

2º CONGRESSO IBEROAMERICANO / 6º IBÉRICO DE CIANOTOXINAS

# EXPLORAR - Exploring the Aquatic Resistome

Tânia Rosado, Vera Manageiro, Duarte Balata, Carina Menezes, Eugénia Ferreira, Octávio Paulo, Manuela Caniça, Elsa Dias

Múrcia, Espanha, 3-5 Julho 2019



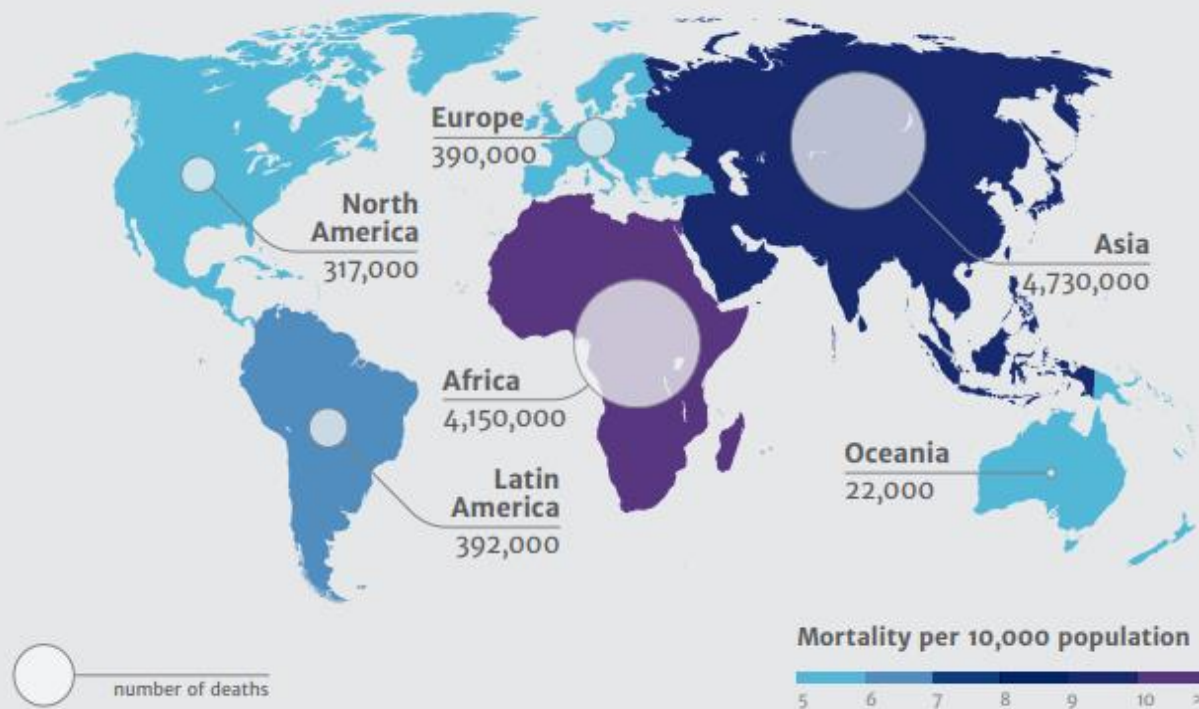
Instituto **Nacional de Saúde**  
*Doutor Ricardo Jorge*



**FCT** Fundação  
para a Ciência  
e a Tecnologia

# THE PROBLEM

## Deaths attributable to AMR every year by 2050



The Review on Antimicrobial Resistance, Chaired by Jim O'Neill

SALUD · Se venden casi tres veces más fármacos que en Alemania

### España encabeza el consumo de antibióticos veterinarios críticos para la salud humana

Su uso incrementó casi un tercio entre 2013 y 2014. A mayor uso de antibióticos, mayores probabilidades de desarrollar bacterias resistentes a los fármacos.



Assinar

INICIO Política Economía País Mundo Desporto Fact Check Cultura Lifestyle Auto

### Portugal está a perder a luta contra as bactérias resistentes

15/11/2017, 16:57 306

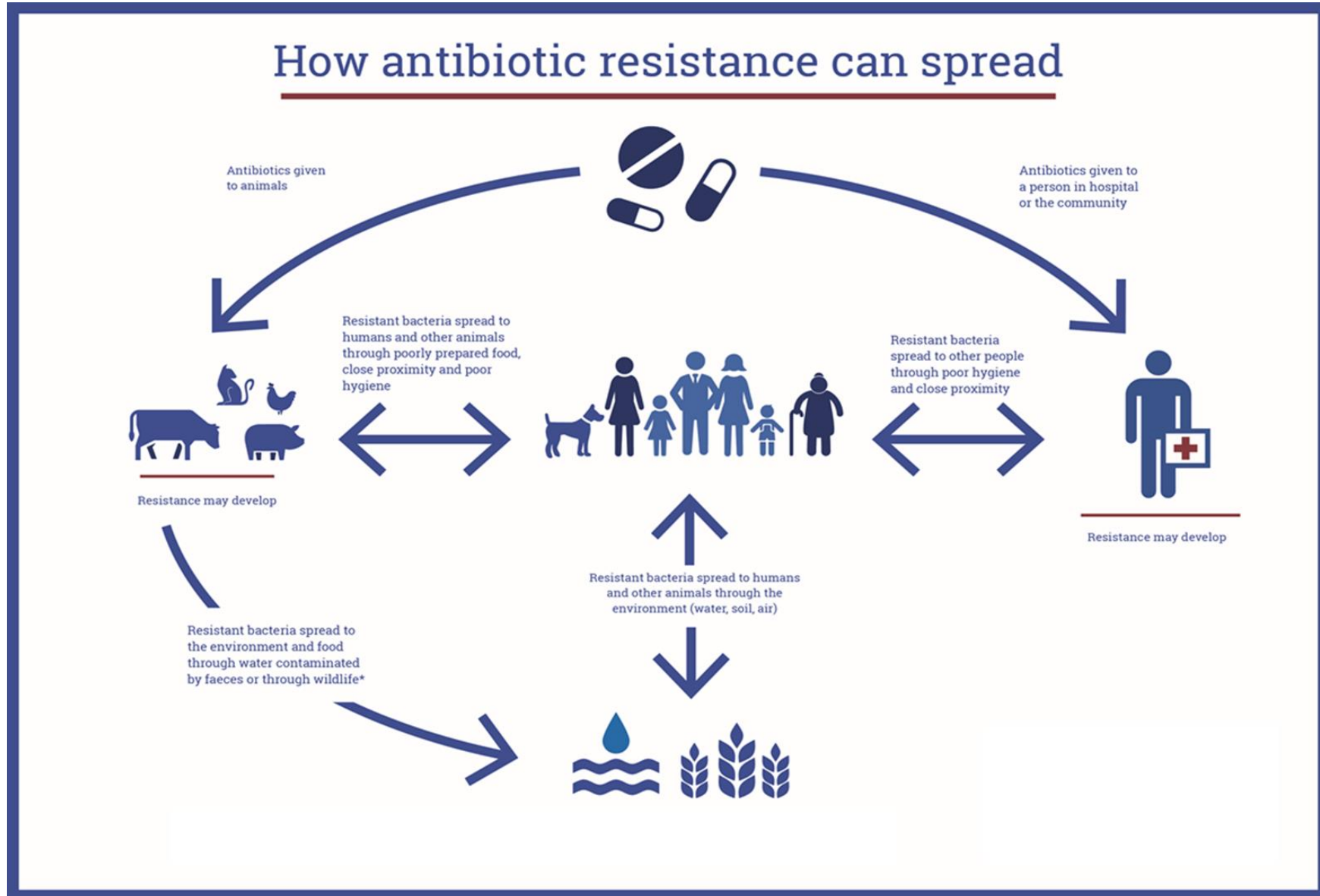
Portugal, tal como os países do sul e sudeste da Europa, não está a conseguir fazer frente ao aumento da percentagem de bactérias resistentes aos antibióticos de última linha.

