bla$_{\text{CTX-M-2}}$ and *bla*$_{\text{CTX-M-28}}$ extended-spectrum β-lactamase genes and class 1 integrons in clinical isolates of *Klebsiella pneumoniae* from Brazil

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Twenty-eight Klebsiella pneumoniae *clinical isolates that exhibited an extended-spectrum cephalosporin-resistance profile from a city in the Northeast of Brazil were analysed by PCR and DNA sequencing in order to determine the occurrence of* bla$_{CTX\text{-}M}$ *genes and class 1 integrons. We determined the occurrence of the* bla$_{CTX\text{-}M\text{-}2}$ *gene in six* K. pneumoniae *isolates and describe the first detection of the* bla$_{CTX\text{-}M\text{-}28}$ *gene in South America. Seven isolates carried class 1 integrons. Partial sequencing analysis of the 5'-3'CS variable region in the class 1 integrons of three isolates revealed the presence of* aadA1, bla$_{OXA\text{-}2}$ *and* dfr22 *gene cassettes.*

Key words: *Klebsiella pneumoniae* - *bla*$_{\text{CTX-M}}$ - integrons - multidrug resistance

Klebsiella pneumoniae is a Gram-negative bacterium that accounts for a significant portion of hospital and community acquired infections worldwide (Souza Lopes et al. 2005, Minarini et al. 2007, Yu et al. 2007). The introduction of oxyimino-cephalosporins into clinical practice for the treatment of resistant gram-negative bacterial infections was soon followed by the emergence of extended-spectrum β-lactamases (ESBLs). The CTX-M-type enzymes, which are non-TEM and non-SHV derivatives, represent a rapidly growing family of ESBLs. The first CTX-M β-lactamase (CTX-M-1/ MEN-1) was characterised in *Escherichia coli* strains isolated from German and Italian patients (Bauernfeind et al. 1990, Barthélémy et al. 1992). In the past 15 years, more than 60 different CTX-M-type β-lactamases have been identified in most parts of the world (www.lahey.org/studies) (Livermore et al. 2007). On the basis of amino acid sequence similarities, CTX-M enzymes have been classified into five distinct phylogenetic groups: CTX-M-1 (> 97% identity), CTX-M-2 (> 94% identity), CTX-M-8 (> 98% identity), CTX-M-9 (> 98% identity) and CTX-M-25 (> 98% identity) (Bonnet 2004).

Members of CTX-M groups evolved by the capture of chromosomal genes from various *Kluyvera* species (Rodriguez et al. 2004). Once mobilised, *bla*$_{\text{CTX-M}}$ genes can be hosted by many mobile elements, most often by large multiresistance plasmids that are responsible for the rapid dissemination of these genes. Insertion sequences (IS), such as IS*Ecp1*, IS*10*, IS*26* and IS*903*, might be involved in the mobilisation of *bla*$_{\text{CTX-M}}$ genes (Bonnet et al. 2000, Saladin et al. 2002). *bla*$_{\text{CTX-M}}$ genes have also been associated with IS*CR1* (IS common region 1, previously also called *orf513*), which is often found downstream of complex class 1 integrons (Partridge & Hall 2003).

CTX-M enzymes are replacing TEM and SHV mutants in many European countries, with *E. coli* joining *K. pneumoniae* as a major host (Livermore et al. 2007). In South America, studies from Argentina have shown that *bla*$_{\text{CTX-M-2}}$ seems to be the most frequent ESBL among Enterobacteriaceae (Quinteros et al. 2003). In the Brazilian Southeast Region, *bla*$_{\text{CTX-M}}$ genes were identified in *E. coli*, *Citrobacter amalonaticus*, *Enterobacter aerogenes* and *Enterobacter cloacae* (Bonnet et al. 2000, 2001, Minarini et al. 2007), but *bla*$_{\text{CTX-M}}$ was only recently detected in *K. pneumoniae* (Do Carmo et al. 2008, Garcia et al. 2008). In this paper we describe the first detection of *bla*$_{\text{CTX-M}}$ genes and class 1 integrons in clinical isolates of *K. pneumoniae* from the Northeast of Brazil.

MATERIALS AND METHODS

Bacterial isolates - Bacterial strains analysed in this study included 28 *K. pneumoniae* clinical isolates that have an ESBL phenotype (resistant isolates to cefotaxime and ceftazidime or aztreonam) (Table I), selected from a collection of 50 resistant isolates from different patients from the city of Recife, state of Pernambuco (PE), from 1998-2008. The isolates were collected from two public hospitals, one private hospital and one private laboratory. All isolates were identified using the API ID 20E (bioMérieux, Marcy l'Etoile, France).

