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Current key topics in fosfomycin

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New microbiological aspects of fosfomycin

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ABSTRACT

The discovery of fosfomycin more than 40 years ago was an important milestone in antibiotic therapy. The antibiotic's usefulness, alone or in combination, for treating infections caused by multidrug-resistant microorganisms is clearer than ever. Both the European Medicines Agency and the US Food and Drug Administration have open processes for reviewing the accumulated information on the use of fosfomycin and the information from new clinical trials on this compound. The agencies' objectives are to establish common usage criteria for Europe and authorize the sale of fosfomycin in the US, respectively. Fosfomycin's single mechanism of action results in no cross-resistance with other antibiotics. However, various fosfomycin-resistance mechanisms have been described, the most important of which, from the epidemiological standpoint, is enzymatic inactivation, which is essentially associated with a gene carrying a *fosA3*-harboring plasmid. Fosfomycin has been found more frequently in Asia in extended-spectrum beta-lactamase-producing and carbapenemase-producing *Enterobacterales*. Although fosfomycin presents lower intrinsic activity against *Pseudomonas aeruginosa* compared with that presented against *Escherichia coli*, fosfomycin's activity has been demonstrated in biofilms, especially in combination with aminoglycosides. The current positioning of fosfomycin in the therapeutic arsenal for the treatment of infections caused by multidrug-resistant microorganisms requires new efforts to deepen our understanding of this compound, including those related to the laboratory methods employed in the antimicrobial susceptibility testing study.

Keywords: Fosfomycin; Mechanisms of resistance; Susceptibility testing study; Biofilms; Antimicrobial combinations.

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BACKGROUND

Fosfomycin, a bactericidal antibiotic produced by, among others, *Streptomyces fradiae*, was discovered by a Spanish team from the Spanish Penicillin and Antibiotics Company (*Compañía Española de Penicilina y Antibióticos*) in 1969. Since then, fosfomycin has been employed in numerous countries for various indications, both in its intravenous (disodium salt) and oral formulations (calcium salt or trometamol). In recent years, the use of fosfomycin has increased spectacularly due to the considerable incidence of multidrug-resistant microorganisms for which fosfomycin constitutes, alone or in combination, a treatment alternative [1,2]. Due to the considerable usage differences worldwide, the need to establish common criteria and the need to expand the knowledge on this antibiotic, the European Medicines Agency has opened a process that seeks to collect evidence supporting fosfomycin's indications and authorize and harmonize its usage criteria in Europe (https://www.ema.europa.eu/en/medicines/human/referrals/fosfomycin-containing-medicinal-products). Moreover, the US Food and Drug Administration included fosfomycin (according to the laboratory that conducts clinical trials of this antibiotic) in the list of drugs with antimicrobial activity (qualified infectious disease product), which facilitates a priority review of the results of the clinical trials and an accelerated registration process (https://www.nabriva.com/pipeline-research).

The implementation of epidemiological surveillance studies that include fosfomycin, the new clinical trials of this antimicrobial, as well as the pharmacokinetics-pharmacodynamics (PK-PD) studies necessary to support its formulation and to understand the significance of the possible development of resistances have deepened our microbiological understanding of this drug. The aim of this article is to review this new evidence from a microbiological standpoint that supports its clinical use.

MECHANISM OF ACTION AND PHARMACODYNAMICS OF FOSFOMYCIN

Fosfomycin has a single mechanism of action: blocking the first step of peptidoglycan synthesis. The transport of fosfomycin to the interior of the bacteria is performed through permeases, such as the glycerol-3-phosphate transporter (GlpT) and glucose-6-phosphate [G6P] transporter (UhpT). While GlpT maintains baseline activity without being induced, UhpT lacks activity in the absence of G6P. Once inside the bacterial cell, fosfomycin inhibits the UDP-N-acetylglucosamine enolpyruvyl transferase (MurA) enzyme, responsible for catalyzing the formation of N-acetylmuramic acid (precursor of peptidoglycan) through the binding of N-acetylglucosamine and phosphoenolpyruvate. Fosfomycin is an analog of phosphoenolpyruvate, with an epoxide ring (essential in its mechanism of action) and a phosphonic group. Fosfomycin binds covalently with MurA, inhibiting the latter and thereby causing lysis of the bacterial cells (figure 1).

Fosfomycin is therefore a bactericidal compound that acts on bacteria in the growth phase. The fact that Gram-positive and Gram-negative bacteria require the formation of N-acetylmuramic acid for the synthesis of peptidoglycan means that fosfomycin's spectrum of action is very broad. Likewise, there is no possibility of crossed resistances with this compound. Fosfomycin has therefore been employed for treating infections by multidrug-resistant pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant coagulase-negative staphylococci (MRCNS), vancomycin-resistant enterococci (VRE), penicillin-resistant *Streptococcus pneumoniae*, extended-spectrum beta-lactamase (ESBL)-producing *Enterobacterales*, carbapenemase-producing *Enterobacterales* (CPE) and multidrug-resistant *Pseudomonas aeruginosa* [3].