Partilhe

Antibiotic-resistant infections spread through Europe

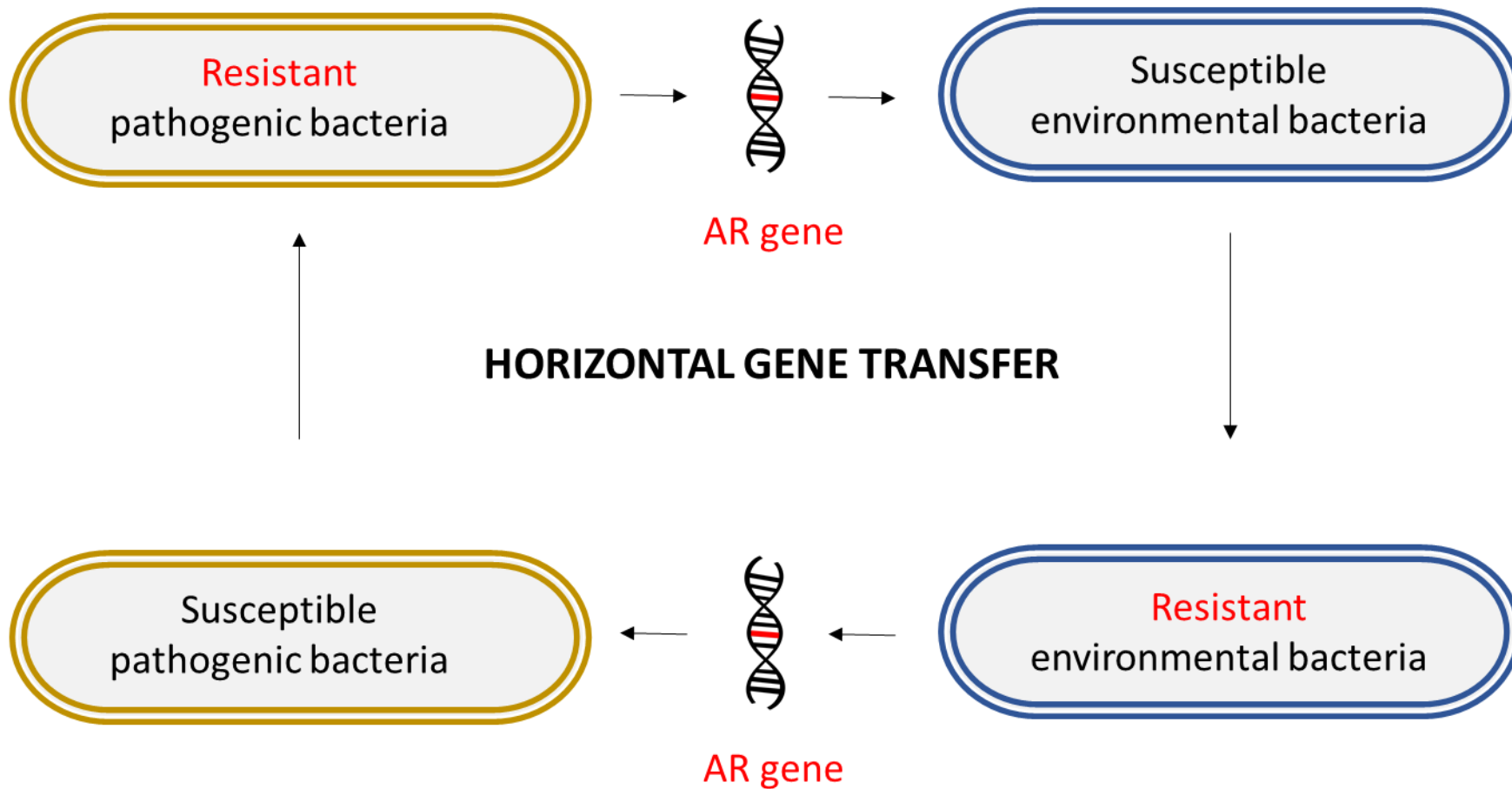
# THE PROBLEM

CIC-2019



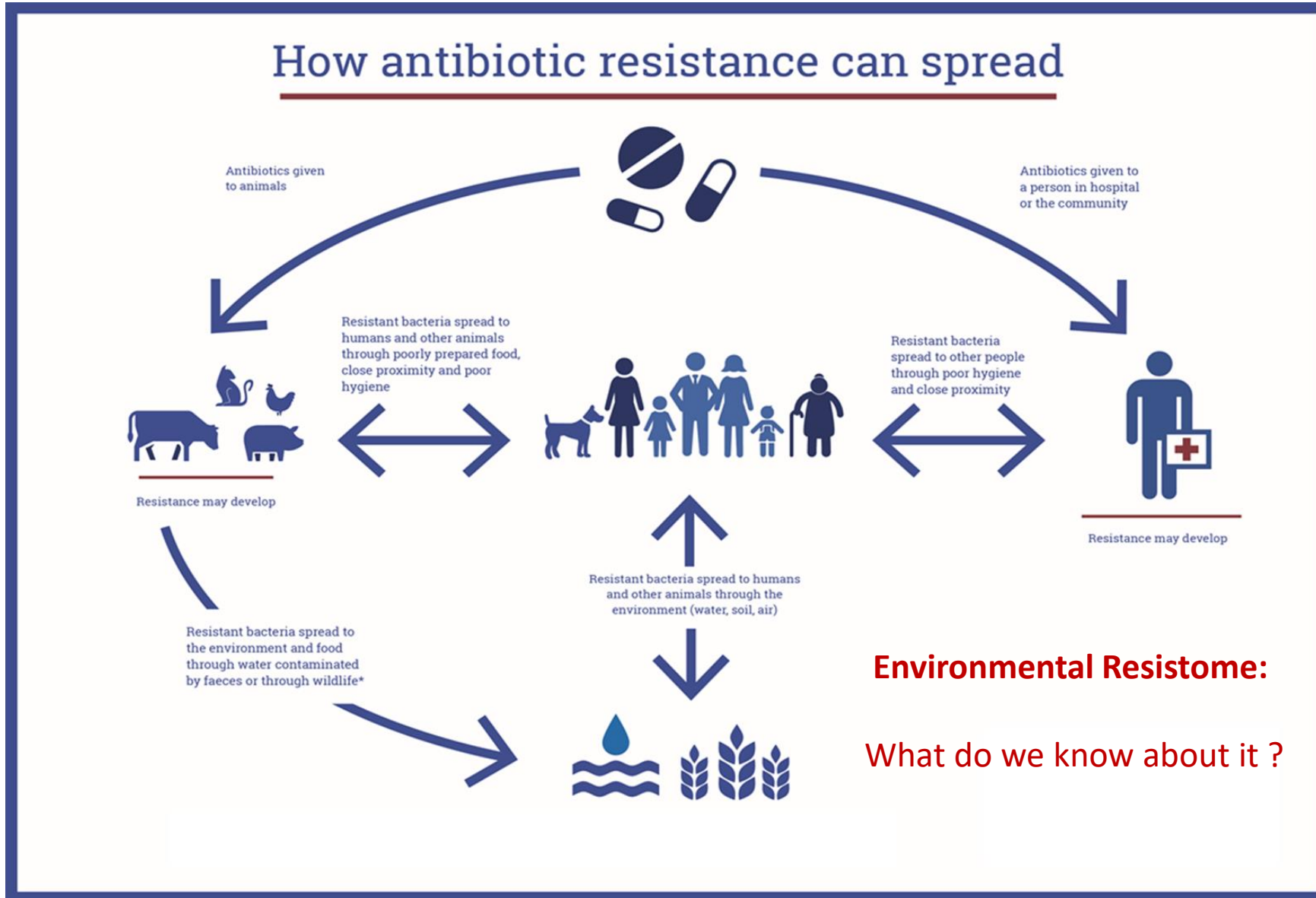
<https://www.amr.gov.au/resources/infographic-how-antibiotic-resistance-can-spread>

