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First Detection of Conjugative Plasmid-Borne Fosfomycin Resistance Gene *fosA3* in *Salmonella* Isolates of Food Origin

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Fosfomycin is a naturally occurring antibacterial agent with a broad spectrum of antimicrobial activity against both Gram-positive and Gram-negative bacteria (1).

Recently, a fosfomycin resistance gene, *fosA3*, was detected in *Escherichia coli* and *Klebsiella pneumoniae* isolates (2–6). This gene is normally plasmid mediated, surrounded by IS*26* transposase genes, and often detectable in CTX-M-producing and multidrug-resistant *E. coli* isolates (2–6). It has been suggested that the increasing prevalence of *fosA3* is due to dissemination of the IncI and IncN plasmids rather than clonal expansion of specific strains (4). This study aimed to determine whether *fosA3*-borne plasmids can disseminate among other closely related *Enterobacteriaceae* species such as *Salmonella* and to identify the underlying mechanisms regulating its dissemination potential.

Two *Salmonella* isolates, S76 and S79, obtained from meat samples purchased from supermarkets and wet markets in Hong Kong in 2013, were shown to be resistant to quinolones and third-generation cephalosporins (Table 1). Surprisingly, these two isolates were also resistant to fosfomycin. *Salmonella* strain S76 was determined to be *Salmonella enterica* serovar Derby; this strain was isolated from chicken product purchased in supermarket on February 22, 2013, whereas strain S79, which was found to be *S. enterica* serovar Enteritidis, was isolated on the same day from a different chicken product purchased from a different supermarket in Hong Kong. Multilocus sequence typing (MLST) analysis of these two isolates showed that S76 belonged to ST11, whereas S79 belonged to ST460. Previous studies had shown that ST11 was associated with invasive *S.* Enteritidis and ceftriaxone-resistant *S.* Enteritidis strains carrying CTX-M-14 and CTX-M-15 on IncI1 and IncFII plasmids, respectively (7–9). In contrast, ST460 had not been reported to be associated with CTX-M-producing *S.* Derby previously.

The gene *fosA3* was detectable in both strains by PCR screening of fosfomycin resistance genes and transferrable to *E. coli* J53 (4, 10). Conjugative plasmids in *E coli* J53 that harbored the *fosA3* gene were shown to be IncFII. S1 pulsed-field gel electrophoresis (PFGE) confirmed that plasmids of two different sizes (~80 kb and ~45 kb) were detectable in both isolates, with the conjugative plasmid being the larger one (Fig. 1A). Southern hybridization showed that the *fosA3* gene was present in the ~80-kb conjugative plasmid (Fig. 1A). Genetic-environment analysis showed that the *fosA3* gene was surrounded by two IS*26* elements with a genetic structure of IS*26*-*fosA3*-*orf1*-*orf2*-*orf3*-IS*26*. An identical structure was first reported for plasmids from clinical *E. coli* isolates in Japan, South Korea, and Hong Kong (3, 4, 6, 11).

Importantly, a PCR assay revealed the presence of a β-lactamase gene, $bla_{\text{CTX-M-55}}$, on the conjugative plasmids recovered from strains S76 and S79; the result was confirmed by Southern hybridization (Fig. 1B). Primers targeting IS*26* and $bla_{\text{CTX-M-55}}$ identified a gene cassette, IS*26*-$bla_{\text{TEM-1}}$-*orf20*-$bla_{\text{CTX-M-55}}$-IS*26*, in the conjugative plasmids pS76 and pS79 (Fig. 1C). The genetic structures of *fosA3* and $bla_{\text{CTX-M-55}}$ were very similar to that of a previously identified plasmid, pHK23a, except that the $bla_{\text{CTX-M-55}}$ and $bla_{\text{CTX-M-4}}$ elements were found in pHK23a and pS76/pS79, respectively (11). However, the length or nature of linkage between *fosA3* and $bla_{\text{CTX-M-55}}$ genetic structures in pS76 and pS79 was different from that of pHK23a, since an attempt to amplify the linkage region between the *fosA3* and $bla_{\text{CTX-M-55}}$ structures in

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TABLE 1 Characteristics of strains used in this study

Strain	Isolation date (day/mo/yr)	Source	Serotype	MLST	Replicon type	MIC (mg/liter)[a]										
						FOS	CIP	ENR	NAL	OLA	AMP	CTX	CRO	CAZ	CTO	FOX
E. coli J53						<4	<0.125	<0.125	2	1	1	<0.125	<0.125	<0.125	<0.125	<0.125
Salmonella strains																
S76	22/02/2013	Chicken	Derby	ST11	FII, FIV	>512	0.25	≤0.25	>64	16	>64	>16	>16	>64	>128	2
TC-S76					FII	>512	0.015	≤0.25	16	8	>64	>16	>16	≤0.5	32	2
S79	22/02/2013	Chicken	Enteritidis	ST460	FII, FIV	>512	1	1	>64	>256	>64	>16	>16	32	>128	2
TC-S79					FII	>512	0.06	≤0.25	>64	128	>64	>16	>16	4	32	2

[a] FOS, fosfomycin; CIP, ciprofloxacin; ENR, enrofloxiacin; NAL, nalidixic acid; OLA, olaquindox; AMP, ampicillin; CTX, cefotaxime; CRO, ceftriaxone; CAZ, ceftazidime; CTO, ceftiofur; FOX, cefoxitin.