In terms of its physical-chemical properties, fosfomycin is a low-molecular-weight, water-soluble compound with low plasma protein binding that disseminates easily to most tissues and to the interstitial fluid. Studies have shown that fosfomycin penetrates and reaches relevant concentrations in inflamed tissues, aqueous and vitreous humor, bones and lungs [4]. Likewise, fosfomycin actively accesses the interior of polymorphonuclear leukocytes. The compound is excreted almost exclusively in urine in a nonmetabolized form [5].

The PK-PD parameter associated with the compound's bacteriological activity is not clearly defined and appears to depend on the microorganism. Recent studies have established that the PK-PD parameter that best predicts fosfomycin activity in Gram-negative bacilli (*P. aeruginosa*, *Escherichia coli* and *Proteus* spp.) is area under the curve (AUC)/minimum inhibitory concentration (MIC) [6, 7], while in *S. aureus* and enterococcus, fosfomycin has a time-dependent (T>MIC) behavior [8]. A study also demonstrated a high postantibiotic effect, even at subinhibitory concentrations [9].

Various studies have been published that have sought to elucidate the PK-PD parameter that determines fosfomycin activity in *P. aeruginosa*, with a number of conflicting results. A study using a murine model observed that AUC/MIC is the parameter that best fits fosfomycin activity [6], while another study showed that the antibiotic is time-dependent [10]. Bilal et al. determined that the PK-PD parameter that determines the total bactericidal activity of fosfomycin in *P. aeruginosa* is AUC/MIC, while T>MIC is related to resistance suppression [11].

MECHANISMS OF FOSFOMYCIN RESISTANCE

Fosfomycin resistance can be produced by 3 separate mechanisms: 1) transport impairment, 2) impairment of the target of action and 3) enzymatic inactivation (table 1) [5, 12, 13]. The first of these mechanisms is produced by mutants in chromosomal genes of the transporters GlpT and UhpT or in their regulator genes, impeding fosfomycin from reaching its location of action. This mechanism has been essentially described in *E. coli* and *P. aeruginosa* isolates. In *Acinetobacter baumannii*, it has been shown that mutants in the chromosomal gene *abrp* (essential for the bacteria's growth and involved in wall patency) determine the resistance to fosfomycin, tetracyclines and chloramphenicol.

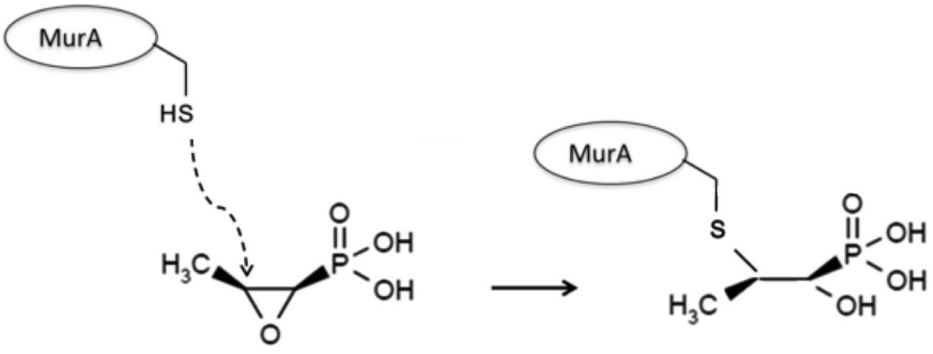

Figure 1 | **Mechanism of action of fosfomycin**

Table 1 Mechanisms of fosfomycin resistance

Process	Resistance mechanism	Microorganism	Localization
Transport reduction	Mutants in transporter genes *glpT* or *uhpT*	*Escherichia coli*	Crom
	Mutants in regulator genes of *glpT* or *uhpT*	*Escherichia coli*	Crom
	Mutants in *cyaA* and *ptsI* (regulate cAMP for *glpT*)	*Escherichia coli*	Crom
	Mutants in *abrp*	*Acinetobater baumannii*	Crom
Change in target or expression	Mutants in *murA*	*Mycobaterium tuberculosis*[a], *Vibrio fischeri*[a], *Escherichia coli*	Crom
	Increased *murA* expression	*Escherichia coli*	Crom
	Alternative pathways to MurA in peptidoglycan synthesis	*Pseudomonas aeruginosa*[b,c], *Pseudomonas putida*[b]	Crom
	Limited participation of MurA in the biological cycle	*Chlamydia trachomatis*[a]	
Inactivation	Inactivation by metalloenzymes by incorporating:		
	-glutathione (FosA, FosA2, FosA3, FosA4, FosA5, FosA6, etc.)	*Enterobacterales*[c], *Pseudomonas* spp[b,c]	Crom / Pl
		Acinetobacter spp.	Crom
	-Bacillithiol or l-cysteine (FosB)	*Staphylococcus* spp., *Enterococcus* spp.	Crom / Pl
		Bacillus subtillis[a]	Crom
	-water (FosX)	*Listeria monocytogenes*[a]	Crom
	Phosphorylation of the phosphonate group by kinases and formation of:		
	-diphosphates and triphosphates (FomA and FomB)	*Streptomyces* spp.	Crom
	-monophosphate (FosC)	*Pseudomonas syringae*	Crom
	(FosC2)	*Escherichia coli*	Pl

