JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2011, p. 1134–1136 Vol. 49, No. 3
0095-1137/11/$12.00 doi:10.1128/JCM.02514-10

Klebsiella pneumoniae Strains Producing Extended-Spectrum β-Lactamases in Spain: Microbiological and Clinical Features[▽]

C. Ruiz de Alegría,[1] J. Rodríguez-Baño,[2,3] M. E. Cano,[1] J. R. Hernández-Bello,[3] J. Calvo,[1] E. Román,[1] M. A. Díaz,[4] A. Pascual,[4,5] and L. Martínez-Martínez[1,6]*
for the Spanish Group for Nosocomial Infections (GEIH)

Service of Microbiology, University Hospital Marqués de Valdecilla-IFIMAV, Santander,[1] Infectious Disease Unit[2] and Service of Microbiology,[4] University Hospital Virgen Macarena, Seville, Departments of Medicine[3] and Microbiology,[5] University of Seville, Seville, and Department of Molecular Biology, University of Cantabria, Santander,[6] Spain

Received 11 December 2010/Accepted 18 December 2010

Extended-spectrum β-lactamases (ESBL) of the CTX-M, SHV, and TEM families were recognized in 76 (67%), 31 (27%), and 6 (5%) isolates, respectively, among 162 ESBL-producing *Klebsiella pneumoniae* (ESBL-Kp) strains obtained in a multicenter study in Spain. Predisposing factors for ESBL-Kp acquisition included invasive procedures, mechanical ventilation, and previous antimicrobial use.

Extended-spectrum β-lactamases (ESBL) currently represent a major problem, antibiotic resistance in enterobacteria (20). During the last decade, strains of both *Escherichia coli* and *Klebsiella pneumoniae* producing CTX-M enzymes (particularly CTX-M-15) have been increasingly isolated on all continents (1, 5–7, 9, 10, 15–17). In a multicenter study on ESBL-producing *E. coli* and ESBL-producing *K. pneumoniae* (ESBL-Kp) carried out in 2000 in Spain (12, 13), the mean prevalence of ESBL production in *K. pneumoniae* was 2.7%. The most common enzymes detected were TEM-derived β-lactamases, and only three isolates producing CTX-M enzymes (specifically CTX-M-10) were identified (12).

A multicenter study was repeated in 2006 (1 February to 31 March) (GEIH-BLEE-2006) (8). Consecutively obtained *K. pneumoniae* strains from clinical samples (1 per patient) with an ESBL production phenotype were obtained in 44 centers. Organisms were confirmed to be *K. pneumoniae* with API 20E strips (bioMérieux, France). Confirmation of ESBL production and testing of susceptibility to aztreonam, cefepime, cefpodoxime, ceftriaxone, cefoxitin, cefotetan, imipenem, and meropenem were performed with ESBL-Plus panels (Microscan; Dade). Susceptibility to other agents indicated in Table 1 was determined by broth microdilution according to CLSI guidelines (3). Clinical categories were defined according to the 2010 document from the CLSI (4).

Clonal relationships of the isolates were determined by repetitive extragenic palindromic PCR (REP-PCR) as described elsewhere (2). Pulsed-field gel electrophoresis (PFGE) (14, 21) was also performed for 29 organisms from the two centers with more than 10 isolates and for 56 isolates presenting identical REP-PCR patterns isolated in different centers or with just one single band of difference. Transmission of resistance by conjugation to *E. coli* J-53 (azide resistant) was evaluated according to REP-PCR/PFGE patterns, including (i) all isolates representing patterns with single isolates, (ii) one isolate of every different antibiogram type (>4-fold difference in the MICs of at least two agents of different biochemical groups) for patterns including 2 to 4 isolates, and (iii) two isolates of every different antibiogram type (as defined above) for patterns including ≥5 isolates. Selection was done on plates containing either cefotaxime (2 mg/liter) or ampicillin (100 mg/liter). ESBL production was confirmed in transconjugants by disk diffusion (disks from Oxoid, United Kingdom) according to CLSI guidelines (4). β-Lactamase genes were detected by PCR and sequencing in the parental isolates selected for conjugation studies or in derived transconjugants, using previously described primers and conditions for TEM (19), SHV (19), and CTX-M (18) enzymes. CTX-M groups were determined according to the method of Woodford et al. (22). CTX-M-15 was differentiated from CTX-M-28 by PCR as described previously (13a). Epidemiological and clinical data from patients in whom ESBL-producing *Klebsiella pneumoniae* (ESBL-Kp) was isolated were collected using a structured questionnaire. ESBL-Kp strains isolated after 48 h of hospital admission were considered nosocomially acquired (NA) (11). Among the rest, ESBL-Kp strains were considered health care associated (HCA) if the patient was admitted to an acute- or long-term-care center or received hemodialysis, specialized home care, or care in a day hospital during the preceding 3 months. All other isolates were considered to be community acquired (CA). Qualitative variables were compared using the chi-square test or the Fisher exact test as appropriate. The project was approved by the Ethic Committee of the Hospital Universitario Virgen Macarena, which waived the need for informed consent due to the observational nature of the study.

