Evolution of carbapenemase-producing*Enterobacteriaceae* at the global and national level: What should be expected in the future?

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Abstract

In recent years, *Enterobacteriaceae*isolates have increased their potential to become highlydrug resistant by acquiring resistance to carbapenems, primarily due to the production of acquiredcarbapenemases.The carbapenemases detected in *Enterobacteriaceae*are largely of the KPC, VIM, NDM, IMP and OXA-48 types. Although the epidemiological origin and geographic distribution of carbapenemasesare clearly different, they all first appeared in the late 20thCentury.Only a decade later, these enzymes have already become established and have expanded globally.

An important epidemiological change has occurred in Spainin recent years, characterized by a rapid increase in the number of cases of carbapenemase-producing *Enterobacteriaceae* (CPE), causing both nosocomial outbreaks and single infections. The impact of CPE in Spain is primarily due to OXA-48-producing and VIM-1-producing *Klebsiellapneumoniae* isolates, although other species such as*Escherichia coli* and *Enterobacter cloacae* are also increasing. The emergence of CPE as a principal cause of community-onset infections is a matter of great concern. Taking into account recent experience, and considering the fact that increasing numbers of patients are becoming infected by CPE and reservoirs of carbapenemases are growing globally, the trend of the CPE epidemic points toward a rise in its incidence.

To prevent a massive CPE pandemic, a well-coordinated responsefrom all health professionals and national and supranational authorities is clearly needed.

Keywords: Carbapenemases, *Enterobacteriaceae*, Spread

Evolution of β-lactam resistance in *Enterobacteriaceae*

Since β-lactam antibiotics became available for the treatment of Gram-negative infections, the greatest threat to these antibiotics has been β-lactamase production. At the beginning of 21stCentury, the primary concern regarding antimicrobial resistance in *Enterobacteriaceae* was the development and spread of resistance to third-generation cephalosporins due to the increase of extended-spectrum β-lactamase (ESBL). ESBL producers now exist globally and have a great clinical impact because they frequently show co-resistance to other antibiotic families such as aminoglycosides and fluoroquinolones[1]. According to the European Antibiotic Resistance Net (EARS-Net), resistance to third-generation cephalosporins in invasive isolates of *Escherichia coli* has increased in Spain from 0.5% in 2001 to 13.9% in 2013 (http://ecdc.europa.eu/en/healthtopics/antimicrobial_resistance/database/Pages/database.aspx). ESBLs have expanded globally through both mobile genetic

elements and clonal expansion, making the eradication of these enzymesdifficult. A mechanism of resistance, once it has widely spread and has exceeded a certain threshold, appears impossible to eradicate [2]. In recent years, concern has resurfaceddue to the emergence of enzymes capable of degrading carbapenems, the only remaining β-lactams effective against ESBL-carrying bacteria.

Carbapenems have been considered one of the first-line antibiotics for the treatment of severe infections due to ESBL-producing *Enterobacteriaceae*. Consequently, the burden of third-generation resistance in these bacteria has been followed by a significant increase incarbapenem use [3-6]. Growing carbapenemuse has selected for resistance to these antibiotics, leading to the emergence of extensivelydrug-resistant (XDR) strains. The emergence of carbapenemresistance during treatment has been reported due to the combination of ESBLs and/or cephalosporinases and decreased drug permeability [7]. Most of these isolates are unique, with limited clonal dissemination, making them less competitive in the absence of antibiotics. However, the greatestcurrent threat concerning antimicrobial resistance is the rapid dissemination of XDR *Enterobacteriaceae*, primarily*Klebsiellapneumoniae* strains, producing carbapenemases encoded by transmissible plasmids [8]. We have recently observed the evolution from sporadic and anecdotal cases to a universal spread of variousenzymes from diverse genetic origins able to hydrolyze carbapenems.

This article aims to describe the global evolution of carbapenemase-producing *Enterobacteriaceae*(CPE), focusing particularlyonSpain (Figures 1 and 2).The tracking ofcarbapenemase expansion by type has been developed according to published data; the rates of resistance in some countries might be underestimated due to the lack of organized reporting structures and limited resources [9]. In addition, the frequent epidemic nature of these CPE strains can significantly influence the local prevalence data.

