# How is fosfomycin resistance developed in Escherichia coli?

Vincent Cattoir, Francois Guerin

**How is fosfomycin resistance developed in *Escherichia coli*?**

Vincent Cattoir[1,2,3]* & François Guérin[4,5]

[1]*Université de Rennes 1, Inserm U1230, Rennes, France*

[2]*CHU de Rennes, Service de Bactériologie-Hygiène hospitalière, Rennes, France*

[3]*CNR de la Résistance aux Antibiotiques, Rennes, France*

[4]*CHU de Caen, Service de Microbiologie, Caen, France*

[5]*Université de Caen Normandie, EA4655, Caen, France*

*__Correspondence:__ Prof. Vincent Cattoir, CHU de Rennes, Service de Bactériologie-Hygiène hospitalière, 2 rue Henri Le Guilloux, 35033 Rennes Cedex, France. +33-2-99-28-98-28, Fax: +33-2-99-28-41-59, E-mail: vincent.cattoir@chu-rennes.fr.

Keywords: *E. coli*; Fosfomycin; MurA; GlpT; UhpT; UhpA; UhpB; UhpC; FosA3.

Word count: Text = **1,300**; **20** References.

## Introduction

Discovered in 1969, fosfomycin is a phosphonic acid-derived bactericidal antibiotic that has been used in clinics for >40 years, especially for the single-dose (3 g) oral therapy of uncomplicated lower urinary tract infections (UTIs) [1]. It exhibits a broad spectrum of antimicrobial activity that comprises Gram-positive cocci, *Enterobacteriaceae* (incl. *Escherichia coli*) and *Pseudomonas aeruginosa* [1]. Due to the worldwide spread of antimicrobial resistance and the paucity of novel drugs in the development pipeline, there has been a renewed interest of fosfomycin as an alternative option for the treatment of infections caused by multidrug-resistant (MDR) Gram-negative bacilli [1].

## Mechanism of action

Fosfomycin acts by inhibiting the initial enzymatic step of peptidoglycan biosynthesis, which takes place into the cytoplasm [2]. As a phosphoenolpyruvate (PEP) analogue, it covalently binds to the key residue Cys115 in the active site of the UDP-*N*-acetylglucosamine-3-*O*-enolpyruvyl transferase (named MurA), preventing the formation of UDP-*N*-acetylglucosamine-3-*O*-enolpyruvate from UDP-*N*-acetylglucosamine and PEP [2]. In *E. coli*, fosfomycin actively enters the cell via two nutrient transporters belonging to the major facilitator superfamily (MFS): 1) the glycerol-3-phosphate transporter (GlpT) that is constitutively expressed and; 2) the hexose-6-phosphate transporter (UhpT) that is induced by extracellular glucose-6-phosphate (G6P) [3]. Moreover, the full expression of both *glpT* and *uhpT* genes requires high levels of cyclic AMP (cAMP) along its receptor protein complex (CRP) [3]. In *Enterobacteriaceae*, cAMP synthesis depends on the activity of the adenyl cyclase (CyaA), while intracellular cAMP levels are also regulated by the phosphotransferase enzyme PstI, which is a component of the PEP sugar phosphotransferase transport system

[3]. Furthermore, the expression of *uhpT* is locally controlled by *uhpA*, *uhpB* and *uhpC* genes [3]. The integral membrane protein UhpC detects the extracellular signal (i.e. G6P) and activates UhpB by phosphorylation. UhpB is a sensor histidine kinase that is part of the two-component regulatory system UhpAB. UhpA is the cognate DNA-binding response regulator that binds to the *uhpT* promoter. Then, activation of UhpB leads to the phosphorylation of UhpA that binds to the *uhpT* promoter and then induces *uhpT* transcription.

**Antimicrobial susceptibility testing**

Concerning antimicrobial susceptibility testing (AST), MICs of fosfomycin should be determined using the agar dilution reference method on Mueller-Hinton supplemented with 25 mg/L of G6P [2]. According to EUCAST, an isolate of *E. coli* is categorized as susceptible or as resistant with an MIC of ≤32 mg/L and >32 mg/L, respectively. Note that CLSI breakpoints for *E. coli* are different: ≤64 mg/L for susceptibility and ≥256 mg/L for resistance. Susceptibility testing for fosfomycin can also be performed using the disk diffusion method knowing that squatter inner colonies that emerge *in vitro* should not be taken into account and that such *E. coli* strains can be interpreted as susceptible to fosfomycin [4].

**Mechanisms of fosfomycin resistance**

Due to the unique structure and mechanism of action of fosfomycin, there is no cross-resistance with other antibacterial agents. Three mechanisms of fosfomycin resistance have been described in *E. coli* so far: impaired drug uptake, enzymatic drug inactivation and target modification [2, 3, 5].

