The role of environmental stressors and genetic information on the antimicrobial tolerance in *Ralstonia pickettii*

Pompeyo Ferro[(1)]*, Ivone Vaz-Moreira[(1)], Javier Tamames[(2)], José L. Martínez[(2)] and Célia M. Manaia[(1)]

(1) Escola Superior de Biotecnologia. Universidade Católica Portuguesa, Porto, Portugal.
(2) Centro Nacional de Biotecnología (CSIC), Consejo Superior de Investigaciones Científicas, Cantoblanco, Madrid, Spain
*poferro@yahoo.com

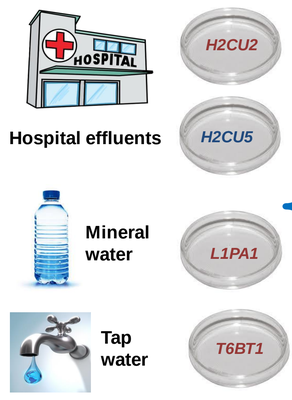
Scope and objectives

Ralstonia pickettii **is a ubiquitous *Betaproteobacteria* species, found in water and occasionally capable of causing infections in humans.**

The ubiquity and opportunistic character, combined with the resistance to different antibiotics, make the members of this species interesting models to study antibiotic resistance evolution. In particular, to assess the possible association between antibiotic and biocide resistance or the potential to acquire new resistance phenotypes. These were the major objectives of work presented in this poster.

Methods

Four *Ralstonia pickettii* strains differring on aminoglycosides susceptibility phenotype



- **Minimum inhibitory concentrations (MICs)** (gentamicin; tetracycline; meropenem; ceftazidime; sulfamethoxazole; streptomycin, As^{3+}, Ni^{2+} and Cu^{2+})
- **Capacity to grow and form biofilm in the presence of stressors** (gentamicin and As^{3+})
- **Survival in the presence of different disinfectants** (UV radiation and chlorine)
- **Whole genome sequencing and comparative analyses**
- **Detection and characterization of Integrative Conjugative Elements (ICEs)**
- ***Ralstonia* strains adaptation to adverse conditions** (presence of inhibitory concentrations of gentamicin and UV-radiation)

Results

Figure 1. Members of the species *Ralstonia pickettii* are ubiquitous.

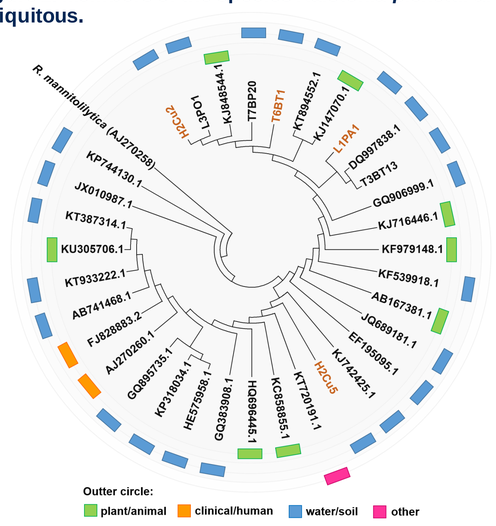


Table 1. *Ralstonia pickettii* minimum inhibitory concentration

Strain	MICs								
	GEN	STR	TET	MER	CEF	SUL	As^{3+}	Ni^{2+}	Cu^{2+}
	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mM	mM	mM
H2Cu2	> 256	>1024	1	>32	6	24	1.4	4	12
H2Cu5	6	56	0.25	6	6	4	0.05	4	8
L1PA1	> 256	>1024	1	>32	6	24	1.4	4	8
T6BT1	> 256	>1024	1	16	8	24	1.4	4	12

GEN, gentamicin; STR, streptomycin; TET, tetracycline; MER, meropenem; CEF, ceftazidime; SUL, sulfamethoxazole and metals salts of As3+, Ni2+ and Cu2+

Aminoglycosides resistance and increased tolerance to arsenite and some antibiotics (TET, SUL and MEM) were associated

