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SHORT RESEARCH AND DISCUSSION ARTICLE

Genetic background of novel sequence types of CTX-M-8- and CTX-M-15-producing *Escherichia coli* and *Klebsiella pneumoniae* from public wastewater treatment plants in São Paulo, Brazil

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Abstract The release of extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* to the environment is a public health issue worldwide. The aim of this study was to investigate the genetic background of genes encoding ESBLs in wastewater treatment plants (WWTPs) in São Paulo, southeastern Brazil. In 2009, during a local surveillance study, seven ESBL-producing *Enterobacteriaceae* strains were recovered from five WWTPs and screened for ESBL genes and mobile genetic elements. Multilocus sequence typing (MLST) was carried out, and wild plasmids were transformed into electrocompetent *Escherichia coli*. S1-PFGE technique was used to verify the presence of high molecular weight plasmids in wild-type strains and in bla_{ESBL}-containing *E. coli* transformants. Strains harbored $bla_{CTX\text{-}M\text{-}8}$, $bla_{CTX\text{-}M\text{-}15}$, and/or $bla_{SHV\text{-}28}$. Sequencing results showed that $bla_{CTX\text{-}M\text{-}8}$ and $bla_{CTX\text{-}M\text{-}15}$ genes were associated with IS*26*. MLST revealed new sequence types for *E. coli* (ST4401, ST4402, ST4403, and ST4445) and *Klebsiella pneumoniae* (ST1574), except for one *K. pneumoniae* from ST307 and *Enterobacter cloacae* from ST131. PCR and S1-PFGE results showed CTX-M-producing *E. coli* transformants carried heavy plasmids sizing 48.5–209 kb, which belonged to IncI1, IncF, and IncM1 incompatibility groups. This is the first report of CTX-M-8 and SHV-28 enzymes in environmental samples, and the present results demonstrate the plasmid-mediated spread of CTX-M-encoding genes through five WWTPs in São Paulo, Brazil, suggesting WWTPs are hotspots for the transfer of ESBL genes and confirming the urgent need to improve the management of sewage in order to minimize the dissemination of resistance genes to the environment.

Keywords Resistance · Sewage · Brazil · ESBL · CTX-M-8 · CTX-M-15 · SHV-28

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The increased prevalence of extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* in the environment is a serious global health issue. In this regard, anthropogenic activities related to the overuse of antimicrobial agents in medicine (human and veterinary) and agriculture have contributed to the environmental reservoirs of resistant bacteria, which can be directly or indirectly transmitted to humans and other animals (Amos et al. 2014; Reinthaler et al. 2013). Previous studies have shown that intestinal carriage of ESBL-producing *Enterobacteriaceae* is frequent, in both hospital and community settings (Valverde et al. 2008; Lim et al. 2014). ESBL-producing bacteria can be released into the wastewater network and public wastewater treatment plants

(WWTPs) (Bréchet et al. 2014). WWTPs can process sewage from several sources, providing a hotspot for horizontal transfer of ESBL-encoding genes between bacteria from many origins. Thus, ESBL variants found in bacterial populations in wastewaters represent the ESBL dominating in environments in contact with broad-spectrum cephalosporins and other beta-lactams used in human and veterinary medicine. Among all ESBL families, CTX-M enzymes have been the most frequent ESBLs reported worldwide. In Brazil, although the production of CTX-M enzymes has become the most prevalent mechanism of acquired resistance to broad-spectrum cephalosporins in gram-negative bacteria from clinical samples (Fernandes et al. 2009; Tollentino et al. 2011; Carvalho-Assef et al. 2014), poultry (Fernandes et al. 2009; Silva et al. 2013; Ferreira et al. 2014), food-producing and companion animals (Aizawa et al. 2014; Leigue et al. 2015), retail chicken meat (Casella et al. 2015), hospital wastewater (Chagas et al. 2011), and urban rivers (Oliveira et al. 2014), there is no data available regarding the detection of CTX-M-producing bacteria in public WWTPs. So, the aim of this study was to investigate the presence of ESBL-producing bacteria and the genetic background of genes encoding ESBLs in public WWTPs.

