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


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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,
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 activity against the tested isolates. ESBL-Kp isolates in Spain in 2006 were more resistant than those with SHV-12 present in 14 centers. CTX-M-14 and CTX-M-1 genes in 15 isolates, CTX-M-14 genes in 14 isolates, CTX-M-15 genes in 40 isolates, CTX-M-15 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 neonatal services (15 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

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

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 neonatal units with cases. This study confirms the relevant role of ESBL-Kp as a pathogen in neonatal units. The fact that most neonatal units with cases.

REFERENCES