

Supplementary Materials: Improving the Efficacy of Antimicrobials against Biofilm-Embedded Bacteria using Bovine Hyaluronidase Azoximer (Longidaza®)

Elena Trizna, Diana Baydamshina, Anna Gorshkova, Valentin Drucker, Mikhail Bogachev, Anton Tikhonov and Airat Kayumov

Table S1. Minimum Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) values of antimicrobials.

	Ciprofloxacin		Cefuroxime	
	MIC, μg/mL	MBC, μg/mL	MIC, μg/mL	MBC, μg/mL
<i>S. aureus</i>	0.5	32	8	64
<i>E. faecalis</i>	8	32	8	32
<i>E. coli</i>	0.5	1	4	8
<i>K. pneumonia</i>	0.25	16	8	64
<i>P. aeruginosa</i>	0.5	1	8	64

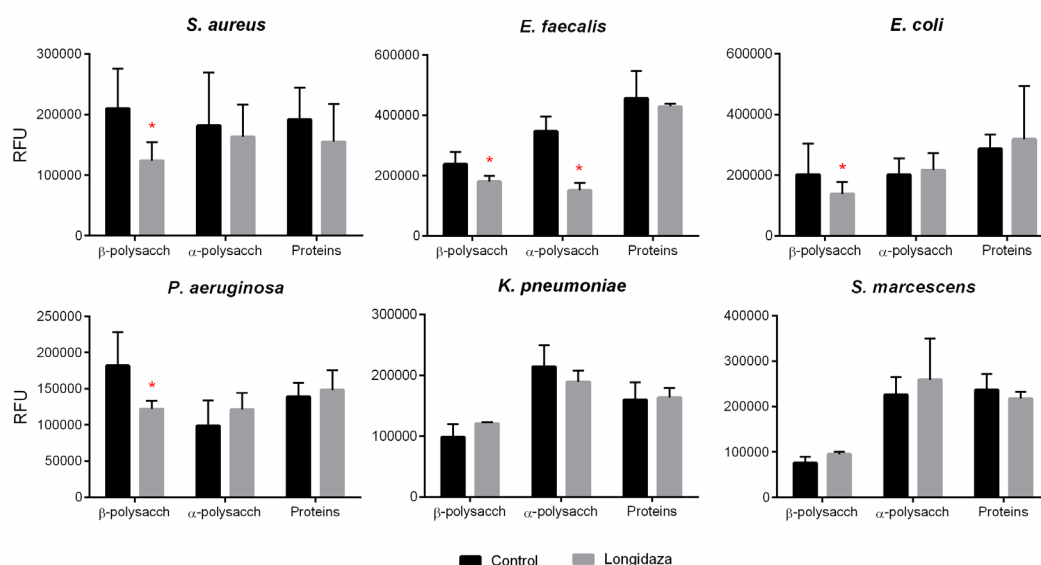


Figure S1. Differential evaluation of the fractions of biofilm matrix components of various bacteria before and after 24 h treatment with Longidaza®. Mature 48 h old biofilms were treated with Longidaza® (750 IU/mL) and stained with Concanavalin A, Calcofluor, and SyproOrange to evaluate the changes of α-polysaccharides, β - polysaccharides and proteins composition, respectively. Asterisks (*) denote statistically significant difference of fluorescence in untreated wells and wells treated with Longidaza® ($p < 0.05$). The text continues here (Figure 2 and Table 2).