*Impaired Drug Uptake*

Reduced drug uptake is the most frequent mechanism of resistance found both in *in vitro* mutants and clinical isolates [2, 3, 5]. It results from chromosomal mutations that alter the functionality of one or both of the two transport systems. Chromosomal mutations (mutations, insertions, deletions) arise in *glpT* and *uhpT* genes or their local regulators, especially *uhpA* [6-10]. Note that it has recently been shown that mutations in *uhpB* and *uhpC* appear to be more frequent than those in *uhpA* among both *in vitro* mutants and clinical isolates [11]. By lowering intracellular levels of cAMP, mutations in *cyaA* or *pstI* can also downregulate the expression of both transporters and be responsible for fosfomycin resistance [6, 7, 10]. Note that the latter mutations have a pleiotropic effect with a decrease in pili biosynthesis and in ability to adhere to epithelial cells [6].

*Enzymatic Drug Inactivation*

More recently, there is the emergence of fosfomycin-modifying enzymes that inactivate the drug, which are usually plasmid mediated [2]. Three types of enzymes have been described to date in bacterial pathogens: 1) $K^+$- and $Mn^{2+}$-dependent glutathione S-transferase (GST) encoded by *fosA*-like genes; 2) $Mg^{2+}$-dependent thiol S-transferase encoded by *fosB*-like genes, and; 3) $Mn^{2+}$-dependent epoxide hydrolase encoded by *fosX* [2, 3]. These enzymes catalyze the addition of glutathione, L-cysteine/bacillithiol or $H_2O$ to the $C_1$ of the oxirane ring, respectively. In *Enterobacteriaceae*, these are GST enzymes (FosA and its subtypes; FosC2) that are responsible for fosfomycin resistance among clinical isolates. Out of the six FosA subtypes (FosA, FosA2, FosA3, FosA4, FosA5 and FosA6), FosA3 is by far the most frequently found variant in *E. coli* [12, 13]. Initially identified from Japan in 2006, it has mainly been reported from Asian countries, especially in China [13]. However, it has also emerged in other parts of the World, such as in the USA and in Europe [14,15]. Note that

*fosA3* has been frequently associated with IS*26*-type composite transposons located on conjugative plasmids that co-harbor $bla_{CTX\text{-}M}$ genes [13]. A co-occurrence of *fosA3* with genes conferring resistance to other antibiotic classes, such β-lactams, aminoglycosides, fluoroquinolones, sulphonamides or tetracyclines has also been reported [1, 13]. Finally, the *fosC2* gene has been found in plasmid-borne class 1 integrons [13].

*Target Modification*

Much more uncommonly, fosfomycin resistance can be mediated by qualitative and/or quantitative modifications of MurA [2, 3, 5]. These mutants have mostly been obtained *in vitro*. For instance, the substitution of cysteine with an aspartate residue at the position 115 using site-directed mutagenesis leads to fosfomycin resistance [3]. The *in vitro* overexpression of *murA* can also increase MICs of fosfomycin with a fitness cost significantly lower than that measured in permeability mutants (5% and 20%, respectively) [16]. While only two *murA*-mutant *E. coli* clinical isolates were reported in Japan [8], enhanced expression of *murA* contributing to fosfomycin resistance has not been reported yet in *E. coli* clinical isolates [2].

**Resistance in Clinical Isolates**

In clinical isolates, the main mechanism for the development of fosfomycin resistance is reduced drug uptake due to chromosomal mutations. Even though fosfomycin-resistant mutants can easily develop *in vitro* with high mutation frequencies (ca. $10^{-8}$-$10^{-7}$), the prevalence of fosfomycin resistance in *E. coli* clinical isolates remains very low (usually <2%) even among ESBL-producing isolates [1,5,17,18]. This is likely due to a high fitness cost of chromosome-encoded fosfomycin resistance. Indeed, most of *in vitro*-selected mutants have a reduced growth rate in laboratory media and urine, irrespective of the absence or

presence of fosfomycin [6]. Also, the presence of fosfomycin could be significant since resistance develops at a lower frequency *in vitro* under higher concentrations (1,000-2,000 mg/L) [18]. This should have a clinical impact since there is a high interindividual variability in urinary concentrations among healthy women receiving a single dose (3 g) of fosfomycin-trometamol [19]. Fosfomycin-induced reduced adhesion of *E. coli* to uroepithelial cells could also prevent bacterial establishment of clinical isolates [6]. Urinary tract physiological conditions (urine acidification and anaerobiosis) that induce expression of GlpT and UhpT after activation of fumarate and nitrate reducatase may also increase fosfomycin activity [10]. Finally, the biological cost of fosfomycin resistance in *E. coli* was demonstrated *in vivo* in a murine model of ascending urinary tract infection with significant reductions in infection rates with fosfomycin-resistant isolates (47-59%) as compared with fosfomycin-susceptible ones (77-94%) [20]. However, some fosfomycin-resistant isolates can combine both high virulence and elevated MICs, suggesting that resistance may be associated with compensatory mutations, allowing bacterial cells to overcome the fitness cost of resistance and adapt to environmental conditions [20].

**Conclusion**

Fosfomycin is an old antibiotic that is very useful for the treatment of uncomplicated UTIs. Even after its extensive use in this indication, the prevalence of resistance remains surprisingly low, likely due to the fitness cost of chromosomal mutations and high urinary drug concentrations. On the other hand, the worldwide spread of fosfomycin-modifying enzymes should be monitored since: 1) the biological cost of this emerging mechanism of resistance is much lower than that induced by chromosomal mutations and; 2) the co-occurrence of *fosA*-like genes on plasmids with other resistance genes.

