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Plasmid-mediated fosfomycin resistance in *Escherichia coli* isolates of worldwide origin

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ABSTRACT

Objectives: Fosfomycin is a first-line treatment for uncomplicated urinary tract infections (UTIs) in several European countries, and it is increasingly becoming the treatment of choice globally. Resistance to fosfomycin in *Escherichia coli* can be exerted through several mechanisms, including the acquisition of fosfomycin-modifying enzymes, of which the FosA-type enzymes are the most common. This study analysed, both phenotypically and genotypically, an international collection of *E. coli* strains harbouring acquired *fosA* genes.

Methods: Thirty-one *fosA*-positive *E. coli* isolates were obtained from both clinical and environmental sources, from seven countries (Portugal (n = 12), Switzerland (n = 9), China (n = 3), France (n = 2), Nepal (n = 2), South Africa (n = 2), Kuwait (n = 1)). MICs were determined according to EUCAST guidelines. Whole genome sequencing (WGS) was performed on 23 isolates, and complete *fosA* plasmid sequences were determined for 12. Conjugation assays were performed on seven isolates.

Results: All isolates exhibited high-level resistance to fosfomycin (64 to >256 mg/L). WGS of 23 isolates identified 17 sequence types (STs), and 16 harboured *fosA3*, four *fosA4*, two *fosA8*, and one *fosA10*. ESBLs, pAmpC, or carbapenemase genes were present in 15, four, and three isolates, respectively. The *fosA* plasmids of 12 isolates were determined and were diverse in size (~67 kb to ~235 kb), resistance gene carriage, and replicon types. Six *fosA* plasmids additionally carried ESBL or carbapenemase genes. Conjugation assays, performed on seven isolates harbouring diverse plasmids, identified that all were capable of being transmitted.

Conclusion: This study highlights the necessity of the surveillance and close monitoring of fosfomycin resistance in *E. coli*, essential to maintain the optimal use of this treatment option.

1. Introduction

Urinary tract infections (UTIs) are the most common bacterial infection type in both primary and hospital care settings [1] and they are associated with a considerable burden on both patients and healthcare systems [2]. Previous studies of community-onset UTIs in several mainland European countries have shown that *Escherichia coli* was the most commonly isolated uropathogen, accounting for more than half of all isolates across all studies [3–5]. Fosfomycin is a broad-spectrum phosphonic antibiotic, first discovered in Spain in 1969, which exhibits bactericidal activity against both Gram-positive and Gram-negative organisms [6], and it is commonly used as a first-line treatment for uncomplicated UTIs (uUTIs) in Europe [7]. The activity of fosfomycin is exerted by disrupting bacterial cell wall synthesis via the binding and inhibition of the UDP-N-acetylglucosamine enolpyruvyl transferase, MurA, which is essential for cell survival [6]. In addition to being first-line treatment for uUTIs, fosfomycin has also been licenced

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for intravenous administration to treat bloodstream and soft tissue infections in several European countries, including the United Kingdom [8]. Furthermore, studies have shown that fosfomycin can be useful in the treatment of infections caused by multidrug-resistant (MDR) and carbapenem-resistant bacteria, particularly when administered as a combination therapy [9,10]. This antimicrobial benefits from a lack of cross-resistance to other antimicrobial classes and exerts excellent activity against AmpC, ESBL, and carbapenemase-producing bacteria [11]. Resistance rates to fosfomycin in *E. coli* remains relatively low [7]; however, in recent years reports of fosfomycin resistance have steadily increased [12].

Resistance to fosfomycin in *E. coli* can be exerted through several mechanisms, including mutational resistance, usually resulting from impaired drug uptake via mutations in transporters or regulators (eg, *glpT, uhpT*, and *uhpA*), mutations within the fosfomycin target, MurA, and perhaps most importantly, the acquisition of fosfomycin-modifying enzymes [6]. Plasmid-mediated Fos enzymes are members of the glyoxalase superfamily and, in *Enterobacterales*, three main enzymes groups are found: FosA (FosA1–10), FosC2, and FosL (FosL1–2) [13]. The FosA-type metalloenzymes act by catalysing the conjugation of glutathione to fosfomycin, thereby inactivating fosfomycin activity [14]. The most common FosA variant is the FosA3 enzyme, which has been reported globally and is usually found encoded in conjugative plasmids and often those that carry other resistance determinants, conferring resistance to additional antibiotic classes [15]. The less frequently reported FosA-type gene variants, *fosA4, fosA5*, and *fosA6* genes, have also been identified in *E. coli* [16–18]. FosL1 was recently described encoded on an IncX1 plasmid in an ESBL-producing *E. coli* strain from Switzerland [19], and FosC2 has been described in ESBL-producing strains from China [20].

This study analysed, both phenotypically and genotypically, an international collection of *E. coli* strains harbouring *fosA* genes.

