KLEBSIELLA PNEUMONIAE RESISTANT TO THIRD GENERATION CEPHALOSPORINS IN A HOSPITAL IN VENEZUELA: SPREAD OF ESBL-PRODUCING ST70 AND ST1261 CLONES

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Abstract

Objectives. To characterize *K. pneumoniae* isolates presenting decreased susceptibility to third generation cephalosporins, and isolated in two high risk units of the University of the Andes Hospital (HULA).

Methods. We studied all the *K. pneumoniae* strains non-susceptible to third generation cephalosporins isolated from patients and the environment in the Neonatal High Risk Unit (NHRU) and Adult Intensive Care Unit (AICU) of the HULA in Merida, Venezuela, from February to March 2007, and from June to November 2009. Antibiotic susceptibility was tested by the disk-diffusion method and by the E-test method. Detection of $bla_{\beta LEE}$ and bla_{AmpC} genes was carried out by PCR and sequencing. The genetic relationship between the isolates was established by pulsed field gel Electrophoresis (PFGE) and Multilocus Sequence Typing (MLST).

Results. In total 39 isolates non-susceptible to third generation cephalosporins were identified, 30 of them from the NHRU and 9 from the AICU; 20 isolates (51,28%) produced CTX-M-15, 8 (20,51 %) CTX-M-2, 8 (20,51%) SHV-12, 2 (5,12%) SHV-1 overproduction and 1 (2.56%) EBC. By PFGE, ten clusters were found. By MLST, the two more prevalent PFGE clusters belonged to ST70, (here reported for the first time in Venezuela), and ST126.

Conclusions. Its to seem to be the first time, the dissemination of the ST70 clone producing CTX-M-15 in different risk units of LAUH is being described in Venezuela; also this is the first worldwide description the ST1261 clone producing CTX-M-2 and SHV-2.

Keywords: *Klebsiella pneumoniae*, BLEE, PFGE, MLST

Introduction

Klebsiella pneumoniae is one of the primary agents of pneumonia, septicemia, and other frequent nosocomial infections (Perlman et al., 2007; Alonso et al., 2005). The rapid spread of *K. pneumoniae* strains that are resistant to multiple families of antibiotics, such as carbapenems and third-generation cephalosporins, is of great concern worldwide (Lynch et al., 2013). Initially, this resistance was associated with SHV-1 or TEM-1 extended-spectrum beta-lactamase (ESBL)-producing strains that caused hospital outbreaks. However, the increased production of CTX-M–type ESBL-producing strains has been reported in recent years. The CTX-M type is associated with certain genetic elements, such as insertion sequences, transposons, and plasmids (Vignoli et al., 2006).In Mérida, Venezuela, the simultaneous presence of TEM-1 and SHV-5 was reported in *K. pneumoniae* strains isolated during a nosocomial outbreak in 2000 (Araque and Rivera, 2004). A 2010 study also from Mérida detected CTX-M-1 and CTX-M-2 ESBLs in *K. pneumoniae* strains isolated from high-risk newborn infants (Millan et al., 2013). Other studies in Caracas reported the presence of TEM-1, SHV-1, and CTX-M-2 in Enterobacteriaceae of hospital origin (Guzman and Alonso, 2009; Torres et al., 2006). During 2007 and 2009 in a university hospital in Mérida, researchers isolated multidrug resistant *K. pneumoniae* strains producing ESBLs, TEM, SHV, and CTX (González et al., 2011; González et al., 2013).

The objective of this study was to characterize the genotypes of *K. pneumoniae* strains with reduced sensitivity to third-generation cephalosporins, which were isolated from the neonatal high-risk unit (NHRU) in 2007 and 2009 and from the adult intensive care unit (AICU) in 2009 at the Los Andes University Hospital (LAUH) in Mérida, Venezuela. The overall aim was to study the distribution and spread of these strains by using specific molecular techniques

Materials and methods

Study Design and Bacterial Strains

Strains were isolated in February 2007 during a10-day outbreak in the High-Risk Neonatology Unit (HRNU) of the Hospital of the University of the Andes (HULA) in Venezuela. *K. penumoniae* strains with diminished sensitivity to cefotaxime or ceftazidime were selected from newborns hospitalized between February and March 2007. Strains were also isolated during environmental microbiologic sampling and from the hands of staff members, to determine possible sources of infection (González et al., 2011). In total,16 *K. pneumonia* strains were isolated from the HRNU in 2007.

