Journal of Asian Scientific Research, 2013, 3(9):882-890

Journal of Asian Scientific Research

journal homepage: http://aessweb.com/journal-detail.php?id=5003

RESISTANCES TO THE OXYIMINO-CEPHALOSPORINS BY CTX-M-15 PRODUCING K*LEBSIELLA* ISOLATED FROM THE URINES SAMPLES OF PATIENTS IN THE UNIVERSITY HOSPITAL COMPLEX PAEDIATRIC CHARLES DE GAULLE (CHUP-CDG) OF OUAGADOUGOU IN BURKINA FASO

METUOR DABIRE Amana

Laboratoire d'Enzymologie de la Chimio-Résistance Bactérienne, Université de Ouagadougou (U.O), Burkina Faso, Ouagadougou.

ZONGO K Jacob

Laboratoire d'Enzymologie de la Chimio-Résistance Bactérienne, Université de Ouagadougou (U.O), Burkina Faso, Ouagadougou.

ZEBA Boukaré

Laboratoire d'Enzymologie de la Chimio-Résistance Bactérienne, Université de Ouagadougou (U.O), Burkina Faso, Ouagadougou.

MOUSSAWI Jihad

Laboratoire de Biotechnologie Végétale, Université libre de Bruxelles, Professeurs Jeneer et Brachet, Gosselies, Belgium

BAUCHER Marie

Laboratoire de Biotechnologie Végétale, Université libre de Bruxelles, Professeurs Jeneer et Brachet, Gosselies, Belgium

EL JAZIRI Mondher

Laboratoire de Biotechnologie Végétale, Université libre de Bruxelles, Professeurs Jeneer et Brachet, Gosselies, Belgium

ABSTRACT

Seventeen oxyimino-cephalosporins-resistant Klebsiella strains were isoleted from urines clinical samples from patients from various service units of University Hospital complex Paediatric Charles De Gaulle (CHUP-CDG) in Burkina Faso. These strains were resistant to at least one oxyimino-cephalosporin. They were identified as producer of extended spectrum β-lactamases (ESBL) by double-disk synergy test between amoxicillin–clavulanate and cefotaxime, ceftriaxone or ceftazidime. The ESBL was identified as CTX-M-15 for the 17 strains by sequencing of PCR

products amplified with primers designed for bla_{CTX-M} *genes. This is the first description of this enzyme in Burkina Faso.*

Keywords: β-lactamases, CTX-M-15; ESBL, Oxyimino-cephalosporins, *Klebsiella.*

1. INTRODUCTION

Cefotaximase first isolate in Munich (CTX-M)-type extended-spectrum β-lactamases (ESBL) constitutes a worldwide growing group of enzymes encoded by bla_{CTX-M} genes located on diverse plasmids belonging to the IncFII group (Eckert *et al.*, 2004; Pieboji *et al.*, 2005; Carattoli Alessandra, 2009). This family of plasmid-mediated ESBL belongs to Ambler class A and functional group 2be of the Bush-Jacoby and Medeiros classification (AMBLER, 1980; BUSH *et al.*, 1995). They are capable of hydrolyzing expanded-spectrum cephalosporins and are inhibited by clavulanic acid, sulbactam, and tazobactam. In addition, they confer a high level resistance to cefotaxime but have a low level activity towards ceftazidime (Bonnet, 2004; Eckert *et al.*, 2004). The CTX-M β-lactamases are the most widespread ESBL enzymes (Mena *et al.*, 2006), distributed both over wide geographic areas and among a wide range of clinical bacteria, in particular, members of the family of Enterobacteriaceae. They were initially reported in the second half of the 1980s, and their rate of dissemination among bacteria and in most parts of the world has increased dramatically since 1995 mostly due to positive selection exerted by use of antimicrobials (Radice *et al.*, 2002; Quinteros *et al.*, 2003; Hernandez *et al.*, 2005; Pieboji *et al.*, 2005; Tumbarello *et al.*, 2006). At present, the CTX-M family comprises 142 enzymes (www.lahey.org/studies/other.asp). A phylogenic study revealed five major groups of CTX-M enzymes according to their amino acid sequences (CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9 and CTX-M-25 group) (Bonnet, 2004; Munday *et al.*, 2004; Oliver *et al.*, 2005; Mena *et al.*, 2006).

