

Fosfomycin: evaluation of the published evidence on the emergence of antimicrobial resistance in Gram-negative pathogens

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Fosfomycin has attracted renewed interest for the treatment of lower urinary tract and even systemic infections caused by Gram-negative pathogens with resistance to traditionally used agents. The main concern regarding the clinical utility of fosfomycin refers to the potential for the emergence of resistance during therapy. In this review, we evaluate the available published evidence regarding the mechanisms and the frequency of *in vitro* mutational resistance to fosfomycin in Gram-negative pathogens. We also review data regarding the emergence of resistance in clinical studies of fosfomycin therapy in various infectious syndromes and data from studies that evaluate the evolution of fosfomycin resistance over time. There appears to be discordance between the high frequency of mutational resistance to fosfomycin *in vitro* and the lower extent of this phenomenon in clinical studies. This discordance could at least partly be attributed to a biological cost associated with common mutations that confer resistance to fosfomycin, including decreased growth rate and low adherence to epithelial cells for the resistant mutants. The development of resistance appears to be more frequent both *in vitro* and in clinical studies for *Pseudomonas aeruginosa* in comparison with *Escherichia coli*, whereas relevant data for other Enterobacteriaceae are relatively scarce. The urinary tract seems to provide a favourable environment for the use of fosfomycin with a low associated likelihood for the emergence of resistance, owing to high drug concentrations and acidic pH. Additional data are needed to further clarify the optimal use of fosfomycin for different infectious syndromes caused by contemporary multidrug-resistant pathogens.

Keywords: biological transport, cell wall, Enterobacteriaceae, microbial drug resistance, mutation, urinary tract infections, virulence

Introduction

Fosfomycin is a natural antibacterial agent that has been known for >40 years and that has mainly been used in the treatment of uncomplicated urinary tract infections.¹ It has a broad spectrum of antimicrobial activity, including against Gram-positive cocci (such as *Staphylococcus aureus* and *Enterococcus faecalis*) and Gram-negative bacteria (such as Enterobacteriaceae and *Pseudomonas aeruginosa*, with the main exception being *Acinetobacter baumannii*).^{2,3} Recently, there has been renewed interest in the use of fosfomycin for the treatment of both urinary and systemic infections caused by multidrug-resistant Gram-negative bacteria, especially Enterobacteriaceae that are resistant to traditionally used agents.⁴ This is mainly because fosfomycin seems to have retained antimicrobial activity against a substantial percentage of these isolates. However, clinical data regarding the use of fosfomycin for infections by such resistant pathogens are still limited. One important consideration for the clinical application of fosfomycin in the above respect is

the potential for the emergence of resistance during therapy and for the selection of resistant mutants.

Mechanism of action of fosfomycin

Fosfomycin is a cell wall-acting agent that inhibits the first committed enzymatic step in peptidoglycan biosynthesis.⁵ Specifically, fosfomycin binds to the enzyme UDP-N-acetylglucosamine enolpyruvyl transferase (MurA), inhibiting the formation of N-acetylmuramic acid (a precursor of peptidoglycan) from N-acetylglucosamine and phosphoenolpyruvate. The above reaction takes place in the cytosol.

Mechanisms of resistance to fosfomycin

Several mechanisms of resistance to fosfomycin have been described, including decreased drug uptake, modification of the target site and inactivation of the antibiotic. Chromosomal

mutations can influence the function of fosfomycin membrane transport systems, resulting in low intracellular levels of the drug. Two transport systems for the uptake of fosfomycin into cells have been described in *Escherichia coli*, involving the glycerol-3-phosphate transporter (GlpT) and a hexose phosphate transporter (UhpT), respectively.^{6,7} The former transporter is constitutively expressed, while the latter is inducible, mainly in the presence of glucose-6-phosphate.^{8,9} Mutations affecting either of these transporters can result in fosfomycin resistance.

Resistance to fosfomycin can also arise as a result of alterations in biological systems that regulate the expression of the transporters mentioned above. Specifically, mutations in the *ptsI* gene can affect the function of the phosphoenolpyruvate: sugar phosphotransferase transport system and result in reduced intracellular levels of cyclic adenosine monophosphate (cAMP).^{8,10} Low levels of cAMP can also arise from mutations in the *cyoA* gene that codes for adenyl cyclase.¹¹ Low intracellular cAMP down-regulates the expression of the fosfomycin transporters GlpT and UhpT.^{6,12} The reduced expression of UhpT can also result from mutations in the *uhpA* gene, which encodes a response regulator protein for the transcriptional activation of the *uhpT* promoter in response to specific stimuli.¹³

Another mechanism of resistance to fosfomycin involves the modification of MurA, the target of the drug's action. In *E. coli*, fosfomycin covalently binds to the cysteine-115 residue of MurA. The substitution of cysteine with aspartate in this active site has been shown to result in resistance to fosfomycin.^{14,15} Closely related alterations in the MurA structure exist in organisms with intrinsic resistance to fosfomycin, including *Vibrio fischeri*, *Mycobacterium tuberculosis* and *Chlamydia trachomatis*.^{13,16} Additional amino acid substitutions in the MurA enzyme of *E. coli* (Asp369Asn and Leu370Ile) have recently been found to relate to resistance to fosfomycin.¹³ The overexpression of MurA is another mechanism that can contribute to the development of a fosfomycin-resistant phenotype.¹⁷

Moreover, resistance to fosfomycin can often be associated with the presence of enzymes that inactivate the antibiotic.^{18,19} Three main mechanisms of this type have been described in pathogenic bacteria. Specifically, *fosA* encodes a glutathione S-transferase, *fosB* encodes an L-cysteine thiol transferase and *fosX* encodes an epoxide hydrolase.²⁰ These enzymes catalyse the addition of glutathione, L-cysteine and H₂O, respectively, to C1 of the oxirane ring of the antibiotic. The *fosA* and *fosB* genes are typically found in plasmids of Gram-negative and Gram-positive bacteria, respectively, while *fosX* is a chromosomal enzyme of *Listeria monocytogenes*. Novel resistance determinants that mediate transferable resistance to fosfomycin through inactivation of the antibiotic have also been described.²¹

Fosfomycin kinases that are involved in the degradation of fosfomycin have been identified in fosfomycin-producing bacteria, such as FomA and FomB in *Streptomyces* spp. and FosC in *Pseudomonas syringae*.²² These kinases appear to protect fosfomycin producers from the harmful effect of the antibiotic. FomA catalyses the phosphorylation of fosfomycin to fosfomycin monophosphate and FomB catalyses the phosphorylation of the latter product to fosfomycin diphosphate. FosC has a similar function to FomA, with which it has shown 25.8% homology.²³ The above reactions require energy (ATP). Whether these kinases have a role in fosfomycin resistance in pathogenic

bacteria is not known. Interestingly, ATP-dependent fosfomycin resistance has been identified in *P. aeruginosa*.²⁴

