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Review Article

Update on fosfomycin-modified genes in *Enterobacteriaceae*

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Abstract The long-used antibiotic fosfomycin has recently been re-evaluated as a potential regimen for treating extended-spectrum β-lactamases (ESBLs) and carbapenem-resistant *Enterobacteriaceae* (CRE). Fosfomycin is known for its robust bactericidal effect against ESBL-producing *Enterobacteriaceae* and CRE. However, fosfomycin-modified genes have been reported in transposon elements and conjugative plasmids, resulting in fosfomycin resistance in parts of East Asia. Here we review reports of fosfomycin-modified (*fos*) genes in *Enterobacteriaceae* and assess the efficacy of fosfomycin against multidrug-resistant *Enterobacteriaceae* infections. At least 10 kinds of *fos* genes have been identified in the past decade; of these, *fosA* (and *fosA* subtypes) and *fosC2* are primarily found in *Enterobacteriaceae*. All *fosA* subtypes except *fosA2* are found in plasmids and transposons, nearby insertion sequence elements, or integrons, indicating that mobilizing elements also play an important role in plasmid-mediated *fos* genes in *Enterobacteriaceae*. *fosA3*, which is prevalent in East Asia, has been transmitted (mostly by animals) within and across continents via IS*26* mobile elements. The acquisition of multiple antibiotic resistance genes via plasmids and mobile elements has resulted in a need for combined treatments for *Enterobacteriaceae* cases. The combination of fosfomycin and carbapenem has been the focus of many *in vitro* studies, but there is a clear need for additional *in vivo* investigations involving pharmacokinetics.

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Introduction

Extended-spectrum β-lactamases (ESBLs) and carbapenem-resistant *Enterobacteriaceae* (CRE), including *E. coli* and *Klebsiella pneumoniae*, represent a serious treatment problem with high in-hospital mortality rates.[1,2] Treatments generally emphasize tigecycline or colistin,[3] although the long-established antibiotic fosfomycin also has treatment potential against these bacterial strains.[4] Fosfomycin is used orally for simple urinary tract infections caused by ESBL-producing *Enterobacteriaceae*, or as a parenteral CRE formulation. Due to the increasing prevalence of ESBL-producing *Enterobacteriaceae* and CRE, physicians and epidemiologists need to test and measure the effectiveness of fosfomycin to treat systemic infections in different world regions.

Fosfomycin is a bactericidal antibiotic that targets UDP-N-acetylglucosamine enolpyruvyl transferase (MurA), an enzyme responsible for peptidoglycan synthesis.[5] Although researchers have reported high fosfomycin susceptibility in ESBL-producing *Enterobacteriaceae* and CRE,[6–8] increasing resistance rates have been observed over the past decade.[9,10] Fosfomycin resistance has been linked to three mechanisms: amino acid substitutions for antibiotic targets (MurA), the inactivation of GlpT and UhpT transporters and their *uhpA*, *cyaA* and *ptsI* regulatory genes, and fosfomycin-modified enzymes.[5] Fosfomycin-modified genes have been reported in transposon elements and conjugative plasmids, resulting in the rapid dissemination of these genes throughout East Asia.[11–13] After consolidating the most recent data for fosfomycin-modified genes in *Enterobacteriaceae*, we evaluated the potential of fosfomycin for treating multidrug-resistant *Enterobacteriaceae* infections.

Fosfomycin-modified enzymes (*fos* genes) in *Enterobacteriaceae*

At least 10 kinds of *fos* genes have been described.[5,11,14–19] The first, *fosA*, which encodes Mn^{2+}-dependent glutathione-S-transferase (GST), was reported in *Serratia marcescens* in 1980. The subtypes *fosA2* through *fosA6* have been identified over the past decade.[11,15–18] *fosB*, which encodes thiol-S-transferase response resistance to fosfomycin, is commonly found in the plasmids and chromosomes of fosfomycin-resistant gram-positive bacteria such as *Staphylococcus epidermidis* and *Bacillus subtilis*.[5] First reported in *Pseudomonas syringae* in 1995, *fosC* inactivates fosfomycin via phosphorylation.[20] One research team has described differences between *fosC2* and *fosC* in *P. syringae* and similarities between *fosC2* and *fosG* in *Achromobacter denitrificans* (Fig. 1).[11] *fosA* and its subtypes, and *fosC2* were mainly responsible for fosfomycin resistance in *Enterobacteriaceae*. Since other *fos* genes have not been identified in *Enterobacteriaceae*, we will not discuss them.[19]

fosA

fosA was first discovered in 1980 on Tn*2921* in plasmids; its sequence and characteristics were determined in 1990.[12,14] 63% (53/84) multidrug-resistant *S. marcescens* isolates were found to be susceptible to fosfomycin, and 9 fosfomycin-resistant *S. marcescens* isolates were further analyzed and identified 2 types of fosfomycin-resistant conjugative plasmids (Table 1).[12,14] Epidemiological survey data from Italy indicated fosfomycin susceptibility in >97% of over 7400 urinary pathogen isolates.[21] Among 30 gram-negative and 30 gram-positive fosfomycin-resistant bacterial isolates selected for the Italian study, plasmid-mediated *fosA* and *fosB* genes were found in three *Enterobacteriaceae* and two staphylococci isolates, respectively. One research team has described the coexistence of *fosA* and bla_{KPC} in an epidemic *K. pneumoniae* sequence Type 11 (ST11) strain located in China.[10] Another Chinese study that focused on pets and their owners found that 88.9% (152/171) of *Enterobacteriaceae* isolates were susceptible to fosfomycin.[22] In that study, 3 of 19 fosfomycin-resistant isolates tested positive for the *fosA* gene.

fosA2

Xu et al. identified *fosA2*-producing *Enterobacter cloacae* in aquatic samples taken from the Salmon River in western Canada.[15] Data from antibiotic susceptibility tests indicate isolate resistance to ampicillin, erythromycin, fosfomycin, and rifampicin. Pulsed-field gel electrophoresis of total DNA digested via I-CeuI endonuclease or S1 nuclease with *fosA2*-probe southern hybridizations indicated a chromosomal location.

fosA3

In the past decade, *fosA3* (in plasmids) has been the most frequently found *fosA* subtype (Table 1). First report (2010, from Japan) involved CTX-M-producing *E. coli* and efficaciously modified fosfomycin via GST activity.[11] In separate studies, Hou et al. reported 89.9% (290/323) and 98.7% (880/892) susceptibility to fosfomycin *E. coli* isolates in pet and food-animal samples collected in Guangdong, China.[23,24] Their PCR screening data indicated that 29 (9%) and 10 isolates (1.1%) respectively contained *fosA3* and different types of bla_{CTX-M} in plasmids. According to these data, both pets and animals for human consumption may be involved in plasmid-borne resistance. In studies involving fowl, Sun et al. reported that 92.4% (207/224) of the *E. coli* isolates collected from the livers of diseased chickens and ducks in multiple locations in China were susceptible to fosfomycin. The 17 fosfomycin-resistant isolates that were identified contained a novel multidrug-resistant plasmid (pXZ) harboring four resistance genes: *rmtB*, *fosA3*, bla_{TEM-1} and $bla_{CTX-M-24}$.[25]

