



Early OXA-48-Producing *Enterobacterales* Isolates Recovered in a Spanish Hospital Reveal a Complex Introduction Dominated by Sequence Type 11 (ST11) and ST405 *Klebsiella pneumoniae* Clones

Desirèe Gijón,^{a,b} Ana P. Tedim,^{a,c} Aránzazu Valverde,^{a,d} Irene Rodríguez,^{a,c} María-Isabel Morosini,^{a,b} Teresa M. Coque,^{a,c} Marina Manrique,^e Eduardo Pareja,^e Raquel Tobes,^e Patricia Ruiz-Garbajosa,^{a,b}  Rafael Cantón^{a,b}

^aServicio de Microbiología, Hospital Universitario Ramón y Cajal and Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain

^bSpanish Network for Research in Infectious Diseases (REIPI), Instituto de Salud Carlos III, Madrid, Spain

^cCentro de Investigación Biomédica en Red de Epidemiología y Salud Pública (CIBER-ESP), Barcelona, Spain

^dCentro de Vigilancia Sanitaria Veterinaria (VISAVET), Universidad Complutense, Madrid, Spain

^eOh no sequences! Research Group, Era7 Bioinformatics, Granada, Spain

ABSTRACT Carbapenemase-producing *Enterobacterales* (CPE) have become an important public health concern. In our hospital, VIM enzymes were first detected in 2005, *Klebsiella pneumoniae* carbapenemase (KPC) enzymes in 2009, and OXA-48 enzymes in 2012. We assess the population biology of the first OXA-48-producing *Enterobacterales* isolates recovered in our hospital (2012 to 2013) where infections by other carbapenemases had been endemic for several years. Over a 21-month period, 71 isolates (61 *Klebsiella pneumoniae*, 5 *Escherichia coli*, 2 *Klebsiella aerogenes*, and 1 each of *Enterobacter cloacae*, *Klebsiella oxytoca*, and *Citrobacter amalonaticus*) recovered from clinical and surveillance specimens from 57 patients (22.8% nonhospitalized) were investigated for OXA-48-like-producing enzymes. Analyses for characterization and determination of the location of the *bla*_{OXA-48} gene, plasmid transferability, sequence, and clonal relatedness were performed. Most of the isolates also coproduced CTX-M-15 (57/71, 80.3%) and/or VIM-1 (7/71, 9.8%). *K. pneumoniae* was predominantly identified as sequence type 11 (ST11) (63.4%) and ST405 (9.8%) and *E. coli* as ST540, ST1406, ST3163, and ST4301. The *bla*_{OXA-48} gene was part of Tn1999.2 located at the *tir* gene of plasmids (ca. ≥50 kb) of the IncL/M group, also carrying *bla*_{VIM-1} and *bla*_{CTX-M-15} genes. We selected one ST11 *K. pneumoniae* isolate for whole-genome sequencing in which we studied the plasmid containing the *bla*_{OXA-48} gene. This plasmid was compared with indexed plasmids existing in NCBI database by the use of BRIG and MAUVE. Our data suggest a rapid spread of *bla*_{OXA-48} genes between commonly isolated high-risk clones of *Enterobacterales* species, frequently associated with antibiotic resistance. Moreover, the emergence of the multiresistant ST11 *K. pneumoniae* clone among nonhospitalized patients emphasizes the difficulty of preventing its dissemination into the community.

IMPORTANCE We present results of microbiological analysis of the first *Enterobacterales* isolates that were isolated in 2012 in our institution expressing OXA-48 carbapenemase. This enzyme confers resistance to carbapenems, an important group of antibiotics widely used in the hospitals. OXA-48 carbapenemase is currently present in many parts of the world, but it is found particularly frequently in the Mediterranean area. It was disseminated at the Ramón y Cajal Hospital and found to be associated with a particular *Klebsiella pneumoniae* strain, so-called high-risk clone ST11, which was previously found in our institution in association with other enzymes such as CTX-M-15, VIM-1, and KPC-3. This clone might have acquired a plasmid

Citation Gijón D, Tedim AP, Valverde A, Rodríguez I, Morosini M-I, Coque TM, Manrique M, Pareja E, Tobes R, Ruiz-Garbajosa P, Cantón R. 2020. Early OXA-48-producing *Enterobacterales* isolates recovered in a Spanish hospital reveal a complex introduction dominated by sequence type 11 (ST11) and ST405 *Klebsiella pneumoniae* clones. *mSphere* 5:e00080-20. <https://doi.org/10.1128/mSphere.00080-20>.

