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ORIGINAL ARTICLE

Molecular characterisation of fosfomycin resistance genes in *Escherichia coli* isolates

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Abstract
Fosfomycin is a well-known antibiotic that exhibits broad-spectrum activity against various bacterial pathogens, including gram-negative strains and some gram-positive strains such as staphylococci. The use of parenteral fosfomycin has been recently revised because the antibiotic has been found to effectively manage serious infections caused by multidrug-resistant pathogens. The occurrence of fosfomycin resistance could threaten the reintroduction of this antibiotic for the treatment of bacterial infections. In this study, a total of 24 fosfomycin-resistant *Escherichia coli* isolates obtained from urine samples were used to investigate the prevalence and molecular epidemiology of plasmid-mediated fosfomycin resistance genes. The replication origins of the conjugative and transformant plasmids obtained from the isolates were examined using the replication origin determination method based on the polymerase chain reaction (PCR). Through the PCR process performed with the *fosA*, *fosA3*, *fosB*, *fosC*, *fosC2*, and *fosX* genes to determine fosfomycin resistance, one out of 24 samples was found to be *fosA3* gene-positive. A Class-1 integron gene was detected in three fosfomycin-resistant *E. coli* isolates, while no Class-2 integrons were detected in any isolate. The conjugation experiments demonstrated that the *fosA3* gene was transferable in one isolate that also carried the *blaTEM*, *blaCTX-M-15*, and *aac(6′)-ib-cr* genes. Through plasmid isolation in the transconjugant *E. coli* isolates, it was determined that the *E. coli* isolate FF21 carried fosfomycin resistance on the plasmid. To ensure the continued effective use of fosfomycin as a treatment option, fosfomycin resistance needs to be detected and closely monitored. Given the global rise in plasmid-transmissible genes, we anticipate a growing resistance to fosfomycin in the near future.

Keywords Fosfomycin · *Escherichia coli* · FosA3 · Antimicrobial resistance · IncF · IncL/M

Introduction

Urinary tract infections (UTIs) are quite common worldwide and can recur often. Resistance to the antibiotics frequently used for treating UTIs, such as beta-lactam, the beta-lactam/beta-lactamase inhibitor combination, trimethoprim-sulfamethoxazole, quinolones, and aminoglycosides, is increasing rapidly, thus making it necessary to develop new antibiotics (Koken et al. 2008). Fosfomycin, which is a derivative of phosphonic acid, has become a preferred alternative to other antibiotics for treating uncomplicated UTIs because of its advantage of requiring only a single dose, having fewer side effects, and having a low resistance rate for *Enterobacterales* (Falagas et al. 2010).

Fosfomycin is a fermentation product of *Streptomyces fradiae*, *Streptomyces viridochromogenes*, and *Streptomyces wedmorensis*, and it is a phosphoenolpyruvate analogue. Fosfomycin irreversibly binds covalently to the 115 th cysteine residue of the MurA enzyme, thus inhibiting the MurA enzyme, preventing the formation of uridine diphosphate N-acetyl muramic acid and demonstrating its antibacterial activity by inhibiting the synthesis of the peptidoglycan layer (Baylan 2010).

Three separate mechanisms of fosfomycin resistance have been reported in the literature: target site modification (MurA), modifying enzymes (*FosA*, *FosB*, and *FosC*), and decreased permeability (GlpT or UhpT) (Castaneda-Garcia et al. 2013). Fosfomycin resistance mechanisms are either chromosomal or plasmid-mediated, and most chromosomal

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mutations occur in structural and regulatory genes that encode bacterial proteins that enable the transport of the drug into the cell. Plasmid-mediated fosfomycin resistance occurs with fosfomycin-modifying enzymes (fos genes). Fos genes catalyse the opening of the fosfomycin epoxide ring, and the drug is inactivated (Zurfluh et al. 2020). *FosA* and *fosB*, the plasmid-encoded glutathione S-transferase genes, contribute to fosfomycin resistance. *FosA* and its derivatives (*fosA2*, *fosA3*, *fosA4*, *fosA5*, and *fosA6*) can be found in extended-spectrum beta-lactamases (ESBL)-positive *Escherichia coli* plasmids. It is crucial to follow the resistance mechanisms because *fosA3*, in particular, spreads horizontally with the plasmid (Falagas et al. 2019; Benzerara et al. 2017; Cattoir and Guerin 2018; Díez-Aguilar and Cantón 2019).