# THE PROBLEM



# THE PROBLEM

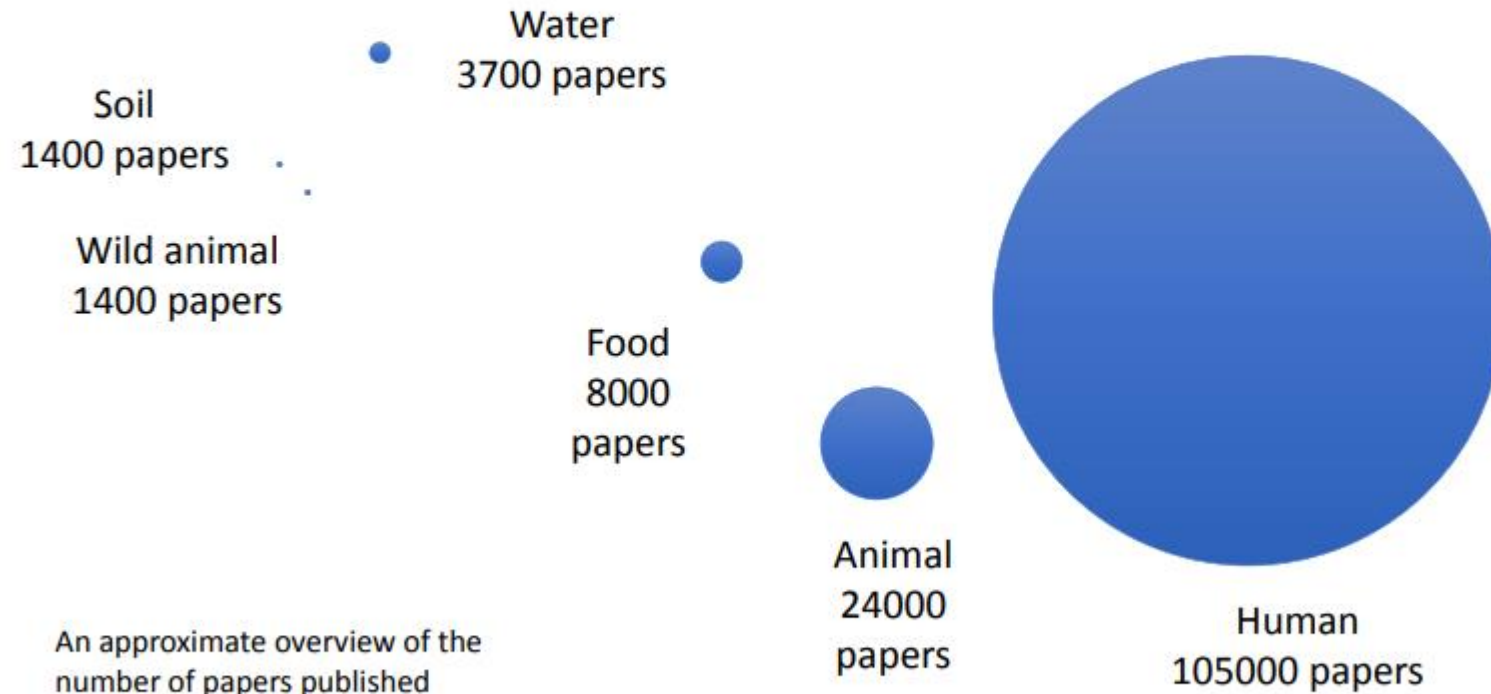
CIC-2019



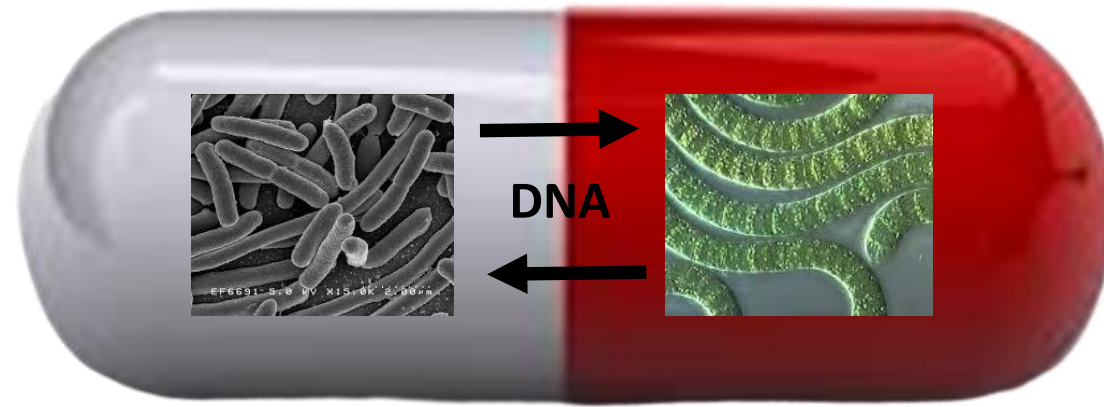
**Environmental Resistome:**

What do we know about it ?

# Antibiotic resistance research Knowledge



## THE HIPOTHESIS

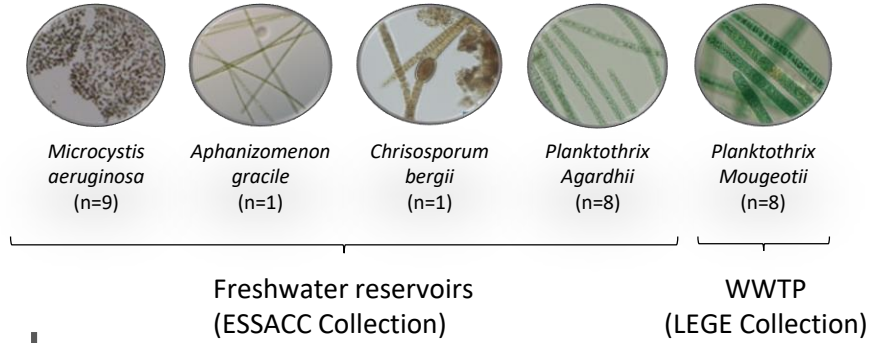


**Cyanobacteria may be resistant to antibiotics**

**Cyanobacteria may acquire and disseminate antibiotic resistance genes**

**Cyanobacteria may