blaCTX-M genes have been found to originate from the chromosomal β-lactamase genes of *Kluyvera.* For instance, CTX-M-2, CTX-M-8 and CTX-M-9 clusters derive from *Kluyveraascorbata's KLUA-1, K. georgiana's KLUG-1,* and *K. georgiana's KLUY* enzymes, respectively (Humeniuk *et al.*, 2002; Poirel *et al.*, 2002; Olson *et al.*, 2005). Besides, a chromosome encoded CTX-M-3 from a *Kluyveraascorbata* strain seems to be the closest enzyme and most probable origin of the CTX-M-1 group (Rodríguez *et al.*, 2004).

The aim of the study is the characterization of ESBL among clinical isolates of 17 different oxyimino-cephalosporin-resistant *Klesiella* strains isolated from samples of urinesfrom CHUP-CDG in Burkina Faso.

2. MATERIALS AND METHODS

2.1. Bacterial Strains

Twelve *K. pneumoniae,* 2 *K. oxytoca and* 3*Klebsiella sp.* strains were collected between July 2010 and Mars 2012, from samples of urines of various service units of CHUP-CDG in Burkina Faso. Isolates were identified using an API 20 E system (bio-Mérieux, Marcy-l'Étoile, France).

2.2. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed using the disk diffusion method on Müeller-Hinton agar (Bio Rad, France) as recommended by the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2006). The double-disk synergy test for confirmation of ESBL activity was carried out as described previously (Jacoby and Han, 1996; Livermore *et al.*, 2001), by using amoxicillin–clavulanate against cefotaxime (CTX), ceftriaxone (CRO), ceftazidime (CAZ) or aztreonam (ATM). Minimal inhibitory concentrations (MIC) of CRO, CTX, CAZ, cefuroxim (CXM), cefepim (FEP) and imipenem (IPM) were determined by Method of dilution in liquid medium for strains according to CLSI guidelines (CLSI, 2006).

2.3. Bacterial DNA Extraction

Genomic DNA was extracted from bacteria using DNAzol®Reagent (Invitrogen/DNA by life technologies) following instructions of the manufacturer.

2.4. Polymerase Chain Reaction (PCR) Amplification of bla_{CTX-M} Genes

Detection of CTX-M encoding genes was performed by PCR. The pair of primers CTX-M F: 5'-GTTACAATGTGTGAGAAGCAG-3' and CTX-M R: 5'-CCGTTTCCGCTATTACAAAC-3'(Pagani *et al.*, 2003) was used to amplify bla_{CTX-M} sequences. The DNA amplification program consisted of an initial denaturation step 5 min at 96°C, followed by 30 cycles of denaturation for 1 min at 96°C, annealing for 1 min at 50°C and 1 min at 72°C for polymerization. Final products were extended by incubation for 10 min at 72 °C. PCR products were visualized by agarose gel electrophoresis. Amplicons of 953bp were sequenced by the Company GATC Biotech in Europe, and the resulting sequences were then compared with the sequences from GenBank database.

3. RESULTS

3.1. β-Lactam Susceptibility Profile and Minimum Inhibitory Concentraction Determination

The different *Klebsiella*strains showed a significant degree of multiresistance to various antibiotics (Table 1). All the 17 *Klebsiella* strains were resistant to CXM, CTX and CRO and with a lower degree to CAZ and FEP but were susceptible to IPM.The disk diffusion method showed synergy betweenceftazidime, cefotaxime, ceftriaxone, and amoxicillin–clavulanic acid against the strains, suggesting the presence of a class A ESBL (Jarlier *et al.*, 1988; Jacoby and Han, 1996; Livermore *et al.*, 2001) (Fig.1)

3.2. Amplification of β-lactamase-encoding bla_{CTX-M} Genes and Sequence Analysis

PCR analysis confirmed the presence of a 953 bpbla_{CTX-M}gene in all *Klebsiella* strains. Sequences analyses of the nucleotide sequence showed the occurrence of $bla_{CTX-M-15}$ (Sequence ID: gb|JQ686199.1|) in all samples.