Global evolution of class Acarbapenemase-producing *Enterobacteriaceae*

Among the varioustypes of class A carbapenemases described (KPC, GES, NMC-A, IMI, SME, SFC and BIC), only 2, the KPC and GES enzymes, have become an important epidemiological issue. They have been reported in variousplasmids, clones and enterobacterial species. Genes encoding the remaining class A carbapenemases appear to have only a chromosomal location, and they have been reported in such species as *Enterobacter* spp. (*bla*$_{\text{NMC-A}}$ and *bla*$_{\text{IMI}}$), *Serratia* spp. (*bla*$_{\text{SME}}$ and *bla*$_{\text{SFC}}$), and *Pseudomonas* spp. (*bla*$_{\text{BIC}}$)[8,9].

Sixteen subtypes of KPC carbapenemaseshave been reported. KPC-2 and KPC-3 have been the types most frequentlyfound in *Enterobacteriaceae*, primarily in *K. pneumoniae*. KPC enzymes have been detected in a large number of *K*. *pneumoniae* sequence types (ST), although the majority belongs to ST14 or ST258 [9,10].

The first KPC-producing *K*. *pneumoniae* strain was reported in the northeastern part of the United Statesin 2001 [2,11]. Five years later, various hospitals in the New York area reported nosocomial outbreaks of KPC-producing *K*. *pneumoniae*, with one finding a prevalence rateas high as 24%; whereas, in the same area, 0.5% of *E.coli* strains acquired this enzyme [9,11]. At the same time, isolates carrying KPC were reported in South America, Israel and China. In Colombia, the prevalence of KPC-producing strains has been increasing since 2006, including nosocomial outbreaks in which 30% of hospital patients have been affected [12,13]. In Brazil, Monteiro*et al*. [14] reported the first KPC-2-producing *K*. *pneumoniae* strain in 2006, and only two years later the expansion of this enzyme had affected various hospitals in different areas, with a prevalence of approximately 6% [15]. In Israel, Leavitt *et al*. [16] studied 51 carbapenem-resistant *K. pneumoniae*isolates collected from January 2004 to December 2006. The pulsed-field gel electrophoresis results of this study revealed a major clone (affecting 31 cases, 60%), with a pattern similar to the pandemic clone ST258 described in the USA outbreaks [16]. These data suggest importation from the USA to Israel, but it could not be proven[2]. In

contrast, the prevalence of KPC remained low in Argentina[17]andChina[18] in 2007.

In Europe, the first KPC-producing strain was reported in Greece in 2008 [19], where KPC-carbapenemases have since become endemic [2,20]. In fact, 89.5% of 270 carbapenem-resistant *Enterobacteriaceae* isolates were KPCproducers according to a national survey carried out in 2011 [21]. Multihospital outbreaks have also been reported in Italy, where KPC-producing *K*. *pneumoniae*have had a rapid and extensive dissemination [22,23]. In Northern and Western European countries, KPC prevalence remains low. In these countries, most reports concern sporadic outbreaks introduced by patients from high-prevalence areas [10,24].

GES enzymes with carbapenemase activity (GES-2, GES-5, GES-6, GES-12, GES-13, GES-14, GES-16, GES-18 and GES-20) haveprimarilybeen recovered from *Pseudomonasaeruginosa* and *Acinetobacterbaumannii*[25,26]. Nevertheless, few *Enterobacteriaceae* strains carrying these enzymes have been reported. Jeong*et al*. [27]noted in 2004 a nosocomial outbreak due to GES-5-producing *K*. *pneumoniae* in the Republic of Korea.