From June to December of 2009, ten sludge and ten sewage samples from five different public WWTPs, from São Paulo, Brazil, were screened for the presence of ampicillin-resistant *Enterobacteriaceae*, using LB broth and MacConkey agar containing 100 μg/mL of ampicillin. Two hundred strains were recovered, from which seven (3.5 %) were ESBL-producing *Enterobacteriaceae*, detected through the double-disk synergy method (CLSI 2012), using LB agar amended with 250 μg/mL of cloxacillin for natural AmpC-producing bacteria (Picão et al. 2009). No carbapenemase-producing bacteria were detected, and MIC measures for nine beta-lactam compounds were determined using Etest® strips (Biomérieux, France). ESBL-encoding genes, class 1 integrons, and insertion sequences (IS*26*, IS*CR1*, and IS*Ecp1*) were screened by PCR and direct DNA sequencing (Table 1). Mapping of ESBL genetic environment was assessed through PCR reactions combining bla_{ESBL} forward and reverse primers with forward and reverse primers targeting the mobile elements. Amplification products were purified and directly sequenced. Transformation experiments, using One Shot® TOP 10 Electrocomp™ *Escherichia coli* (Invitrogen, CA, USA) as recipients, were carried out, and transformed cells were selected in Luria Bertani agar containing cefotaxime 4 μg/mL. PCR and sequencing (Table 1) were carried out to confirm the presence of bla_{ESBL} genes and mobile elements in *E. coli* transformants. PCR-based replicon typing (PBRT) was carried out (Carattoli et al. 2005, 2015), and plasmids were extracted and characterized by S1-PFGE as previously described (Dropa et al. 2015). Finally, multilocus sequence typing (MLST) was carried out to characterize *E. coli*, *Klebsiella pneumoniae*, and *Enterobacter cloacae* sequence types (STs) (http://mlst.

Table 1 Primers used in this study for PCR and sequencing

Primer	5′-3′ Sequence	Target	Product (bp)	Reference
TEM-P F	TAAAATTCTTGAAGACGAA	$bla_{\text{TEM-group}}$	1066	This study
TEM R	CCAAWGCTTAATCAGTGAG			
SHV-P F	GTGCTTAGGCAGGGCTAGAT	$bla_{\text{SHV-group}}$	1017	This study
SHV R	TTAGCGTTGCCAGTGYTCGA			
CTX-M-1 F	AAATCACTGCGYCAGTTCA	$bla_{\text{CTX-M-1-group}}$	854	Dropa et al. 2015
CTX-M-1 R	GGTGACGATTTTAGCCGCCG			
CTX-M-2 F	GACTCAGAGCATTCGCCGC	$bla_{\text{CTX-M-2-group}}$	870	Dropa et al. 2015
CTX-M-2 R	TCAGAAACCGYGGGTTACGA			
CTX-M-8 F	GATGAGACATCGCGTTAAG	$bla_{\text{CTX-M-8-group}}$	861	Dropa et al. 2015
CTX-M-8 R	GGTGACGATTTTCGCGGCA			
CTX-M-9 F	TGACAAAGAGARTGCAACGG	$bla_{\text{CTX-M-9-group}}$	857	Dropa et al. 2015
CTX-M-9 R	CGATGATTCTCGCCGCTGAA			
CTX-M-25 F	ATGAGAAAAAGCGTAAGGCGGG	$bla_{\text{CTX-M-25-group}}$ and other $bla_{\text{CTX-M}}$ genes	865	Dropa et al. 2015
CTX-M-25 R	CCGTCGGTGACWATTCTG			
IntI 1 F	ACATGTGATGGCGACGCACGA	*intI 1*	569	Pan et al. 2006
IntI 1 R	ATTTCTGTCCTGGCTGGCGA	Class 1 integrase gene		
IS*Ecp1 F*	GCAGGTCTTTTTCTGCTCC	IS*Ecp1 tnpA* gene	535	Park et al. 2009
IS*Ecp1 R*	TTTCCGCAGCACCGTTTGC			
IS*CR1* F	AAGGAACGCCACGGCGAGTCAA	IS*CR1 tnpA* gene	1200	Park et al. 2009
IS*CR1* R	TGCAAAGACGCCGTGGAAGC			
IS*26* F	CAGCGTGACATCATTCTGTG	IS*26 tnpA* gene	662	This study
IS*26* R	TCTGCTTACCAGGCGCATTT			