[a]Intrinsic resistance; [b]Reduced susceptibility; [c]Some species of *Enterobacterales* (*Serratia marcescens, Klebsiella* spp., *Enterobacter* spp, *Kluyvera georgiana*, etc. have homologous chromosomal *fosA* genes and can present reduced fosfomycin susceptibility); Crom: chromosome; Pl: plasmid

The target of action can be altered intrinsically or by *murA* gene mutants that affect the structure of MurA, with fosfomycin incapable of acting as a substrate. *Mycobacterium tuberculosis* naturally presents MurA with an aspartate residue instead of cysteine in position 117 and is incapable of interacting with fosfomycin, thereby resulting in its intrinsic resistance. Mutants with an altered active center of MurA are found relatively frequently in *E. coli*. The overproduction of MurA also results in insufficient inhibition by fosfomycin, with the microorganism non-susceptible to the action of this antibiotic. In some microorganisms such as *P. aeruginosa* and *Pseudomonas putida*, alternative metabolic pathways independent of MurA have been described in the synthesis of the peptidoglycan that explain the low fosfomycin susceptibility presented by these microorganisms. The lack of susceptibility of *Chlamydia trachomatis* to this antibiotic is due to the lack of importance of MurA in its biological cycle.

However, the mechanism that has attracted the most attention due to its greater epidemiological importance is fosfomycin inactivation, which can be caused by metalloenzymes that efficiently impare this antibiotic, blocking its inhibitory action on MurA. Various metalloenzymes have been described, including FosX and FosA, which inactivate fosfomycin by opening the epoxide ring by incorporating a water and glutathione molecule, respectively. FosB, another metalloenzyme, inactivates fosfomycin by adding a cysteine or bacillithiol molecule, the latter of which is used by Gram-positive microorganisms (Firmicutes) that do not produce glutathione. The incorporation of *fosA* in plasmids and their transformation in *E. coli* raises the MIC values of fosfomycin.

FosX has been found in environmental microorganisms with intrinsic fosfomycin resistance such as *Mesorhizobium loti* and *Desulfitobacterium hafniense* and in pathogens such as *Listeria monocytogenes, Brucella melitensis* and *Clostridium botulinum*. FosA and FosB have an approximate amino acid sequence homology of 48%, and their corresponding genes have been found in the case of *fosB* in plasmids and in the chromosomes of Gram-positive microorganisms (*Staphylococcus epidermidis* and *Bacillus subtilis*) and occasionally associated with plasmids in *Enterobacterales* [14]. The *fosA* gene and its various homologous genes, such as *fosA2, fosA3, fosA4, fosA5* and *fosA6*, have been associated with plasmids in isolates of ESBL-producing *E. coli* and in carbapenemase-producing *Klebsiella pneumoniae*. For *Klebsiella* spp., *Enterobacter* spp., *Serratia marcescens, Kluyvera* spp. and *P. aeruginosa*, *fosA* variants have been identified in their chromosome, with differing sequences but preserving the active center, which could explain the low fosfomycin activity (modal MIC, 4-64 mg/L) in these species when compared with that presented against *E. coli* (modal MIC, 2-4 mg/L) (https://mic.eucast.org/Eucast2/). It has been shown that the deletion of

these chromosomal genes reduces the MIC values of fosfomycin and that its insertion into a plasmid and transformation in *E. coli* confers an increase in MIC values.

Studies have also described kinases (FomA and FomB) that phosphorylate the phosphonate group of fosfomycin, forming diphosphate and triphosphate compounds that lack antimicrobial activity. Another reported kinase is FosC, a homologous phosphotransferase of FomA, which in *Pseudomonas syringae* (another microorganism able to synthesize fosfomycin) converts fosfomycin to fosfomycin monophosphate, which is non-susceptible to MurA.