In a Korean study, 92.9% (157/165) of ESBL-producing *E. coli* and 95.2% (169/182) ESBL-producing *K. pneumoniae* isolates were susceptible to fosfomycin.[26] Although only 7 isolates harbored the *fosA3* gene, all 7 were found to contain bla_{CTX-M} genes in the same IS*26*-composite transposons. In Hong Kong, Ho et al. gathered statistics on the prevalence of fosfomycin resistance from animals and human isolates, and found that 93.6% (1584/1693) of the *E. coli* isolates from fecal specimens from different animals were susceptible to fosfomycin.[27,28] Among 101

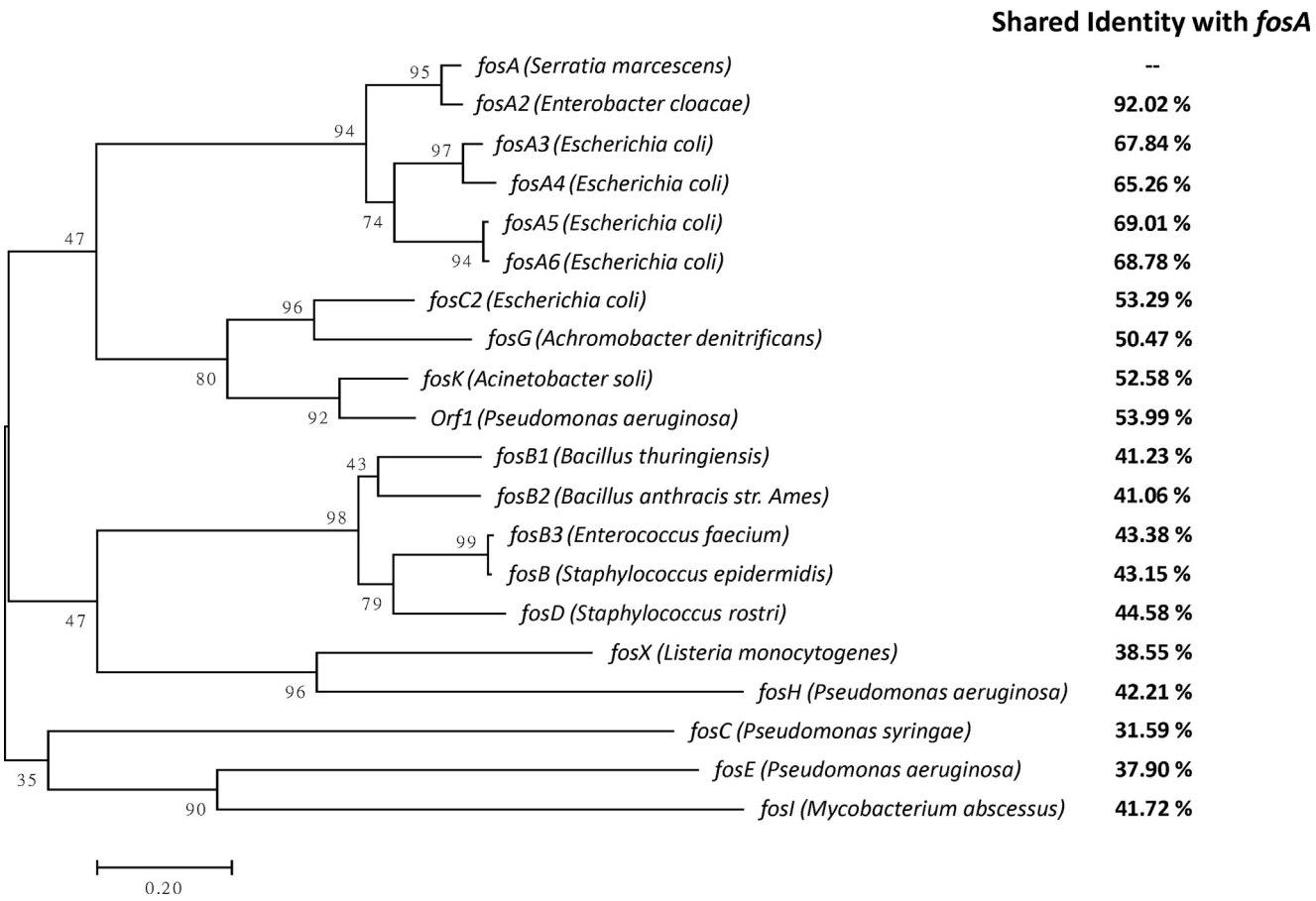

Figure 1. *fos* gene phylogenetic tree from all reports to date. Neighbor-joining MEGA7 software was used for tree generation. Numbers next to nodes indicate confidence levels, expressed as percentages of occurrence over 2000 bootstrap samples. Scale bar indicates evolutionary distance. Represented sequences are *fosA* (GenBank accession no. M85195), *fosA2* (EU487198), *fosA3* (KT199757), *fosA4* (AB908992), *fosA5* (KP143090), *fosA6* (KU254579), *fosC2* (AB522969), *fosG* (NG_047895.1), *fosK* (NG_047898.1), *Orf1* (AY294333.1), *fosB1* (NC_014171.1), *fosB2* (NC_003997.3), *fosB3* (HQ219726.1), *fosB* (X54227.1), *fosD* (NG_047892.1), *fosX* (NG_047899.1), *fosH* (NG_047896.1), *fosC* (Z33413), *fosE* (NG_051850.1), and *fosI* (CP003505.2).

fosfomycin-resistant *E. coli* isolates, 97 (96.0%) and 94 (93.1%) contained *fosA3* and bla_{CTX-M} genes, respectively. In the second of these two Hong Kong studies, 99.0% (1860/1878) of clinical *E. coli* isolates collected from multiple locations were fosfomycin-susceptible,[28] with 6 and 2 isolates containing *fosA3* and *fosA5*, respectively. PCR data indicate the co-presence of *fosA3* and bla_{CTX-M} in plasmids of different sizes.

In a study focused on chickens from various locations in China, Yang et al. found that 91.2% (603/661) of the *E. coli* isolates they tested were fosfomycin-susceptible.[29] The 58 fosfomycin-resistant *E. coli* isolates that harbored *fosA3* were clonally unrelated, indicating the spread of *fosA3* due to horizontal dissemination. In a Japanese study involving stool specimens from healthy humans, 93.5% (129/138) of the tested CTX-M-producing *E. coli* isolates were fosfomycin-susceptible. In 8 fosfomycin-resistant *E. coli* isolates, 5 contained both *fosA3* and bla_{CTX-M} in different types of plasmids.[30] Similar to previous studies, the data for 5 ESBL-producing *E. coli* isolates revealed *fosA3* insertions in different CTX-M-positive plasmids via IS*26* mobile elements.