Frequency of development of fosfomycin-resistant mutants *in vitro*

Mutants that are resistant to fosfomycin typically develop rapidly *in vitro*.²⁵ Several studies have quantitatively assessed the frequency of mutation to fosfomycin resistance for Gram-negative pathogens. We present the relevant data extracted from these studies in Table 1.^{12,26–33}

One study evaluated 109 isolates of various species that were collected at a hospital laboratory in France during 1974–75.³³ This study found that only 7.3% of the isolates did not develop any notable mutation to fosfomycin, whereas >50% of the isolates had a mutation frequency of 1×10^{-7} to 1×10^{-6} cells. Other studies have also demonstrated a relatively high frequency of fosfomycin-resistant mutants, particularly for strains of *P. aeruginosa* or *Klebsiella pneumoniae*, as compared with *E. coli* strains.^{31,32} In two studies that provided specific relevant data, the frequency of fosfomycin-resistant mutants for non-mutator *E. coli* strains was higher in comparison with rifampicin, with a difference in the magnitude of >2 log₁₀ values.^{28,29} Another study that evaluated a *P. aeruginosa* reference strain found that fosfomycin resistance mutations emerged as frequently as for imipenem, but considerably more frequently than for tobramycin (Table 1).²⁶

Although the development of resistance to fosfomycin through single-step mutation in *E. coli* has been considered possible,³² this appears likely only for hypermutable (mutator) strains.²⁸ Hypermutable strains of *E. coli* or *P. aeruginosa*, such as those with deficient methyl-directed DNA mismatch repair systems, have a 10- to 100-fold higher frequency of development of fosfomycin-resistant mutants.^{26,28,29} Of note, a microbiological method that has been developed for the identification of hypermutable *E. coli* strains is based on the number of colonies that appear in the inhibition zone around fosfomycin and rifampicin discs.³⁴ Strains with >70 squatter colonies around the fosfomycin disc and >10 colonies around the rifampicin disc are considered to be strong mutators, although these data have not been adequately verified.^{28,35}

One study has assessed the frequency of emergence of fosfomycin-resistant mutants both *in vitro* and *in vivo* using a *P. aeruginosa* lung infection model.²⁶ The *in vivo* mutation frequency was similar to that observed *in vitro* ($\sim 10^{-7}$). If the rate of mutation for fosfomycin resistance that is observed *in vitro* is applied in models simulating clinical data, then the ensuing probability of emergence of fosfomycin resistance would be considerably high.^{12,36} One relevant study that has used an *in vitro* model for the treatment of bacterial cystitis with fosfomycin found a respective probability of $>10^{-2}$.¹²

Clinical data for the emergence of fosfomycin resistance

Almost all randomized controlled trials that have evaluated treatment with fosfomycin refer to lower urinary tract infections and, particularly, the use of fosfomycin as a single-dose regimen. A recent meta-analysis of these trials reported that the

Table 1. *In vitro* mutation frequency of Gram-negative isolates to fosfomycin, according to data from different studies

Reference	Country, period of isolation	Isolates studied	Resistance characteristics	Medium for selection of mutants	Frequency of fosfomycin-resistant mutants	Frequency of mutants resistant to other antibiotics
Rodríguez-Rojas <i>et al.</i> , ²⁶ 2010	NA	<i>P. aeruginosa</i> PA14 strain, derivative <i>mutS</i> -deficient (hypermutable) mutant strain PA14mutS::MAR2xT7	both strains were susceptible to FOF, tobramycin and imipenem	MH agar with 128 mg/L FOF, 4 mg/L imipenem or 4 mg/L tobramycin	PA14: 1.56×10^{-6} PA14mutS::MAR2xT7: 1.1×10^{-4}	imipenem: PA14: 2.3×10^{-6} , PA14mutS::MAR2xT7: 9.1×10^{-4} tobramycin: PA14: 2.2×10^{-9} , PA14mutS::MAR2xT7: 1.4×10^{-7}
MacLeod <i>et al.</i> , ²⁷ 2009	USA	<i>P. aeruginosa</i> strains from patients with cystic fibrosis, other clinical <i>P. aeruginosa</i> strains and a reference <i>P. aeruginosa</i> strain	NR	MH agar containing 4x MIC of each antibiotic	C002: 6.5×10^{-3} C003: 1.1×10^{-6} C013: 9.2×10^{-3} C014: 1.4×10^{-4} ATCC 27853: 7.2×10^{-4}	tobramycin: C002: 1.1×10^{-5} C003: 4.2×10^{-5} C013: 1.4×10^{-6} C014: 1.3×10^{-6} ATCC 27853: 3.0×10^{-5}
Ellington <i>et al.</i> , ²⁸ 2006	UK, 2003–04	220 <i>E. coli</i> UT isolates submitted to reference laboratory	all isolates ESBL (CTX-M), FOF S, NIT S, RIF non-R	Agar with 4x MIC FOF (with 100 mg/L G-6-P) and 4x MIC RIF	non-mutators ($n=6$) $3.0-6.7 \times 10^{-6}$ weak mutators ($n=5$) $0.8-2.6 \times 10^{-5}$ strong mutators ($n=4$) $0.15-1.5 \times 10^{-4}$	RIF: $1.0-3.9 \times 10^{-8}$ RIF: $1.0-3.0 \times 10^{-7}$
Nilsson <i>et al.</i> , ¹² 2003	NA	<i>E. coli</i> strain NU14 (originally isolated from a patient with UTI)	NR	Luria–Bertani agar with 200 mg/L FOF or with 50 mg/L FOF and 50 mg/L G-6-P	200 mg/L FOF: $\sim 10^{-7}$ 50 mg/L FOF + 50 mg/L G-6-P: $\sim 10^{-8}$	NA
Denamur <i>et al.</i> , ²⁹ 2002	France (clinical isolates), various countries (commensal isolates), NR	21 mutator and 47 non-mutator isolates out of a collection of 603 human <i>E. coli</i> or <i>Shigella</i> sp. commensal or pathogenic isolates	NR	869 medium, 30 mg/L FOF, 100 mg/L RIF, 40 mg/L nalidixic acid	mean mutation frequencies ^a non-mutator strains 7×10^{-6} mutator strains 6×10^{-5}	RIF: 1.8×10^{-8} nalidixic acid: 3×10^{-9} RIF: 1×10^{-6} nalidixic acid: 1.2×10^{-7}
Talarmin <i>et al.</i> , ³⁰ 1996	France (17 hospitals), other countries (2 hospitals), NR	10 FOF-S isolates of serotype O12 and 5 FOF-S isolates of other serotypes out of 214 <i>P. aeruginosa</i> clinical isolates (serotype O12, 25; other serotypes, 189)	serotype O12: MDR, 18/25 (72%); β -lactamase production, 25/25 (100%)	MH agar, 35 mg/L FOF	mutation frequency, mean (range): serotype O12: 8.1×10^{-6} ($6 \times 10^{-7}-2.2 \times 10^{-5}$) other serotypes: 7.7×10^{-6} ($5.2 \times 10^{-8}-3 \times 10^{-5}$)	NA

