Phage-Antibiotic Combinations Against Multidrug-resistant *S. aureus, E. faecium* and *P. aeruginosa*: Protein Synthesis Inhibitors Antagonize Phage Activity

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Background

- Bacterio (phage) to augment antibiotic efficacy is a possible therapeutic option with increasing antimicrobial resistance.
- Ideally, phage in combination with antibiotics (PAC) would retain their pharmacodynamic activity, despite some antibiotics interfering with bacterial metabolism components required for full phage activity.
- Limited studies have assessed phage-antibiotic combinations (PAC) and associated synergy (PAS); however, evaluations of antibiotic mechanism of action affecting PAC efficacy and subsequent phage susceptibility are lacking.
- Objective: To evaluate PAS and antagonism (PAA) among PAC with protein synthesis inhibitors and cell wall active agents against clinical strains of multidrug-resistant (MDR) *S. aureus, E. faecium* and *P. aeruginosa*.

Methods

- Strains of MDR *S. aureus* (684, D712, N315), *E. faecium* (R497, HOU503, SF12047), and *P. aeruginosa* (R9010, 10266) were each evaluated against phages (*S. aureus*: Intesti13 and Sb1, *E. faecium*: NV-497, NV-503-01, NV-503-02, *P. aeruginosa*: 14207, EM) in PAC with cell wall active agents (daptomycin, ceftaroline, cefepime) or protein synthesis inhibitors (linezolid, minocycline, rifampin, gentamicin, azithromycin).
- PAS and PAA were evaluated with modified checkerboard (CB) MIC followed by 24h TKA.Synergy and antagonism were defined as a fractional inhibitory concentration (FIC) of ≤0.5 and >4 in CB analyses and a ≥2 CFU/mL reduction from baseline or a PAC with CFU/mL higher than the most effective single treatment in 24h TKA, respectively.
- Antibiotic impact on phage infection efficiency was evaluated with efficiency of plating (EOP).
- Data were compared by one-way ANOVA and Tukey (HSD) test (P<0.05).

Results

Figure 1 A-C. Modified checkerboard MIC and 24h time kill analyses of multidrug-resistant strains of (A) *S. aureus*, (B) *E. faecium*, and (C) *P. aeruginosa* against phage-antibiotic combinations containing cell wall active antibiotics or protein synthesis pathway inhibitors.

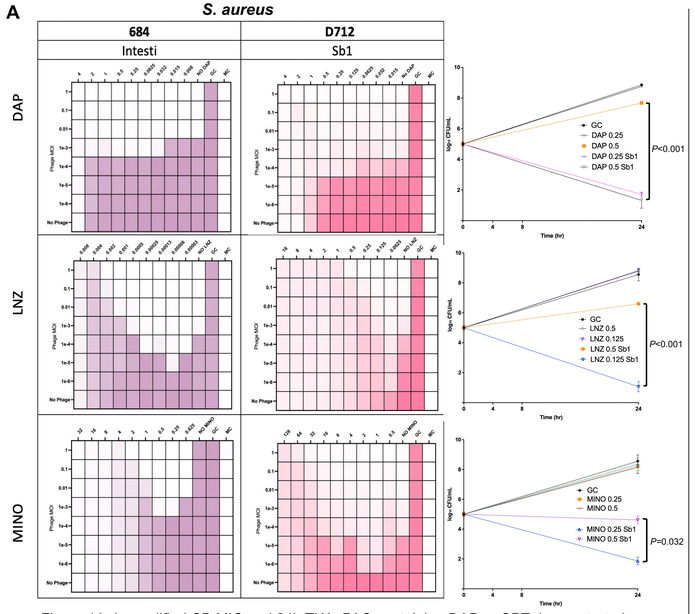

Figure 1A. In modified CB MIC and 24h TKA, PAC containing DAP or CPT demonstrated synergistic killing of *S. aureus* strains 684, D712, and N315 compared to DAP or CPT monotherapy (FIC 0.5, ANOVA range of mean difference 0.98 to 6.7 log10 CFU/mL; P<0.001); however, PAC with LNZ, MINO, or RIF demonstrated antagonism at 0.5x MIC compared to 0.25x MIC (FIC >4, ANOVA range of mean difference 3.2 to 6.4 log10 CFU/mL; P<0.001) (all data not shown). Phage EOP results demonstrated that LZD, MINO, and RIF, but not DAP or CPT impeded phage infection in 24h TKA compared to untreated controls (0.07±0.02 vs. 0.87±0.06 plaque forming units, respectively; P<0.001) (all data not shown).

Abbreviations: CB, checkerboard; MIC, minimum inhibitory concentration; TKA, time kill analysis; PAC, phage-antibiotic combination; DAP, daptomycin; CPT, ceftaroline; LNZ, linezolid; MINO, minocycline; RIF, rifampin; FIC, fractional inhibitory concentration; EOP, efficiency of plating.