In a study involving 12 Chinese hospitals, Jiang et al. reported fosfomycin-susceptibility in 109/278 (39.2%) KPC-producing *K. pneumoniae* isolates and 70/80 (87.5%) ESBL-producing *K. pneumoniae* isolates. PCR data indicate that 94/278 (33.8%) KPC-producers and 7/80 (8.8%) ESBL-producers harbored the *fosA3* gene.[9] Unlike previous reports indicating that the *fosA3* gene can be transferred via IS*26* transposons or plasmids in genetically diverse *Enterobacteriaceae*,[26,30] this study found that the *fosA3*-positive KPC producers were clonally related, indicating fosfomycin-resistant clonal dissemination in China. In another Chinese study, Xiang et al. found that 41.2% (40/97) of the KPC-producing *K. pneumoniae* isolates they tested were susceptible to fosfomycin, and that 44 *fosA3*-positive isolates belonged to ST11 with the same pulsotype.[10] In a separate study, Li et al. reported 1023/1109 (92.2%) fosfomycin-susceptible *E. coli* isolates in 20 widely dispersed Chinese tertiary hospitals.[31] Among the 86 non-fosfomycin susceptible *E. coli* isolates they analyzed, 69 contained *fosA3*; 29/69 (42%) of these isolates were determined to be capable of transmitting *fosA3* among different *E. coli* strains. In Taiwan, Tseng et al. reported that 115/123 (93.5%) human

Table 1 Characterization of fosfomycin-resistant genes (*fos*) in *Enterobacteriaceae*.

Fosfomycinase	Year	Country	Organism with Emerging Resistance (n)	Source (n)	Susceptibility to Fosfomycin % (n/total)	Number of *fos*-positive isolates with other emerging resistance	GenBank Accession No.	Ref
fosA	1980	Spain	*Serratia marcescens* (84)	Patient (84)	63% (53/84)	Only 9 isolates were further analyzed	—	14
	1990						M85195.1 (Tn*2921*)	12
	1997	Italy	Of over 7400 urinary pathogens, 30 *Enterobacteriaceae* were studied	Patiant (>7400)	>97% (>7181/7400)	3	—	21
	2015	China	KPC-producing *K. pneumoniae* (97)	Patient (97)	41.2% (40/97)	1 (KPC)	—	10
	2016	China	*Enterobacteriaceae* (171)	Pet owner (21) Pet (150)	88.9 (152/171)	3	—	22
fosA2	2011	Canada	MDR *E. cloacae* (1)	Aquatic environment (1)	N/A[a]	1 (MDR)	EU487198	15
fosA3	2010	Japan	ESBL-producing *E. coli* (192)	Patient (192)	96.4% (185/192)	2 (ESBL)	AB522970	11
	2012	China	*E. coli* (323)	Healthy pet (136) Diseased pet (187)	89.9% (290/323)	29 (ESBL)	JF411006—JF411008	23
	2012	China	*E. coli* (224)	Fowl (224)	92.4% (207/224)	17 (MDR)	JF927996.1	25
	2012	Korea	ESBL-producing *E. coli* (165) ESBL-producing *K. pneumoniae* (182)	Patient (347)	92.9% 157/165 95.2% 169/182	5 (ESBL) 2 (ESBL)	JQ343849—JQ343851	26
	2013	China	*E. coli* (1693)	Domestic animal (1693)	93.6% (1584/1693)	97 (94 ESBL)	JQ823170.2 JX442751—JX442753	27
	2013	China	*E. coli* (892)	Domestic animal (892)	98.7% (880/892)	10 (10 ESBL)	JX017363	24
	2013	China	*E. coli* (1878)	Patient (1878)	99% (1860/1878)	6 (6 ESBL)	JX627737	28
	2013	Japan	ESBL-producing *E. coli* (138)	Health individual (138)	93.5% (129/138)	5 (5 ESBL)	AB778291 AB778503	30
	2014	China	*E. coli* (661)	Fowl (661)	91.2% (603/661)	58 (57 ESBL)	KJ668701—2	29
	2015	China	KPC-producing *K. pneumoniae* (278) ESBL-producing *K. pneumoniae* (80)	Patient (358)	39.2% (109/278) 87.5% (70/80)	101 (94 KPC and 7 ESBL)	KJ653815	9
	2015	China	*E. coli* (1109)	Patient (1109)	92.2% (1023/1109)	69	—	31
	2015	Taiwan	ESBL-producing *E. coli* (145)	Patient (123) Livestock (22)	93.5% for human (115/123) 77.3% for pig (17/22)	4 (4 ESBL)	KT199757	32
	2015	China	KPC-producing *K. pneumoniae* (97)	Patient (97)	41.2% (40/97)	44 (44 KPC)	KP893385	10
	2016	China	Among 80 collected *E. coli* isolates, 55 resistant to cefotaxime were studied further.	Retail meat (80)	78.2% (43/55)	12 (12 ESBL)	—	34

	2016	China	*Enterobacteriaceae* (171)	Pet owner (21) Pet (150)	88.9 (152/171)	16 (16 MDR)	–	22
	2017	Taiwan	carbapenem-resistant *K. pneumoniae* (642)	Patient (642)	63.6% (408/642)	35 (4 IMP8 and 25 KPC)	–	33
fosA4	2014	Japan	fosfomycin-nonsusceptible *E. coli* (25)	Patient & health individual (25)	N/A[a]	1	AB908992	16
fosA5	2013	China	*E. coli* (1878)	Patient (1878)	99% (1860/1878)	2[b] (1 ESBL)	KC960485	28
	2015	China	ESBL-producing *E. coli* (1)	Patient (1)	N/A[a]	1 (ESBL)	KP143090	17
	2017	Taiwan	carbapenem-resistant *K. pneumoniae* (642)	Patient (642)	63.6% (408/642)	27 (5 KPC)	–	33
fosA6	2016	USA	ESBL-producing *E. coli* (1)	Patient (1)	N/A[a]	1 (ESBL)	KU254578–81	18
fosC2	2010	Japan	ESBL-producing *E. coli* (192)	Patient (192)	96.4% (185/192)	1 (ESBL)	AB522969	11
	2015	China	MDR-*E. cloacae* (1)	Patient (1)	N/A[a]	1 (bla_{IMP-34}, *fosC2*, and *aacC2*)	KM877517	13

[a] N/A: Not applicable.
[b] Original designated as *fosKP96*.

and 17/22 (77.3%) pig ESBL-producing *E. coli* isolates they tested were susceptible to fosfomycin.[32] Further, they observed *fosA3* inserted in both IncN- and IncB/O-type plasmids via IS*26* mobile elements in 4 CTX-M-producing *E. coli* isolates.