Continued

Table 1. Continued

Reference	Country, period of isolation	Isolates studied	Resistance characteristics	Medium for selection of mutants	Frequency of fosfomycin-resistant mutants	Frequency of mutants resistant to other antibiotics
Ferrara <i>et al.</i> , ³¹ 1988	Italy, NR	17 out of 193 recent isolates from UT or bronchial secretions: <i>S. aureus</i> , 3; <i>E. coli</i> , 3; <i>K. pneumoniae</i> , 3; <i>P. aeruginosa</i> , 3; indole-positive <i>Proteus</i> , 3; <i>P. mirabilis</i> , 2	NR	nutrient agar, 250 mg/L FOF, pH 7.4	mutation frequency for each studied isolate: <i>S. aureus</i> : 10^{-8} , 10^{-8} , 10^{-7} <i>E. coli</i> : no resistance, 10^{-9} , 10^{-9} <i>K. pneumoniae</i> : 10^{-7} , 10^{-6} , 10^{-6} <i>P. aeruginosa</i> : 10^{-7} , 10^{-6} , 10^{-6} indole-positive <i>Proteus</i> : 10^{-8} , 10^{-7} , 10^{-6} <i>P. mirabilis</i> : 10^{-8} , 10^{-7}	NA
Lerner <i>et al.</i> , ³² 1988	USA, NR	6 out of 100 consecutive UT isolates (from outpatient clinics): <i>E. coli</i> , 2; <i>K. pneumoniae</i> , 2; <i>P. mirabilis</i> , 2	all 6 isolates FOF S (MICs ≤ 16 mg/L)	MH agar with 25 mg/L G-6-P, 4 \times MIC FOF nutrient agar with 25 mg/L G-6-P, 4 \times MIC FOF	mutation frequency (MH agar/nutrient agar) for each strain: <i>E. coli</i> 61: 1.4×10^{-7} / 7.7×10^{-8} <i>E. coli</i> 118: 8.8×10^{-8} / 1.6×10^{-9} <i>K. pneumoniae</i> 90: 4.8×10^{-7} / 9.6×10^{-7} <i>K. pneumoniae</i> 116: 5×10^{-5} / 5×10^{-8} <i>P. mirabilis</i> 87: 1.8×10^{-6} / 3.2×10^{-6} <i>P. mirabilis</i> 125: 7.2×10^{-5} / 8×10^{-7}	NA
Courtieu <i>et al.</i> , ³³ 1977	France, 1974, 1975	109 out of 760 isolates at a hospital laboratory that were FOF S: <i>S. aureus</i> , 46; <i>Proteus</i> spp., 33; <i>P. aeruginosa</i> , 17; <i>Serratia</i> spp., 11; <i>E. coli</i> , 2	all isolates FOF S	MH agar with 250 mg/L FOF (without G-6-P)	mutation frequencies for all 109 isolates: $<10^{-8}$: 8/109 (7.3%) $1-9 \times 10^{-8}$: 39/109 (35.8%) $1-9 \times 10^{-7}$: 46/109 (42.2%) $1-9 \times 10^{-6}$: 16/109 (14.7%)	NA

FOF, fosfomycin; G-6-P, glucose-6-phosphate; MDR, multiple drug resistance; MH, Mueller-Hinton; NA, not available; NR, not reported; RIF, rifampicin; S, susceptible; R, resistant; UT(I), urinary tract (infection).

^aApproximate data extracted from a bar chart.

emergence of resistance to fosfomycin was not observed in the included trials, although this issue was specifically addressed in only a few of the trials.³⁷

In Table 2 we present data from 10 clinical studies or trials that have evaluated the evolution of resistance to fosfomycin during therapy.^{38–47} The emergence of resistance to fosfomycin has been noted in 2.3%–6.7% of cases when this agent has been used for the treatment of infections other than uncomplicated cystitis, such as respiratory tract infections or osteomyelitis. Only one study, which evaluated the use of fosfomycin for chronic suppurative otitis, noted an apparently greater frequency of emergence of resistance (13.3%).⁴⁶ In all the above-mentioned studies, the pathogens that developed resistance to fosfomycin were *P. aeruginosa*, *Proteus* spp., *Klebsiella* spp. or *Enterobacter* spp. Of note, none of the above-reported cases of resistance emergence involved an *E. coli* isolate.

The emergence of resistance to fosfomycin appears to be relatively frequent for systemic infections with *P. aeruginosa*; values between 7% and 20% have been reported in four relevant studies.^{41,45–47} In the most comprehensive of these studies, fosfomycin was used in various dosages and routes of administration for the treatment of various infectious syndromes caused by Gram-negative and Gram-positive pathogens in a single-arm, multicentre trial in Spanish hospitals. The emergence of resistance to fosfomycin was noted for 3% of the 959 cases treated in total; this figure was considerably higher (10%) for the 86 cases of *P. aeruginosa* infection.⁴⁵

Effect of fosfomycin on faecal flora

Certain studies have evaluated the effect of fosfomycin administration on the resistance characteristics of the faecal flora.^{48–50} Specifically, one study has sequentially assessed the changes in the resistance of faecal isolates after a single oral dose of 3 g of fosfomycin trometamol administered in eight healthy volunteers.⁵⁰ Fosfomycin-resistant coliform bacteria were isolated in three (37.5%) of the volunteers, but they disappeared by days 7–14. In another study, fosfomycin calcium was administered at a total daily dosage of 2 g for 28 days.⁴⁹ The number of *E. coli* in faeces decreased markedly during treatment and the surviving isolates remained susceptible to fosfomycin. However, there was a substantial increase in the number of *Klebsiella* and *Enterobacter* organisms isolated from faeces. After 2 weeks from the discontinuation of fosfomycin, the above changes tended to return to baseline. Lastly, in a clinical trial, 62 adult women with acute uncomplicated cystitis were randomized to receive orally 3 g of fosfomycin as a single dose, 250 mg of ciprofloxacin twice daily for 3 days or 100 mg of nitrofurantoin twice daily for 7 days.⁴⁸ No resistance to the administered agent, until the following 28–40 days, was noted for the *E. coli* faecal isolates in the fosfomycin and nitrofurantoin groups. In contrast, ciprofloxacin-resistant bacteria were isolated in 2/25 (8%) of the patients in the ciprofloxacin group.