2. Materials and methods

2.1. Bacterial isolates

Thirty-one *fosA*-positive *E. coli* isolates were obtained from both clinical (n = 29) and environmental sources (n = 2), between 2017 and 2022. Isolates were chosen from seven countries (Portugal (n = 12), Switzerland (n = 9), China (n = 3), France (n = 2), Nepal (n = 2), South Africa (n = 2), and Kuwait (n = 1)) to be internationally representative. Species identification was confirmed using API-20E tests (bioMérieux). FosA positivity had previously been determined by PCR and subsequent Sanger sequencing analyses as previously described [21].

2.2. Susceptibility testing

MICs of antimicrobial agents were determined on all 31 isolates by broth microdilution, according to CLSI guidelines [22], and interpreted according to EUCAST guidelines [23], or CLSI guidelines for cefoxitin and tetracycline [22]. Fosfomycin MICs were determined by agar doubling dilution method, using glucose-6-phophate (25 mg/L) supplemented Mueller Hinton agar. Sodium phosphonoformate (PPF) (5 mM) was incorporated in Mueller Hinton agar to assess the inhibition of Fos enzymes.

2.3. Whole genome sequencing (WGS)

WGS was performed on a subset of 23 isolates, selected to representative of all FosA-types found in this study, using both short- (Illumina) and long-read (Oxford Nanopore) sequencing technologies. Illumina (short-read) sequencing was performed on all 23 isolates and Nanopore (long-read) sequencing was additionally performed on 12 isolates to obtain complete FosA plasmid sequences.

Total genomic DNA (gDNA) of isolates was extracted from a bacterial culture grown overnight using the QIAamp DNA Mini Kit (Qiagen, Germany). WGS was performed using either a HiSeq 4000 (Illumina, San Diego, CA) or MinION Mk1b (Oxford Nanopore Technologies, United Kingdom) platforms. For short-read sequencing, gDNA was sheared with a Covaris E220 ultrasonicator. The TruSeq DNA Nano kit from Illumina (Illumina, San Diego, CA) was used for library preparation with 100 ng of fragmented DNA as input. Library molarity and quality were assessed with Qubit (ThermosFisher Scientific, Waltham, MA) and Tapestation using a DNA high sensitivity chip (Agilent Technologies). A paired-end 100 sequencing run was performed with the TruSeq SBS chemistry. For MinION sequencing, sequencing libraries were prepared following the sequencing by ligations protocol using a native barcoding kit (EXP-NBD104; Oxford Nanopore Technologies). Sequencing was performed on a MinION Mk1b sequencer (Oxford Nanopore Technologies, UK) using an R10.3 MinION Flow Cell (FLO-MIN112; Oxford Nanopore Technologies, UK).

Long read base calling was performed using Guppy (v. 6.1.2) with a high accuracy (hac) model on the high-performance computing clusters at the University of Geneva. The resulting fastq files were used to produce assemblies with Flye software (v. 2.9) and were polished with short-read data using Pilon (v. 1.24). Alternatively, hybrid assembles, using both short- and long-read data, were performed using UniCycler (v. 0.4.9b) [24]. Short paired-end reads assemblies were performed using the Shovill pipeline (https://github.com/tseemann/shovill), and contigs were annotated using Prokka [25]. Sequence types (STs), the presence of resistance genes and plasmid replicon types were determined using MLST 2.0, ResFinder 4.1 [26], and PlasmidFinder 2.1 [27], on the Center for Genomic Epidemiology platform (https://cge.cbs.dtu.dk/services/).

2.4. Conjugation studies

Mating-out assays of seven *fosA*-harbouring plasmids (three *fosA3*, two *fosA8*, and one each with *fosA4* and *fosA10*) were attempted using a sodium azide–resistant *E. coli* J53 as recipient. Briefly, overnight cultures of recipient and donor cells were mixed in a 4:1 ratio and centrifuged at 3000 × *g* for 10 min before being transferred to sterile 0.22 µM filters (Merck Millipore, Ireland) and incubated on LB agar plates (Carl Roth, Karlsruhe, Germany) at 37°C for 4 hours. Cells were washed away from the filters using 0.85% NaCl, and the mixture was vortexed. Serial dilutions were plated onto LB agar plates containing either 16 mg/L fosfomycin (to quantify donors) or both 100 mg/L sodium azide and 16 mg/L fosfomycin (to quantify transconjugants). Conjugation frequencies were calculated by dividing the number of transconjugants by the number of donors.

2.5. Data availability

Sequence data from this study were submitted to the National Center for Biotechnology Information's Sequence Read Archive (BioProject no. PRJNA999080).