have a role on water resistome**

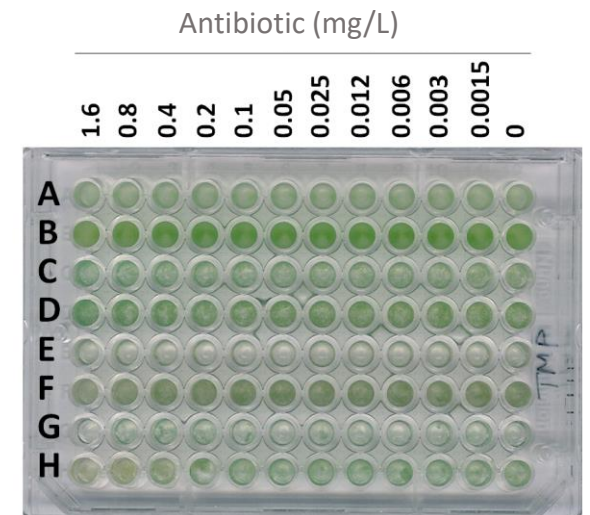
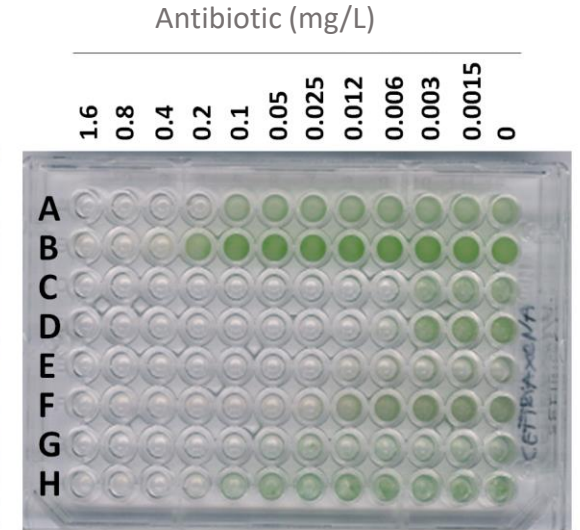
**PREVIOUS WORK** (Dias et al. 2015. *Front. Microbiol.* 6: 799; Dias E. et al. 2018. *STOTEN* 652: 447-454).



- Aminoglycosides
  - Kanamycin
  - Gentamicine
  - Streptomycin
  - Tetracycline
- Beta-lactams
  - Ceftazidime
  - Ceftriaxone
  - Amoxicillin
- Quinolones
  - Norfloxacin
  - Nalidixic acid
  - Trimethoprim

