Occurrence of β-lactamases, namely GES-5 carbapenemase, among Gram-negative isolates from wastewater samples in Northern Portugal

Caniça, M.[1], Manageiro, V.[1], Ferreira, E.[1], Figueira, V.[2], and Manaia, C. M.[2]

[1] National Reference Laboratory of Antimicrobial Resistances, National Institute of Health Dr. Ricardo Jorge, Lisbon, Portugal

[2] CBQF/Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Porto, Portugal

Abs1943

Introduction & Objectives

Antimicrobial resistant pathogens are profoundly relevant to human health and many were the studies that focused on their spread.

The aquatic environment may be considered a reservoir for dissemination of antibiotic resistance determinants. Indeed, resistant bacteria have been isolated frequently from bacterial communities residing in lakes, rivers, or wastewater treatment plants (Lupo *et al*., 2012). However, natural and human associated environmental reservoirs of antibiotic resistance are yet poorly understood.

The main goal of this study was to evaluate some antibiotic resistance mechanisms in Gram-negative bacteria isolates from surface and raw and treated waste water environments.

Materials & Methods

Bacterial Strain. Forty-eight Gram-negative isolates (Figure 1) were collected in water samples from different aquatic environments within an urban water cycle (Figure 2) in the region of Northern Portugal , as previously described (Figueira *et al,* 2011 and 2012).

Antimicrobial susceptibility tests. Antimicrobial susceptibility testing was performed by standard disk diffusion method, according to French Society of Microbiology (SFM) guidelines, by using 32 commercial disks (Bio-Rad), after culture in a simple agar medium.

Characterization of antibiotic resistance genes. PCR and sequencing were used to screen and identify *bla* genes (bla_{TEM}, bla_{SHV}, bla_{OXA}, bla_{CTX-M} and plasmid-mediated *ampC*), as well as PMQR genes (*qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *qepA* and *aac(6')Ib-cr*), using previously described primers (Manageiro *et al*, 2012). All isolates were also screened for the presence of class 1 integrons.

PCR-based replicon typing (PBRT). PBRT was used to type the resistance plasmids of the bla_{GES-5}-producing isolate. The major incompatibility (Inc) groups, specifically FIA, FIB, FIC, HI1, HI2, I1-Iγ, L/M, N, P, W, T, A/C, K, B/O, X, Y, F, and FIIA were detected as previously described (Carattoli *et al*, 2005).

Molecular epidemiology. GES-5 *K. pneumoniae*-producing isolate was studied by multilocus sequence typing (MLST) according to the Institut Pasteur scheme for *K. pneumoniae* (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html.

Conclusions

- This study provides the first description of a class A carbapenemase-producing *Enterobacteriaceae* recovered in an environmental setting in Portugal and worldwide, in addition to several other β-lactam resistance mechanisms (through 31 other β-lactamases-producing strains, from 3 families: AmpC, TEM and SHV). The study highlights the need of surveillance of these antibiotic resistance mechanisms in environmental backgrounds, since it represents a liable reservoir of potential pathogenic resistant bacteria.

References

Carattoli A, et al. J Microbiol Methods 2005; 63:219-228.

Figueira V et al. 2011. Sci Total Environ, 409:1017-23.

Figueira V et al. 2012. J Water Health 10:1-10.

Girlich D, et al. Appl Environ Microbiol 2012; 78:1292-1295

Lupo A, et al. Front Microbiol 2012; 3:18

Manageiro V, et al. Diagn Microbiol Infect Dis 2012; 72:272-277.

Acknowledgments

V. Manageiro was supported by grant SFRH/BPD/77486/2011 from Fundação para a Ciência e a Tecnologia (FCT), Lisbon, Portugal. This study was supported financially by the PTDC/AAC/AMB/113840/2009 grant from the Fundação para a Ciência e a Tecnologia. We thank the team of curators of the Institut Pasteur MLST system (Paris, France) for importing novel alleles, profiles and/or isolates at http://www.pasteur.fr/mlst.