A second sampling was conducted atthe HRNU with the same selection criteria from June to November 2009 (González et al., 2013). A

total of 14 *K. pneumoniae* strains were selected from this sampling. In addition, 9 strains with the same resistance pattern that were isolated from patients admitted to the Adult Intensive Care Unit (AICU) from June to November 2009 were included in the study. The AICU strains were included to detect the possible spread of *K. pneumoniae* strains with diminished sensitivity to third-generation cephalosporins across other HULA units. The microbiologic study was conducted at the "Dr. Roberto Gabaldón" Bacteriology Laboratory of the Department of Microbiology and Parasitology of the School of Pharmacy and Bioanalysis of the University of the Andes. The study of resistance mechanisms and molecular epidemiology was performed at the National Center of Microbiology of the Carlos III Health Institute.

Susceptibility to antimicrobial agents

Antimicrobial sensitivity was assessed with the disk-diffusion sensitivity test in Mueller-Hinton agar. The minimum inhibitory concentration (MIC) was determined via Etest® (BioMérieux, France),in accordance with the manufacturer's instructions. Strain sensitivity was determined via cut-off points. Strains with intermediate sensitivity or with resistance to cefotaxime or ceftazidime based on CLSI, (2013) were considered to have diminished sensitivity to third-generation cephalosporins. To confirm production of ESBL,cefotaxime and ceftazidime slides were used, alone or in combination with clavulanic acid, according to the manufacturers' instructions (CLSI., 2013).

AmpC production was suspected for strains that were resistant to cefoxitin and amoxicillin/clavulanic acid and that did not regain cefotaxime or ceftazidime activity with clavulanic acid. For phenotype confirmation of AmpC,the DDT test was performed, combined with phenylboronic acid as an inhibitor and cefoxitin (Yagi et al., 2005).

Characterization of antibiotic resistance genes

Specific primers were used to amplify the various bla_{BLEE} genes ($bla_{\text{CTX-M}}$, bla_{SHV}, and bla_{TEM}) (Oteo et al., 2006). Multiple PCR analysis was used to study genes that code for AmpC-type plasma β-lactamases (Pérez-Pérez and Hanson, 2002).

Sequencing

Amplified DNA was obtained by PCR and placed in a sequencer. For each PCR run, the corresponding primer was used at 10 μM. Sequences were analyzed via the program SeqMan análysis sofware DNA and pág web BLAST® (Basic Local Alignment Search Tool).

Molecular epidemiology

Genetic correlation was performed between the *K. pneumoniae* isolates by pulsed-field gel electrophoresis (PFGE) after complete digestion of DNA with *Xba*I. Some strains representing the primary clusters detected by PFGE were typed via MLST, following instructions from the Pasteur Institute(http://www.Pasteur.Fr/recherche/genopole/PF8/Mist/*K.pneumoniae*. html; last accessed December, 2013).

Results

In total, we studied 39 isolates of third-generation cephalosporin-resistant *K. pneumoniae* strains. Of the 39 isolates, 16 were isolated from the NHRU between February and June 2007, including nine from infants with sepsis, six from environmental samples, and one from hospital staff. Fourteen strains were isolated from the NHRU between June and December 2009, all from infants with sepsis. The remaining nine strains were isolated from the AICU between June and December 2009, from six adult patients with sepsis and three from adult patients with pneumonia.

