



The polyamino-isoprenic potentiator NV716 revives the disused antibiotic rifampin against biofilm infection by Gram-negative ESKAPE pathogens

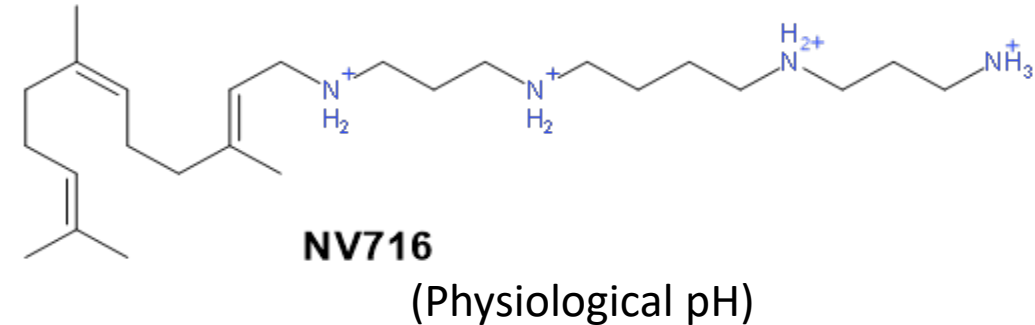
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Context of the study

1. **Rifampin** is - highly active on Gram-positive bacteria, including in biofilms
- not active on most Gram-negative bacteria, because of low penetration
2. **NV716** potentiator shows synergy with rifampin against *P. aeruginosa* by
- increasing the outer membrane permeability
- increasing antibiotic accumulation inside bacteria



Aim of the study

Examine the effect of NV716 on the activity of rifampin

1. against planktonic bacteria (MIC testing)
2. against biofilm form of infection

by *P. aeruginosa*, *E. coli*, *K. pneumoniae*, and *A. baumannii*

Results: MICs

MIC (mg/L) of rifampin against Gram-negative bacteria in the absence of or presence of NV716.

Strains	MIC ($\mu\text{g/mL}$)
	NV716 alone
<i>P. aeruginosa</i> PAO1	20
<i>E. coli</i> ATCC 47076	20
<i>K. pneumoniae</i> ATCC 43816	40
<i>K. pneumoniae</i> ATCC 700603	40
<i>A. baumannii</i> ATCC 19606	40

Results: MICs

MIC (mg/L) of rifampin against Gram-negative bacteria in the absence of or presence of NV716.

Strains	MIC ($\mu\text{g}/\text{mL}$)	
	NV716 alone	RIF alone
<i>P. aeruginosa</i> PAO1	20	16
<i>E. coli</i> ATCC 47076	20	16
<i>K. pneumoniae</i> ATCC 43816	40	32
<i>K. pneumoniae</i> ATCC 700603	40	32
<i>A. baumannii</i> ATCC 19606	40	2