3. Results and discussion

3.1. Susceptibility testing

All 31 isolates exhibited high-level resistance to fosfomycin with MICs ranging from 64 to >256 mg/L. The addition of 5 mM PPF restored the susceptibility of 22.6% (7/31) isolates, although 23/31 (74.2%) isolates exhibited 2–128-fold reductions in MICs,

Table 1
MIC distribution of FosA-positive *E. coli* (n = 31) from this study

Antimicrobial agent	Breakpoint (mg/L)	Isolates (No./%)													No./% Resistant
		≤0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	≥256	
Fosfomycin	>32											1/3.2	3/9.7	27/87.1	31/100.0
Fosfomycin/5mM PPF[a]	>32[b]						1/3.2		2/6.5		4/12.9	4/12.9	9/29.0	11/35.5	24/77.4
Amoxicillin	>8													31/100.0	31/100.0
Temocillin	>16						4/12.9	7/22.6	14/45.2	5/16.1			1/3.2		1/3.2
Cefoxitin	>16[c]						2/6.5	5/16.1	9/29.0	7/22.6	3/9.7	2/6.5	2/6.5	1/3.2	8/25.8
Cefotaxime	>2		9/29.0					1/3.2	2/6.5	1/3.2			3/9.7	15/48.4	22/71.0
Ceftazidime	>4			7/22.6	2/6.5	4/12.9	2/6.5	3/9.7	2/6.5	4/12.9	4/12.9	2/6.5		1/3.2	13/41.9
Cefepime	>4	7/22.6	2/6.5	1/3.2		2/6.5	3/9.7	3/9.7	1/3.2	2/6.5	2/6.5	4/12.9	4/12.9		13/41.9
Ertapenem	>0.5	28/90.3								2/6.5				1/3.2	3/9.7
Imipenem	>4		7/22.6	20/64.5	1/3.2					2/6.5	1/3.2				3/9.7
Meropenem	>8	28/90.3							2/6.5		1/3.2				1/3.2
Chloramphenicol	>8							2/6.5	12/38.7	2/6.5		1/3.2	2/6.5	12.38.7	17/54.8
Gentamicin	>2					14/45.2	5/16.1	6/19.4				1/3.2	3/9.7	2/6.5	12/38.7
Ciprofloxacin	>0.5		9/29.0	1/3.2	1/3.2			3/9.7	3/9.7	4/12.9	2/6.5	5/16.1	2/6.5	1/3.2	20/64.5
Tetracycline	>8[c]					3/9.7	1/3.2	1/3.2			2/6.5	2/6.5	2/6.5	20/64.5	26/83.9
Tigecycline	>0.5	14/45.2	13/41.9	2/6.5	2/6.5										0/0.0
Trimethoprim	>4			3/9.7	4/12.9		1/3.2							23/74.2	23/74.2
Colistin	>2	19/61.3	5/16.1	1/3.2				1/3.2	4/12.9	1/3.2					6/19.4

Vertical lines indicate epidemiological cutoff values by EUCAST recommendations.
[a] PPF; sodium phosphonoformate.
[b] EUCAST fosfomycin breakpoint used.
[c] Breakpoints taken from CLSI 28th edition.

confirming the role of the *fosA* genes in conferring the fosfomycin-resistant phenotype. All isolates were resistant to amoxicillin, and resistance to the cephalosporins cefotaxime, ceftazidime, and cefepime was observed in 71.0% (22/31), 41.9% (13/31), and 41.9% (13/31) of isolates, respectively (Table 1). Carbapenem resistance (resistance to >1 tested carbapenems) was observed in three isolates (9.7%), and six isolates (19.4%) exhibited resistance to colistin. This high level of resistance to colistin is uncommon in *E. coli*, suggesting that those colistin-resistant isolates may result from selection in the environment or in veterinary medicine, where colistin is still frequently used.

3.2. Whole genome sequencing of fosA-positive isolates

WGS of 23 isolates identified 17 different STs, all of which were represented by a single isolate with the exceptions of ST69 (n = 4), ST226 (n = 3), and ST57 (n = 2). Only a single member of the globally dominant high-risk clone, ST131, was identified. Amongst the 23 isolates, 16 harboured *fosA3*, four *fosA4*, two *fosA8*, and one isolate harboured *fosA10*. ESBLs or pAmpC genes were present in 16 isolates, comprising $bla_{\text{CTX-M-15}}$ (n = 4), $bla_{\text{CTX-M-55}}$ (n = 4), $bla_{\text{CMY-2}}$ (n = 4), $bla_{\text{CTX-M-3}}$ (n = 3), $bla_{\text{CTX-M-14}}$ (n = 3), and $bla_{\text{CTX-M-65}}$ (n = 1). The relatively high frequency of association between ESBL and *fosA* gene carriage has been already reported [12,13]. Genes encoding KPC-2 carbapenemases were also detected in two isolates, both from China, and one isolate from Switzerland encoded NDM-5. Interestingly, all six isolates that were colistin-resistant (MICs ranging from 4–16 mg/L) were found to harbour *mcr-1* genes. These six isolates were geographically diverse (two from South Africa, two from Nepal, one from Kuwait, and one from Portugal) and represented five different STs.