Editor Patricia A. Bradford, Antimicrobial Development Specialists, LLC

Copyright © 2020 Gijón et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Rafael Cantón, rafael.canton@salud.madrid.org.

Received 14 March 2020

Accepted 19 March 2020

Published 8 April 2020

harboring the *bla*_{OXA-48} gene. Our results point out the importance of local epidemiology in the dissemination and maintenance of multidrug-resistant bacteria.

KEYWORDS OXA-48, carbapenemase, VIM-1, CTX-M-15, *Enterobacterales*, ST11

Carbapenemase-producing *Enterobacterales* (CPE) isolates have become an important public health concern worldwide. They have been increasingly reported in Europe, particularly in recent years, with current classification of Spain as a country with “interregional spread” of CPE according to the European Centre for Disease Prevention and Control (ECDC) (1–6).

Carbapenemases include enzymes belonging to Ambler classes A (*Klebsiella pneumoniae* carbapenemase [KPC] type), B (VIM, IMP, and NDM types, among others), and D (mainly OXA-48). The OXA-48 carbapenemase was first identified in 2003 from a *Klebsiella pneumoniae* clinical isolate recovered in Turkey (7). Since then, OXA-48 producers have increasingly been detected worldwide but particularly in Europe (8, 9). This enzyme weakly hydrolyses carbapenems and spares extended-spectrum cephalosporins (10). OXA-48-producing isolates often carry genes encoding extended-spectrum β -lactamases (ESBLs) or other carbapenemases, hindering their accurate detection, particularly in hospitals with a high prevalence of different carbapenemase enzymes (11).

In our hospital in Madrid (Spain), VIM enzymes were first detected in 2005 (1), KPC enzymes in 2009 (12, 13), and OXA-48 enzymes in 2012. It is of note that large OXA-48 outbreaks were described in other hospitals in Madrid (2) and in other Spanish regions (3, 14, 15) before the emergence in our center. Epidemiology of CPE has been investigated not only locally but also at a national level in the Spanish reference center (16, 17). The aim of this work was to characterize the microbiological and epidemiological scenario, through classical and genomic approaches, represented by the first OXA-48-producing *Enterobacterales* recovered in our hospital (2012 to 2013), where infections by strains producing other carbapenemases (mainly VIM and KPC) had been endemic for several years.

RESULTS

Patient characteristics and carbapenemase characterization. A total of 57 patients (33 males) were infected ($n = 41$) or colonized ($n = 16$) with OXA-48-producing *Enterobacterales* during the studied period. The majority of patients were elderly (median age, 69 years; range, 18 to 92 years). In this period, the prevalence of carbapenemase in our institution was 0.4% and the predominant carbapenemase was OXA-48. Patients were admitted to medical ($n = 20$; 4%) and surgical ($n = 21$; 4%) wards and intensive care units (ICU) ($n = 4$; 7%). It should be noted that 13 isolates were recovered from 13 nonhospitalized patients at the time of sampling, although 5 of them had been previously admitted to our institution (Table 1). The first OXA-48-producing *Enterobacterales* isolates recovered in our institution were obtained from patients admitted to the urology ward in 2012. In that ward, most of the *K. pneumoniae* isolates belonged to the same clone (KP-A) identified as sequence type 11 (ST11). Overall, from the 57 patients, we recovered 71 isolates (61 *K. pneumoniae*, 5 *Escherichia coli*, 2 *Klebsiella aerogenes*, and 1 each of *Klebsiella oxytoca*, *Enterobacter cloacae*, and *Citrobacter amalonaticus*). Apart from the OXA-48 enzyme, 51 isolates (48 *K. pneumoniae*, 1 *K. oxytoca*, 1 *K. aerogenes*, and 1 *C. amalonaticus*) coproduced CTX-M-15, 6 isolates (4 *K. pneumoniae* and 2 *E. coli*) coproduced VIM-1 and CTX-M-15, and 1 *E. coli* isolate also coproduced VIM-1. It is of note that the double-disk synergy test (using EDTA) was negative in 5 of 7 isolates harboring VIM-1.