Changes in the susceptibility to fosfomycin over time

Despite the high *in vitro* frequency of fosfomycin resistance mutations, susceptibility rates have remained relatively stable since the introduction of this agent in clinical practice. In

Japan, one of the countries where fosfomycin has been used clinically for the treatment of systemic infections, two studies have shown that the susceptibility of *E. coli* and *P. aeruginosa* isolates to fosfomycin did not change considerably after >20 years of use.^{24,51}

In Table 3 we present data extracted from 12 European studies referring to temporal trends in the susceptibility to fosfomycin of various pathogens.^{52–63} Eight of these studies were performed in Spain, three were performed in France and the remaining one in Italy. In all three countries, fosfomycin has been used as an intravenously administered agent for the treatment of systemic infections, in addition to its oral use for lower urinary tract infections. Cumulatively, data from these studies cover a relatively long period of time, starting from the introduction of fosfomycin in clinical practice in the early 1970s. Most of these studies refer to urinary isolates, of which *E. coli* isolates constitute the majority. However, other Gram-negative and Gram-positive pathogens are also evaluated.

None of the above-mentioned studies has shown a major difference in the susceptibility to fosfomycin between the first and the last year of the study period, considering all of the pathogens evaluated. Particularly regarding *E. coli*, the susceptibility to fosfomycin did not decrease by >2.2% in any of the studies that provided specific relevant data. Regarding *P. aeruginosa*, one study showed a decline in the susceptibility to fosfomycin by 4% over 5 years,⁵⁴ whereas the remaining three studies that provided specific relevant data showed a slight increase in the susceptibility to fosfomycin over time.^{58,59,61}

The above data should be interpreted with caution, since most are not adjusted for the consumption of fosfomycin during the time period evaluated in each study. Of note, one of the above-presented studies, which was performed in a French hospital, reported a very low level of fosfomycin consumption between 1999 and 2005, in comparison primarily with fluoroquinolones and, secondly, with nitrofurantoin.⁵⁵ More importantly, a study from an urban community healthcare centre in Spain showed an increasing trend in the resistance to fosfomycin among urinary *E. coli* isolates, which was related to a >50% increase in the consumption of fosfomycin.⁵² This could be related to the selection pressure for isolates, particularly extended-spectrum β -lactamase (ESBL) producers, carrying fosfomycin resistance determinants. However, another study performed at a single institution in Spain over a 3 year period (1973–75) did not identify an association of fosfomycin resistance with the consumption of this antibiotic in the institution.⁶³

Biological cost of fosfomycin resistance

According to the data presented above, mutational resistance to fosfomycin appears to emerge rather frequently *in vitro* for Gram-negative bacteria. However, this does not absolutely coincide with the frequency of resistance development reported from clinical studies that evaluated fosfomycin for the treatment of human infections, as well as with the evolution of susceptibility to fosfomycin over time, which has remained relatively stable in different settings. This could imply that the development of resistance to fosfomycin may also confer a biological cost.

Certain *in vitro* studies have found that fosfomycin-resistant mutants of Enterobacteriaceae have a reduced growth rate

Table 2. Emergence of resistance to fosfomycin during therapy, according to data from clinical studies

Reference	Country, study design	Study population (n, sex, age)	Type of infection; origin of isolation	Pathogens isolated (n); resistance characteristics	Treatment (n) ^a	Emergence of resistance n/N (%)
Naber <i>et al.</i> , ³⁸ 1992	Germany, microbiological study from RCT data	284, F, 18–75 years	acute uncomplicated lower UTI	<i>E. coli</i> (239), <i>Klebsiella</i> spp. (8), <i>Staphylococcus</i> spp. (16), <i>P. mirabilis</i> (13), other Gram-negative bacilli (4), <i>Enterococcus</i> spp. (3)	group I (140 pts): 3 g FOF po single dose group II (63 pts): 1.92 g SXT po single dose group III (81 pts): 200 mg OFX po single dose	none for all 3 treatment groups
Meissner <i>et al.</i> , ³⁹ 1989	Germany, clinical trial	60, 48 (80.0%) M, mean age 37.4 years	chronic osteomyelitis	<i>S. aureus</i> (34), coagulase-negative staphylococci (15), streptococci (10), <i>P. aeruginosa</i> (10)	10 g FOF iv pre-operatively, then 15 g daily iv	4/60 (6.7) pts
Nissen <i>et al.</i> , ⁴⁰ 1986	Denmark, RCT	32, 14 (43.8%) F, >18 years	severe pneumonia	41 isolates, coagulase-positive staphylococci (9), <i>E. coli</i> (7), <i>S. pneumoniae</i> (6), <i>M. catarrhalis</i> (4), <i>K. pneumoniae</i> (4), <i>P. aeruginosa</i> (mixed infection) (3), β-haemolytic streptococci (2), α-haemolytic streptococci (2), <i>H. influenzae</i> (2), <i>E. cloacae</i> (1), coagulase-negative staphylococci (1)	group I (17 pts): 4 g FOF iv q8h + 1 g AMP iv q6h group II (15 pts): 80 mg GEN iv q8h + 1 g AMP iv q6h	group I: 1/17 (5.9) pts [K. pneumoniae: 1/2 (50.0) isolates] group II: 0/15 (0) pts
Bacardi <i>et al.</i> , ⁴¹ 1977	Spain, retrospective case-series	29, 14 (48.3%) M, 27 adults and 2 children	serious respiratory infections [pneumonia (10), chronic bronchitis (8), chronic bronchopathies (6), sepsis or pulmonary dissemination (3), empyema (1), pulmonary abscess (1)]	<i>P. aeruginosa</i> (14), <i>E. coli</i> (7), <i>Klebsiella/Enterobacter</i> (4), <i>S. pneumoniae</i> (4)	3 g FOF po + 3 g im daily (for 7–14 days)	1/29 (3.4) [<i>P. aeruginosa</i> : 1/14 (7.1)] isolates
Bonora <i>et al.</i> , ⁴² 1977	Spain, retrospective study	40, 31 (77.5%) M, mean age 49 years	respiratory bacterial infections (40) [pneumonia (27), chronic broncho-pneumopathy (10), lung abscess (2), acute bronchitis (1)]	<i>S. viridans</i> (13), β-haemolytic <i>Streptococcus</i> (1), <i>S. pneumoniae</i> (8), <i>Enterobacter</i> sp. (8), <i>S. aureus</i> (6), <i>E. coli</i> (4), <i>Klebsiella</i> sp. (3), <i>P. aeruginosa</i> (2), <i>Serratia</i> (1), <i>P. mirabilis</i> (1), <i>Clostridium welchii</i> (1)	60 (45–185) mg/kg FOF daily iv	2/34 (5.9) ^b [K. pneumoniae: 1/3 (33.3), <i>Enterobacter</i> sp.: 1/8 (12.5)] isolates