The degree of susceptibility depends on the specie and/or the antibiotic

None of the strains are susceptible to NAL and TMP



Minimum inhibitory concentrations (mg/L) of antibiotics on cyanobacteria strains

<i>M. aeruginosa</i>	AMX	CAZ	CRO	KAN	GEN	STR	TET	NOR	NAL	TMP
LMEXCYA 7	0.8	0.4	0.2	0.2	0.2	-	>0.8	0.05	>1.6	>1.6
LMEXCYA 50	0.2	0.8	0.8	0.2	0.2	0.1	>1.6	0.2	>1.6	>1.6
LMEXCYA 91B	0.1	0.1	1.6	0.2	0.2	0.4	>1.6	0.05	>1.6	>1.6
LMEXCYA 108	0.2	0.8	0.8	0.1	0.2	0.025	>1.6	0.1	>1.6	>1.6
LMEXCYA 113	0.1	0.4	0.8	0.4	0.4	0.05	>1.6	0.05	>1.6	>1.6
LMEXCYA 142	0.2	0.2	0.8	0.2	0.2	0.05	>1.6	0.2	>1.6	>1.6
LMEXCYA 151	1.6	0.2	0.8	0.2	0.2	0.05	>1.6	0.1	>1.6	>1.6
LMEXCYA 159	0.1	0.2	0.4	0.2	0.2	0.025	>1.6	0.05	>1.6	>1.6
LMEXCYA 167	0.2	0.8	0.8	0.2	0.2	0.025	>1.6	0.2	>1.6	>1.6
<i>Planktothrix agardhii</i>	AMX	CAZ	CRO	KAN	GEN	STR	TET	NOR	NAL	TMP
LMEXCYA 153A	0.1	1.6	1.6	0.2	0.2	0.1	0.4	>1.6	>1.6	>1.6
LMEXCYA 230	0.05	0.8	0.8	0.2	0.1	0.05	0.8	>1.6	>1.6	>1.6
LMEXCYA 257	0.025	1.6	0.8	0.2	0.1	0.025	0.4	>1.6	>1.6	>1.6
LMEXCYA 269	0.025	0.4	0.4	0.2	0.1	0.025	0.2	>1.6	>1.6	>1.6
LMEXCYA 280	0.8	1.6	1.6	0.2	0.1	0.05	0.8	>1.6	>1.6	>1.6
LMEXCYA 283	0.05	1.6	0.8	0.2	0.1	0.025	0.2	>1.6	>1.6	>1.6
LMEXCYA 292	0.05	1.6	0.8	0.4	0.2	0.1	0.4	1.6	>1.6	>1.6
LMEXCYA 303	0.05	0.8	0.4	0.2	0.1	0.025	0.2	>1.6	>1.6	>1.6
<i>Planktothrix mougeotii</i>	AMX	CAZ	CRO	KAN	GEN	STR	TET	NOR	NAL	TMP
LEGE 06224	>1.6	0.4	0.2	0.2	0.8	0.05	>1.6	>1.6	>1.6	>1.6
LEGE 06225	>1.6	0.8	0.4	0.2	0.8	0.1	>1.6	>1.6	>1.6	>1.6
LEGE 06226	>1.6	0.8	0.8	0.4	0.8	0.05	>1.6	>1.6	>1.6	>1.6
LEGE 06233	>1.6	0.4	0.2	0.2	0.4	0.05	1.6	>1.6	>1.6	>1.6
LEGE 07227	>1.6	0.8	0.8	0.4	0.4	0.05	>1.6	>1.6	>1.6	>1.6
LEGE 07229	>1.6	0.8	0.4	0.4	0.8	0.1	>1.6	>1.6	>1.6	>1.6
LEGE 07230	>1.6	0.8	0.4	0.8	0.8	0.05	>1.6	>1.7	>1.6	>1.6
LEGE 07231	>1.6	0.4	0.4	0.4	0.8	0.1	>1.6	>1.6	>1.6	>1.6
<i>Aphanizomenon gracile</i>	AMX	CAZ	CRO	KAN	GEN	STR	TET	NOR	NAL	TMP
LMECYA 40	0.012	0.025	0.012	0.4	0.4	-	1.6	0.2	>1.6	>1.6
<i>Anabaena berghii</i>	AMX	CAZ	CRO	KAN	GEN	STR	TET	NOR	NAL	TMP
LMECYA 246	0.012	0.4	0.1	0.8	0.4	-	1.6	0.8	>1.6	>1.6

All strains have reduced susceptibility to nalidixic acid and trimethoprim.

Intrinsic resistance?

*P. mougeotii* (WWTP) have reduced susceptibility to antibiotics of 4 ≠ classes.

Acquired resistance?



Cyanobacteria specie	Strain	Antibiotic resistance genes (n=17) and MGE (n=4)			
		<i>int1</i>	<i>sul1</i>	<i>qacΔE</i>	<i>strA-strB</i>
M. aeruginosa	LMECYA 50	-	-	-	+
	LMECYA 91B	-	-	-	-
	LMECYA 108	-	-	-	-
	LMECYA 113	+	+	-	-
	LMECYA 142	-	-	-	-
	LMECYA 151	-	+	-	-
	LMECYA 159	-	+	-	-
	LMECYA 167	+	+	+	-
P. agardhii	LMECYA 153A	+	+	+	+
	LMECYA 230	-	-	-	-
	LMECYA 257	-	-	-	-
	LMECYA 269	-	-	-	-
	LMECYA 280	+	+	-	+
	LMECYA 283	-	-	-	-
	LMECYA 292	-	-	-	-
	LMECYA 303	+	+	-	+
P. mougeotii	LEGE 06224	-	-	-	-
	LEGE 06225	+	+	-	+
	LEGE 06226	+	+	-	+
	LEGE 06233	-	+	-	-
	LEGE 07227	-	-	-	+
	LEGE 07229	-	+	-	+
	LEGE 07230	+	+	-	-
	LEGE 07231	-	-	-	-

- 33% of cyano cultures w/ MGE and 25% w/ ARGs
- Higher % ARGs in WWTP CB
- Phenotype  $\neq$  Genotype
- Genes conferring resistance to antibiotics for witch cyanobacteria are resistant (TMP, NAL) were not detected.

### Intrinsic resistance?

### Does cyanobacteria harbours new ARGs?

- Cyano cultures w/ genes conferring resistance to antibiotics for witch they are susceptible.

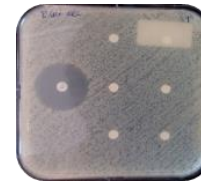
### Low expression level of Cyano ARGs?

### Are ARGs from cyanobacteria-associated bacteria?

PREVIOUS WORK (Dias et al. 2013. ECCMID).