4. DISCUSSION

In this report, we mentioned the occurrence of several *Klebsiella* strains carrying the $bla_{CTX\text{-}M\text{-}15}$ gene for the first time in Burkina Faso. *Klebsiella* were found to be resistant to oxyimino-cephalosporins, FEP and exhibited a positive double-disc synergy test, indicating the presence of an ESBL (Jarlier *et al.*, 1988). Multiresistance has often been described for ESBL (and particularly CTX-M) producing clinical isolates (Rice *et al.*, 1996; Shannon *et al.*, 1998; Asensio *et al.*, 2000; Nathisuwan *et al.*, 2001; Bradford, 2001 ; Kang *et al.*, 2004). When a PCR assay for CTX-M-type genes was used, 953 bp long amplicons were detected ineach strain of *Klebsiella.* Sequence analysis showed that all PCR products correspond to $bla_{CTX\text{-}M\text{-}15}$.Like the majority of the CTX-M enzymes, CTX-M-15 has been shown to hydrolize the CTX preferentially to CAZ (Sturenburg *et al.*, 2004).CTX-M-15 was reported in several countries but never in Burkina Faso. For instance at the Charles-Nicolle Hospital in Tunis (Tunisia), 62 enterobacterial strains producing CTX-M β-lactamase were collected between March 2000 and June 2003. All of isolates produce CTX-M-15 or CTX-M-16 (Mamlouk *et al.*, 2006). CTX-M-15 is also found in other bacterial species in other countries. As such *E. coli* strains CTX-M-15 have been described in Canada, in India, in Kuwait, in France, in Switzerland, in Portugal and in Spain (Coque *et al.*, 2008). In some cases $bla_{CTX\text{-}M\text{-}15}$disseminationis related to dispersion of a same plasmid and to the propagation of some clones of *Klebsiella*. The exchange of plasmids between *E. coli* and *K. pneumonia* with high epidemic potential is most probable.Results in favor of these genetic exchanges between bacterial species were obtained in Poland with CTX-M-3 (Baraniak *et al.*, 2002). In this report, the plasmids present in the *Klebsiella* strains isolated in Burkina Faso were not characterized but many studies showed that dissemination of organisms that produce CTX-M-9, CTX-M-14, CTX-M-15 and CTX-M-32 have been linked with epidemic plasmids associated to those of the incompatibility group IncFII (Lavollay *et al.*, 2006; Novais *et al.*, 2006) . Studies realized in Spain and Israel indeed showed that the rate of CTX-M-15 producing *E. coli* found in saddles of in-patients is 11,8 % and 10,8 % respectively (Valverde *et al.*, 2004; Ben-Ami *et al.*, 2006), which would constitute a tank very significant of $bla_{CTX\text{-}M}$ plasmids carrying with strong potential of inter-species transfer. In China, in contrast to the diversity of clonal relationships, many local isolates harbored a 90-kb *IncFII* plasmid carrying $bla_{CTX\text{-}M\text{-}15}$, suggesting that this plasmid appeared to be a major vehicle mediating the local dissemination of $bla_{CTX\text{-}M\text{-}15}$ in *K. pneumonia* (Zhuo et al., 2013). Indeed, previous reports (Canton and Coque, 2006) suggested that plasmid is one major factor responsible for the worldwide spread of $bla_{CTX\text{-}M\text{-}15}$. For example, in *E. coli*, many plasmids carrying $bla_{CTX\text{-}M\text{-}15}$ found in France, Tunis, Bangui and India (Coque *et al.*, 2008) (Karim *et al.*, 2001), shared common features with pC15-1a from Canada (Nicolas-Chanoine *et al.*, 2008). Furthermore, emergence of *K. pneumoniae* isolates producing CTX-M-15 were also found in European countries and $bla_{CTX\text{-}M\text{-}15}$ transfer were mediated by IncFII-related plasmids with different sizes among part of them (Machado *et al.*, 2006).

