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BIOTECNOLOGIA

Antibiotic resistance in urban wastewater: environmental contamination and risks of water reuse

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WASTEWATER REUSE APPLICATIONS AND CONTAMINANTS OF EMERGING CONCERN
13-14 September 2012 - Limassol, Cyprus

Summary

1. Five adjectives to characterize antibiotic resistance
2. Evidences of antibiotic resistance in wastewater systems
3. Wastewater: a bioreactor for antibiotic resistance enrichment?
4. Wastewater and environmental contamination

Five adjectives to characterize antibiotic resistance

Natural

Widespread

Unknown

Plastic

Pollutant

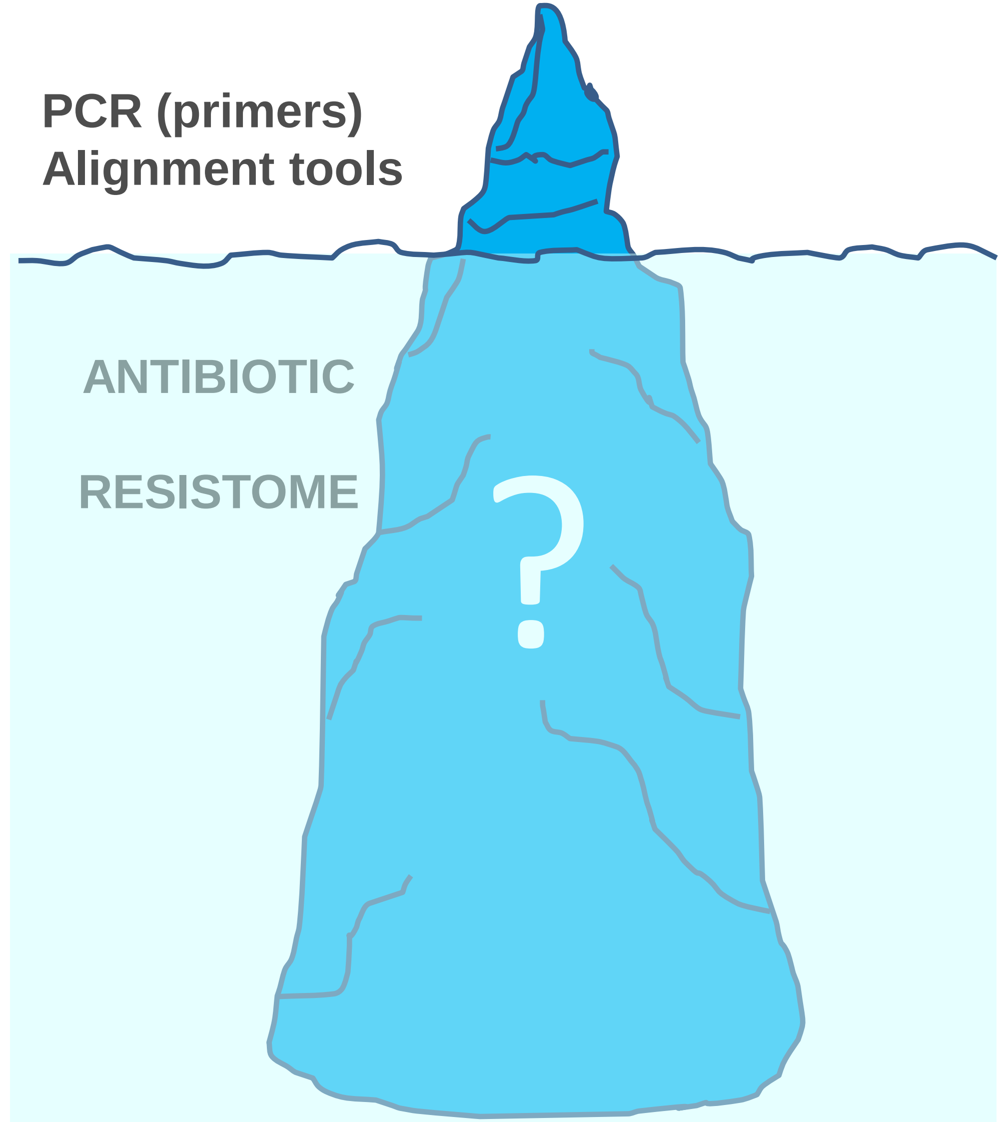
Natural and ancient

Multi-resistant spore-forming bacteria (actinomyces-like) in urban, agricultural and forest soils D' Costa et al., Science, 2006
Resistance genes in 30,000-year-old permafrost sediments (beta-lactam, tetracycline and glycopeptides) D' Costa et al., Nature, 2011
Soil bacteria able to use natural and synthetic antibiotics as sole carbon source Dantas et al., Science, 2008

Widespread

Habitats	References (examples)
Soils	Riesenfeld et al. 2004; D'Costa et al., 2006; Dantas et al., 2008
Surface water	Hamelin et al., 2007; Dolejská et al., 2009; Figueira et al., 2011 a; b.
Drinking water	Faria et al., 2009; Figueira et al., 2011c; Vaz-Moreira et al., 2011; Falcone-Dias et al., 2012; Gomez-Alvarez et a., 2012
Food products	Johnston & Jaykus, 2004; Wang et al., 2006
Wild life	Poeta et al., 2008; Dolejská et al., 2009; Literak et al., 2010; Allen et al., 2011; Gonçalves et al., 2011
Remote Earth zones	D' Costa et al., 2011; Hernández et al., 2012

Unknown



SOIL

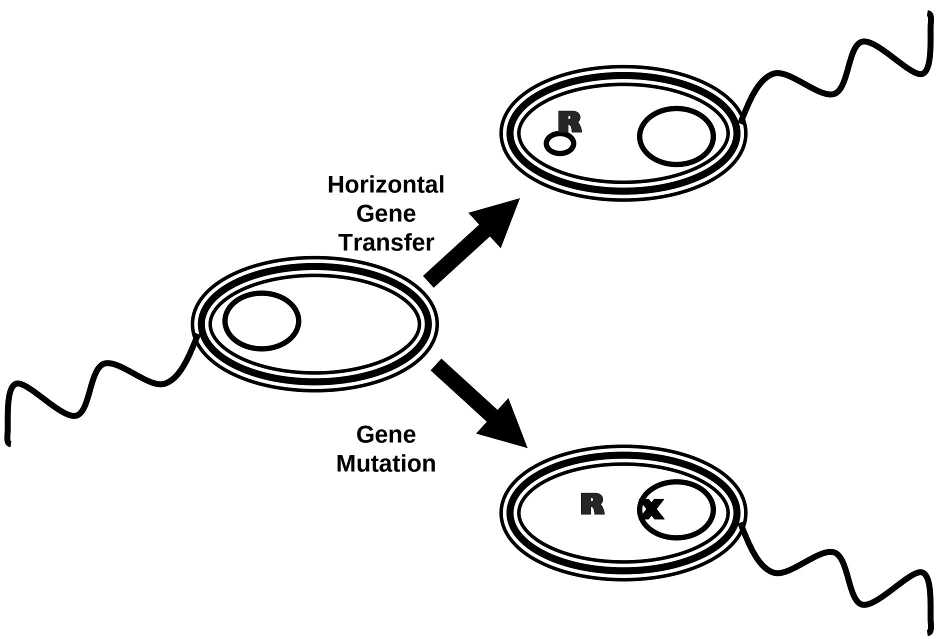
"...resistance proteins ...deduced amino acid sequences with < 60% identity to previously published sequences...".
Riesenfeld et al., 2004