Table 2 Characteristics and genetic background of CTX-M-8- and CTX-M-15-producing *Enterobacteriaceae* in public wastewater treatment plants in southeastern Brazil

Strain	WWTP location (latitude/longitude)	Sample/date (m/y)	Antimicrobial susceptibility MIC (μg/mL)									bla_{ESBL} genes	Plasmid analysis[a]		MLST
			FOX	CTT	CAZ	CTX	ATM	FEP	AMC	MEM	ERT		mol wt (kb)	Inc group	
Kp 1271	WWTP 1 S 23°36′ 44.6 W 046° 34′ 52.3″	Sewage water/06/09	3	0.25	64	>256	48	48	4	0.064	0.094	CTX-M-15, SHV-28	130.5, **209**	Non-typeable	ST307
Ec 1314	WWTP 1 S 23°36′ 44.6 W 046 °34′ 52.3″	Sewage water/08/09	6	0.50	128	>256	256	48	8	0.023	0.023	CTX-M-15	**112**	**FIA**, **FIB**	ST4401[b]
Ec 1312	WWTP 1 S 23°36′ 44.6 W 046° 34′ 52.3″	Sewage water/08/09	4	0.25	2	12	3	8	6	0.047	0.016	CTX-M-8	**48.5**	**I1**	ST4445[b]
Ec 1333	WWTP 2 S 23°30′40.7″ W 46° 50′ 54.7″	Sewage water/08/09	4	0.25	1	12	3	96	6	0.047	0.016	CTX-M-8	**82**	**I1**	ST4402[b]
K p 1356	WWTP 3 S 23° 26′ 21.0″ W 046° 52′ 10.5″	Sewage water/09/09	4	0.19	8	128	12	96	8	0.094	0.064	CTX-M-8	**63.5**, 160.5	**M1**	ST1574[b]
Ec 1373	WWTP 4 S 22° 42′ 48.1″ W 047° 20′ 12.4″	Sludge/10/09	4	0.38	1	12	3	96	8	0.047	0.016	CTX-M-8	97, **112**	**I1/F**	ST4403[b]
Ecl 1471	WWTP 5 S 23° 00′ 23.6″ W 046° 59′ 07.8″	Sludge/12/09	>256	96	3	48	16	>256	192	0.094	0.75	CTX-M-8	**63.5**, 145.5	**M1**	ST131

Kp Klebsiella pneumoniae, *Ec Escherichia coli*, *Ecl Enterobacter cloacae*, *WWTP* public wastewater treatment plant, *FOX* cefoxitin, *CTT* cefotetan, *CAZ* ceftazidime, *CTX* cefotaxime, *ATM* aztreonam, *FEP* cefepime, *AMC* amoxicillin/clavulanic acid, *MEM* meropenem, *ERT* ertapenem

[a] Size and Inc group of the transferable plasmid carrying $bla_{CTX\text{-}M\text{-}15}$- or $bla_{CTX\text{-}M\text{-}8}$-type genes were set in bold

[b] Novel sequence types (STs) found in this study

warwick.ac.uk/mlst/dbs/Ecoli; http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html; http://pubmlst.org/ecloacae/).