Results: MICs

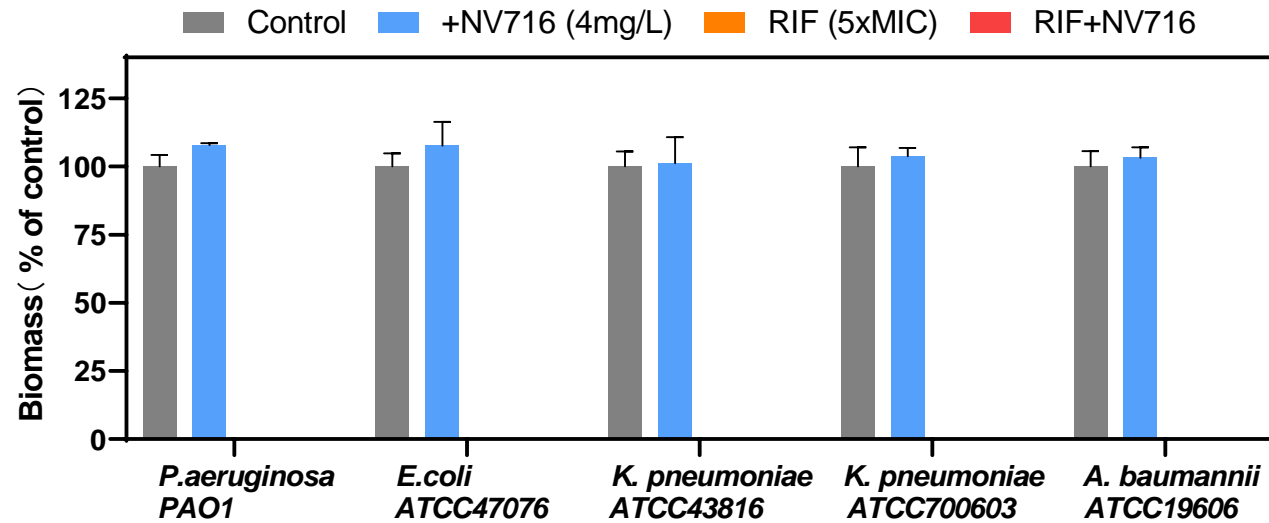
MIC (mg/L) of rifampin against Gram-negative bacteria in the absence of or presence of NV716.

Strains	MIC ($\mu\text{g/mL}$)			
	NV716 alone	RIF alone	RIF+NV716 (4mg/L)	Fold reduction in MIC (RIF alone/combo)
<i>P. aeruginosa</i> PAO1	20	16	0.25	64
<i>E. coli</i> ATCC 47076	20	16	0.03	512
<i>K. pneumoniae</i> ATCC 43816	40	32	0.03	1024
<i>K. pneumoniae</i> ATCC 700603	40	32	2	16
<i>A. baumannii</i> ATCC 19606	40	2	0.13	16

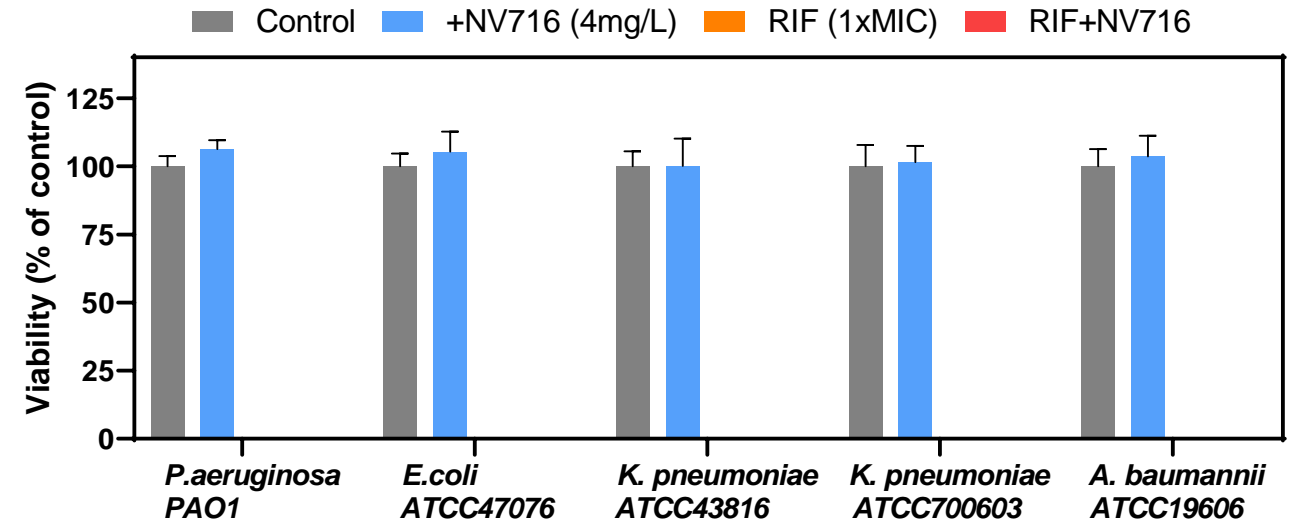
➤ **NV716** markedly decreased the MIC of rifampin against all strains