Of the 16 strains obtained from the NHRU in 2007, 12 (75%) presented the same resistance profile, characterized by resistance to amoxicillin/clavulanic acid, ampicillin/sulbactam, cefotaxime, ceftazidime, cefepime, aztreonam, gentamicin, and tobramycin, as well as sensitivity to cefoxitin, piperacillin-tazobactam, amikacin, ciprofloxacin, cotrimoxazole, and carbapenems. The remaining four strains (25%) presented a different antibiotype, although all strains showed a phenotype compatible with ESBL production. The 14 isolates from infant bacteremia in 2009 showed different antibiotypes than those of strains isolated in 2007, but most of them (12/14, 85.7%) presented a phenotype typical of ESBL production. Eight of the nine strains (88.8%) isolated from patients in the AICU had an ESBL-producing phenotype. The remaining one isolate (11.2%) presented a profile compatible with AmpC beta-lactamase production.

Using PFGE, we detected 12 clusters with over 85% genetic homology. Two of them, cluster 1 (C1) and cluster 2 (C2), included 25 of the 39 strains studied (62.5%). C1 comprised 14 identical strains, including 12 isolated from the NHRU in 2007 and two isolated from the AICU in 2009. C2 comprised 11 strains from the NHRU, including four from 2007 (PFGE C2c variant) and seven from 2009 (three PFGE C2a and four C2b variants). C3 comprised three strains isolated from the NHRU in 2009. Clusters 4 to 12 represented individual strains or groups of two strains unrelated to the primary clusters (Figure 1).

An MLST study of strains C1, C2 (with variants C2a, C2b, and C2c), and C3 revealed that they belonged to sequence type (ST) 70, ST1261, andST23, respectively. This study represents the first reported isolation of

ST1261.Of the 39 strains studied, 20 strains (51.28%) produced CTX-M-15, eight strains (20.61%) produced CTX-M-2, eight strains (20.61%) produced SHV-12, two strains (5.12%) showed hyperproduction of SHV-1, and one strain (2.56%) produced EBC.

In terms of the ESBLs identified in the NHRU, the 12 C1 strains (ST70) from 2007 were carriers of $bla_{\text{CTX-M-15}}$. Four were isolated from infants, one was isolated from a staff member's hands, and seven were isolated from the environment. The four blood isolates of C2 (ST1261) from 2007 were carriers of $bla_{\text{CTX-M-2}}$. Of the 14 strains isolated in 2009 from the NHRU, five strains (35.7%) were carriers of $bla_{\text{SHV-12}}$ (twoC2 and one each of C8, C9, and C12), four strains (28.6%) were carriers of $bla_{\text{CTX-M-15}}$ (threeC3 and oneC9), and three strains were carriers of $bla_{\text{CTX-M-2}}$ (C2). Finally, two strains were carriers of $bla_{\text{SHV-1}}$ and presented a profile compatible with hyperproduction, with C2 predominating (ST1261) (Table 1, Figure 1).

Regarding the ESBLs identified in the AICU, four of the nine strains were carriers of $bla_{\text{CTX-M-15}}$ (two C1, namely ST70, prevalent in the NHRU in 2007; and two C7), three strains were carriers of $bla_{\text{SHV-12}}$(C5, C11, and C12), one strain contained $bla_{\text{CTX-M-2}}$ (C6), and one strain contained bla_{EBC} (C4), which codes for the plasmid AmpC beta-lactamase (Table 1, Figure 1).

Table 1. Characteristics of *K. pneumoniae* strains isolated in newborns and adults with nosocomial infection in the NHRU and AICU between February and June 2007, and between June and November 2009.