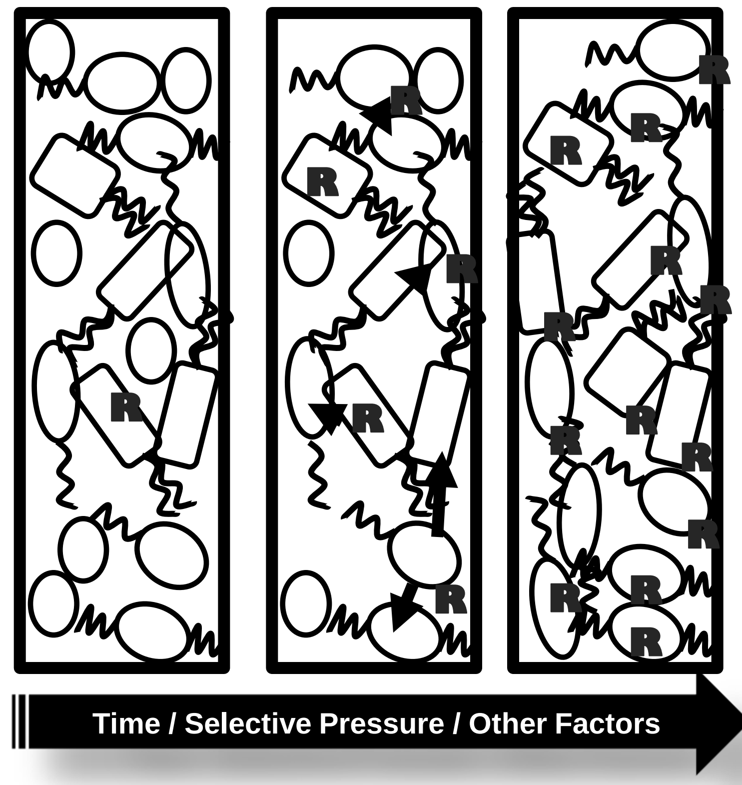
GUT HUMAN MICROBIOME

"...most of the resistance genes we identified with culture independent metagenomic sampling ... were novel when compared to all known genes in public databases. " ***Sommer et al., 2010***

Plastic

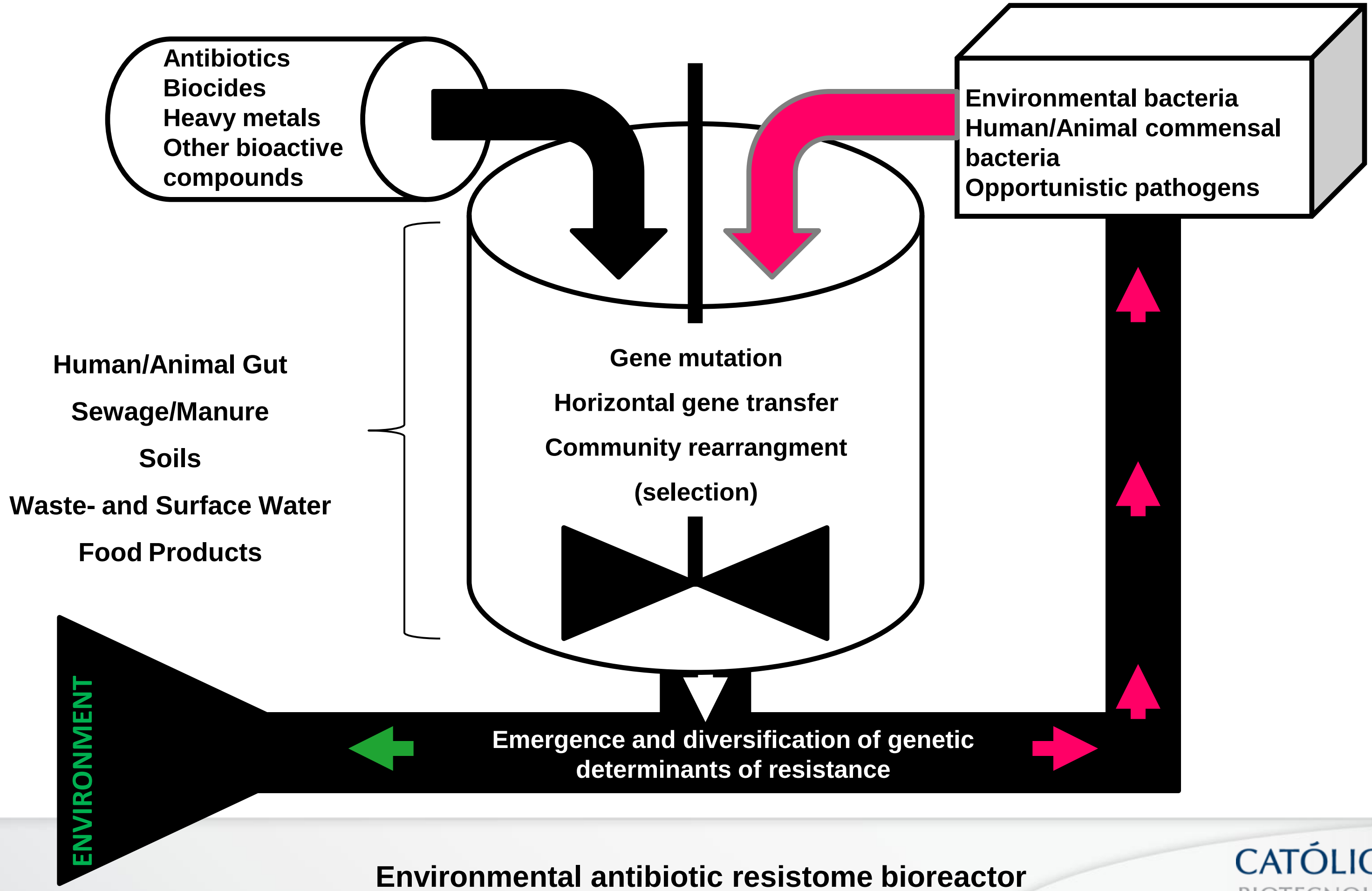
Bacterial genomes and bacterial communities are dynamic





Plastic

Dynamics (genomes + communities) = reaction to environmental conditions



Environmental antibiotic resistome bioreactor

Pollutant

"A pollutant is a substance that is present in concentrations that may harm organisms (humans, plants and animals) or exceed an environmental quality standard. The term is frequently used synonymously with contaminant." **Organisation for Economic Co-operation and Development**

70 Years antibiotics
Heavy metals
Other pollutants (biocides, pharmaceuticals)
???

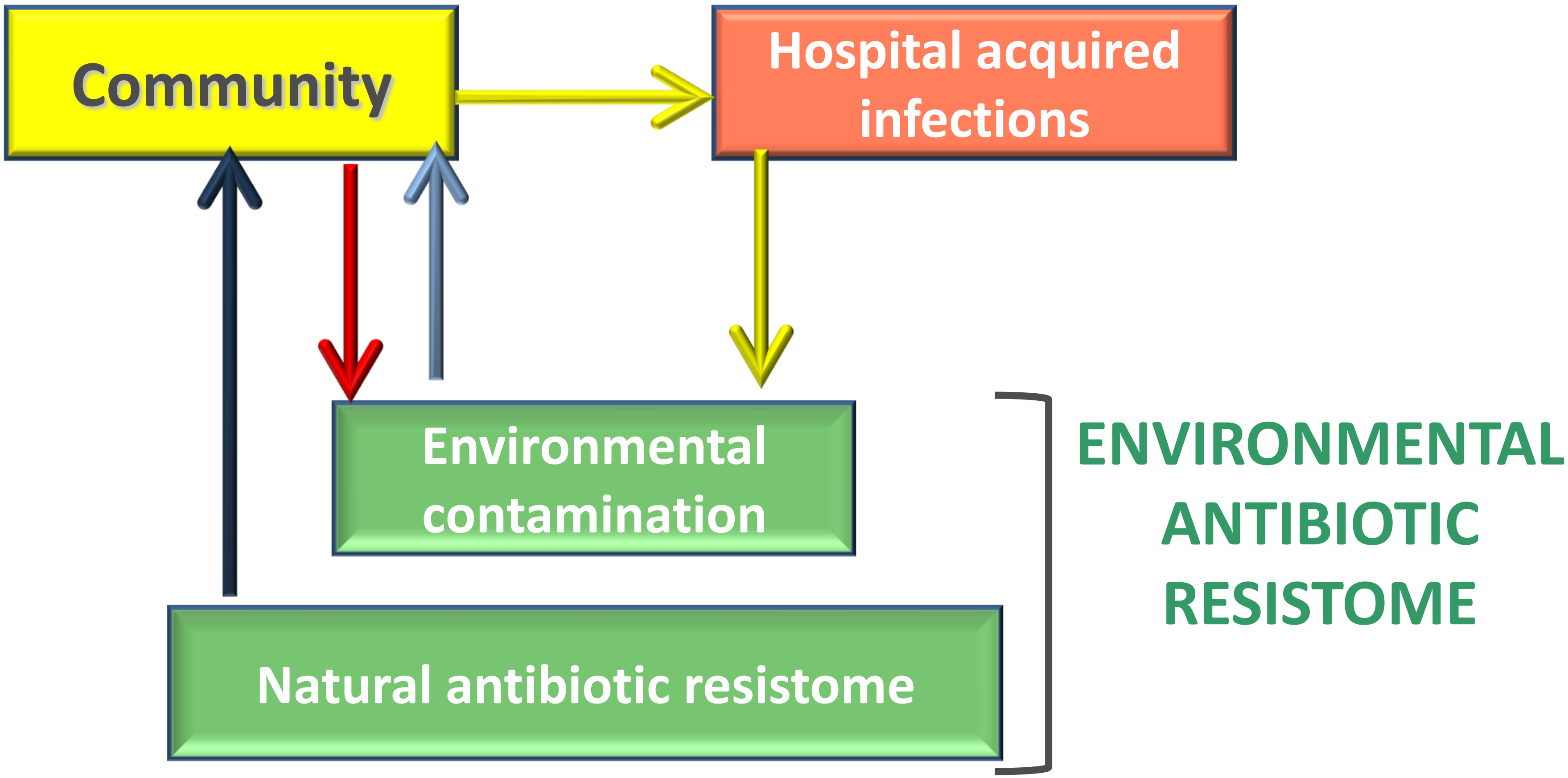
Human activities disturbed the natural equilibrium

