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Original Article

Prevalence of silent plasmid-mediated fosfomycin-resistance genes among clinical isolates of fosfomycin-susceptible *Escherichia coli*

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Abstract

Introduction: Infections caused by *Escherichia coli* place a considerable burden on both patients and healthcare facilities. This study aims to investigate the prevalence and molecular detection of silent plasmid-mediated fosfomycin-resistance (PMFR) genes among fosfomycin-sensitive, extended-spectrum β-lactamase (ESβL)- producing *E. coli* isolates.

Methodology: Clinical samples of *E. coli* were collected from various sources and hospitals in Duhok, Iraq, from December 2020 to April 2021. Standard microbiological techniques and the Vitek II system were used to identify *E. coli,* which was further confirmed through species-specific genes. The Kirby-Bauer method was employed for antimicrobial sensitivity testing, followed by the detection of targeted PMFR and genes using conventional polymerase chain reaction (PCR).

Results: High resistance levels were observed against ampicillin, ceftriaxone, tetracycline, and ciprofloxacin, whereas a lower resistance rate was detected for carbapenems. Notably, all the isolates were susceptible to fosfomycin. Four isolates tested positive for *fosA3*, four were positive for *fosA*, and 16 were positive for the $bla_{CTX\text{-}M9}$ gene. Within the isolates, three co-harbored *fosA3, fosA*, and $bla_{CTX\text{-}M9}$ genes, while one isolate co-expressed *fosA3* and $bla_{CTX\text{-}M9}$ genes. The distribution of these genes, both individually and co-harbored, was observed across all clinical samples analyzed, except those derived from blood and sputum.

Conclusions: The dissemination of silent PMFR genes could pose a future risk for public health under the selective pressure of antibiotics, especially extended-spectrum beta-lactams, for a long time, or transfer to other bacteria, potentially leading to the activation of these genes.

Key words: clinical samples; silent genes; *E. coli*; fosfomycin.

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Introduction

Fosfomycin was identified in 1969 and has a broad range of bactericidal effects against multiple Gram-positive and Gram-negative bacteria. This drug suppresses the formation of peptidoglycan by irreversibly attacking UDP-N acetylglucosamine enolpyruvyl transferase (MurA) [1]. Recently, however, fosfomycin has been receiving renewed worldwide attention as one of the most active agents for preserving carbapenems in ESβL-producing isolates [2]. A study of fosfomycin in pediatrics suggests it for treating lower urinary tract infections (UTIs) in children up to 6 years old and in adults, as well as for osteomyelitis, particularly in cases due to methicillin-resistant *Staphylococcus aureus* [3]. Furthermore, it is recommended for treating bacteremia caused by multidrug-resistant Gram-negative bacilli, especially those involving *Enterobacteria* that carry carbapenemases [4].

The clinical use of fosfomycin has indeed increased; this increase has been associated with the emergence of resistance mechanisms among bacteria, especially when utilized as monotherapy [5]. These mechanisms include decreased drug uptake, drug inactivation by modification of the target enzyme, and enzymes (*fos* genes) that can inactivate the antibiotic molecule as four main types [6]. The four main types of these enzymes are *fosA, fosB, fosC,* and *fosX,* each of which may have various subtypes. Notably, the *fosA, fosA2, fosA3, fosA4,* and *fosC2* genes have been identified in fosfomycin-resistant *Enterobacteriaceae* [7]. The *fosA* is for a glutathione S-transferase that facilitates the conjugation of glutathione to the C1 position of the epoxide ring of the fosfomycin, thereby assisting in the antibiotic resistance mechanism [8].

Plasmid-mediated *fosA* was recognized for the first time in 1980 in clinical strains of *Serratia marcescens* and has since been detected in various *Enterobacteriaceae*, *Pseudomonas* spp., and *Acinetobacter* spp. [9]. It is a metalloenzyme that is transferred via plasmids within *Enterobacterales,* with the *fosA3* variant being the most prevalent identified determinant present as an acquired mechanism in *E. coli* [10]. In addition, the *fosA3* gene is often found on

conjugative plasmids that also harbor genes responsible for producing *CTX-M*-type ESβL-encoding genes [11]. Since 2006, researchers in several countries in East Asia have described plasmid-mediated *fosA3* and, less frequently, *fosA5,* which are mostly associated with *CTX-M* and co-harbored on a conjugative plasmid. In 2016, Portugal reported the first imported case of a travel-related infection in Europe involving an *E. coli* strain co-expressing *fosA3* and *CTX-M*-15 [12]. Several studies focus on silent antibiotic resistance genes in phenotypically sensitive bacteria [13,14]. The concept of an antibiotic resistance groups encompasses all known and unknown resistance-related genes-whether expressed, silent, or proto-resistance-into a single framework [15]. Silent resistance genes, which are typically not expressed, can become active through recombination or mutation, potentially conferring resistance if mobilized into a new host [13]. Despite the global increase in fosfomycin use, susceptibility testing is not routine, and data on resistance profiles are limited in this context. Diagnosing bacterial resistance based solely on phenotypic resistance provides limited insight into bacterial evolution. This study aims to investigate the prevalence of silent PMFR genes (*fosA* and *fosA3*) among fosfomycin-sensitive, ESβL-producing *E. coli* isolates in Duhok City, Iraq.

