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Letters to the Editor

Frequency of Spontaneous Resistance to Fosfomycin Combined with Different Antibiotics in *Pseudomonas aeruginosa*▽

Chronic infections caused by *Pseudomonas aeruginosa* are probably the most recurrent clinical situation where antibiotic treatment fails (5). The presence of hypermutable strains exacerbates this phenomenon and appears to be an important factor for the development of multiple-antimicrobial resistance (6, 8). The shortage of new antimicrobials led to reconsideration of old antibiotics, including fosfomycin, as appealing alternatives for treatments (2). The good effectiveness of fosfomycin combined with other antibiotics has been reported (2, 3, 7, 9, 11, 15). However, *P. aeruginosa* has a very high mutant frequency for fosfomycin resistance *in vitro* (12) and *in vivo* (13), suggesting an elevated risk of resistance to combined treatments.

We analyzed the frequency of mutants resistant to fosfomycin in combination with other antimicrobials currently used in *P. aeruginosa* infections, including tobramycin, amikacin, imipenem, meropenem, ceftazidime, ciprofloxacin, and colistin. PA14 and its hypermutable *mutS*::*MAR2xT7* derivative (4) were used as model strains. Experiments were performed in quintuplicate in all cases as described previously (12). Antibiotic concentrations were chosen according to each drug clinical breakpoint, as established by EUCAST (www.eucast.org/clinical_breakpoints/), except for fosfomycin, which was used at 128 μg/ml to avoid the background on plates (no significant differences were found in mutant frequencies at 32, 64, and 128 μg/ml [not shown]).

The mutant frequencies of PA14 for individual antibiotics were very high for fosfomycin, high for imipenem and meropenem, moderate for ceftazidime, and relatively low for ciprofloxacin, tobramycin, and amikacin (Table 1). The frequencies of the hypermutable strain were, as expected, 100- to 1,000-fold higher. However, the mutant frequencies of the wild-type (WT) strain for the combinations were below the limit of detection ($<1 \times 10^{-10}$) for all antibiotics, except for imipenem plus fosfomycin (1.1×10^{-9}). For the hypermutable strain, the mutant frequencies for combinations of fosfomycin with tobramycin, amikacin, meropenem, ciprofloxacin, and colistin were below the limit of detection. However, combinations with ceftazidime or imipenem yielded a higher-than-expected number of mutants resistant to both antibiotics (higher than the product of the frequencies for each single antibiotic), with values of 1.0×10^{-8} and 1.1×10^{-7}, respectively. These interesting results remain to be explained, although antagonism between fosfomycin and these antibiotics *in vitro* could not be demonstrated (data not shown). These results suggest that the combinations of fosfomycin with ceftazidime or imipenem are less appropriate, in terms of probability of mutant occurrence, than those with tobramycin or ciprofloxacin.

TABLE 1. Frequency of mutants of *P. aeruginosa* PA14 and its hypermutable *mutS* derivative resistant to single antibiotics and their combinations with fosfomycin

Antibiotic(s)	MIC[a] (μg/ml)	Concn[b] (μg/ml)	Mutant frequency	
			WT	*mutS*
Fosfomycin	8	128	2.3×10^{-6}	2.1×10^{-4}
Tobramycin	1	4	2.2×10^{-9}	1.4×10^{-7}
Amikacin	1	16	2.6×10^{-9}	1.2×10^{-7}
Imipenem	0.5	8	1.5×10^{-7}	1.6×10^{-5}
Meropenem	0.25	8	4.2×10^{-8}	1.3×10^{-6}
Ceftazidime	1	8	1.3×10^{-8}	1.3×10^{-6}
Ciprofloxacin	0.1	1	1.1×10^{-9}	9.0×10^{-7}
Colistin	0.5	2	$<1.0 \times 10^{-10}$	$<1.0 \times 10^{-10}$
Tobramycin + fosfomycin		4 + 128	$<1.0 \times 10^{-10}$	$<1.0 \times 10^{-10}$
Amikacin + fosfomycin		16 + 128	$<1.0 \times 10^{-10}$	$<1.0 \times 10^{-10}$
Imipenem + fosfomycin		8 + 128	1.1×10^{-9}	1.1×10^{-7}
Meropenem + fosfomycin		8 + 128	$<1.0 \times 10^{-10}$	$<1.0 \times 10^{-10}$
Ceftazidime + fosfomycin		8 + 128	$<1.0 \times 10^{-10}$	1.0×10^{-8}
Ciprofloxacin + fosfomycin		1 + 128	$<1.0 \times 10^{-10}$	$<1.0 \times 10^{-10}$
Colistin + fosfomycin		2 + 128	$<1.0 \times 10^{-10}$	$<1.0 \times 10^{-10}$

[a] The MICs for PA14 (WT) and its *mutS* derivative were identical in all cases. For all antibiotics, the MIC was determined as recommended by the CLSI (1).
[b] Final concentration used for calculation of mutant frequency.

In cases of chronic infection, such as that involving cystic fibrosis, the bacterial load of *P. aeruginosa* can be as high as 10^7 to 10^9 CFU per ml of mucus secretion (14), with a high frequency of hypermutable strains (10). According to our results, the probability of finding mutants resistant to the combination of fosfomycin with ceftazidime or imipenem is dangerously high.

Antibiotic combinations must be carefully considered to minimize the selection of strains with double resistance. Further studies on combinations need to be done considering different criteria, including pharmacological activity and the possibility of emergence of resistant mutants.

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