Financial & competing interests disclosure:

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

## References

1. Falagas ME, Vouloumanou EK, Samonis G, Vardakas KZ. Fosfomycin. *Clin. Microbiol. Rev*. 29(2), 321-347 (2016).
2. Silver LL. Fosfomycin: mechanism and resistance. *Cold Spring Harb. Perspect. Med*. 7(2), a025262 (2017).
3. Castaneda-Garcia A, Blazquez J, Rodriguez-Rojas A. Molecular mechanisms and clinical impact of acquired and intrinsic fosfomycin resistance. *Antibiotics (Basel)*. 2(2), 217-236 (2013).
4. Lucas AE, Ito R, Mustapha MM *et al.* Frequency and mechanisms of spontaneous fosfomycin nonsusceptibility observed upon disk diffusion testing of *Escherichia coli*. *J. Clin. Microbiol*, 56(1), e01368-17 (2018).
5. Karageorgopoulos DE, Wang R, Yu XH, Falagas ME. Fosfomycin: evaluation of the published evidence on the emergence of antimicrobial resistance in Gram-negative pathogens. *J. Antimicrob. Chemother*. 67(2), 255-268 (2012).
6. Nilsson AI, Berg OG, Aspevall O, Kahlmeter G, Andersson DI. Biological costs and mechanisms of fosfomycin resistance in *Escherichia coli*. *Antimicrob. Agents Chemother*. 47(9), 2850-2858 (2003).
7. Castaneda-Garcia A, Rodriguez-Rojas A, Guelfo JR, Blazquez J. The glycerol-3-phosphate permease GlpT is the only fosfomycin transporter in *Pseudomonas aeruginosa*. *J. Bacteriol*. 191(22), 6968-6974 (2009).
8. Takahata S, Ida T, Hiraishi T *et al.* Molecular mechanisms of fosfomycin resistance in clinical isolates of *Escherichia coli*. *Int. J. Antimicrob. Agents*. 35(4), 333-337 (2010).
9. Ballestero-Tellez M, Docobo-Perez F, Portillo-Calderon I *et al.* Molecular insights into fosfomycin resistance in *Escherichia coli*. *J. Antimicrob. Chemother*. 72(5), 1303-1309 (2017).
10. Martin-Gutierrez G, Docobo-Perez F, Rodriguez-Beltran J *et al.* Urinary tract conditions affect fosfomycin activity against *Escherichia coli* strains harboring chromosomal mutations involved in fosfomycin uptake. *Antimicrob. Agents Chemother*. 62(1), e01899-17 (2018).

11. Guérin F, Magnan M, Pourbaix A *et al.* Novel mechanisms of fosfomycin resistance in *Escherichia coli*. 28th ECCMID, 21-24 April 2018, Madrid. Poster P1625.
12. Suarez JE, Mendoza MC. Plasmid-encoded fosfomycin resistance. *Antimicrob. Agents Chemother*. 35(5), 791-795 (1991).
13. Yang TY, Lu PL, Tseng SP. Update on fosfomycin-modified genes in *Enterobacteriaceae*. *J. Microbiol. Immunol. Infect*. (2017).
14. Alrowais H, McElheny CL, Spychala CN *et al.* Fosfomycin resistance in *Escherichia coli*, Pennsylvania, USA. *Emerg. Infect. Dis*. 21(11), 2045-2047 (2015).
15. Benzerara Y, Gallah S, Hommeril B *et al.* Emergence of plasmid-mediated fosfomycin-resistance genes among *Escherichia coli* isolates, France. *Emerg. Infect. Dis*. 23(9), 1564-1567 (2017).
16. Couce A, Briales A, Rodriguez-Rojas A, Costas C, Pascual A, Blazquez J. Genomewide overexpression screen for fosfomycin resistance in *Escherichia coli*: MurA confers clinical resistance at low fitness cost. *Antimicrob. Agents Chemother*. 56(5), 2767-2769 (2012).
17. Vardakas KZ, Legakis NJ, Triarides N, Falagas ME. Susceptibility of contemporary isolates to fosfomycin: a systematic review of the literature. *Int. J. Antimicrob. Agents*. 47(4), 269-285 (2016).
18. Falagas ME, Athanasaki F, Voulgaris GL, Triarides NA, Vardakas KZ. Resistance to fosfomycin: mechanisms, frequency and clinical consequences. *Int. J. Antimicrob. Agents*. S1684-1182(17), 30257-8 (2018).
19. Wijma RA, Koch BCP, van Gelder T, Mouton JW. High interindividual variability in urinary fosfomycin concentrations in healthy female volunteers. *Clin. Microbiol. Infect*. 24(5), 528-532 (2018).
20. Pourbaix A, Guerin F, Lastours V *et al.* Biological cost of fosfomycin resistance in *Escherichia coli* in a murine model of urinary tract infection. *Int. J. Med. Microbiol*. 307(8), 452-459 (2017).