Methodology

Bacterial strains and susceptibility testing

A total of 110 *E. coli* clinical isolates (comprising 85 from urine, 12 from wound swabs, 2 from blood culture, 2 from sputum, 7 from vaginal swabs, and 2 from semen fluid samples) collected from the study site in Duhok city, Iraq, between December 2020 and the end of April 2021, were investigated retrospectively. The sources and patient demographics for the isolate collections have been reported previously by Albazaz and Yassin [16]. Regarding clinical samples, all participants provided written informed consent before the commencement of the study, and the privacy of the collected information was safeguarded. The study process received approval from the relevant ethics committees at the University of Duhok and the General Health Directorate. The approval is associated with a specific protocol number, ET-P 8/11/2020. Inclusion criteria included patients who were not taking antibiotics 3 days prior and those who provided formal consent to participate in the study, while exclusion criteria involved patients who had taken antibiotics 3 days prior and those who refused to participate in the study.

Identification of Isolates

All purified strains were initially identified using traditional biochemical tests, followed by confirmation with the VITEK-2 Compact system. Species-level identification was further verified through amplification of the *uid*A gene using the primers F: AAAACGGCAAGAAAAAGCAG and R: ACGCGTGGTTACAGTCTTGCG. The amplification resulted in a product of 147 bp with an annealing temperature of 58°C [17].

Antibiotic-Susceptibility testing

A susceptibility test was conducted using a panel of 16 antibiotics from various classes, including Ampicillin (Am), Amoxicillin+clavulanic acid (AMC), Piperacillin-tazobactam (TZP), Ceftriaxone (CRO), Ceftazidime (CAZ), Cefepime (FEP), Cefoxitin (CFM), Cefotaxime (CTX), Meropenem (MRP), Imipenem (IMI), Gentamicin (CN), Tetracycline (TE), Trimethoprim/Sulfamethoxazole (SXT), Chloramphenicol (C), Ciprofloxacin (CIP), and Fosfomycin (F), using the agar disk diffusion method. The results were primarily categorized as resistant, intermediate, or susceptible based on the observed responses [18]. Breakpoints for fosfomycin (200 ug disc) were applied for *E. coli* clinical isolates and the sensitivity of the isolates was assessed using the Kerby-Bauer method, and the results were interpreted based on the criteria established by the Clinical and Laboratory Standards Institute (inhibition zone diameters: ≥ v16 mm, sensitive; intermediate, 13-15 mm; and ≤ 12, resistant [18].

Extended-spectrum β-lactamase production determination

In accordance with Drieux *et al.* (2008), all isolates were tested for the production of phenotypic extended-spectrum beta-lactamases (ESβLs) using a double disc synergy test (DDST) with four antibiotics: amoxicillin/clavulanic acid (AMC, 20/10 g), cefotaxime (30 g), ceftazidime (30 g), and aztreonam (ATM, 30 g).

Preparation of DNA templates for PCR testing

The purified clinical *E. coli* isolates were subsequently analyzed using polymerase chain reaction (PCR) with specific primers to determine the existence of *fosA*, *fosA3,* and *CTX-M* genes. For genomic extraction, the heat shock method was used, and crude lysates were used as templates for PCR assays as described by Yuan *et al.* [19]. The crude DNA samples were used for the amplification of those genes using the

Table 1. Primers used in this study.

Target gene	Primer	Sequence, 5------3'	Annealing Temp, C°	Amplicon size, bp	Reference
fosA	F	ATCTGTGGGTCTGCCTGTCGT	58	217	[20]
	R	ATGCCCGCATAGGGCTTCT			
fosA3	F	CCTGGCATTTTATCAGCAGT	57	282	[21]
	R	CGGTTATCTTTCCATACCTCAG			
BlaCTX-M9	F	GCT GGA GAA AAG CAG CGG AG	57	474	[22]
	R	GTA AGC TGA CGC AAC GTC TG			

corresponding primers listed in Table 1. PCR reactions were performed in a total volume of 25 µl, containing 1 µL of each primer (10 pmol/µL), 10 µL of hot start master mix, 3 µl of DNA with a concentration of 30-100 ng/ µL, and 10 µL of nuclease-free water. After amplification, 10 µl aliquots of the PCR products were subjected to 2% agarose gel electrophoresis, followed by gel staining and DNA safe staining. The sizes of the amplicons were evaluated by comparing them to a 100bp DNA marker. UV-induced fluorescence was employed to visualize DNA fragments of a specific molecular weight following amplification.

Statistical analysis

The data was tabulated and analyzed using SPSS software version 25. Frequency and chi-square test were used to analyze the data. A $p < 0.05$ was considered significant.

Results

Distribution of E. coli isolates

The majority of clinical Escherichia coli isolates were derived from urine samples, totaling 85 isolates, which represents 77.3% of the overall sample. Among these, 71.8% were obtained from outpatients, 74.2% were female, and individuals aged 21 to 30 years constituted 20% of the isolates. Conversely, the lowest prevalence of isolates was observed in wound swabs, with 12 isolates (10.9%), followed by high vaginal swabs (HVS) with 7 isolates (6.4%), sputum with 2 isolates (1.8%), blood with 2 isolates (1.8%), and semen fluid with 2 isolates (1.8%).