ESBL-Kp strains were isolated in 32 out of the 44 (72.7%) participating centers. In all, 162 isolates (1 to 16 per center), corresponding to 80 clones (1 to 9 per center), were obtained. A predominant clone was present in a specific hospital in most cases, although in 12 and 8 centers, ≥3 and 2 clones were identified, respectively.

The results of susceptibility testing are presented in Table 1. One (0.6%) isolate was susceptible to cefotaxime, 7 (4.3%) and 12 (7.4%) isolates were intermediate and susceptible to ceftazidime,

* Corresponding author. Mailing address: Service of Microbiology, University Hospital Marqués de Valdecilla, Avda. Valdecilla s/n, 39008 Santander, Spain. Phone: 34 942 202580. Fax: 34 942 203462. E-mail: lmartinez@humv.es.

▽ Published ahead of print on 29 December 2010.

TABLE 1. *In vitro* activities of antimicrobial agents against ESBL-producing *K. pneumoniae* isolates in the GEIH-BLEE-2006 study ($n = 162$)[a]

Agent	MIC (mg/liter)			% susceptible isolates	
	Range	50%	90%	2006 study	2000 study[b]
Cefotaxime	≤0.5–>128	>128	>128	0.6	—
Ceftazidime	≤0.5–>128	>128	64	7.4	—
Cefepime	≤1–>32	>32	>32	8.0	—
Aztreonam	≤0.5–>64	>64	>64	6.8	—
Cefoxitin	≤2–>32	4	16	87.1	94
Cefotetan	≤1–4	≤1	≤1	100	98.5
Imipenem	≤0.5–1	≤0.5	≤0.5	100	100
Meropenem	≤0.5–2	≤0.5	≤0.5	100	100
Ertapenem	≤0.008–16	0.125	0.5	98.2	Not tested
Amoxicillin-clavulanate[c]	8–>128	64	>128	4.3	40
Piperacillin-tazobactam[d]	1–>1,024	16	>1,024	55.6	74
Nalidixic acid	2–>1,024	>1,024	>1,024	28.0	Not tested
Ciprofloxacin	≤0.008–>128	32	>128	37.8	88.5
Gentamicin	≤0.125–>128	8	128	49.4	33.0
Tobramycin	≤0.125–>128	16	64	35.9	38.5
Amikacin	0.25–64	1	8	98.1	91.0
Tigecycline	≤0.008–8	0.5	4	Not applicable	Not tested
Co-trimoxazole[e]	≤0.062–>32	>32	>32	27.2	40.0

[a] Susceptibility defined by 2010 CLSI breakpoints (4). Data on percentages of susceptibility from isolates obtained in the study performed in 2000 ($n = 70$) are also presented for comparison.
[b] —, not determined (all organisms considered resistant because of ESBL production).
[c] The concentrations of amoxicillin are indicated.
[d] The concentrations of piperacillin are indicated.
[e] The concentrations of trimethoprim are indicated.

respectively, 14 (8.6%) and 13 (8.0%) were intermediate and susceptible to cefepime, respectively, and 4 (2.5%) and 11 (6.8%) were intermediate and susceptible to aztreonam, respectively. However, whether the patients from whom ESBL-Kp strains susceptible to expanded-spectrum cephalosporins or aztreonam were isolated may respond satisfactorily when treated with these agents is not yet completely defined. Carbapenems were very active, and only 2 (1.2%) and 1 (0.6%) isolates were resistant and intermediate to ertapenem, respectively. Amikacin and tigecycline also presented good *in vitro* activity against the tested isolates. ESBL-Kp isolates in Spain in 2006 were more resistant than the isolates studied in 2000 to amoxicillin-clavulanate, piperacillin-tazobactam, ciprofloxacin, and co-trimoxazole (Table 1). Increased resistance to amoxicillin-clavulanate might be related to the dissemination of plasmids coding for CXT-M-15 (see below) that usually also contain other determinants of resistance to this combination. The decreased activity of ciprofloxacin may be related in part to the presence of *aac*(*6′*)*-Ib-cr*, which was detected in 34.2% of 114 isolates studied (unpublished data).