Results: biofilms

Effects on biomass (crystal violet staining)



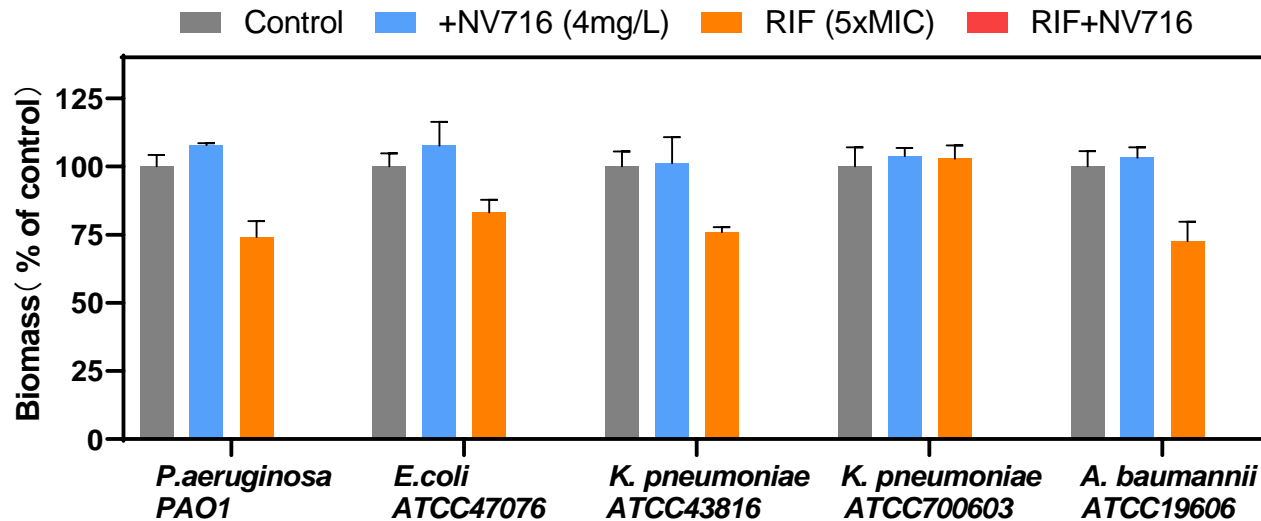
Effects on viability (metabolic test)



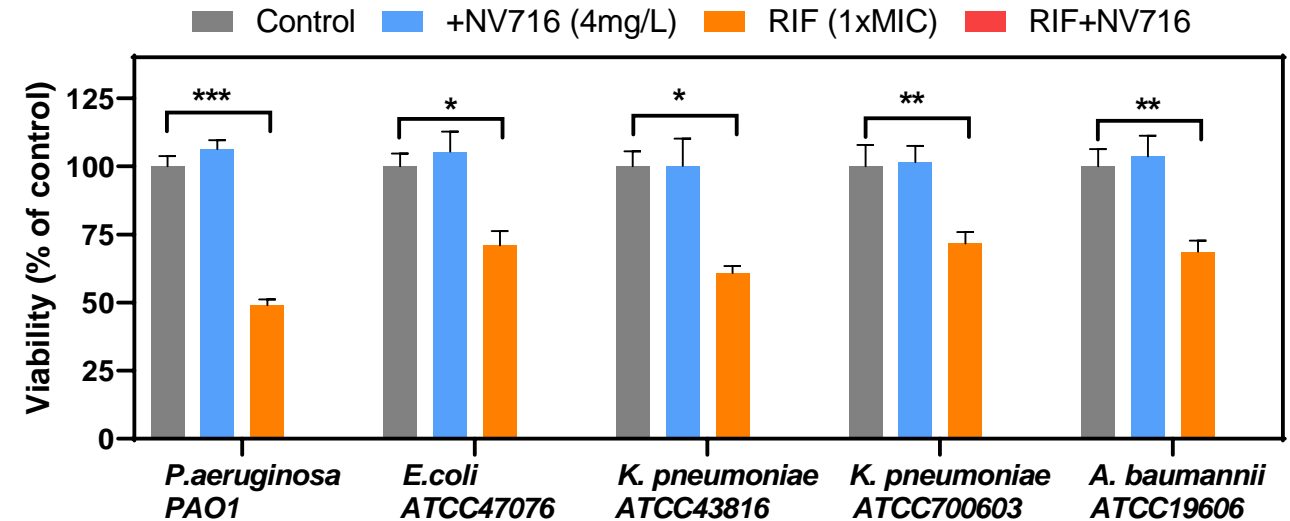
➤ In the biofilm model, **4 mg/L NV716** alone was not active to reduce biomass and viability.