In conclusion, our study highlighted the CTX-M-15 type ESBL responsible for resistance to the oxyimino-cephalosporins of *Klebsiella* strains isolated from the urines from sick children in CHUP-CDG of Ouagadougou.The increase of consumption of cefotaxime and ceftazidime could

have contributed to the emergence of CTX-M enzymes encoding genes among *Klebsiella* strains in Burkina hospitals. It is anticipated that CTX-M-15 producing *Klebsiella* strains will become an eventual epidemiological problem in CHUP-CDG in Burkina Faso.

The bladder is a site of the human organism where high concentrations are observed in certain antibiotics (quinolones and β-lactamines) (NCCLS, 1999). ESBL producing strains were highlighted in the urines. This result corroborates those of Philippon *et al.* (1993) and of Bermudes *et al.* (1997) which reports that the majority of strains ESBL come from the urine.

The dissemination of *Klebsiella* strains CTX-M-15 type ESBL producing represents a world problem of public health insofar as this species is very easily implied in nosocomial epidemics. Carbapenems often represent the single therapeutic alternative. However, the emergence of resistance to carbapenems was already reported in *K.pneumoniae*strains and other species of Enterobacteria producing different variable of CTX-M-type (Elliott *et al.*, 2006; Woodford *et al.*, 2007; Chen *et al.*, 2008; Oteo *et al.*, 2008); these resistances complicate the assumption of responsibility of the patients.

5. ACKNOWLEDGEMENTS

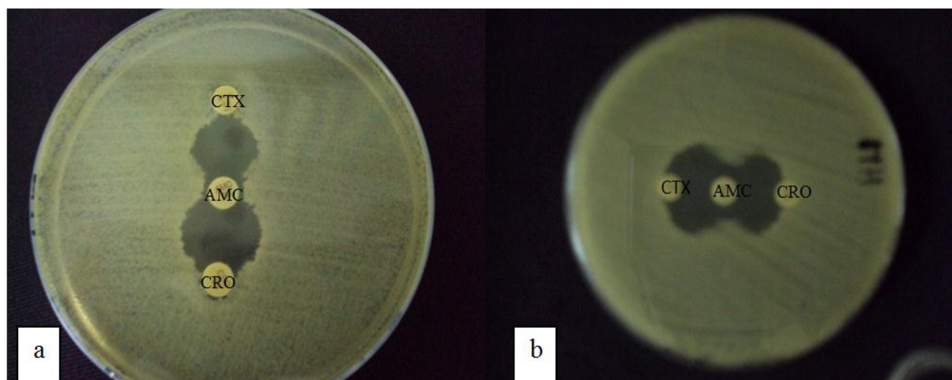
This study was done with the financial support of the University Commission for the Development (CUD).

Table-1. Bacterial isolates and minimum inhibitory concentration determination of 6 antibiotics