According to data from a national study in Taiwan, almost two-thirds (63.6%, 408/642) of tested carbapenem-resistant *K. pneumoniae* (CRKP) isolates were susceptible to fosfomycin, with 71.4% (25/35) of *fosA3*-positive isolates containing both *fosA3* and bla_{KPC-2}.[33] In two other Chinese studies, livestock and pets were found to be potential reservoirs for *fosA3*-carrying *Enterobacteriaceae* isolates.[22,34] Yao et al. found 16 *fosA3*-positive isolates among 171 *Enterobacteriaceae* isolates collected from 150 pets and 21 pet owners. Of these, PCR data indicate that 15 and 7 isolates harbored bla_{CTX-M} and *rmtB*, respectively.[22] According to Xie et al., meat products sold in several retail wet markets in Shenzhen, China were contaminated with *E. coli* containing *fosA3* and bla_{CTX-M}, with the potential for dissemination to other human pathogens.[34] Combined, these studies indicate the prevalence of *fosA3* throughout East Asia, with the potential for intra- and intercontinental transmission via animals and other sources.

fosA4

Nakamura et al. found *fosA4* (sharing 94% amino acid identity with *fosA3*) in *E. coli* isolates in Japan.[16] They used a simple two-disk diffusion method based on a combination of fosfomycin and the $FosA^{PA}$ inhibitor sodium phosphonoformate to detect *fos*-carrying *E. coli* isolates.

fosA5

Ma et al. found plasmid-mediated *fosA5* in an ESBL-producing *E. coli* isolate in China.[17] FosA5 shares 69% and 80% amino acid sequences with FosA and FosA3, respectively. According to BLASTN search results, a putative gene was reported in a pKP96 plasmid in 2008; it was designated *fosKP96* in 2013.[28] In a Taiwanese study, Tseng et al. reported that 63.6% (408/642) of the CRKP isolates they examined were susceptible to fosfomycin.[33] Only 5 of 27 *fosKP96*-positve CRKP isolates also contained KPC-2.

fosA6

Guo et al. describe a new plasmid-mediated *fosA6* that was determined in a CTX-M-2-producing *E. coli* isolate in urine samples from a US hospital.[18] According to a BLASTN search, this gene is widely distributed in *K. pneumoniae* chromosomes. Data from analyses of adjacent sequences indicate that the gene can be mobilized from *K. pneumoniae* chromosomes to *E. coli* plasmids via IS*10* mobile elements.

fosC2

Located on plasmids, FosC2 shares 72% and 56% amino acid sequences with FosG and FosA, respectively. Although different sequences exist between FosA and FosC2, both modify fosfomycin via GST activity.[11] Two research teams

have described *fosC2* in class 1 integrons with different antibiotic resistance genes.[11,13] Wanchino et al. describe a class 1 integron carrying *fosC2*, *dfrA17* and *aadA5* in a conjugative plasmid, and Wang et al. describe *fosC2* and bla_{IMP-34} in multidrug-resistant plasmids. These data indicate that *fosC2* can be acquired by integrons in response to antibiotic challenges.

In summary, various *fos* genes contribute to fosfomycin resistance in *Enterobacteriaceae*, with *fosA* subtypes and *fosC2* sharing nucleotide sequence identities of between 53% and 92% with *fosA* (Fig. 1). In one study, the fosfomycin MIC for *fosA2* (>1024 μg/ml) exceeded that of *fosA* (192 μg/ml) in *E. coli* JM109, with respective DNA fragments cloned into pGEM-T.[15] Others have reported that *fosA3*, the dominant *fosA* subtype, confers a wide range of MIC values (from 256 to >1024 μg/ml). The same researchers also reported a wide range of MIC values in transconjugants carrying *fosA3*-harboring plasmids (256 to >1024 μg/ml).[10,11,35–38] One *fosA5*-carrying clinical *E. coli* isolate and its transconjugant were found to have a significantly high fosfomycin MIC value of 512 μg/ml.[17] A *fosA6*-carrying clinical *E. coli* isolate, its transformant, and its transconjugant had fosfomycin MIC values of 128 to >1024 μg/ml.[18] To our knowledge, MIC data for *fosA4* have not yet been reported.

Surrounding environments and possible *fos* gene transmission pathways in *Enterobacteriaceae*

All *fos* genes except *fosA2* are located in plasmids (Table 1). They have also been observed in transposons, insertion sequences (ISs), and integrons,[11–13,17,18] indicating that surrounding mobilization elements play important roles in plasmid-mediated *fos* genes in *Enterobacteriaceae*. The first-ever described *fos* gene, *fosA*, was located on Tn*2921* in plasmids, and has also been linked to conjugative plasmids in *S. marcescens* isolates (Table 2).[12,14] Unlike other *fos*-related genes, *fosA2* has been found in the chromosomes of *E. cloacae* isolates and flanked by Tn*2961* sequences, indicating dissemination potential within *Enterobacter* spp.[15] Further, IS*26*-composite transposons have been identified as dominant mobile elements carrying *fosA3* in *Enterobacteriaceae* (Table 2).

The first report of a *fosA3* region flanked by two IS*26* elements came from a Japanese laboratory.[11] According to sequence analysis data, plasmid-mediated *fosA3* shared 80% amino acid sequence identity with a related gene in the chromosome of *K. pneumoniae* strain 342, suggesting that chromosomal FosA may be an ancestor of plasmid-mediated FosA3. Since this initial report, numerous descriptions of IS*26* transposon-like structures carrying *fosA3* associated with antibiotic-resistant gene-carrying plasmids have been published.[10,23,25,26,36,37,39] Hou et al.[23] noted the co-location of three types of IS*26*-composite transposons carrying *fosA3* with conjugative plasmids carrying bla_{CTX-M}, and *rmtB* in China. Lee et al. have described the simultaneous presence of 7 isolates (5 *E. coli* and 2 *K. pneumoniae*) carrying IS*26*-composite transposons with *fosA3* and either $bla_{CTX-M-1\ group}$ ($bla_{CTX-M-3}$ or $bla_{CTX-M-55}$) or $bla_{CTX-M-9\ group}$ ($bla_{CTX-M-14}$) in plasmids.[26] Ho et al. observed 7 types of IS*26*-composite transposons carrying *fosA3* co-located in conjugative plasmids with $bla_{CTX-M-1\ group}$ ($bla_{CTX-M-3}$ or $bla_{CTX-M-55}$), $bla_{CTX-M-9\ group}$ ($bla_{CTX-M-14}$ or $bla_{CTX-M-65}$), or both in 97 *E. coli* isolates collected in Hong Kong.[27] In a later Chinese study, Hou et al. found evidence of $bla_{CTX-M-9\ group}$ (n = 10) (one $bla_{CTX-M-14}$ and nine $bla_{CTX-M-65}$) or *rmtB* and $bla_{CTX-M-55}$ (n = 1) co-localized with *fosA3* genes in the same plasmids.[24]

Ho et al. used PCR mapping and RFLP analyses to identify 3 genetic environments containing *fosA3* and IS*26*.[28] In the same study, they found 2 types of IS*26*-composite transposons carrying *fosA3* and bla_{CTX-M} in the plasmids of 6 *E. coli* isolates. A Japanese research team reported the co-location of 5 *fosA3*-positive conjugative plasmids with different forms of bla_{CTX-M} (three with $bla_{CTX-M-14}$, one $bla_{CTX-M-3}$, and one $bla_{CTX-M-55}$). They also observed that *fosA3* was flanked by two different genetic IS*26* elements.[30] Yang et al. found 58 fosfomycin-resistant *E. coli* isolates carrying *fosA3* from chickens raised in different locations in China.[29] In addition to identifying 5 genetic IS*26* elements carrying *fosA3*, they also reported the successful transfer of *fosA3* from 50 isolates via conjugation, and the co-transference of bla_{CTX-M}, *rmtB* and *floR* with *fosA3*. Yao et al. describe 16 instances of *fosA3*-positive *Enterobacteriaceae* isolates collected from pets and their owners in China, including two types of IS*26*-composite transposons carrying *fosA3* in conjugative plasmids.[22] They found that two *fosA3*-positive isolates collected from dogs were genetically identical to isolates collected from pet owners, indicating a need to better understand and, if possible, control pet-human bacterial transmission. In Taiwan, Tseng et al. found IS*26*-composite transposons carrying 2 and 5 surrounding types of *fosA3* in ESBL-producing *E. coli* isolates and CRKP isolates, respectively.[32,33] Although they found evidence of conjugative plasmids carrying *fosA3* and $bla_{CTX-M-9\ group}$ in ESBL-producing *E. coli* isolates, they did not find similar evidence when investigating CRKP isolates. According to these results, *fosA3* transmission in CRKP isolates mostly occurs via mobile elements.