Results: biofilms

Effects on biomass (crystal violet staining)



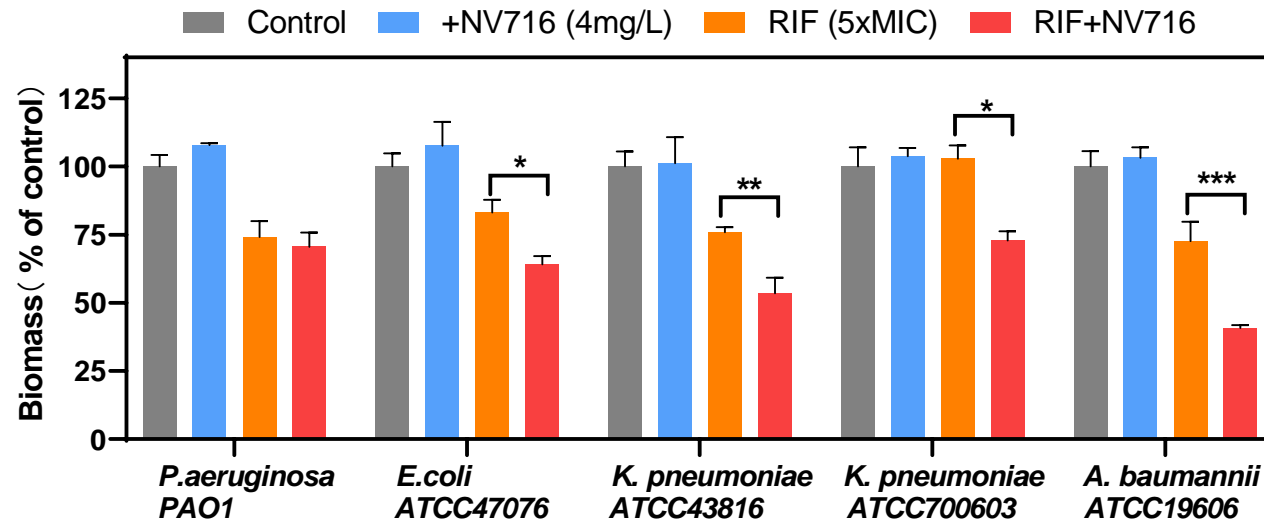
Effects on viability (metabolic test)



