Game of Research Season Four, 4June 2021

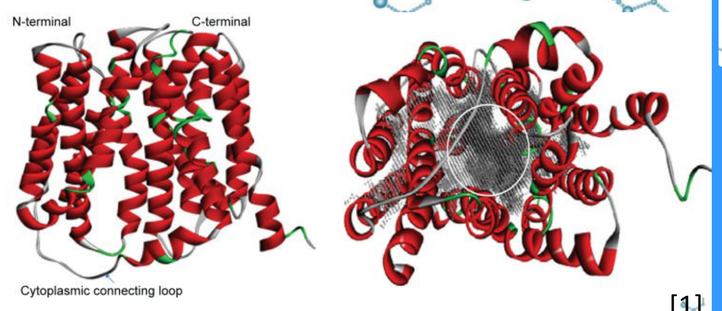
Major Facilitator Superfamily (MFS) transporters, a druggable target for antibacterial therapy

Rampacci Elisa[1]✝

[1] Dipartimento di Medicina Veterinaria, Università degli Studi di Perugia, Via San Costanzo, 4, 06126, Perugia, IT

✝e-mail: elisa.rampacci@gmail.com

Background



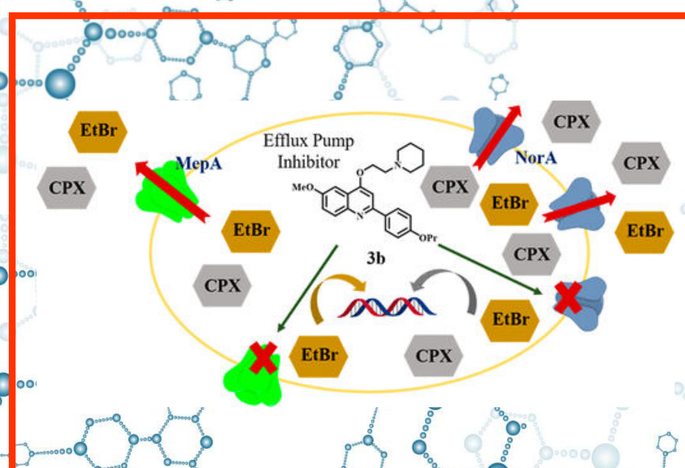
[1]

Major Facilitator Superfamily (MFS) transporters are membrane proteins found in all domains of life. Anomalous functions of MFS proteins in eukaryotic cells have been associated with debilitating diseases, including **cancer** and **degenerative processes**. In bacteria, MFS transporters are involved in the uptake of nutrients and extrusion of toxic compounds, including antibiotics and biocides. Because of this function, they were identified as **multidrug resistance efflux pumps (MDR EPs)**. As MDR EPs, MFS transporters have been a major focus of investigations aimed at developing new drugs against antibiotic efflux systems of pathogenic microorganisms.

Long-term objectives

The long-term objective of our researches is to elucidate the **fundamental mechanisms** by which relevant animal and human **pathogens extrude antibiotics and biocides** and to provide **novel therapeutic means** to overcome drug resistance by targeting their MFS efflux transporters.

Methodology



[3]

To study efflux-mediated resistance in bacteria, two approaches can be applied: ***in vitro* building up of resistance** by stimulation of isogenic strains with increasing concentrations of ethidium bromide (EtBr), [2] and selection of **antimicrobial-resistant clinical isolates**.

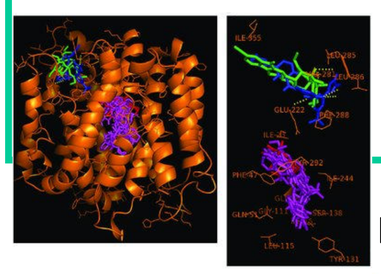
Evaluation of efflux activity:
> Fluorometry
> MIC in the presence/absence of **efflux pump inhibitors (EPIs)**

Molecular mechanisms:
> EP gene expression analysis
> DNA sequencing of **promoter regions** and **transcriptional regulators**

Preliminary results

Role of EPIs Thioridazine (TZ) and Reserpine (RES)

		MIC in combination with inhibitor (mg/L)			
Drugs	**No inhibitor** (mg/L)	**TZ**	**FICI**	**RES**	**FICI**
EtBr	50 (128x)	6.25	**0.25**	6.25	**0.19**
AZM	2 (4x)	0.5	**0.5**	0.5	**0.31**
CIP	2 (4x)	0.5	**0.5**	1	0.52



[4]

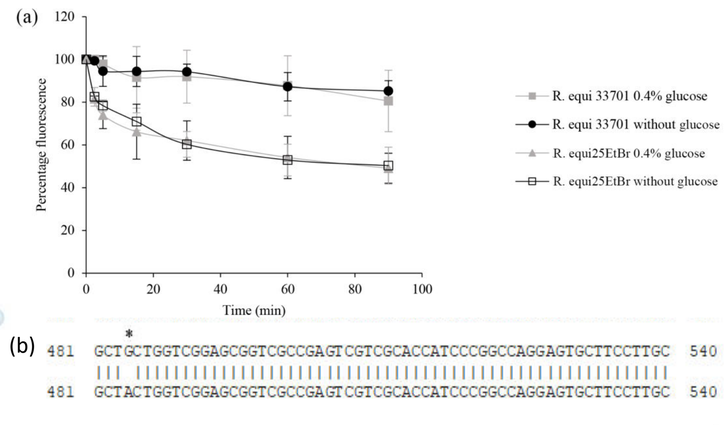
[5]

Exposure of ***Rhodococcus equi*** to EtBr unmasked an efflux-mediated defense **(a)** against azithromycin and ciprofloxacin

MIC increased 4x

14-fold overexpression of the **MFS** transporter ***REQ_RS13460***

G→A transition in the transcriptional repressor ***tetR/acrR*** adjacent to *REQ_RS13460* **(b)**



[4]

Perspectives

So far, few genes coding for efflux proteins have been identified in ***Staphylococcus pseudintermedius***, the primary cause of canine cutaneous infections.
Finding that overexpression of MFS efflux systems in *Staphylococcus pseudintermedius* is responsible for resistance to antibiotics commonly used to treat skin infections could justify the **REPOSITIONING** of EPIs optimized against MSF transporters of *Staphylococcus aureus* [3] and nontuberculous mycobacteria [6] for both *Rhodococcus equi* and *Staphylococcus pseudintermedius* disease in animals and humans.

REFERENCES
[1] Bhaskar B. V. Homology modeling, molecular dynamics, and virtual screening of NorA efflux pump inhibitors of Staphylococcus aureus. Drug Des Devel Ther 10 3237–3252, 2016. [2] Couto I et al. Efflux-mediated response of Staphylococcus aureus exposed to ethidium bromide. J Antimicrob Chemother, 62:504–513, 2008 . [3] Felicetti T. 2-Phenylquinoline S. aureus NorA Efflux Pump Inhibitors: Evaluation of the Importance of Methoxy Group Introduction. J Med Chem 61, 7827−7848, 2018. [4] Rampacci E. Ethidium bromide exposure unmasks an antibiotic efflux system in Rhodococcus equi. J Antimicrob Chemother dkab121., 2021. [5] Zimmermann S. Clinically Approved Drugs Inhibit the Staphylococcus aureus Multidrug NorA Efflux Pump and Reduce Biofilm Formation. Front Microbiol 10:2762, 2019. [6] Felicetti T et al. 2-Phenylquinoline S. aureus NorA Efflux Pump Inhibitors: Evaluation of the Importance of Methoxy Group Introduction. J Med Chem, 61:7827–48, 2018.