Isolation of bacteria



SUSCEPTIBILITY OF CYANOBACTERIA-ASSOCIATED BACTERIA TO DISTINCT CLASSES OF ANTIBIOTICS

Cyanobacteria	Bacterial isolate	Identification	Amoxicillin	Ticarcillin	Cephalotin	Cefuroxime	Ceftazidime	Cefotaxime	Cefpodoxime	Ceftriaxone	Cefepime	Aztreonam	Cefoxitin	Imipinem	Meropenem	Ertapenem	Pieracillin + TZP	Amoxicillin + CLA	Nalidixic acid	Ciprofloxacin	Norfloxacin	Streptomycin	Kanamycin	Gentamicin	Amikacin	Trimethoprim/sulfamethoxazole	Nitrofurantoin	Fosfomicin	Colistin	Tigecycline	Chloramphenicol	Tetracycline
Microcystis spp (LMECYA 7)	CI7-1a	<i>Hydrogenophaga</i> spp	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	CI7-1b	<i>Hydrogenophaga</i> spp	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	CI7-2a	<i>Rhizobium radiobacter</i>	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Red	Blue	Blue	Blue	Red	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	CI7-2b	<i>Rhizobium radiobacter</i>	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Red	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	CI7-2c	<i>Rhizobium radiobacter</i>	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Red	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	CI7-3	<i>Hydrogenophaga atypica</i> spp	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
Aphanizomenon spp (LMECYA 40)	CI40-1	<i>Limnobacter thioxidans</i>	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	CI40-2	<i>Limnobacter thioxidans</i>	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	CI 40-3b	<i>Limnobacter thioxidans</i>	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	CI 40-3c	<i>Limnobacter thioxidans</i>	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	CI 40-4	<i>Hydrogenophaga</i> spp	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	CI40-5	<i>Hydrogenophaga</i> spp	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	CI40-6	<i>Hydrogenophaga</i> spp	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	CI40-7	<i>Runella slithyformis</i>	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	CI40-8	<i>Novosphingobium panipatense</i>	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Red	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	CI40-9	<i>Enhydrobacter aerosaccus</i>	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
CI40-10	<i>Afipia</i> spp	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
Anabaena spp (LMECYA 246)	CI246-1	<i>Hydrogenophaga palleronii</i>	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	CI246-2	<i>Methylobacterium radiotolerans</i>	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	CI246-3	<i>Novosphingobium panipatense</i>	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	CI246-4	<i>Novosphingobium panipatense</i>	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	CI246-5	<i>Novosphingobium panipatense</i>	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	CI246-6	<i>Novosphingobium panipatense</i>	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
Planktothrix spp (LMECYA 260)	CI 260-1	<i>Sphingobium</i> spp	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	CI 260-2	<i>Brevundimonas lenta</i>	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue
	CI 260-3	<i>Brevundimonas lenta</i>	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue

Blue Susceptible Red Resistant Grey Intermediate susceptibility

Multiresistant pattern!

CIC-2019

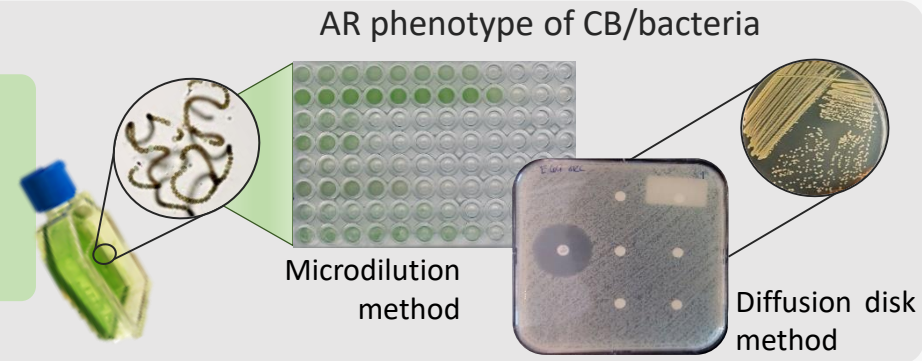
AIMS

TASKS

1

Evaluate the AR phenotype of CB and their associated bacteria from fresh- and wastewaters

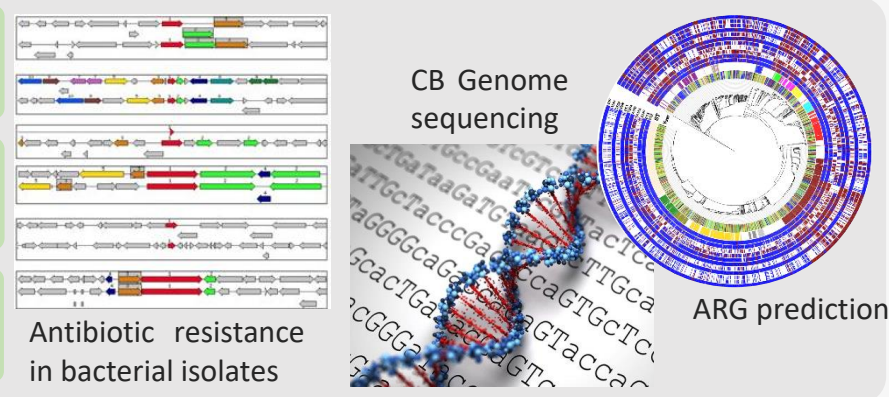
I - Antibiotic susceptibility patterns of CB/bacterial isolates



2

Search/predict AR genes (and related mobile genetic elements-MGE) in CB and associated bacterial strains isolated from fresh- and wastewaters

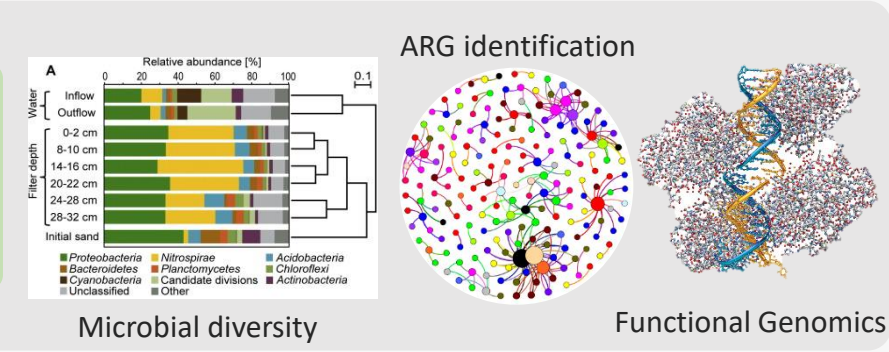
II - Antibiotic resistance genes in bacterial isolates  
 III - WGS of cyanobacteria strains by HTS approaches  
 IV - Data processing of putative ARG in CB genomes



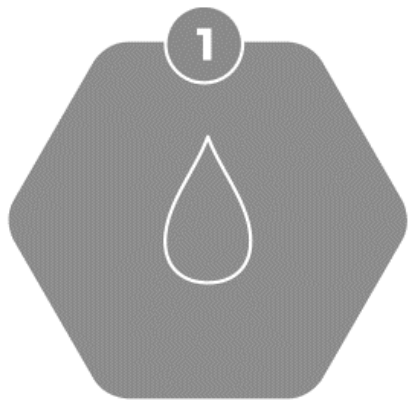
3

Identify the presence of AR genes (and related MGE) in the whole DNA from fresh- and wastewater samples and characterize their CB/bacterial communities