Genome sequencing is a powerful tool for analyzing complete plasmids. Sun et al. used it to identify a composite IS*26* transposon-like structure carrying *fosA3*, $bla_{CTX-M-24}$, *rmtB* and bla_{TEM-1} in a 76-kb conjugative plasmid labeled pXZ.[25] He et al. also found four similar antibiotic-resistant genes in a 76-kb plasmid labeled pHN7A8.[39] Ho et al. provide details for a 73-kb conjugative plasmid (pHK23a) carrying *fosA3*, Δbla_{TEM-1} and $bla_{CTX-M-3}$ in an *E. coli* isolate collected from pigs.[36] The *fosA3* was carried by an IS*26* mobile element, and the Δbla_{TEM-1} and $bla_{CTX-M-3}$ were both flanked by two other IS*26* elements, thus forming a composite IS*26* transposon-like structure.

Unlike previous reports of *fosA3* and ESBLs located on the same plasmids, Jiang et al. describe a pFOS18 plasmid containing *fosA3* and bla_{KPC-2} on IS*26* mobile elements and in the integrative structures of Tn*3*-Tn*4401*.[9] Two subsequent studies describe the co-location of *fosA3* and bla_{KPC-2} on the same transposons.[10,37] Li et al. observed 2 bacterial isolates (*E. coli* and *Enterobacter aerogenes*) carrying conjugative plasmids with both *fosA3* and bla_{KPC-2}.[37] Both bacterial isolates had composite Tn*1721*-Tn*3* transposons on associated plasmids, as well as IS*26* mobile elements carrying *fosA3* inserted in the Tn*1721-tnpA* gene, resulting

Table 2 Surrounding genetic element of fosfomycin-modified enzymes and possible transmission pathways in *Enterobacteriaceae*.

Fosfomycinase	Year and Location	Organism	No. of *fos*-pos isolates	Surrounding Element (number)	Gene Location	Successful Transfer Method (n)	Co-transmission Resistant Genes[a]	Ref
fosA	1980 Spain	*Serratia marcescens*	9	–	Plasmid; Tn*2921*	Conjugation (n = 3)	NR[b]	14
	1990 Spain			Tn*2921*- *fosA* (n = 1)				12
fosA2	2011 Canada	*Enterobacter cloacae*	1	Tn*2961*-*fosA2* (n = 1)	Chromosome (flanked by Tn*2961*)	NR[b]	NR[b]	15
fosA3	2010 Japan	*E. coli*	2	IS*26*-*fosA3*-*orf1*-*orf2*-Δ*orf3*-IS*26* (n = 2)	Plasmid	Conjugation (n = 2)	bla_{CTX-M}	11
	2012 China	*E. coli*	29	IS*26*-*fosA3*-*orf1*-*orf2*-Δ*orf3*-IS*26* (n = 8) IS*26*-*fosA3*-*orf1*-Δ*orf2*-IS*26* (n = 18) IS*26*-*fosA3*-Δ*orf1*-IS*26* (n = 3)	Plasmid	Conjugation (n = 23) Transformation (Method not reported) (n = 4)	bla_{CTX-M} and *rmtB*	23
	2012 China	*E. coli*	17	IS*26*-$bla_{CTX-M-24}$-ΔIS*903*-*iroN*-IS*26*-*fosA3*-*orf1*-Δ*orf2*-IS*26*-IS*1294*-bla_{TEM-1}-*rmtB*-IS*26* (n = 1)	Plasmid	Conjugation (n = 1)	$bla_{CTX-M-24}$, *rmtB* and bla_{TEM-1}	25
	2012 Korea	*E. coli*	5	IS*26*-ΔIS*Ecp1*-$bla_{CTX-M-14}$-ΔIS*903*-*fosA3*-*orf1*-Δ*orf2*-IS*26* (n = 1)	Plasmid	Conjugation and Transformation (Heat shock method) (n = 6)	$bla_{CTX-M-1\ group}$ ($bla_{CTX-M-3}$ or $bla_{CTX-M-55}$) or $bla_{CTX-M-9\ group}$ ($bla_{CTX-M-14}$) or Δbla_{TEM}	26
		K. pneumoniae	2	IS*26*-ΔIS*Ecp1*-$bla_{CTX-M-3}$-*orf477*-Δbla_{TEM}-IS*26*-*fosA3*-*orf1*-*orf2*-Δ*orf3*-IS*26* (n = 1) IS*26*-ΔIS*Ecp1*-$bla_{CTX-M-55}$-*orf477*-Δbla_{TEM}-IS*26*-*fosA3*-*orf1*-*orf2*-Δ*orf3*-IS*26* (n = 1)				
	2013 China	*E. coli*	97	IS*26*-*fosA3*-*orf1*-*orf2*-Δ*orf3*-IS*26* (n = 71) IS*26*-*fosA3*-*orf1*-Δ*orf2*-IS*26* (n = 9) IS*26*-*fosA3*-Δ*orf1*-IS*26* (n = 1) IS*26*-ΔIS*Ecp1*-$bla_{CTX-M-14}$-ΔIS*903*-*fosA3*-*orf1*-Δ*orf2*-IS*26* (n = 10) IS*26*-ΔIS*Ecp1*-IS*10*-ΔIS*Ecp1*-$bla_{CTX-M-14}$-ΔIS*903*-*fosA3*-*orf1*-*orf2*-Δ*orf3*-IS*26* (n = 4) *fosA3*-*orf1*-Δ*orf2*-IS*26*[c] (n = 1) *fosA3*-*orf1*-*orf2*-Δ*orf3*-IS*26*[c] (n = 1)	Plasmid	Conjugation (n = 11)	$bla_{CTX-M-1\ group}$ ($bla_{CTX-M-3}$ or $bla_{CTX-M-55}$) or $bla_{CTX-M-9\ group}$ ($bla_{CTX-M-14}$ or $bla_{CTX-M-65}$)	27
	2013 China	*E. coli*	1	IS*26*-$bla_{CTX-M-65}$-ΔIS*903*-*iroN*-IS*26*-*fosA3*-*orf1*-Δ*orf2*-IS*26*-IS*1294*-bla_{TEM-1}-*rmtB*-IS*26* (n = 1)	Plasmid	Transformation (Method not reported) (n = 1)	$bla_{CTX-M-65}$, *rmtB*	39
	2013 China	*E. coli*	1	IS*26*-*fosA3*-*orf1*-*orf2*-Δ*orf3*-IS*26*-Δ*intI1*-ΔIS*26*-Δbla_{TEM-1}-*orf20*-$bla_{CTX-M-3}$-ΔIS*Ecp1*-IS*26* (n = 1)	Plasmid	Conjugation (n = 1)	Δbla_{TEM-1}, $bla_{CTX-M-3}$	36
	2013 China	*E. coli*	10	*fosA3*-*orf1*-*orf2*-Δ*orf3* (441 bp)-IS*26*[c] (n = 6) (1758 bp) *fosA3*-*orf1*-*orf2*-Δ*orf3* (326 bp)-IS*26*[c] (n = 1) (1643 bp) *fosA3*-*orf1*-Δ*orf2*-IS*26*[c] (n = 2) Unknown (n = 1)	Plasmid	Conjugation and Transformation (Method not reported) (n = 8)	$bla_{CTX-M-9\ group}$ ($bla_{CTX-M-14}$ or $bla_{CTX-M-65}$) or *rmtB*	24