3.3. fosA plasmids

Using a combination of long- and short-read sequence methodologies, the entire *fosA* plasmids of 12 isolates were sequenced to closure. The isolates selected for plasmid sequencing represented 11 different STs and were obtained from four countries: eight from Switzerland, two from South Africa, and one each from China and Kuwait. These comprised eight plasmids encoding FosA3, one encoding FosA4, two FosA8 plasmids, and one encoding FosA10. The sequenced plasmids were diverse in size (ranging from ~67 kb to ~235 kb), resistance gene carriage, and replicon types (Table 2). Amongst the 12 plasmids, 5/8 FosA3-encoding plasmids carried a $bla_{\text{CTX-M}}$ ESBL gene, comprising $bla_{\text{CTX-M-55}}$ (n = 2), $bla_{\text{CTX-M-3}}$, $bla_{\text{CTX-M-15}}$, and $bla_{\text{CTX-M-65}}$; one harboured $bla_{\text{KPC-2}}$, and the FosA10-encoding plasmid harboured $bla_{\text{CMY-2}}$. The plasmid harbouring *fosA3* and $bla_{\text{KPC-2}}$ (pFos_R4667) was found in an ST609 *E. coli* isolate from China and was identical to a plasmid previously described in an ST46 clinical *E. coli* isolate (GenBank Accession No. KF701335) [28]. Subsequent mapping analyses identified that the *fosA3* plasmid in strain R3022 (ST69, also from China) also exhibited >99% identity to this same plasmid. Interestingly, two isolates, both ST226, but one from South Africa (R2745) and one from Portugal (R6245), were found to harbour a highly similar 235 033 bp IncHI2/IncHI2A plasmid which also encoded $bla_{\text{CTX-M-55}}$ and *mcr-1*, despite being isolated on separate continents. Plasmid pFos_R3023, found in an ST58 isolate obtained from China, was shown to exhibit >99% similarity to 70 388 bp IncFII plasmid, pFos_UN95, obtained from Switzerland. Furthermore, the plasmid from ST3107, obtained from a chicken in Nepal, exhibited a high level of similarity (>96%) to a clinical ST3944 isolate obtained from Kuwait. The plasmid encoding FosA10 was found in an ST6870 isolate and was encoded on an IncI1 101 398 bp plasmid. Previously, *fosA10* has only been described on an IncFII 53 kb plasmid from China [29]. These similarities observed between FosA-encoding plasmids found in isolates of different STs and from different countries illustrates the transmission of *fosA* plasmids and, importantly, the potential for co-resistance to the beta-lactams upon the inheritance of a FosA-encoding gene. This pattern of association of resistance may be therefore the source of difficult-to-treat urinary tract infections.

3.4. FosA genetic environment

Examination of the immediate genetic environments in which the *fosA* genes were encoded identified that *fosA3*, *fosA4*, and *fosA8* genes were flanked by IS*26* elements (Fig. 1). This indicates the key role in mobilisation of *fosA* genes played by IS*26* as has been extensively reported previously [13]. However, *fosA10* was found to be flanked by two copies of IS*10R*, similarly to what was previously

Table 2
Genotypic characteristics of the 23 sequenced isolates and 12 FosA-encoding plasmids

