Off-target activity of spiramycin disarms *Pseudomonas aeruginosa* by inhibition of biofilm formation, pigment production and phenotypic differentiation

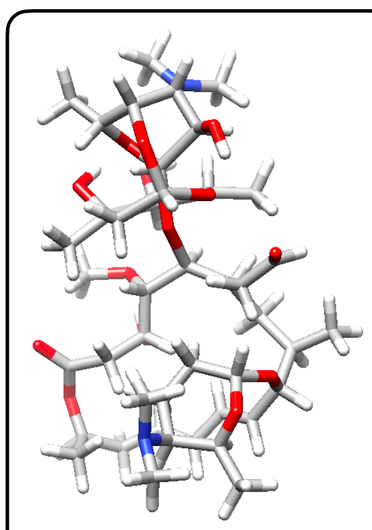
Matteo Calcagnile and Pietro Alifano.

Contact: *matteo.calcagnile@unisalento.it*

UNIVERSITÀ DEL SALENTO

ECA 2022

Introduction



3D structure of Spiramycin

Spiramycin is a 16-membered macrolide antibiotic effective against parasites and bacteria. *Pseudomonas aeruginosa* is considered inherently resistant to macrolides, but some studies suggest that macrolides can be used as adjunct therapy against infections of this bacterium. We tested spiramycin as a compound effective against virulence.

Biofilm inibition

We used hydroxyapatite as a biomimetic material to allow *P. aeruginosa* to form biofilms. Spiramycin inhibits biofilm formation (**A and B**) and increases the number of planktonic cells (**C**).

	Units	Control	Spiramycin (60 µg/ml)
Biofilm	CFU/support	$1x10^6$	$5x10^3$
Planktonic cells	Total protein (µg/µL)	1.5	2
Pyocianin	Absorbance at 520 nm	4.2	9.22

In vivo models

P. aeruginosa was injected into *Galleria mellonella* larvae with or without spiramycin. After 24 hours, the mortality of the larvae treated with *P. aeruginosa* was >90% (**A**), while in the presence of spiramycin it was around 30% (**B**). These preliminary data suggest that spiramycin can disarm *P. aeruginosa*.

A

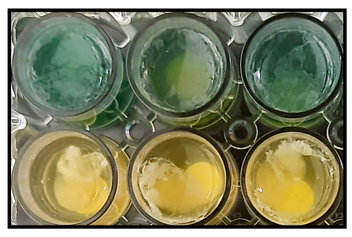
B

Conclusion

Spiramycin affects the *P. aeruginosa* phenotype by reducing the secretion of virulence-related molecules and inhibiting biofilm formation. The preliminary data we obtained with the *in vivo* model confirm this hypothesis. Further studies are needed to clarify the mechanism of action.

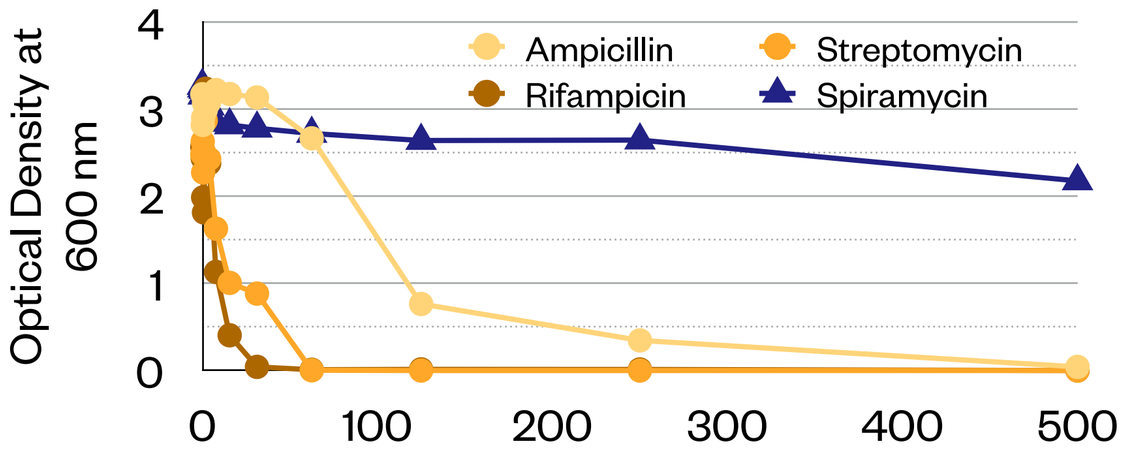
Phenotype of *P. aeruginosa* traded with Spiramycin