Strains N°	Date	Room	Sample	Type of ESBL	Perfil PFGE	ST
1(24)	18/02/2007	NHRU (A)	Blood	CTX-M-15	1	70
2(25)	19/02/2007	NHRU (A)	Blood	CTX-M-15	1	70
3(26)	19/02/2007	NHRU (A)	Blood	CTX-M-15	1	70
4(27)	19/02/2007	NHRU (A)	Blood	CTX-M-15	1	70
5(28)	21/02/2007	NHRU (A)	Blood	CTX-M-15	1	70
6 (Soap G)	27/02/2007	Depósito	Environment	CTX-M-15	1	70
7 (Soap T)	27/02/2007	NHRU (t)	Environment	CTX-M-15	1	70
8 (Soap A)	27/02/2007	NHRU (A)	Environment	CTX-M-15	1	70
9 (Soap B)	27/02/2007	NHRU (B)	Environment	CTX-M-15	1	70
10 Hands	27/02/2007	Personal	Personal	CTX-M-15	1	70
11 Sink	27/02/2007	NHRU (A)	Environment	CTX-M-15	1	70
12 handle	27/02/2007	NHRU (A)	Environment	CTX-M-15	1	70
13(263)	09/05/2007	NHRU (T)	Blood	CTX-M-2	2C	1261
14(268)	11/05/2007	NHRU (A)	Blood	CTX-M-2	2C	1261
15(270)	16/06/2007	NHRU (A)	Blood	CTX-M-2	2C	1261
16(273)	20/07/2007	NHRU (A)	Blood	CTX-M-2	2C	1261
17(79)	18/06/2009	NHRU (A)	Blood	SHV-12	9A	-
18(88)	19/06/2009	NHRU (A)	Blood	CXT-M-15	9B	-
19(123)	21/06/2009	NHRU (A)	Blood	SHV-12	10	-
20(162)	25/06/2009	NHRU (A)	Blood	SHV-12	2B	1261
21(164)	28/06/2009	NHRU (A)	Blood	SHV-12	2B	1261
22(218)	29/06/2009	NHRU (A)	Blood	CTX-M-15	3B	23
23(220)	13/07/2009	NHRU (A)	Blood	CTX-M-15	3A	23
24(228)	17/07/2009	NHRU (A)	Blood	CTX-M-15	3A	23

25(234)	27/07/2009	NHRU (B)	Blood	HIP.SHV-1	2A	1261
26(416)	15/08/2009	NHRU (T)	Blood	CTX-M-2	2B	1261
27(577)	20/08/2009	NHRU (A)	Blood	CTX-M-2	2A	1261
28(578)	23/08/2009	NHRU (A)	CSF	CTX-M-2	2A	1261
29(580)	09/09/2009	NHRU (T)	Blood	HIP. SHV-1	2B	1261
30(620)	11/10/2009	NHRU (A)	Blood	SHV-12	8	-
31(09)	03/07/2009	AICU	BS	CTX-M-2	6	-
32(016)	05/07/2009	AICU	Blood	EBC	4	-
33(022)	07/07/2009	AICU	Blood	CTX-M-15	1	70
34(023)	21/07/2009	AICU	Blood	CTX-M-15	1	70
35(025)	28/07/2009	AICU	Blood	CTX-M-15	7	-
36(028)	04/08/2009	AICU	BS	CTX-M-15	7	-
37(040)	13/08/2009	AICU	Blood	SHV-12	5	-
38(041)	16/11/2009	AICU	Blood	SHV-12	11	-
39(044)	25/11/2009	AICU	Blood	SHV-12	12	-

NHRU (A): Neonatal intensive care unit A room
NHRU (B). Neonatal intensive care unit B room
NHRU (T): Neonatal intensive care unit therapy room
HIP. SHV-1: Hyperproduction of SHV-1.
AICU: Adult intensive care unit
CSF: Cerebrospinal fluid
BS: Bronchial secretion