Results & Discussion

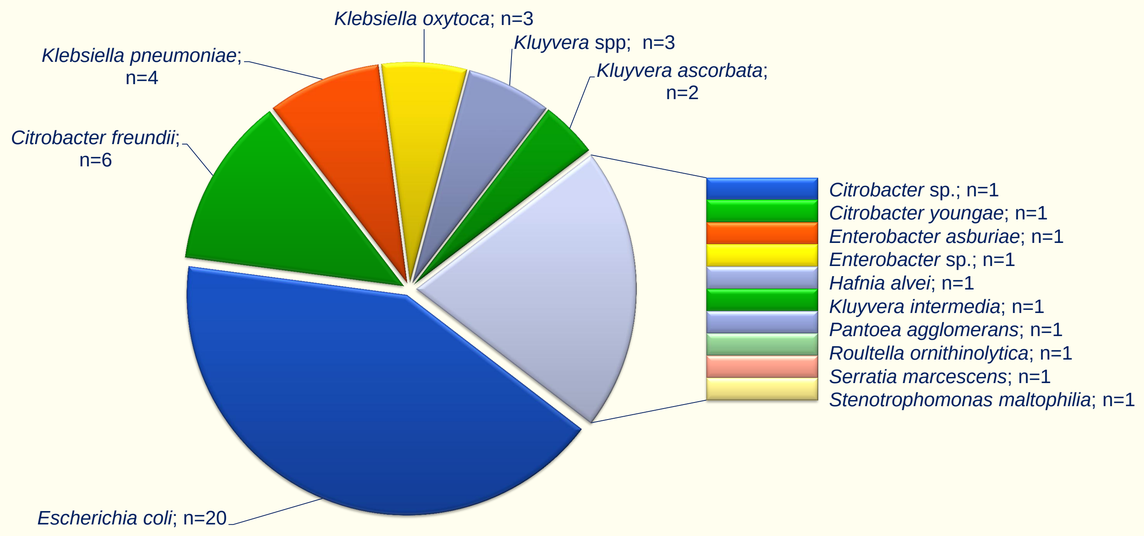


Figure 1: Gram-negative isolates collected from waste water samples

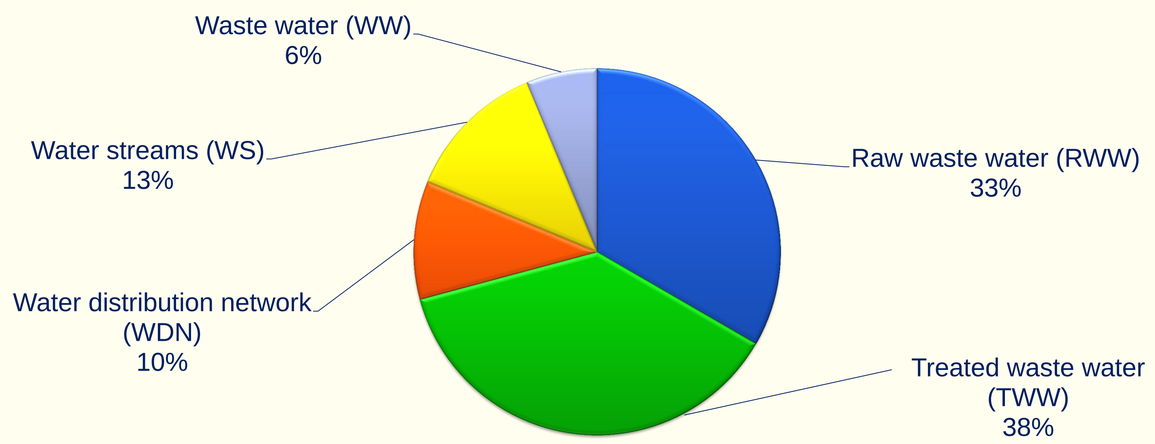
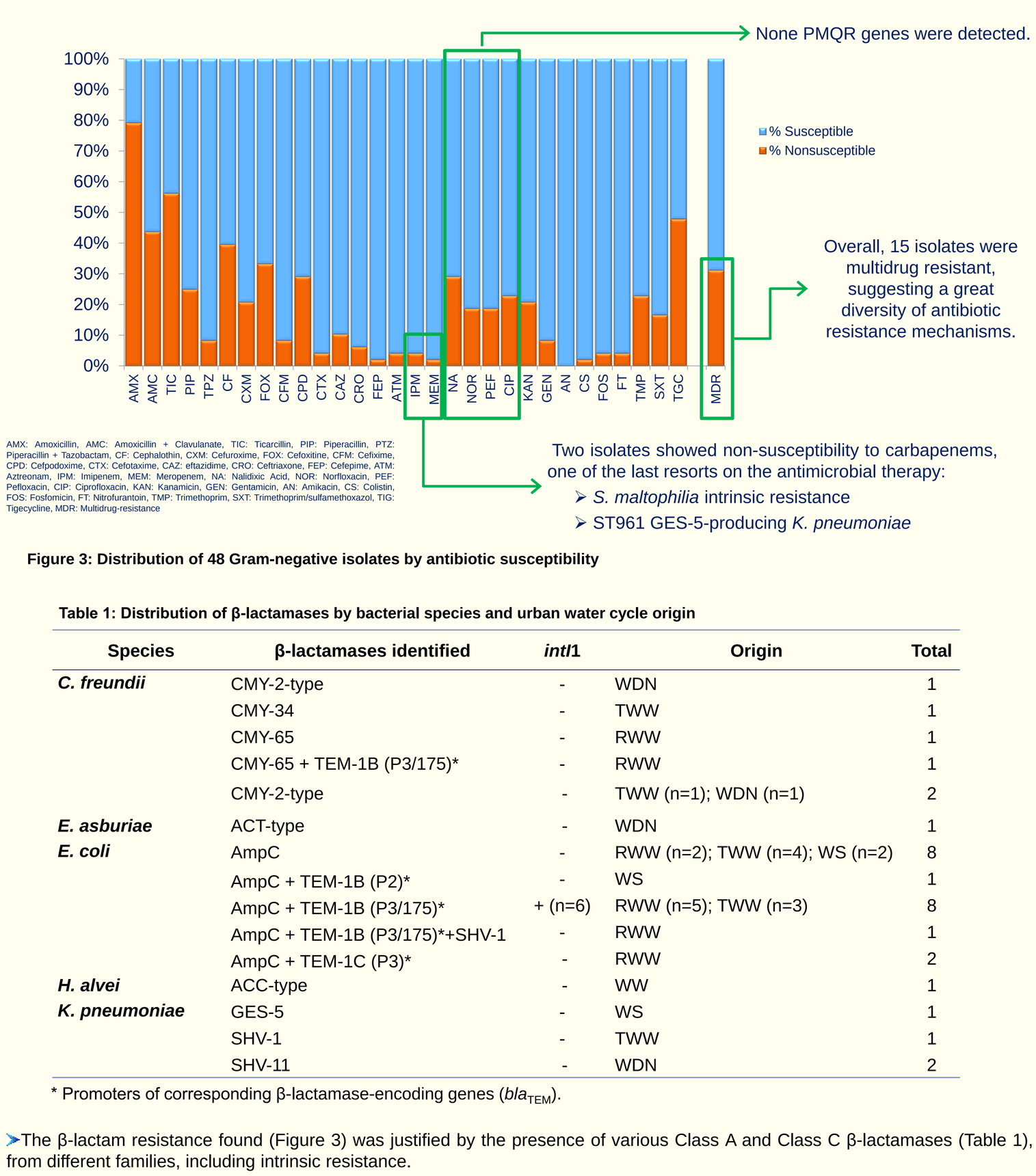


