Carbapenemase-Producing Enterobacteriaceae in Spain in 2012

Jesús Oteo,1 David Saez,1 Verónica Bautista,1 Sara Fernández-Romero,1 Juan Manuel Hernández-Molina,1 María Pérez-Vázquez,1 Belén Aracil,2 José Campos,1,2,6 the Spanish Collaborating Group for the Antibiotic Resistance Surveillance Program

Antibiotic Laboratory, Bacteriology Department, Centro Nacional de Microbiología, Majadahonda, Madrid, Spain; Microbiology Department, Hospital Universitario Carlos Haya, Málaga, Spain; Consejo Superior de Investigaciones Científicas, Madrid, Spain

We report the epidemiological impact of carbapenemase-producing Enterobacteriaceae (CPE) in Spain in 2012. Of the 237 carbapenemases detected, 163 were from the OXA-48 group, 60 were from VIM-1, 8 were from KPC-2, 5 were from IMP, and 1 was from NDM-1. Interhospital spread of carbapenemase-producing Klebsiella pneumoniae was due to a limited number of multilocus sequence types (MLST) and carbapenemase types, including ST15–VIM-1, ST11–OXA-48, ST405–OXA-48, ST101–KPC-2, and ST11–VIM-1. The number of CPE cases in Spain has increased sharply in recent years, due mainly to the emergence of OXA-48.

In recent years, Enterobacteriaceae isolates, mainly Klebsiella pneumoniae, have increased their potential to become extensively drug resistant by acquiring resistance to carbapenems (1–3), due mainly to the production of carbapenemases.

In general, carbapenemases hydrolyze all β-lactam antibiotics (1–3). The most clinically important carbapenemases produced by Enterobacteriaceae are the class B metallo-β-lactamases (MBLs), represented by VIM, IMP, and NDM types, the class A enzymes of the KPC type, and the class D enzymes, represented by the OXA-48 type (3). In Spain, the number of reports on carbapenemase-producing Enterobacteriaceae (CPE) has increased in recent years (4–10). However, comprehensive assessment of the impact of CPE in Spain is still missing.

Our institute has run an active and unrestricted Antibiotic Resistance Surveillance Program at the national level since 2009. When this program was launched, all Spanish clinical microbiology laboratories and health-associated professionals were personally contacted and encouraged to submit their carbapenem-resistant Enterobacteriaceae isolates to our antibiotic reference lab for molecular and epidemiological characterization.

Enterobacteriaceae isolates were identified by standard microbiological methods and a MicroScan semiautomated system (MicroScan; Siemens Healthcare Diagnostics, Deerfield, IL, USA). If necessary, species identification was confirmed by 16S ribosomal DNA sequencing.

Antibiotic susceptibility testing was carried out by broth microdilution (panel type Neg MIC 31; MicroScan) and by the disc diffusion method according to the EUCAST guidelines (11). Isolates were considered nonsusceptible to carbapenems if they were either resistant or intermediate to at least one of the three carbapenem antibiotics tested (imipenem, meropenem, and ertapenem) according to EUCAST breakpoints (11). A modified Hodge test using an ertapenem disk was performed on all isolates. Inhibition of carbapenemase activity was carried out by comparing the inhibition zones obtained from ertapenem disks, with or without EDTA (10 μl 0.5 M solution) and phenyl-boronic acid (400 μg).

The presence of genes encoding carbapenemases, blaKPC, blaVIM, bladOXA, and blaNDM, was confirmed by PCR and DNA sequencing (5, 12, 13). Specific primers for PCR amplification and sequencing of blaOXA-48-like genes were designed according to GenBank (National Center for Biotechnology Information, National Institutes of Health, Bethesda, MD, USA) database entry AY236073 (OXA-48-TOT-F, 5’-TGGTGTTAGGCTTATC G-3’; OXA-48-TOT-R, 5’-TTTTCTGTTTGTAGCCTTC-3’).