The present study aimed to conduct molecular analyses in relation to acquired fosfomycin resistance and its genetic determinants in uncomplicated UTI *E. coli* isolates.

Materials and methods

Bacterial strains

Fosfomycin-resistant *E. coli* ($n = 24$) isolated from the urine samples of patients with a preliminary diagnosis of UTIs from various clinics in the Ahi Evran University Education and Research Hospital were included in this study.

Bacterial identification and antibiotic susceptibility testing

The urine samples used in this study were inoculated on 5% sheep blood agar (bioMérieux, Craponne, France) and eosin methylene blue agar (EMB; bioMérieux). The inoculated Petri dishes were incubated overnight at 37 °C, after which the colonies were evaluated. *Enterobacterales* were identified at the species level using the Vitek MS (bioMérieux) automated system. Two methods were used to determine the susceptibility of the isolates to fosfomycin following the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST; European Committee on Antimicrobial Susceptibility Testing breakpoints 2019). First, as the reference, the agar dilution method was carried out using Mueller Hinton Agar (MHA), to which 25 µg/mL glucose-6-phosphate was added. The susceptibility results were interpreted according to the available breakpoints defined by the EUCAST (susceptible at an minimum inhibitory concentration of ≤ 32 µg/mL for *Enterobacterales*). Second, the Kirby–Bauer disk diffusion method was employed for MHA plates using a fosfomycin disk (Oxoid Limited, Hampshire, UK). The disk contained 200 µg of fosfomycin and 50 µg of glucose-6-phosphate. The disk diffusion results for *Enterobacterales* were interpreted according to the zone diameter breakpoints for *E. coli* defined by the EUCAST (susceptible if ≥ 24 mm; resistant if < 24 mm).

Determining antimicrobial resistance genes

DNA was extracted from the *E. coli* isolates using the boiling method (Queipo-Ortuño et al. 2008). Plasmid-derived *fosA*, *fosA3*, *fosB*, *fosC*, *fosC2*, and *fosX*, which are known to be responsible for fosfomycin resistance; *blaTEM*, *blaSHV*, *blaOXA-1*, *blaPER-1*, and *blaCTX-M*, which are responsible for resistance to beta-lactams and antibiotics; *qnrA*, *qnrB*, *qnrS*, *aac-(6^')-ib-cr*, which are responsible for plasmid-mediated resistance to quinolone-group antibiotics; and the presence of Class 1 and Class 2 integrons were screened by means of polymerase chain reaction (PCR) methods using specific primers. All PCR products were subjected to DNA sequencing to determine the subtypes of *blaCTX-M*.

Bacterial genotyping

Repetitive element sequence–based PCR (rep-PCR) analysis, which is a DNA fingerprinting method, was used to determine the genotypic diversity of the 24 fosfomycin-resistant *E. coli* isolates. The amplified fragments of the isolates were examined, and a matrix was created from the existent and non-existent fragments among them. This matrix was then converted into a dendrogram using the unweighted pair group mean average (UPGMA) method in MEGA 6.0.

