

Introduction

Antimicrobial resistant pathogens are profoundly relevant to human health and many were the studies that focused on their spread. Water has been recognized as a reservoir for antibiotic resistance genes (ARG), where the presence of mobile genetic elements, including plasmids, favors their dissemination (Allen *et al.*, 2010; Stalder *et al.*, 2012). 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 the presence of ARGs, related with β -lactam and quinolone resistance, in Gram-negative bacteria isolates from surface and raw and treated waste water environments.

Materials and Methods

Bacterial Strain. Fifty-six Gram-negative isolates (Figure 1) were collected in water samples from different aquatic environments within an urban water cycle in the region of Northern Portugal, as previously described (Figueira *et al.*, 2011a, 2011b and 2012; Narciso-da-Rocha *et al.*, 2012; Vaz-Moreira *et al.*, 2011).

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. The 4 isolates identified as members of the family were studied for their antibiotic resistance phenotypes, by using the ATB PSE5 panel (bioMérieux), according to the manufacturer's instructions.

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-Iy, 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>).

Discussion and Conclusions

> The β -lactam resistance found (Figure 2) 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).

> 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 38 other β -lactamases-producing strains, from different types: AmpC, TEM, SHV, carbapenemases). 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

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

Figure 1
Gram-negative isolates collected from waste water samples

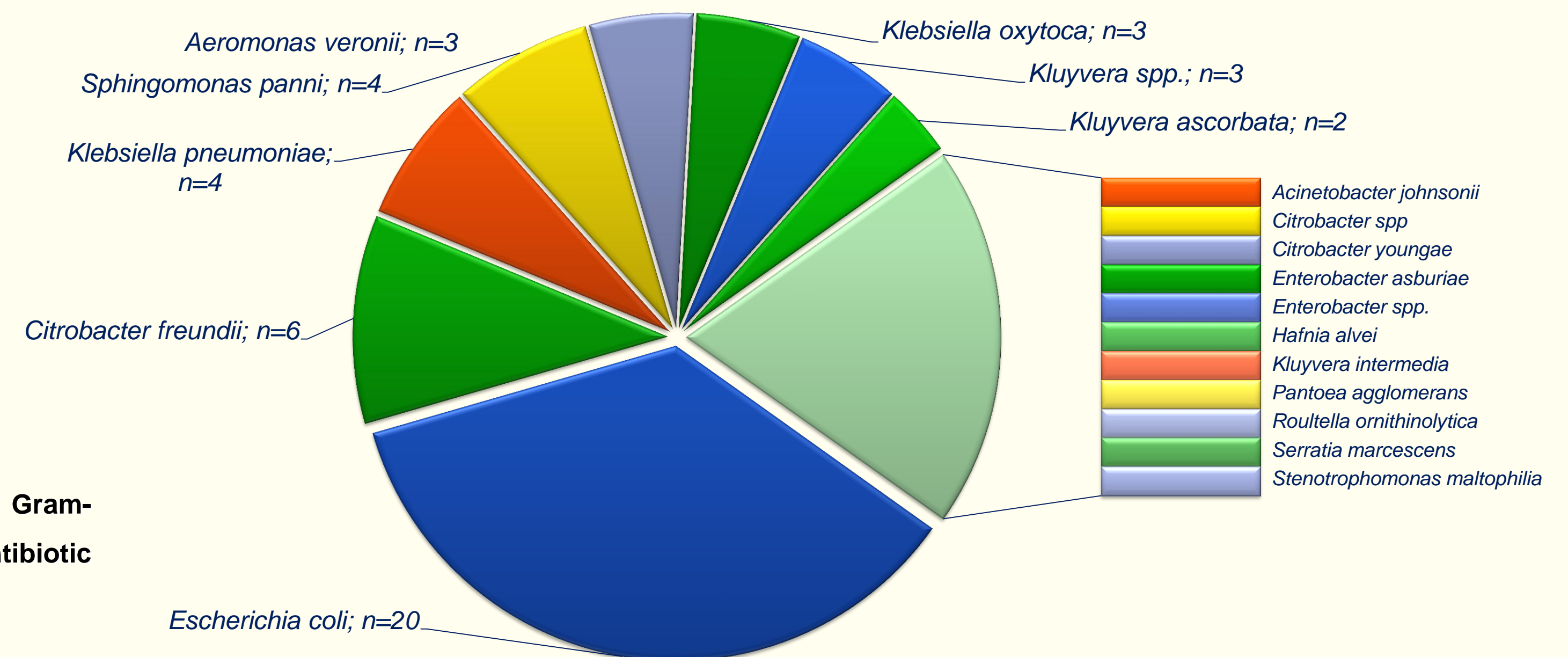
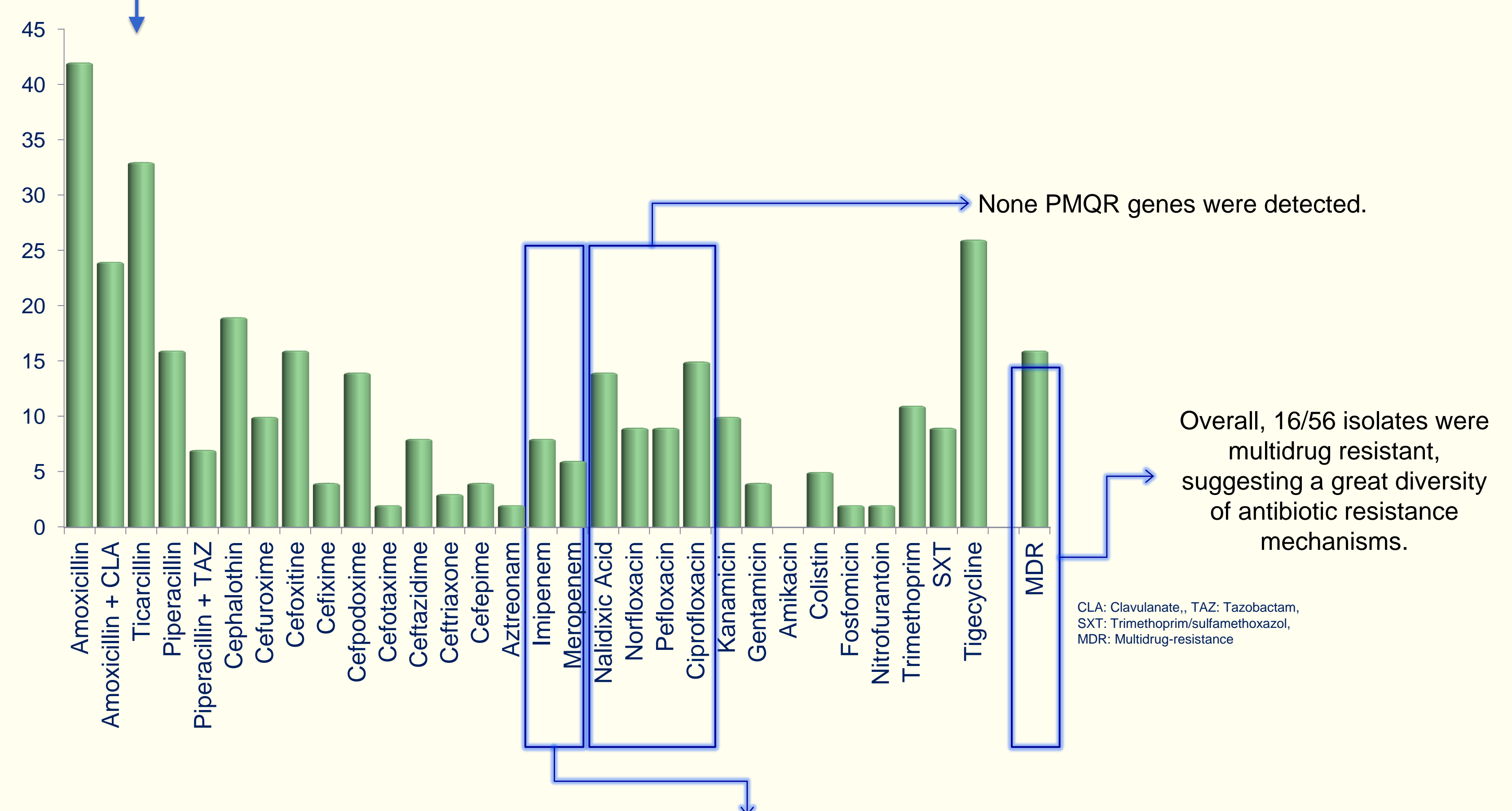