(*continued on next page*)

Table 2 (*continued*)

Fosfomycinase	Year and Location	Organism	No. of *fos*-pos isolates	Surrounding Element (number)	Gene Location	Successful Transfer Method (n)	Co-transmission Resistant Genes[a]	Ref
	2013 China	*E. coli*	6	IS*26*-*fosA3*-*orf1*-*orf2*-Δ*orf3*-IS*26* (n = 5) IS*26*-*fosA3*-*orf1*-Δ*orf2*-IS*26* (n = 1)	Plasmid	Conjugation (n = 6)	$bla_{CTX\text{-}M\text{-}3}$, or $bla_{CTX\text{-}M\text{-}9\ group}$ ($bla_{CTX\text{-}M\text{-}14}$ or $bla_{CTX\text{-}M\text{-}65}$)	28
	2013 Japan	*E. coli*	5	IS*26*-IS*Ecp1*-$bla_{CTX\text{-}M\text{-}14}$-ΔIS*903*-*fosA3*-*orf1*-Δ*orf2*-IS*26* (n = 2) IS*26*-ΔIS*Ecp1*-$bla_{CTX\text{-}M\text{-}55}$-*orf477*-Δ$bla_{TEM\text{-}1}$-IS*26*-*fosA3*-*orf1*-*orf2*-Δ*orf3*-IS*26* (n = 1) Unknown (n = 2)	Plasmid	Conjugation (n = 5)	$bla_{CTX\text{-}M\text{-}14}$ or $bla_{CTX\text{-}M\text{-}55}$ Δ$bla_{TEM\text{-}1}$	30
	2014 China	*E. coli*	58	IS*26*-316bp-*fosA3*-*orf1*-*orf2*-Δ*orf3*-IS*26* (n = 37) IS*26*-252bp-*fosA3*-*orf1*-*orf2*-Δ*orf3*-IS*26* (n = 1) IS*26*-*fosA3*-*orf1*-Δ*orf2*-IS*26* (n = 7) $bla_{CTX\text{-}M\text{-}14}$-ΔIS*903*-*fosA3*-*orf1*-*orf2*-Δ*orf3*-IS*26* (n = 3) IS*26*-IS*Ecp1*-$bla_{CTX\text{-}M\text{-}14}$-ΔIS*903*-*fosA3*-*orf1*-Δ*orf2*-IS*26* (n = 5) Unknown (n = 2)	Plasmid	Conjugation (n = 50)	$bla_{CTX\text{-}M\text{-}1\ group}$ ($bla_{CTX\text{-}M\text{-}3}$ or $bla_{CTX\text{-}M\text{-}55}$) or $bla_{CTX\text{-}M\text{-}9\ group}$ ($bla_{CTX\text{-}M\text{-}14}$ or $bla_{CTX\text{-}M\text{-}65}$) or $bla_{CTX\text{-}M\text{-}123}$, *rmtB*, *floR*	29
	2015 China	*K. pneumoniae*	94 + 7	IS*26*-*fosA3*-*orf1*-*orf2*-Δ*orf3*-IS*26* (n = 1) IS*26*-*fosA3*-variable region (1.4–3 kb)-IS*26* (n = 91)	Plasmid	NR[b]	NR[b]	9
	2015 China	*E. coli* *E. aerogenes*	1 1	IS*26*-*fosA3*-*orf1*-*orf2*-Δ*orf3*-IS*26* (n = 2)	Plasmid	Conjugation (n = 2)	$bla_{KPC\text{-}2}$	37
	2015 Taiwan	*E. coli*	4	IS*26*-*fosA3*-*orf1*-*orf2*-Δ*orf3*-IS*26* (n = 3) IS*26*-IS*Apl1*-*fosA3*-*orf1*-*orf2*-Δ*orf3*-IS*26* (n = 1)	Plasmid	Conjugation (n = 4)	$bla_{CTX\text{-}M\text{-}9\ group}$ or $bla_{CMY\text{-}2}$	32
	2015 Germany	*Salmonella enterica*	1	IS*26*-*fosA3*-*orf1*-*orf2*-Δ*orf3*-IS*26* (n = 1)	Plasmid	Conjugation (n = 1)	$bla_{NDM\text{-}1}$, $bla_{CMY\text{-}16}$, *sul1*, *sul2*, *strA*, *strB*, *aac(6′)-Ib*, *aadA5*, *aphA6*, *tetA*(A), *mph(A)*, *floR*, and *dfrA7*	38
	2015 China	*K. pneumoniae*	44	ΔIS*1294*-IS*26*-*fosA3*-*orf1*-Δ*orf2*-IS*26*-ΔIS*1294* (n = 1)	Plasmid	Transformation (Heat shock method) (n = 1)	$bla_{CTX\text{-}M\text{-}65}$, $bla_{SHV\text{-}12}$, $bla_{KPC\text{-}2}$, *rmtB*, *catA2*	10

	2016 China	*Enterobacteriaceae*	16	IS*26*-*fosA3*-*orf1*-*orf2*-Δ*orf3*-IS*26* (n = 11) IS*26*-*fosA3*-Δ*orf1*-IS*26* (n = 5)	Plasmid	Conjugation (n = 11)	NR[b]	22
	2017 Taiwan	*K. pneumoniae*	35	IS*26*-*fosA3*-*orf1*-*orf2*-Δ*orf3*-IS*26* (n = 2) IS*26*-*fosA3*-*orf1*-Δ*orf2*-IS*26* (n = 23) *fosA3*-*orf1*-Δ*orf2*-IS*26*[c] (n = 1) IS*26*-*fosA3*-*orf1*-Δ*orf2*[c] (n = 4) *fosA3*-*orf1*-Δ*orf2* (n = 5)	Plasmid	NR[b]	NR[b]	33
fosA5 (*fosKP96*)	2013 China	*E. coli*	2	IS*10*-Δ*lysR*-*fosA5*-Δ*orf* -IS*10* (n = 2)	Plasmid	Conjugation (n = 2)	NR[b]	28
fosA5	2015 China	ESBL-producing *E. coli*	1	*insA*-*insB*-Δ*lysR*-*fosA5*-Δ*orf*-IS*10*[d] (n = 1)	Plasmid	Conjugation (n = 1)	*sul1*, *strA*, *strB*	17
fosA6	2016 USA	ESBL-producing *E. coli*	1	Δ*orf*-ΔIS*26*-IS*10*-Δ*lysR*-*fosA6*-Δ*yjiR_1*-ΔIS*26*-Δ*resA* (n = 1)	Plasmid	Conjugation and Transformation (Electroporation) (n = 1)	*floR*	18
fosC2	2010 Japan	CTX-M-producing *E. coli*	1	IS*26*-Δ*intl1*-*fosC2*-*dfrA17*-*aadA5*-*qacE*Δ*1*-*sul1* (n = 1)	Class 1 integron in Plasmid	Conjugation (n = 1)	bla_{CTX-M}, *dfrA17*, *aadA5*, *qacE*Δ*1* and *sul1*	11
	2015 China	*E. cloacae*	1	Δ*tnpA*-IS*26*-Δ*tnpA*-*tnpR*-*tnpM*-*intl1*-*fosC2*-bla_{IMP-34}-*tniR*-*tniQ*-*tniB*-*tniA* (n = 1) (Tn*5075*-In1070 including *fosC2*, bla_{IMP-34})	Class 1 integron in Plasmid	Conjugation (n = 1)	bla_{TEM-1}, bla_{IMP-34}, *aacC2*, *strA*, *strB*, *dfrA14*, and *mph*(A)	13