Determining plasmid-mediated fosfomycin resistance

Conjugation experiments were conducted using the broth-mating method to determine whether the plasmid-mediated fosfomycin resistance genes were present on the conjugative plasmid. For the PCR procedures, isolates containing one or more fos genes were used as donor cells, and a rifampicin-resistant *E. coli* isolate J53-2 was used as recipient cells (Alpay-Karaoglu et al. 2007). The donor and recipient cells were cultured in 3 mL of antibiotic-free Luria–Bertani broth and incubated overnight at 37 °C while shaking, while the cultures of these cells were mixed in equal volumes (1:1) and incubated overnight at 37 °C without shaking (Alpay-Karaoglu et al. 2007). To select transconjugants, a 100 µL 10^{-1} dilution of the conjugation mixture was dropped onto the EMB agar surface, to which 150 µL/mL rifampicin (*E. coli* J53-2 is resistant) and 32 µg/mL fosfomycin antibiotic (donor cell is resistant) were added using an automatic pipette, spread onto the agar surface with a sterile glass rod, and incubated at 37 °C for 18–24 h. Colonies with metallic blue-green highlights were phenotypically presumed to be transconjugants, and replica cultures were developed on EMB and Minimal Agar having four different types of content (Alpay-Karaoglu

et al. 2007). The plasmid transfers were confirmed by once again isolating the plasmid DNA from the transconjugants. To determine the number of viable donor cells required to calculate the conjugation efficiency, 10^{-5}, 10^{-6}, and 10^{-7} dilutions were made beforehand, and 100 µL of each was dropped onto the Luria Bertani agar surface and inoculated through spreading using a sterile glass rod. Next, the bacterial colonies were counted, and the number of viable bacteria was determined by multiplying the inoculated volume (mL) by the dilution coefficient using the following formula: number of viable bacteria = number of colonies in agar/inoculated volume (ml) × dilution coefficient (Alpay-Karaoglu et al. 2007).

Plasmid isolation

The isolation of the plasmids of the transconjugant *E. coli* isolates was conducted using the GeneJET Plasmid Miniprep Kit (Thermo Fisher Scientific, K0502) according to the manufacturer's instructions. The fosfomycin and other resistance genes carried by the isolated plasmids were identified using PCR and primers.

Determining PCR-based plasmid replication origin

The plasmids in the transconjugant cells were categorised using a PCR-based replicon-type procedure (Carattoli et al. 2005). The main mismatch groups of FIA, FIB, FIC, HI1, HI2, I1-I_γ, L/M, N, P, W, T, A/C, K, B/O, X, Y, F, and FIIA replicons were identified using 18 pairs of primers as well as five multiplex and three simplex PCRs. The primers used for the identification of the plasmid groups are presented in Table 2. The five multiplex and three simplex PCRs were conducted using these specific primers. The replicons that this study attempted to amplify were as follows: first multiplex: PCR, HI1, HI2, and I1-I_γ; second multiplex: PCR, X, L/M, and N; third multiplex: PCR, W, FIA, and FIB replicons; fourth multiplex: PCR, P, Y, and FIC; and fifth multiplex: PCR, T, A/C, and FIIA. In addition to the multiplex PCRs, the K, B/O, and FIIA replicons were amplified using gene-specific simplex PCRs.

Data analyses

The DNA sequences of *blaCTX-M* identified during the study were processed using BioEdit, and the similarity percentages that had closely related sequences were calculated using the Basic Local Alignment Search Tool (BLAST) from the NCBI GenBank database (Altschul et al. 1990). The rep-PCR data were arranged into a matrix according to the existence and non-existence of bands in the FASTA format. This matrix was then analysed using the UPGMA method in MEGA-X (Kumar et al. 2018).

Results

All 24 *E. coli* isolates studied here were found to be resistant to fosfomycin and susceptible to carbapenems, but their resistance rates for other antibiotics were high. These are presented in Table 1.

Through the PCR technique using *fosA*, *fosA3*, *fosB*, *fosC*, *fosC2*, and *fosX* to determine fosfomycin resistance, one (FF25) of 24 samples was found to be positive for *fosA3*. The presence of *blaTEM* was detected in 11 samples, *blaOXA*−1 was detected in six samples, and *blaSHV* was found in three samples. After the PCR technique was utilised to detect other *blaCTX-M* content in the samples, the products were sent for sequence analysis, and all 14 positive results were found to belong to the *CTX-M15* group. No *blaPER-1* s were detected in any of the isolates. The presence of *qnrA*, *qnrB*, *qnrS*, and *aac-(6^')-ib-cr*—which are responsible for plasmid-mediated resistance to quinolone group antibiotics—was verified using specific primers. Of the *E. coli* isolates, six included *qnrB* and *qnrS*, while nine included *aac-(6^')-ib-cr*. Further, none of the isolates included *qnrA*. Through the analyses of the contents of Class 1 and Class 2 integrons, a Class 1 integron was detected in three of the isolates (FF8, FF20, and FF23), whereas no Class 2 integrons were detected in any of the isolates. The antibiotic resistance genes of the isolates are summarised in Table 2.