Figure 2: Distribution of 48 Gram-negative isolates by antibiotic susceptibility



Ten isolates showed non-susceptibility to carbapenems, one of the last resorts on the antimicrobial therapy:

- > 1 *A. johnsonii*, 3 *A. veronii*, and 1 *S. maltophilia* intrinsic resistance;
- > ST961 GES-5-producing *K. pneumoniae*;
- > No carbapenemases were found in 4 *S. panni* isolates, although this study reveals the first description of a β -lactamase (SHV-type) within this bacterial specie.

Table 1: Distribution of β -lactamases by 38 bacterial species and urban water cycle origin

Species	Origin ^a	β -lactamases identified	<i>int1</i>
<i>A. johnsonii</i> (n=1)	TW	OXA-211	-
<i>A. veronii</i> (n=3)	RWW (n=2); AO (n=1)	ImiS	-
<i>C. freundii</i> (n=5)	WDN	CMY-90 ^b	-
	TWW	CMY-34	-
	RWW	CMY-89 ^b	-
	RWW	CMY-65 ^b + TEM-1B (P3/175) ^c	-
	TWW	CMY-2	-
	TWW	CMY-2	-
<i>E. asburiae</i> (n=1)	WDN	ACT-13 ^b	-
<i>E. coli</i> (n=20)	RWW (n=2); TWW (n=4); WS (n=2)	AmpC	-
	WS	AmpC + TEM-1b (P2) ^c	-
	RWW (n=5); TWW (n=3)	AmpC + TEM-1b (P3) ^c	+ (n=6)
	RWW	AmpC + TEM-1b (P3) ^c + SHV-1	-
	RWW (n=2)	AmpC + TEM-1b (P3) ^c	-
<i>H. alvei</i> (n=1)	WW	ACC-5 ^b	-
<i>K. pneumoniae</i> (n=4)	WS	GES-5	-
	TWW	SHV-1	-
	WDN (n=2)	SHV-11	-
<i>S. panni</i> (n=2)	TW	SHV-type	-
<i>S. maltophilia</i> (n=1)	RWW	L1	-

^a AO, after ozonation; RWW, raw wastewater; TP, tap water; TWW, treated wastewater; WDN, water distribution network; WS, water streams; and WW, wastewater

^b β -lactamases here firstly described: *bla*_{CMY-65} (JF780936), *bla*_{CMY-89} (HE819403), *bla*_{CMY-90} (HE819404), *bla*_{ACT-13} (HE819402) and *bla*_{ACC-5} (HE819401).

^c Promoters of corresponding TEM-encoding gene.

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