Biological Pollutant

Unlike chemical pollution…

Stable…
Proliferates
Spreads

Antibiotic resistance sources



Urban wastewater treatment plants

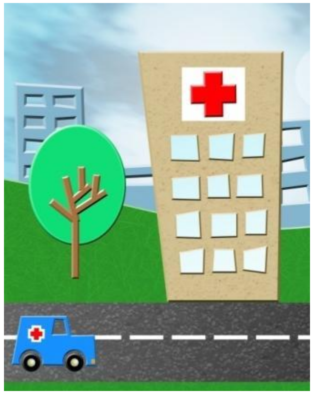
a bioreactor for antibiotic resistance enrichment?

Antibiotics and wastewaters

Germany

0.5 g *per capita* per year

25 % used in the Hospital



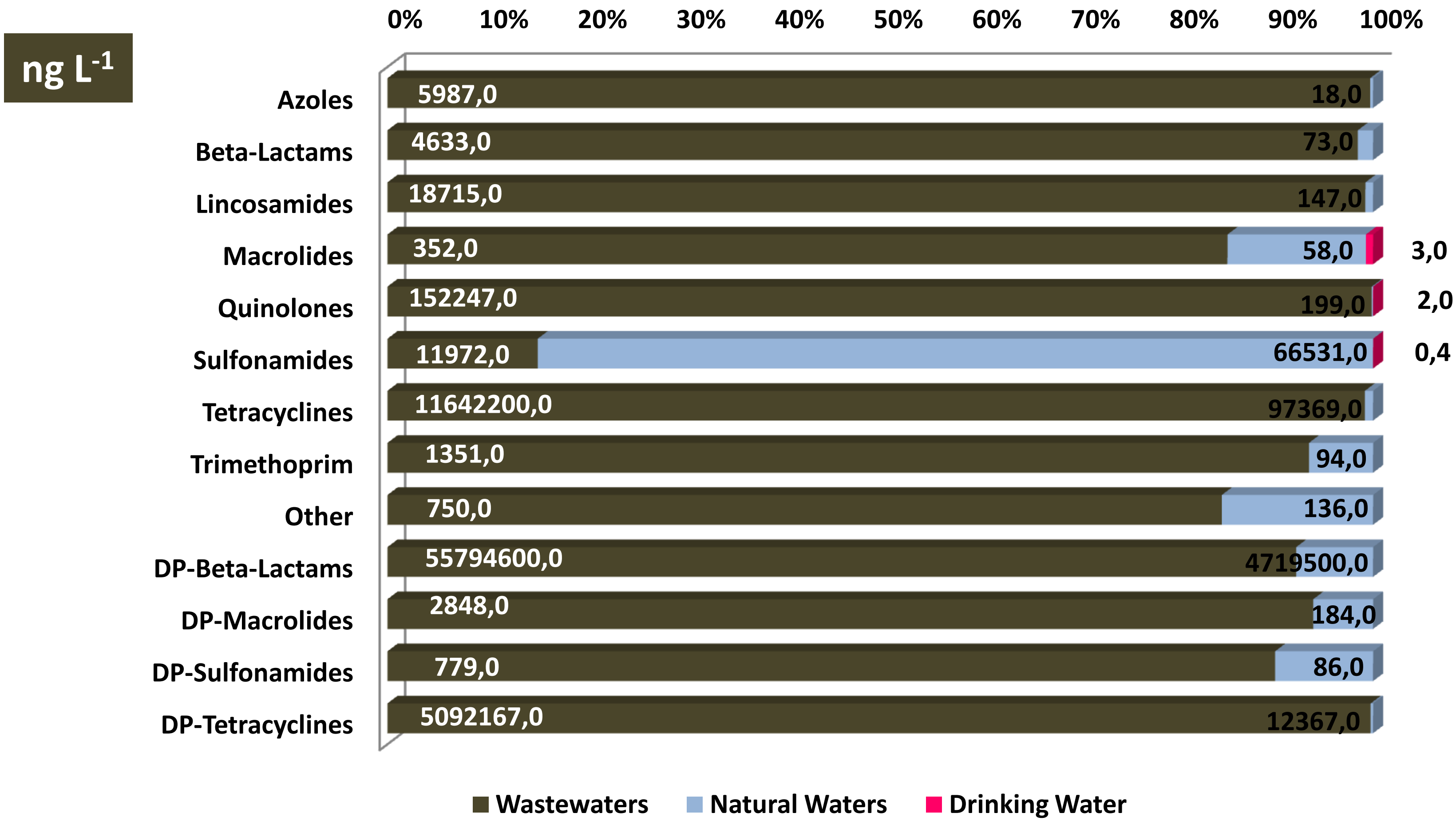
~75 % EXCRETED TO UWWTP

Kümmerer and Henninger. 2003. Clin Microbiol Infect 9:1203-1214

ANTIBIOTICS IN WASTEWATERS

Web of Science and Google Scholar search for articles published in peer-reviewed journals written in the English language since 1984

Up to 1 g/L



Segura et al., 2009, Environ Health Perspect. 117(5):675-84

Evidences of antibiotic resistance in urban wastewaters

VRE
MRSA

	Percentage of resistance (concentration of antibiotic, µg or µ/mL)										
	Total heterotrophs	*Acinetobacter*		*E. coli*					*Enterococcus*		
Aminopenicillins **Ampicillin (AP)** **Amoxicillin (AX)**	22.4-26.1 (32)[AX]	0.0-0.6 (10)[AP]	19.5 (30)[AX1]	30.9 (25)[AX]	15 (10)[AP]	36.0-41.0 (32)[AP]	16.5-23.5 (32)[AP]	34 (2-16)[AP]	7.0 (0.5-32.0)[AP]	0.4-0.9 (32)[AX]	2.0 (25)[AX]
Tetracycline	1.1-1.8 (16)	5.5-10.4 (30)	n.a.	34.1 (30)	10.0 (30)	19-23 (16)	6.8 (4)	23.5 (1-8)	20.0 (0.5-8.0)	14.5-22.8 (16)	32.0 (30)
Sulfamethoxazole (+trimethoprim, T)	n.a.	0.6-2.4 (25)	11.2 (250)[2]	22.3 (25)[T]	17.0 (300)[3]	34-37 (350)	17.5 (256)	11.1 (10-40)[T]	n.a.	n.a.	1.0 (25)[T]
Quinolones **Nalidixic acid (NA)** **Ciprofloxacin (C)**	1.7-2.8 (4)[C]	0.0-5.5 (5)[C] 0.6-5.6 (30)[NA]	7.5 (5)[C]	5.5 (5)[C]	5.0 (30)[NA]	5.0-7.0 (4)[C]	0.7-0.9 (4)[C]	10.5 (0.5-2)[C]	28.6 (0.5-4.0)[C]	2.4-5.0 (4)[C]	18.0 (5)[C]
Gentamycin	n.a.	0.0-0.6 (10)	0.8 (10)	4.4 (10)	0.0 (10)	n.a.	n.a.	2.0 (1-8)	3.2 (synergy) (500)	n.a.	50.0 (10)
Method (antibiotic concentration)	AIA (µg/mL)	DDM (µg)	DDM (µg)	DDM (µg)	DDM (µg)	AIA (µg/mL)	MMPN (µg/mL)	MD (µg/mL)	MD (µg/mL)	AIA (µg/mL)	DDM (µg)
Region (Observations)	Portugal FE, RV3	Danmark RV2, TT	USA TT	Portugal	Australia AV5	Australia FE, RV2	Ireland FE, RV2	Poland	Poland	Portugal FE, RV3	Portugal
Reference	Novo and Manaia, 2010	Guardabassi et al., 2002	Zhang et al., 2009	Ferreira da Silva et al., 2007	Watkinson et al., 2007a	Watkinson et al., 2007b	Galvin et al., 2010	Łuczkiewicz et al., 2010	Łuczkiewicz et al., 2010	Novo and Manaia, 2010	Ferreira da Silva et al., 2006