Antibiotic sensitivity patterns

All 110 *Escherichia coli* isolates were determined to be susceptible to fosfomycin as assessed by the disk diffusion method. In contrast, the resistance patterns exhibited by these isolates varied across other antibiotic classes, with significant resistance observed particularly against penicillins, cephalosporins, aminoglycosides, and fluoroquinolones. The specific resistance rates recorded were 86% for ampicillin, 74% for ceftriaxone, 72% for tetracycline, and 48% for ciprofloxacin. Conversely, the lowest resistance rates were noted for imipenem and meropenem, at 4% and 2%, respectively, suggesting that carbapenems are the most effective antibiotics against these isolates (Table 2).

Detection of antibiotic resistance genes

CTX-M9 gene

According to the CLSI guidelines, phenotypic double-disc synergy testing revealed that 41.4% of *E. coli* isolates (n = 46) were ESβL producers. The majority is detected in urine samples compared to other clinical sources. Furthermore, when the isolates were examined for the presence of the *CTX-M9* gene revealed that the $bla_{CTX\text{-}M9}$ ESβL gene was detected in 14.5% of the *E. coli* isolates (n = 16), as detailed in Table 3. A significant association was found between the source of the isolates and the presence of the *CTX-M9* gene $p = 0.006$. The majority of these positive

Table 2. Antibiotics sensitivity patterns of clinical *E. coli* isolates.

Antibiotics	Resistant	Intermediate	Susceptible
Fosfomycin (F)	0 (0)	0 (0)	110 (100)
Ampicillin (Am)	95 (86)	0 (0.0)	15 (13)
Amoxillin + clavulanic acid (AMC)	61 (55)	16 (14)	33 (30)
Piperacillin-tazobactam (TZP)	25 (23)	10 (9)	75 (68)
Ceftriaxone (CRO)	82 (74)	0 (0.0)	28 (25)
Ceftazidime (CAZ)	84 (76)	0 (0.0)	26 (24)
Cefepime (FEP)	83 (75)	0 (0.0)	27 (25)
Cefoxitin (CFM)	53 (48)	5 (4)	52 (47)
Cefotaxime (CTX)	73 (66)	1 (0.9)	36 (33)
Meropenem (MRP)	4 (3)	0 (0.0)	106 (96)
Imipenem (IMI)	5 (4)	1 (0.9)	104 (94)
Gentamicin (CN)	37 (33)	0 (0.0)	73 (66)
Tetracyclin (TE)	79 (72)	1 (0.9)	30 (27)
Trimethoprim/ Sulphamethoxazole (SXT)	59 (54)	1 (0.9)	50 (45)
Chloramphenicol (C)	33 (30)	2 (2)	75 (68)
Ciprofloxacin (CIP)	53 (48)	2 (2)	55 (50)

Table 3. The correlation between phenotypic ESBL and CTXM.

Sample Type	No. of isolates (%)	ESβL-producer's, No. (%)	Gene amplification *bla*CTX-M
Urine	85 (71.8)	27 (31.4)	9
Wound	12 (10.9)	12 (10.9)	-
HVS	7 (6.4)	7 (6.4)	2
sputum	2 (1.8)	2 (100)	1
Blood	2 (1.8)	2 (100)	2
semen	2 (1.8)	2 (100)	2
Total	110	46 (41.4)	16 (14.4)

isolates originated from urine samples (13 isolates), which represent the most prevalent source of the gene, followed by semen fluid and HVS.