Samples	Isolates	MIC (µg/ml)					
		CXM	CTX	CRO	CAZ	FEP	IPM
Urines 44	*K.pneumoniae*	200< MIC ≤ 300	>300	100< MIC ≤ 200	≤ 25	25< MIC ≤ 50	≤ 25
Urines 46	*K.pneumoniae*	200< MIC ≤ 300	200< MIC ≤ 300	100< MIC ≤ 200	≤ 25	≤ 25	≤ 25
Urines 120	*K. pneumoniae*	>300	>300	200< MIC ≤ 300	25< MIC ≤ 50	50< MIC ≤ 100	≤ 25
Urines 130	*K.pneumoniae*	100< MIC ≤ 200	>300	100< MIC ≤ 200	25< MIC ≤ 50	25< MIC ≤ 50	≤ 25
Urines 213	*K.pneumoniae*	>300	200< MIC ≤ 300	200< MIC≤ 300	50< MIC ≤ 100	50< MIC ≤ 100	≤ 25
Urines 292	*K.pneumoniae*	200< MIC ≤ 300	200< MIC ≤ 300	>300	≤ 25	50< MIC ≤ 100	≤ 25
Urines 466	*K.pneumoniae*	200< MIC ≤ 300	200< MIC≤ 300	>300	25< MIC≤ 50	≤ 25	≤ 25
Urines 534	*K.pneumoniae*	200< MIC ≤ 300	200< MIC ≤ 300	200< MIC ≤ 300	25< MIC≤ 50	50< MIC≤ 100	≤ 25
Urines 538	*K.pneumoniae*	100< MIC ≤ 200	100< MIC ≤ 200	100< MI C≤ 200	50< MIC ≤ 100	50< MIC ≤ 100	≤ 25
Urines 736	*K.pneumoniae*	>300	>300	>300	50< MIC ≤ 100	50< MIC ≤ 100	≤ 25
Urines 774	*K.pneumoniae*	>300	200< MIC ≤ 300	100< MIC ≤ 200	50< MIC ≤ 100	50< MIC ≤ 100	≤ 25
Urines 778	*K.pneumoniae*	100< MIC ≤ 200	200< MIC ≤ 300	100< MIC ≤ 200	50< MIC≤ 100	50< MIC≤ 200	≤ 25
Urines 203	*Klesiella sp*	200< MIC ≤ 300	200< MIC≤ 300	200< MIC ≤ 300	25< MIC ≤ 50	≤ 25	≤ 25
Urines 336	*Klesiella sp*	200< MIC ≤ 300	200< MIC ≤ 300	>300	25<MIC ≤ 50	≤ 25	≤ 25
Urines 715	*Klesiella sp*	200< MIC ≤ 300	100< MIC≤ 200	>300	50< MIC ≤ 100	50< MIC ≤ 100	≤ 25
Urines 362	*K.oxytoca*	50< MIC ≤ 100	100< MIC ≤ 200	200< MIC ≤ 300	25< MIC ≤ 50	≤ 25	≤ 25
Urines 613	*K.oxytoca*	>300	>300	>300	25< MIC≤ 50	25< MIC ≤ 50	≤ 25

MIC : Minimal inhibitory concentrations;CXM : Cefuroxim ; CTX : Cefotaxim ; CRO: Ceftriaxon; CAZ: Ceftazidim; FEP: Cefepim; IPM: Imipenem

Fig-1. Double-disk synergy testfor confirmation of ESBL activity: *K. pneumoniae* isolated from urine 736 (a) and *K. pneumoniae* isolated from urine 120 (b)



REFERENCES

AMBLER, R.P., 1980. The structure of β-lactamases. Philos. Trans. R. Soc.London Ser.B, 289(1036): 321- 331.

Asensio, A., A. Oliver, P. Gonzalez-Diego, B. F., J. Perez-Diaz and P. Ros, 2000. Outbreak of a multiresistant klebsiella pneumoniae strain in an intensive care unit: Antibiotic use as a risk factor for colonization and infection. Clin Infect Dis, 30:(1) 55–60.

Baraniak, A., J. Fiett, A. Sulikowska, W. Hryniewicz and M. Gniadkowski, 2002. Countrywide spread of ctx-m-3 extended-spectrum b-lactamase-producing microorganisms of the family enterobacteriaceae in poland. Antimicrob Agents Chemother, 46(1): 151–159.

Ben-Ami, R., M. Schwaber, S. Navon-Venezia, D. Schwartz, M. Giladi and I. Chmelnitsky, 2006. Influx of extended-spectrum b-lactamase-producing enterobacteriaceae into the hospital. Clin Infect Dis, 42(7): 925–934.