Antibiotic susceptibility. All OXA-48-positive isolates were resistant to β -lactam- β -lactamase inhibitor combinations and to broad-spectrum cephalosporins. However, some OXA-48-positive isolates (43/71, 60.5%) were susceptible to imipenem (41/71, 58%) and ertapenem (2/71, 3%). Phenotypes of resistance to non β -lactam antibiotics are presented in Table 1. Most of the isolates were resistant to gentamicin (52/71, 73%),

TABLE 1 Epidemiological data for OXA-48-producing *Enterobacteriaceae* isolates^a

Species (no. of isolates)	PFGE type (no. of isolates)	Sequence type	Plasmid size(s) (Kb)	RFLP pattern	Rep type	Co-resistance	Ward(s) and/or patient status	Other β-lactamase	
<i>K. pneumoniae</i> (n = 61)	A (n = 40)	11	50 (n = 38) 220 (n = 2)	A ND	L/M (n = 17) Flk (n = 2)	Gm, Tb, Cp, Nx, S/T, Fm, Nf	Urology (n = 10), general surgery (n = 5), pneumology (n = 1), vascular surgery (n = 1), preventive medicine (n = 5), internal medicine (n = 3), hematology (n = 2), cardiology (n = 1), gastroenterology (n = 1), nephrology (n = 1), neurosurgery (n = 2), ICU (n = 2), outpatients (n = 6)	CTX-M-15 (n = 38), VIM-1 (n = 3)	
		D (n = 2)	11	50 (n = 1) 60 (n = 1)	A ND	L/M (n = 1)	Tb (n = 2), Cp (n = 2), Gm (n = 1), Fm (n = 1)	Outpatients (n = 2)	CTX-M-15 (n = 2)
	I (n = 1) K (n = 1) M (n = 1) F (n = 1) H (n = 1) O (n = 1) P (n = 1) B (n = 5)	11	11	65	A	L/M	Gm, Tb, Cp, S/T	Hematology (n = 1)	CTX-M-15
		11	11	60	A	L/M	Gm, Tb, Cp, S/T	General surgery (n = 1)	CTX-M-15
		11	11	65	A	L/M	Tb, Nx, Cp, S/T, Fm	Outpatient (n = 1)	CTX-M-15
		15	15	40	B	L/M	Cp, Fm	Pneumology (n = 1)	
		15	15	50	B	L/M	Gm, Cp, S/T	Outpatient (n = 1)	
		307	307	60	B	L/M	Gm, Tb, Cp, S/T	Otorhinolaryngology (n = 1)	CTX-M-15 (n = 2)
		307	307	60	C (n = 3)	L/M (n = 4)	Gm (n = 5), Tb (n = 5), Cp (n = 5), S/T (n = 5), Fm (n = 1)	Neurosurgery (n = 1)	CTX-M-15 (n = 4)
		405	405	50 (n = 2) 60 (n = 2) 70 (n = 1) 60 (n = 1) 40 (n = 1)	ND	L/M (n = 1)	Gm (n = 1), Tb (n = 1), Cp (n = 1), S/T (n = 2)	Vascular surgery (n = 2) General surgery ICU (n = 2) Outpatient (n = 1) Geriatrics (n = 1) Gastroenterology (n = 1)	CTX-M-15 (n = 1)
E (n = 2)	405	405	60 (n = 1) 40 (n = 1)	ND	L/M (n = 1)	Gm (n = 1), Tb (n = 1), Cp (n = 1), S/T (n = 2)	Urology (n = 1) Traumatology (n = 1)	CTX-M-15 + VIM-1	
	405	405	60	C	L/M	Gm, Tb, Cp, S/T	Internal medicine (n = 1)	CTX-M-15	
	487	487	50	A	L/M	Gm, Tb, Cp, S/T	Oncology (n = 1)		
	712	712	60	A	L/M	Gm, Tb, Cp, S/T, Fm	Outpatient (n = 1)		
	971	971	50	A	L/M	Gm, Tb, Cp, S/T, Fm	Outpatient (n = 1)		
E. coli (n = 5)	C (n = 1) B (n = 2)	540	50	D	L/M	S/T	Outpatient (n = 1)	CTX-M-15 (n = 1)	
		1406	60	A	L/M (n = 2)	Gm (n = 1), Tb (n = 1), S/T (n = 2)	General surgery (n = 2)	VIM-1 (n = 2) CTX-M-15 + VIM-1	
	A (n = 1) D (n = 1)	3163	50	ND	L/M	Gm, Tb, Cp, S/T	Urology (n = 1)		
		4301	50	A	L/M	Cp, S/T	Oncology (n = 1)		
		540	50	ND	L/M (n = 2)	L/M (n = 2)	Gm (n = 1), Tb (n = 1), Cp (n = 1), S/T (n = 1)	Urology (n = 1), general surgery ICU (n = 1)	CTX-M-15 (n = 1)
K. aerogenes (n = 2)	540	50	ND	L/M (n = 2)	L/M (n = 2)	Gm (n = 1), Tb (n = 1), Cp (n = 1), S/T (n = 1)	Urology (n = 1), general surgery ICU (n = 1)	CTX-M-15 (n = 1)	
	1406	60	A	L/M (n = 2)	L/M (n = 2)	Gm, Tb, Cp, S/T	Traumatology (n = 1)	CTX-M-15	
E. cloacae (n = 1)	540	50	ND	L/M	L/M	Gm, Tb, Cp, S/T	Oncology (n = 1)	CTX-M-15	
	1406	60	A	L/M	L/M	Gm, Tb, Cp, S/T	Infectious disease (n = 1)	CTX-M-15	
K. oxytoca (n = 1)	540	50	ND	L/M	L/M	Gm, Tb, Cp, S/T	Oncology (n = 1)	CTX-M-15	
	1406	60	A	L/M	L/M	Gm, Tb, Cp, S/T	Infectious disease (n = 1)	CTX-M-15	
C. amalonaticus (n = 1)	540	50	ND	L/M	L/M	Gm, Tb, Cp, S/T	Oncology (n = 1)	CTX-M-15	
	1406	60	A	L/M	L/M	Gm, Tb, Cp, S/T	Infectious disease (n = 1)	CTX-M-15	