The presence of fosfomycin resistance genes in a transferable plasmid in the 24 *E. coli* fosfomycin-resistant isolates was examined using conjugation experiments. The results indicated that the *E. coli* isolate FF25 transferred *fosA3* with 10^{-7} efficiency. Thereby, the conjugative plasmid detected was named pFF25. As a result of the conjugation, *blaTEM*, *blaCTX-M-15*, and *aac(6')-ib-cr* were detected in the transconjugant *E. coli* J53-2 (FF25) cell along with *fosA3* (Table 3).

Transformation experiments were conducted to determine whether the 24 fosfomycin-resistant isolates, which were found to be negative after the conjugation experiments, carried resistance to fosfomycin in their plasmids. These experiments suggested that the *E. coli* isolate FF21 carried fosfomycin resistance in its plasmid, which was consequently named "pFF21". No fosfomycin-resistant genes were detected in the wild-type or transformant cells; however, *blaSHV*, *aac(6')-ib-cr*, and *qnrS* were detected in the transformant plasmid (Table 3).

The clonal relationships of the isolates were studied using rep-PCR. Using the obtained dendrogram, fosfomycin-resistant *E. coli* isolates were found to have gathered in five groups. Specifically, isolates F22 and F23 from Group 1 A and F13 and F14 from Group 2 exhibited the same clonal similarities. The clonal relationships of the isolates are illustrated in the dendrogram in Fig. 1.

Table 1 Resistance rate of antibiotics

	Ampicillin	Amoxicillin-clavulanic acid	Piperasiline tazobactam	Cefixime	Cefuroxime	Cefotixin	Ceftazidime	Ceftriaxone	Trimethoprim/sulfamethoxazole	Fosfomycin	Nitrofurantoin	Amikacin	Gentamicin	Ciprofloxacin	Ertapenem	Imipenem	Meropenem
R%	87.5	41.6	29	33.3	60	12.5	29	41.6	62.5	100	8	12.5	50	37.5	0	0	0

The origin of replication of the conjugative pFF25 and transformant pFF21 plasmids obtained from the isolates was identified according to the PCR-based origin-of-replication determination method. It revealed that the pFF25 plasmid had an IncF replication origin. Through the transformation experiments, the replication origin of pTf21, another fosfomycin-resistant plasmid, was determined to be IncL/M.

Discussion

Fosfomycin has been successfully used in both the treatment of uncomplicated UTIs and as a part of combination therapies for infections caused by multidrug-resistant (MDR) bacteria. It is crucial to investigate fosfomycin resistance and the underlying mechanisms to be able to continue its use because of the difficulties associated with developing new antibiotics (Castaneda-Garcia et al. 2013).