In 2006, ESBL were identified in 105 out of the 114 (92.1%) isolates. The ESBL identified in 2006 clearly differ from those observed in 2000 (12, 13). An SHV amplicon was obtained in 107 (93.8%) of these 114 parental isolates, but an SHV-type ESBL was identified in only 31 isolates, of which 21 contained SHV-12, 6 contained SHV-2, 3 contained SHV-5, and 1 contained SHV-33. The remaining 76 SHV amplicons presumably correspond to the non-ESBL variants of the chromosomal SHV enzyme of *K. pneumoniae*. Sequencing in 37 randomly chosen isolates from which SHV-type enzymes were not identified in transconjugants confirmed in all cases the presence of either SHV-1 (21 isolates; 56.8%) or SHV-11 (16 isolates; 43.2%). A gene coding for a TEM enzyme was detected in 55 (48.2%) out of the 114 parental isolates, but a TEM-type ESBL was demonstrated in only 6 (5.3%) transconjugants (TEM-4 in 3 cases and TEM-3, TEM-15, and TEM-74 in 1 case each). Sequencing of the gene in the remaining 49 cases demonstrated the presence of TEM-1. Genes coding for CTX-M ESBL were detected in 76 (66.7%) of the 114 isolates, with the following distribution: CTX-M-15 genes in 40 isolates, CTX-M-1 genes in 15 isolates, CTX-M-14 genes in 14 isolates, CTX-M-32 genes in 4 isolates, and CTX-M-9 genes in 3 isolates. Isolates with CTX-M-15 were present in 18 hospitals, and those with SHV-12 were present in 14 centers. CTX-M-14 and CTX-M-1 were also broadly distributed (each enzyme in strains from 8 centers). This distribution of ESBL among *K. pneumoniae* strains mirrors that obtained in the *E. coli* strains isolated during the same period (9).

ESBL-Kp strains were obtained from 133 adults and 29 pediatric patients (of whom 26 were from neonates). Complete clinical data were available for 102 adults and 23 neonates (Table 2). Acquisition was considered nosocomial for 85 (64%) adult patients admitted to medical services (35 patients), surgical services (25 patients), or intensive care units (ICUs) (25 patients). Acquisition of the pathogen was health care associated for 28 (21%) adults (22 received specialized ambulatory care, 10 had previous hospital admission, 3 were nursing home residents, and 1 was on hemodialysis). For the remaining 17 (13%) adults, a strict community origin was considered. One hundred three out of the 133 (77%) adult patients were considered to have an infection. Clustered isolates were more frequently nosocomial than nonnosocomial (77% versus 59%; $P = 0.01$), while sporadic isolates were more frequently strictly community acquired than health care related or nosocomial (17% versus 6%; $P = 0.01$). The most frequent ESBL in adults were CTX-M-15 (47 isolates), SHV-12 (29 isolates), and CTX-M-14 (18 isolates). Empirical therapy was inappropriate in about half of adult patients. However, since our series included

TABLE 2. Sites of infection, therapies, and prognoses of 127 patients with infection due to ESBL-producing *K. pneumoniae*

Variable	Adults (n = 104)	Neonates (n = 23)
No. (%) of patients with:		
Infection type		
Urinary tract	50 (49)[a]	9 (39)
Respiratory tract	32 (22)[b]	5 (22)
Skin and soft tissue	19 (18)[c]	1 (5)
Primary bacteremia	7 (7)	5 (22)
Other	3 (3)[d]	1 (5)[e]
Appropriate empirical therapy	58 (56)	14 (88)
Median hospital stay after infection (no. of days [interquartile range])	24 (13–47)	19 (5–40)
Mortality during admission (no. [%] of patients)	14 (14)	2 (9)

[a] Infections were cystitis (n = 48), prostatitis (n = 1), and pyelonephritis (n = 1).
[b] Infections included pneumonia (n = 14) and tracheobronchitis (n = 9).
[c] Infections were cellulitis (n = 11), chronic ulcer infection (n = 7), and necrotizing cellulitis (n = 1).
[d] Infections were osteomyelitis (n = 2) and cholangitis (n = 1).
[e] Intra-abdominal infection.

many patients with noninvasive infections (mostly cystitis), mortality in this series was low.