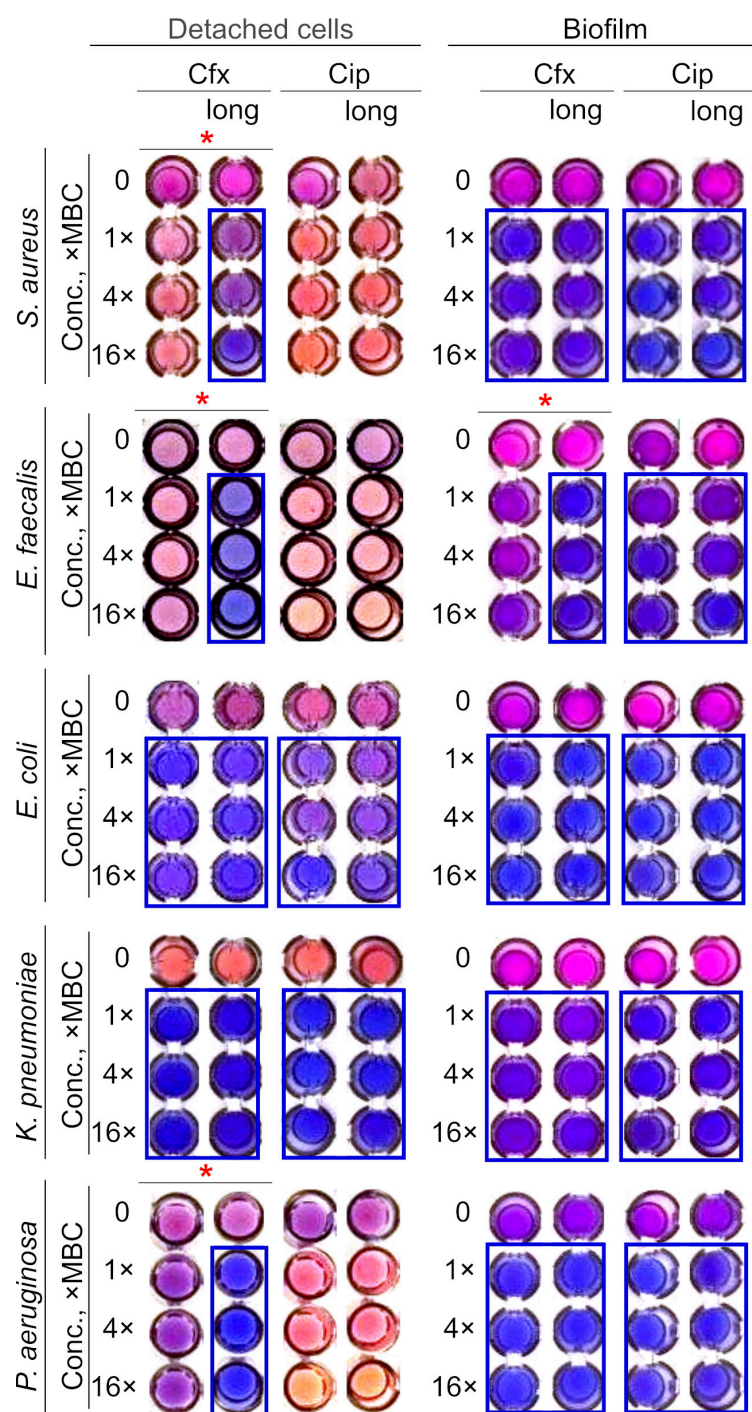


Figure S2. The effect of Longidaza® on the susceptibility of biofilms-embedded bacteria to antimicrobials. Longidaza® was added to 48 h old biofilms until final concentration of 750 IU/mL. Ciprofloxacin and cefuroxime were added up to final concentrations of 1-16× MBC (see Table S1 for values). After 24 h incubation, the biofilms were washed twice with sterile 0.9% NaCl. The viability of adherent cells was analyzed with resazurine test. Blue boxes denote wells with non-viable cells. * shows where there was a difference between treated and untreated wells.