Antibiotic susceptibility - The susceptibility to antimicrobial agents was tested on Mueller-Hinton agar by the disk diffusion method (CLSI 2006). Commercially available disks (Oxoid) loaded with the following antibi-

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TABLE I

Origin, antimicrobial susceptibility and presence of bla_{CTX-M} genes in isolates of *Klebsiella pneumoniae*, resistant or intermediate to cephalosporins, from Recife, Pernambuco, Brazil

Isolates[a]	Date of isolation (month/year)	Specimen/origin	Resistance phenotypes diffusion disk	PCR bla_{CTX-M}
K6R	Mar/98	tracheal aspirate/hospital	AMP, AMO, TET, CTX (Int.)	-
K10R	Mar/98	tracheal aspirate/hospital	AMP, AMO, CLO, TET, EST, CTX (Int.)	-
K13R	April/98	tracheal aspirate/hospital	AMP, AMO, CLO, TET, CTX (Int.)	-
K15R	April/98	blood/hospital	AMP, AMO, AMC, CLO, TET, CTX,	-
K16R	April/98	urine/hospital	AMP, AMO, AMC, CLO, EST, TSU, CTX, CAZ	+
K19R	April/98	urine/hospital	AMP, AMO, CLO, TET, CTX, ATM	-
K20R	April/98	urine/hospital	AMP, AMO, TET, CTX (Int.)	-
K3C	Oct/99	urine/hospital	AMP, AMO, TET, CTX (Int.)	-
K4C	Oct/99	wound/hospital	AMP, AMO, CLO, TET, CAZ	-
K6C	Oct/99	urine/hospital	AMP, AMO, CLO, TET, CTX (Int.)	-
K7C	June/99	urine/hospital	AMP, AMO, CTX (Int.)	-
K10C	Nov/99	urine/hospital	AMP, AMO, AMC, CLO, TET, NAL, CTX (Int.), CAZ (Int.), ATM	-
K12C	Nov/99	urine/hospital	AMP, AMO, CLO, TET, CTX (Int.)	-
K13C	Dec/99	wound/hospital	AMP, AMO, TET, EST, NAL, CTX	-
K14C	Dec/99	wound/hospital	AMP, AMO, CLO, TET, CTX (Int.)	-
K15C	Dec/99	urine/hospital	AMP, AMO, TET, NAL, CTX (Int.)	-
K16C	Dec/99	urine/hospital	AMP, AMO, TET, EST, NAL, CTX (Int.)	-
K18C	Jan/00	urine/hospital	AMP, AMO, CLO, TET, CAZ	-
K2CM	Sep/04	peritoneal fluid/ private laboratory	AMP, AMO, TSU, CAZ, CTX, ATM	-
K3CM	Sep/04	urine/ private laboratory	AMP, AMO, AMC, CLO, NAL, CIP, TSU, CTX, CAZ, ATM	+
K11CM	June/05	urine/ private laboratory	AMP, AMO, AMC, CLO, TET, NAL, CFO, CIP, TSU, CTX, CAZ,ATM	+
K2P	Mar/08	urine/hospital	AMP, AMO, TET, NAL, TSU, CAZ, ATM	-
K6P	Mar/08	blood/hospital	AMP, AMO, AMC, CLO, TSU, CTX, CAZ, ATM	+
K7P	Mar/08	urine/hospital	AMP, AMO, AMC, CLO, TET, NAL, CFO, CIP, TSU, CTX, CAZ, ATM	-
K11P	April/08	urine/hospital	AMP, AMO, AMC, CLO, TET, NAL, CIP, EST, TSU, CTX, CAZ, ATM	+
K17P	May/08	urine/hospital	AMP, AMO, AMC, TET, NAL, CIP, TSU, CTX, CAZ, ATM	+
K18P	May/08	urine/hospital	AMP, AMO, AMC, CLO, TET, NAL, CIP, EST, TSU, CAZ, ATM	+
K21P	May/08	peritoneal fluid/ hospital	AMP, AMO, AMC, TET, NAL, CFO, CIP, TSU, CTX, CAZ, ATM	-

a:*K. pneumoniae* isolates resistant or intermediate to cephalosporin by the disk diffusion method; AMC: amoxicillin-clavulanate; AMO: amoxicillin; AMP: ampicillin; ATM: aztreonam; CAZ: ceftazidime; CFO: cefoxitin; CIP: ciprofloxacin; CLO: chloramphenicol; CTX: cefotaxime; EST: streptomycin; Int.: intermediate; NAL: nalidixic acid; TET: tetracycline; TSU: trimethoprim/sulfamethoxazole.