In Table 2 are the quoted MIC values for β-lactams and ESBL genotypes. According to sequencing results, three *E. coli*, one *K. pneumoniae*, and one *Enterobacter cloacae*, each one from a different WWTP, carried the $bla_{\text{CTX-M-8}}$ gene while one *E. coli* and one *K. pneumoniae*, from the same WWTP, harbored the $bla_{\text{CTX-M-15}}$ gene. ESBL-encoding $bla_{\text{SHV-28}}$ gene was also identified in the *K. pneumoniae* strain harboring the $bla_{\text{CTX-M-15}}$ ESBL gene. All strains carried class 1 integrase genes and insertion sequences IS*26* and IS*Ecp1*. Mapping of genetic environment revealed that ESBL genes were not associated with class 1 integrons, and that $bla_{\text{CTX-M-8}}$ and $bla_{\text{CTX-M-15}}$ genes were actually associated with IS*26*. For the IS*26*-$bla_{\text{CTX-M-8}}$ array, the 5′-3′ $bla_{\text{CTX-M-8}}$ was flanked upstream by a 3′-5′ IS*26* truncating a 3′-5′ IS*10*, whereas for the IS*26*-$bla_{\text{CTX-M-15}}$, the 5′-3′ $bla_{\text{CTX-M-15}}$ was flanked upstream by a 3′-5′ IS*26* interrupting a 5′-3′ IS*Ecp1* (Fig. 1). $bla_{\text{SHV-28}}$ was not associated with neither class 1 integrons nor any of the studied insertion sequences.

MLST results revealed that *E. coli* strains belonged to new STs. Regarding *K. pneumoniae*, while the strain co-producing CTX-M-15 and SHV-28 belonged to the ST307, the CTX-M-8-producing strain belonged to the new ST1574 (Table 2). MLST also revealed that *E. cloacae* FSP1471/09 belonged to ST131.

S1-PFGE results showed that all wild-type strains possessed one or two high molecular weight plasmids, ranging from 48.5 to 209 kb (Table 2), which were successfully electroporated into *E. coli* Top 10 recipient strains. Each electrocompetent *E. coli* received only one of the high molecular weight plasmids, harboring $bla_{\text{CTX-M}}$ genes and their respective genetic platforms. In *K. pneumoniae*, the $bla_{\text{SHV-28}}$ gene was not co-transferred with $bla_{\text{CTX-M-15}}$, suggesting chromosomal location. Otherwise, *E. coli* strains harbored IncI1 plasmids carrying $bla_{\text{CTX-M-8}}$, whereas the IncM1 backbone was detected in CTX-M-8-producing *K. pneumoniae* and *E. cloacae*. On the other hand, CTX-M-15-producing *E. coli* carried IncFIA and IncFIB, and the CTX-M-15-producing *K. pneumoniae* strain was not typeable by PBRT.

This study shows the presence of CTX-M-type extended-spectrum β-lactamases among members of the *Enterobacteriaceae* family recovered from public WWTPs in Brazil. The genetic background of $bla_{\text{CTX-M}}$ genes was associated with IS*26* insertion sequences, harbored by high molecular weight plasmids. MLST analysis revealed new STs for CTX-M-15-producing *E. coli*, different from STs usually identified in clinical settings. So, the spread of this gene through sewage could be mediated by transferable IncF-type plasmids, which could be part of CTX-M-15 route from hospitals to the environment. An elegant comparison of resistance patterns in clinical- and sewage sludge-originated *E. coli* (Reinthaler et al. 2013) showed that resistance patterns found in medical environment are reflected in sludge samples, and also that the spread of cephalosporin resistance to the environment could be more effective than other kinds of resistance, due to strains' fitness and/or changes in their genetic characteristics, like the presence of mobile elements.

Regarding the CTX-M-8 enzyme, this ESBL variant was first described in Brazil and originated a new cluster of CTX-M enzymes (Bonnet et al. 2000). Curiously, CTX-M-8-producing *Enterobacteriaceae* have been sporadically identified in clinical settings (Minarini et al. 2009; García-Fulgueiras et al. 2011; Sennati et al. 2012), whereas it has been prevalent among isolates from food-producing animals and chicken meat (Jouini et al. 2007; Dhanji et al. 2010; Aizawa et al. 2014; Eller et al. 2014; Kawamura et al. 2014; Casella et al. 2015), suggesting that the studied WWTPs include waste from animal sources. Indeed, the presence of CTX-M-8-producing bacteria was confirmed in the collected samples from all five WWTPs, showing new STs for *E. coli* and *K. pneumoniae*.

