# Vancomycin and ciprofloxacin resistance in enterococci from a Hospital effluent and in the receiving Municipal Wastewater Treatment Plant

### Ana Rita Varela<sup>\*1</sup>, Giovanna Ferro<sup>1,2</sup>, Jana Vredenburg<sup>1</sup>, Melike Yanik<sup>1</sup>, Luigi Rizzo<sup>2</sup>, Celia M Manaia<sup>1</sup>

1- CBQF/Escola Superior de Biotecnologia – Universidade Católica Portuguesa, Porto, Portugal 2-Department of Civil Engineering, University of Salerno, 84084, Fisciano (SA), Italy \*avarela@porto.ucp.pt

# **Scope and objectives**

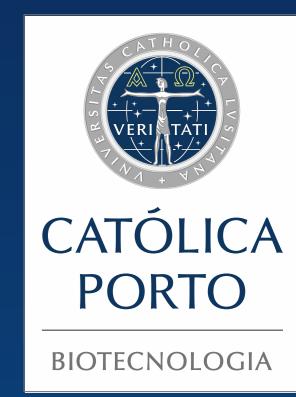
•Hospital effluents can be discharged to municipal wastewater treatment plants /MWWTP) without previous treatment;

•Enterococci are indicators of faecal contamination and recognized harbours of clinically relevant resistance phenotypes;

### Approach

Water samples: Hospital – MWWTP (raw & treated) **Comparison of: Cultivable counts** 





The major objectives in this study were:

•Assess if hospital effluents may be a source of ciprofloxacin and vancomycin resistant enterococci;

• Compare the enterococci loads and respective resistance rates in the untreated hospital effluent and in the raw inflow of the receiving municipal wastewater treatment plant (MWWTP);

• Characterize the most relevant enterococci species and antibiotic resistance patterns observed in hospital effluents and in municipal wastewater.

#### **Antibiotic resistance prevalence Species diversity**

-Isolation on mEnterococcus agar and on mEnterococcus agar supplemented with vancomycin or ciprofloxacin

-CFU/mL and percentage of resistance

-16S rRNA gene sequence analysis

-Antibiotic resistance phenotypes - disk diffusion method -- Detection of vancomycin-resistance associated genes

a

**Hospit**a

Inflow

MWWTP

**MWWTP** Effluent

Others

**WWTP** Membrane **Filtration** Method

ERY

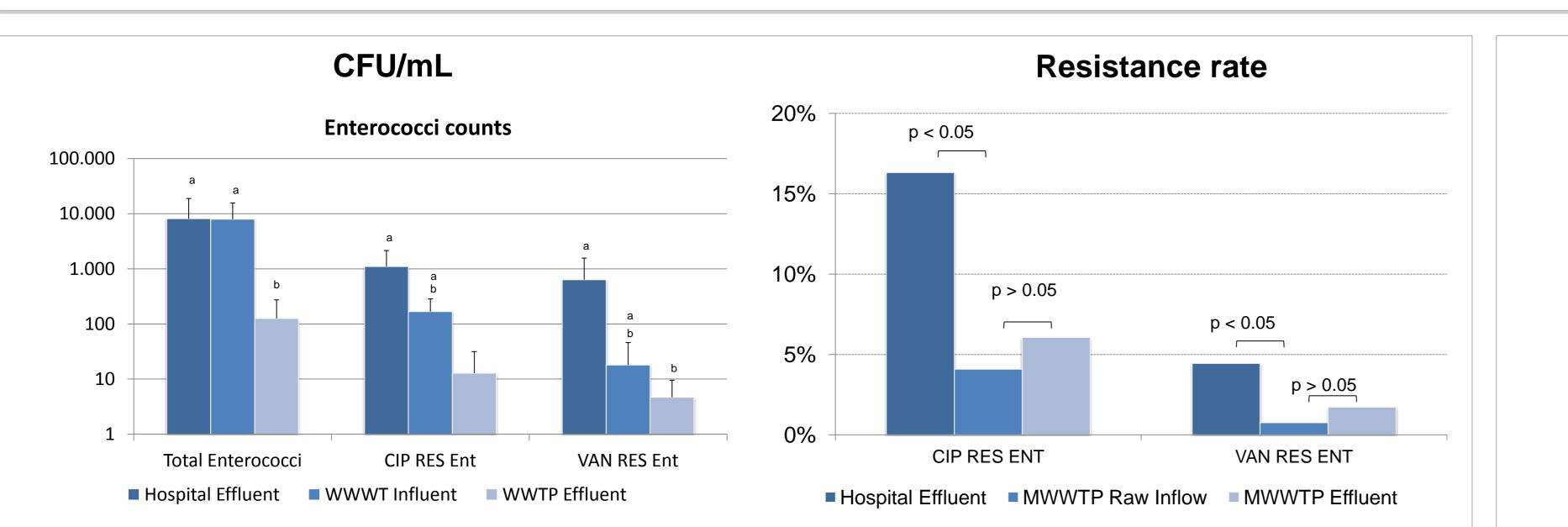
SXT TET

S

S

n/d

# Results



Species (>99 % 16S rRNA sequence similarity)	(n)	AML	VAN	GEN	CIP
	1	S	R	R	R
	1	S	R	R	R
	1	S	R	R	I
	1	S	R	R	I
	1	S	R	R	R
	3	S	R	R	R
	1	S	S	R	R
	2	S	R	R	R

Figure 1 - Colony forming units of total and resistant enterococci in hospital effluent and MWWTP raw and treated wastewater; a-b, significantly different values (p>0,05).

Figure 2 - Percentage of bacteria able to grow in antibioticsupplemented media in the hospital effluent and MWWTP raw and treated wastewater.