Gobernado <i>et al.</i> , ⁴³ 1977	Spain, non-comparative study	58, F [45 (77.6%) pregnant women], mean age 34 years	obstetric-gynaecological infections (UTI, 30; abdominal wall post-laparotomy infection, 13; perineal dehiscence, 9; endometritis, 5; post-abortion sepsis, 5)	70 isolates: <i>E. coli</i> (33), <i>P. mirabilis</i> (12), <i>Klebsiella-Enterobacter- Serratia</i> (7), <i>E. faecalis</i> (5), <i>S. aureus</i> (4), <i>P. rettgeri</i> (3), <i>P. aeruginosa</i> (3), <i>S. viridans</i> (1), <i>C. welchii</i> (1), <i>A. dispar</i> (1)	~4 g FOF daily im (32 pts) or po (26 pts) + nitroxoline (8 pts) or GEN (2 pts)	3/70 (4.3) [<i>P. aeruginosa</i> : 1/3 (33.3), <i>P. rettgeri</i> : 1/3 (33.3)] isolates
Honorato <i>et al.</i> , ⁴⁴ 1977	Spain, prospective clinical study, NR	25 pts, 11 (44%) F, 19– 74 years	acute exacerbation of chronic bronchopathy (12), bronchopneumonia (10), acute bronchitis (3)	44 isolates: <i>S. pneumoniae</i> (9), <i>Klebsiella</i> spp. (9), <i>Enterobacter</i> (5), <i>P. aeruginosa</i> (5), <i>E. coli</i> (5), other (7)	4 g/day FOF im for 8–15 days (mean duration of treatment: 10 days)	1/44 (2.3%) [<i>Klebsiella</i> spp.: 1/9 (11.1)] isolates
Rodríguez <i>et al.</i> , ⁴⁵ 1977	Spain, multicentre clinical trial	959 pts, NR, NR	various infectious diseases: gonococcal urethritis (87), typhoid fever (54), <i>E. coli</i> enterocolitis (146), acute (40) and chronic (193) UTIs, osteomyelitis (41), chronic otorrhoea (24), septicaemia (34), meningitis (17), surgical and suppurative infections (61), bronchitis or pneumonia (73), pharyngo-amygdalitis (24), burns (12), endometritis (31), ocular infection (20), whooping cough (15)	<i>E. coli</i> (297 pts), <i>S. aureus</i> (133 pts), <i>Proteus</i> spp. (88 pts), <i>P. aeruginosa</i> (86 pts), <i>Streptococcus</i> spp. (73 pts), <i>K. pneumoniae</i> or <i>Enterobacter</i> (67 pts), <i>S. typhi</i> (54 pts), <i>S. marcescens</i> (31 pts), <i>N. gonorrhoeae</i> (87 pts), other (43 pts)	FOF po, im, iv or topically in various dosages	average resistance development: 3% for all pathogens (<i>P. aeruginosa</i> : 10%)
Sole Puyo and Poch Viñals, ⁴⁶ 1977	Spain, non-comparative study	24 pts, 17 (70.8%) F, 15– 57 years	chronic suppurative otitis (otitis media in 22 pts, otitis externa in 2 pts)	<i>P. aeruginosa</i> (12), <i>S. aureus</i> (7), <i>P. mirabilis</i> (3), <i>E. coli</i> (2), <i>E. faecalis</i> (2), <i>Klebsiella</i> (2), <i>Citrobacter</i> (1), <i>Bacillus</i> (1)	2–8 g FOF daily im (+FOF otic drops in 7 pts)	4/30 (13.3) [<i>P. aeruginosa</i> : 2/12 (16.7), <i>P. mirabilis</i> : 2/3 isolates (66.7)]
Fernandez-Valencia <i>et al.</i> , ⁴⁷ 1976	Spain, non-comparative study	37, 35 (94.6%) M, 4– 75 years	osteomyelitis	<i>S. aureus</i> (35), <i>P. aeruginosa</i> (5), coagulase-negative staphylococci (2), <i>Klebsiella</i> sp. (2), <i>E. faecalis</i> (1), <i>E. coli</i> (1)	4–8 g FOF im daily in 32 pts (+FOF po in 19 pts), 3–4 g FOF po daily in 5 pts	1/37 (2.7) pts [<i>P. aeruginosa</i> : 1/5 (20.0) isolates]

AMP, ampicillin; F, female; FOF, fosfomycin; GEN, gentamicin; im, intramuscular; iv, intravenous; M, male; NIT, nitrofurantoin; NR, not reported; OFX, ofloxacin; po, orally; pts, patients; q6h, every 6 h; q8h, every 8 h; RCT, randomized clinical trial; SXT, trimethoprim/sulfamethoxazole; UTI, urinary tract infection.

^aDenotes the number of patients receiving fosfomycin alone or in combination out of the total number of patients evaluated in the study.

^bEmergence of resistance includes increase in fosfomycin MICs for subsequent isolates compared with baseline isolates.

Table 3. Evolution in susceptibility to fosfomycin over time, according to data from different European studies