Global evolution of metallo-β-lactamase-producing (MBL) *Enterobacteriaceae*

The more geographically widespread MBLs include IMP, VIM and NDM [9]. To date, 48 IMPvariants have been reported (http://www.lahey.org/Studies/),primarily in *P.aeruginosa* from Asia. The first IMP-producing *Enterobacteriaceae* strain was a *Serratiamarcescens* strain carrying $bla_{\text{IMP-1}}$, isolated in Japan in 1991 [28]. Also in Japan, IMP-1 and IMP-2 enzymes have been described in various*Enterobacteriaceae* species such as *Citrobacterfreundii*, *Morganellamorganii*and*Enterobactercloacae*, with a prevalence of approximately 5% [29]. IMP-producing *Enterobacteriaceae* strains have also been reported in Australia, where in 2004 a prospective study revealed 19 isolates recovered from 16 patients: *S. marcescens*(10 isolates), *K.*

pneumoniae(4 isolates), *P. aeruginosa*(3 isolates), *E. coli* (1 isolate) and *E. cloacae* (1 isolate) [30]. A number of sporadic cases of IMP-1- and IMP-4-*K*. *pneumoniae*-producing strains have been reported in SingaporeandChina, respectively [10]. In Europe, IMP-producing *Enterobacteriaceae* strains have been found in Turkey (*K*. *pneumoniae* and *E.cloacae*) [31], the United Kingdom (*K. pneumoniae*) Poland (*K. pneumoniae*) [2] and Spain (*K*. *pneumoniae*) [55,65]. Among the strains carrying IMP enzymes, the predominant ST was ST11, related to IMP-1 and IMP-8 [32,33]. ST11 is frequent in *K*. *pneumoniae*, primarily in ESBL- or plasmid-AmpC- producing strains. Nevertheless, other STs of *K. pneumoniae* have been reported to carry IMP enzymes; for example, IMP-35 was reported in strains with sequence type ST622 [34].

Veronaintegron-encoded MBLs (VIM) are reportedglobally, with the exception of Northern Europe and the United States, where outbreak rates remain low [29]. Nevertheless, VIM-producing *Enterobacteriaceae* isolates were frequently found in the Mediterranean area, primarily in Greece[2,10]. The most frequently found enzymes were VIM-1 in *Enterobacteriaceae* and VIM-2 in *P*. *aeruginosa*.
The most significant public health impact MBLs provoke is disease caused by NDM-producing *Enterobacteriaceae*, primarily the*E*. *coli* and *K*. *pneumoniae* species. This enzyme was first described in New Delhi[10], where these MBLs are now endemic [10,29,35]. The enzyme was later reported in the Middle East [10]. It is nowobserved primarily in Europe, except in Great Britain and the Balkans, with only a few cases being reported on the American continent [36]. In contrast to KPC-producing *K*. *pneumoniae*, there are no predominant clones related to the rapid dissemination of NDM-producing strains. A variety of studies showed a great diversity of *E*. *coli* strains harboring the$bla_{\text{NDM-1}}$ gene, although ST101 and ST131 appear to be the most frequent sequence type identified [37-39]. A high number of resistance genes have been associated with NDM, including OXA-48 types, VIM-type genes, plasmid-mediated AmpC genes, ESBL genes, aminoglycoside resistance genes andsulfamethoxazole resistance genes [28].

Global evolution of OXA-48-likecarbapenemase-producing *Enterobacteriaceae*

The class D carbapenemases present in *Enterobacteriaceae* reduce to OXA-48-related enzymes. The *bla*$_{\text{OXA-48}}$ gene has always been reported in *Enterobacteriaceae*, and never in *Pseudomonas* or *Acinetobacter*;*bla*$_{\text{OXA-48}}$ genehas primarily been found in *K*. *pneumoniae,* but it has also been foundin *E*. *coli*and*E*. *cloacae.* The first OXA-48 producer was a *K*. *pneumoniae* strain isolated in Turkey in 2003, where this enzyme has persisted and caused significant nosocomial outbreaks [40]. After Turkey, the OXA-48 enzyme spread throughout the Mediterranean area, including Lebanon, Tunisia, Egypt, Morocco and Senegal[41-44]. The prevalence of these enzymes is increasing in Europe (France, Germany, Spain, the Netherlands and the United Kingdom), where an increasing number of outbreaks have been reported[2]. The SENTRY antimicrobial surveillance program showed an increase from 3% in 2007 to 27% in 2009 [45]. Only a few cases have been reported in North America[46], however, and no OXA-48 producers have been reported in Canada[28]. The OXA-181 enzyme is the variant of OXA-48 most prevalent in India: among the 1445 *Enterobacteriaceae* strains studied between 2006 and 2007 in India, 10 (0.7%) harbored *bla*$_{\text{OXA-181}}$[47]. In 2008, two *bla*$_{\text{OXA-163}}$-producing isolates of *K*. *pneumoniae* and *E*. *cloacae*were identified in Argentina[48]. Two cases of infection caused by two genetically unrelated OXA-163-carrying *K*. *pneumoniae* strains were reported in Cairo, Egypt, in 2009 and 2010 [49]. Another OXA-48-derivative is OXA-204 carbapenemase, thus far only reported in one *K*. *pneumoniae*strain isolated in Tunisia[50].