TABLE II

Primers used in PCR amplification and sequencing to detect bla_{CTX-M}-like genes and class 1 integrons

Primer	Primer sequence (5'-3')	Annealing temperature (°C)	Position	Reference
CTX-MA1	SCSATGTGCAGYACCAGTAA	60	270-289	Saladin et al. (2002)
CTX-MA2	CCGCRATATGRTTGGTGGTG		794-813	
CTX-M13U	GGTTAAAAAATCACTGCGTC	60	65-84	Saladin et al. (2002)
CTX-M13L	TTGGTGACGATTTTAGCCGC		909-928	
CTX-M25U	ATGATGACTCAGAGCATTCG	62	6-25	Saladin et al. (2002)
CTX-M25L	TGGGTTACGATTTTCGCCGC		852-871	
CTX-M8-IF1	ACGAACTGGGTGTGGCGTTG	60	138-156	This study
CTX-M8-IR1	TGTCATCGTGCCATTGACGTG		343-363	
CTX-M9U	ATGGTGACAAAGAGAGTGCA	60	112-131	Saladin et al. (2002)
CTX-M9L	CCCTTCGGCGATGATTCTC		957-975	
CTX-M-G25-F	CCGTCGGTGACAATTCTGGC	60	7-26	This study
CTX-M-G25-R	AGAAAAAGCGTAAGGCGGGC		850-859	
5'-CS-F	GGCATCCAAGCAGCAAG	65	1190-1206	Bissonnette & Roy (1992)
3'-CS-R	AAGCAGACTTGACCTGA		1342-1326	

otics were used: ampicillin, amoxicillin, amoxicillin-clavulanate, chloramphenicol, tetracycline, nalidixic acid, streptomycin, amikacin, trimethoprim/sulfamethoxazole, ciprofloxacin, cefoxitin, cefotaxime, ceftazidime, aztreonam, meropenem and imipenem. Minimum inhibitory concentrations of ceftazidime, cefotaxime and aztreonam (Sigma Aldrich) were determined by a microdilution test in accordance with the criteria of the CLSI (2006).

DNA preparation and identification by PCR of the bla_{CTX-M} genes - Genomic DNA was extracted from direct colony suspensions in 200 µL of distilled water. The suspensions were heated to 100°C for 10 min, centrifuged (5 min/10,000 *g*) and 150 µL of the recovered supernatant was frozen at -20°C until use.

bla_{CTX-M} genes were first investigated by PCR using group-specific primers (Table II). The bla_{CTX-M} gene was investigated in 28 isolates of *K. pneumoniae* that were resistant to either third generation cephalosporin or aztreonam. The amplification reactions were prepared in a total volume of 25 µL containing 1 ng of genomic DNA, 2.0 U of *Taq* DNA polymerase (Promega), 200 µM of deoxynucleoside triphosphates (Invitrogen), 1.5 mM of $MgCl_2$, 1 µM of each primer and 1X reaction buffer (final concentration). The PCR amplifications of the bla_{CTX-M} gene were performed in a thermocycler (MJ Research) as follows: 95ºC for 5 min and 30 cycles of 1 min at 95ºC, 1 min at 60ºC and 1 min at 72ºC. A final extension step of 10 min at 72ºC was performed. Afterwards, more specific primers were used for each group of bla_{CTX-M} genes, as described in Table II, using the same PCR conditions described above.

Detection of class 1 integron - The presence of class 1 integron was investigated using PCR amplification and sequencing of the 5' and 3' CS variable regions in the bla_{CTX-M}-positive isolates. These primers (Table II) amplify the region between the 5'-CS-3'-CS conserved segments, generating products of variable sizes depending on the numbers and lengths of the inserted gene cassettes.