Besides CTX-M-type ESBLs, this study describes for the first time the detection of an environmental SHV-28-producing *K. pneumoniae*. This ESBL has already been reported in clinical samples from Brazilian studies (Cergole-Novella et al. 2010; Tollentino et al. 2011; Veras et al. 2011), supporting their dissemination from clinical to water environment.

In summary, we hereby report the spread of plasmidial-located IS*26*-associated $bla_{\text{CTX-M}}$ genes through five public WWTPs in Brazil, along with the first description of CTX-M-8 and SHV-28 in environmental samples. Identification of novel STs of CTX-M-type-producing *E. coli*

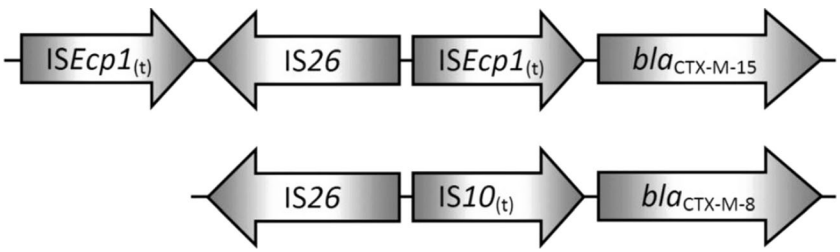

Fig. 1 Genetic context of $bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-8}}$ genes in *Enterobacteriaceae* isolated from public wastewater treatment plants in Brazil. *Top*, the association of $bla_{\text{CTX-M-15}}$ with IS*26* and IS*Ecp1*. *Bottom*, the association of $bla_{\text{CTX-M-8}}$ with IS*26* and IS*10*. *(t)* truncated sequence

and *K. pneumoniae* suggests that WWTPs provide an environmental hotspot for the transfer of bla_{CTX-M} genes.

Nucleotide sequence accession numbers. KT001471 to KT001477.

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Compliance with ethical standards

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Conflict of interest The authors declare that they have no competing interests.

References

Aizawa J, Neuwirt N, Barbato L, Neves PR, Leigue L, Padilha J, Pestana de Castro AF, Gregory L, Lincopan N (2014) Identification of fluoroquinolone-resistant extended-spectrum β-lactamase (CTX-M-8)-producing *Escherichia coli* ST224, ST2179 and ST2308 in buffalo (*Bubalus bubalis*). J Antimicrob Chemother 69:2866–2869

Amos GC, Hawkey PM, Gaze WH, Wellington EM (2014) Waste water effluent contributes to the dissemination of CTX-M-15 in the natural environment. J Antimicrob Chemother 69:1785–1791

Bonnet R, Sampaio JL, Labia R, De Champs C, Sirot D, Chanal C, Sirot J (2000) A novel CTX-M beta-lactamase (CTX-M-8) in cefotaxime-resistant *Enterobacteriaceae* isolated in Brazil. Antimicrob Agents Chemother 44:1936–1942

Bréchet C, Plantin J, Sauget M, Thouverez M, Talon D, Cholley P, Guyeux C, Hocquet D, Bertrand X (2014) Wastewater treatment plants release large amounts of extended-spectrum β-lactamase-producing *Escherichia coli* into the environment. Clin Infect Dis 58:1658–1665

Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ (2005) Identification of plasmids by PCR-based replicon typing. J Microbiol Methods 63:219–228

Carattoli A, Seiffert SN, Schwendener S, Perreten V, Endimiani A (2015) Differentiation of IncL and IncM plasmids associated with the spread of clinically relevant antimicrobial resistance. PLoS One 10:e0123063

Carvalho-Assef AP, Pereira PS, Albano RM, Berião GC, Tavares CP, Chagas TP, Marques EA, Timm LN, Da Silva RC, Falci DR, Asensi MD (2014) Detection of NDM-1-, CTX-M-15-, and qnrB4-producing *Enterobacter hormaechei* isolates in Brazil. Antimicrob Agents Chemother 58:2475–2476