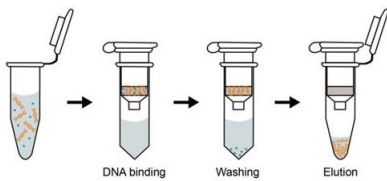
V - Metagenomic analyses of fresh and wastewater



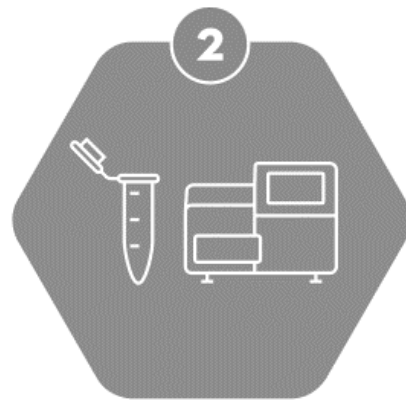
# HIGH-THROUGHPUT SEQUENCING



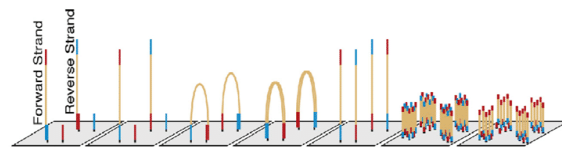
**gDNA extraction from CB**



DNA amount/quality



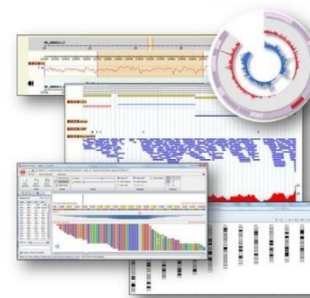
**MiSeq (Illumina®)**



Library quality



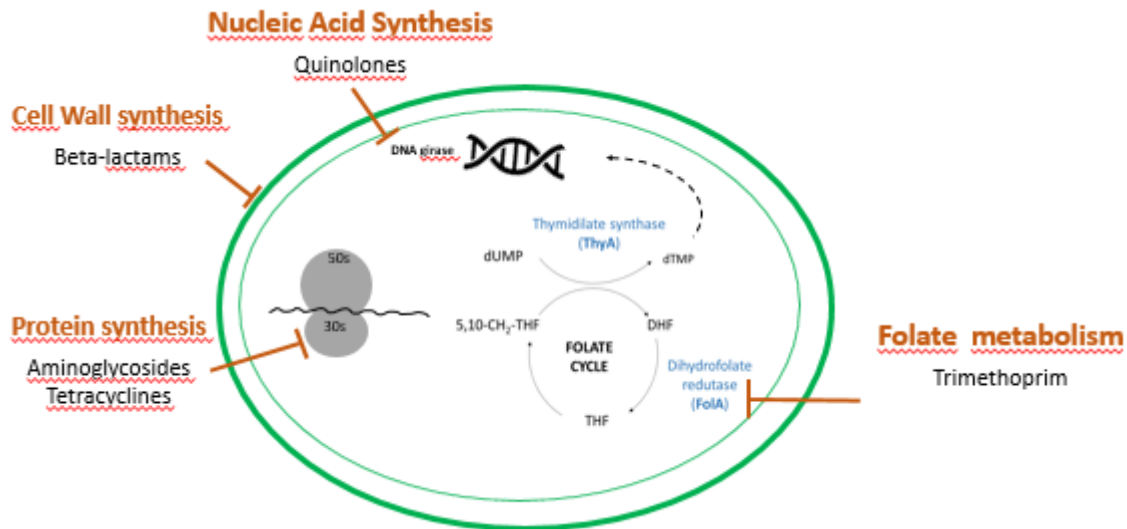
**Data analysis**



Incomplete databases  
Computational errors or biases

- CB/bacterial community ID
- ARG prediction/identification
- Microbial characterization diversity
- Population assessment Dynamics

# PRELIMINARY RESULTS (Dias et al. 2019. 11th ICTC)



## Intrinsic resistance of cyanobacteria to TMP

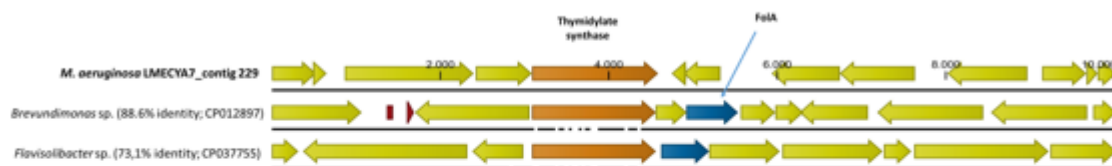
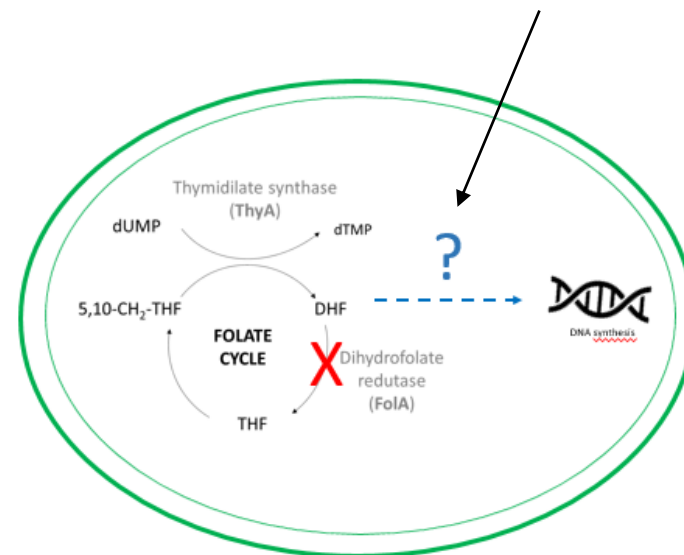


Figure 1. Comparison of genomic sequences harboring *thyA* gene (in orange, coding to thymidylate synthase EC 2.1.1.45) and/or *folA* gene (in blue, coding to bacterial dihydrofolate reductase EC 1.5.1.3). The CLC genomics workbench 10.0 (Qiagen) and the Basic Local Alignment Search Tool (BLAST) were used to compare the similarity of *Microcystis aeruginosa* sequence harboring *thyA* gene (LMECYA7\_contig 229) with other LMECYA7 contigs mapping other bacteria species.