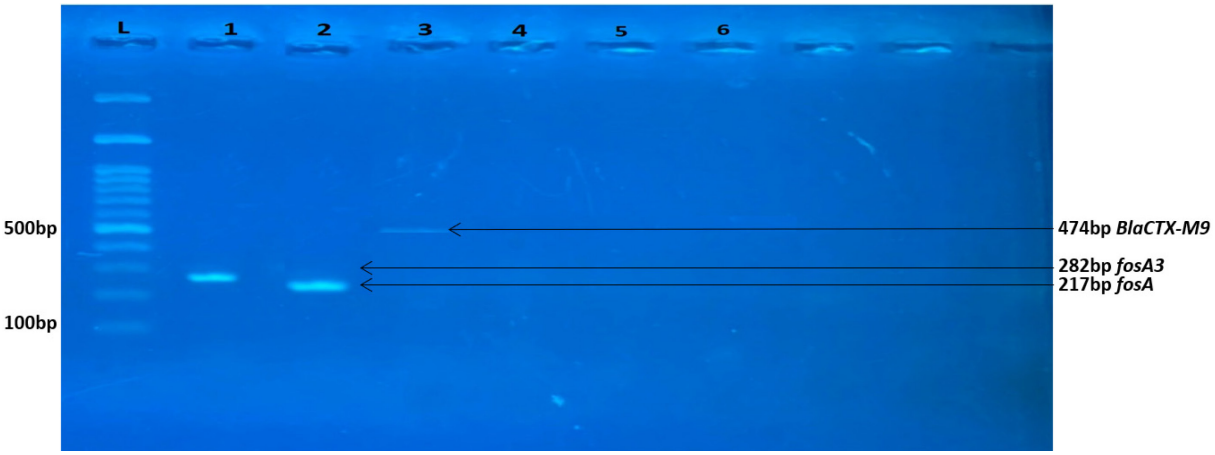
fosA and *fosA3* genes

Among the 110 fosfomycin-susceptible *E. coli* screened for plasmid-mediated fosfomycin resistance genes, specifically *fosA* and *fosA3*, it was found that only 8 strains (7.2%) harbored these PMFR genes. Each gene was identified in 4 strains, corresponding to a frequency of 3.6% for each. The remaining 102 isolates, which were phenotypically susceptible to fosfomycin, did not possess the investigated PMFR genes. Notably, two urine isolates and an isolate each from high vaginal swabs (HVS) and semen fluid tested positive for both *fosA* and *fosA3* in clinical specimens. The bands of *fosA*, *fosA3*, and *CTX-M9* genes visualized under UV-transilluminator are illustrated in Figure 1. The genes of interest showed co-expression, particularly in instances of two-co-harbored genes (*fosA* and *fosA*3 in 3 isolates) and three co-harbored genes (*fosA*, *fosA*3, and *CTX-M9* in 3 isolates) (Table 4).

Discussion

Although fosfomycin is rarely used as an antibiotic, and as a result, susceptibility testing may not be commonly performed in all labs. This can lead to a gap in information on the sensitivity profiles of *E. coli* isolates from clinical settings. In this study, 110 clinical *E. coli* isolates were collected, all were fully phenotypically susceptible to fosfomycin (100%) using the disc-diffusion method. In a recent study in our

Figure 1. PCR products of *fosA3*, *fosA* and bla_{CTX-M9} genes.



Lane L: 100 bp DNA ladder, Lane 1: 282bp positive *fosA3* gene, Lane 2:217bp positive *fosA*, Lane 3:474bp positive bla_{CTX-M9} gene, and Lane (4,5,6) negative isolates from urine.

Table 4. Association co-harboring genes of PMFR and *CTX-M9* genes among clinical *E. coli* isolates from different sources.

Genes patterns	No. (%)	Urine	Wound	HVS	Semen	Blood	Sputum	*p*
fosA	4 (3.6)	2	-	1	1	-	-	0.008
fosA3	4 (3.6)	2	-	1	1	-	-	0.008
CTX-M9	16 (14.5)	9	-	2	2	2	1	0.006
Total	24 (21.8)	13		4	4	2	1	
Co-carriage								
fosA, fosA3	3	1	-	1	1	-	-	
fosA, CTX-M9	1	1	-	-	-	-	-	
fosA3, CTX-M9	0	-	-	-	-	-	-	
fosA, fosA3, CTX-M9	3	1	-	1	1	-	-	
Total	7 (29.1)	3	-	2	2	-	-	

neighboring country, Turkey, bloodstream-infected *E. coli,* the fosfomycin susceptibility was 64.3%, whereas no fosfomycin-susceptible isolates were found in the *K. pneumoniae* isolates [23]. Generally, global studies report a high susceptibility of *E. coli* clinical isolates to fosfomycin, with rates of 96.5% in Japan [24], 99% in China [25], 90% in Pakistan [26], 99.1% in France [12], 88% in Spain [27], 97.9% in Uruguay [28], 99.9% in Australia [29], and 97.8 % in South Africa [30]. In contrast, a fosfomycin resistance rate of 38.5% was observed among uropathogenic *E. coli* isolates in Egypt [31]. The antibiotic-susceptibility results of the isolates showed that the least effective antibiotics against isolates were ampicillin, amoxicillin-clavulanic acid, ceftazidime, trimethoprim-sulfamethoxazole, cefioxitine, ceftriaxone, cefepime, and cefotaxime, with respective percentages of 86%, 55%, 76%, 54%, 48%, 74%, 75%, and 66%. These antibiotics could cause problems in clinical practice. This research, in line with findings of Ahmed *et al.* [32], while contradicting the results of Maleki *et al.* [33] in Iran, reported resistance rates for ceftazidime and cefotaxime of 26.1% and 30%, respectively. The ongoing study revealed that 41.4% of the isolates exhibited a phenotypic ESβL-producing profile, while 14.4% carried the *CTX-M9* gene. Urine samples had the highest ESβL production and gene amplification rates, while wound isolates showed relatively high ESβL-production but no detectable amplification of the resistance gene. This may indicate other resistance mechanisms, especially among phenotypic ESβL-producing isolates that lack the *CTX-M9* gene. A recent study in Iran reported that 51% of the fosfomycin-resistant isolates carried the *CTX*-M gene [34]. Fosfomycin-resistant ESβL-producing *E. coli* strains carrying *CTX-M-14, CTX-M-15,* or *CTX-M-27* have been previously reported in both Spain and Japan [35,36]. However, those isolates carried the plasmid-mediated *fosA3* gene and exhibited high fosfomycin MIC values. However, infections caused by ESβL-producing *E. coli* are typically linked to significantly longer hospital stays and increased healthcare costs, even though the evidence suggesting a higher rate of fosfomycin resistance among these strains remains hypothetical [36]. Such an association of resistance genes was not observed in another study conducted in Iran [34]. In considering ESβL-producing isolates, patients in our community frequently utilize third-generation cephalosporin antibiotics, while fosfomycin usage is low. This reduced use of fosfomycin may be linked to a decrease in resistance [37] as well as differences in geographic dispersion and sample size restrictions [31]. A study done by Oteo *et al.* [36] found that fosfomycin resistance in ESβL-producing *E. coli* rose from 2.2% in 2003 to 21.7% in 2008, coinciding with a 50% increase in fosfomycin usage.

