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Pharmacodynamics of fosfomycin against extended spectrum beta lactamase and/or carbapenemase producing Enterobacteriaceae

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Introduction

The increase of antibiotic resistance in Gram-negative bacteria and the limited therapeutic options due to the shortage of new antibiotics has increased the interest of the "old" antibiotic fosfomycin in the treatment of infections. However there are contradictory reports on the pharmacodynamics of and emergence of resistance to fosfomycin.

Aim of the study

To assess the pharmacodynamics (PD) of fosfomycin in a collection of Enterobacteriaceae using extensive time-kill assays, including analysis of subpopulations.

Methods

Strains

- Eleven ESBL+ producing and three ESBL- strains. MIC 0.5-64 mg/L.

Antibiotic and susceptibility testing

- Phosphomycin disodium salt (FOS). MIC determined by agar dilution (ISO).

Time-Kill assay and Analysis

- Time-kill assays were performed at 37°C at two-fold increasing concentrations from 0.125 up to 32xMIC.
- The sigmoid maximum effect (E_{max}) model was fitted to the time-kill curves data.
- Amplification of resistance over time was evaluated under various conditions of selective pressure by plating on 16x MIC plates
- Kill rate ($log_{10}CFU/mL \times h^{-1}$) was determined by linear regression analysis for the time interval of 0-6h.
- The maximal kill-rate (*Emax*) and the concentration corresponding to 50% of *Emax* (EC50/MIC) was determined.

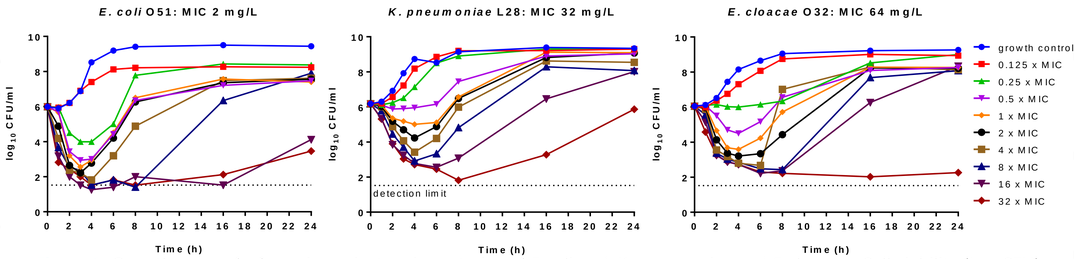

Figure 1. Growth curves fosfomycin against various strains of *E.coli and K.pneumoniae* and *E.cloacae* . Cell viability (Log CFU) plotted for cultures grown at different concentrations of fosfomycin relative to strain-specific MICs.

Table 1. Pharmacodynamic parameter estimates after 6 hours of fosfomycin exposure against different strains of *E. coli*, *K. pneumoniae*, *E. cloacae* and *C. freundii*

Species	Strain	Growth rate ($log_{10}cfu/mL \times h^{-1}$)	Max killrate (h^{-1})	Hill slope	EC50/MIC	Stasis (mg/L)	C_{stasis}/MIC	R^2
E. coli	O5	0.505	0.420	4.02	0.71	0.36	0.72	0.971
	O39	0.567	0.406	3.41	0.33	0.37	0.37	0.969
	O41	0.564	0.502	2.65	0.36	0.38	0.38	0.958
	O51	0.615	0.573	1.09	0.15	0.44	0.22	0.918
	ATCC 25922	0.581	0.786	0.87	0.63	0.61	0.61	0.965
	All (mean ± SD)	**0.567 ± 0.039**	**0.537 ± 0.155**	**2.41 ± 1.39**	**0.44 ± 0.23**	**0.43 ± 0.10**	**0.46 ± 0.20**	
K. pneumoniae	O6	0.525	0.322	1.24	0.14	4.02	0.25	0.997
	O20	0.593	0.597	1.18	0.40	3.69	0.46	0.976
	O58	0.647	0.683	0.78	0.31	14.05	0.44	0.991
	L28	0.477	0.631	1.14	0.72	21.22	0.66	0.977
	All (mean ± SD)	**0.561 ± 0.075**	**0.558 ± 0.162**	**1.09 ± 0.21**	**0.39 ± 0.24**	**10.75 ± 8.48**	**0.45 ± 0.17**	
E. cloacae	O21	0.581	0.615	1.79	0.37	5.98	0.37	0.988
	O32	0.493	0.623	1.41	0.31	18.59	0.29	0.993
	O94	0.483	0.612	1.95	0.27	0.49	0.24	0.992
	Am30966C	0.470	0.801	0.82	0.21	11.84	0.16	0.989
	All (mean ± SD)	**0.507 ± 0.050**	**0.663 ± 0.092**	**1.49 ± 0.50**	**0.29 ± 0.068**	**9.22 ± 7.78**	**0.27 ± 0.080**	
C. freundii	O26	0.437	0.535	2.94	0.31	0.29	0.29	0.976

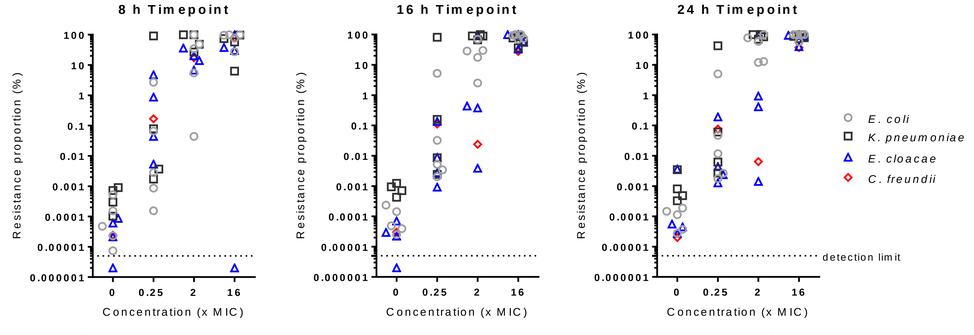

Figure 2. The resistance proportion (%), between the resistant subpopulation and total population exposed to different FOS concentrations (0x, 0.25x, 2x and 16x MIC) for 14 strains after 8, 16 and 24h. Species are indicated by different symbols.

Results

- The mean log-linear growth rates in the drug-free control were similar (0.51-0.57 $log_{10}CFU/ml \times h^{-1}$), except for *C.freundii* (0.44).
- For all strains a fast bactericidal effect within 6-8h was observed at a concentration of ≥4-8x MIC.
- Using the E_{max} model, no significant differences between strains were observed for the pharmacodynamic parameters.
- Large variation in Hill's slope factors for *E. coli* of 0.87 → 4.02 indicates that the killing behaviour appears to be more time dependent for some strains but concentration dependent for others.
- After initial fast killing, regrowth started to occur from 6-8h onwards and full growth of resistant (sub)populations was observed
- In the fosfomycin exposed cultures under low and high selective pressure (≥2x MIC) the median resistance proportions between the resistant and total population increased from ≤ 0.0002 % (T=0h) to 65.2-89.9 % (T=24h).
- Resistance appeared stable after repeated subculturing

Conclusion

- Killing behavior of fosfomycin does not only differ between species but also within species and may have an impact on the design of optimal dosing regimens.
- Although fosfomycin was bactericidal against all strains (re)growth of resistant sub-populations occurred relatively fast. This may limit the use of fosfomycin as a single drug therapy.

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