The OXA-48 enzyme has been detected in variousclones of *K*. *pneumoniae;* however, ST101 was the sequence type most frequently described, followed by ST14, ST15, ST147 and ST395. ST395 was reported in Morocco, France and the Netherlands, indicating a clonal dissemination [40]. In the USA, the first clinical cases were associated with ST199 and ST43 [51].

Emergence and spread of CPE in Spain

The first cases of CPE in Spain were VIM-1-producing isolates reported in a study performed in Barcelona in 2003 [52]. One strain of *E. coli* from a urinary tract infection and one strain of*K. pneumoniae* from a fecal carrier were detected among 4,345 clinically relevant isolates and 2,398 isolates from stools, respectively [52].

Since this first discovery, both sporadic cases and significant outbreaks of MBL-producers, primarily concerning the VIM type, have been reported[53-61].
In 2007, the first Spanish outbreak involving VIM-1 was reported in a hospital in Madrid[54]. The *bla*$_{\text{VIM-1}}$ gene was detected in various species, including *K. pneumonia*e, *E. cloacae*, *E. coli* and *Klebsiellaoxytoca*.The clonal analysis revealed a complex population structure, including clonal and polyclonal dissemination [54]. A wide clonal spread of the VIM-1-producing *K. pneumoniae* strain belonging to ST15 was detected in another Spanish hospital in 2009 [59]. Fifty-five patients from surgical wards and medical ICUs were infected and/or colonized by this clonal strain,which also produced the new ESBL SHV-134 [59]. A total of 14 pediatric patients were infected by VIM-1-producing *Enterobacteriaceae* in 2 Spanish hospitals inMadrid (8 cases of *K. pneumoniae*, 2 of *E. coli* and 1 of *K. oxytoca*) [57] and Vizcaya (3 cases of *E. cloacae*) [53]. VIM-1 *Enterobacteriaceae* producers have been progressively increasing in recent years and now constitute the second most frequent type of carbapenemase in Spain [62,63].

Other MBL carbapenemase types, such as IMP and NDM, are much less frequent than the VIMtype, and only single cases and a few small outbreaks have been reported[55, 62-68].

In a national multicenter survey performed in Spain in 2009, Miró*et al*. [55] detected 10 IMP-producing *Enterobacteriaceae*: 9 clonal *K. pneumoniae* isolates producing IMP-22 and 1*K. oxytoca* producing IMP-28, first described in this study [55,64].Conejo*et al*. [65]described a prolonged clonal outbreak of nosocomial infections due to a strain of IMP-8-producing *K. oxytoca* in a

community hospital in Seville, emphasizing that identifying and isolating the environmental reservoir was essential for eradicating this outbreak [69]. According to data from the Spanish Antibiotic Resistance Surveillance Program of the National Center of Microbiology (PVRA-CNM), the IMP-producing *Enterobacteriaceae* isolates submitted in 2012 were 3 IMP-8-producing *K. pneumoniae*isolates and 2 IMP-22-producing *E. cloacae* isolates (2.1% of the total CPE submitted) [62].

Despite the concern generated by the worldwide spread of NDM-type carbapenemases, few cases have been detected in Spain[62, 66-68]. All had an established origin in India: an *E. coli* isolate recovered from a stool specimen from a Spanish patient with diarrhea who had traveled to India [66]; an abdominal abscess due to *K. pneumoniae* in a Spanish patient after hospitalization in India for a perforated appendix[67]; and a *K. pneumoniae* isolated from urine in a three-month-old child adopted from India [68].