This is the first study to investigate the prevalence of plasmid-mediated *fosA* and *fosA3* genes among susceptible-fosfomycin isolates. Out of 110 phenotypically susceptible isolates, the *fosA* and *fosA3* genes each appeared in 4 samples (8 isolates), representing 3.6% of each gene. These were primarily found in urine (2 samples each) and in HVS and semen (1 sample each) for each gene. The presence of these genes among susceptible isolates suggests that they may have been acquired from abroad in our region, as the use of these antibiotics is not routine in our region. Considering silent antibiotic-resistance genes, another study conducted by our team in Iraq, it was found that 17 out of 32 fluoroquinolone-sensitive *K. pneumoniae* isolates carried silent plasmid-mediated quinolone resistance genes [38]. A recently published retrospective study conducted in China on *Salmonella enterica* isolates from various sources between 2019 and 2022 found that 10.7% of positive *fosA*7 were fosfomycin-susceptible [39]. The spread of silent fosfomycin-resistance genes in *Klebsiella pneumoniae* isolates with high priority was also noted by Monte & de Oliveira, 2024 [40]. The frequency of *fosA* and *fosA3* in fosfomycin-resistant clinical isolates of *E. coli* has been documented in several investigations conducted both regionally and globally. In the neighbored country Iran, neither *fosA3* nor *fosA* were detected among *E. coli* isolates [41], in Turkey 5.8% isolates were *fosA*-positive, but *fosA3* was not detected [23], 3.6 % *fosA3* positive was in Japan [24] in China, *fosA3* and *fosA* (each four genes from urine and blood) accounted for 6 and 2 of the 18 fosfomycin-resistant clinical *E. coli* isolates, respectively [25], 7 out of 1354 screened isolates were *fos*A3-positive in France [12], 60.5% was *fosA*-positive in Mexico [42], 15 % was *fosA*-positive in Portugal [43], *fosA3* (47.8%) and *fosA* (34.8%) were positive in Egypt [31]. These results demonstrate the value of molecular testing in situations when phenotypic testing for antibiotic susceptibility may not yield a conclusive result. *FosA* and *fosA3* may not represent a major public health concern at this time in our setting; however, if they are carried by a high-copy plasmid or mobile genetic elements that result in enhanced gene expression, there is a possibility that fosfomycin resistance could arise. Consequently, care should be used while assessing this fosfomycin resistance gene's clinical significance [14,20]. Moreover, the bacteria in the animals (particularly pets)

in the area that contain and spread this resistance gene may be the cause of this outcome [44].

*CTX-M*9 is the most prevalent gene, found in 16 samples (14.5% of total), as in the current day. This gene is predominantly detected in urine (9 samples), followed by HVS (2 samples), semen (2 samples), blood (2 samples), and sputum (1 sample). This suggests that *CTX-M9* is commonly found in urine samples, which might indicate its association with UTIs or its persistence in the urinary tract. Furthermore, the co-occurrence of *fosA*, *fosA*3, and *CTX-M*9 is observed in 3 samples (1 each from urine, HVS, and semen).

To the best of our knowledge, this study is the first instance of detecting *fosA, foA3*/ *CTX-M-9* in *E. coli* isolates derived from human sources in this context. This suggests that the simultaneous presence of these three genes could indicate a higher level of antibiotic resistance or a more complex infection, which may involve multiple resistance mechanisms. Nevertheless, even though none of the patients had ever been treated with fosfomycin, this investigation found genetic evidence of fosfomycin resistance genes (*fosA* and *fosA3*) in *E. coli* isolates recovered from patients. Initially, from 2006, scientists from a number of East Asian nations have reported plasmid-mediated *fos*A3, which is co-harbored on a conjugative plasmid and mostly linked to *CTX-M* [45]. Studies have concentrated on human clinical *E. coli* strains in China [25], South Korea [46], and Japan [24]. While some research has examined veterinary *E. coli* strains isolated from pets across China [44]. Portugal announced in 2016 that an *E. coli* strain co-expressing *fosA3* and *CTX-M-15* was the first imported instance of a travel-related infection in Europe [45]. Given that additional resistance genes, like *CTX-M,* enable co-selection of *fosA3* and *fosA* by cephalosporins and/or aminoglycosides (particularly amikacin and gentamicin), which have been widely used for UTI therapy in current times, the association of *fosA3* and *fosA* with other resistance determinants (*CTX-M*) has likely favored the dissemination and maintenance of these antibiotic resistance determinants among bacteria. This study finding suggested that the genes *fosA*, *fosA3,* and *CTX-M9* were found on the same conjugative plasmids and consistent with these reports [23,24,38,43,44]. Our results corroborated that *fosA3* and $bla_{CTX\text{-}M9}$ genes were co-located on the same conjugative plasmids, thus allowing the simultaneous dissemination of these antibiotic resistance determinants among bacteria.

In conclusion, despite fosfomycin not being used by our patients, all recovered clinical *E. coli* isolates were fosfomycin-susceptible, but the presence of silent fosfomycin-resistance genes (*fosA* and *fosA3*) was detected and mainly limited to urine, HVS, and semen. The *CTX-M9* gene is the most prevalent across samples, particularly in urine. Co-carriage is most common in urine and semen, with *fosA*, *fosA3*, and *CTX-M9* co-occurring in 3 samples, indicating a higher level of antibiotic resistance or a more complex infection that may involve multiple resistance mechanisms. The frequency of acquired fosfomycin resistance genes in *E. coli* isolates from both community and hospital settings should be emphasized. However, because fosfomycin is effective against carbapenemase *Enterobacteria* and ESβL, its use is not advised when there are more limited treatment choices available. As its use grows, resistance levels may also rise.