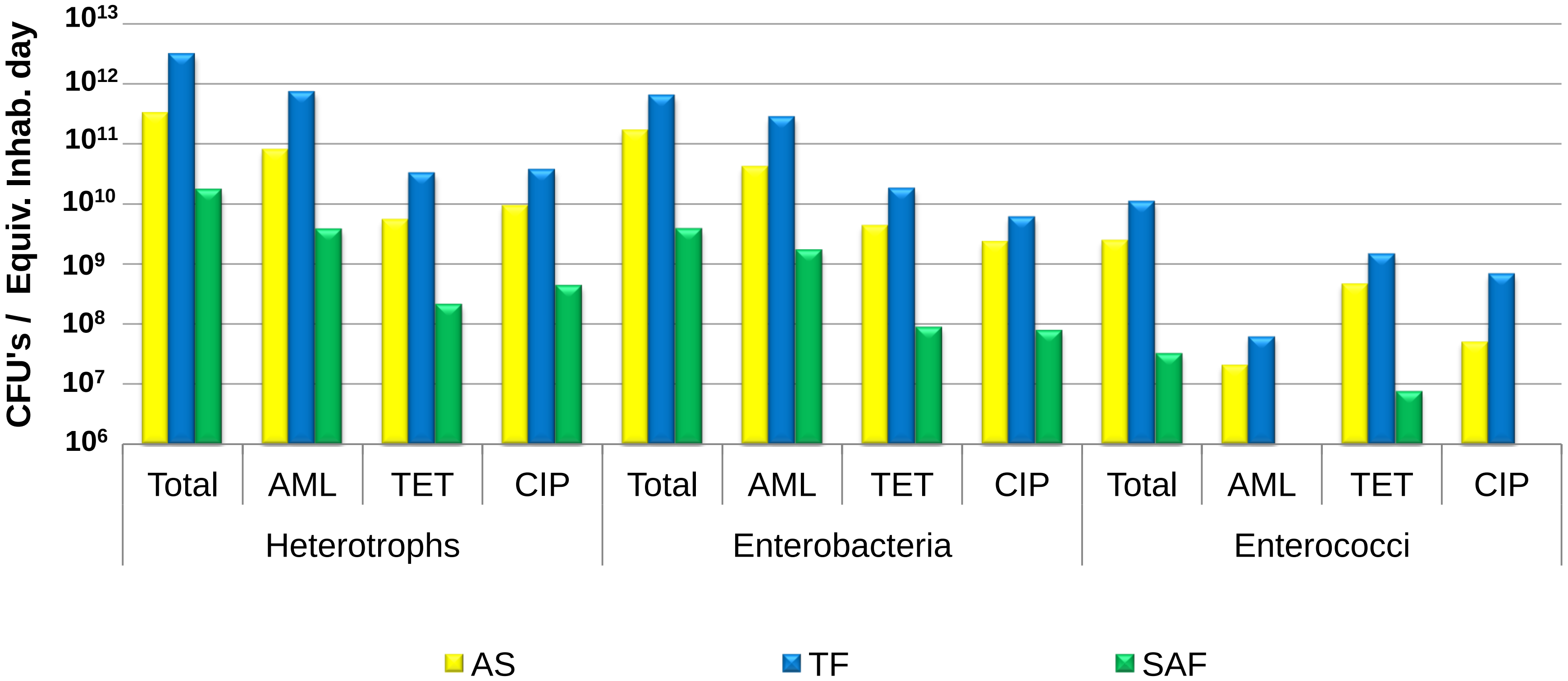
n.a. not /available; -, not applicable; 1, with clavulanic acid; 2, Sulfisoxazole; 3, Sulfafurazole; AIA, Antibiotic impregnated agar; DDM, Disk diffusion method; MMPN, Modified Most Probable Number; MD, Microdilution method; FE, Final effluent; RVn, Range values of n plants; AVn, Average values of n plants; TT, tertiary treatment implemented.

Manaia et al. 2012. In *The Handbook of Environmental Chemistry*, Springer, Volume 20/2012, 173-212