^aAbbreviations: ICU, intensive care unit; Gm, gentamicin; Tb, tobramycin; Cp, ciprofloxacin; Nx, nalidixic acid; S/T, trimethoprim-sulfonamide; Fm, fosfomicin; NF, nitrofurantoin.

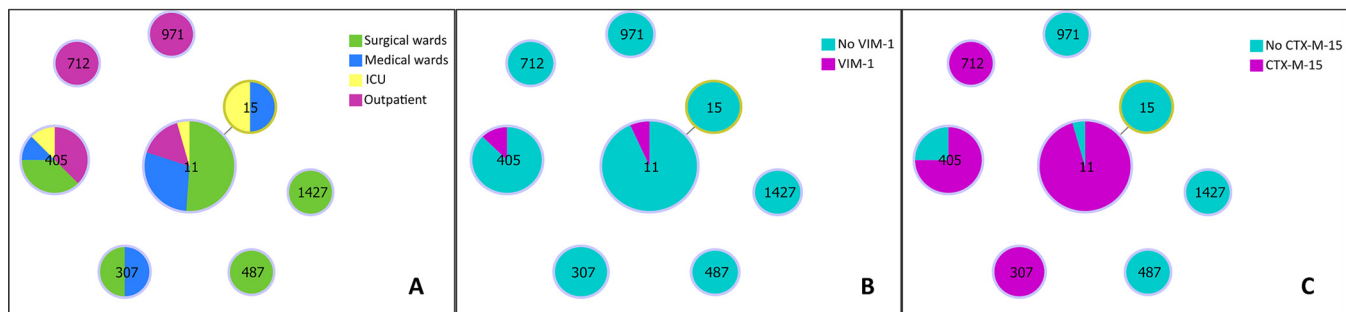
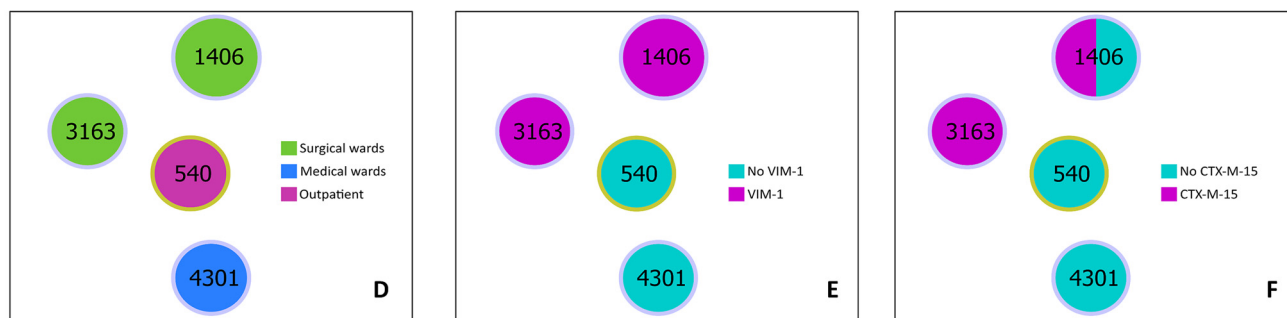
Klebsiella pneumoniae producing OXA-48*E. coli* producing OXA-48