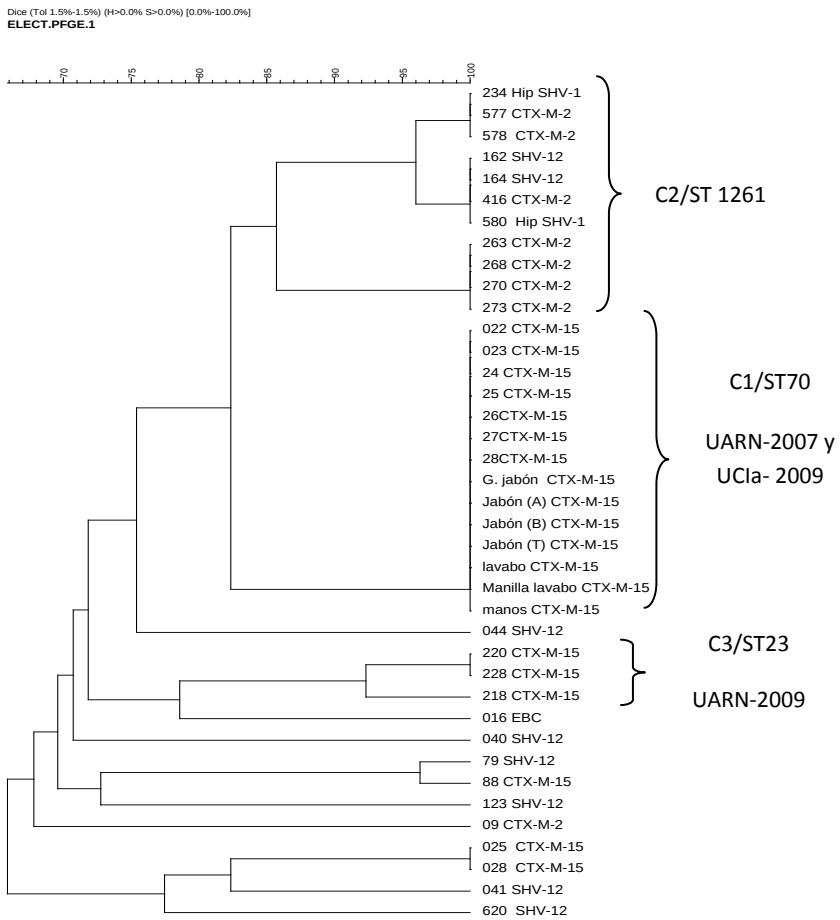

Figure 1. Tree diagram illustrating the genetic relationship between *K. pneumoniae* strains producing CTX-M-15, CTX-M-2, and SHV-12 beta-lactamases, as well as SHV-1 and plasmid EBC hyperproduction in the NHRU and AICU at LAUH.

Discussion

K. pneumoniae has been one of the principal pathogens causing neonatal sepsis in the NHRU at LAUH. Despite the implementation of measures for controlling and preventing nosocomial infection, the rate of *K. pneumonia* isolation continues to increase due to patient overcrowding (the NHRU of LAUH is the only public neonatology service available in the Andes region of the country). Thus, periodic outbreaks continue to occur, such as the outbreak at the end of February 2007 that affected five infants hospitalized in the same location over a period of 10 days (González et al., 2011).

The ESBL-producing C1/ST70 CTX-M-15 clone was responsible for the 2007 outbreak. The C2/ST1261 CTX-M-2–producing clone also circulated and was isolated as the predominant clone in the unit in 2009, in this case producing different ESBLs. There was no predominant clone in the AICU. However, the ST70 clone isolated in the NHRU did spread to the AICU, where an EBC AmpC-producing *K. pneumoniae* strain was also isolated. In prior studies in the NHRU, *K. pneumoniae* strains isolated during a 1998–1999 outbreak carried genes coding for the production of two β-lactamases, one SHV-5 and one TEM-1, as well as others determining resistance to aminoglycosides, chloramphenicol, and tetracycline (Araque and Rivera, 2004) . A subsequent study in the AICU found that three *K. pneumoniae* strains isolated from infected infants were carriers of CTX-M-1 and CTX-M-2 (Millan et al., 2013) Other national studies in Venezuela reported that four *K. pneumoniae* strains isolated from patients with nosocomial infection in the "Antonio Patricio de Alcalá" University Hospital in Cumaná were CTX-M-2 producers (Guzman et al., 2009). This same enzyme was reported in Enterobacteriaceae isolated from hospitals in Caracas (Torres et al., 2006).

Some strains isolated from both the NHRU and AICU were CTX-M-2 producers. However, this is the first time that strains producing both CTX-M-15 and SHV-12 are reported together in the two most important units of LAUH. These two enzymes have frequently been reported in Europe, where they are more prevalent than in the United States (Coque et al., 2008). CTX-M-15 predominates in Western Europe, the United Kingdom, Italy, France, and Portugal, where its presence has grown rapidly (Cantón et al., 2008; Livermore et al., 2007). A recent study in Venezuela on the frequency and diversity of CTX enzymes in Enterobacteriaceae isolates, found that CTX-M-1 was the most common (91%) followed by CTX-M-2, whereas there was a lower percentage of CTX-M-15 and CTX-M-14 . This recent study was the first to report CTX-M-15– and CTX-M-14–producing Enterobacteriaceae in Caracas, Venezuela (Redondo et al., 2013).