Acquisition of ESBL-Kp was considered to be nosocomial for all neonates, of which 23 (88%) were considered to be infected. Among neonates, the most frequent ESBL was again CTX-M-15 (19 patients). Clusters of related isolates were found in 5 of 7 neonatal units with cases. This study confirms the relevant role of ESBL-Kp as a pathogen in neonatal units. The fact that most neonates received appropriate therapy (mostly carbapenems) indicates a high level of suspicion within units affected by outbreaks. However, such extensive use of carbapenems might facilitate the spread of carbapenem resistance, and thus control of ESBL-Kp within neonatal units should be considered a priority.

The study group GEIH-BLEE-2006 (Spain) is represented by C. Martínez Peinado (Villajoyosa, Alicante), J. F. Ordás (Cangas de Nancea, Asturias), E. Garduño (Badajoz), M. A. Domínguez (Barcelona), F. Navarro (Barcelona), G. Prats (Barcelona), F. Marco (Barcelona), E. Ojeda (Burgos), P. Marín (Cádiz), R. Carranza (Alcazar de San Juan, Ciudad Real), F. Rodríguez (Córdoba), C. García Tejero (Figueras, Gerona), F. Artiles (Gran Canaria), B. Palop (Granada), I. Cuesta (Jaén), M. Cartelle (A Coruña), M. D. Rodríguez (Ferrol, A Coruña), I. Fernández (León), E. Ugalde (Logroño), R. Cantón (Madrid), E. Cercenado (Madrid), F. Chaves (Madrid), J. J. Picazo (Madrid), A. Delgado (Alcorcón, Madrid), C. Guerrero (Murcia), B. Fernández (Orense), A. Fleites (Oviedo), A. Oliver (Palma de Mallorca), J. J. García (Pamplona), M. García (Pontevedra), J. Elías (Salamanca), L. Martínez-Martínez (Santander), M. Treviño (Santiago de Compostela), M. Ruiz (Seville), M. A. Díaz (Seville), M. Lara (Tenerife), L. Torres (Teruel), E. García (Toledo), D. Navarro (Valencia), M. Gobernado (Valencia), A. Tenorio (Valladolid), I. Otero (Vigo), L. Michaus (Vitoria), and J. Castillo (Zaragoza).

This study was partially supported by an unrestricted grant from Wyeth Laboratories (Spain), by the Ministerio de Sanidad y Consumo, Instituto de Salud Carlos III-FEDER, by the Spanish Network for Research in Infectious Diseases (grant REIPI RD06/0008), by FIS (grants PI070190 and INT08/151), and by the Junta de Andalucía (grants 0048/2008, CTS-5209, and 0034/2009). E.R. is supported by IFIMAV, Cantabria, Spain.

J.R.-B. reports that he has been a consultant for Wyeth, Merck, and Pfizer, has served as speaker for Wyeth, Merck, Pfizer, AstraZeneca, and GlaxoSmithKline, and has received research support from Merck and Wyeth. A.P. reports that he has been a consultant for Merck and Pfizer, has served as a speaker for Wyeth, AstraZeneca, Merck, and Pfizer, and has received research support from Merck, Pfizer, and Wyeth. L.M.-M. reports that he has been a consultant for Wyeth and Pfizer, has served as speaker for Wyeth, Merck, Pfizer, and Janssen-Cilag, and has received research support from Merck, Wyeth, and Janssen-Cilag. All other authors report no conflicts.