MICROBIOLOGICAL CONSEQUENCES AND CLINICAL SIGNIFICANCE OF THE DEVELOPMENT OF FOSFOMYCIN RESISTANCE DEVELOPMENT

Despite the considerable ease with which fosfomycin-resistant mutants can be obtained, the clinical repercussion of such mutants has not been sufficiently tested [13]. In some cases, the mechanisms of fosfomycin resistance reduce the fitness of the bacteria that present fosfomycin resistance, and in numerous occasions reduce the bacterial virulence. Such is the case for some mutants in genes that participate in fosfomycin transport, such as *cysA* or *pstI*, which, in *E. coli,* reduce the formation of pili that limit its virulent nature by reducing its ability to adhere to epithelial cells and synthetic materials such as catheters. Lower fitness has also been observed in isolates with MurA overproduction, and its relationship with clinical failure has not been demonstrated. A noteworthy example is that of *L. monocytogenes*, which, *in vitro*, is considered inherently fosfomycin-resistant, not only because it has FosX, which inactivates fosfomycin but also because it is unable of transporting fosfomycin and accessing its location of action. However, *in vivo*, *L. monocytogenes* expresses a permease (Hpt) of G6P, which facilitates the entry of the antibiotic and its susceptibility.

The phenomenon of heteroresistance has been reported in various microorganisms, such as *E. coli*, *A. baumannii, P. aeruginosa* and even *S. pneumoniae*, which indicates the presence of bacterial subpopulations with lower fosfomycin susceptibility. This phenomenon would partly explain the high frequency of mutation for fosfomycin. Resistant mutants can be obtained in up to 40% of *E. coli* isolates at a rate of 10^{-7}-10^{-5}. These mutants present MCIs of 32-64 mg/L, with occasional mutants in genes *glpT* and *uhpT*. Their *in vitro* stability in laboratory media and urine is low, and the typical MIC values can be recovered in successive passages (2-4 mg/L). In approximately 1% of isolates, resistant mutants can be obtained at a lower rate (10^{-11}-10^{-7}) by deletions or insertions in genes *uhpT* and *uhpA*. These mutants present high MICs (512-1,024 mg/L) and lower growth stability than the isogenic strains but greater than that of the *glpT* and *uhpT* mutants [15-17]. These mutants are obtained more frequently in hypermutator strains. However, in all cases, their lower fitness, absence of stability and lower likelihood of selection in acidic environments (e.g., in urine) would also explain the low *in vivo* repercussion of fosfomycin resistance observed *in vitro* [18]. It should be noted that the high concentrations that fosfomycin reaches in some locations, such as urine, and its good penetration in biofilms minimize the possible selection of these mutants. This fact has been demonstrated in *in vitro* models in which the mutant selection window (the concentration range in which resistant mutants would be selected) has been able to be defined. This selection window can be avoided with therapeutic regimens higher than 4 g/8 h [19].

A recent meta-analysis estimated that the risk of selecting resistant mutants during fosfomycin monotherapy in various types of infections (urinary, respiratory, bacteremia, central nervous system and bone) with the involvement of various microorganisms was 3.4% [20]. Resistant mutants were obtained at a higher rate in *Klebsiella* spp., *Proteus* spp., *Enterobacter* spp. and *P. aeruginosa*, the latter of which can reach 20%. This fact could be due to fosfomycin's lower intrinsic activity than that it presents against *E. coli,* which would facilitate its entry into the selection window and justify the administration of fosfomycin in combination with other antimicrobials for infections caused by *P. aeruginosa*. Additionally, a fitness cost associated with fosfomycin resistance in isolates of fosfomycin-resistant *P. aeruginosa* has not been demonstrated, which could reinforce the need for combined therapy in infections caused by this pathogen. These combinations would reduce the selection window in which resistant mutants could be selected [21].

Regardless of the mechanisms detailed earlier, the most important from the epidemiological and clinical standpoint is the enzymatic inactivation associated with *fos* genes. The most important of these genes due to its greater dispersion, plasmid characteristics and presence in ESBL-producing and carbapenemase-producing *Enterobacterales* is *fosA3* [14]. Initially described in 2010, *fosA3* has been found more frequently in Asia, in human and animal isolates, although it is also present in Europe [22, 23]. The rate of *fosA3* varies according to the studied collection but can be present in 90% of fosfomycin-resistant isolates (3-15% of all isolates) that produce ESBL or carbapenemase.

Recently, the origin of the *fosA3* gene in *Kluyvera georgina* has been confirmed. Its integration into plasmids of various incompatibility groups could be related with composite transposons with the insertion sequence IS26 [24].

FOSFOMYCIN SUSCEPTIBILITY TESTING STUDY IN THE LABORATORY, CLINICAL AND EPIDEMIOLOGICAL BREAKPOINTS

The study of *in vitro* fosfomycin susceptibility has always been a challenge in the laboratory due to the lack of unanimous criteria on how it should be conducted for all microorganisms involved in infections for which fosfomycin is indicated. In addition, not all microorganisms currently have interpretive breakpoints (table 2). This situation could change due to the growing interest in this antimicrobial and the need to study it against multidrug-resistant microorganisms in which fosfomycin represents a treatment option.