Urban wastewater treatment

~ 10^7 - 10^{11} CFU/IE.DAY OF ANTIBIOTIC RESISTANT BACTERIA

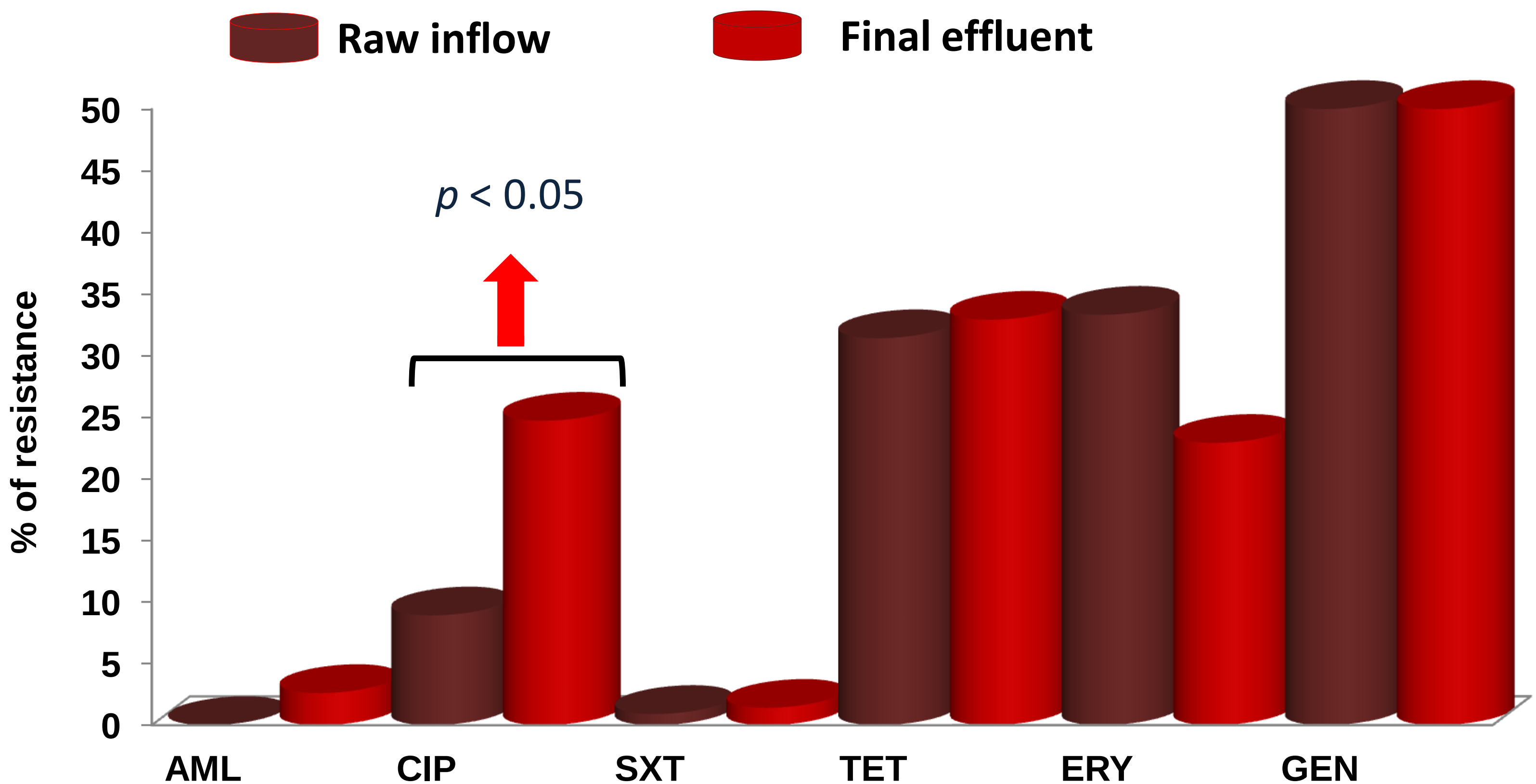
IN THE FINAL EFFLUENT



AS =activated sludge ;TF = trickling filter; SAF = submerged aerated filter

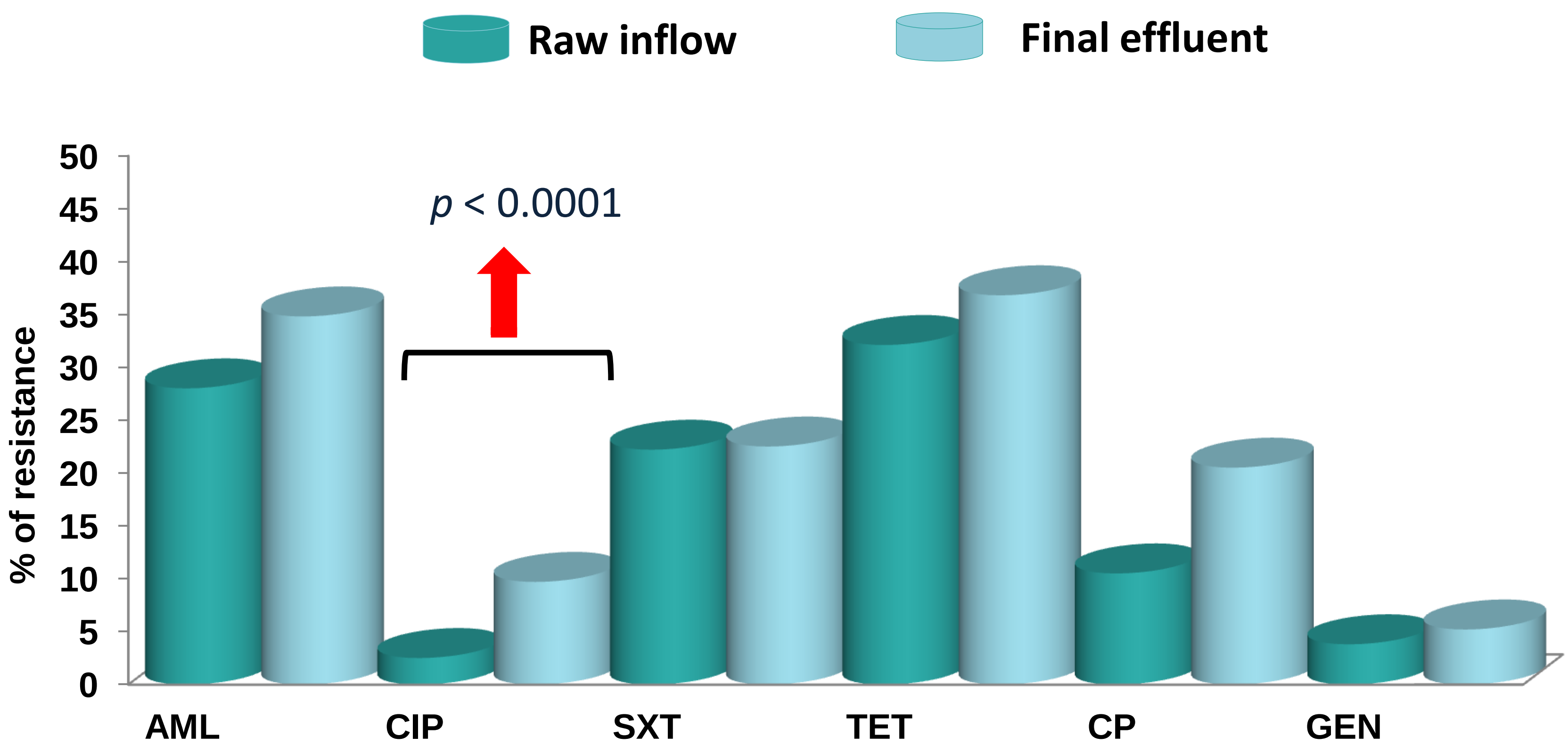
Novo & Manaia, 2010. Appl Microbiol Biotechnol 87:1157–1166

Wastewater treatment
Enterococci



Ferreira da Silva et al. 2006. FEMS Microbiology Ecology, 55:322-329.

Wastewater treatment
Escherichia coli



Ferreira da Silva, et al. 2007. FEMS Microbiology Ecology, 60:166-176

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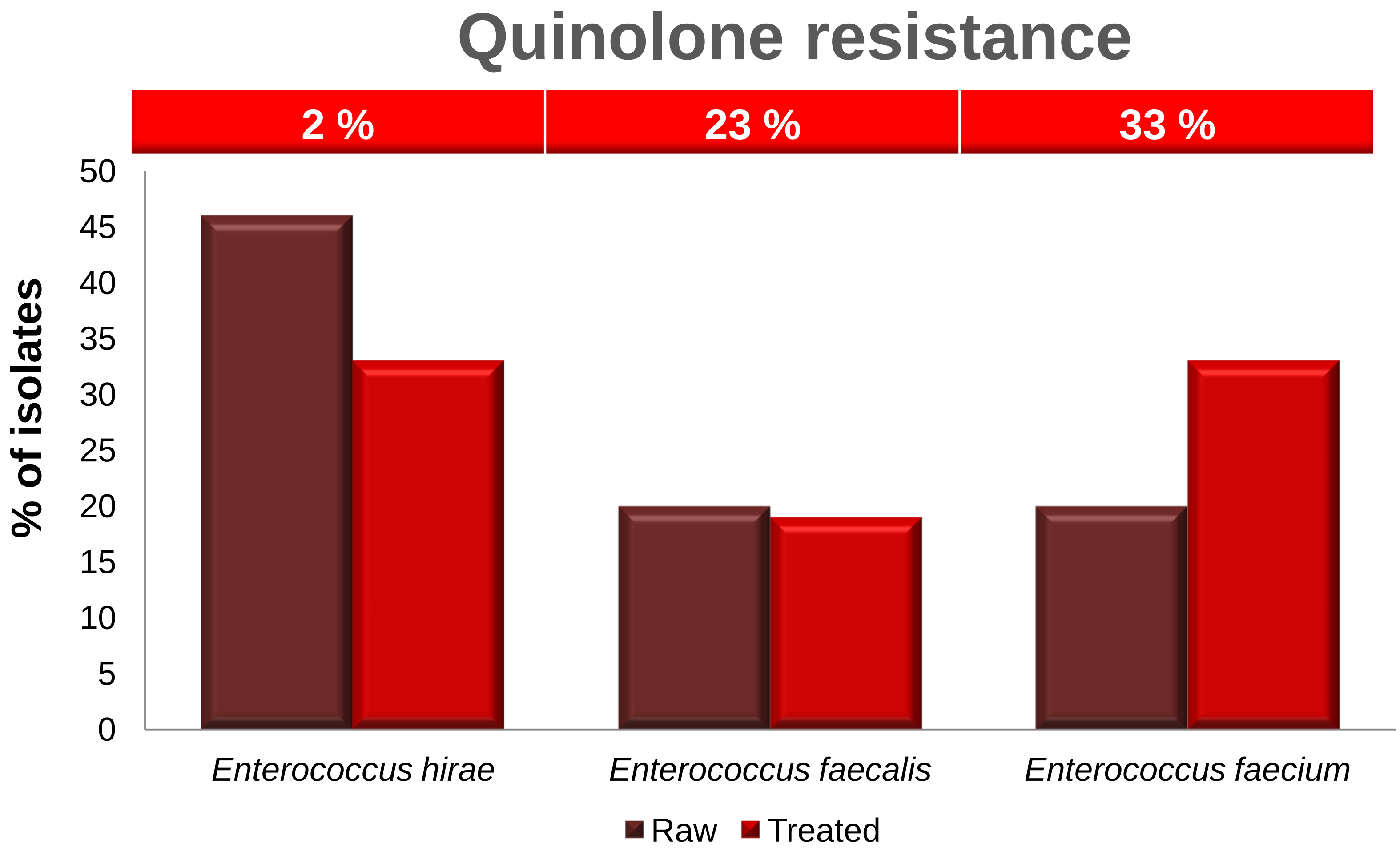
Quinolone resistance and enterococci species distribution