REFERENCES

1. **Blanco, M., et al.** 2009. Molecular epidemiology of *Escherichia coli* producing extended-spectrum β-lactamases in Lugo (Spain): dissemination of clone O25b: H4-ST131 producing CTX-M-15. J. Antimicrob. Chemother. **63:**1135–1141.
2. **Cano, M. E., et al.** 2009. Detection of plasmid-mediated quinolone resistance genes in clinical isolates of *Enterobacter* spp. in Spain. J. Clin. Microbiol. **47:**2033–2039.
3. **Clinical and Laboratory Standards Institute.** 2009. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard, 8th ed. M07–A8. Clinical Laboratory Standards Institute, Wayne, PA.
4. **Clinical and Laboratory Standards Institute.** 2010. Performance standards for antimicrobial susceptibility testing; 20th informational supplement. M100–S20. Clinical Laboratory Standards Institute, Wayne, PA.
5. **Coelho, A., et al.** 2009. Detection of three stable genetic clones of CTX-M-15-producing *Klebsiella pneumoniae* in the Barcelona metropolitan area, Spain. J. Antimicrob. Chemother. **64:**862–864.
6. **Coque, T. M., et al.** 2008. Dissemination of clonally related *Escherichia coli* strains expressing extended-spectrum beta-lactamase CTX-M-15. Emerg. Infect. Dis. **14:**195–200.
7. **Dedeic-Ljubovic, A., et al.** 2010. Emergence of CTX-M-15 extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* isolates in Bosnia and Herzegovina. Clin. Microbiol. Infect. **16:**152–156.
8. **Díaz, M. A., J. R. Hernández, L. Martínez-Martínez, J. Rodríguez-Baño, and A. Pascual.** 2009. Extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in Spanish hospitals: 2nd multicenter study (GEIH-BLEE project, 2006). Enferm. Infecc. Microbiol. Clin. **27:**503–510.
9. **Díaz, M. A., et al.** 2010. The diversity of *Escherichia coli* producing extended-spectrum β-lactamases in Spain: second nationwide study. J. Clin. Microbiol. **48:**2840–2845.
10. **Diestra, K., et al.** 2008. Characterization and molecular epidemiology of ESBL in *Escherichia coli* and *Klebsiella pneumoniae* in 11 Spanish hospitals (2004). Enferm. Infecc. Microbiol. Clin. **26:**404–410.
11. **Garner, J. S., W. R. Jarvis, T. G. Emori, T. C. Horan, and J. M. Hughes.** 1988. CDC definitions for nosocomial infections. Am. J. Infect. Control **16:**128–140.
12. **Hernández, J. R., et al..** 2005. Nationwide study of *Escherichia coli* and *Klebsiella pneumoniae* producing extended-spectrum beta-lactamases in Spain. Antimicrob. Agents Chemother. **49:**2122–2125.
13. **Hernández, J. R., A. Pascual, R. Cantón, L. Martínez-Martínez, and Grupo de Estudio de Infección Hospitalaria (GEIH).** 2003. Extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in Spanish hospitals (GEIH-BLEE project 2002). Enferm. Infecc. Microbiol. Clin. **21:**77–82.

13a. **Leflon-Guibout, V., et al.** 2004. Emergence and spread of three clonally related virulent isolates of CTX-M-15-producing *Escherichia coli* with variable resistance to aminoglycosides and tetracycline in a French geriatric hospital. Antimicrob. Agents Chemother. **48:**3736–3742.

14. **Maslow, J. N., et al.** 1993. Relationship between indole production and differentiation of *Klebsiella* species: indole-positive and -negative isolates of *Klebsiella* determined to be clonal. J. Clin. Microbiol. **31:**2000–2003.
15. **Nicolas-Chanoine, M. H., et al.** 2008. Intercontinental emergence of *Escherichia coli* clone O25:H4-ST131 producing CTX-M-15. J. Antimicrob. Chemother. **61:**273–281.
16. **Oteo, J., et al.** 2009. Emergence of CTX-M-15-producing *Klebsiella pneumoniae* of multilocus sequence types 1, 11, 14, 17, 20, 35 and 36 as pathogens and colonizers in newborns and adults. J. Antimicrob. Chemother. **64:**524–528.
17. **Oteo, J., et al..** 2008. Antibiotic-resistant *Klebsiella pneumoniae* in Spain: analyses of 718 invasive isolates from 35 hospitals and report of one outbreak caused by an SHV-12-producing strain. J. Antimicrob. Chemother. **61:**222–224.
18. **Pagani, L., et al.** 2003. Multiple CTX-M-type extended-spectrum beta-lactamases in nosocomial isolates of *Enterobacteriaceae* from a hospital in northern Italy. J. Clin. Microbiol. **41:**4264–4269.
19. **Rasheed, J. K., et al.** 1997. Evolution of extended-spectrum beta-lactam resistance (SHV-8) in a strain of *Escherichia coli* during multiple episodes of bacteremia. Antimicrob. Agents Chemother. **41:**647–653.
20. **Rodríguez-Baño, J., and A. Pascual.** 2008. Clinical significance of extended-spectrum beta-lactamases. Expert Rev. Anti Infect. Ther. **6:**671–683.
21. **Tenover, F. C., et al.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33:**2233–2239.
22. **Woodford, N., E. J. Fagan, and M. J. Ellington.** 2006. Multiplex PCR for rapid detection of genes encoding CTX-M extended-spectrum (beta)-lactamases. J. Antimicrob. Chemother. **57:**154–155.