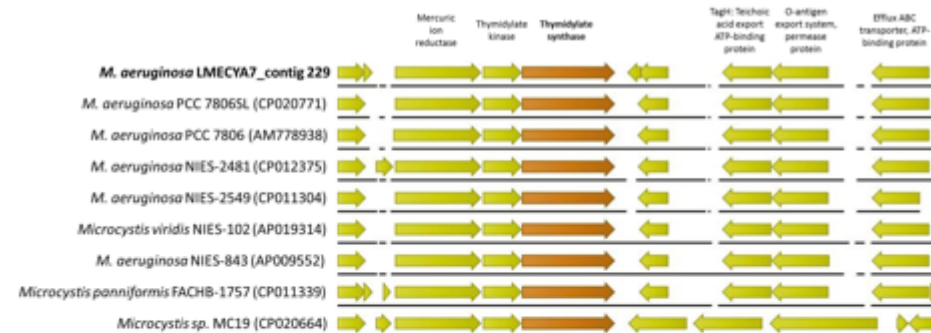


Figure 2. Comparison of genomic sequences harboring *thyA* gene (in orange). The CLC genomics workbench 10.0 (Qiagen) and the Basic Local Alignment Search Tool (BLAST) were used to compare the similarity of *Microcystis* spp. sequences harboring *thyA* gene (coding to Thymidylate synthase EC 2.1.1.45).

- *M. aeruginosa* LMECYA 7 lack the gene codifying dihydrofolate reductase (FolA) (Fig. 1).
- The gene *folA* is also absent in the genomes of other *M. aeruginosa* strains previously sequenced (Fig. 2).

## Microcystis-associated bacteria

(WGS of *M. aeruginosa* cultures)

UNDISCLOSED

# THE PROJECT



## Water resistome



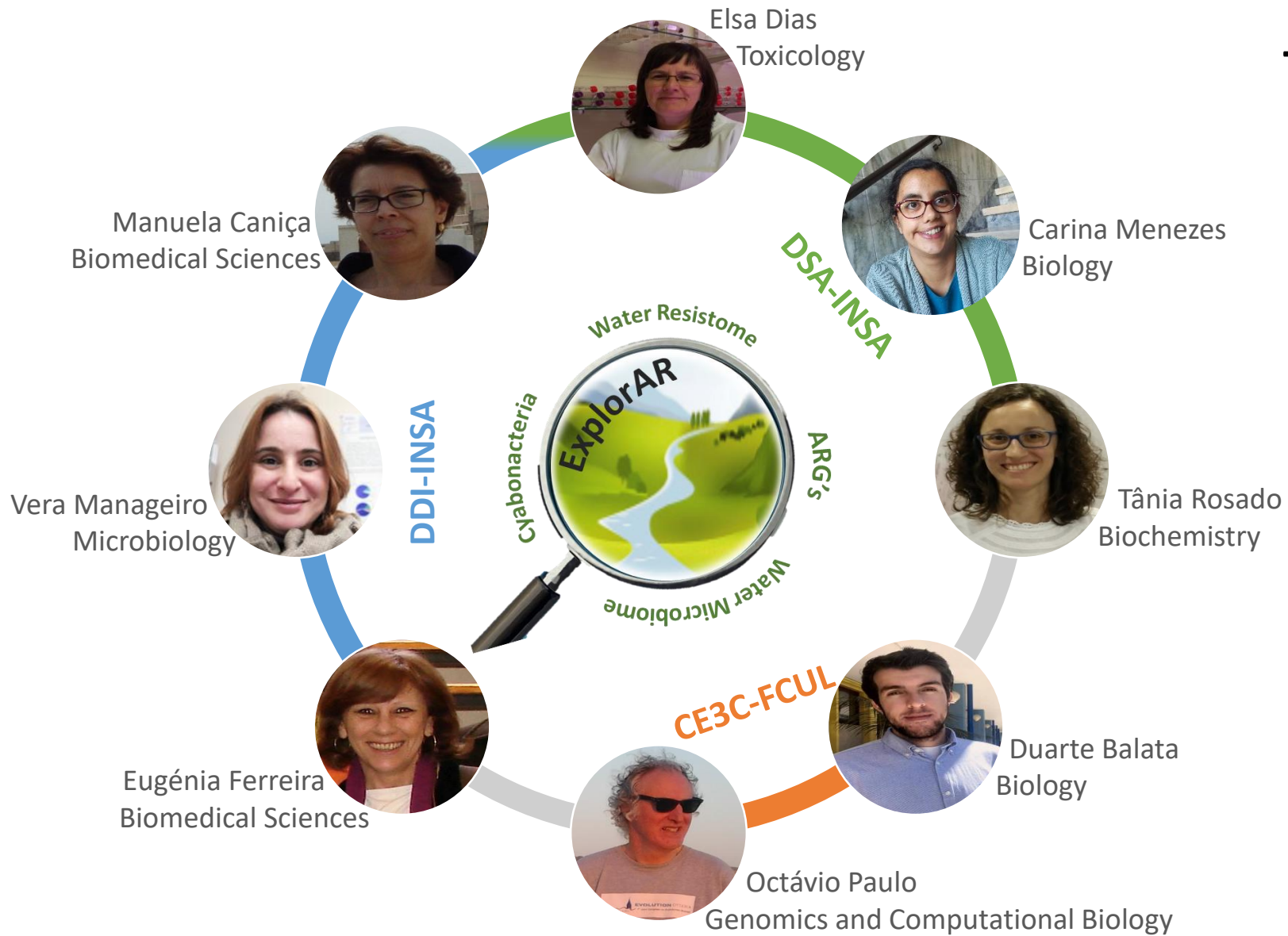
- Understand how aquatic ecosystems are impacted by antibiotic pollution.

- Map the AR profiles of a representative number of freshwater resources.
- Identify/characterize ecological niches associated with the occurrence of resistant microorganisms.

- Define monitoring/preventive/control measures against the dissemination of AR in the environment.



# Team members



**DSA-INSA:** Laboratory of Biology and Ecotoxicology, Department of Environmental Health, Lisbon

**DDI-INSA:** National Reference Laboratory of Antimicrobial Resistances and Healthcare Associated Infections, Department of Infectious Diseases, Lisbon

**CE3C-FCUL:** Centre for Ecology, Evolution and Environmental Changes, Faculty of Sciences, University of Lisbon, Lisbon

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Luís Vieira

Catarina Silva

Sílvia Duarte

Daniel Sampaio

Joana Mendonça

CE3C- FCUL/Univ. Lisbon

Francisco Martins

