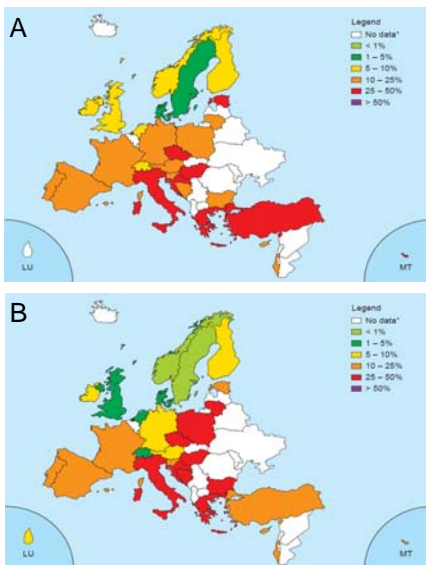


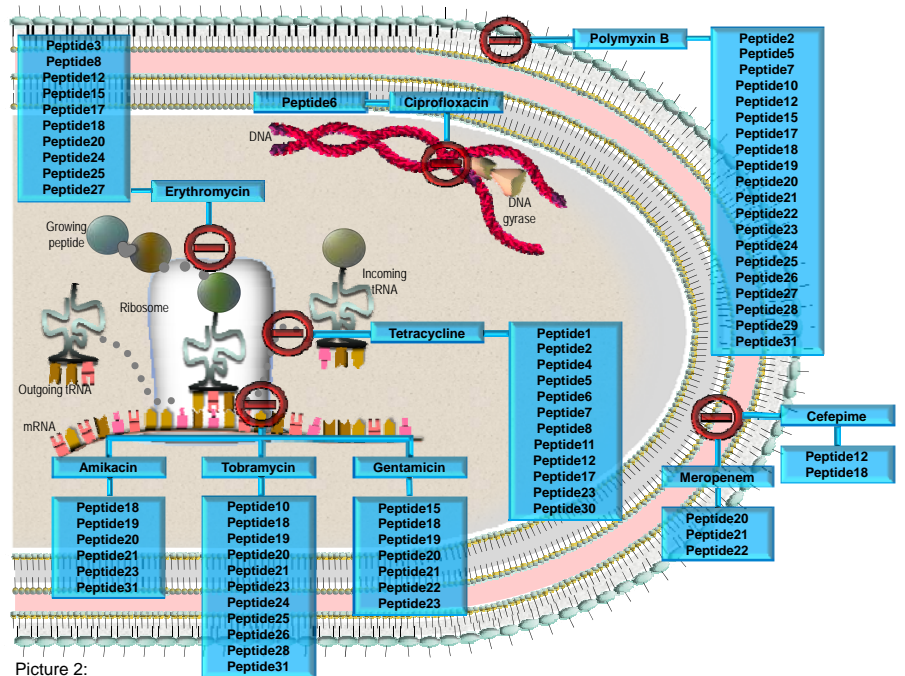
Synergy as a strategy to kill multidrug-resistant *Pseudomonas aeruginosa* with common antibiotics

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Introduction
Worldwide increase of bacterial resistance to medically used antibiotics presents a serious concern in modern medicine. Especially the nosocomial infections with the germs of *Pseudomonas aeruginosa* (*P. aeruginosa*) are extremely hard to cure. *P. aeruginosa* has natural insensibility to different classes of antibiotics as well as the ability to adopt new resistance genes and therefore to develop multidrug-resistance very rapidly. Considering such critical situation, many efforts are done to develop novel drugs and therapies against multidrug-resistant (MDR) microbes. A promising candidate for a lead structure to design new drugs against MDR pathogens is the class of cationic host defense peptides (HDPs). HDPs show *in vitro* antimicrobial broad-spectrum activity, also against MDR microbes. Here, we studied the synergy between short cationic HDPs and different clinical antibiotics, which were only weak active against MDR *P. aeruginosa*.



Picture 1: The proportion of invasive isolates of *P. aeruginosa* resistant to A) carbapenems (e.g. imipenem, meropenem), B) aminoglycosides (e.g. amikacin, gentamicin), within the EU in 2008 [EARSS Annual Report 2008].



Picture 2: Targets of different antibiotics, that showed synergistic effects in combination with different HDPs.

Table 1:
Fractional inhibitory concentration (FIC) indices obtained for the combination of 13 antibiotics and 31 HDPs, determined by a checkerboard assay. FIC index values above 2.0 indicate antagonistic effects, values between 0.5 and 2.0 indicate additive effects, values between 0.5 and 0.4 indicate weak synergistic effects (shown in grey) and values of less than 0.4 indicate strong synergistic effects (shown in dark grey).