Reference	Country	Time period examined	Origin of isolation	Number and type of isolates: susceptibility to fosfomycin (%) ^a		Absolute change in the percentage susceptibility to fosfomycin (last – first year)
				first year of study	last year of study	
Oteo <i>et al.</i> , ⁵² 2009	Spain	2003–08	17 602 <i>E. coli</i> isolates (575 were ESBL producers) from UTIs in one large community urban health area ^b	all <i>E. coli</i> : 98.4 ESBL <i>E. coli</i> : 97.8	all <i>E. coli</i> : 96.2 ESBL <i>E. coli</i> : 78.3	all <i>E. coli</i> : –2.2 ESBL <i>E. coli</i> : –19.5
Andreu <i>et al.</i> , ⁵³ 2008	Spain	2000 and 2006	uropathogens from patients with community-acquired lower urinary tract infections collected at 15 microbiology laboratories in 9 regions	745 <i>E. coli</i> : 99.4	2189 <i>E. coli</i> : 98.3	–1.1
Gamero Delgado <i>et al.</i> , ⁵⁴ 2007	Spain	2000–05	3019 <i>P. aeruginosa</i> isolates (2532 from inpatients, 487 from outpatients) at a single hospital	NR: 43	NR: 39	–4
Honderlick <i>et al.</i> , ⁵⁵ 2006	France	2000–05	17 176 uropathogens at a single hospital: 15 042 (87.6%) Enterobacteriaceae (<i>E. coli</i> : 10 711), 1469 (8.6%) <i>Staphylococcus</i> spp., 665 (3.9%) <i>Enterococcus</i> spp. – <i>Streptococcus</i> spp.	4633 isolates: 88.5 2248 Enterobacteriaceae: 94.3 (1675 <i>E. coli</i> : 99) 265 <i>Staphylococcus</i> spp.: 76	4859 isolates: 92.8 2415 Enterobacteriaceae: 89.4 (1657 <i>E. coli</i> : 98.7) 218 <i>Staphylococcus</i> spp.: 62	all isolates: 4.3 Enterobacteriaceae: –4.9 <i>E. coli</i> : –0.3 <i>Staphylococcus</i> spp.: –14
Junquera <i>et al.</i> , ⁵⁶ 2005	Spain	1994–2001	14 319 <i>E. coli</i> uropathogens collected at a single hospital	1056 total <i>E. coli</i> : 97.6 192 <i>E. coli</i> outpatient isolates: 97.4 ^c 1842 <i>E. coli</i> nosocomial isolates: 99.0 ^c	1666 total <i>E. coli</i> : 98.4 586 <i>E. coli</i> outpatient isolates: 97.6 1080 <i>E. coli</i> nosocomial isolates: 98.8	total <i>E. coli</i> : 0.8 <i>E. coli</i> outpatient isolates: 0.2 <i>E. coli</i> nosocomial isolates: –0.2
Lorente Garin <i>et al.</i> , ⁵⁷ 2005	Spain	1997–2001	consecutive urinary isolates from patients with community-acquired urinary tract infection collected at a reference laboratory	1184 <i>E. coli</i> : 99.4 31 <i>P. mirabilis</i> : 80.7 63 <i>K. pneumoniae</i> : 88.9	1289 <i>E. coli</i> : 98.7 61 <i>P. mirabilis</i> : 90.2 93 <i>K. pneumoniae</i> : 84.9	<i>E. coli</i> : –0.7 <i>P. mirabilis</i> : 9.5 <i>K. pneumoniae</i> : –4
Rodríguez Lopez <i>et al.</i> , ⁵⁸ 2005	Spain	1997–2003	urinary isolates from outpatients at a reference hospital laboratory	592 total Gram-negative isolates: 95.8 479 <i>E. coli</i> : 99 51 <i>P. mirabilis</i> : 94 42 <i>K. pneumoniae</i> : 93 20 <i>P. aeruginosa</i> : 29 ^d	1519 total Gram-negative isolates: 92.9 1210 <i>E. coli</i> : 98 140 <i>P. mirabilis</i> : 88 118 <i>K. pneumoniae</i> : 72 51 <i>P. aeruginosa</i> : 33	total Gram-negative isolates: –2.9 <i>E. coli</i> : –1 <i>P. mirabilis</i> : –6 <i>K. pneumoniae</i> : –21 <i>P. aeruginosa</i> : 4

Gobernado, ⁵⁹ 2003	Spain	1973, 1977, 1981, 1985, 1992 and 2002	pathogens isolated at a single hospital ^e	<i>S. aureus</i> : 88 <i>E. faecalis</i> : 90 <i>E. coli</i> : 84 <i>Klebsiella</i> spp.: 79 <i>Enterobacter</i> spp.: 44 <i>S. marcescens</i> : 97 <i>Salmonella</i> spp.: 99 <i>P. mirabilis</i> : 82 <i>M. morgani</i> : 40 <i>P. aeruginosa</i> : 71 576 <i>E. coli</i> : 99	<i>S. aureus</i> : 94 <i>E. faecalis</i> : 89 <i>E. coli</i> : 99 <i>Klebsiella</i> spp.: 87 <i>Enterobacter</i> spp.: 68 <i>S. marcescens</i> : 87 <i>Salmonella</i> spp.: 99 <i>P. mirabilis</i> : 87 <i>M. morgani</i> : 37 <i>P. aeruginosa</i> : 77 387 <i>E. coli</i> : 99	<i>S. aureus</i> : 6 <i>E. faecalis</i> : –1 <i>E. coli</i> : 15 <i>Klebsiella</i> spp.: 8 <i>Enterobacter</i> spp.: 24 <i>S. marcescens</i> : –10 <i>Salmonella</i> spp.: 0 <i>P. mirabilis</i> : 5 <i>M. morgani</i> : –3 <i>P. aeruginosa</i> : 6 0
Schito, ⁶⁰ 2003	Italy	1990 ^f and 2000	urinary isolates	590 <i>P. aeruginosa</i> : 17.1 98 ^h	423 <i>P. aeruginosa</i> : 25.1 99 ^h	8 1
Bert et al., ⁶¹ 1997	France	1989–96	3876 <i>P. aeruginosa</i> isolates at a single hospital (47% were from ICU patients) from various infection sites ^g	590 <i>P. aeruginosa</i> : 17.1	423 <i>P. aeruginosa</i> : 25.1	8
Philippon et al., ⁶² 1996	France	1991–95	11 816 <i>E. coli</i> isolates at a single hospital (urinary isolates, 90.5%; blood isolates, 9.5%)	98 ^h	99 ^h	1
Dámaso et al., ⁶³ 1977	Spain	1973–75	5329 isolates at a single institution (Gram-negative, 77%; Gram-positive, 23%)	249 Gram-positive: 63 742 Gram-negative: 36	278 Gram-positive: 57.5 957 Gram-negative: 32.6	Gram-positive: –5.5 Gram-negative: –3.4

ICU, intensive care unit; NR, not reported.

^aFor some of the presented data, we calculated % susceptibility as 100 – % resistance.

^bSpecific number of isolates per year was not reported.

^cData are for 1996.

^d*P. aeruginosa* data are for 1999.

^eNumber of isolates was not reported.

^f1990 data are from a previous study.

^gRespiratory tract (30.1), urinary tract (17.6), wound (15.8), ear, nose or throat (11), drainage fluid (10.7), stool (9.5), blood (2.9) and NR (2.4).