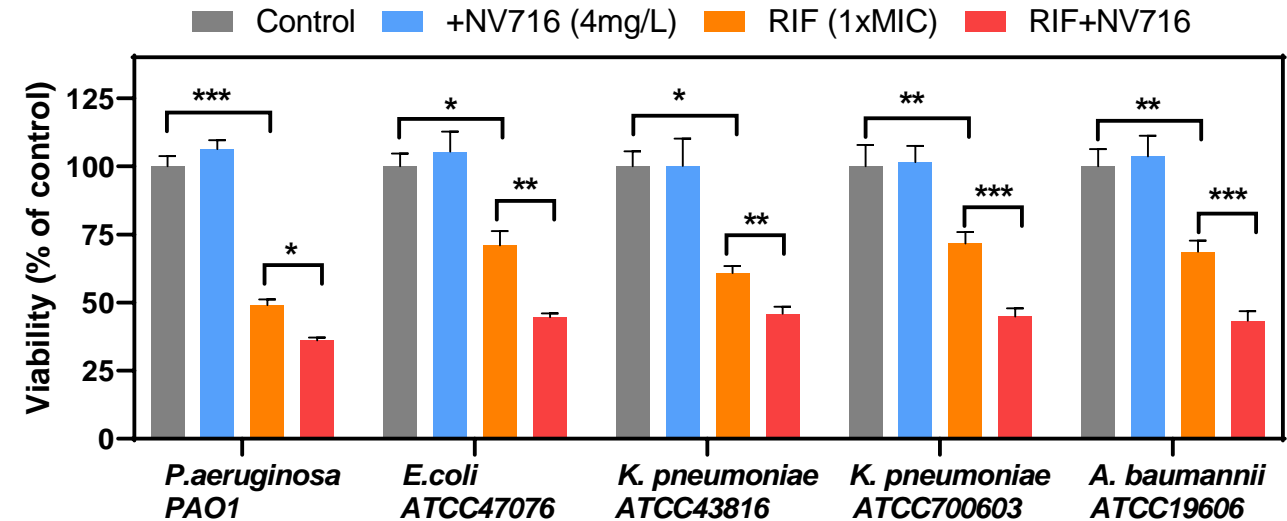
- In the biofilm model, **4mg/L NV716** alone was not active to reduce biomass and viability.
- Rifampin has no effect on biomass at 5 x MIC and is only marginally effective to reduce viability at 1 x MIC.

Results: biofilms

Effects on biomass (crystal violet staining)