A

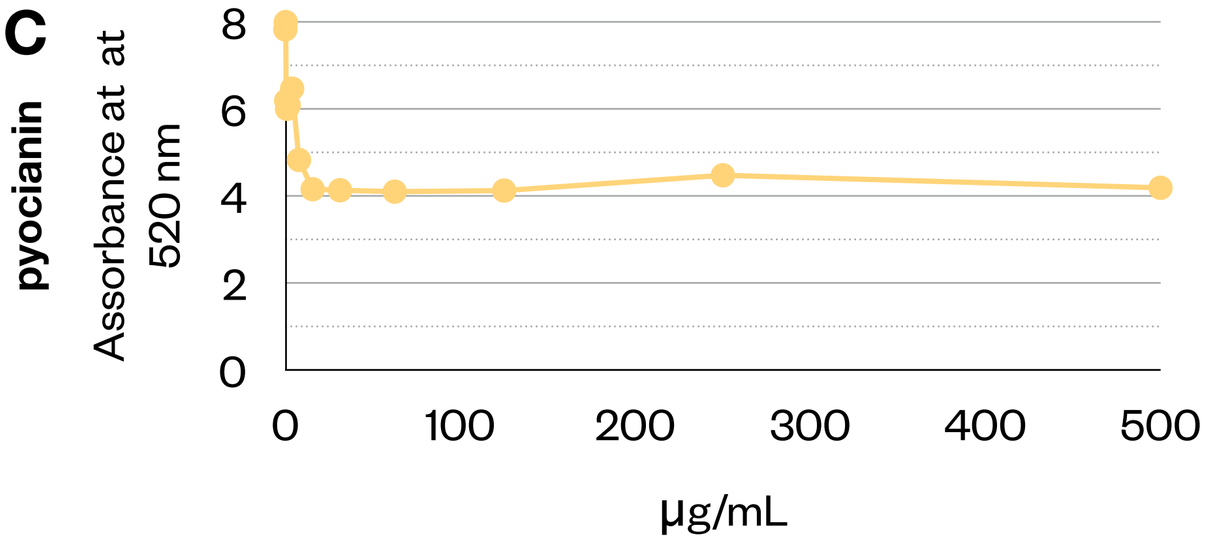


Spiramycin, both in liquid and solid media, inhibits the production of pigments (e.g., pyocianin), (**A and C**) but does not affect growth (**A and B**)

B



C

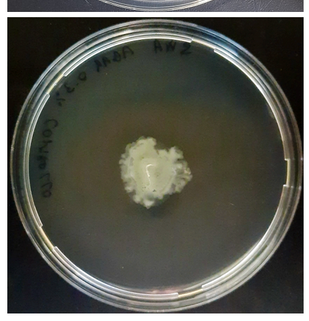


Motility and rhamnolipids production

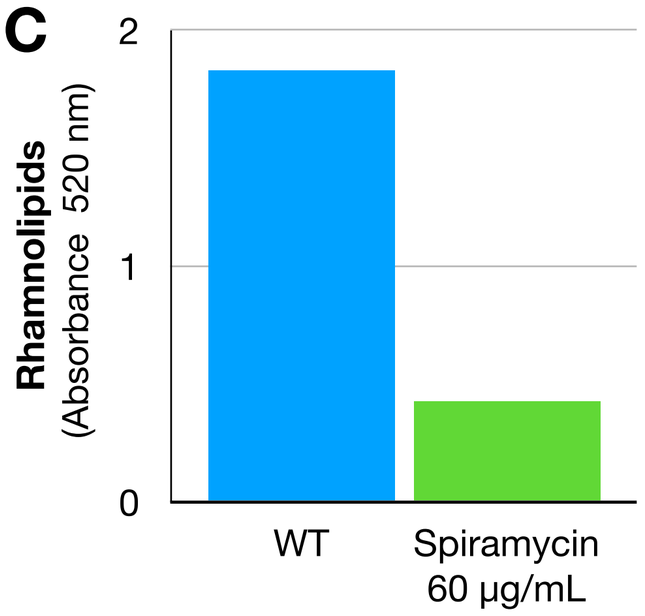
Spiramycin reduces swarming motility on the agar surface (**A**, controls; **B**, spiramycin 60 µg/ml). Furthermore, spiramycin negatively affects the production of rhamnolipids during growth in the liquid medium LB (**C**).

A

B



C



References

1. Chalmers, JD. 2017 doi: 10.1183/13993003.00689-2017.
2. Mustafa, MH; et al. J. 2017 doi: 10.1183/13993003.01847-2016
3. Kobayashi, H.; et al. 1995.doi: 10.1016/s0002-9343(99)80282-4.
4. Hirakata, Y.; et al. 1992. doi: 10.1128/AAC.36.6.1198.
5. Imamura, Y., et al. 2004. doi: 10.1128/AAC.48.9.3457-3461.2004.