Strain	Country of Origin	Source	ST	Resistance genes	Replicon types	Fos plasmid genotypes			
						Fos plasmid	Plasmid size (bp)	Replicon types	Resistance genes
L780	Switzerland	Human	174	*fosA3*	FII, B/O/K/Z	pFos_L780	66,839	FII	*fosA3*
R2742	South Africa	Human	57	*fosA3*, $bla_{CTX-M-55}$, bla_{TEM-1}, *aac(6')-Ib3*, *aadA2*, *aadA5*, *oqxAB*, *floR*, *mcr-1*, *tetA*, *sul1*, *sul2*, *dfrA12*, *dfrA17*, *mphA*	HI2, HI2A, N, Y, FIB(AP001918), FIC(FII), I2	pFos_R2742	247,864	HI2, HI2A, N	*fosA3*, $bla_{CTX-M-55}$, *aadA5*, *aac(6')-lb3*, *oqxAB*, *sul2*, *floR*, *mcr-1*, *dfrA17*
R2745	South Africa	Human	226	*fosA3*, $bla_{CTX-M-55}$, bla_{TEM-1}, *aac(6')-Ib3*, *aadA1*, *floR*, *mcr-1*, *tetA*, *sul1*, *sul2*, *dfrA15*	HI2, HI2A, FIB(AP001918), FII(pCoo), I1	pFos_R2745	235,033	HI2, HI2A	*fosA3*, $bla_{CTX-M-55}$, *mcr-1*, *aac(6')lb3*, *floR*, *sul2*
R4667	China	Human	609	*fosA3*, bla_{KPC-2}	P1	pFos_R4667	69,478	P1	*fosA3*, bla_{KPC-2}
UN82	Switzerland	Human	14622	*fosA3*, bla_{OXA-1}, *aac(3)-IId*, *aph(6)-Id*, *aph(3")-Ib*, *aadA5*, *aac(6')-Ib-cr*, *floR*, *tetA*, *sul1*, *sul2*, *dfrA17*, *arr-3*, *mphA*, *catB3*	FIA, FIB(AP001918), B/O/K/Z	pFos_UN82	85,381	FIA, FIB(AP001918)	*fosA3*, bla_{OXA-1}, *aac(3)-lld*, *aac(6')-lb-cr*, *aadA5*, *mphA*, *sul1*, *dfrA17*, *arr-3*, *catB3*
UN95	Switzerland	Human	117	*fosA3*, $bla_{CTX-M-3}$, bla_{TEM-1}, *aadA1*, *aph(3")-Ib*, *aph(6)-Id*, *aph(3')-Ia*, *tetB*, *sul1*, *sul2*, *mphB*	FII, FIB(AP001918), Q1, Col156	pFos_UN95	70,388	FII	*fosA3*, $bla_{CTX-M-3}$
UN195	Switzerland	Human	359	*fosA3*, $bla_{CTX-M-65}$, bla_{TEM-1}, *aph(3')-Ia*, *aph(3")-Ib*, *aph(6)-Id*, *catA2*, *tetA*, *sul2*, *dfrA14*	FII, FIB(AP001918), Y	pFos_UN195	129,323	FII, FIB(AP001918)	*fosA3*, $bla_{CTX-M-65}$, bla_{TEM-1}, *aph(6)-Id*, *aph(3")-Ib*, *aph(3')-Ia*, *tetA*, *sul2*, *dfrA14*
UN515	Switzerland	Human	69	*fosA3*, $bla_{CTX-M-15}$, bla_{TEM-1}, *aph(3")-Ib*, *aph(6)-Id*, *tetA*, *sul2*, *dfrA14*	FII, FIB(AP001918), FIC(FII)	pFos_UN515	72,056	FII	*fosA3*, $bla_{CTX-M-15}$
Fos126	France	Human	117	*fosA3*, bla_{TEM-1}, $bla_{CTX-M-55}$, bla_{CMY-2}, *aph(3")-Ib*, *aph(6)-Id*, *aadA1*, *InuF*, *tetA*, *sul2*, *sul3*	FIA, FIB(AP001918), FIC(FII), HI2, HI2A, Q1, Col440II	NA	ND	ND	ND
R3022	China	Human	69	*fosA3*, bla_{KPC-2}, $bla_{CTX-M-14}$, *aac(6′)lb-cr*, *cmlA1*, *ermB*, *mphA*	FII, P1, R, Col440II	NA	ND	ND	ND
R3023	China	Human	58	*fosA3*, $bla_{CTX-M-3}$, bla_{TEM-1}	B/O/K/Z, FII, I1	NA	ND	ND	ND
R6147	Switzerland	Human	14314	*fosA3*, bla_{TEM-1}, $bla_{CTX-M-14}$, *aac(3)-IV*, *aadA2*, *floR*, *tetA*, *sul2*, *dfrA12*, *mphA*	FIB(AP001918), FIC(FII)	NA	ND	ND	ND
R6207	Portugal	Human	69	*fosA3*, $bla_{CTX-M-15}$, bla_{TEM-1}, *aph(6)-Id*, *aph(3'')-Ib*, *tetA*, *sul1*, *sul2*, *dfrA7*	FIA, FIB(AP001918), FII, Q1, Col440II	NA	ND	ND	ND
R6239	Portugal	Human	131	*fosA3*, $bla_{CTX-M-3}$, bla_{TEM-1}, *aph(6)-Id*, *aph(3'')-Ib*, *aac(3)-IId*, *tetA*, *sul2*, *mphA*	FIA, FIB(AP001918), FII(pRSB107)	NA	ND	ND	ND
R6245	Portugal	Human	226	*fosA3*, $bla_{CTX-M-55}$, bla_{TEM-1}, *aac(6′)-Ib3*, *aadA1*, *mcr-1*, *floR*, *tetA*, *sul1*, *sul2*, *dfrA15*	FIB(AP001918), FII(pCOO), HI2, HI2A, I1	NA	ND	ND	ND
R6351	Portugal	Human	372	*fosA3*, $bla_{CTX-M-14}$, bla_{TEM-1}, *aadA1*, *sul1*, *dfrA1*	Y	NA	ND	ND	ND
R4880	Kuwait	Human	3944	*fosA4*, bla_{CMY-2}, *qnrS1*, *tetA*, *InuF*, *mcr-1*, *sul3*, *dfrA12*, *mphA*	I1, N, p0111, I2, FII, FIC(FII), FIB(AP001918), B/O/K/Z	pFos_R4880	106,834	I1	*fosA4*, *dfrA12*, *mphA*
Fos90	France	Human	226	*fosA4*, bla_{TEM-1}, *qnrS1*, *floR*, *tetA*, *tetM*, *sul3*, *dfrA14*, *mphA*	FII(pCOO), p0111	NA	ND	ND	ND
R5371	Nepal	Chicken	3107	*fosA4*, bla_{TEM-1}, *aph(3")-Ib*, *aph(6)-Id*, *aac(3)-IId*, *aadA1*, *mcr-1*, *floR*, *tetA*, *sul2*, *sul3*, *dfrA17*, *I*, *mphA*	FIB(AP001918), FIC(FII), FII(pCOO), I1, I2	NA	ND	ND	ND
R5377	Nepal	Chicken	48	*fosA4*, bla_{CMY-2}, bla_{TEM-1}, *catA2*, *floR*, *aph(3′)-Ia* *aadA1*, *aadA2*, *mcr-1*, *qnrS1*, *tetA*, *tetM*, *sul1*. *sul3*, *dfrA5*, *dfrA12*, *dfrA14*, *mphA*	FII(pCOO), I1, I2, HI2, HI2A, X1, p0111, ColpVC	NA	ND	ND	ND
N1345	Switzerland	Human	69	*fosA8*, bla_{TEM-1}, *aadA1*, *aadA2*, *aac(3)-IId*, *aph(3')-IIa*, *aph(3')-Ia* *floR*, *cmlA1*, *tetA*, *tetM*, *sul2*, *sul3*, *dfrA1212*	FII, FIA, FIB(AP001918), p0111	pFos_N1345	85,690	FII	*fosA8*, *aph(3')-lla*
R4390	Switzerland	Human	410	*fosA8*, bla_{OXA-1}, $bla_{CTX-M-15}$, *aph(3')-lla*, aac(6')-Ib-cr, *tetA*, *dfrA14*	N, X1, FIA, FIB(AP001918), Col156	pFos_R4390	67,907	X1, N	*fosA8*, *aph(3')-lla*
N2736	Switzerland	Human	6870	*fosA10*, bla_{NDM-5}, $bla_{CTX-M-15}$, bla_{OXA-1}, bla_{CMY-2}, *aadA2*, *aac(6')-Ib-cr*, *mphA*, *tetA*, *sul1*, *dfrA12*	FII, FIA, FIB(AP001918), FIB(H89-PhagePlasmid), I1	pFos_N2736	101,398	I1	*fosA10*, bla_{CMY-2}