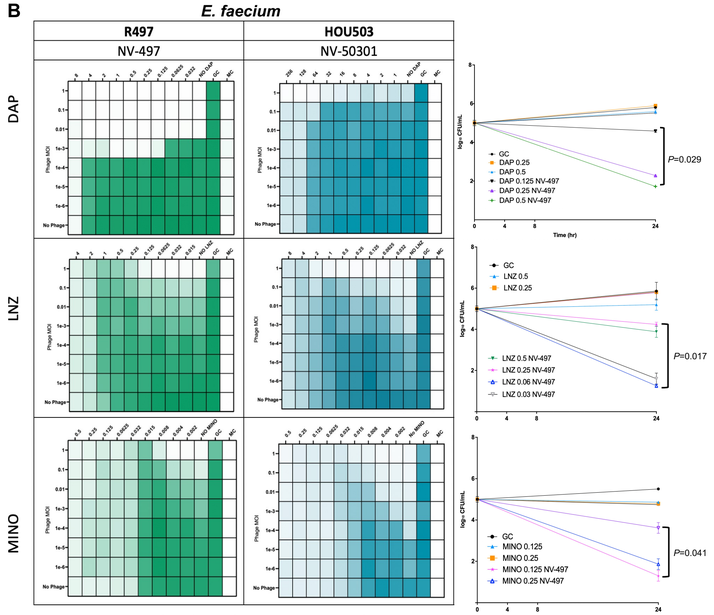

Figure 1B. In modified CB MIC and 24h TKA, PAC containing DAP or CPT demonstrated synergy against *E. faecium* strains R497, HOU503, and SF12047 at 0.5x and 0.25x MIC (FIC 0.5, ANOVA range of mean difference 0.9 to 3.2 log10 CFU/mL; P<0.001); however, PAC with LNZ, MINO, or RIF demonstrated antagonism at higher antibiotic concentrations of 0.5x MIC compared to 0.25x and 0.125x MIC (FIC >4, ANOVA range of mean difference 1.6 to 4.7 log10 CFU/mL; P<0.001) (all data not shown). Efficiency of plating results demonstrated that LZD, MINO, and RIF, but not DAP or CPT impeded phage infection in 24h TKA compared to untreated controls (0.13±0.07 vs. 0.59±0.04; P<0.001) (all data not shown).

Abbreviations: CB, checkerboard; MIC, minimum inhibitory concentration; TKA, time kill analysis; PAC, phage-antibiotic combination; DAP, daptomycin; CPT, ceftaroline; LNZ, linezolid; MINO, minocycline; RIF, rifampin; FIC, fractional inhibitory concentration; EOP, efficiency of plating.

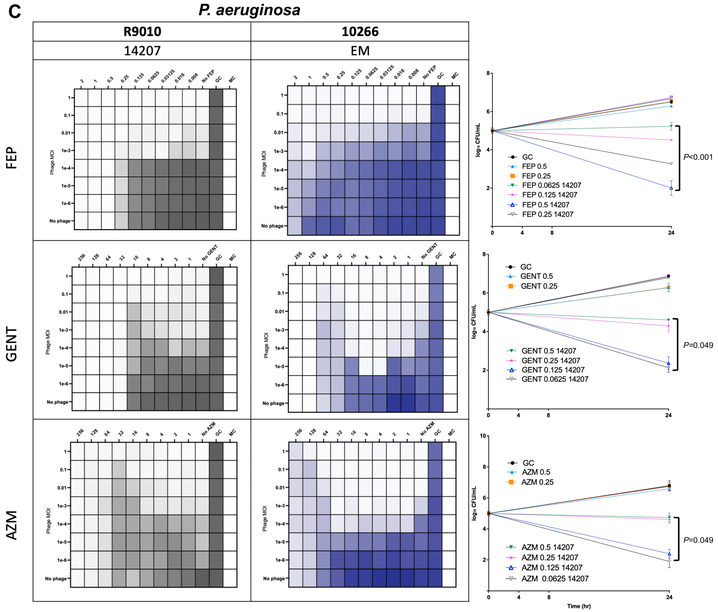

Figure 1C. In modified CB MIC and 24h TKA, PAC FEP at 0.5x MIC demonstrated synergistic killing against *P. aeruginosa* strains R9010 and 10266 compared to FEP monotherapy or FEP at lower concentrations (FIC 0.5, ANOVA range of mean difference 1.8 to 5.3 log10 CFU/mL; P<0.001); however, PAC with GENT or AZM demonstrated antagonism at 0.5x and 0.25x MIC compared to at lower antibiotic concentrations (FIC >4, ANOVA range of mean difference 1.4 to 5.1 log10 CFU/mL; P<0.001) (all data not shown). Efficiency of plating results demonstrated that GENT and AZM impeded phage infection in 24h TKA compared to untreated controls (0.05±0.04 vs. 0.79±0.14; P<0.001) (all data not shown).

Abbreviations: CB, checkerboard; MIC, minimum inhibitory concentration; TKA, time kill analysis; PAC, phage-antibiotic combination; FEP, cefepime; FIC, fractional inhibitory concentration; CFU, colony forming units; GENT, gentamicin; AZM, azithromycin.

Conclusions

- These data highlight significant PAA as demonstrated by PAC containing protein synthesis inhibitors (linezolid, minocycline, rifampin, gentamicin, azithromycin) in modified CB MIC and 24h TKA compared to PAS demonstrated with cell wall active agents (daptomycin, ceftaroline, cefepime) in PAC.
- Phage infection efficiency was also impacted by PAC containing protein synthesis pathway inhibitors compared to cell wall active agents as demonstrated by 24h TKA sample EOP results.
- Studies assessing the impact of phage activity, location of ribosomal protein synthesis inhibition, and bactericidal vs. bacteriostatic antibiotic activity are warranted.

Disclosures

The views expressed in this poster reflect the results of research conducted by the authors and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, nor the United States Government.

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