Study Limitations

Due to the small number of fosfomycin resistance genes studied, the small sample size, and the lack of target fosfomycin-resistant gene sequencing, the generalizability of the results is restricted.

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Authors Contributions

Concept, Design, Analysis, and writing: Professor Dr. Najim A. Yassin 30%, Data Collection or Processing: Rojan I. Albazaz 35 %. Literature Search: Haliz S. Hasan 35%.

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Conflict of interest

No conflict of interest is declared.

References

1. Raina D, Kumar C, Kumar V, Khan IA, Saran S (2021) Potential inhibitors targeting *Escherichia coli* UDP-N-acetylglucosamine enolpyruvyl transferase (MurA): an overview. Indian J Microbiol 62: 1-12. doi: 10.1007/s12088-021-00988-6.
2. Coppola N, Maraolo A, Onorato L, Scotto R, Calò F, Atripaldi L, Borrelli A, Corcione A, De Cristofaro MG, Durante-Mangoni E, Filippelli A, Franci G, Galdo M, Guglielmi G, Pagliano P, Perrella A, Piazza O, Picardi M, Punzi R, Trama U, Gentile I (2022) Epidemiology, mechanisms of resistance

and treatment algorithm for infections due to carbapenem-resistant Gram-negative bacteria: an expert panel opinion. Antibiotics 11: 1263. doi: 10.3390/antibiotics11091263.
3. Tran MT (2023) New ways of using old antibiotics in pediatrics: focus on fosfomycin. Pharmacotherapy 43: 705-12. doi: 10.1002/phar.2780.
4. Paul M, Carrara E, Retamar P, Tängdén T, Bitterman R, Bonomo R, de Waele J, Daikos G, Akova M, Harbarth S, Pulcini C, Garnacho-Montero J, Seme K, Tumbarello M, Lindemann P, Gandra S, Yu Y, Bassetti M, Mouton J, Tacconelli E, Rodríguez-Baño J (2022) European Society of Clinical Microbiology and Infectious Diseases (ESCMID) guidelines for the treatment of infections caused by multidrug-resistant Gram-negative bacilli (endorsed by European society of intensive care medicine). Clin Microbiol Infect 28: 521-47. doi: 10.1016/j.cmi.2021.11.025.
5. Seok H, Choi J, Wi Y, Park D, Peck K, Ko K (2020) Fosfomycin resistance in *Escherichia coli* isolates from South Korea and in vitro activity of fosfomycin alone and in combination with other antibiotics. Antibiotics 9: 112. doi: 10.3390/antibiotics9030112.
6. Vaiyapuri M, Kusunur A, Badireddy M (2023) Molecular mechanisms of antimicrobial resistance. Handbook on antimicrobial resistance: current status, trends in detection and mitigation measures. Springer.
7. Sorlozano-Puerto A, Lopez-Machado I, Albertuz-Crespo M, Martinez-Gonzalez L, Gutierrez-Fernandez J (2020) Characterization of fosfomycin and nitrofurantoin resistance mechanisms in *Escherichia coli* isolated in clinical urine samples. Antibiotics 9: 534. doi: 10.3390/antibiotics9090534.
8. Garallah E, Al-Jubori S (2020) Surveillance of murA and the plasmid-mediated fosfomycin resistance fosA gene in uropathogenic *Escherichia coli* isolates from UTI patients. Gene Report 21: 100872. doi: 10.1016/j.genrep.2020.100872.
9. Mattioni Marchetti V, Hrabak J, Bitar I (2023) Fosfomycin resistance mechanisms in Enterobacterales: an increasing threat. Front Cell Infect Microbiol l13: 1178547. doi: 10.3389/fcimb.2023.1178547.
10. Ibrahim A, Mohammed R (2024) Bacteriological and molecular study of fosfomycin resistance in uropathogenic *Escherichia coli*. Braz J Microbiol 2024: 1-7. doi: 10.1007/s42770-024-01272-7.
11. Hapuarachchi I, Hannaway R, Roman T, Biswas A, Dyet K, Morgan X, Ussher J (2020) Genetic evaluation of ESBL *Escherichia coli* urinary isolates in Otago, New Zealand. JAC Antimicrob Resist 3: dlab147. doi: 10.1093/jacamr/dlab147.
12. Benzerara Y, Gallah S, Hommeril B, Genel N, Decré D, Rottman M, Arlet G (2017) Emergence of plasmid-mediated fosfomycin-resistance genes among *Escherichia coli* isolates, France. Emerg Infect Dis 23: 1564. doi: 10.3201/eid2309.170560.