HDPs	Antibiotics												
	Polymyxin B	Piperacillin	Ceftazidime	Cefepime	Meropenem	Imipenem	Ciprofloxacin	Tetracycline	Erythromycin	Kanamycin	Tobramycin	Amikacin	Gentamicin
Peptide1	0.51	1.00	2.00	0.75	2.00	1.32	1.08	0.44	0.69	0.71	1.00	1.00	0.75
Peptide2	0.49	0.56	2.00	0.63	0.63	1.00	0.59	0.48	0.64	0.56	0.53	1.00	0.75
Peptide3	1.09	0.56	2.00	0.51	0.75	1.00	0.63	0.71	0.42	0.63	2.00	1.00	0.75
Peptide4	1.17	0.63	2.00	0.63	0.78	0.63	0.63	0.38	0.67	0.67	2.00	0.51	0.56
Peptide5	0.43	0.56	0.63	1.00	0.76	0.75	0.75	0.38	1.04	0.56	0.63	2.00	0.75
Peptide6	0.78	1.00	0.56	0.69	1.26	0.63	0.41	0.46	0.66	1.00	2.00	0.88	0.63
Peptide7	0.38	1.00	2.00	1.00	0.57	0.63	0.53	0.41	0.53	0.75	2.00	2.00	0.75
Peptide8	0.67	0.53	0.56	0.56	0.57	2.00	0.75	0.46	0.50	0.63	2.00	0.53	1.00
Peptide9	1.00	0.75	2.00	0.63	0.55	0.75	0.56	0.60	0.53	0.63	0.51	0.75	1.00
Peptide10	0.44	0.63	2.00	1.00	0.58	1.00	1.00	0.52	0.75	0.60	0.75	0.53	0.54
Peptide11	0.97	0.56	1.00	0.75	0.64	0.51	0.63	0.34	0.51	0.56	1.32	0.63	0.63
Peptide12	0.39	1.00	0.53	0.50	0.67	0.75	0.75	0.50	0.43	0.75	1.38	0.51	0.63
Peptide13	0.55	0.63	2.00	0.51	1.00	1.00	0.75	0.55	0.53	0.75	2.00	0.51	0.75
Peptide14	0.60	0.63	2.00	2.00	1.00	0.75	0.63	0.75	0.75	0.56	2.00	2.00	2.00
Peptide15	0.41	0.56	0.53	0.75	1.02	0.75	0.63	0.71	0.44	1.00	0.63	0.75	0.50
Peptide16	1.00	2.00	2.00	2.00	0.63	2.00	0.75	0.56	0.63	0.75	0.75	1.00	1.00
Peptide17	0.38	0.51	0.56	0.71	0.55	0.51	1.00	0.48	0.45	2.00	2.00	1.00	0.63
Peptide18	0.35	0.63	0.56	0.50	0.59	0.63	1.00	0.56	0.43	0.63	0.38	0.38	0.21
Peptide19	0.38	0.56	0.56	1.00	0.54	0.63	0.69	0.75	0.75	0.75	0.29	0.32	0.19
Peptide20	0.33	0.63	0.56	1.00	0.38	0.63	0.64	1.00	0.46	0.67	0.33	0.37	0.23
Peptide21	0.29	0.53	2.00	1.00	0.48	0.75	0.63	0.52	0.64	0.75	0.27	0.31	0.23
Peptide22	0.33	0.75	2.00	1.19	0.47	1.00	0.78	0.66	0.64	0.75	0.52	0.55	0.36
Peptide23	0.38	0.75	0.63	2.00	0.47	0.75	0.75	0.50	0.88	0.55	0.38	0.38	0.27
Peptide24	0.38	2.00	0.53	1.00	0.53	2.00	0.75	0.75	0.50	2.00	0.50	0.55	0.75
Peptide25	0.29	0.56	2.00	0.75	0.54	0.75	0.75	0.56	0.45	1.00	0.50	0.63	0.75
Peptide26	0.50	0.75	2.00	2.00	0.63	2.00	0.75	0.75	0.75	0.75	0.43	0.52	0.63
Peptide27	0.40	1.00	2.00	0.56	0.52	0.63	1.00	1.00	0.38	2.00	0.63	0.75	0.63
Peptide28	0.46	0.53	2.00	1.00	1.00	0.75	1.00	0.63	0.56	0.56	0.47	0.75	0.75
Peptide29	0.43	0.51	2.00	1.00	1.00	1.00	1.00	2.00	0.59	1.00	0.69	0.63	0.75
Peptide30	0.57	2.00	2.00	0.75	0.54	1.00	0.51	0.40	0.51	1.00	2.00	2.00	2.00
Peptide31	0.44	0.56	2.00	0.51	0.82	0.63	1.00	0.50	0.69	0.75	0.50	0.44	0.57

Conclusion:
We investigated the synergistic effects of 31 different HDPs with 13 clinically used antibiotics, resulting in 403 combinations. 20 HDPs showed synergistic activity both with the polymyxin B, a membrane-disrupting antibiotic, and at the same time several antibiotics which affect the bacterial ribosome. Total of 6 HDPs showed additionally synergy with beta-lactam antibiotics meropenem and cefepime. In addition 1 HDP was acting synergistically only with the antibiotic polymyxin B, and 3 HDPs were acting only with several ribosome-affecting antibiotics.

Outlook:
The results from our study demonstrate a new strategy to treat infections with MDR microbes. Antibiotics, that are clinically approved, but not active against MDR bacteria, can be reactivated by the combination with novel HDPs. Such synergisms can also help to reduce the development of new resistant bacteria.