DNA sequencing and analysis of bla_{CTX-M} and class 1 integron genes - All positive amplicons were purified with Wizard SV Gel and PCR Clean-up System (Promega, USA) and sequenced. DNA sequences were determined in both strands with an automated sequencer ABI Prism 3700 (Applied Biosystems), using the same primers as for the PCR (Table II). The sequencing was partial for the integron variable regions. The nucleotide sequences and deduced protein sequences were analysed by the programs BLAST (http://www.ncbi.nlm.nih.gov/) and Clustal W of the European Bioinformatics Institute (http://www.cbi.ac.uk /). The sequences of the $bla_{CTX-M-2}$ and $bla_{CTX-M-28}$ genes and class 1 integron genes (bla_{OXA-2}, *aadA1* and *dfr22)* were deposited in the GenBank Database under the following accession: FJ973565, FJ973566, FJ973567, FJ973568, FJ973570, FJ973571, FJ973572, FJ985262, FJ976897, FJ976898.

RESULTS

Twenty-eight clinical isolates of *K. pneumoniae* from the Brazilian Northeast Region (Recife, PE) that exhibited resistance to extended-spectrum cephalosporins or aztreonam were also resistant to ampicillin and amoxicillin and showed a high rate of resistance to amoxicillin-clavulanate, chloramphenicol, tetracycline and cefotaxime. No isolate exhibited resistance to amikacin, imipenem or meropenem (Table I). Three bla_{CTX-M}-positive isolates were more resistant to cefotaxime than to ceftazidime and aztreonam (Table III).

The results of the PCR with consensus primers for the 28 isolates of *K. pneumoniae* showed that seven were positive for the presence of bla_{CTX-M} genes. The PCR results with more specific primers for each group of the bla_{CTX-M} genes demonstrated that six isolates were positive for the group $bla_{CTX-M-2}$ and one isolate was positive for $bla_{CTX-M-1}$. None of the isolates were positive for the groups $bla_{CTX-M-8}$, $bla_{CTX-M-9}$ or $bla_{CTX-M-25}$. The entire coding sequences of the seven bla_{CTX-M} genes were subsequently sequenced with the specific

TABLE III

Minimum inhibitory concentrations (MICs), bla_{CTX-M} genes and class 1 integron in CTX-M positives *K. pneumoniae* isolates from Recife, Pernambuco, Brazil

Isolates	Specimen/origin	MICs (µg/mL) of			Others drugs to which resistance was shown (diffusion disk)	bla_{CTX-M} gene	Size (bp) of amplicons for class 1 integron (and the gene cassete identified)
		CTX	CAZ	ATM			
K16R	urine/hospital	> 256	32	16	AMP, AMO, AMC, CLO, EST, TSU	$bla_{CTX-M-2}$	2000 (bla_{OXA-2})
K3CM	urine/community	> 256	64	64	AMP, AMO, AMC, CLONAL, CIP, TSU	$bla_{CTX-M-2}$	1800 (ND)
K11CM	urine/community	> 256	64	> 256	AMP, AMO, AMC, CLO, TET, NAL, CFO, CIP, TSU	$bla_{CTX-M-2}$	1800 (ND)
K6P	blood/hospital	64	32	32	AMP, AMO, AMC, CLO, TSU	$bla_{CTX-M-2}$	1800 (ND)
K11P	urine/hospital	64	32	32	AMP, AMO, AMC, CLO, TET, NAL, CIP, EST, TSU	$bla_{CTX-M-28}$	750 (*dfr22*), 2000 (ND)
K17P	urine/hospital	64	32	32	AMP, AMO, AMC, TET, NAL, CIP, TSU	$bla_{CTX-M-2}$	1800 (ND)
K18P	urine/hospital	32	32	32	AMP, AMO, AMC, CLO, TET, NAL, CIP, EST, TSU	$bla_{CTX-M-2}$	1000 (*aadA1*), 1800 (ND)

AMC: amoxicillin-clavulanate; AMO: amoxicillin; AMP: ampicillin; ATM: aztreonam; CAZ: ceftazidime; CFO: cefoxitin; CIP: ciprofloxacin; CLO: chloramphenicol; CTX: cefotaxime; EST: streptomycin; NAL: nalidixic acid; ND: not determined; TET: tetracycline; TSU: trimethoprim/sulfamethoxazole.

primers for groups $bla_{CTX-M-2}$ and $bla_{CTX-M-1}$. The analysis of the nucleotide sequences and deduced protein sequences with BLAST and Clustal W showed that six isolates of *K. pneumoniae* harboured $bla_{CTX-M-2}$ genes and one isolate had $bla_{CTX-M-28}$ (Table III).