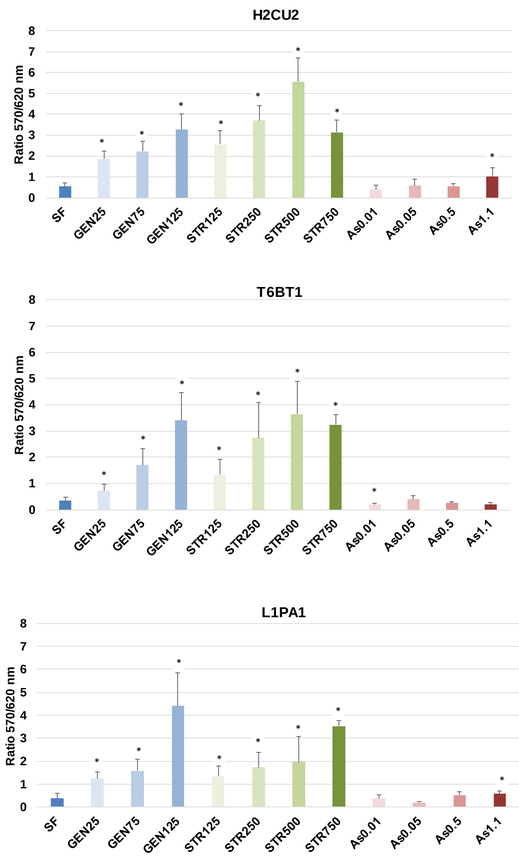
Table 2. Bacterial growth kinetics in the presence of sub-inhibitory concentrations of gentamicin or arsenite

Strain	Growth rate (h^{-1})						Phase Lag (hours)						Yield (OD)					
	SF		As^{3+}		GEN		SF		As^{3+}		GEN		SF		As^{3+}		GEN	
H2Cu2	0.4±0.03	α;a,b	0.3±0.02	β;a	0.1±0.01	γ;a	0.9±0.2	α;a	3.4±0.5	β;a	2.0±0.7	α,β;a	2.9±0.1	α;a	2.2±0.2	β;a	0.5±0.1	γ;a
H2Cu5	0.3±0.01	α;a	0.3±0.04	α;a	0.1±0.01	β;b	0.7±0.4	α;a	1.0±0.3	α;b	2.2±2.5	α;a	2.5±0.3	α;a	2.0±0.3	α;a	0.9±0.2	β;a
L1PA1	0.4±0.04	α;a,b	0.3±0.04	α;a,b	0.2±0.01	β;c	0.7±0.4	α;a	3.0±0.9	β;a	0.8±0.1	α;a	2.9±0.4	α;a	2.5±0.5	α;a	0.8±0.1	β;a
T6BT1	0.4±0.02	α;a,b	0.3±0.01	β;a	0.2±0.01	γ;b	0.9±0.3	α;a	2.4±0.3	β;a	1.4±0.4	α;a	2.7±0.1	α;a	2.4±0.3	α;a	0.7±0.1	β;a

SF: stressor free; OD, optical density at 610 nm; As3+, arsenite; GEN, gentamicin. Significant differences between stresses conditions are indicated by the symbols: α, β, γ; and significant differences between strains are indicated by the letters: a, b, c, d.

Sub-inhibitory concentrations of gentamicin or arsenite were observed to significantly decrease the growth rate and yield, while arsenite but not gentamicin caused a significant increase of the lag phase.

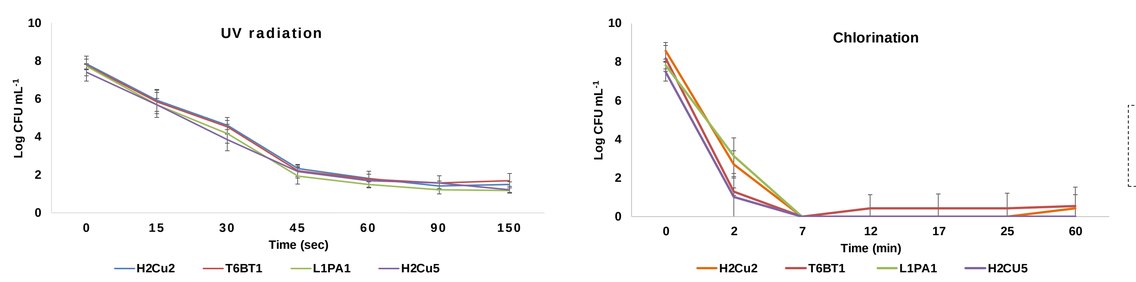
Figure 2. Variation on the capacity to form biofilm in the presence of different stressors



* Significantly different from the ability to form biofilm in stressor free (SF) conditions. For the strain H2Cu5 were not observed significant differences. The metals Ni and Cu did not affect the capacity to form biofilm, for all the strains.