fosA gene variants shown in bold.
NA, not applicable; ND, not determined.

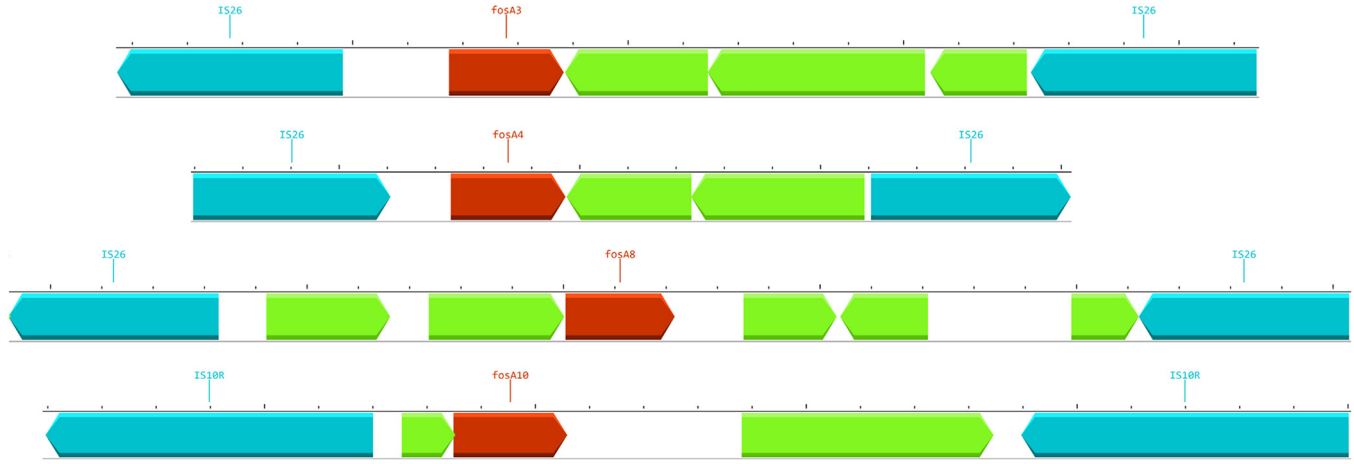

Fig. 1. The genetic environment surrounding *fosA3* (R4667), *fosA4* (R4880), *fosA8* (N1345), and *fosA10* (N2736) genes. Blue arrows: insertion sequences elements; red arrows: *fosA* genes; green arrows: hypothetical proteins.