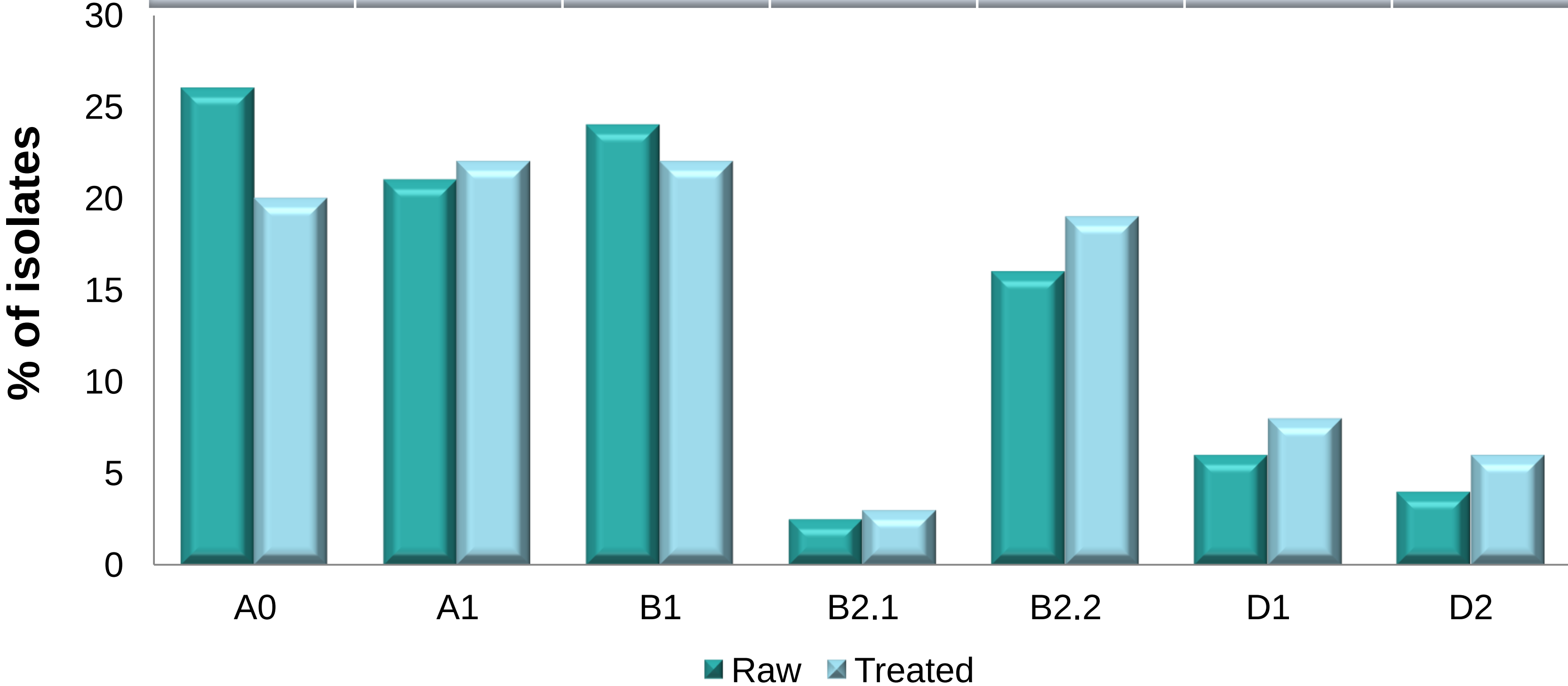


Ferreira da Silva et al. 2006. FEMS Microbiology Ecology, 55:322-329

Quinolone resistance and *Escherichia coli* genotypic groups distribution

Quinolone resistance (100 % chromosome encoded)

0 %	4.2 %	9.6 %	0 %	4.2 %	13.9 %	3.0 %



Figueira et al. 2011 Science of the Total Environment, 409:1017–1023

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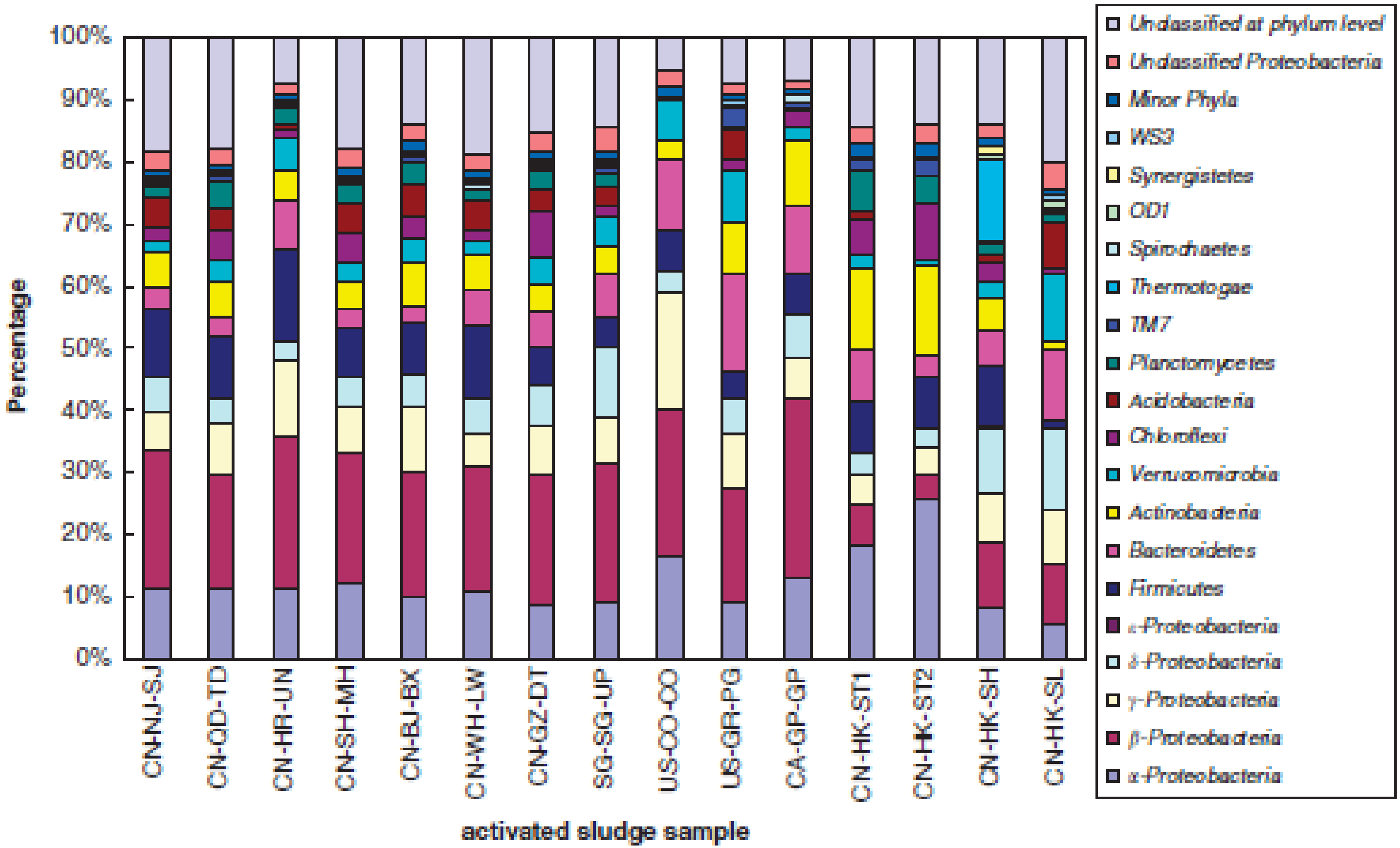
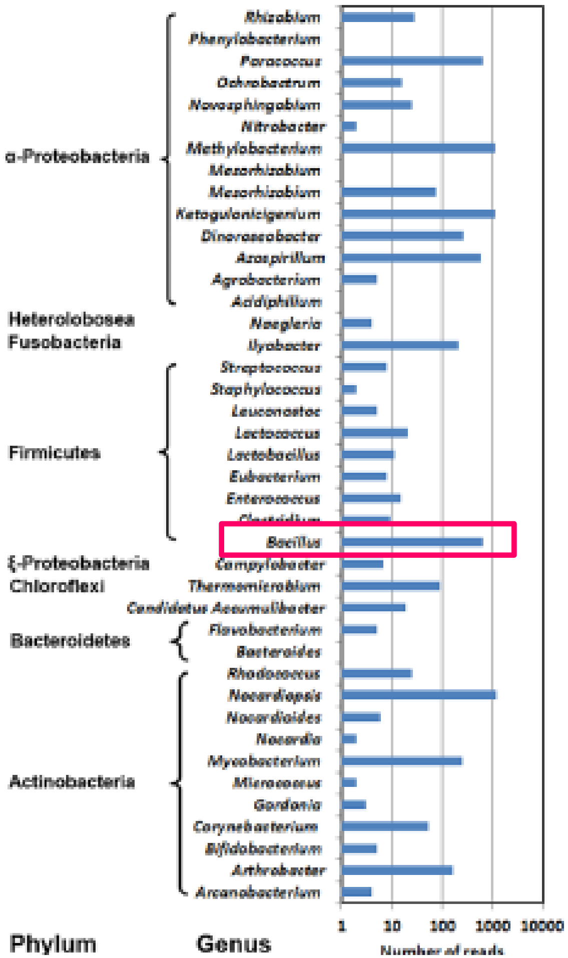
Wastewater systems: hiden microbial worlds