The emergence of the KPCtype in Spain was first detected between September 2009 and February 2010 in ST384 and ST388 *K. pneumoniae* clones isolated from a tertiary hospital in Madrid, both harboring *bla*$_{KPC-3}$[70]. At approximately the same time, there were 3 clonallyrelated *C. freundii* isolates detected in a nearby hospital that harbored*bla*$_{KPC-2}$[71]; all the strains were isolated in different patients with no apparent epidemiological links. In a recent study concerning the PVRA-CNM data [62], 8 KPC-producing isolates were studied in 2012 (3.4% of the total CPE submitted). Six were *K. pneumoniae* belonging to ST101 and ST11 from Madrid and Ciudad Real, and the 2 remaining cases were *E. cloacae* and *S. marcescens;* all produced KPC-2 carbapenemase[62]. The majority of the KPC-producing *K. pneumoniae*inSpain do not belong to the epidemic *K. pneumoniae* ST258 high-risk clone [62, 70, 72].A single ST258 isolate producing KPC-2 has recently been collected from a patient in Spain who had previously been hospitalized in the intensive care unit of a hospital in Greece[73]. In Spain, KPC prevalence in *Enterobacteriaceae* is low, although a few hospital outbreaks have been detected. A significant outbreak due to a KPC-3-producing *K. pneumoniae* belonging to ST512 has recently been

reported in a hospital in Córdoba; the index case was a patient transferred from an Italian hospital [74]. Dissemination to other community hospitals in the same province has occurred. The epidemiological status of KPC-producers in Spain can be characterized as “sporadic hospital outbreaks” [20].

The greatest clinical and epidemiologic impact of CPE in Spain is due to OXA-48-producing isolates. Since their emergence in *K. pneumoniae* in 2009 [75], multidrug-resistant *K. pneumoniae* isolates producing OXA-48 carbapenemase are emerging as significant pathogens in Spain due to intra- and inter-hospital, clonal and non-clonal dissemination[62,75-79]. The first report was of an outbreak due to the ST101 *K. pneumoniae* strain coproducing OXA-48 and CTX-M-15; the index case was a patient transferred from an ICU in a hospital in Marrakech (Morocco) to Barcelona[75].

Subsequently, several outbreaks due to various*K. pneumoniae* sequence types (primarily ST11, ST405, ST15 and ST16) were detected in various hospitals throughout the country [62,63,76,77], including prolonged and widespread outbreaks associated with significantly high in-hospital mortality [77]. In addition, 2 novel variants of the OXA-48-type carbapenemase, OXA-244 and OXA-245, have been reported in a single hospital inMalaga in the context of a nosocomial outbreak [76]. In 2012, 68.8% of the 237 CPE isolates submitted to the PVRA-CNM were OXA-48-like producers [62], and 93.8% were *K. pneumoniae*. According to this study, the OXA-48-producing *Enterobacteriaceae* isolates submitted to the PVRA-CNM have increased from no cases in 2010 to 160 in 2012 [62] and 523 in 2013 (unpublished data, Oteo J *et al.*).

A multicenter study performed in hospitals in the Cataloniaregion of Spainshowed a prevalence of*K.pneumoniae*strainsproducingOXA-48 of approximately2.2% (87/3.901). Clonalstudiesrevealed fivepulsetypes(A to E, with 76% homology). The 2 mostprevalentcloneswereST405(78%) and ST101(19%), whosestrainscoexpressedthe*bla*$_{\text{CTX-M-15}}$ and *bla*$_{\text{SHV-76}}$ genesandshowed resistance toaminoglycosides and fluoroquinolones[80]. Considering all the carbapenemase types, the CPE cases in Spain have quickly

increased. One multicenter study performed in 2009 in 35 Spanish hospitals detected only 43 CPE cases (0.04%), primarily VIM-1 and IMP-22 [55]. The data obtained from a recent Spanish study performed in 2013 with 80 hospitals participating showed a significant evolution, with a total of 382 CPE cases, chiefly OXA-48- and VIM-producing *K. pneumonia*e isolates (71.5% and 25.4%, respectively) [63].