To date, the reference method recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI) for the study of fosfomycin susceptibility is agar dilution, adding G6P to the medium (25 mg/L). The justification for this recommendation is that fosfomycin uses 2 types of transporters to penetrate bacterial cells. The first transporter, which has constitutive expression, uses glycerol 3-phosphate. This transporter reduces its activity in culture media that contain glucose or phosphate, which occurs with Mueller-Hinton agar, increasing fosfomycin's MIC values compared with other culture media. The second transporter is induced by the presence of G6P; therefore, when G6P is added to the medium, fosfomycin enters the bacteria more effectively, and its MIC values are drastically reduced. The addition of 25 mg/L of G6P mimics the physiological situation of bacteria at the site of the infection; the MIC values would therefore approach the theoretical values. An increase in the amount of G6P above 25 mg/L in the medium has little effect on the MIC values.

Some microorganisms, such as *P. aeruginosa*, lack a G6P-dependent transporter and only present the glycerol 3-phosphate-dependent transporter. In this case, the addition of G6P to the medium does not change the MIC values [25]. It has recently been shown that fosfomycin activity is increased (lower MIC values) in this microorganism when studied in conditions with limited oxygen availability. This is explained by higher expression of the glycerol-3-phosphate-dependent transporter GlpT, which would resemble that of growth conditions in biofilms and would explain the strong fosfomycin activity against *P. aeruginosa* when grown in these conditions [26].

Although broth microdilution is not recommended for the study of fosfomycin susceptibility testing, a number of authors have demonstrated in *P. aeruginosa* the equivalence of agar dilution and broth microdilution [25]. In *Enterobacterales*, there is a very low correlation between the various methods, including the automatic systems and agar dilution, and are therefore not recommended for the susceptibility study [27, 28].

In the diffusion methods, G6P is added to the disc or to the gradient strips. The disc load recommended by EUCAST and CLSI is 200 µg with 50 µg of G6P. The reading of inhibition zone or ellipses is usually problematic because colonies can appear inside the inhibition zone in up to 41% of *E. coli* isolates. EUCAST has standardized its reading for *E. coli,* proposed that colonies considered susceptible within the inhibition zone should be ignored and has planned to offer recommendations for *K. pneumoniae* and *P. aeruginosa.* Using whole genome sequencing, Lucas et al. [17] recently studied the colonies observed inside the inhibition zone, estimating that only 0.8% of cases were considered resistant when re-examined by disc diffusion. These colonies are mutants whose resistance is due to deletions or nonsense mutants in the *uhpT* gene associated with G6P-dependent fosfomycin transport.

To facilitate reading the inhibition zones or ellipses, reducing the standard inoculum from $1.5x10^8$ to $1.5x10^6$ colony-forming units/mL has been proposed for *P. aeruginosa* [29]. This reduction decreases the presence of inner colonies and improves the correlation with the agar dilution MIC values to better define the wild-type population [MIC less than or equal to the epidemiological cutoff (ECOFF), 128 mg/L]. This approach should also be explored in *Enterobacterales*.

Table 2 **Clinical breakpoints for intrepreting fosfomycin susceptibility testing results**

	EUCAST				CLSI			
	MIC (mg/L)		Inhibition zone (mm)		MIC (mg/L)		Inhibition zone (mm)	
	≤S	>R	≥S	<R	≤S	≥R	≥S	≤R
Enterobacterales	32[a]	32[a]	24[a]	24[a]	64[b]	256[b]	16[b]	12[b]
Pseudomonas spp.	128[c]	128[c]	12[c]	12[c]	-	-	-	-
Enterococcus spp.	-	-	-	-	64[d]	256[d]	16[d]	12[d]
Staphylococcus spp.	32[e]	32[e]	-	-	-	-	-	-
Streptococcus pneumoniae	IE	IE	IE	IE	-	-	-	-
Haemophilus influenzae	IE	IE	IE	IE	-	-	-	-
Moraxella catarrhalis	IE	IE	IE	IE	-	-	-	-

EUCAST, European Antimicrobial Suceptiblity Testing Committee; CLSI, Clinical and Laboratory Standards Institute; IE: insufficient evidence to establish breakpoint values.

