

# Carbapenemase-Producing *Enterobacteriaceae* in Spain in 2012

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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  $\beta$ -lactam antibiotics (1–3). The most clinically important carbapenemases produced by *Enterobacteriaceae* are the class B metallo- $\beta$ -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  $\mu$ l 0.5 M solution) and phenyl-boronic acid (400  $\mu$ g).

The presence of genes encoding carbapenemases, *bla*<sub>KPC</sub>, *bla*<sub>VIM</sub>, *bla*<sub>IMP</sub>, and *bla*<sub>NDM</sub>, was confirmed by PCR and DNA sequencing (5, 12, 13). Specific primers for PCR amplification and sequencing of *bla*<sub>OXA-48</sub>-like genes were designed according to GenBank (National Center for Biotechnology Information, National Institutes of Health, Bethesda, MD, USA) database entry

TABLE 1 Distribution of carbapenemase-producing *Enterobacteriaceae* in 2012 in Spain according to the national surveillance program of the Instituto de Salud Carlos III

Species	No. of isolates (%)					
	With CBP <sup>a</sup>	OXA-48 group	VIM group	KPC group	IMP group	NDM group
<i>K. pneumoniae</i>	203 (85.6)	153	40	6	3	1
<i>K. oxytoca</i>	6 (2.5)	0	6	0	0	0
<i>E. cloacae</i>	16 (6.8)	7	6	1	2	0
<i>E. aerogenes</i>	2 (0.8)	2	0	0	0	0
<i>E. coli</i>	4 (1.7)	1	3	0	0	0
<i>S. marcescens</i>	1 (0.4)	0	0	1	0	0
<i>M. morgani</i>	1 (0.4)	0	1	0	0	0
<i>C. freundii</i>	1 (0.4)	0	1	0	0	0
<i>Enterobacter</i> spp.	3 (1.3)	0	3	0	0	0
Total	237	163	60	8	5	1

<sup>a</sup> CBP, carbapenemases.

AY236073 (OXA-48-TOT-F, 5'-TGCGTGTATTAGCCTTATC G-3'; OXA-48-TOT-R, 5'-TTTTTCCTGTTTGAGCACTTC-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-

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

Carbapenemase type (no. of isolates)	Carbapenem	MIC ( $\mu\text{g/ml}$ )			% <sup>a</sup>		
		Range	50%	90%	S	I	R
OXA-48 like ( <i>n</i> = 163)	Ertapenem	2->4	4	>4	0	0	100
	Imipenem	$\leq 1$ ->8	2	>8	66.3	20.2	13.5
	Meropenem	$\leq 1$ ->8	4	>8	30	47.9	22.1
VIM-1 ( <i>n</i> = 60)	Ertapenem	1->4	4	>4	0	10	90
	Imipenem	$\leq 1$ ->8	4	>8	15	55	30
	Meropenem	$\leq 1$ ->8	8	>8	18.3	40	41.7
IMP-like ( <i>n</i> = 5)	Ertapenem	4->4	>4	>4	0	0	100
	Imipenem	$\leq 1$ -2	2	2	100	0	0
	Meropenem	2->8	8	>8	20	60	20
KPC-like ( <i>n</i> = 8)	Ertapenem	>4->4	>4	>4	0	0	100
	Imipenem	4->8	4	>8	0	75	25
	Meropenem	2->8	8	>8	25	25	50

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

graphic areas. The estimated catchment population was about 21.5%, corresponding to approximately 10.5 million. Of these 357 isolates, 237 (66.4%) had a carbapenemase gene positively identified and distributed as follows: 203 (74.4%) carbapenemases were identified in 273 carbapenem-nonsusceptible *K. pneumoniae* isolates, 6 (75%) in 8 *Klebsiella oxytoca* isolates, 16 (36.4%) in 44 *Enterobacter cloacae* isolates, 2 (25%) in 8 *Enterobacter aerogenes* isolates, 4 (22.2%) in 18 *Escherichia coli* isolates, and 6 (100%) in 6 single isolates of *Serratia marcescens*, *Morganella morganii*, *Citrobacter freundii*, and *Enterobacter* spp. (Table 1).