13. Stasiak M, Maćkiw E, Kowalska J, Kucharek K, Postupolski J (2021) Silent genes: Antimicrobial resistance and antibiotic production. Polish J Microb 70: 421-9. doi: 10.33073/pjm-2021-040.
14. Deekshit V, Srikumar S (2022) 'To be, or not to be'-The dilemma of 'silent' antimicrobial resistance genes in bacteria. J Appl Microbiol 133: 2902-14. doi: 10.1111/jam.15738.
15. Ma L, Yang H, Guan L, Liu X, Zhang T (2022) Risks of antibiotic resistance genes and antimicrobial resistance under chlorination disinfection with public health concerns. Environ Int 158: 106978. doi: 10.1016/j.envint.2021.106978.
16. Albazaz R, Yassin N (2024) Prevalence and molecular detection of virulence genes among multidrug-resistant *Escherichia coli* from human clinical samples and poultry in Duhok City, Iraq. Med J Babylon 21: S81-S7. doi: 10.4103/mjbl.mjbl_204_23.
17. Omolajaiye S, Afolabi K, Iweriebor B (2020) Research article pathotyping and antibiotic resistance profiling of *Escherichia coli* isolates from children with acute diarrhea in Amatole District Municipality of Eastern Cape, South Africa. Biomed Res Int 2020: 4250165. doi: 10.1155/2020/4250165.
18. Clinical and Laboratory Standards Institute (2020) Performance standards for antimicrobial susceptibility testing. Available: https://www.nih.org.pk/wp-content/uploads/2021/02/CLSI-2020.pdf. Accessed: 1 January 2020.
19. Yuan S, Cohen DB, Ravel J, Abdo Z, Forney L (2012) Evaluation of methods for the extraction and purification of DNA from the human microbiome. PloS one 7: e33865. doi: 10.1371/journal.pone.0033865.
20. Zhang X, Ma M, Cheng Y, Huang Y, Tan Y, Yang Y, Qian Y, Zhong X, Lu Y, Si H (2022) Spread and molecular characteristics of *Enterobacteriaceae* carrying fosA-like genes from farms in China. Micro Spectr 10: e00545-22. doi: 10.1128/spectrum.00545-22.
21. Bahy R, Abu El-Wafa W, Abouwarda A (2023) Molecular mechanisms of fosfomycin resistance in MDR *Escherichia coli* isolates from urinary tract infections. Egypt J Med Microbiol 32: 25-9. doi: 10.21608/ejmm.2023.279741.
22. Loras C, González-Prieto A, Pérez-Vázquez M, Bautista V, Ávila A, Campoy P, Oteo-Iglesias J, Alós JI. (2021) Prevalence, detection and characterisation of fosfomycin-resistant *Escherichia coli* strains carrying fosA genes in Community of Madrid, Spain. J Glob Antimicrob Resist 25: 137-41. doi: 10.1016/j.jgar.2021.02.032.
23. Gezer Y, Tanriverdi E, Otlu B, Yilmaz S, Bayindir Y (2022) Screening for fosfomycin resistance genes in carbapenem-resistant isolates of Enterobacterales from the bloodstream of liver transplant patients. Mediterr J Infect Microb Antimicrob 11: 40. doi: 10.4274/mjima.galenos.2022.2022.40.
24. Wachino J, Yamane K, Suzuki S, Kimura K, Arakawa Y (2010) 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 54: 3061-4. doi: 10.1128/AAC.01834-09.
25. Ho P, Chan J, Lo W-U, Lai E, Cheung Y, Lau T, Chow K (2013) Prevalence and molecular epidemiology of plasmid-mediated fosfomycin resistance genes among blood and urinary *Escherichia coli* isolates. J Med Microbiol 62: 1707-13. doi: 10.1099/jmm.0.062653-0.
26. Ali I, Rafaque Z, Ahmed S, Malik S, Dasti J (2016) Prevalence of multi-drug resistant uropathogenic *Escherichia coli* in Potohar region of Pakistan. Asian Pacific J Tropical Biomed 6: 60-6. doi: 10.1016/j.apjtb.2015.09.022.
27. Rodríguez-Lozano J, de Malet A, Cano M, de la Rubia L, Wallmann R, Martínez-Martínez L, Calvo J (2018) Antimicrobial susceptibility of microorganisms that cause urinary tract infections in pediatric patients. Enferm Infecc Microbiol Clin 36: 417-22. doi: 10.1016/j.eimc.2017.08.003. [Article in Spanish]
28. Garcia-Fulgueiras V, Caiata L, Bado I, Giachetto G, Robino L (2022) Antibiotic susceptibility and fosfomycin resistance characterization in a cohort of children older than 6 years of