In the present study, we provide the first report of CTX-M-15–producing *K. pneumoniae* strains associated with an NHRU outbreak in Mérida, which spread to another intensive care unit at LAUH. The predominance of the C1/ST70 clone among strains isolated in the NHRU during the 2007 outbreak and its presence in two AICU strains in 2009 show that this epidemic clone circulated in the hospital at different times and in different units, indicating its ability to produce periodic outbreaks.

The possible generalization of CTX-M-15 has been reported in Argentina. This clone often associates with other clones across the world, such as *E. coli* ST131 and *K. pneumoniae* ST11 (Sennati *et al*., 2012; Diestra *et al*., 2009). Other STs capable of producing and spreading CTX-M-15 have been described, such as ST15, which produced epidemic outbreaks in Hungary (Damjanova *et al*., 2008). Likewise, in studies on CTX-M-15–producing *K. pneumoniae* strains from infants and adults in Spain, the most prevalent STs were ST1, ST11, ST13, ST14, ST15, ST16, ST20, ST35, ST36, and ST134 (Oteo et al., 2009). Here, we describe the C2/ST126 clone for the first time at a global level. Strains of this clone produce different types of ESBLs, suggesting the possible plasmidic dissemination of *bla* genes in a previously predominant sensitive clone.

Conclusion

In conclusion, the results of our study indicate that different ESBL-producing *K. pneumoniae* clones (ST70, ST1261, and ST23) circulated in the NHRU and AICU of LAUH in Mérida, Venezuela. Clones ST70 and ST23 produced the CTX-M-15 ESBL, described for the first time in Venezuela. The CTX-M-2– and SHV-12–producing clone ST1261 is described for the first time at a global level.

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Conflicts of interest. All authors report no conflicts of interest relevant to this article

References:

Perlman E, Saiman L, Larson R. (2007). Risk Factors for Late-Onset Health Care-Associated Bloodstream Infections in Patients in Neonatal Intensive Care Units. Am J Infect Control 35(3): 177–182.

Alonso G, Malaver E, Guzmán M, Rodríguez V. (2005). Caracterización de Plásmidos e Integrones Presentes en Bacterias Multirresistentes Aisladas en Diferentes Ambientes de Venezuela. MIBE 4, 81-4.

Lynch JP, Clark NM, Zhanel GG. (2013). Evolution of antimicrobial resistance among Enterobacteriaceae (focus on extended spectrum β-lactamases and carbapenemases). Expert Opin Pharmacother 14(2): 199-210.

Vignoli R, Cordeiro N, Seija V, Schelotto F, Radice M, Ayala J. (2006). Entorno Genético de CTX-M-2 en Aislamientos de *Klebsiella pneumoniae* Provenientes de Pacientes Hospitalizados en Uruguay. Rev Argent Microbiol 38, 84-88.

Araque M & Rivera I. (2004). Simultaneus Presence of bla_{TEM} and bla_{SHV} Genes on a Large Conjugative Plasmid Carried by Extended Spectrum β-lactamase-Producing *Klebsiella pneumoniae*. Am J Med Sci 327, 118-122.

Millán B, Castro D, Araque M, Ghiglione B, Gutkind G. (2013). ISCR1 asociado con genes blaCTX-M-1 y blaCTX-M-2 en plásmidos IncN e IncFIIA aislados en *Klebsiella pneumoniae* de origen nosocomial en Mérida, Venezuela. Bioméd 33, 12-16.

Guzmán M & Alonso G. (2009). Characterization of extended-spectrum ß-lactamases in nosocomial strains of *Klebsiella pneumoniae*. Sucre-Venezuela. Invest Clin 50, 419 – 431.