Figure 2: Frequency of isolates collected from the different aquatic environments within an urban water cycle



AMX: Amoxicillin, AMC: Amoxicillin + Clavulanate, TIC: Ticarcillin, PIP: Piperacillin, PTZ: Piperacillin + Tazobactam, CF: Cephalothin, CXM: Cefuroxime, FOX: Cefoxitine, CFM: Cefixime, CPD: Cefpodoxime, CTX: Cefotaxime, CAZ: eftazidime, CRO: Ceftriaxone, FEP: Cefepime, ATM: Aztreonam, IPM: Imipenem, MEM: Meropenem, NA: Nalidixic Acid, NOR: Norfloxacin, PEF: Pefloxacin, CIP: Ciprofloxacin, KAN: Kanamicin, GEN: Gentamicin, AN: Amikacin, CS: Colistin, FOS: Fosfomicin, FT: Nitrofurantoin, TMP: Trimethoprim, SXT: Trimethoprim/sulfamethoxazol, TIG: Tigecycline, MDR: Multidrug-resistance

Figure 3: Distribution of 48 Gram-negative isolates by antibiotic susceptibility

Table 1: Distribution of β-lactamases by bacterial species and urban water cycle origin

Species	β-lactamases identified	*intI*1	Origin	Total
C. freundii	CMY-2-type	-	WDN	1
	CMY-34	-	TWW	1
	CMY-65	-	RWW	1
	CMY-65 + TEM-1B (P3/175)*	-	RWW	1
	CMY-2-type	-	TWW (n=1); WDN (n=1)	2
E. asburiae	ACT-type	-	WDN	1
E. coli	AmpC	-	RWW (n=2); TWW (n=4); WS (n=2)	8
	AmpC + TEM-1B (P2)*	-	WS	1
	AmpC + TEM-1B (P3/175)*	+ (n=6)	RWW (n=5); TWW (n=3)	8
	AmpC + TEM-1B (P3/175)*+SHV-1	-	RWW	1
	AmpC + TEM-1C (P3)*	-	RWW	2
H. alvei	ACC-type	-	WW	1
K. pneumoniae	GES-5	-	WS	1
	SHV-1	-	TWW	1
	SHV-11	-	WDN	2

* Promoters of corresponding β-lactamase-encoding genes (bla_{TEM}).

- The β-lactam resistance found (Figure 3) was justified by the presence of various Class A and Class C β-lactamases (Table 1), from different families, including intrinsic resistance.

- bla_{GES-5} gene was identified in a ST961 (18-22-18-90-142-13-179) *K. pneumoniae* isolate. PCR-based replicon typing indicated the presence of a non-typable plasmid. A recent work had described this gene in a *Pseudomonas knackmussii* B13 isolate recovered from an activated sludge bacterial community of a municipal wastewater treatment plant in Germany (Girlich *et al*, 2012).