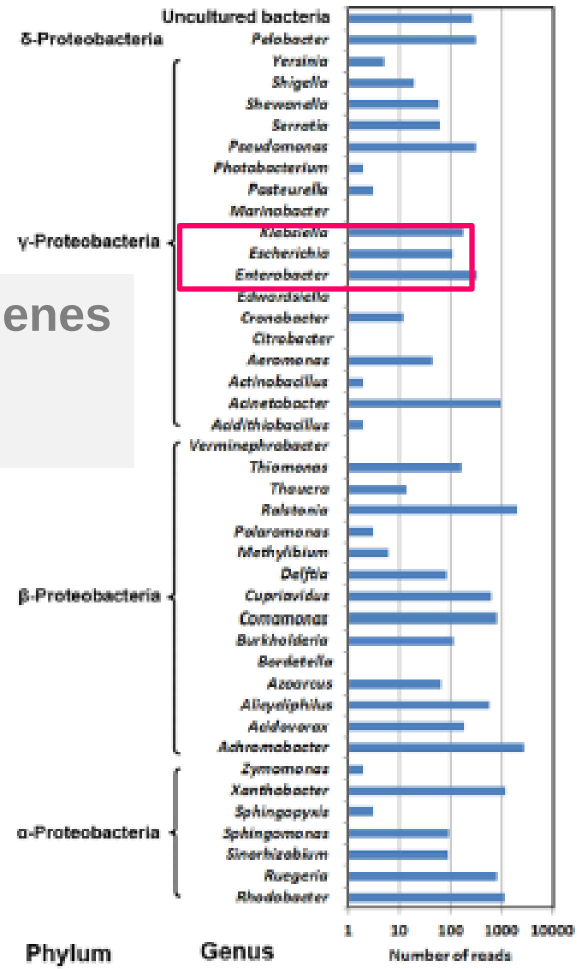


Zhang et al., 2012. 454 ISME J., 6(6):1137-47.

Plasmid hosts in activated sludge



Antibiotic resistance genes
Integrons
Transposons



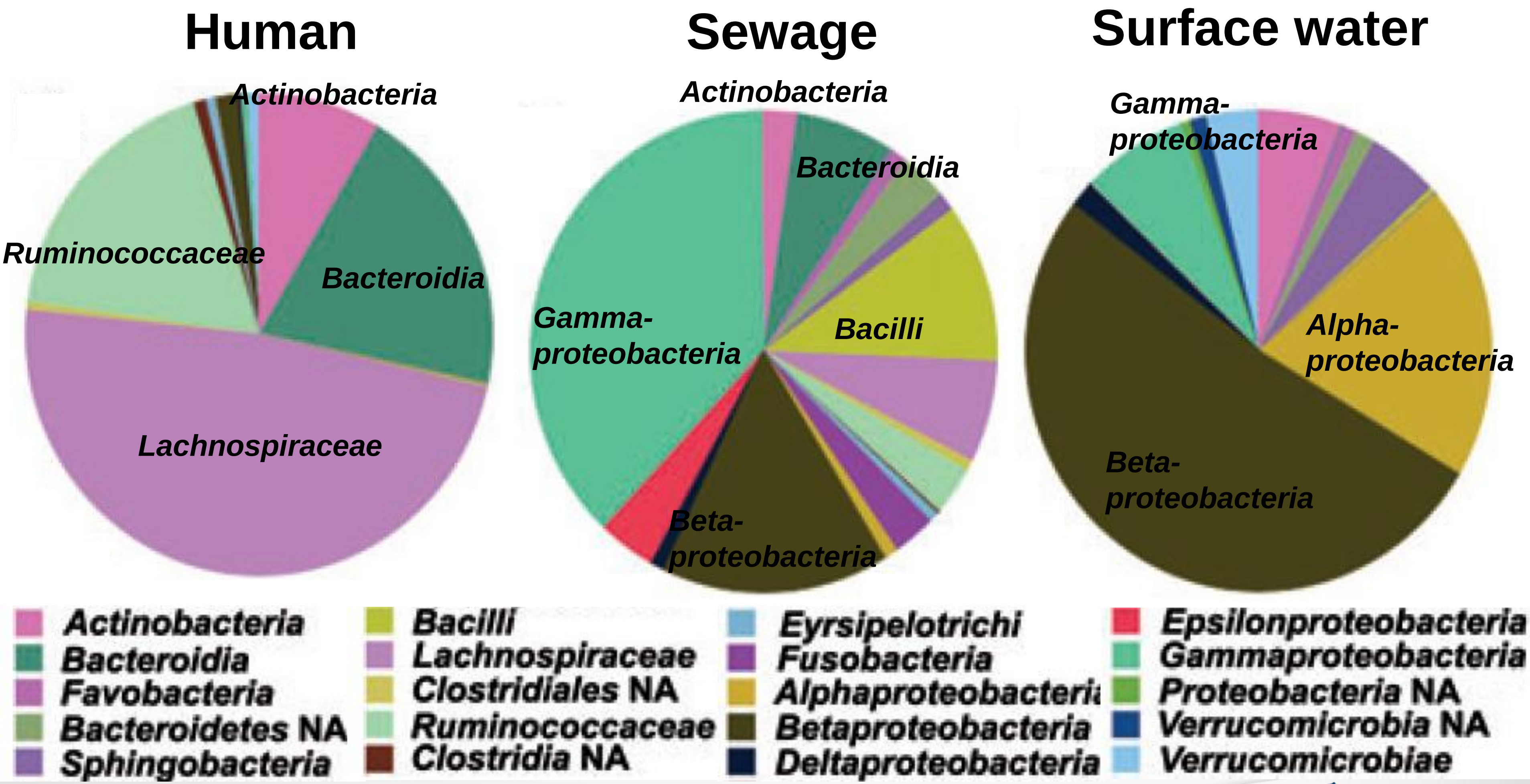
AFTER WASTE WATER TREATMENT?
Environmental contamination

Fate of antibiotic resistant

bacteria and genetic determinants

?