Torres L, Gagliotta V, Torres O, Benítez M, Domínguez M, Pedroza R. (2006). Extended-Spectrum β-Lactamases in Enterobacteriaceae isolated in Health Centres of Caracas . Rev Soc Venez Microbiol 26,80-88.

González A, Nieves B, Solórzano M, Cruz J, Puig J, Moreno M. (2013). Characterization of extended-spectrum β-lactamases-producing *Klebsiella pneumoniae* isolates of two intensive care units. Rev Chil Infect 30, 374-380.

González A, Gil F, Solórzano M, Cruz J, Puig J, Suárez M. & other authors. (2011). Outbreak of Multiresistant and Extended Spectrum β-lactamase Producing *Klebsiella pneumoniae* In a High Risk Neonatal Unit. Rev Chil Infect 28, 28-34.

Clinical and Laboratory Standards Institute. (CLSI). (2013). Performance and standards for antimicrobial susceptibility testing. Twentieth informational supplement. Document M100-S23. Wayne, PA.

Yagi T. (2005). Practical methods using boronic acid compounds for identification of class C β-lactamase-producing *Klebsiella pneumoniae* and Escherichia coli. J Clin. Microbiol 43, 2551–2558.

Oteo J, Navarro C, Cercenado E, Delgado-Iribarren A, Wilhelmi I, Orden B. & other authors . Spread of *Escherichia coli* strains with high-level cefotaxime and ceftazidime resistance between the community, long-term care facilities, and hospital institutions. J Clin Microbiol 44, 2359-66.

Pérez-Pérez J & Hanson N. (2002) Detection of Plasmid-Mediated AmpC β-Lactamase Genes in Clinical Isolates by Using Multiplex PCR. J. Clin. Microbiol 40(6), 2153.

Coque TM, Baquero F, Canton R. (2008). Increasing prevalence of ESBL-producing Enterobacteriaceae in Europe. Euro Surveill 20, 13.

Cantón R, Novais A, Valverde A, Machado E, Peixe L, Baquero F & other authors. (2008). Prevalence and spread of extended-spectrum beta-lactamase-producing Enterobacteriaceae in Europe. Clin Microbiol Infect 14, 144-53.

Livermore D, Canton R, Gniadkowski M, Nordmann P, Rossolini G, Arlet G & other authors (2007). CTX-M: changing the face of ESBLs in Europe. J Antimicrob Chemother 59, 165-74.

Redondo C, Chalbaud A, Alonso G. (2013). Frequency and diversity of CTX-M enzymes among extended-spectrum β-lactamase-producing Enterobacteriaceae isolates from Caracas, Venezuela. Microb Drug Resist. 19, 42-7.

Sennati S, Santella G, Di Conza J, Pallecchi L, Pino M, Ghiglione B & other authors.(2012). Changing Epidemiology of Extended-Spectrum β-Lactamases in Argentina: Emergence of CTX-M-15. Antimicrob Agents Chemother 56, 6003–6005.

Diestra K, Juan C, Curiao T, Moyá B, Miró E, Oteo J, & other authors (2009). Characterization of plasmids encoding blaESBL and surrounding genes in Spanish clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae.* J Antimicrob Chemother 63, 60-6.

Damjanova I, Tóth A, Pászti J, Hajbel-Vékony G, Jakab M, Berta J & other authors. (2008). Expansion and countrywide dissemination of ST11, ST15 and ST147 ciprofloxacin-resistant CTX-M-15-type beta-lactamase producing *Klebsiella pneumoniae* epidemic clones in Hungary in 2005--the new 'MRSAs'? J Antimicrob Chemother 62, 978-85.

Oteo J, Cuevas O, López-Rodríguez I., Banderas-Florido A, Vindel A, Pérez-Vázquez M., & other authors. (2009). Emergence of CTX-M-15-producing *Klebsiella pneumoniae* of multilocus sequencetypes 1, 11, 14, 17, 20, 35 and 36 as pathogens and colonizers in newborns and adults. J Antimicrob Chemother 64, 524-8.