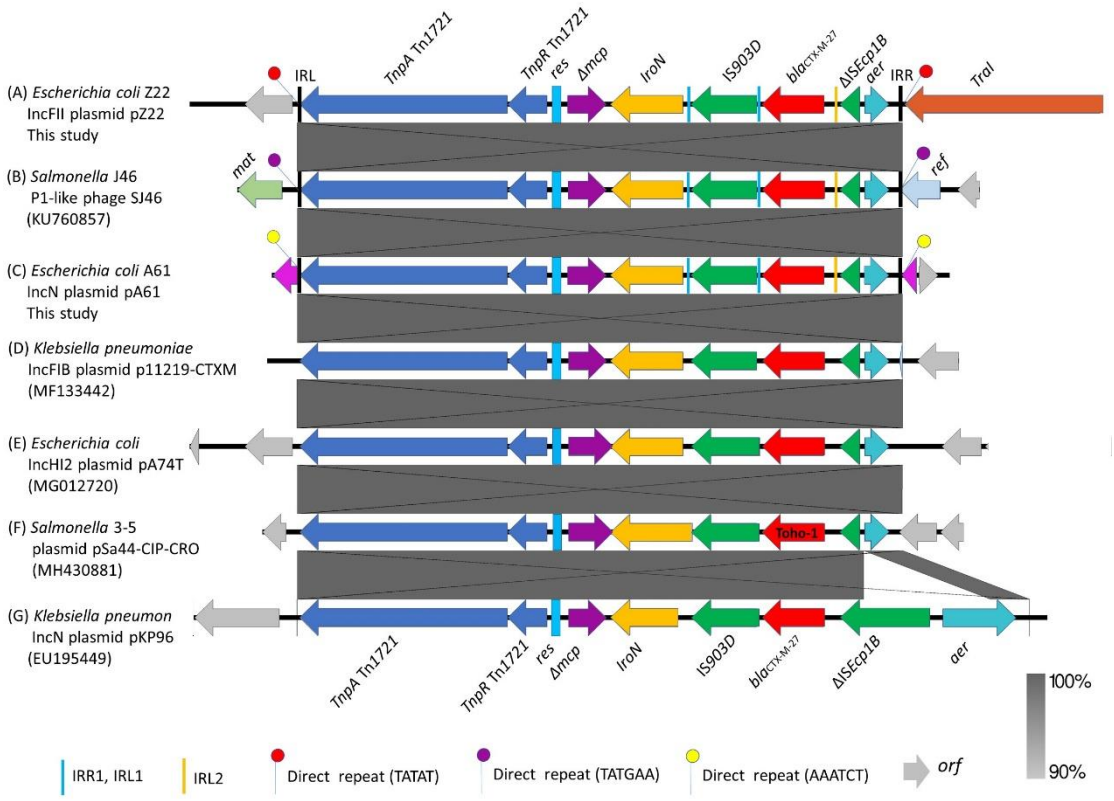

Figure S3. Genomic environment of the *bla*$_{\text{CTX-M-27}}$ gene in *Escherichia coli* isolates and *Salmonella* isolates.

(A) Genetic environment of *bla*$_{\text{CTX-M-27}}$ gene of plasmid IncFII in *Escherichia coli* Z22. (B) Genetic environment of *bla*$_{\text{CTX-M-27}}$ gene of P1-like bacteriophage in *Salmonella* J46 (KU760857). (C) Genetic environment of *bla*$_{\text{CTX-M-27}}$ gene of IncN plasmid in *Escherichia coli* A61. (D) Genetic environment of *bla*$_{\text{CTX-M-27}}$ gene in the IncFIB plasmid of *Klebsiella pneumoniae* (MF133442). (E) Genetic environment of *bla*$_{\text{CTX-M-27}}$ gene in the IncHI2 plasmid of *Escherichia coli* (MG012720). (F) Genetic environment of *bla*$_{\text{Toho-1}}$ gene in *Salmonella* (MH430881). (G) Genetic environment of *bla*$_{\text{CTX-M-27}}$ gene in the IncN plasmid of *Klebsiella pneumoniae* (EU195449).

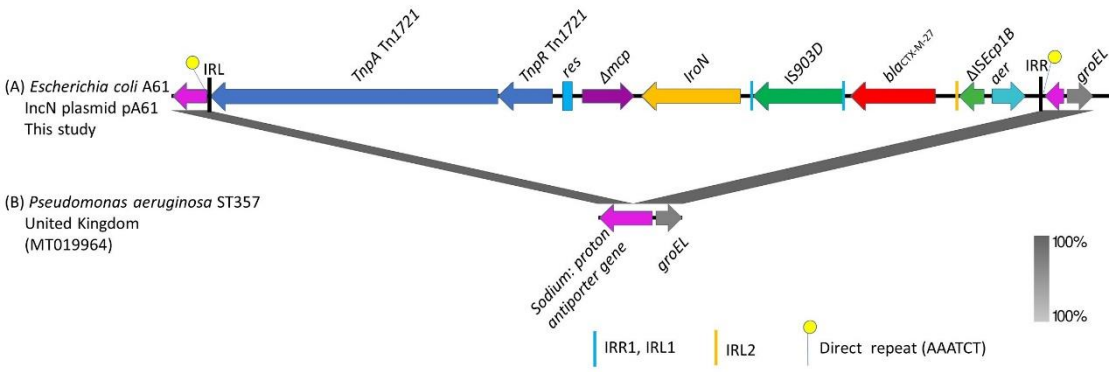

Figure S4. Schematic representation of insertion of transposition units harboring *bla*$_{\text{CTX-M-27}}$ in the Sodium proton antiporter gene from *Escherichia coli* A61.

The 8633-bp Tn1721-like structure sequence is indicated in color arrows, and the duplicated sequences generated during the transposition events are highlighted with lollipop shape (AAATCT).