^hPercentages were read from a bar graph. The exact number of isolates for each studied year was not reported.

compared with their fosfomycin-susceptible parent strains. This has been shown mainly for *E. coli*,^{12,15,64,65} but also for *K. pneumoniae* and *Proteus mirabilis*.^{64,65} Two of these studies have additionally shown that fosfomycin-resistant mutants of the above organisms have a decreased capacity to adhere to uroepithelial cells.^{64,65} These data could imply that fosfomycin-resistant mutants of *E. coli* and possibly of other Enterobacteriaceae might have decreased virulence, at least when urinary tract infections are considered. This is because the growth rate and adherence of bacteria to endothelial cells are important properties for an organism to be able to persist in the environment of the urinary bladder.⁶⁶

The development of mutational resistance to fosfomycin, through inactivation of *glpT*, does not appear to confer a biological fitness cost in *P. aeruginosa*.^{26,67} This has been shown in terms of the bacterial growth rate and lethality, and in competition experiments in a mouse lung infection model.²⁶ Interestingly, though, the presence of either of two fosfomycin resistance mechanisms (overexpression of *fosA* or mutation in *glpT*) resulted in lower numbers of persister cells of *P. aeruginosa* after exposure to ofloxacin.⁶⁸ Persister cells are important for the perpetuation of biofilm infections despite antibiotic treatment.⁶⁹ The above finding was observed in the absence of fosfomycin and could be explained by inactivation by FosA or decreased uptake through GlpT of a structural analogue of fosfomycin that can promote the persister phenotype.

Further *in vivo* and clinical data also appear to support the idea that the development of mutational resistance to fosfomycin might have a biological cost for *E. coli*. In an experimental study, two fosfomycin-resistant strains of *E. coli* demonstrated decreased virulence against mice, compared with their fosfomycin-susceptible parent strains. Additionally, in a multicentre, randomized clinical trial, fosfomycin was compared with doxycycline (both administered in combination with metronidazole) as antibiotic prophylaxis in elective colorectal surgery.⁷⁰ Although in the final compared with the first study period the percentage of fosfomycin-resistant aerobic Gram-negative isolates identified in preoperative faecal cultures increased considerably (from 9% to 17%), the percentage of fosfomycin-resistant isolates identified in cultures of infected surgical wounds remained constant at 10%. This may imply that the fosfomycin-resistant faecal isolates had relatively lower virulence for causing wound infections. Notably, the same phenomenon was not observed with doxycycline, for which the wound isolates had resistance rates that increased 2-fold during the study.

Interpretation of the biological cost of fosfomycin resistance

The biological cost of the mutations that confer resistance to fosfomycin in Enterobacteriaceae could be attributed to the loss of important cellular functions.⁷¹ Mutations in the fosfomycin transporter *glpT* are thought to result in the decreased utilization of glycerol-3-phosphate as a carbon source.⁷² Glycerol-3-phosphate is essential for many metabolic functions in *E. coli*, including glycolysis and phospholipid biosynthesis.⁷³ Additionally, mutations in the *ptsI* gene can result in disruption of the transport of multiple carbohydrates.⁷² These

types of mutations have been associated with decreased virulence for certain bacteria, including *Shigella flexneri* and *Salmonella typhimurium*.⁷⁴ Mutations in the *ptsI* gene and other genes such as *cyaA* can also cause a lower intracellular concentration of cAMP, with a subsequent decrease in pilus biosynthesis and in the ability to adhere to epithelial cells.¹²

These effects have not been confirmed for *P. aeruginosa*, which primarily utilizes glycerol as a carbon source.²⁶ Moreover, there appears to be only one fosfomycin transport system in *P. aeruginosa* (the one related to GlpT); mutations in other transport systems are not associated with fosfomycin resistance.⁶⁷

Mutations leading to fosfomycin resistance through alterations in the affinity of binding with MurA, the drug's target of action, have been shown to result in impaired cell wall synthesis, leading to morphological changes in the cell.^{15,64} Moreover, MurA is considered essential for many cellular functions in *E. coli*.⁷⁵ Lastly, resistance to fosfomycin related to mechanisms of inactivation of the drug can be energy-dependent, decreasing the energy sources that are available for other cellular functions.^{22,24}

Relation of *in vitro* data to clinical data

There are many aspects that relate to the development of mutational resistance to fosfomycin *in vitro*, which could differ when this drug is used clinically. First of all, the spectrum of mutations that are observed in fosfomycin-resistant clinical isolates may be different from those observed *in vitro*.¹² Greater alterations in the genetic structure have been noted among clinical isolates.^{8,13} The development of high-level resistance to fosfomycin may require acquisition of mutations in more than one biological system, which might not be very likely to occur during fosfomycin therapy.^{17,28} Fosfomycin-resistant clinical isolates may also need to acquire additional mutations to compensate for the biological cost associated with the presence of fosfomycin resistance mechanisms.¹²

Certain clinically relevant factors can influence the rate of development of fosfomycin-resistant mutants. A lower frequency of resistance development has been observed at higher fosfomycin concentrations.^{31,36} A study that evaluated the frequency of emergence of fosfomycin-resistant mutants for different pathogens *in vitro*, at three different fosfomycin selection concentrations (250, 1000 and 2000 mg/L), showed an inverse association between resistance frequency and fosfomycin concentration.³¹ Under a low pH of 6, no fosfomycin-resistant mutants were seen at a fosfomycin concentration of 2000 mg/L. In contrast, at a fosfomycin concentration of 250 mg/L, resistant mutants were observed, with decreasing frequency, for all three *K. pneumoniae* strains, all three *P. aeruginosa* strains, three of five *Proteus* spp. strains, but none of the three *E. coli* strains tested. In another study that tested the exposure of four *E. coli* strains with different levels of susceptibility to two doses of fosfomycin according to a simulated *in vitro* model of bacterial cystitis, resistant mutants were observed with peak fosfomycin concentrations of 50 and 250 mg/L, even for the two fully susceptible strains.³⁶ In contrast, no resistant mutants were observed when the peak fosfomycin concentration was 2500 mg/L.

Moreover, the frequency of development of mutational resistance to fosfomycin could be lower at acidic pH.³¹ The above

factors may translate into a low probability for the emergence of resistance to fosfomycin when this drug is used for the treatment of urinary tract infections. With a single 3 g dose of fosfomycin trometamol, peak urine concentrations are in the range of 1053–4415 mg/L, while drug levels >128 mg/L can persist for >24–48 h.⁷⁶ The short period of exposure of uropathogens to this drug with the single-dose regimen used for uncomplicated cystitis is an additional factor against the development of resistance to fosfomycin.^{38,77}

Achievable fosfomycin concentrations in other body compartments or sites are substantially lower than in the urine. According to data from several studies, after intravenous administration of a 4 to 8 g fosfomycin dose, mean peak serum levels are commonly in the range of 200 to 400 mg/L, respectively.⁷⁸ Site to serum concentration ratios can be 0.32–0.54 for lung tissue, 0.23–0.26 for cortical bone, 0.17–0.20 for CSF, 0.39–0.69 for muscle and 0.39–0.49 for subcutaneous tissue.^{78,79} The clinical implications of the lower penetration of fosfomycin in body sites other than the urine can be important, but this issue has not been well evaluated. In general, the exposure of pathogens to different concentrations of antibiotics may induce different types of mutations that can confer variable levels of resistance.⁸⁰ The exposure of pathogens to concentrations of certain antibiotics that are close to the MIC can also induce an SOS response, promoting mutagenesis.⁸¹ In this context, the use of the highest recommended fosfomycin dose is warranted for the treatment of systemic infections.⁷⁹