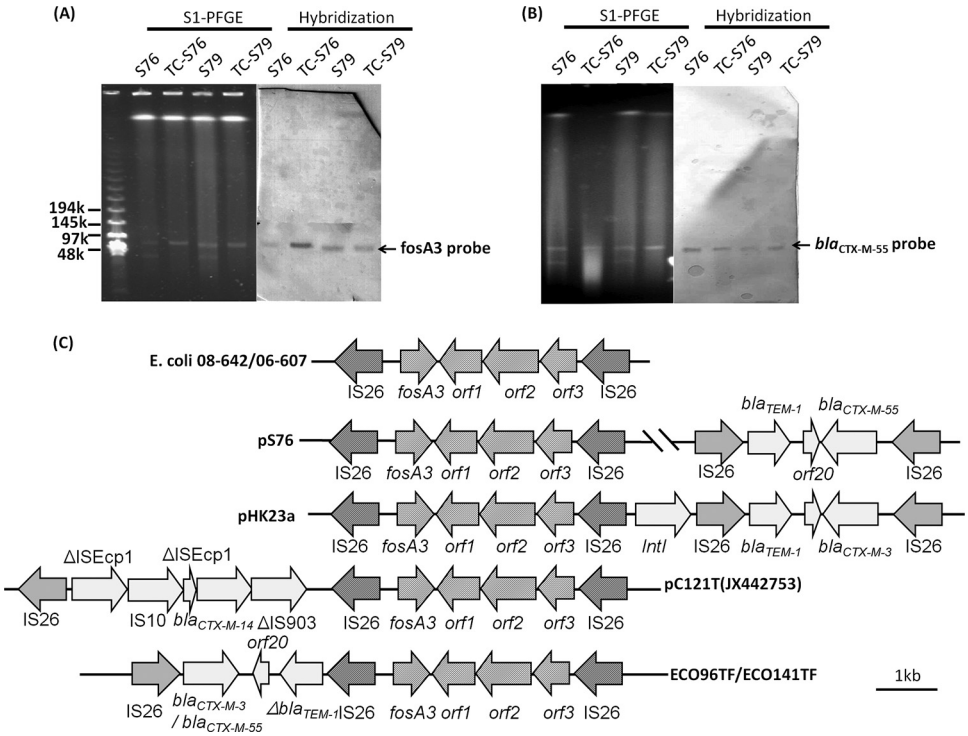

FIG 1 Analysis of *fosA3*-borne plasmids and the *fosA3*-$bla_{CTX\text{-}M\text{-}55}$ genetic environment. (A) *Salmonella* isolates S76 and S79 and the corresponding transconjugants, TC-S76 and TC-S79, were subjected to S1 PFGE and Southern hybridization analysis using the *fosA3* probe. (A) *Salmonella* isolates S76 and S79 and transconjugants TC-S76 and TC-S79 were subjected to S1 PFGE and Southern hybridization analysis using the $bla_{CTX\text{-}M\text{-}55}$ probe. (C) Representative genetic structures of *fosA3* and $bla_{CTX\text{-}Ms}$ in different conjugative plasmids. The genetic structures and the corresponding references are as follows: *E. coli* 08-642/06-607 (6), pS76 (this study), pHK23a (11), pC121T (GenBank no. JX442753) (3), and ECO96TF/ECO141TF (4).

pS76 and pS79 was not successful. Restriction fragment length polymorphism (RFLP) analysis confirmed that pS76 and pS79 were structurally different from pHK23a (data not shown).

So far, four different genetic structures representing a typical *fosA3* cassette, IS*26*-*fosA3*-*orf1*-*orf2*-*orf3*-IS*26*, and cefotaxime resistance determinants, respectively, have been identified. In pHK23a, the IS*26*-$bla_{TEM\text{-}1}$-*orf20*-$bla_{CTX\text{-}M\text{-}55}$-IS*26* cassette is located downstream of the *fosA3* cassette, whereas in the other two genetic structures, the $bla_{CTX\text{-}Ms}$ cassette was upstream of the *fosA3* cassette. In one of the genetic structures, the $bla_{CTX\text{-}M}$ cassette is truncated. These data suggest that a typical *fosA3* cassette could be randomly inserted into the adjacent area of the $bla_{CTX\text{-}Ms}$ cassette, forming different genetic structures. In pS76 and pS79, the two cassettes were separated by a distance (Fig. 1C), suggesting that an IS*26*-flanked antimicrobial resistance cassette can be inserted into the hot spots in plasmids forming multiple resistance gene clusters in the plasmid. In addition to the typical *fosA3* cassette IS*26*-*fosA3*-*orf1*-*orf2*-*orf3*-IS*26*, several C-terminally truncated forms, such as IS*26*-*fosA3*-*orf1*-*orf2*-IS*26*, IS*26*-*fosA3*-*orf1*-Δ*orf2*-IS*26*, and IS*26*-*fosA3*-Δ*orf1*-IS*26*, have been detected in plasmids recovered from *E. coli* isolates, suggesting that IS*26* transposition activities mediated the formation of the *fosA3* cassette (2, 10).

In conclusion, this study has for the first time identified and characterized a conjugative FII plasmid carrying both IS*26*-mediated $bla_{CTX\text{-}M\text{-}55}$ and *fosA3* gene cassettes in two *Salmonella* isolates. The fact that this genetic arrangement is similar to that observable in *E. coli* suggests the possibility that this plasmid can circulate among members of *Enterobacteriaceae*. The association of the *fosA3* gene with $bla_{CTX\text{-}Ms}$ could eliminate the possibility of using fosfomycin as an alternative treatment approach, which may be effective for treatment of multidrug-resistant *Salmonella* infection. Further monitoring of transmission of the *fosA3* gene is necessary.

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