[a] Extended-spectrum β-lactamases (ESBL) genes: bla_{CTX-M}, bla_{TEM-1}, bla_{CMY}, and bla_{SHV}; carbapenemase genes: bla_{KPC-2}, bla_{NDM-1}, and bla_{IMP}; aminoglycosides-resistant genes: *rmtB*, *strA*, *strB*, *aac(6′)-Ib*, *aadA5*, and *aphA6*; chloramphenicol-resistant genes: *floR* and *catA2*; sulfonamide-resistant genes: *sul1*, and *sul2*; tetracycline-resistant gene: *tetA*(A); macrolide-resistant gene: *mph(A)*; trimethoprim-resistant genes: *dfrA7*, *dfrA14*, and *dfrA17*; antiseptic-resistance gene: *qacE*Δ*1*.

[b] NR; not reported.

[c] Upstream elements of *fosA3* undefined.

[d] Upstream elements of *insA* undefined.

in the simultaneous location of *fosA3* and bla_{KPC-2} in the same Tn*1721*-Tn*3*—like composite transposons. Xiang et al. reported their finding of a 136-kb plasmid (labeled pKP1034) carrying *fosA3*, *rmtB*, bla_{KPC-2}, bla_{TEM-1}, $bla_{CTX-M-65}$ and bla_{SHV-12} in a sample collected in China.[10] In addition to harboring a composite Tn*1721*-Tn*3* transposon, this plasmid contained bla_{KPC-2} and an IS*26* mobile element carrying *fosA3* inserted in IS*1294*. There have been few reports of *fosA3* detection in Europe, although one German study describes a wild bird carrying a *Salmonella enterica* strain with a conjugative *fosA3*-positive plasmid, thus raising the possibility of *fosA3* transmission in Europe.[38] Results from complete plasmid sequencing indicate 14 antibiotic resistance genes (including bla_{NDM-1} and an IS*26*-composite transposon carrying *fosA3*) located on a 187-kb IncA/C plasmid.

Although the *fosA4* sequence has been identified, its genetic environment has yet to be determined.[16] In contrast, the surrounding region of the plasmid-mediated *fosA5* gene (1169 bp) has been shown to share a 98% nucleotide identity with a *K. pneumoniae* CG4 chromosomal sequence, suggesting that *K. pneumoniae* CG4 is a likely candidate for the origin of the plasmid-mediated *fosA5* gene.[17] Unlike *fosA3*, *fosA5* is likely mobilized via IS*10*, taking *fosA5* from the *K. pneumoniae* chromosome and integrating it into plasmids.[17,28] The first report of *fosA6* involved ESBL-producing *E. coli* isolates collected in the US.[18] Similar to *fosA5*, *fosA6* was likely captured from a *K. pneumoniae* chromosome sequence and mobilized to conjugative plasmids via IS*10*. Other researchers have reported observations of *fosC2* (accompanied by other resistant genes) in class 1 integrons.[11,13] In a Japanese study, Wanchino et al. found a class 1 integron carrying *fosC2*, *dfrA17* and *aadA5* in a conjugative pHPA plasmid.[11] In another Chinese study, Wang et al. describe *fosC2* and bla_{IMP-34} on Tn*5075*-In1070 composite transposons in multidrug-resistant pIMP-HB623 plasmids found in *E. cloacae* isolates.[13] Combined, the data suggest that plasmid-mediated *fos* genes may originate in bacterial chromosomes, where they are captured by insertion sequences or other mobile elements.

Possible combined antibiotic treatments with fosfomycin in *Enterobacteriaceae*

Multidrug-resistant *Enterobacteriaceae* is one of many examples of rapidly evolving resistance to antibiotics being reported worldwide.[40—43] Fosfomycin has long been the antibiotic of choice for treating MDR *Enterobacteriaceae* infections (including those involving ESBL and carbapenemase producers), but monotherapy resistance rates have been steadily increasing over the past decade (Table 1), leading physicians and researchers to experiment with combination antibiotic therapies in response to MDR *Enterobacteriaceae* isolates (Table 3).[33,44—48] Bercot et al. have reported *in vitro* synergistic activity (checkerboard method) for fosfomycin plus either colistin or tigecycline against 8 NDM-1-producing *Enterobacteriaceae* isolates collected in various global locations.[44] Only 3 exhibited fractional inhibitory concentrations between 0.5 and 1.1 (indicating variation in terms of synergy and no interaction) for colistin and fosfomycin. No antagonistic results have been reported. Lingscheid et al. examined the efficacy of fosfomycin plus doripenem against clinical blood isolates (checkerboard method and time-kill curve tests).[45] Checkerboard-based synergistic effects were observed in 80% (8/10) of *E. coli*, 100% (5/5) of *K. pneumoniae*, and 33% (1/3) of *E. cloacae* isolates. Time-kill curve-based synergistic effects using 1/4 X MIC doripenem and fosfomycin have been observed in 2 *E. coli* and 2 *K. pneumoniae* isolates.[45] Corvec et al. used *in vitro* time-kill curve tests and animal experiments to evaluate the effects of various antibiotic combinations against a fluoroquinolone-resistant CTX-M15-producing *E. coli* (Bj HDE-1) isolate with an implant-associated infection.[46] Their data indicate synergistic effects from combinations of fosfomycin plus either colistin or tigecycline. They also found evidence of regrowth following treatment with a combination of gentamycin plus fosfomycin. In their animal experiments, Corvec et al. observed a low cure rate following fosfomycin-only treatments against biofilm-forming bacteria (2/12, 17%), but significantly better results for the combined therapies of fosfomycin plus colistin (67%), tigecycline (50%) or gentamycin (42%).

