

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

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

	Ciprofloxacin		Cefuroxime	
	MIC, $\mu\text{g/mL}$	MBC, $\mu\text{g/mL}$	MIC, $\mu\text{g/mL}$	MBC, $\mu\text{g/mL}$
<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

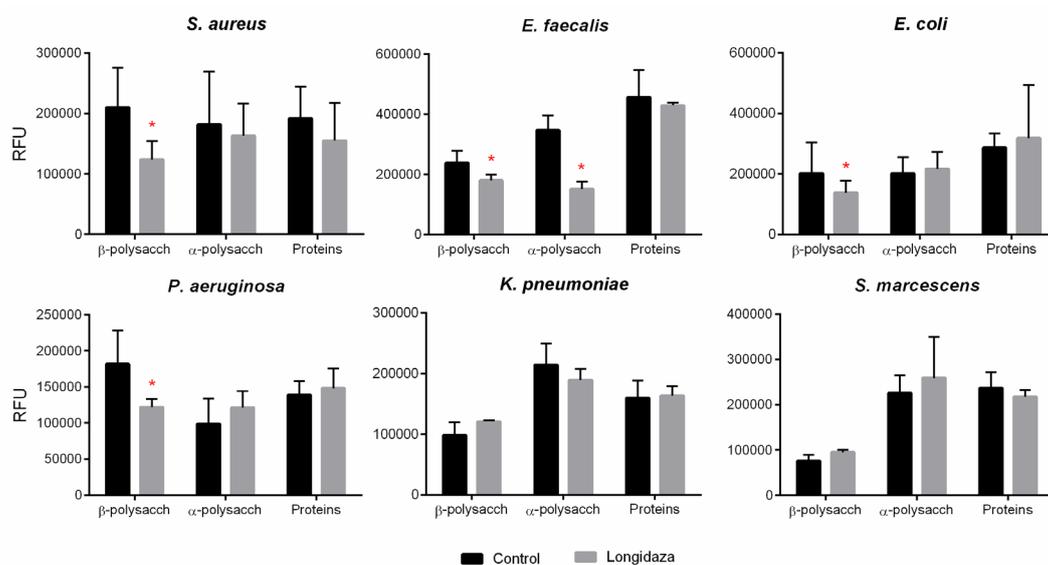


Figure S1. Differential evaluation of the fractions of biofilm matrix components of various bacteria before and after 24 h treatment with Longidaza®. Mature 48 h old biofilms were treated with Longidaza® (750 IU/mL) and stained with Concanavalin A, Calcofluor, and SyproOrange to evaluate the changes 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).