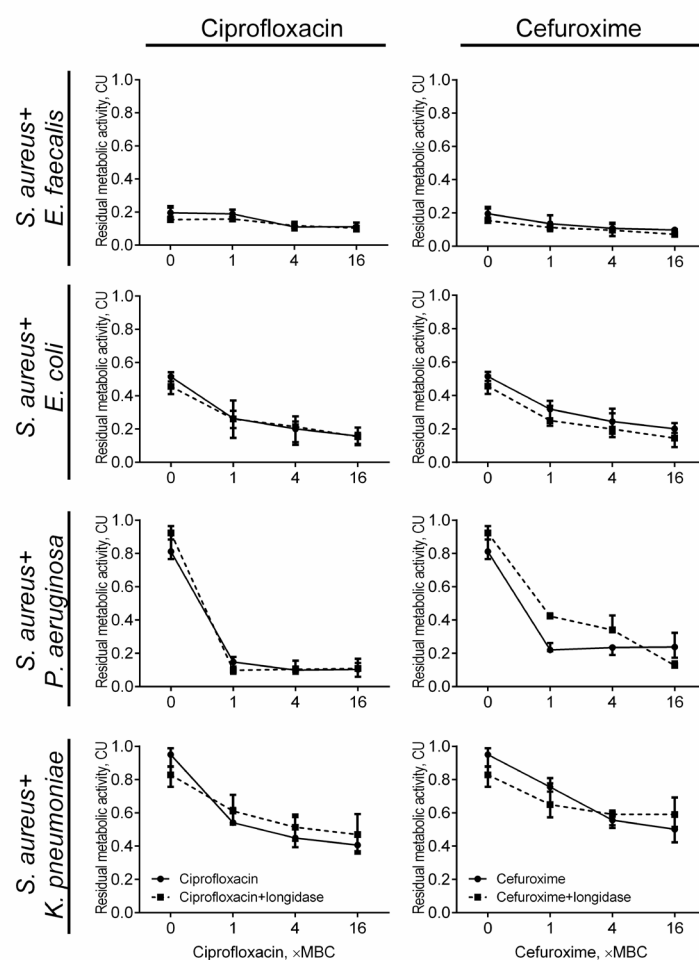


Figure S3. The effect of Longidaza® on the susceptibility of biofilms-embedded dual species bacterial consortia to antimicrobials. Longidaza® was added to 48 h old biofilms until final concentration of 750 IU/mL. Ciprofloxacin and cefuroxime were added up to final concentrations of 1–16×MBC (see Table S1 for values). After 24 h incubation, biofilms were washed twice with sterile 0.9% NaCl and the viability of adherent cells was analyzed with MTT assay.

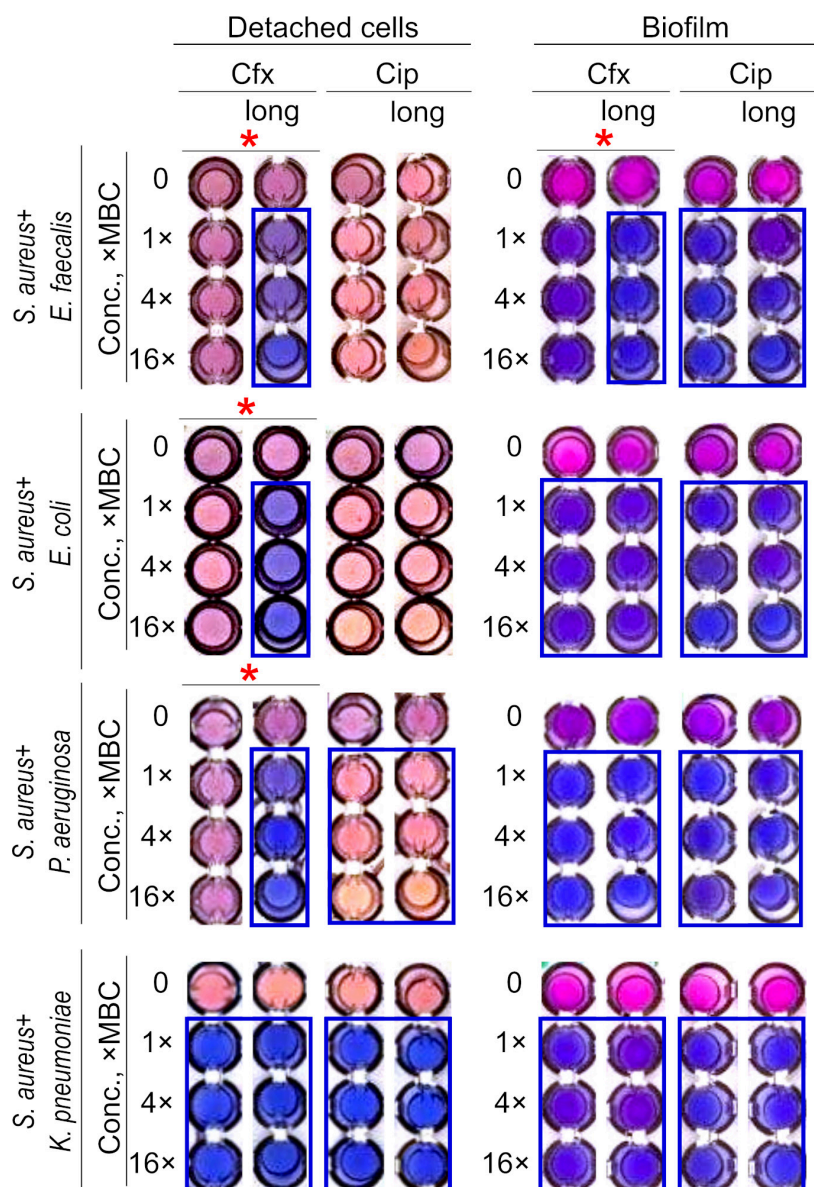


Figure S4. The effect of Longidaza® on the susceptibility of biofilms-embedded dual species bacterial consortia to antimicrobials. Longidaza® was added to 48 h old biofilms until final concentration of 750 IU/mL. Ciprofloxacin and cefuroxime were added up to final concentrations of 1-16xMBC (see Table S1 for values). After 24 h incubation, biofilms were washed twice with sterile 0.9% NaCl and the viability of adherent cells was analyzed with resazurine test. Blue boxes denote wells with non-viable cells. * shows where there was a difference between treated and untreated wells.