Casella T, Rodríguez MM, Takahashi JT, Ghiglione B, Dropa M, Assunção E, Nogueira ML, Lincopan N, Gutkind G, Nogueira MC (2015) Detection of bla_{CTX-M}-type genes in complex class 1 integrons carried by *Enterobacteriaceae* isolated from retail chicken meat in Brazil. Int J Food Microbiol 197:88–91

Cergole-Novella MC, Guth BE, Castanheira M, Carmo MS, Pignatari AC (2010) First description of $bla_{CTX-M-14}$- and $bla_{CTX-M-15}$-producing *Escherichia coli* isolates in Brazil. Microb Drug Resist 16:177–184

Chagas TP, Seki LM, Cury JC, Oliveira JA, Dávila AM, Silva DM, Asensi MD (2011) Multiresistance, beta-lactamase-encoding genes and bacterial diversity in hospital wastewater in Rio de Janeiro, Brazil. J Appl Microbiol 111:572–581

Clinical and Laboratory Standards Institute (2012) Performance standards for antimicrobial susceptibility testing; twenty-second informational supplement M100-S22, vol 32. Clinical and Laboratory Standards Institute, Wayne

Dhanji H, Murphy NM, Doumith M, Durmus S, Lee SS, Hope R, Woodford N, Livermore DM (2010) Cephalosporin resistance mechanisms in *Escherichia coli* isolated from raw chicken imported into the UK. J Antimicrob Chemother 65:2534–2537

Dropa M, Balsalobre LC, Lincopan N, Matté GR, Matté MH (2015) Complex class 1 integrons harboring CTX-M-2-encoding genes in clinical *Enterobacteriaceae* from a hospital in Brazil. J Infect Dev Ctries 9:890–897

Eller C, Leistner R, Guerra B, Fischer J, Wendt C, Rabsch W, Werner G, Pfeifer Y (2014) Emergence of extended-spectrum β-lactamase (ESBL) CTX-M-8 in Germany. J Antimicrob Chemother 69:562–564

Fernandes SA, Paterson DL, Ghilardi-Rodrigues AC, Adams-Haduch JM, Tavechio AT, Doi Y (2009) CTX-M-2-producing *Salmonella* Typhimurium isolated from pediatric patients and poultry in Brazil. Microb Drug Resist 15:317–321

Ferreira JC, Penha Filho RA, Andrade LN, Berchieri A Jr, Darini AL (2014) Detection of chromosomal $bla_{CTX-M-2}$ in diverse *Escherichia coli* isolates from healthy broiler chickens. Clin Microbiol Infect 20:O623–626

García-Fulgueiras V, Bado I, Mota MI, Robino L, Cordeiro NF, Varela A, Algorta G, Gutkind G, Ayala JA, Vignoli R (2011) Extended-spectrum β-lactamases and plasmid-mediated quinolone resistance in enterobacterial clinical isolates in the paediatric hospital of Uruguay. J Antimicrob Chemother 66:1725–1729

Jouini A, Vinué L, Slama KB, Sáenz Y, Klibi N, Hammami S, Boudabous A, Torres C (2007) Characterization of CTX-M and SHV extended-spectrum beta-lactamases and associated resistance genes in *Escherichia coli* strains of food samples in Tunisia. J Antimicrob Chemother 60:1137–1141

Kawamura K, Goto K, Nakane K, Arakawa Y (2014) Molecular epidemiology of extended-spectrum β-lactamases and *Escherichia coli* isolated from retail foods including chicken meat in Japan. Foodborne Pathog Dis 11:104–110

Leigue L, Warth JF, Melo LC, Silva KC, Moura RA, Barbato L, Silva LC, Santos AC, Silva RM, Lincopan N (2015) MDR ST2179-CTX-M-15 *Escherichia coli* co-producing RmtD and AAC(6′)-Ib-cr in a horse with extraintestinal infection, Brazil. J Antimicrob Chemother 70:1263–1265

Lim CJ, Cheng AC, Kennon J, Spelman D, Hale D, Melican G, Sidjabat HE, Paterson DL, Kong DC, Peleg AY (2014) Prevalence of multidrug-resistant organisms and risk factors for carriage in long-term care facilities: a nested case–control study. J Antimicrob Chemother 69:1972–1980

