Combined effect of vankomycin and emodin on *Staphylococcus aureus* MSSA and MRSA isolates

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Introduction

Staphylococcus aureus is the most common cause of acute, chronic and nosocomial biofilm-associated infections. According to increasing antibiotic resistance, combination of natural products and antibiotics could be considered as promising strategy against persistent infections. Emodin, antraquionone isolated from *Frangula alnus* posses various biological activities, but its antibiofilm activity in combination with antibiotics is poorly investigated.

Aim of the study

The aim of the study was to investigate the effect of vankomycin and emodin in combination on biofilm formation of two *Staphylococcus aureus* clinical isolates derived from wound **(Gp41, MSSA)** and blood culture **(Gp29, MRSA)**. Furthermore, the effect on bacterial respiration was monitored.

Methods

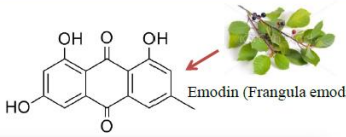
In order to determine minimal inhibitory concentrations (MICs) of single and combined treatments microdilution and checkerboard assays were applied. The effect against biofilm formation was investigated by crystal violet staining of biofilm biomass. O_2 consumption and CO_2 production in treated and untreated bacterial suspensions was monitored using Micro-Oxymax respirometer.

Results

Based on the microdilution and checkerboard assays concentrations that should be combined in screening of antibiofilm activity and respiration were selected **(Table 1)**. Biofilm formation of both strains was inhibited by combined treatments up to 89% **(Figure 1)**. Furthermore, test substances disturb respiration of both strains **(Figure 2)**.

Table 1. The obtained MIC values and selected concentration for combined threatment

	Vankomycin (μg/mL)		Emodin (μg/mL)	
	MIC	In combination	MIC	In combination
Gp41	2.5	0.156	12.5	3.125
Gp29	1.25	0.078	3.125	3.125



Emodin (Frangula emodin)

Micro-Oxymax respirometer

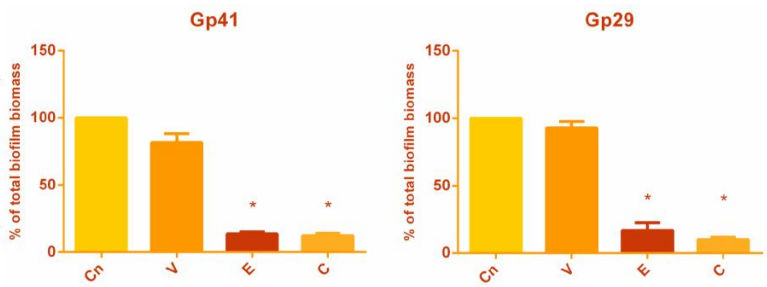


Figure 1. The effect of vankomycin/emodin combination on biofilm formation of *S. aureus* strains. Cn-control; V-vankomycin; E-emodin; C-combination; *statistical significance ($p<0.05$)

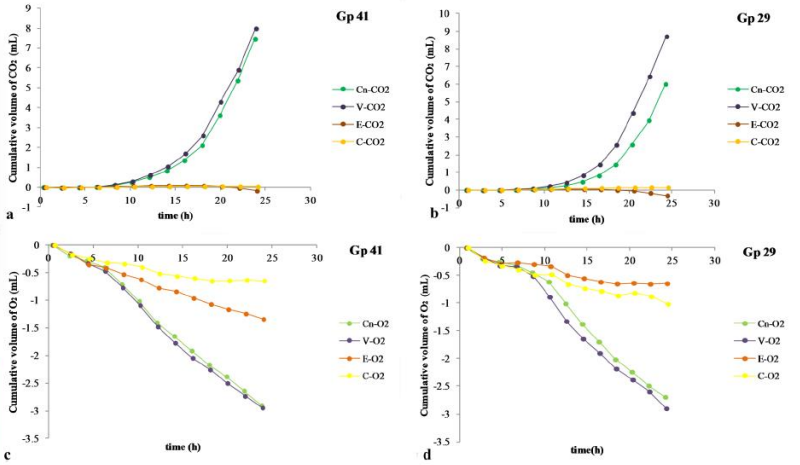


Figure 2. The effect of vankomycin/emodin combination and single threatments on cumulative production of CO_2 **(a,b)** and consuption of O_2 **(c,d)** in planctonic form of *S. aureus* strains during 24h. Cn-control; V-vankomycin; E-emodin; C-combination

Conclusion

Based on the obtained results it could be noticed that combination successfully inhibited biofilm formation of both strains. Moreover, aerobic respiration was disturbed in both strains, but mode of action was not the same. Obtained results encourage further investigation, especially on preformed biofilms and respiration inside them.