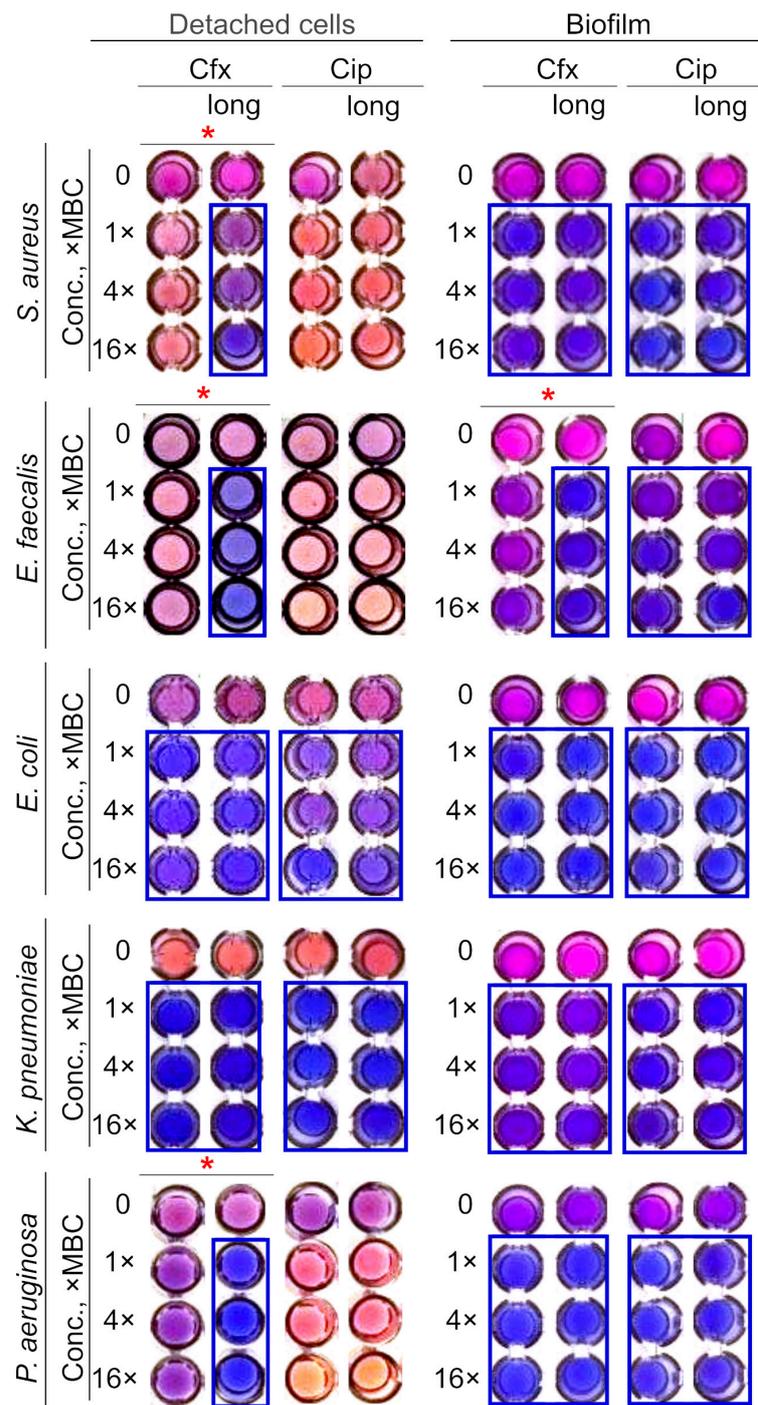


Figure S2. The effect of Longidaza® on the susceptibility of biofilms-embedded bacteria to antimicrobials. Longidaza® was added to 48 h old biofilms until final concentration of 750 IU/mL. Ciprofloxacin and cefuroxime were added up to final concentrations of 1-16× MBC (see Table S1 for values). After 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.

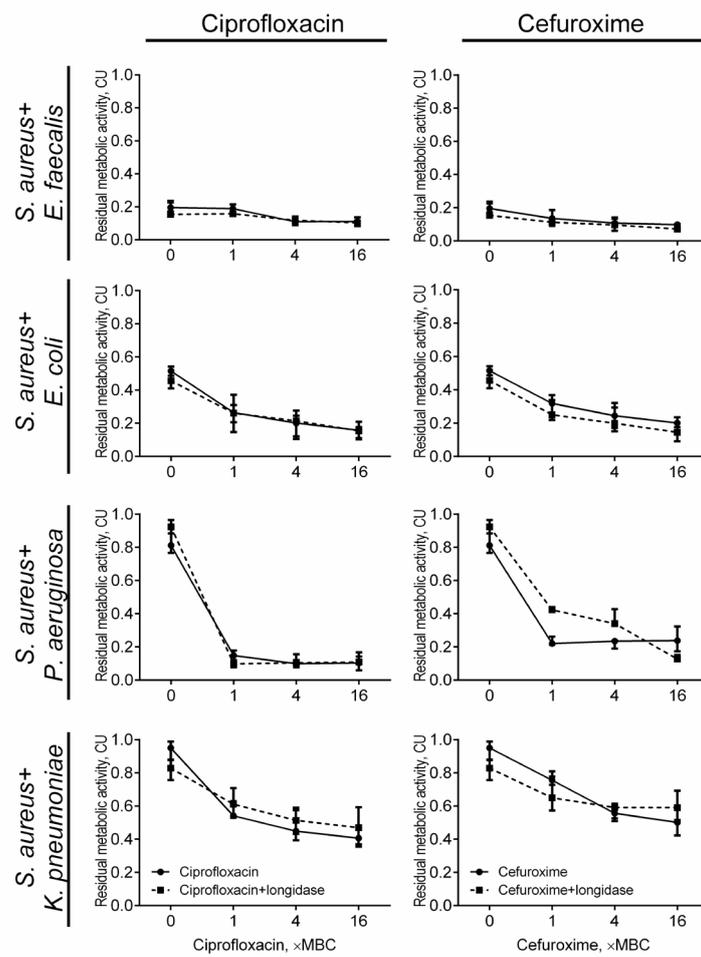


Figure S3. The effect of Longidaza® on the susceptibility of biofilms-embedded dual species bacterial consortia to antimicrobials. Longidaza® was added to 48 h old biofilms until final concentration of 750 IU/mL. Ciprofloxacin and cefuroxime were added up to final concentrations of 1-16×MBC (see Table 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.