Minarini LAR, Poirel L, Trevisani NAC, Darini ALC, Nordmann P (2009) Predominance of CTX-M-type extended-spectrum β-lactamase genes among enterobacterial isolates from outpatients in Brazil. Diagn Microbiol Infect Dis 65:202–206

Oliveira S, Moura RA, Silva KC, Pavez M, McCulloch JA, Dropa M, Matté MH, Mamizuka EM, Sato MIZ, Pestana de Castro AF, Lincopan N (2014) Isolation of KPC-2-producing *Klebsiella pneumoniae* strains belonging to the high-risk multiresistant clonal complex 11 (ST437 and ST340) in urban rivers. J Antimicrob Chemother 69:849–852

Pan JC, Ye R, Meng DM, Zhang W, Wang HQ, Liu KZ (2006) Molecular characteristics of class 1 and class 2 integrons and their relationships to antibiotic resistance in clinical isolates of *Shigella sonnei* and *Shigella flexneri*. J Antimicrob Chemother 58:288–296

Park YJ, Kim SY, Yu JK, Kim SI, Uh Y, Hong SG, Jongwook L, Kwak HS (2009) Spread of *Serratia marcescens* coharboring *aac(6′)-Ib-cr*,

bla$_{CTX-M}$, *armA*, and *bla*$_{OXA-1}$ carried by conjugative IncL/M type plasmid in Korean hospitals. Microb Drug Resist 15:97–102

Picão RC, Poirel L, Gales AC, Nordmann P (2009) Diversity of beta-lactamases produced by ceftazidime-resistant *Pseudomonas aeruginosa* isolates causing bloodstream infections in Brazil. Antimicrob Agents Chemother 53:3908–3913

Reinthaler FF, Galler H, Feierl G, Haas D, Leitner E, Mascher F, Melkes A, Posch J, Pertschy B, Winter I, Himmel W, Marth E, Zarfel G (2013) Resistance patterns of *Escherichia coli* isolated from sewage sludge in comparison with those isolated from human patients in 2000 and 2009. J Water Health 11:13–20

Sennati S, Santella G, Di Conza J, Pallecchi L, Pino M, Ghiglione B, Rossolini GM, Radice M, Gutkind G (2012) Changing epidemiology of extended-spectrum β-lactamases in Argentina: emergence of CTX-M-15. Antimicrob Agents Chemother 56:6003–6005

Silva KC, Fontes LC, Moreno AM, Astolfi-Ferreira CS, Ferreira AJ, Lincopan N (2013) Emergence of extended-spectrum-β-lactamase CTX-M-2-producing *Salmonella* enterica serovars Schwarzengrund and Agona in poultry farms. Antimicrob Agents Chemother 57: 3458–3459

Tollentino FM, Polotto M, Nogueira ML, Lincopan N, Neves P, Mamizuka EM, Remeli GA, De Almeida MT, Rúbio FG, Nogueira MC (2011) High prevalence of *bla* CTX-M extended spectrum beta-lactamase genes in *Klebsiella pneumoniae* isolates from a tertiary care hospital: first report of *bla*$_{SHV-12}$, *bla*$_{SHV-31}$, *bla*$_{SHV-38}$, and *bla*$_{CTX-M-15}$ in Brazil. Microb Drug Resist 17:7–16

Valverde A, Grill F, Coque TM, Pintado V, Baquero F, Cantón R, Cobo J (2008) High rate of intestinal colonization with extended-spectrum-beta-lactamase-producing organisms in household contacts of infected community patients. J Clin Microbiol 46:2796–2799

Veras DL, Alves LC, Brayner FA, Guedes DR, Maciel MA, Rocha CR, de Souza Lopes AC (2011) Prevalence of the *bla*$_{SHV}$ gene in *Klebsiella pneumoniae* isolates obtained from hospital and community infections and from the microbiota of healthy individuals in Recife, Brazil. Curr Microbiol 62:1610–1616