Multilocus sequence type (MLST) was determined in all carbapenemase-producing K. pneumoniae isolates according to the Institut Pasteur scheme (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html; data last accessed in May 2013). Escherichia coli isolates were typed by MLST according to the University College Cork (Cork, Ireland) scheme (http://mlst.ucc.ie/mlst/dbs/Ecoli; data last accessed in May 2013).

In 2012, 357 isolates of Enterobacteriaceae nonsusceptible to carbapenems were studied in detail, and only one isolate per patient was considered. They came from 49 Spanish hospitals (about 20% of all Spanish microbiology laboratories) located in 24 geo-
infections (UTI), 37 (22.8%) blood infections, 28 (17.3%) respiratory tract infections, 13 (8%) wound infections, and 13 (8%) other infections. The remaining 75 isolates (31.6%) were obtained from carriers, mainly from rectal samples.

The carbapenemases detected were from the following groups: 163 from OXA-48 (84 from OXA-48 and 79 from OXA-245), 60 from VIM-1, 8 from KPC-2, 5 from IMP (2 from IMP-22 and 3 from IMP-8), and 1 from NDM-1 (Table 1). These CPE isolates came from 30 Spanish hospitals (average of 8.1 CPE isolates per hospital, range of 1 to 83) located in 14 geographic areas. Six hospitals had more than 10 CPE cases; the remaining 24 hospitals had between one and nine cases.

Susceptibility to carbapenem antibiotics is depicted in Table 2; all carbapenemase-producing isolates were ertapenem nonsusceptible, but of the OXA-48-like and VIM-1 producers, 66.3% and 15% were susceptible to imipenem, respectively.

From 2009 to 2012, we observed an increase in the number of CPE isolates submitted to the surveillance program: 15 isolates in 2009, 38 in 2010, 112 in 2011, and 237 in 2012 (16-fold increase). The number of hospitals submitting cases increased from 6 in 2009 to 30 in 2012 (5-fold increase) (Fig. 1). Although VIM-1 was the first carbapenemase described in Spain (14), its frequency has been widely surpassed by the abrupt emergence of OXA-48 in the last 2 years (Fig. 1).

The frequency and distribution of carbapenemases are distinct in different countries. A rapid dissemination of KPC-producing K. pneumoniae was first noticed in the United States (3). Later, isolates producing KPC-2 and KPC-3 also emerged in Latin America, Israel, and Greece (1, 3, 16). A recent study showed that KPC enzymes were the most common (89.5%) found in Italy (15). Outbreaks caused by OXA-48-producing K. pneumoniae have been described in several countries (1, 3, 16).

According to our data, OXA-48 is by far the most common carbapenemase type circulating in Spain in K. pneumoniae (75.4% in this study), followed by VIM (19.7%) (Table 1). The carbapenemase-producing K. pneumoniae isolates belonged to 12 different sequence types (STs) (Table 3), although most of them (88.7%) were carried by four major clones: ST11, ST15, ST16, and ST405.

### TABLE 2 Susceptibility to carbapenem antibiotics in carbapenemase-producing Enterobacteriaceae isolated in Spain (2012)