[a]Intravenous and oral use (uncomplicated UTI); [b]*E. coli* isolates from the urinary tract; [c]Epidemiological cutoff values (ECOFF) use in combination with other antimicrobials; [d]*E. faecalis* isolates from the urinary tract; [e]Intravenous use

Table 3 **Fosfomycin activity in pathogens with various resistance mechanisms**

Author, date of publication	Microorganism, resistance, (n)	% Fosfomycin susceptibility	Other susceptibility data	Methodology (Breakpoints)	Source of isolate	Country	Ref.
Flamm, R., 2018	*E. coli* (22)/ ESBL *K. pneumoniae* (21)	81.8%/91.7%	MIC_{50}, MIC_{90}= 0.5, 2 mg/L / MIC_{50}, MIC_{90}= 4, 8 mg/L	Agar dilution (CLSI)	SENTRY study	USA	(30)
	E. coli (11)/ Carbapenemase *K. pneumoniae* (12)	92%	MIC_{50}, MIC_{90}= 8, 64 mg/L / MIC_{50}, MIC_{90}= 1, >256 mg/L				
Falagas, M.,2009	MDR/XDR Enterobacterales (152)	98%		Gradient strips (CLSI)	Clinical isolates	Greece	(31)
Bouxom, H., 2018	ESBL *E. coli* and *K. pneumoniae* (100)	92.7%		Agar dilution (EUCAST)	Urinary-bacteremia isolates	France	(35)
Bi, W. 2017	ESBL *E. coli* (356)	92,7%	MIC_{50}, MIC_{90}= 0.5, 32 mg/L	Agar dilution (CLSI)	Urinary isolates	China	(34)
Mezzatesta ML., 2017	ESBL *E. coli* (24)/ KPC *K. pneumoniae* (53)	100%/78%	MIC_{50}, MIC_{90}= 0.5, 1 mg/L / MIC_{50}, MIC_{90}= 32, 128 mg/L	Agar dilution/microdilution/ gradient diffusion (CLSI)	Urinary isolates	Italy	(32)
Flamm, R., 2018	*P. aeruginosa* not susceptible to CAZ-AVI (21)	85.7%	MIC_{50}, MIC_{90}= 32, 128 mg/L	Agar dilution (CLSI)	SENTRY study	USA	(30)
	P. aeruginosa not susceptible to MER (20)	75%	MIC_{50}, MIC_{90}= 32, 128 mg/L				
Walsh C., 2015	MDR and non-MDR *P. aeruginosa* (64)	61%	MIC_{50}, MIC_{90}= 64, 512 mg/L	Agar dilution/microdilution (CLSI)	Cystic fibrosis, bacteremia	Australia	(10)
Perdigao-Neto LV., 2014	MDR *P. aeruginosa* (15)	7%		Agar dilution/microdilution (CLSI)	Urinary, bacteremia and respiratory isolates	Brazil	(38)
Flamm, R., 2018	MRSA (101)	100%	MIC_{50}, MIC_{90}= 4, 8 mg/L	Agar dilution (CLSI)	SENTRY study	USA	(30)
Falagas M., 2010	MRSA (130)	99.2%		Disc diffusion (200) (CLSI)	Nonurinary	Greece	(40)
Lu CL., 2011	MRSA (100)	89%		Agar dilution (NE)	Clinical isolates	Taiwan	(41)
López Díaz MC., 2017	MRSA (55)	43.6%	MIC_{50}, MIC_{90}= 128, 512 mg/L	Agar dilution (NE)	Clinical isolates	Spain	(42)
Wu D., 2018	MRSA (293)	46.8%		Agar dilution (CLSI)	Urinary, bacteremia and respiratory isolates	China	(43)
Guo Y., 2017	VRE (890)	85.1% susceptible 13.4% intermediate		Agar dilution (CLSI)	Rectal swabs	USA	(44)
Tang HJ., 2013	VR *E. faecium* (19) VR *E. faecalis* (21)	30% 44%	MIC_{50}, MIC_{90}=128 mg/L	Agar dilution (CLSI)	Clinical isolates	Taiwan	(45)

CAZ/AVI, ceftazidime/avibactam; CLSI, Clinical and Laboratory Standards Institute; ESBL, extended-spectrum beta-lactamase; EUCAST, European Committee on Antimicrobial Susceptibility Testing; KPC, *Klebsiella pneumoniae* carbapenemase; MER, meropenem; MIC, minimum inhibitory concentration; MDR, multidrug-resistant; MRSA, methicillin-resistant *S. aureus*; NS, not specified; VR, vancomycin-resistant; VRE, vancomycin-resistant enterococcus; XDR, extremely drug-resistant

NEW DATA FROM EPIDEMIOLOGICAL SURVEILLANCE STUDIES

The revaluation of fosfomycin in recent years is due to the scarcity of new antibiotic options and the increased incidence of infections by multidrug-resistant microorganisms. Fosfomycin's unique mechanism of action results in no crossed resistances with other antibiotics. Fosfomycin is therefore situated as one of the few therapeutic options for infections by multidrug-resistant microorganisms. The latest studies that detail fosfomycin activity in pathogens with various mechanisms of resistance are listed in table 3.