age with urinary tract infection. Rev Argent Microbiol 54: 120-4. doi: 10.1016/j.ram.2021.04.002.
29. Mowlaboccus S, Daley D, Birdsall J, Gottlieb T, Merlino J, Nimmo G, George N, Korman T, Streitberg R, Robson J, Peachey G, Collignon P, Bradbury S, Rogers B, Coombs G (2021) Molecular characterization of fosfomycin-resistant *Escherichia coli* urinary tract infection isolates from Australia. Clin Microbiol Infect 27: 1360-1. doi: 10.1016/j.cmi.2021.04.029.
30. Mosime L, Newton-Foot M, Nel P (2022) Fosfomycin resistance in community-acquired urinary pathogens from Western Cape, South Africa. South African J Infect Dis 37: 321. doi: 10.4102/sajid.v37i1.321.
31. Abdelraheem W, Mahdi W, Abuelela I, Hassuna N (2023) High incidence of fosfomycin-resistant uropathogenic *E. coli* among children. BMC Infect Dis 23: 475. doi: 10.1186/s12879-023-08449-9.
32. Ahmed A, Al-Masoodi A, Abdullah A (2022) Study of antibiotic resistance to *Escherichia coli* bacteria isolated from urinary tract ingress. Infekc bolezni 20: 63–68. doi: 10.20953/1729-9225-2022-4-63-68. [Article in Russian]
33. Maleki D, Jahromy S, Karizi S, Eslami P (2016) The prevalence of acrA and acrB genes among multiple-drug resistant uropathogenic *Escherichia coli* isolated from patients with UTI in Milad Hospital, Tehran. Avicenna J Clin Microbiol Infect 4: 39785. doi: 10.17795/ajcmi-39785.
34. Najafikhah A, Hakemi-Vala M, Samavat S, Nasiri M (2023) Molecular analysis of fosfomycin resistance among *Escherichia coli* isolates from urinary tract infections in kidney transplant patients during 2019 - 2020. Arch Clin Infect Dis 18: e132120. doi: 10.5812/archcid-132120.
35. Loras C, Mendes AC, Peixe L, Novais A, Alos JI (2020) *Escherichia coli* resistant to fosfomycin from urinary tract infections: Detection of the fosA3 gene in Spain. J Glob Antimicrob Resist 21: 414–6. doi: 10.1016/j.jgar.2020.01.023.
36. Oteo J, Orden B, Bautista V, Cuevas O, Arroyo M, Martínez-Ruiz R, Pérez-Vázquez M, Alcaraz M, García-Cobos S, Campos J (2009) CTX-M-15-producing urinary *Escherichia coli* O25b-ST131-phylogroup B2 has acquired resistance to fosfomycin. J Antimicrob Chemother 64: 712-7. doi: 10.1093/jac/dkp288.
37. Lee H, Pharm D, Le J (2018) Urinary tract infections. PSAP. Infectious Diseases.
38. Abdulkareem M, Yassin N, Abdulrahman M (2023) Prevalence of silent plasmid-mediated quinolone resistance (PMQR) genes among clinical isolates fluoroquinolone-sensitive *Klebsiella pneumoniae*. Passer J Basic Appl Sci 5: 207-212. doi: 10.24271/psr.2023.386813.1269.
39. Wang J, Li Q, Jiang Y, Wang Z, Jiao X (2024) fosA7: a silent fosfomycin resistance gene in *Salmonella*. Lancet Microbe 5: e211. doi: 10.1016/S2666-5247(23)00342-7.
40. Monte DF, de Oliveira CJB (2024) Silent dissemination of fosfomycin resistance genes in critical-priority *Klebsiella pneumoniae*. Int J Antimicrob Agent 63: 107139. doi: 10.1016/j.ijantimicag.2024.107139.
41. Ghanavati R, Ohadi E, Kazemian H, Yazdani F, Torki A, Kalani B, Irajian G (2018) Evaluation of fosfomycin activity against extended spectrum beta lactamase (ESBL) producing *Enterobacteriaceae* isolated from three centers of Tehran, Iran. Recent Pat Antiinfect Drug Discov 13: 180-6. doi: 10.2174/1574891X13666180517075803.
42. Galindo-Méndez M, Navarrete-Salazar H, Baltazar-Jiménez F, Munoz-de La Paz E, Sánchez-Mawcinitt MF, Gómez-Pardo A, Garza-González E, Ponce-de-León-Garduño LA, Franco-Cendejas R, Morfín-Otero R, Rojas-Larios F, Mena-Ramírez JP, Morales-de-la-Peña CT, García-Mendoza L, Choy-Chang EV, Avilés-Benítez LK, López-Gutiérrez E, Canizales-Oviedo JL, Barlandas-Rendón NE, Maldonado-Anicacio JY, Rosales-García AA, Ostos-Cantú HL (2022) Emergence of fosfomycin resistance by plasmid-mediated fos genes in uropathogenic ESBL-producing *Escherichia coli* isolates in Mexico. Antibiotics 11: 1383. doi: 10.3390/antibiotics11101383.
43. Grilo T, Freire S, Miguel B, Martins LN, Menezes MF, Nordmann P, Poirel L, Sousa MJR, Aires-de-Sousa M (2023) Occurrence of plasmid-mediated fosfomycin resistance (fos genes) among *Escherichia coli* isolates, Portugal. J Glob Antimicrob Resist 35: 342-346. doi: 10.1016/j.jgar.2023.08.001.
44. Hou J, Huang X, Deng Y, He L, Yang T, Zeng Z, Chen Z, Liu J (2012) Dissemination of the fosfomycin resistance gene fosA3 with CTX-M β-lactamase genes and rmtB carried on IncFII plasmids among *Escherichia coli* isolates from pets in China. Antimicrob Agents Chemother 56: 2135-8. doi: 10.1128/AAC.05104-11.
45. Mendes AC, Rodrigues C, Pires J, Amorim J, Ramos MH, Novais Â, Peixe L (2016) Importation of fosfomycin resistance fosA3 gene to Europe. Emerg Infect Dis 22: 346. doi: 10.3201/eid2202.151301.
46. Lee S, Park Y, Yu J, Jung S, Kim Y, Jeong S, Arakawa Y (2012) Prevalence of acquired fosfomycin resistance among extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* clinical isolates in Korea and IS 26-composite transposon surrounding fosA3. J Antimicrob Chemother 67: 2843-7. doi: 10.1093/jac/dks319.