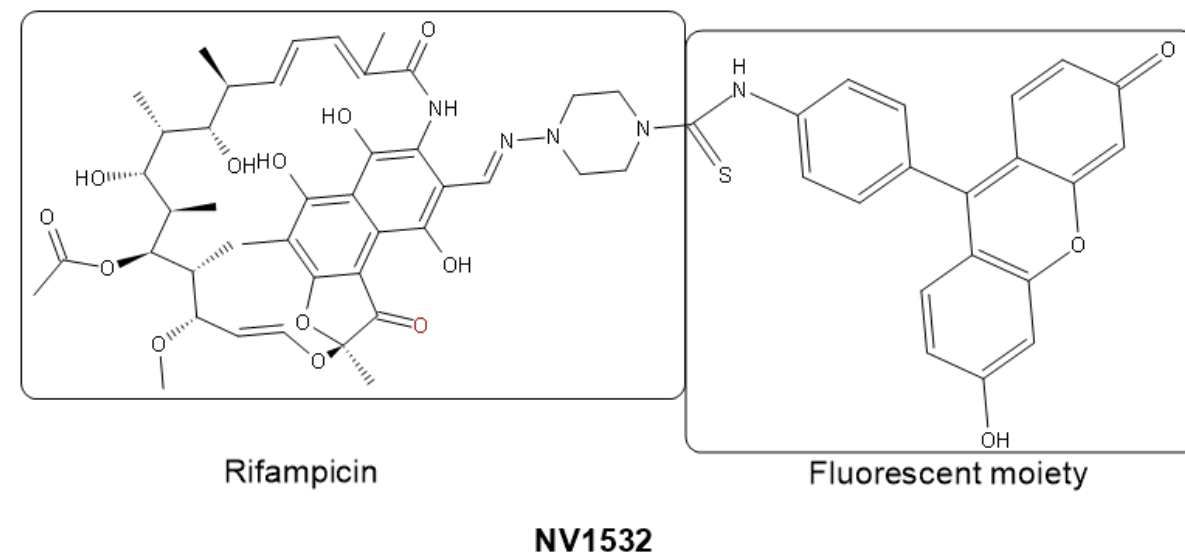
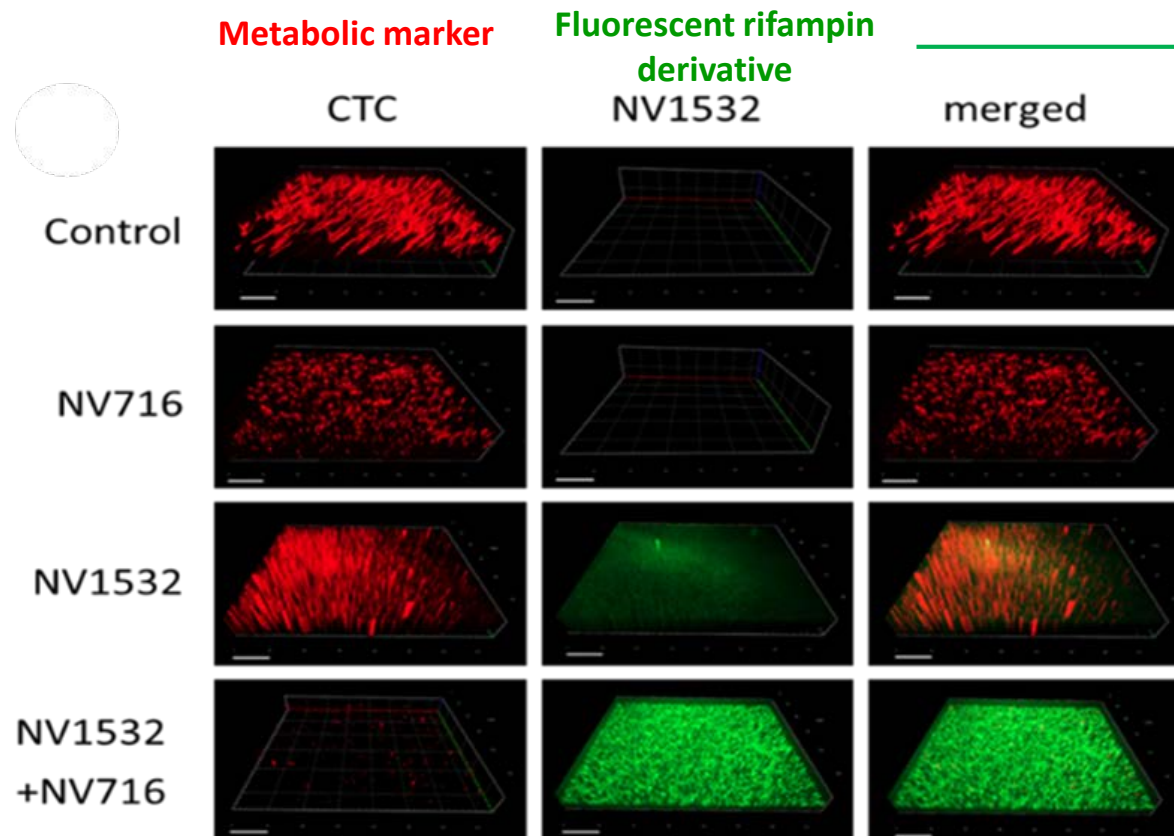


Effects on viability (metabolic test)



- In the biofilm model, **4 mg/L NV716** alone was not active to reduce biomass and viability.
- Rifampin has no effect on biomass at 5 x MIC and is only marginally effective to reduce viability at 1 x MIC.
- In combination, the biomass and viability were further significantly decreased for all strains (except PAO1 for biomass)

Results: confocal microscopy of biofilms



- NV716 markedly improves the penetration of the fluorescent rifampin derivative NV1532 in PAO1 biofilm
- The combination allows to markedly reduce the abundance of living cells.

Conclusions

NV716 not only reduced MICs but also increased the effect of rifampin on biomass and viability of biofilms for different ESKAPE pathogens by increasing its penetration in the biofilm.

→ This data suggests to further evaluate the interest of this potentiator to revive more old antibiotics against biofilm infections by Gram-negative bacteria.

Thank You For Your Attention

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