Bacterial communities are dynamic

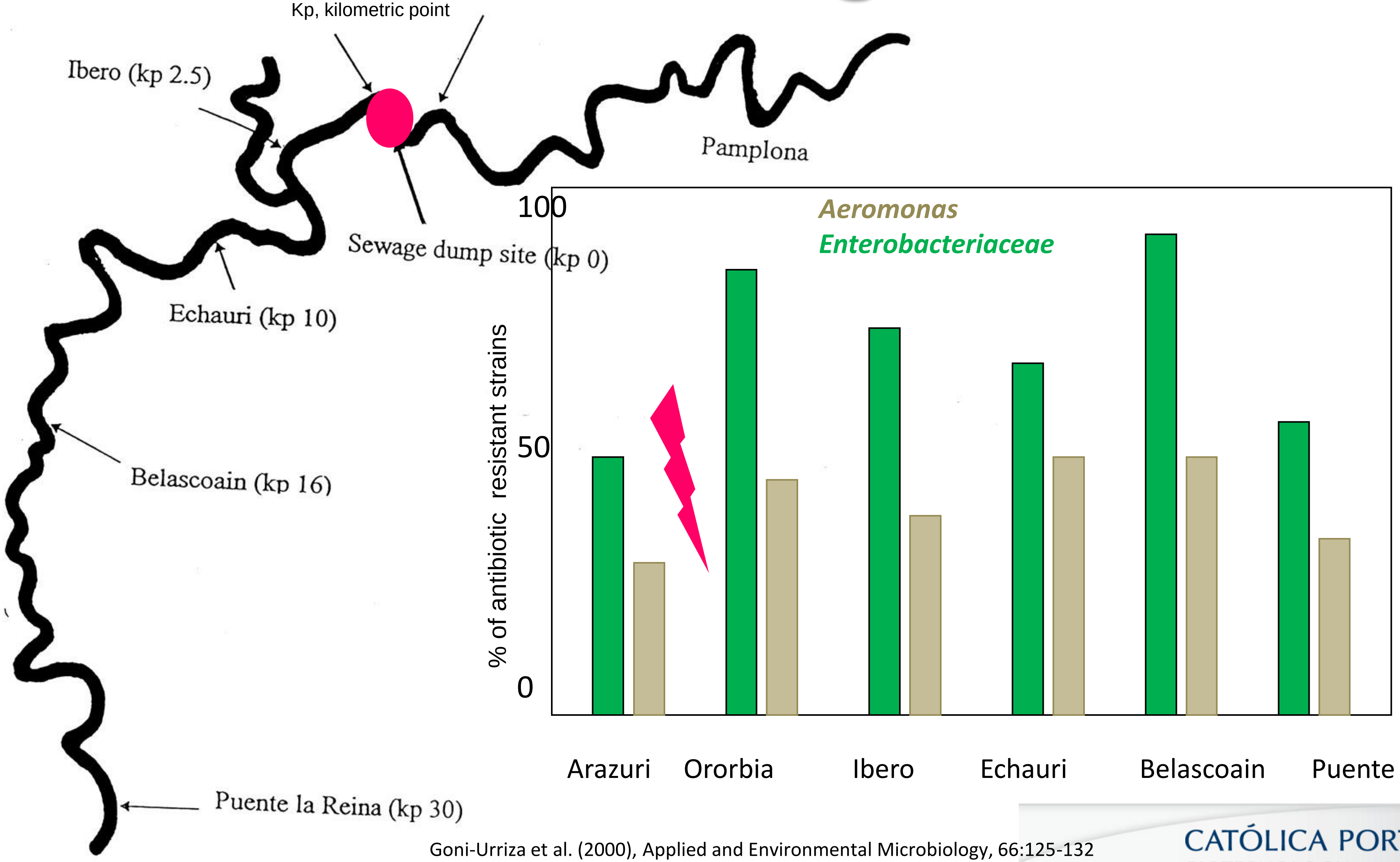


McLellan et al., Environmental Microbiology (2010) **12**(2), 378–392

Examples of antibiotic resistance genes detected in wastewater of UWWTP

Class	Mechanism (Type)[a]	Examples of genes[b]	Approach[c]	Reference[d]
Aminoglycosides	Modification by adenylylation (DM)	aad(A1, A2, A13, B)	CD	4; 8
	Modification by phosphorylation (DM)	aph(A, A-3, A-6, 2); str(A, B)	CI	11
		strB	CD	9
Beta-lactams	Beta-lactam ring cleavage (beta-lactamase production) (DM)	Class A: CTX, GES, NPS,PER, SHV, TEM,TLA, VEB	CI	3; 6; 11; 12
		Class B: IMP, VIM	CI	11
		Class C: ampC, CMY	CI	11
		Class D: OXA	CI	11
		Class D: OXA	CD	8
	Penicillin binding protein (TP)	mecA	CI	2; 3; 10
Glycopeptides	Modified peptodoglycan pentapeptide (TM)	vanA	CD	1
			CI	10
Macrolides	Macrolide-efflux protein (DE)	mel	CI	11
	Erythromycin inactivation (DM)	ereA2	CI	11
	Modification by 23S rRNA methylation (TP)	ermB	CD	1
		erm(B, F)	CI	11
	Macrolide phosphotransferase (DM)	mph(A, B)	CI	11
Quinolones	Modification by acetylation (DM)	aacA6-ib-cr	CD	5
	DNA gyrase protection (TP)	qnr(A3, B1, B2, B4, B5, S2)	CI	11
		qnrVC; qnrS	CD	5; 12
Sulfonamides	Modified dihydropteroate synthase (TM)	sul2	CD	9
		sul(1, 2, 3)	CI	11
Tetracyclines	Other	tetU	CI	11
	Tetracycline efflux pump (DE)	tetA	CD	9
		tet(A, B, D, G, H, Y, 31, 35, 36, 39)	CI	2; 7; 11
	Ribosomal protection protein (TP)	tetM	CD	1
		tet(M, S)	CI	7; 11
	Tetracycline modification (DM)	tetX	CI	7; 11
Trimethoprim	Dihydrofolate reductase (DM)	dfr(A1, A12, 18)	CD	4; 8; 9
		dfr (II, V, VII, XII, 13, 16, 17, A19, B2, D); dhfr (I, VIII, XV)	CI	11
Multidrug	Multidrug resistance efflux pump (DE)	acr(B, D), mex(B, D, F, I, Y)	CI	11

Effect of UWWTP effluent discharge



Goni-Urriza et al. (2000), Applied and Environmental Microbiology, 66:125-132

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Selectors for antibiotic resistance genes in a river

Antibiotic resistance gene abundances associated with waste discharges to the Almendares River near Havana, Cuba

TABLE 3. Antibiotic Resistance Genes (ARG) Correlated with Sediment Heavy−Metal and Water−Column Antibiotic Levels[a]

parameter	number of correlated ARG	specific ARG significantly correlated with indicated parameter
Cu	7	*tet*(M), *tet*(O), *tet*(Q), *tet*(W), *erm*(B), *erm*(E), bla_{OXA}
Cd	0	–
Cr	0	–
Pb	2	*tet*(M), bla_{OXA}
Co	2	*tet*(Q), *erm*(B)
Zn	3	*tet*(M), *tet*(Q), *tet*(W)
tetracyclines	2	*tet*(M), bla_{TEM}
ampicillin	5	*tet*(M), *tet*(O), *tet*(Q), *tet*(W), bla_{OXA}
benzyl−penicillin	0	–

[a] $\alpha = 0.05$ significance level.

Graham et al. 2011. Environmental Science & Technology 45(2), 418-24.

FAQS *vs*. NYAQS

FAQS
(Frequently Asked Questions)

Which bacteria?
Which genes?
Which habitats?

NYAQS
(not yet answered questions)

Clues about the unkown resistome?
How important is the environment?
Factors triggering gene transfer?
What makes a resistance gene a case of success?

In summary…

The question is not where we can find antibiotic resistant bacteria, but which are "relevant resistance types" and the conditions which favour their selective development and/or horizontal gene transfer

Partnership

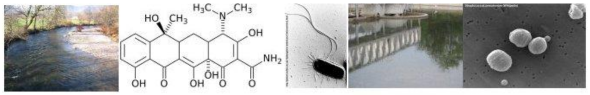
CBQF
Centro de Biotecnologia e Química Fina
LABORATÓRIO ASSOCIADO DO ESTADO

lepae
Laboratory for Process, Environmental and Energy Engineering

AGÊNCIA PORTUGUESA DO AMBIENTE
Ministério do Ambiente e do Ordenamento do Território

cost
EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY

DARE



Detecting evolutionary hot spots of Antibiotic Resistance in Europe

People

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Vânia Figueira
Ana Rita Varela
Carlos Narciso da Rocha
Olga Nunes
Mónica Vieira
Cátia Faria
Maria Fernanda Falcone-Dias
Paula Viana
Sandra André
Edward Moore

Samples

A special thanks for sample suppliers

WWTP/DWTP/Hospital

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