<table>
<thead>
<tr>
<th>Carbapenemase type (no. of isolates)</th>
<th>MIC (µg/ml)</th>
<th>%&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Carbapenem</td>
<td>50%</td>
</tr>
<tr>
<td>OXA-48 like (n = 163)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ertapenem</td>
<td>2–&gt;4</td>
<td>4</td>
</tr>
<tr>
<td>Imipenem</td>
<td>≤1–&gt;8</td>
<td>2</td>
</tr>
<tr>
<td>Meropenem</td>
<td>≤1–&gt;8</td>
<td>4</td>
</tr>
<tr>
<td>VIM-1 (n = 60)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ertapenem</td>
<td>1–&gt;4</td>
<td>4</td>
</tr>
<tr>
<td>Imipenem</td>
<td>≤1–&gt;8</td>
<td>4</td>
</tr>
<tr>
<td>Meropenem</td>
<td>≤1–&gt;8</td>
<td>8</td>
</tr>
<tr>
<td>IMP-like (n = 5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ertapenem</td>
<td>4–&gt;4</td>
<td>&gt;4</td>
</tr>
<tr>
<td>Imipenem</td>
<td>≤1–2</td>
<td>2</td>
</tr>
<tr>
<td>Meropenem</td>
<td>2–&gt;8</td>
<td>8</td>
</tr>
<tr>
<td>KPC-like (n = 8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ertapenem</td>
<td>&gt;4–&gt;4</td>
<td>&gt;4</td>
</tr>
<tr>
<td>Imipenem</td>
<td>4–&gt;8</td>
<td>4</td>
</tr>
<tr>
<td>Meropenem</td>
<td>2–&gt;8</td>
<td>8</td>
</tr>
</tbody>
</table>

<sup>a</sup> S, susceptible isolates according to EUCAST breakpoints; I, intermediate isolates according to EUCAST breakpoints; R, resistant isolates according to EUCAST breakpoints.

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**FIG 1** Yearly evolution (2009–2012) of carbapenemase-producing *Enterobacteriaceae* in Spain and number of individual hospitals reporting cases to the national surveillance program of the Instituto de Salud Carlos III.
ST11, ST15, and ST16 have been described previously to be associated with different outbreaks due to extended-spectrum ß-lactamases (ESBLs) or carbapenemase-producing K. pneumoniae (1, 7, 9, 17). ST405 was recently associated with OXA-48 producing K. pneumoniae in Spain and Belgium (7, 8, 16) and was found in this study in eight hospitals from three geographic regions. These data may suggest that ST405 has been established in Spain and contributes to the dissemination of OXA-48.

The most common ST–carbapenemase associations found are detailed in Table 3. Only two STs carried more than one type of carbapenemase: ST11 (OXA-48, OXA-245, VIM-1, KPC-2, and NDM-1) and ST15 (VIM-1 and OXA-48). It should be emphasized the apparent capacity of ST11 to carry and disseminate different types of carbapenemases (1, 7, 17).

The four carbapenemase-producing E. coli isolates belonged to four different STs: ST10, ST226, and ST1152, with one case each producing VIM-1, and ST131 that produced OXA-48.

Our results are based on a large representative sample of Spanish CPE cases, but reporting of CPE is not mandatory in this country so far. Recent global data about the spread of CPE in Spain are not available; one multicenter study carried out in 2009 in Spain estimated only 43 CPE cases, mainly VIM-1 and IMP-22 (5).

Only 13.5% of the isolates producing carbapenemases in this study were K. pneumoniae or E. coli isolated from blood, suggesting that EARS-Net may underestimate the occurrence of carbapenem-resistant Enterobacteriaceae. From 2011 to 2012, imipenem-nonsusceptible K. pneumonia has increased from <1% to 1.7% according to Spanish EARS-Net databases (unpublished data); similarly, according to Surveillance Program data depicted in Fig. 1, the number of carbapenemase-producing Enterobacteriaceae cases more than doubled between 2011 and 2012.

It is remarkable that, from 2009 to 2012, the number of hospitals reporting CPE increased by a factor of five. This fact may suggest that a recent epidemiological change may have occurred in this country, characterized by a rapid increase in the number of cases of CPE causing both nosocomial outbreaks and single infections (Table 3). A second significant factor explaining this trend may be that hospitals have increased awareness of CPE.

In summary, our data suggest that the impact of CPE in Spain has dramatically increased in the last years. Interhospital spread of several K. pneumoniae clone–carbapenemase combinations have been detected in this study, mainly ST15–VIM-1, ST11–OXA-48, ST405–OXA-48, ST101–KPC-2, and ST11–VIM-1. To address the emergence and spread of CPE, urgent measures are required, including early detection and the rapid implementation of control measures.

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