Table 3
Conjugation frequency in filter matting assays for Fos gene-producing *E. coli*-carrying distinct plasmids

Strain	Fos variant	Plasmid replicon type	Plasmid size (bp)	Plasmid resistance genes	Conjugation frequency
R4667	FosA3	P1	69 478	*fosA3*, bla_{KPC-2}	1.00×10^{-1}
L780	FosA3	FII	66 839	*fosA3*	6.78×10^{-3}
R2745	FosA3	HI2/HI2A	235 033	*fosA3*, $bla_{CTX-M-55}$, *mcr-1*, *aac(6')Ib3*, *aac(6')Ib-cr*, *floR*, *sul2*	1.30×10^{-5}
R4880	FosA4	I1	106 834	*fosA4*, *dfrA12*, *mphA*	4.19×10^{-4}
R4390	FosA8	X1/N	67 907	*fosA8*, *aph(3')-IIa*	4.33×10^{-1}
N1345	FosA8	FII	85 690	*fosA8*, *aph(3')-IIa*	1.88×10^{-2}
N2736	FosA10	I1	101 398	*fosA10*, bla_{CMY-2}	5.31×10^{-5}

described in the first identification of plasmid-mediated *fosA10* on a 53 kb IncFII plasmid in China [29].

3.5. Conjugation assays

Conjugation assays were performed on seven strains (three *fosA3*, one *fosA4*, two *fosA8*, and one *fosA10*) to assess the transmissibility of the FosA-encoding plasmids with diverse replicon types. All seven plasmids were capable of being transmitted with conjugation frequencies ranging from 4.33×10^{-1}–1.30×10^{-5} (Table 3), with the highest frequencies being observed for both FosA8-encoding plasmids (one IncX1/IncN and one IncFII) and the IncP1 FosA3-encoding plasmid. These data confirm the relative ease with which plasmid-mediated fosfomycin resistance can disseminate.

4. Conclusions

This epidemiological study describes an international collection of FosA-encoding plasmids and, notably, highlights the potential for the dissemination of *fosA* genes on MDR plasmids of unrelated structures. Plasmid-mediated antibiotic resistance dissemination in Gram-negative bacteria is a key driver of the epidemic of antibiotic resistant infections worldwide. Tracking plasmid spread may be elusive, mainly due to technical reasons linked to high-complexity regions of DNA that are not possible to assemble using short-read sequencing. By taking advantage of long-read sequencing to circumvent the technical issues, we were able to circularize 12 plasmids in this study, enabling for full plasmid characterization and comparative analyses.

Overall, the findings of this study underpin the necessity for the surveillance and close monitoring of fosfomycin resistance in *E. coli*, which is essential to maintain the optimal use of this treatment option.

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References

[1] Nicolle LE. Epidemiology of urinary tract infections. Clin Microb News 2002;24:135–40. doi:10.1016/j.ccc.2013.03.014.
[2] Foxman B. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. Am J Med 2002;113(Suppl 1A). 5S–13S. doi: 10.1016/s0002-9343(02)01054-9.
[3] Kahlmeter G, ECOSENS. An international survey of the antimicrobial susceptibility of pathogens from uncomplicated urinary tract infections: the ECO·SENS Project. J Antimicrob Chemother 2003;51:69–76. doi:10.1093/jac/dkg028.
[4] Cordoba G, Holm A, Hansen F, Hammerum AM, Bjerrum L. Prevalence of antimicrobial resistant Escherichia coli from patients with suspected urinary tract infection in primary care, Denmark. BMC Infect Dis 2017;17:670. doi:10.1186/s12879-017-2785-y.
[5] Schito GC, Naber KG, Botto H, Palou J, Mazzei T, Gualco L, et al. The ARESC study: an international survey on the antimicrobial resistance of pathogens involved in uncomplicated urinary tract infections. Int J Antimicrob Agents 2009;34:407–13. doi:10.1016/j.ijantimicag.2009.04.012.
[6] Falagas ME, Giannopoulou KP, Kokolakis GN. Rafailidis PI. Fosfomycin: use beyond urinary tract and gastrointestinal infections. Clin Infect Dis 2008;46:1069–77. doi:10.1086/527442.
[7] Tutone M, Bjerklund Johansen TE, Cai T, Mushtaq S, Livermore DM. SUsceptibility and Resistance to Fosfomycin and other antimicrobial agents among pathogens causing lower urinary tract infections: findings of the SURF study. Int J Antimicrob Agents 2022;59:106574. doi:10.1016/j.ijantimicag.2022.
[8] Dijkmans AC, Zacarías NVO, Burggraaf J, Mouton JW, Wilms EB, van Nieuwkoop C, et al. Fosfomycin: pharmacological, clinical and future perspectives. Antibiotics (Basel) 2017;6:24. doi:10.3390/antibiotics6040024.
[9] Oliva A, Volpicelli L, Di Bari S, Curtolo A, Borrazzo C, Cogliati Dezza F, et al. Effect of ceftazidime/avibactam plus fosfomycin combination on 30 day mor-