According to data from the PVRA-CNM, the number of CPE isolates submitted to this program increased from 15 in 2009 to 237 in 2012 [62], including clinical isolates (68.4%) and isolates from carriers (31.6%). The number of hospitals submitting cases also increased from 6 in 2009 to 30 in 2012 [62]. In 2013, the CPE isolates studied by this surveillance program, including both clonal andsingle isolates, significantly rose to 777 CPE isolates in 57 hospitals located in 19 geographic areas (Spanish provinces) (unpublished data, Oteo J *et al.*) (Figure 3).

Multiple epidemiologicallyrelated outbreaks occurredearlyin 2014 in varioushealth districts, suggesting an inter-regional autochthonous spread and inter-institutional transmission (Table 1). This epidemiological stage of inter-regional spread is primarily due to OXA-48-producing *K. pneumoniae*, and it has quickly evolved since 2011 [2] and February 2013 [20], when the Spanish situation regarding CPE was classified as “independent hospital outbreaks” and “regional spread,” respectively [2,20].

What should be expected in the future?

The public health threat due to the global spread of CPE isolates has caught us unprepared, despite recent and similar experiences with ESBL producers. Taking into account this previous experience, and considering that infections of patients by CPE and reservoirs of carbapenemases are growing globally, the future trend of the CPE epidemic will be to increaseits incidence.

Thus far, the greatest epidemiological and clinical impact of CPE has been due to nosocomial infections produced by *K. pneumoniae.* However, the prevalence of carbapenemases in *E. coli*, primarily OXA-48-producers, is increasing (PVRA-CNM unpublished data, Oteo J. *et al.*). A massive transfer of carbapenemases to *E. coli* could introduce a new situation in which a fast clonal and polyclonal spread, including in community settings, would greatly increase the threat of CPE. Of concern is the acquisition of carbapenemases by successful *E. coli* clones such as ST131, as has already occurred[38,81].
The ESBL situation changed greatly with the emergence of CTX-M-type β-lactamases as a significant cause of community-onset infections. Similarly, the emergence of CPE as a major cause of community-onset infections, primarily due to OXA-48 producers, may be changing the epidemiology of CPE.

The co-production of 2 or more carbapenemases in a same isolate is increasing [21,60] making the diagnosis and treatment of these infectionsmore difficult. Finally, the consumption of the few antibiotics available against infection by CPE (e.g., colistin, amikacin, tigecycline, susceptible carbapenems) is rising. The emergence of CPEs with increased MICs to carbapenems, and/or resistance to colistin and/or tigecycline could limit the therapeutic options in the near futureeven further.

The geographic distribution of CPE shows significant variation. Multiple factors could contribute to this varyingdispersion of the carbapenemases, among which we find the genetic structures of the microorganisms themselves and human relations (mobility for economic, commercial, political or recreational purposes).

A well-coordinated and promptresponse from all health professionals and national and supranational authorities is clearly needed to prevent amassive global dissemination of CPE . We have not avoided the CTX-M pandemic; will we be able to avoid a pandemic caused by CPE?

Conflicts of interest

None to declare.

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Figure 1. Representation of the global distribution of carbapenemase-producing *Enterobacteriaceae.*

Figure 2. Representation of the European distribution of carbapenemase-producing *Enterobacteriaceae.*

Figure 3. Yearly evolution (2009-2013) of the number of cases of carbapenemase-producing *Enterobacteriaceae* submitted to the Spanish Antibiotic Resistance Surveillance Program of the National Center of Microbiology (Updated in reference 62).

Table 1. Occurrence of carbapenemase-producing *Enterobacteriaceae* (CPE) in Spain (April 2014)

Carbapenemase types	**Epidemiological stage**
VIM-type	Regional spread
IMP-type	Independent hospital outbreaks
NDM-type	Sporadic occurrence
KPC-type	Sporadic hospital outbreaks
OXA-48-type	Inter-regional spread
All types	Inter-regional spread

According to the epidemiological scale described in Reference 20.