FIG 1 Results of goEburst analysis of *K. pneumoniae* (A to C) and *E. coli* (D to F) producing OXA-48 isolates differentiated by isolate collection location (A and D), presence or absence of VIM-1 (B and E), and presence or absence of CTX-M-15 (C and F).

tobramycin (56/71, 79%), ciprofloxacin (61/71, 86%), and trimethoprim-sulfamethoxazole (60/71, 84.5%).

Clonal background. OXA-48-producing *K. pneumoniae* isolates were classified in 16 pulsed-field gel electrophoresis (PFGE) types (KP-A to KP-P) and 7 sequence types (ST), namely, ST11, ST15, ST307, ST405, ST487, ST712, and ST971 (Fig. 1). ST11 and ST405 were the STs most frequently found, recovered from samples collected from patients in different hospital wards, and *K. pneumoniae* isolates coproducing VIM-1 and CTX-M-15 belonged to these STs (Table 1). Carbapenemase-producing (CP) *E. coli* isolates were classified in four distinct PFGE types identified as ST540, ST1406, ST4301, and ST3163, the last being described for the first time in this report. The *E. coli* isolates coproducing OXA-48 and VIM-1 belonged to ST1406 ($n = 1$), and the isolates coproducing OXA-48, VIM-1, and CTX-M-15 ($n = 2$) belonged to ST1406 and ST3163 (Fig. 1).

Transferability and location of *bla* carbapenemase genes and plasmid typing.

The *bla*_{OXA-48} gene was located on plasmids (50 to 70 kb), most of them being transferable by conjugation (59/71 isolates). They carried *bla*_{OXA-48}, often with *bla*_{VIM-1} (7/71, 9.8%) and/or *bla*_{CTX-M-15} (57/71, 80.3%). OXA-48-carrying plasmids were categorized as IncL/M according to the PCR-based replicon typing (PBRT) scheme (18). In addition, 2 of 71 isolates presented a larger (ca. 220-kb) plasmid unrelated to *bla* genes and typed as IncFIIk by the scheme described previously by Villa et al. (19) (Table 1). Restriction fragment length polymorphism (RFLP) analysis for OXA-48 plasmids revealed a similar band pattern (Fig. S1). These results indicate a similar backbone of the OXA-48 encoding plasmids, a finding also supported by the results produced by PCR and sequencing of their *repA*, *traU*, and *parA* genes (Table 1). More than 98% identity among all these plasmids and with the pOXA-48 plasmids described previously by Poirel et al. (20) was observed.

Characterization of pRYC-OXA-48. The plasmid isolated from strain F64-ST11-OXA-48 was an IncL/M plasmid of 74,686 bp, comprising 92 coding DNA sequences