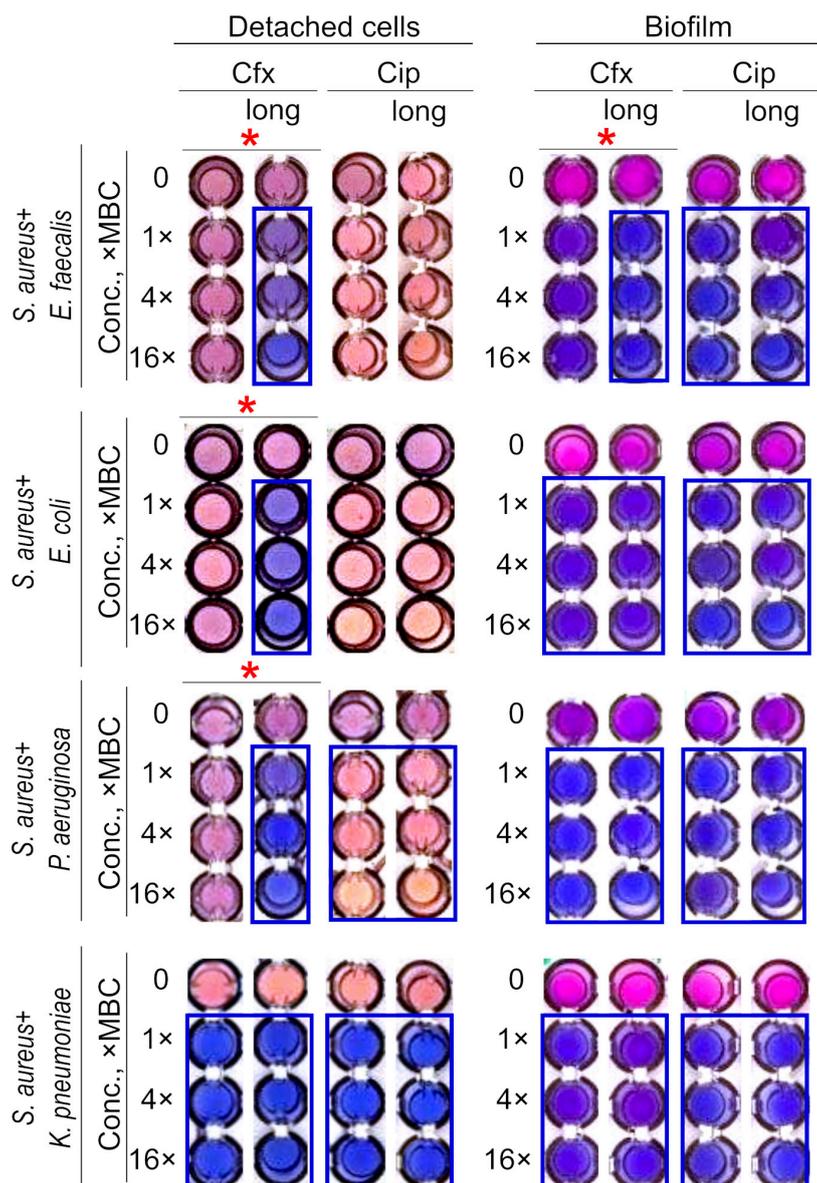


Figure S4. The effect of Longidaza® on the susceptibility of biofilms-embedded dual species bacterial consortia to antimicrobials. Longidaza® was added to 48 h old biofilms until final concentration of 750 IU/mL. Ciprofloxacin and cefuroxime were added up to final concentrations of 1-16×MBC (see Table 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 resazurine test. Blue boxes denote wells with non-viable cells. * shows where there was a difference between treated and untreated wells.