Bermudes, H., C. Arpin, F. Inde, Z. El Harrif, C. Bebear and C. Quentin, 1997. Molecular epidemiology of an outbreak due to expanded spectrum beta-lactamase producing enterobacteriaceae in french hospital. Eur. J. Microbiol. Infect. Dis, 16(7): 523-259.

Bonnet, R., 2004. Growing croup of extended-spectrum b-lactamases:The ctxm enzymes. Antimicrob Agents Chemother, 48(1): 1–14.

Bradford, P.A., 2001 Extended spectrum b-lactamases in the 21st century: Characterisation, epidemiology, and detection of this important resistance threat. Clin Microbilol Rev, 14(4): 933–951.

BUSH, K., G.A. JACOB and A.A. MEDEIROS 1995. A functional classificcation scheme for β-lactamases and its correlation with molecular structure. Antimicrob Agents Chemother, 39(6): 1211-1233.

Canton, R. and T. Coque, 2006. The CTX-M b-lactamase pandemic. Current Opinion in Microbiology, 9(5): 466-475.

Carattoli Alessandra, 2009. Resistance plasmids families in enterobacteriacae. Microbial Agent and Chemotherapy, 53(6): 2227-2238.

Chen, Y., Y. Zhang, Y. Yu, T. Qu, Wei ZQ and P. Shen, 2008. In vivo development of carbapenem resistance in clinical isolates of enterobacter aerogenes producing multiple b-lactamases. Int J Antimicrob Agents, 32(4): 302–307.

CLSI, C.l.s.i., 2006. Performance standards for antimicrobial susceptibility testing; sixteenth informational supplement, clsi document m100-s16. Wayne, PA: CLSI.

Coque, T., A. Novais, A. Carattoli, L. Poirel, J. Pitout and L. Peixe, 2008. Dissemination of clonally related escherichia coli strains expressing extended-spectrum blactamase ctx-m-15. Emerg Infect Dis, 14(2): 195–200.

Eckert, C., V. Gautier, M. Saladin-Allard, N. Hidri, C. Verdet and Z. Ould-Hocine, 2004. Dissemination of ctx-m-type b-lactamases among clinical isolates of enterobacteriaceae in paris, France. Antimicrob Agents Chemother, 48(4): 1249–1255.

Elliott, E., Brink AJ, J. van Greune, Z. Els, N. Woodford and J. Turton, 2006. In vivo development of ertapenem resistance in a patient with pneumonia caused by klebsiella pneumoniae with an extended-spectrum b-lactamase. Clin Infect Dis, 42(1): 95–98.

Hernandez, J., Martnez-Martnez L, Canton R, Coque TM and Pascual A., 2005. The spanish group for nosocomial infections (geih). Nationwide study of escherichia coli and klebsiella pneumonia producing extended-spectrum b-lactamases in Spain. Antimicrob Agents Chemother, 49(7): 2122-2125.

Humeniuk, C., Guillaume A, Gautier V, Grimont P, Labia R and Philippon A., 2002. B-lactamases of kluyvera ascorbata, probable progenitors of some plasmid-encoded ctx-m types. Antimicrob Agents Chemother, 46(9): 3045-3049.

Jacoby, G. and P. Han, 1996. Detection of extended-spectrum b-lactamases in clinical isolates of klebsiella pneumoniae and escherichia coli. J Clin Microbiol, 34(4): 908-911.

Jarlier, V., M.-H. Nicolas, G. Fournier and A. Philippon., 1988. Extended broad -spectrum β-lactamases confering transferable resistance to newer β-lactam agents in enterobacteriaceae : Hospital prevalence and susceptibility patterns. Rev. Infect. Dis, 10(3): 867-878.

Kang, C., Kim SH, Park WB, Lee KD, Kim HB and Kim EC, 2004. Bloodstream infections due to extended-spectrum b-lactamase-producing escherichia coli and klebsiella pneumoniae: Risk factors for mortality and treatment outcome, with special emphasis on antimicrobial therapy. Antimicrob Agents Chemother, 48(4): 4574-4581.