Although plasmids can transfer their antimicrobial resistance genes to other species through conjugation, they can sometimes be integrated into chromosomal DNA (Oliveira et al. 2009). As plasmids can transfer most genes resistant to antimicrobials from species to species, the systematic monitoring of antibiotic resistance, infection control, and prevention is vital (Patwardhan et al. 2017). The plasmid-mediated fosfomycin resistance genes often observed in fosfomycin-resistant *Enterobacterales* isolates are *fosA* and *fosC2*. Both these resistance genes modify fosfomycin through glutathione S-transferase. Among the *fosA*-type resistance genes, *fosA3* is the most frequently reported plasmid-mediated resistance gene, which is commonly found in *E. coli* and other *Enterobacterales* species from East Asia (Ho et al. 2013; Jiang et al. 2015). Studies have attributed the increase in fosfomycin resistance observed in ESBL-producing *E. coli* and *Klebsiella pneumoniae* isolates primarily to the widespread emergence of plasmid-mediated *fosA3* (Lee et al. 2012a, 2012b; Yao et al. 2016; Cao et al. 2017). *FosA3* was first described in clinical *E. coli* isolates producing ESBL in Japan, with a positivity rate of 1.04% (Wachino et al. 2010). Many studies conducted in Asian countries have detected ESBL-producing *Enterobacterales* isolates in clinical samples obtained from farm animals, domestic animals, and humans, as well as in healthy individuals (Lee et al. 2012a, 2012b; Yao et al. 2016; Sato et al. 2013; Hou et al. 2013). Domestic animals have become sources of *Enterobacterales* isolates carrying *fosA3* in Asian countries, and this can be easily transferred through conjugation. Fosfomycin resistance is found to be higher in Asian countries than in European ones because of the transmission of plasmids carrying *fosA3* or clones carrying *fosA3* between domestic animals and humans. Fosfomycin resistance was found to be around 1% in *E. coli* that cause UTIs (Neuzillet et al. 2012; Martín-Gutiérrez et al. 2018). A study conducted in Taiwan found a

Table 2 Antibiotic resistance genes of fosfomycin-resistant *E. coli* isolates

Isolates	*fos*A	*fos*B	*fos*C	*fos*C2	*fos*A3	*fos*X	TEM	OXA-1	*SHV*	*PER-1*	CTX-M	qnrA	qnrB	qnrS	*aac-(6ı)-ib-cr*	Integron 1	Integron 2
FF1	-	-	-	-	-	-	-	-	-	-	**-**	-	-	-	-	-	-
FF2	-	-	-	-	-	-	+	-	+	-	**-**	-	+	+	+	-	-
FF3	-	-	-	-	-	-	-	-	-	-	**CTX-M15**	-	+	+	+	-	-
FF4	-	-	-	-	-	-	+	-	-	-	**-**	-	-	-	-	-	-
FF5	-	-	-	-	-	-	+	-	-	-	**-**	-	-	-	-	-	-
FF6	-	-	-	-	-	-	-	+	-	-	**CTX-M15**	-	-	-	-	-	-
FF7	-	-	-	-	-	-	-	-	-	-	**-**	-	-	-	-	-	-
FF8	-	-	-	-	-	-	+	-	-	-	**CTX-M15**	-	-	-	-	+	-
FF9	-	-	-	-	-	-	-	+	-	-	**CTX-M15**	-	-	-	-	-	-
FF10	-	-	-	-	-	-	+	-	-	-	**CTX-M15**	-	+	+	+	-	-
FF11	-	-	-	-	-	-	+	-	-	-	**-**	-	-	-	-	-	-
FF12	-	-	-	-	-	-	-	-	-	-	**-**	-	-	-	-	-	-
FF13	-	-	-	-	-	-	-	+	-	-	**CTX-M15**	-	-	-	+	-	-
FF14	-	-	-	-	-	-	-	+	-	-	**CTX-M15**	-	-	-	+	-	-
FF15	-	-	-	-	-	-	+	+	+	-	**CTX-M15**	-	+	+	+	-	-
FF17	-	-	-	-	-	-	-	-	-	-	**-**	-	-	-	-	-	-
FF18	-	-	-	-	-	-	-	+	-	-	**CTX-M15**	-	+	+	+	-	-
FF19	-	-	-	-	-	-	-	-	-	-	**-**	-	-	-	-	-	-
FF20	-	-	-	-	-	-	-	-	-	-	**CTX-M15**	-	-	-	-	+	-
FF21	-	-	-	-	-	-	-	-	+	-	**CTX-M15**	-	+	+	+	-	-
FF22	-	-	-	-	-	-	+	-	-	-	**CTX-M15**	-	-	-	-	-	-
FF23	-	-	-	-	-	-	+	-	-	-	**CTX-M15**	-	-	-	-	+	-
FF24	-	-	-	-	-	-	+	-	-	-	**-**	-	-	-	-	-	-
FF25	-	-	-	-	+	-	+	-	-	-	**CTX-M15**	-	-	-	+	-	-

