Resveratrol, a novel inhibitor of the NorA efflux pump and resistance modulator in *Staphylococcus aureus*

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Introduction

- Among the bacterial resistance mechanisms, the active efflux pumps play a role in the extrusion of different molecules, and thus contribute for antimicrobial resistance [1].
- *S. aureus* is a Gram-positive bacterium that can present resistance to various antibiotics, for which NorA, a predominant efflux pump of these strains, is known to promote resistance to fluoroquinolones [2].
- Thus, the inhibition of this efflux pump may modulate resistance in *S. aureus*, namely to fluoroquinolones [3].
- **This study aimed to investigate the ability of a natural compound, resveratrol (RSV), to modulate fluoroquinolones resistance in *S. aureus*.**

Methods

Antimicrobial Activity | Modulation Assay | Ethidium Bromide Accumulation Assay | Impact on Frequency of Resistance | Post-antibiotic Effect (PAE)

Results

Table 1 - Minimum inhibitory concentrations (MIC) of RSV, Norfloxacin (Nor) and ethidium bromide (EtBr) against the *S. aureus* strains.

Bacterial Strains	MIC (µg/mL)		
	RSV	Nor	EtBr
SA1199 (wildtype)	200	0.25	2
SA1199B (norA++)	100	32	16
8325-4 (wildtype)	400	0.25	2
SAK1758 (Δ*norA*)	200	0.125	0.25

Table 2 - Modulation of antimicrobial activity of Nor and EtBr in absence and presence of RSV (at ¼ MIC) against *S. aureus* strains.

Bacterial Strains	MIC (µg/mL)			
	Nor	Nor+RSV	EtBr	EtBr+RSV
SA1199 (wildtype)	0.25 (2*)	0.125	2 (4*)	0.5
SA1199B (norA++)	32 (16*)	2	16 (8*)	2
8325-4 (wildtype)	0.25 (2*)	0.125	2 (32*)	0.0625
SAK1758 (Δ*norA*)	0.125 (1*)	0.125	0.25 (4*)	0.0625

* Fold reduction in MIC.

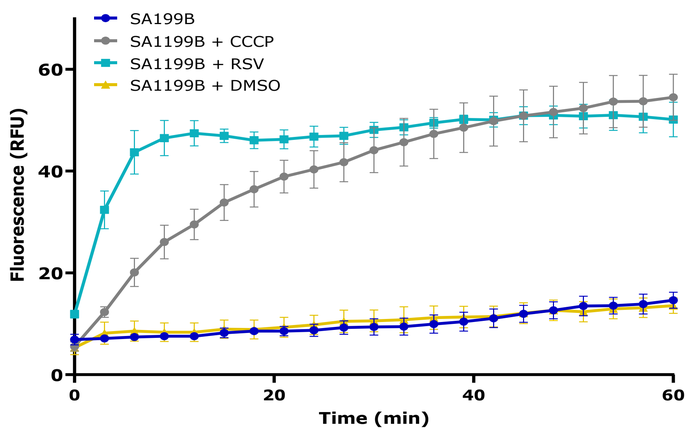

Fig. 1 - Effect of RSV on intracellular accumulation of EtBr, in *S. aureus* SA1199B (norA++).

- The MIC of Nor and EtBr against *S. aureus* strains decreased when in presence of RSV.
- In the presence of RSV, the norA++ strain had an augmented fluorescence, consequence of the accumulation of EtBr.

Table 3 - Mutation frequency of *S. aureus* SA1199.

Resveratrol (µg/mL)	Mutation frequency with norfloxacin		
	4 x MIC (1µg/mL)	8 x MIC (2µg/mL)	16 x MIC (4µg/mL)
0	1.87×10^{-5}	5.27×10^{-7}	4.03×10^{-8}
50	2.16×10^{-7}	1.93×10^{-8}	$< 5.31 \times 10^{-10}$

Table 4 - PAE of Nor alone and in combination with RSV against *S. aureus* SA1199B.

Regimen	Mean PAE (h) ± SD		
	0.25× MIC Nor (8 µg/mL)	0.5× MIC Nor (16 µg/mL)	MIC Nor (32 µg/mL)
Nor	2.19 ± 0.25	2.24 ± 0.22	2.65 ± 0.18
Nor + RSV (25 µg/mL)	2.83 ± 0.10	2.80 ± 0,24	3.02 ± 0.04

- There was a decrease in mutation prevention concentration of Nor when combined with RSV.
- The combination of Nor at 32 mg/L with RSV showed a most extended PAE than the antibiotic alone.

Conclusion

Our findings demonstrated that resveratrol could modulate the norfloxacin-resistance, by inhibition of NorA, increasing the effectiveness of this antibiotic against *S. aureus*.

References

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