Karim, A., L. Poirel, S. Nagarajan and P. Nordmann, 2001. Plasmid-mediated extended-spectrum beta-lactamase (CTX-M-3 like) from India and gene association with insertion sequence ISEcp1. FEMS Microbiol. Lett, 201(2): 237–241.

Lavollay, M., K. Mamlouk, T. Frank, A. Akpabie, B. Burghoffer and R.S. Ben, 2006. Clonal dissemination of a CTX-M-15 betalactamase-producing Escherichia coli strain in the Paris area, Tunis, and Bangui. Antimicrob Agents Chemother, 50(7): 2433—2438.

Livermore, D., D. Brawn, C. Belhadj and Belhadj O., 2001. Detection of blactamase-mediated resistance. J Antimicrob Chemother, 48(1): SI: 59-64.

Machado, E., Coque TM, Canto´n R, Baquero F and Sousa JC, 2006. Dissemination in portugal of ctx-m-15-, oxa-1-, and tem-1-producing enterobacteriaceae strains containing the aac(69)-ib-cr gene, which encodes an aminoglycoside- and fluoroquinolone-modifying enzyme. Antimicrob Agents Chemother, 50(3): 3220-3221.

Mamlouk, K., Ben Boubaker IB, Gautier V, Vimont S, Picard B and Ben Redjeb S, 2006. Emergence and outbreaks of ctx-m b-lactamase-producing escherichia coli and klebsiella pneumoniae strains in a Tunisian hospital. J Clin Microbiol, 44(11): 4049-4056.

Mena, A., Plasencia V, Garca L, Hidalgo O, Ayestaran JI and Alberti S, 2006. Characterization of a large outbreak by ctx-m-1-producing klebsiella pneumoniae and mechanisms leading to in vivo carbapenem resistance development. J Clin Microbiol, 44(8): 2831-2837.

Munday, C., Boyd DA, Brenwald N, Miller M, Andrews JM and Wise R, 2004. Molecular and kinetic comparison of the novel extended-spectrum b-lactamases ctx-m-25 and ctx-m-26. Antimicrob Agents Chemother, 48(12): 4829-4834.

Nathisuwan, S., Burgess DS and Lewis JS., 2001. Extended spectrum b-lactamases: Epidemiology, detection and treatment. Pharmacotherapy, 21(8): 920-928.

NCCLS, N.C.f.C.L.S., 1999. Performance standards for antimicrobial susceptibility testing; ninth informational supplement. Approved standard M100-S9. NCCLS, Wayne, Pa.

Nicolas-Chanoine, M.H., J. Blanco, V. Leflon-Guibout, R. Demarty, M.P. Alonso, M.M. Canica, Y.J. Park, J.P. Lavigne, J. Pitout and J.R. Johnson, 2008. Intercontinental emergence of escherichia coli clone O25: H4-ST131 producing CTX-M-15. J. Antimicrob. Chemother, 61(2): 273-281.

Novais, A., R. Canton, A. Valverde, E. Machado, J. Galan, L. Peixe, A. Caratolli, F. Baquero and T. Coque, 2006. Dissemination and persistance of blaCTX-M-9 are linked to class 1integron containing CR1 assocated with defective trans poson derivatives from Tn402 located in early antibiotic resistance plasmid of IncHI2, IncP1-a, and IncFI Groups, Antimicrobal Agents Chemother, 50(2): 2741-2750.

Oliver, A., Coque TM, Alonso D, Valverde A, Baquero F and Canton R., 2005. Ctxm-10 linked to a phage-related element is widely disseminated among enterobacteriaceae in a Spanish hospital. Antimicrob Agents Chemother, 49(4): 1567-1571.

Olson, A., Silverman M, Boyd DA, McGeer A, Willey BM and Porter VP, 2005. Identification of a progenitor of the ctx-m-9 group of extendedspectrum b-lactamases from kluyvera georgiana isolated in guyana. Antimicrob Agents Chemother, 49(5): 2112-2115.