Table 3 Plasmid-mediated fosfomycin resistance genes of isolates

Wild-type isolate	Resistance genes of wild-type isolate	Transferable type	Transferable isolates	Transferable resistance genes	Effectiveness
FF21	*blaSHV, CTX-M15, qnrS, qnrB, aac(6′)-ib-cr*	Transformation	*E. coli* DH5α (FF21)	*blaSHV, qnrS, aac(6′)-ib-cr*	10^{-8}
FF25	*blaTEM, CTX-M15,ac(6′)-ib-cr, fosA3*	Conjugation	*E. coli* J53-2 (FF25)	*blaTEM, blaCTX-M-15, ac(6′)-ib-cr, fosA3*	10^{-7}

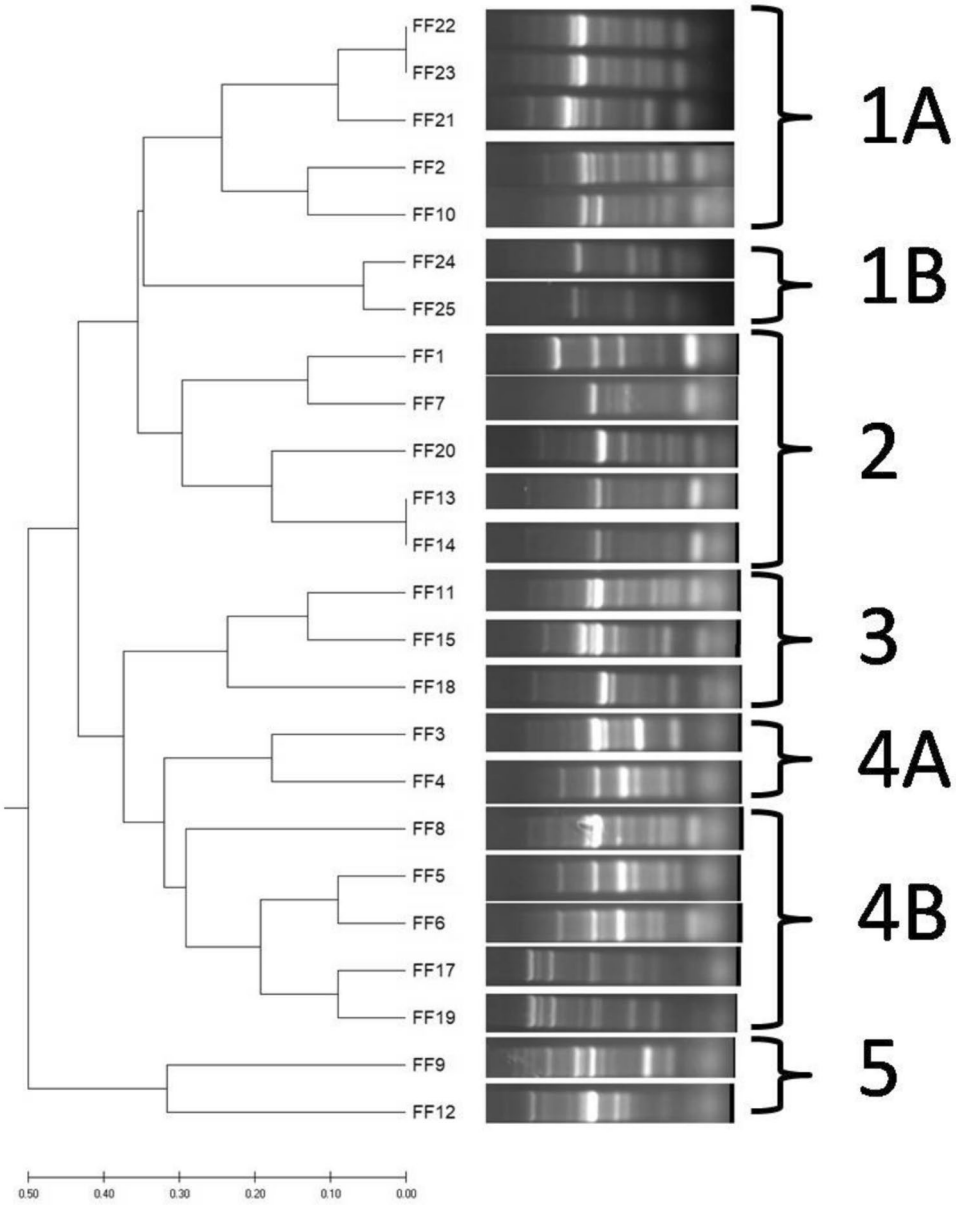

Fig. 1 Dendrogram of relationships among the 24 fosfomycin-resistant *E. coli*

fosfomycin resistance of 4.5% in ESBL-positive *E. coli* (Liu et al. 2011). Another study conducted in China revealed a fosfomycin resistance of 6.7% in *E. coli* isolates, and the presence of *fosA3* was detected in these isolates (Bi et al. 2017). In the present study, *fosA3* was found in only one (FF25) of the 24 isolates resistant to fosfomycin. In addition, 4.8% of the fosfomycin-resistant isolates tested positive for *fosA3*.

FosC2 is another gene that is believed to be responsible for plasmid-mediated fosfomycin resistance in *Enterobacterales* isolates. *fosC2* has an amino acid sequence that is 56% similar to that of *fosA*. Although *fosA* and *fosC2* have

different sequences, both of these resistance genes modify fosfomycin through glutathione S-transferase. *fosC2*, like *fosA3*, was first identified in Japan in clinical *E. coli* isolates producing ESBL (Wachino et al. 2010). *FosC2* was identified along with different resistance genes in Class 1 integrons. Previous research has shown the presence of a Class 1 integron carrying *fosC2*, *dfrA17*, and *aadA5* in a plasmid and *fosC2* and *blaIMP-34* in MDR plasmids (Wachino et al. 2010; Wang et al. 2015). These data indicate that *fosC2* is acquired through integrons to generate a response against antibiotics (Yang et al. 2019). The studies conducted by Bi et al. (2017) and White et al. (2017) using ESBL-producing *E. coli* isolates and carbapenem-resistant *Enterobacter* spp. isolates, respectively, did not detect *fosC2*-positive isolates. In the present study, similar to the results reported in the literature, no *fosC2*-positive isolate was found.

The results of various studies have demonstrated that the clonal spread of *CTX-M15* is associated with fosfomycin resistance. *BlaCTX-M* and *fosA3* can be transmitted by plasmids that have the same replicon types, and previous research has revealed that plasmids can spread two genes simultaneously (Sato et al. 2013; Hou et al. 2013). Lee et al. (2012a, 2012b) found the concurrent presence of *fosA3* and *blaCTX-M* in seven (five *E. coli* and two *K. pneumoniae*) isolates that were not sensitive to fosfomycin. In addition, using sequence analyses, they found that *fosA3* bound to *blaCTX-M* is located between two oppositely oriented IS26 elements, forming an IS26-composite transposon, and that the IS26-composite transposon appears to be the main vehicle for *fosA3* to spread in various clones of *E. coli* and *K. pneumoniae* (Lee et al. 2012a, 2012b).

Nigiz et al. (2022) detected *fosA3* in one of nine *Klebsiella* isolates resistant to fosfomycin and reported that this isolate carried *blaCTX-M* together with *fosA3*; however, no *fosC2*-positive isolate was found.