The investigation of the class 1 integron in the seven bla_{CTX-M}-positive isolates of *K. pneumoniae* by PCR revealed that they carried the following structure. Two isolates (K11P and K18P) had two intregons each, with sizes ranging from 750-2000 bp, while the other five positive isolates amplified only one class 1 integron with sizes ranging from 1800-2000 bp (Table III). Analysis of the partial sequence of integrons 5'-3' CS variable regions in three isolates revealed that one isolate contained the *aadA1* gene cassette, one isolate had bla_{OXA-2}, which confers resistance to aminoglycosides and β-lactams, respectively and the isolate K11P harboured a gene cassette (*dfr22*) coding for trimethoprim resistance (Table III). It is very likely that the integrons variable regions with a size of 1800 bp and 2000 bp harboured more than one gene cassette.

DISCUSSION

The bla_{CTX-M} genes have been increasingly detected worldwide, but this is the first study on the occurrence of bla_{CTX-M} in the Brazilian Northeast. The data presented in this work demonstrate the presence of $bla_{CTX-M-2}$ and $bla_{CTX-M-28}$ genes in *K. pneumoniae* from Recife, Brazil and show that $bla_{CTX-M-2}$ genes were present in *K. pneumoniae* from 1998 until at least 2008.

Recent studies in the Brazilian Southeast Region have demonstrated the presence of $bla_{CTX-M-2}$ and $bla_{CTX-M-59}$ genes in *K. pneumoniae* (Do Carmo et al. 2008, Garcia et al. 2008). Clinical isolates of *Proteus mirabilis, C. amalonaticus, E. cloacae* and *E. aerogenes* collected from hospitals in Rio de Janeiro (Brazil) also demonstrated $bla_{CTX-M-2}$ (Bonnet et al. 2000, Bonnet et al. 2001). In South America, the $bla_{CTX-M-2}$ appears to be dominant in Argentina (Quinteros et al. 2003). Studies carried out in Europe have shown that the occurrence of the $bla_{CTX-M-2}$ group is rare. However, there is a high prevalence of group 1, predominantly $bla_{CTX-M-15}$, in Europe (Livermore et al. 2007).

In the present study, we also report the first $bla_{CTX-M-28}$ gene found in Brazil and, to our knowledge, the first isolation of this gene in South America. $bla_{CTX-M-28}$ was first described in 2003, in clinical isolates of *E. coli* in France (GenBank accession AJ549244.1) and recently it was also detected in China (Yu et al. 2007) and India (Kingsley & Verghese 2008) in *K. pneumoniae* and *E. coli* nosocomial isolates, respectively. Thus, $bla_{CTX-M-28}$, a gene that was detected mainly in Asia is also present in Brazil.

Most bla_{CTX-M} positive isolates exhibited resistance to non-β-lactams antibiotics, mainly streptomycin, sulphonamides and trimethoprim. Among Enterobacteriaceae, these resistances were commonly associated with a few types of integrons, mainly class 1 and 2 (Bissonnette & Roy 1992, Partridge & Hall 2003). In this study, two isolates that carried the gene $bla_{CTX-M-2}$ (K16R and K18P) also contained class 1 integrons with the gene cassettes *aadA1* or bla_{OXA-2}, which encode the enzymes aminoglycoside-3′-adenyltransferase and oxacillinase type 2, respectively. In

Brazil, this is the first report of the occurrence of bla_{OXA-2} in *K. pneumoniae*. This gene has also been described in *Shigella* spp isolates from Brazil (Peirano et al. 2005). The isolate K11P, which contained the gene $bla_{CTX-M-28}$, also harboured the gene cassette *dfr22* that encodes resistance to trimethoprim. This gene showed 100% identity with the gene *dfr22* described in a class 1 integron of *K. pneumoniae* originating from São Paulo, Brazil, in 2008 (GenBank accession EU622041). The different sizes and gene cassettes inserted between the conserved segment regions of the strains studied demonstrate the variable nature of these structures.

In conclusion, the data presented herein illustrate the complexity and extent of the spread of bla_{CTX-M} genes in *K. pneumoniae* and exemplify the diverse mode of spreading of this emerging resistance mechanism all over the world.

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REFERENCES

Barthélémy M, Péduzzi J, Bernard H, Tancréde C, Labia R 1992. Close amino acid sequence relationship between the new plasmid-mediated extended-spectrum β-lactamase MEN-1 and chromosomally encoded enzymes of *Klebsiella oxytoca*. *Biochim Biophys Acta 1122*: 15-22.

Bauernfeind A, Grimm H, Schweighart S 1990. A new plasmidic cefotaximase in a clinical isolate of *Escherichia coli*. *Infection 18*: 294-229.