Oteo, J., Delgado-Iribarren A, Vega D, Bautista V, Rodrı´guez MC and Velasco M, 2008. Emergence of imipenem resistance in clinical escherichia coli during therapy. Int J Antimicrob Agents, 32(6): 534-537.

Pagani, L., Dell'Amico E and Migliavacca R, 2003. Multiple ctx-m-type extended spectrum beta-lactamases in nosocomial isolates of enterobacteriaceae from a hospital in Northern Italy. J Clin Microbiol, 41(9): 4264-4269.

Philippon, A., A. G. and P. H. Lagrange, 1993. B-lactamses à spectre élargi. Rev. Prat, 43(1): 2387-2395.

Pieboji, J., Miriagou V, Vourli S, Tzelepi E, Ngassam P and Tzouvelekis LS., 2005. Emergence of ctx-m-15-producing enterobacteria in cameroon and characterization of a blactx-m-15-carrying element. Antimicrob Agents Chemother, 49(1): 441-443.

Poirel, L., Kämpfer P and Nordmann P., 2002. Chromosome-encoded ambler class a b-lactamase of kluyvera georgiana, a probable progenitor of a subgroup of ctx-m extended-spectrum b-lactamases. Antimicrob Agents Chemother, 46(2): 4038-4040.

Quinteros, M., Radice M, Gardella N, Rodriguez MM, Costa N and Korbenfeld D, 2003. Extended-spectrum b-lactamases in enterobacteriaceae in buenos aires, Argentina, public hospitals. Antimicrob Agents Chemother, 47(9): 2864-2867.

Radice, M., Power P, Di Conza J and Gutkind G., 2002. Early dissemination of ctxm-derived enzymes in South America. Antimicrob Agents Chemother 46(2): 602-604.

Rice, L., Eckstein EC, De Vente J and Shlaes DM., 1996. Ceftazidime-resistant klebsiella pneumoniae isolates recovered at the cleveland department of veterans affairs medical center. Clin Infect Dis, 23(1): 118-124.

Rodríguez, M., Power P, Radice M, Vay C, Famiglietti A and Galleni M, 2004. Chromosome-encoded ctx-m-3 from kluyvera ascorbata: A possible origin of plasmid-borne ctx-m-1-derived cefotaximases. Antimicrob Agents Chemother, 48(12): 4895-4897.

Shannon, K., Stapleton P, Xiang X, Johnson A, Beattie H and El Bakri F, 1998. Extended-spectrum b-lactamase-producing klebsiella pneumoniae strains causing nosocomial outbreaks of infection in the united kingdom. J Clin Microbiol, 36(10): 3105-3110.

Sturenburg, E., Kuhn A, Mack D and Laufs R., 2004. A novel extended-spectrum blactamase ctx-m-23 with a p167t substitution in the active-site omega loop associated with ceftazidime resistance. J Antimicrob Chemother, 54(7): 406-409.

Tumbarello, M., Spanu T, Sanguinetti M, Citton R, Montuori E and Leone F, 2006. Bloodstream infections caused by extended-spectrum-b-lactamaseproducing klebsiella pneumoniae: Risk factors, molecular epidemiology, and clinical outcome. Antimicrob Agents Chemother, 50(2): 498-504.

Valverde, A., Coque TM, Sa´nchez-Moreno MP, Rolla´n A, Baquero F and Canto´n R., 2004. Dramatic increase in prevalence of fecal carriage of extended-spectrum betalactamase-producing enterobacteriaceae during non outbreak situations in Spain. J Clin Microbiol, 42(10): 4769-4775.

Woodford, N., Dallow JW, Hill RL, Palepou MF, Pike R and Ward ME, 2007. Ertapenem resistance among klebsiella and enterobacter submitted in the uk to a reference laboratory. Int J Antimicrob Agents, 29(4): 456-459.

Zhuo, C., Li X-q, Zong Z-y and Zhong N-S, 2013. Epidemic plasmid carrying blactx-m-15 in klebsiella penumoniae in China. PLoS ONE 8(1): E522222. Available from doi:10.1371/journal.pone.0052222.