***Enterobacterales* with extended-spectrum beta-lactamase and carbapenemases.** According to various *in vitro* susceptibility studies, fosfomycin maintains its activity against ESBL-producing and carbapenemase-producing *Enterobacterales*. It has been reported fosfomycin susceptibility rates of more than 80% against these microorganisms. The authors of a recent article that described fosfomycin activity clinical isolates from the US observed 100% (43/43 isolates) susceptibility to fosfomycin in ESBL-producing *E. coli* and *K. pneumoniae* (MIC_{50}/MIC_{90} of 0.5/2 mg/L and 4/8 mg/L, respectively). In terms of CPE, a susceptibility of 81.8% ($MIC_{50/90}$ of 1/>256 mg/L) was observed for *E. coli* isolates and 91.7% ($MIC_{50/90}$ of 8/64 mg/L) for *K. pneumoniae* [30]. A susceptibility of 94.9% was observed in CPE from Greece [31], while 78%

was observed in *K. pneumoniae* with *Klebsiella pneumoniae* carbapenemase (KPC) from Italy [32].

A review by Falagas et al. [33] that collected *in vitro* data calculated a fosfomycin susceptibility of 96.8% and 81.3% for ESBL-producing *E. coli* and *K. pneumoniae*, respectively. In China, a susceptibility of 92.7% was observed in *E. coli* with ESBL from urinary infections. The resistance in most isolates was associated with a plasmid that carries the bla_{fosA} and bla_{CTX-M} genes [34]

In a study that compared the antibiotic susceptibility of fosfomycin with that of other noncarbapenem antibiotics in *Enterobacterales* with ESBL, 98% of the isolates were fosfomycin-susceptible, while 100% were ceftazidime-avibactam-susceptible, 97% were susceptible to amikacin and piperacillin-tazobactam, and 96% were nitrofurantoin-susceptible [35].

Although these data demonstrated high susceptibility rates in this type of microorganism, an increase in fosfomycin-resistant isolate was reported in Spain during a 4-year period, which was attributed to the increased use of this antibiotic in community-acquired urinary tract infections and to the dispersion of epidemic clones [36]. Likewise, PD studies conducted using time-kill curves and *in vitro* models of emergence of resistant mutants in enterobacteria with ESBL and/or carbapenemases showed not only the bactericidal activity of fosfomycin but also a regrowth of resistant subpopulations that varied according to the species and isolate [37].

Multidrug-resistant *Pseudomonas aeruginosa*. Fosfomycin activity against *P. aeruginosa* is controversial due to the mutation freequency rate at which resistant mutants emerge. There is considerable heterogeneity in the *in vitro* susceptibility results, often due to the method employed for reading the susceptibility. In a study conducted in Australia, 61% of multidrug-resistant and nonmultidrug-resistant *P. aeruginosa* isolates were susceptible to fosfomycin (considering the MIC breakpoint as ≤64 mg/L), with a similar MIC distribution in the 2 groups [10]. In *P. aeruginosa* isolates not susceptible to ceftazidime-avibactam and not susceptible to meropenem, a fosfomycin susceptibility of 85.7% and 75%, respectively, was observed [30]. Much lower susceptibility rates (7%) were observed by Perdigao-Net et al. in Brazil [38].

A review of fosfomycin activity against nonfermenting Gram-negative bacilli collected 19 studies that measured a susceptibility rate in multidrug-resistant *P. aeruginosa* of 30.2%, with a considerable variety of methods employed and different mean susceptibility rates for each of them [39]: microdilution, 91.1% (mean 58.1%, range 0-100%, SD ±45%); agar, 90% (mean 70%, range 0-100%, SD ±41%); disc diffusion, 56.3% (mean 51%, range 0-100%, SD ±35%) and MIC gradient test, 11.1% (mean 28.6%, range 0-93.3%, SD ±35%). Given that agar dilution is the reference method for fosfomycin susceptibility testing, our group has proposed an alternative procedure for implementing the diffusion methods, in which the 0.5 McFarland inoculum is diluted 100 times, which significantly improves the correlation with the reference method [29].

Methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus*. While a number of studies have observed good fosfomycin activity in methicillin-susceptible *S. aureus* (MSSA) and in MRSA, with susceptibility rates of up to 99.2% [30, 40, 41], other studies have reported susceptibility readings of less than 50% in MRSA [42], with differences according to the clonal lineage [43]. Likewise, data on fosfomycin activity against *Enterococcus* vary according to the study. Thus, more than 80% of vancomycin-resistant *Enterococcus faecium* have preserved fosfomycin susceptibility [44] versus 30% reported in other studies [45].

ANTIMICROBIAL ACTIVITY IN BIOFILMS

Fosfomycin has shown a high rate of penetration in mature biofilms of *P. aeruginosa* [46]. Likewise, the anaerobic environment present in the interior of these structures favors the expression of the fosfomycin transporter GlpT. A larger quantity of antibiotic therefore penetrates the interior of the bacteria [26]. There are several *in vitro* and animal model studies that have shown that fosfomycin combined with various antibiotics has the capacity to eradicate or reduce the biofilms of Gram-positive and Gram-negative bacteria. An example of this is the published studies on MRSA biofilms, in which good results have been obtained with fosfomycin combined with vancomycin [47], rifampicin [48], linezolid, minocycline, vancomycin or teicoplanin [49, 50] or with *Enterococcus faecalis* in monotherapy and in combination with gentamicin [8]. Likewise, synergy has been demonstrated against *P. aeruginosa* biofilms in combination with tobramycin, enhancing the penetration of this antibiotic to the cell's interior [51-53].