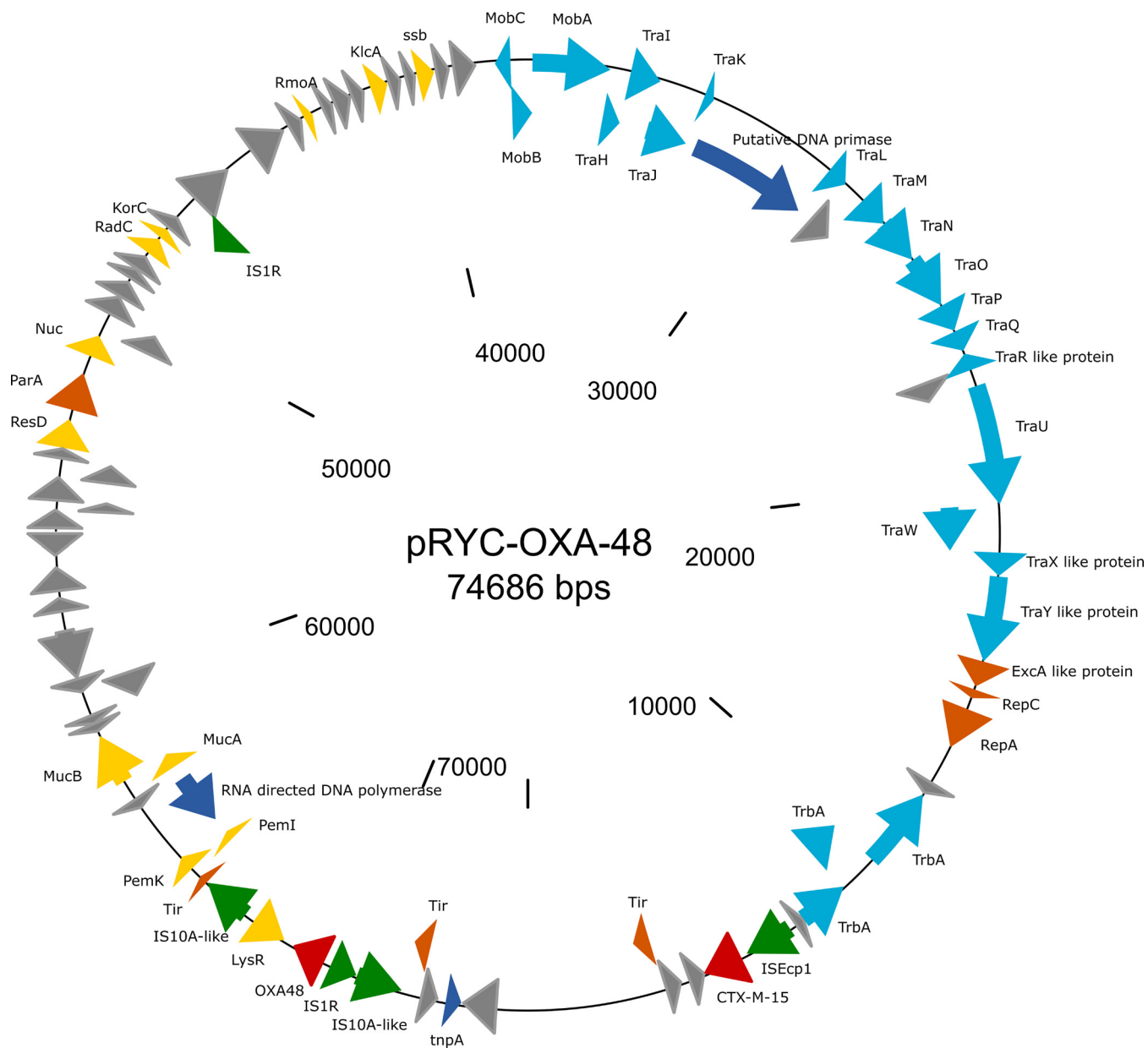


FIG 2 ST11 *K. pneumoniae* clone carrying pRYC-OXA48.

(CDS; GC%, 50.8%) and containing two *bla* genes (*bla*_{OXA-48} and *bla*_{CTX-M-15}) as the only antibiotic resistance markers. The *bla*_{OXA-48} gene was part of Tn1999.2 inserted at the *tir* gene, responsible for inhibition of transfer of the plasmid, whereas the *bla*_{CTX-M-15} gene was located downstream of *ISEcp1* (Fig. 2). The resistance region of the plasmid (including both *bla* resistance determinants) was delimited by two insertion sequences: *ISEcp1* was found to be associated with the *bla*_{CTX-M-15} gene and an *IS10A*-like sequence located upstream of *bla*_{OXA-48} (Fig. 2). *In silico* comparisons with other OXA-48-encoding-plasmids revealed a very similar structure (>99% similarity) with the same backbone as that found in the IncL-OXA-48 plasmids (Fig. 3) (GenBank accession no. [JN626286](#), [KC335143](#), [KC354801](#), and [NC_021502](#)).