In the current study, a Class 1 integron gene cassette was detected in three of the isolates (FF8, FF20, and FF23), and Class 2 integron content was not detected in any isolate. The *E. coli* isolate FF25 was found to transfer *fosA3* and was named "conjugative plasmid pFF25" based on the conjugation experiment. In addition, the transconjugant *E. coli* J53-2 (FF25) cell carried *fosA3* as well as *blaTEM*, *blaCTX_M15*, and *aac(6')-ib-cr*. Consistent with other studies, this result indicates that the clonal spread of *CTX-M15* is associated with fosfomycin resistance. According to our knowledge, it was found that this is the first time the association between *CTX-M15* and *fosA3* for *E. coli* isolates has been reported from Turkey. According to the results of the transformation experiments, the *E. coli* isolate FF21 was found to carry the fosfomycin resistance gene on its plasmid. The identified plasmid was named "pFF21"; however, *blaSHV*, *aac(6')-ib-cr*, and *qnrS* resistance genes were also detected on the transformant plasmid. The result of PCR analysis showed that the replication origin of pFF25 was IncF. Further, the replication origin of pTf21, which was another plasmid that was determined to have fosfomycin resistance, was found to be IncL/M.

IncF are conjugative plasmids with a host range limited to the *Enterobacterales* family. IncF plasmids are heterogeneous with variable sizes and often carry more than one replicon and resistance gene. The most frequently identified resistance genes in IncF plasmids are as follows: ESBL genes, genes encoding carbapenemases, genes encoding aminoglycoside-modifying enzymes, and plasmid-mediated quinolone resistance genes. The global spread of *blaCTX-M-15* in human *E. coli* isolates has been associated with IncFII plasmids. IncL/M is a group of broad-host-range plasmids. The IncL/M type plasmid is associated with the global spread of *blaOXA-48*. IncL/M plasmids can also carry *blaCTX-M-1*, *blaCTX-M-3*, *blaCTX-M-14*, *blaCTX-M-15*, *blaTEM-1*, *blaTEM-10*, *blaTEM-52*, *blaSHV-1*, and *armA* genes (Rozwandowicz et al. 2018).

Wang et al. (2021) examined fosfomycin resistance and molecular characterisation in carbapenem-resistant *Klebsiella* isolates and found that 81.8% of the isolates carried *fosA3*, that 12.7% carried *fosA5*, and that there was clustering in the clone ST11. In their genomic analyses, they have noted that the insertion sequence IS26 predominantly flanked the *fosA3* structure and played an important role in *fosA3* propagation. The authors also found that *fosA3* genes in six isolates were located on plasmids that could be transferred to *E. coli* J53 recipient cells through conjugation (Wang et al. 2021). Previous studies have reported that a *fosA3*-carrying plasmid is classified as mismatch group IncFII, IncN, IncI1, and IncB/O or is not determined. IncF plasmids are heterogeneous, vary in size, and often carry multiple replicons and resistance genes (Jiang et al. 2017; Villa et al. 2010).

Conclusions

The contribution of fosfomycin-inactivating enzymes to the emergence and spread of fosfomycin resistance currently appears to be low; however, their presence in transferable plasmids can enable the potential spread of fosfomycin resistance in the future. They coexist with genes that confer resistance to classes of antibiotics, such as beta-lactams, fluoroquinolones, and aminoglycosides, which threaten the development of multidrug resistance. In our study, we believe it is important to detect the ESBL gene and quinolone resistance gene, along with the fosfomycin resistance gene, in IncF and IncL/M plasmids. These findings suggest that both IncF and IncL/M plasmids are significant carriers of life-threatening resistance genes. Data from recent studies indicate that the therapeutic use of other classes of antibiotics promotes the emergence and spread of

fosfomycin-resistant *Enterobacterales*. Geographically, plasmid types differ; thus, our results will contribute to the literature. Furthermore, understanding the global epidemiology of acquired fosfomycin resistance genes will provide insights into the major transmission routes of these resistance profiles. Some plasmids strongly correlate with specific genes (e.g. IncL/M-*blaOXA-48*), yet these particular relationships remain unclear. Although the efficacy of fosfomycin appears to be high, it is crucial to intermittently monitor the associated resistance rates and molecular mechanisms because of its use as monotherapy or in combination with other antibiotics for the treatment of infections caused by MDR bacteria.

Declarations

Conflict of interest The authors declare no competing interests.

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