Bissonnette L, Roy PH 1992. Characterization of In0 of *Pseudomonas aeruginosa* plasmid pVS1, an ancestor of integrons of multiresistance plasmids and transposons of gram-negative bacteria. *J bacteriol 174*: 1248-1257.

Bonnet R 2004. Growing group of extended-spectrum β-lactamases: the CTX-M enzymes. *Antimicrob Agents Chemother 48*: 1-14.

Bonnet R, Dutor C, Sampaio JL, Chanal C, Sirot D, Labia R, Champs C, Sirot J 2001. Novel cefotaximase (CTX-M-16) with increased catalytic efficiency due to substitution Asp-240→Gly. *Antimicrob Agents Chemother 45*: 2269-2275.

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

CLSI - Clinical and Laboratory Standards Institute 2006. *Performance standards for antimicrobial susceptibility testing. 16th informational supplement,* CLSI M100-S17, Wayne, 177 pp.

Do Carmo FJR, Silva RM, Castanheira M, Tognim MC, Gales AC, Sader HS 2008. Prevalence and genetic characterization of bla_{CTX-M} among *Klebsiella pneumoniae* isolates collected in an intesive care unit in Brazil. *J Chemother 20*: 600-603.

Garcia DO, Doi Y, Szabo D, Adams-Haduch JM, Vaz TM, Leite D, Padoveze MC, Freire MP, Silveira FP, Paterson DL 2008. Multiclonal outbreak of *Klebsiella pneumoniae* producing extended-spectrum β-lactamase CTX-M-2 and novel variant CTX-M-59 in a neonatal intensive care unit in Brazil. *Antimicrob Agents Chemother 52*: 1790-1793.

Kingsley J, Verghese S 2008. Sequence analysis of $bla_{CTX-M-28}$, an ESBL responsible for third-generation cephalosporin resistance in *Enterobacteriaceae*, for the first time in India. *Indian J Pathol Microbiol 51*: 218-221.

Livermore DM, Canton R, Gniadkowski M, Nordmann P, Rossolini GM, Arlet G, Ayala J, Coque TM, Kern-Zdanowicz I, Luzzaro F, Poirel L, Woodford N 2007. CTX-M: changing the face of ESBLs in Europe. *J Antimicrob Chemother 59*: 165-174.

Minarini LA, Gales AC, Palazzo IC, Darini AL 2007. Prevalence of community-occurring extended spectrum beta-lactamase-producing Enterobacteriaceae in Brazil. *Curr Microbiol 54*: 335-341.

Partridge SR, Hall RM 2003. In34, a complex In5 family class 1 integron containing orf513 and *dfrA10*. *Antimicrob Agents Chemother 47*: 342-349.

Peirano G, Agerso Y, Aarestrup FM, Rodrigues DP 2005. Occurrence of integrons and resistance genes among sulphonamide-resistance *Shigella* spp from Brazil. *J Antimicrob Chemother 55*: 301-305.

Quinteros M, Radice M, Gardella N, Rodríguez MM, Costa N, Korbenfeld D, Couto E, Gutkind G 2003. Extended-spectrum β-lactamases in Enterobacteriaceae in Buenos Aires, Argentina, Public Hospitals. *Antimicrob Agents Chemother 47*: 2864-2867.

Rodriguez MM, Power P, Radice M, Vay C, Famiglietti A, Galleni M, Ayala JA, Gutkind G 2004. Chromosome-encoded CTX-M-3 from *Kluyvera ascorbata*: a possible origin of plasmid-borne CTX-M-1-derived cefotaximases. *Antimicrob Agents Chemother 48*: 4895-4897.

Saladin M, Cao VTB, Lambert T, Donay JL, Herrmann JL, Ould-Hocine Z, Verdet C, Delisle F, Philippon A, Arlet G 2002. Diversity of CTX-M β-lactamases and their promoter regions from *Enterobacteriaceae* isolated in three Parisian hospitals. *FEMS Microbiol Lett 209*: 161-168.

Souza Lopes AC, Rodrigues JF, Morais Júnior MA 2005. Molecular typing of *Klebsiella pneumoniae* isolates from public hospitals in Recife, Brazil. *Microbiol Res 160*: 37-46.

Yu Y, Ji S, Chen Y, Zhou W, Wei Z, Li L, Ma Y 2007. Resistance of strains producing extended-spectrum β-lactamases and genotype distribution in China. *J Infect 54*: 53-57.