tality in patients with bloodstream infections caused by KPC-producing Klebsiella pneumoniae: results from a multicentre retrospective study. JAC Antimicrob Resist 2022;4:dlac121. doi:10.1093/jacamr/dlac121.
[10] Hughes S, Gilchrist M, Heard K, Hamilton R, Sneddon J. Treating infections caused by carbapenemase-producing *Enterobacterales* (CPE): a pragmatic approach to antimicrobial stewardship on behalf of the UKCPA Pharmacy Infection Network (PIN). JAC Antimicrob Resist 2020;2:dlaa075. doi:10.1093/jacamr/dlaa075.
[11] Bader MS, Loeb M, Brooks AA. An update on the management of urinary tract infections in the era of antimicrobial resistance. Postgrad Med 2017;129:242–58.
[12] Ríos E, Del Carmen López Diaz M, Culebras E, Rodríguez-Avial I. Rodríguez-Avial C.Resistance to fosfomycin is increasing and is significantly associated with extended-spectrum β-lactamase-production in urinary isolates of Escherichia coli. Med Microbiol Immunol 2022;211:269–72. doi:10.1007/s00430-022-00749-2.
[13] Zurfluh K, Treier A, Schmitt K, Stephan R. Mobile fosfomycin resistance genes in Enterobacteriaceae: an increasing threat. Microbiologyopen 2020;9:e1135. doi:10.1002/mbo3.1135.
[14] Falagas ME, Vouloumanou EK, Samonis G, Vardakas KZ. Fosfomycin. Clin Microbiol Rev 2016;29:321–47. doi:10.1128/CMR.00068-15.
[15] Yang TY, Lu PL, Tseng SP. Update on fosfomycin-modified genes in Enterobacteriaceae. J Microbiol Immunol Infect 2019;52:9–21. doi:10.1016/j.jmii.2017.10.006.
[16] Benzerara Y, Gallah S, Hommeril B, Genel N, Decre D, Rottman M, et al. Emergence of plasmid-mediated fosfomycin-resistance genes among Escherichia coli isolates, France. Emerg Infect Dis 2017;23:564–7. doi:10.3201/eid2309.170560.
[17] Nakamura G, Wachino J, Sato N, Kimura K, Yamada K, Jin W, et al. Practical agar-based disk potentiation test for detection of fosfomycin-nonsusceptible Escherichia coli clinical isolates producing glutathione S-transferases. J Clin Microbiol 2014;52:175–9. doi:10.1128/JCM.01094-14.
[18] Guo Q, Tomich AD, McElheny CL, Cooper VS, Stoesser N, Wang M, et al. Glutathione-S-transferase FosA6 of Klebsiella pneumoniae origin conferring fosfomycin resistance in ESBL-producing Escherichia coli. J Antimicrob Chemother 2016;71:2460–5. doi:10.1093/jac/dkw177.
[19] Kieffer N, Poirel L, Descombes MC, Nordmann P. Characterization of FosL1, a plasmid-encoded fosfomycin resistance protein identified in Escherichia coli. Antimicrob Agents Chemother 2020;64:19. doi:10.1128/AAC.02042-19.
[20] Wachino J, Yamane K, Suzuki S, Kimura K, Arakawa Y. Prevalence of fosfomycin resistance among CTX-M-producing Escherichia coli clinical isolates in Japan and identification of novel plasmid-mediated fosfomycin-modifying enzymes. Antimicrob Agents Chemother 2010;54:3061–4. doi:10.1128/AAC.01834-09.
[21] Nordmann P, Poirel L, Mueller L. Rapid detection of fosfomycin resistance in Escherichia coli. J Clin Microbiol 2019;57;01531:18. doi:10.1128/JCM.01531-18.
[22] Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing, 30th ed. Wayne, PA; 2020.
[23] EUCAST Clinical breakpoint table v.12.0; 2022 https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_13.0_Breakpoint_Tables.pdf.
[24] Wick RR, Judd LM, Gorrie CL, Holt KE. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol 2017;13:e1005595. doi:10.1371/journal.pcbi.1005595.
[25] Seeman T. Prokka: rapid prokaryotic genome annotation. Bioinf 2014;30:2068–9.
[26] Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, et al. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother 2012;67:2640–4. doi:10.1093/jac/dks261.
[27] Carattoli A, Zankari E, García-Fernández A, Voldby Larsen M, Lund O, Villa L, et al. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother 2014;58:3895–903. doi:10.1128/AAC.02412-14.
[28] Li G, Zhang Y, Bi D, Shen P, Ai F, Liu H, et al. First report of a clinical, multidrug-resistant Enterobacteriaceae isolate coharboring fosfomycin resistance gene fosA3 and carbapenemase gene blaKPC-2 on the same transposon. Tn1721. Antimicrob Agents Chemother 2015;59:338–43. doi:10.1128/AAC.03061-14.
[29] Huang Y, Lin Q, Zhou Q, Lv L, Wan M, Gao X, et al. Identification of fosA10, a novel plasmid-mediated fosfomycin resistance gene of Klebsiella pneumoniae origin. Escherichia coli. Infect Drug Resist 2020;13:1273–9. doi:10.2147/IDR.S251360.