In a separate study, Evren et al. analyzed the *in vitro* efficacies of fosfomycin in combination with imipenem, meropenem, colistin or tigecycline against 12 OXA-48-producing *K. pneumoniae* isolates.[47] All 12 isolates were resistant to imipenem, meropenem and fosfomycin when used individually, but robust antimicrobial activity was observed when fosfomycin was used in combination with meropenem (33%, 4/12), imipenem (42%, 5/12) or tigecycline (33%, 4/12). According to Hickman et al., fosfomycin plus either aztreonam or mecillinam and fosfomycin plus both aztreonam and mecillinam were effective against a plasmid-mediated ESBL-producing MDR *K. pneumoniae* isolate and its transconjugant.[48] These studies are representative of renewed interest in "forgotten" antibiotics—especially in re-evaluations of the tri-combination of fosfomycin, aztreonam and mecillinam—for treating MDR *Enterobacteriaceae* infections, especially those involving *K. pneumoniae*. Tseng et al. describe a synergistic effect from fosfomycin combined with meropenem against 25 randomly selected fosfomycin-resistant CRKP isolates recovered from pulsotype XXIII and ST11.[33] Data from treatments of isolates containing fosfomycinase (48%, 12/25) or carbapenemase (96%, 24/25) using *in vitro* checkerboard methods suggest that the combination of fosfomycin and meropenem has potential as an alternative clinical treatment.

In summary, fosfomycin remains a favored (though not the only) choice among physicians for treating MDR *Enterobacteriaceae* infections, either alone or in combination with other antimicrobial agents. The extensive effects of fosfomycin combination treatments for other types of antibiotic-resistant bacteria (e.g., MDR *Pseudomonas aeruginosa* and penicillin-resistant *Streptococcus pneumoniae*) have also been reported.[49,50] However, several issues require detailed investigation, especially *in vivo* pharmacokinetics, other possible effects of treatment combinations, and overall reductions in antibiotic resistance.

Table 3 Synergistic effect of fosfomycin with different antibiotic in *Enterobacteriaceae*.

Species	Total Isolates	Resistant Pattern (No. of isolates)		Antibiotic[a]	Efficacy of Antimicrobial Combination[b] (no. of isolates) (method)	Ref
		Phenotype	Genotype			
E. cloacae	2	FOS (1)	NDM-1 (2)	COL + FOS	S/NI (1), NI (1) (checkerboard)	44
				TGC + FOS	NI (2) (checkerboard)	
K. pneumoniae	3	TGC (1)	NDM-1 (3)	COL + FOS	S/NI (1), NI (2) (checkerboard)	
				TGC + FOS	NI (3) (checkerboard)	
K. oxytoca	1	NR[c]	NDM-1 (1)	COL + FOS	S/NI (1) (checkerboard)	
				TGC + FOS	NI (1) (checkerboard)	
E. coli	2	NR[c]	NDM-1 (2)	COL + FOS	NI (2) (checkerboard)	
				TGC + FOS	NI (2) (checkerboard)	
E. coli	10	MDR	ESBL (8) AmpC (2)	DOR + FOS	S (8), NI (2) (checkerboard) S (2) time-kill curve, 2 synergistic isolates (checkerboard)	45
K. pneumoniae	5	MDR	ESBL (3) AmpC (2)	DOR + FOS	S (5) (Checkerboard) S (2) time-kill curve, 2 synergistic isolates (checkerboard)	
E. cloacae	3	NR[c]	ESBL (1)	DOR + FOS	S (1), NI (2) (checkerboard)	
E. coli	1	CIP	CTX-M-15 Chromosomal AmpC variant	*In vitro*[d]	*In vitro* (time-kill curve)	46
				COL + FOS	S (1)	
				TGC + FOS	S (1)	
				GEN + FOS	NI (1)	
				In vivo[e]	*In vivo* (cured rate; n/N)[f]	
				FOS only	17% (2/12)	
				COL + FOS	67% (8/12)	
				TGC + FOS	50% (6/12)	
				GEN + FOS	42% (5/12)	
K. pneumoniae	12	FOS (11) IMP (12) MEM (12) COL (5) TGC (5)	OXA-48 (12)	IMP + FOS	S (5), NI (7) (checkerboard)	47
				MEM + FOS	S (4), NI (8) (checkerboard)	
				COL + FOS	A (12) (checkerboard)	
				TGC + FOS	S (4), NI (8) (checkerboard)	
K. pneumoniae	1	MDR	pUUH239.1, pUUH239.2 (multiple antibiotic resistance genes)	ATM + FOS	NI (1) (checkerboard)	48
				MEC + FOS	S (1) (checkerboard)	
				ATM + FOS	S (1) (time-kill curve)	
				MEC + FOS	NI (1) (time-kill curve)	
				ATM + MEC + FOS	S (1) (time-kill curve)	
E. coli	1 (Transconjugant)	MDR	pUUH239.2	ATM + FOS	S (1) (checkerboard)	
				MEC + FOS	S (1) (checkerboard)	
				ATM + FOS	S (1) (time-kill curve)	
				MEC + FOS	S (1) (time-kill curve)	
				ATM + MEC + FOS	S (1) (time-kill curve)	
K. pneumoniae	25	FOS (25) MEM (25)	KPC (24) *fosA3* (9) *fosKP96* (6)	MEM + FOS	S (25) (checkerboard)	33

[a] AMK, amikacin; AMP, ampicillin; ATM, aztreonam; CHL, chloramphenicol; CIP, ciprofloxacin; COL, colistin; CPO, cefpirome; CTX, cefotaxime; CXM, cefuroxime; ERY, erythromycin; ETP, ertapenem; FEP, cefepime; FOS, fosfomycin; GEN, gentamicin; KAN, kanamycin; MEC, mecillinam; MEM, meropenem; NAL, nalidixic acid; SMZ, sulfamethoxazole; SPT, spectinomycin; STR, streptomycin; SXT, trimethoprim/sulfamethoxazole (1/19); TET, tetracycline; TGC, tigecycline; TMP, trimethoprim.

[b] S, synergistic effect; NI, no interaction; A, antagonism. In checkerboard method, FICI values of $\leq$0.5 indicate synergism; FICI values of $>$0.5 to 4 indicate no interaction; and FICI values of $>$4 indicate antagonism; in time-kill curve method, synergistic effect was defined as 2-$\log_{10}$ decrease in colony-forming units (CFU)/ml at 24 h by the combination compared to the most active single agent at the same concentration and a 2-$\log_{10}$ decrease in CFU/ml compared to the starting inoculum; in cured rate, synergistic effect was defined as the combination showed the higher cure rate, which was significantly better than that of fosfomycin alone.

[c] NR, not reported.

[d] *In vitro*, *in vitro* assay.

[e] *In vivo*, *in vivo* assay.

[f] n/N, number of cured mice over number of total mice.

Conclusion

Although chromosomal variations of impaired transporters and amino acid substitution of MurA are the primary fosfomycin resistance mechanism, more research is required to monitor the potential for transmitting *fos* genes via plasmids and transposons. Plasmids containing ESBL and *fos* genes may facilitate the dissemination of antibiotic resistance. Recent studies indicate that the recombination of plasmid-encoding carbapenemase and fosfomycinase occurs via mobile elements, thus presenting new treatment challenges. In summary, the therapeutic potential of therapies involving fosfomycin in combination with other antimicrobial agents requires further investigation, with the primary focus being their proper use in the treatment of infections that may be caused by MDR *Enterobacteriaceae*.

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