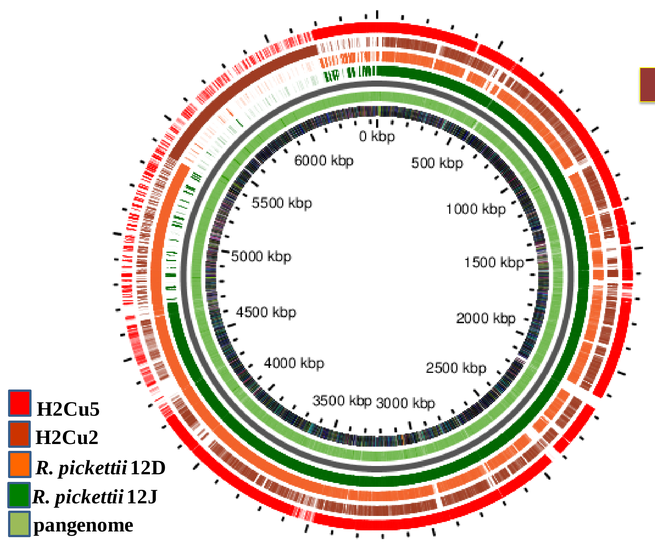
The presence of sub-inhibitory concentrations of aminoglycosides and some heavy metals influence the capacity to form biofilms of the aminoglycoside resistant isolates, but not the aminoglycoside susceptible isolate.

Figure. 3 Survival to disinfection (UV radiation and chlorination)



The survival to UV radiation or chlorination was similar for the four strains

Figure 4. Genome sequencing for two *R. pickettii* with different resistance phenotypes.



The draft genomes of the wild hospital effluent strains evidenced that the gentamicin resistant isolate (H2Cu2) presented some genes related with tolerance to arsenic and toxic compounds, integrative conjugative elements, genes encoding lysozyme inhibitors and genes related with phages/prophages. These genes were not detected in the gentamicin susceptible strain (H2Cu5). The capacity of both strains to acquired new resistance traits, indicative of genome liability, were compared based on successive stress exposure – gentamicin and germicidal UV radiation.

Figure 5. Adaptation assays based on the sucessive exposure to increasing concentrations of gentamicin (A) or longer times to germicidal UV radiation (B)

A.1. Aminoglycoside resistant strain H2Cu2

No. of sucessive transfers	0	15	25	35	65
MIC GEN (mg/L)	>256	>256			
Resistance phenotype	GEN STR (NA) MEM TIC CT	Acquired resistance phenotypes: NONE			

A.2. Aminoglycoside susceptible strain H2Cu5

No. Of sucessive transfers	0	15	60	145
MIC GEN (mg/L)	8	32	>256	>256
Resistance phenotype	(NA) TIC (CAZ) (MEM) CT	Acquired resistance phenotypes: GEN STR		

The aminoglycoside susceptible strain gained some resistance phenotypes (GEN and STR), while the resistant one did not suffer any apparent modification

B. Resistance phenotypes after UV exposition

Strain	Pre-UV		After-UV (280-380 sec)	
	Resistance phenotypes	MIC GEN mg/L	Modifications in the resistance phenotype	MIC GEN mg/L
H2Cu2	GEN STR (NA) MEM TIC CT	>256	NONE	>256
H2Cu5	(NA) TIC (CAZ) (MEM) CT	8	STR MEM	16
L1PA1	GEN STR (NA) MEM TIC CT	>256	NONE	>256
T6BT1	GEN STR (MEM) (TIC) CT	>256	MEM TIC	>256

GEN, gentamicin; STR, streptomycin; TET, tetracycline; MER, meropenem; TIC, ticarcillin; CT, colistin sulphate; NA, nalidixic acid

After successive UV exposure, the aminoglycoside susceptible strain gained increased tolerance to aminoglycosides, while a opposed effect may have occurred in some resistant strains

Conclusions

- **Tolerance to aminoglycosides, to arsenite and possibly to other antibiotics was associated in the examined strains.**
- **Growth kinetic parameters were affected by gentamicin and by arsenite, evidencing their roles as stressors for *R. pickettii* strains.**
- **Aminoglycoside resistance was not observed to be associated with an increased tolerance to disinfection by UV radiation or chlorination.**
- **The genomes of the aminoglycoside-resistant and susceptible strains H2Cu2 and H2Cu5 differed on the presence of integrative conjugative elements and other determinants associated with genetic recombination as well as genes related with the tolerance of toxic compounds, only detected in the resistant strain.**
- **After successive exposure to stress conditions the aminoglycoside-susceptible strain H2Cu5 showed the potential to develop new resistance traits, while the resistant one seemed to have reached already a steady state, which may be explained based on the whole genome sequence analysis**

Acknowledgements

This work was supported by the National Funds from FCT - Fundação para a Ciência e a Tecnologia through projects PEst-OE/EQB/LA0016/2013, NanoDiaBac ENMed/0001/2014 and IVM grant SFRH/BPD/87360/2012, and by FCT and MINECO through the project WaterJPI/0001/2013 STARE – "Stopping Antibiotic Resistance Evolution". The PF grant was supported by Erasmus Mundus, SUD-UE. The authors would also like to acknowledge the financial support provided by COST-European Cooperation in Science and Technology, to the COST Action ES1403: New and emerging challenges and opportunities in wastewater reuse (NEREUS).