A total of 149 (62.9%) isolates were from males and 76 (32.1%) were from patients  $\geq 65$  years old. Of the 237 CPE isolates, 162 (68.4%) produced clinical infections: 71 (43.8%) urinary tract infections (UTI), 37 (22.8%) blood infections, 28 (17.3%) respi-

ratory 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.

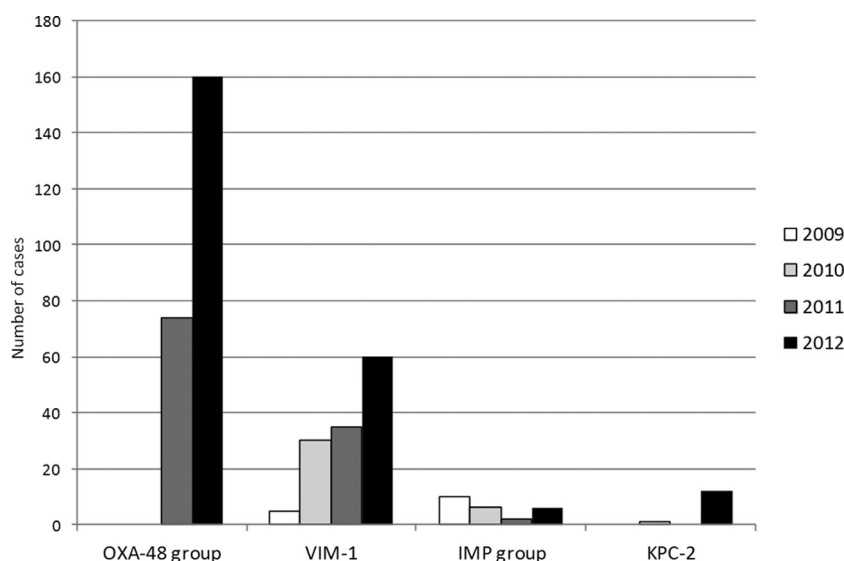
The carbapenemases detected were from the following groups: 163 from OXA-48 (84 from OXA-48 and 79 from OXA-245), 60 from VIM-1, 8 from KPC-2, 5 from IMP (2 from IMP-22 and 3 from IMP-8), and 1 from NDM-1 (Table 1). These CPE isolates came from 30 Spanish hospitals (average of 8.1 CPE isolates per hospital, 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). 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.



**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.

**TABLE 3** Distribution of *Klebsiella pneumoniae* MLST clones producing carbapenemases in Spain in 2012 according to the national surveillance program of the Instituto de Salud Carlos III

ST	Carbapenemase	No. of cases	No. of hospitals	Geographic source(s)
11	OXA-245	76	1	Málaga
	VIM-1	14	6	Madrid, Guadalajara
	OXA-48	12	7	Madrid
	KPC-2	3	3	Madrid, Ciudad Real
	NDM-1	1	1	Alicante
15	VIM-1	15	4	Madrid, Barcelona, Ávila
	OXA-48	4	2	Madrid, Málaga
405	OXA-48	38	8	Madrid, Barcelona, Guadalajara
16	OXA-48	17	2	Asturias
147	VIM-1	5	1	Alicante
340	VIM-1	5	1	Madrid
437	OXA-245	3	1	Málaga
101	KPC-2	3	2	Madrid
464	IMP-8	3	1	Almería
846	OXA-48	2	1	Madrid
13	OXA-48	1	1	Barcelona
1235	VIM-1	1	1	Guadalajara

ST11, ST15, and ST16 have been described previously to be associated with different outbreaks due to extended-spectrum  $\beta$ -lactamases (ESBLs) or carbapenemase-producing *K. pneumoniae* (1, 7, 9, 17). ST405 was recently associated with OXA-48 production 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 detected 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. pneumoniae* 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 infec-

tions (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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