Furthermore, certain biological substances can modify the frequency of development or the level of resistance to fosfomycin.^{12,33} The main example is glucose-6-phosphate, which can induce the alternative hexose transport system of fosfomycin for Enterobacteriaceae strains that have an impairment of fosfomycin transport via GlpT.^{6,8,15} The presence of exogenous cAMP can also induce fosfomycin transport in pathogens with mutations affecting the intracellular concentration of cAMP.⁶ Of note, a higher frequency of mutations for fosfomycin resistance has been detected in Mueller–Hinton agar compared with nutrient agar.³²

Lastly, it should be noted that the evolution of resistance to fosfomycin in a bacterial population has not conclusively been shown to positively correlate with the mutation frequency of the population.^{28–30} This could be attributed to the fact that hypermutable strains have a higher likelihood to develop deleterious mutations, apart from those that confer resistance to an antibiotic.⁸² These mutations may not facilitate the proliferation of the antibiotic-resistant strains.

Critical evaluation of the available evidence

There are many biological mechanisms in Gram-negative pathogens through which mutational resistance to fosfomycin can develop. The one most frequently observed *in vitro* relates to a decrease in fosfomycin uptake into cells. Resistance can also relate to mutations in the drug's target of action, influencing its affinity with the antibiotic or its degree of expression. *In vitro*, the mutation frequency for fosfomycin resistance in Gram-negative pathogens is relatively high, compared with the case for other antibiotics. In clinical studies, though, the degree of development of resistance to fosfomycin during fosfomycin

use for urinary tract infections and other infectious syndromes appears to be considerably lower than that expected from the relevant *in vitro* data. This is also in line with the findings of studies that have evaluated temporal trends in fosfomycin resistance. Specifically, fosfomycin resistance rates in Gram-negative pathogens have been found to be relatively stable, even in countries where fosfomycin has been used as a systemically administered agent.

The above apparent discordance between *in vitro* and clinical data could, at least partly, be attributed to a biological cost associated with most of the mechanisms of development of mutational resistance to fosfomycin. Furthermore, many biological factors can influence the degree of development of resistance to fosfomycin during the treatment of human infections.

It should be noted that most of the above data for fosfomycin resistance have been observed for *E. coli* isolates. Differences may exist regarding other Gram-negative pathogens, particularly *P. aeruginosa*, which appears to have a relatively higher *in vitro* mutation frequency as well as a higher likelihood for the development of resistance to fosfomycin with clinical use. Some studies also indicate that there is no biological cost associated with fosfomycin resistance in *P. aeruginosa*. Differences in the frequency of development of resistance may also be observed regarding the use of fosfomycin for different sites of infection. For lower urinary tract infections, in particular, the likelihood of development of resistance to fosfomycin during therapy appears to be low, because of the high drug concentrations, the acidic pH, short-course therapy and, presumably, the low adherence of fosfomycin-resistant mutants to the epithelial cells.⁷⁸

It is difficult to extrapolate data on the *in vitro* mutant frequency and the *in vitro*- or *in vivo*-determined biological cost of fosfomycin resistance for the estimation of clinical outcomes, such as the probability of development of resistance during fosfomycin therapy for Gram-negative infections and the virulence of fosfomycin-resistant mutants.⁷¹ In a complex biological system like human infection, the probability of resistance developing during fosfomycin therapy plausibly depends on the rapidity of the eradication of the infecting organisms, which in turn depends on the pharmacokinetic and pharmacodynamic parameters of the drug for specific sites and types of infection, as well as the integrity of the host's defences and the efficacy of the host's immune response.⁸³ The level of fosfomycin resistance exhibited by the fosfomycin-resistant mutants can also be an important determinant of the outcome of fosfomycin therapy.

The biological cost of resistance may relate to the specific biological system affected, as well as the genetic background in which the resistance occurs and the environment in which it is expressed.⁸⁴ Thus, it may be species and strain specific or it may depend on the type of infection (e.g. whether it is acute, chronic or foreign-body associated). Whether the magnitude of the biological cost of common fosfomycin resistance mutations is clinically relevant for affecting the treatment outcome has not been determined. Moreover, the probability of development of compensatory mutations during treatment has also not been established.

Given the high spontaneous mutation rate for fosfomycin resistance *in vitro*, the use of additional antimicrobials in combination with fosfomycin is reasonable for preventing the development of fosfomycin resistance during therapy and maximizing treatment outcome.⁸⁵ The choice of candidate drugs to

be combined with fosfomycin should be based on antimicrobial activity against the infecting strain, as well as on potential synergy and specific data for preventing the development of resistance. Carbapenems and aminoglycosides have often been found synergistic in combination with fosfomycin against Gram-negative pathogens.^{86,87} Among these two classes, aminoglycosides may be particularly efficacious in preventing the development of resistance to fosfomycin, but this can be species specific.^{88–90}

It is also difficult to estimate the rapidity of development and the rate of resistance to fosfomycin that will be observed among Gram-negative nosocomial or community isolates, if fosfomycin is used widely for the treatment of such infections.^{83,91} The above parameters depend on the transmissibility of fosfomycin-resistant mutants, the likelihood of integration of chromosomal fosfomycin-resistant determinants into mobile genetic elements and of horizontal gene transfer, the selection and spread of pre-existing plasmid-mediated fosfomycin resistance, as well as the level of antibiotic pressure, and the probability of co-selection of fosfomycin resistance by other antibiotics.⁹²

Conclusions

Although the observed *in vitro* mutation frequency for fosfomycin resistance in Gram-negative pathogens is high, the relevant data from clinical studies and data regarding the evolution of resistance over time could ameliorate concerns related to the potential for the development of resistance to fosfomycin during therapy. This issue may be of greater concern for Gram-negative pathogens other than *E. coli*, particularly for *P. aeruginosa*, and in the treatment of syndromes other than urinary tract infections. Nevertheless, from a clinician's standpoint, the administration of fosfomycin for the treatment of systemic infections in combination antimicrobial regimens with the aim of preventing the emergence of resistance seems prudent until further relevant evidence becomes available. Particularly, data regarding the use of fosfomycin for the treatment of infections caused by contemporary multidrug Gram-negative pathogens are awaited to further delineate issues regarding the optimal clinical use of this revived antimicrobial agent.

Transparency declarations

None to declare.

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