FOSFOMYCIN ACTIVITY IN COMBINATION WITH OTHER ANTIMICROBIALS

One of the main problems presented by fosfomycin is the high rate at which resistant mutants emerge during the treatment, which, coupled with the lack of crossed resistances and antagonism with other families, means that fosfomycin is administered in most cases in combination with other antimicrobials. There are numerous *in vitro* studies that have sought to elucidate the effect of the combinations, against both Gram-negative bacilli and Gram-positive microorganisms.

Combinations against Gram-negative bacteria. Fosfomycin is one of the few alternatives (along with aminoglycosides and colistin) that present MICs within the susceptibility range in CPE. Therefore, the activity of the combinations of these antibiotics has been studied. The effect of the combination of fosfomycin and amikacin or colistin against KPC-2-producing *K. pneumoniae* was determined in a PK-PD model. A lower resistance rate was observed with the use of the fosfomycin-colistin combination than when colistin was employed in monotherapy [54]. This synergistic effect appears to be due

to the fact that colistin facilitates the entry of fosfomycin into the bacteria's interior, thereby increasing fosfomycin's concentration in the active site. The effect of the fosfomycin-colistin combination appears to depend on the type of strain studied. Thus, the bactericidal effect was not boosted with the combination in colistin-heteroresistant or colistin-resistant strains [55, 56]. *In vitro* synergy with imipenem, ertapenem and tigecycline was also demonstrated in time-kill curves and checkerboard models in KPC-producing *K. pneumoniae* [57].

An interesting combination is the one with phosphonoformic acid (foscarnet) derivatives, an antiviral drug that also possess activity as inhibitor of the FosA enzyme which hydrolyze fosfomycin in Gram-negative microorganisms. Fosfomycin activity is thereby increased in bacteria such as *P. aeruginosa*, *K. pneumoniae* and *Enterobacter cloacae*, which have this enzyme encoded in their chromosome [58] .

The combination of temocillin and fosfomycin has also been shown to be synergistic *in vitro* and *in vivo* and prevents the emergence of resistant mutants when used against *E. coli* with KPC carbapenemases and even OXA-48, which confer resistance to temocillin [59].

In *P. aeruginosa*, there is an alternative pathway bound to the recycling of the peptidoglycan, which prevents its *de novo* synthesis. This fact could explain the lower fosfomycin activity in this microorganism. Peptidoglycan recycling inhibitors have been shown to increase fosfomycin susceptibility [60].

In terms of beta-lactam antibiotics, ceftolozane-tazobactam in combination with fosfomycin has demonstrated *in vitro* synergy, which could be useful for treating infections caused by multidrug-resistant *P. aeruginosa* [61]. Likewise, the combination with meropenem in a model of hollow-fiber infection increased the bactericidal effect and prevented the emergence of resistant mutants [62].

Combinations against Gram-positive bacteria. The combination of fosfomycin and daptomycin is one of the most studied strategies against Gram-positive bacteria. In a recent review that collected cases of infection caused by Gram-positive microorganisms treated with different fosfomycin combinations and the results of time-kill curves in MRSA and MSSA, the combination with daptomycin was shown to be the most effective [63]. An animal model of MRSA endocarditis showed the bactericidal and synergistic action of this combination, where the proportion of sterile vegetations and the bacterial inoculum in the vegetations were also improved [64]. Likewise, daptomycin combined with fosfomycin showed synergy *in vitro* and in a PK-PD model in VRE [65]. In MRSA with intermediate susceptibility to glycopeptides, the combination with imipenem or ceftriaxone was synergistic in an animal model and in time-kill curves [66]. Using time-kill curves and checkerboard assays, *in vitro* synergy has also been demonstrated against MRSA for fosfomycin combined with linezolid [67], rifampicin, tigecycline [68], acid fusidic [69] or quinupristin-dalfopristin [70].

CONCLUSIONS

The microbiological understanding and clinical use of fosfomycin has increased in recent years. However, various aspects still need to be defined, such as those related to its *in vitro* susceptibility study and the PK-PD parameters that best predict its clinical efficacy. Despite this need and the introduction of new antimicrobials with activity against multidrug-resistant microorganisms, the empiric and targeted use of fosfomycin (alone or in combination with other antimicrobials) has increased. It is therefore essential to have fosfomycin in countries with the highest resistance rates, as supported by surveillance studies on resistance and the clinical guidelines.

CONFLICTS OF INTEREST

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