### **General comments**

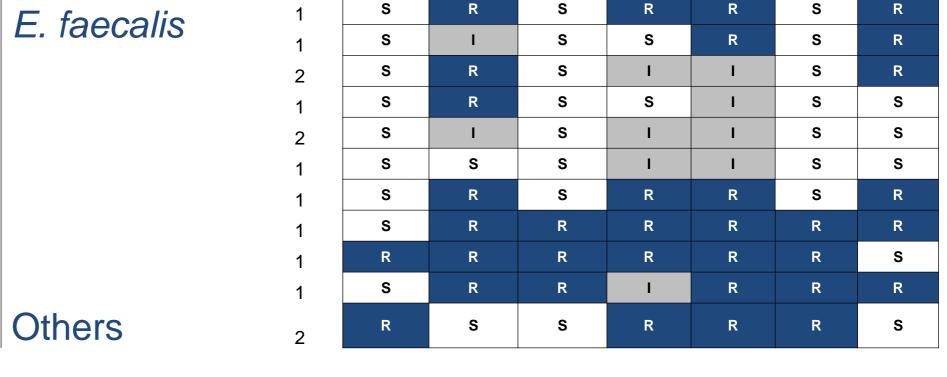
•Strong temporal variations of cultivable counts, mainly in the untreated hospital effluent;

• Enterococci counts, including the ciprofloxacin and vancomycin resistant, were not significantly different in the hospital effluent and in the MWWTP raw inflow;

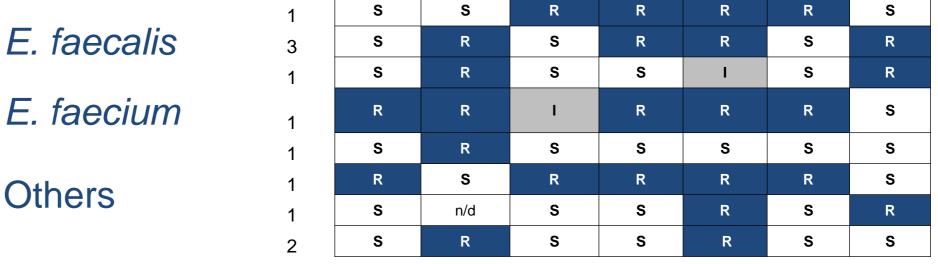
Nevertheless,

• The mean percentage of antibiotic resistant enterococci was at least three times higher in the hospital effluent than in the raw inflow;

• The prevalence of resistance in the raw and treated wastewater was not significantly different;



2 1 1 3 1 1 1 1 1 5 5 6 7 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	S	R	S	I	I	S	S	
	S	R	S	I	I	R	S	
	S	R	R	I	R	S	R	
	S	n/d	R	R	R	S	R	
	S	R	S	R	R	S	R	
	S	I	S	I	I	S	S	
	S	R	R	R	R	R	R	
	S	R	S	R	I	S	R	
	S	R	R	R	R	R	R	
	R	R	I	R	R	S	S	
	S	S	R	R	R	R	R	
	S	S	S	S	S	S	R	
	1	S	S	S	S	S	S	S
	1	S	S	R	R	R	R	S
E. faecalis	3	S	R	S	R	R	S	R
		e	D	e	e	1	6	D

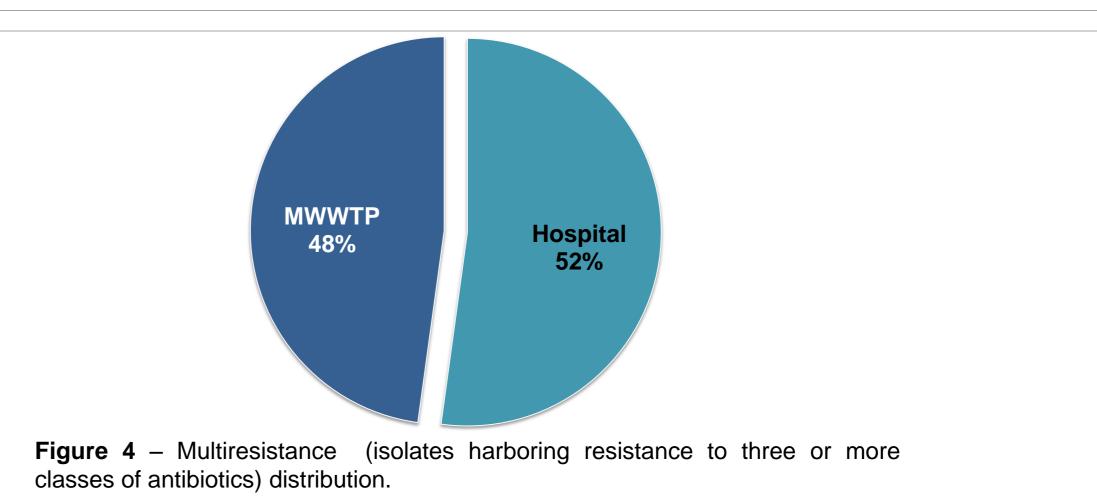


**Figure 3** - Antibiotic resistance profiles: blue, resistant; grey, intermediary; white, susceptible.

• The final treated effluent had lower resistance rates than the hospital effluent.

#### However,

•The vanA gene was found in samples from the three types of water analysed, in 40% of the total isolates.



#### Acknowledgements

The authors gratefully acknowledge the engineers of the MWWTP and the Hospital for their support for samples collection. This study was financed by FCT, project PTDC/ AAC-AMB/113840/2009 and ARBV Grant SFRH/BD/70986/2010. FCT Fundação para a Ciência e a Tecnologia

MINISTÉRIO DA CIÊNCIA, TECNOLOGIA E ENSINO SUPERIOR Portugal