DISCUSSION

Spread of CPE has been increasingly reported worldwide since first description more than 20 years ago, with predominance of metallo-beta-lactamase (MBL) producers in

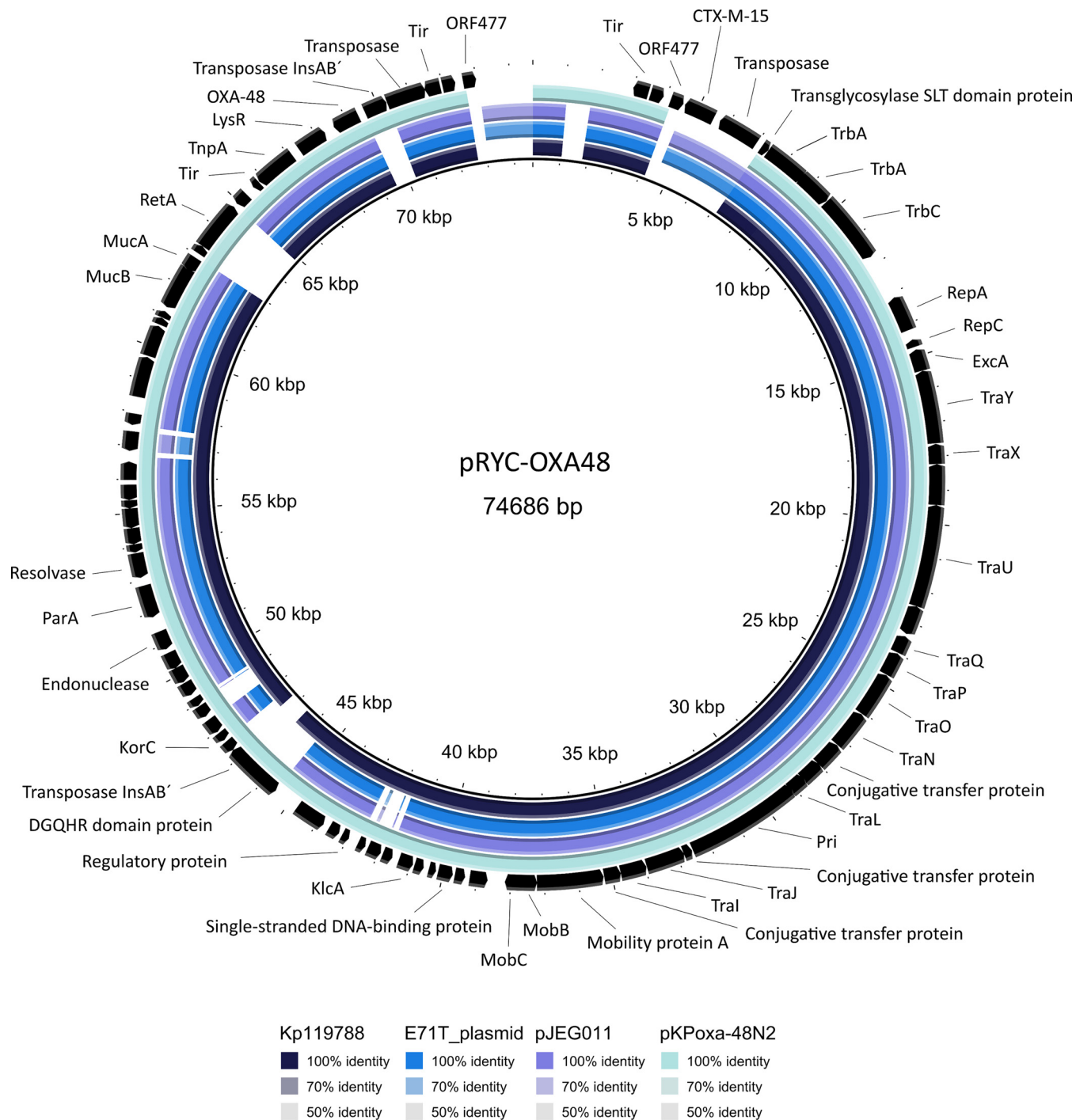


FIG 3 Brig comparison of pRYC-OXA-48 with other OXA-48-carrying plasmids.

Asia and Europe, KPC producers in the United States, and OXA-48 producers in the Mediterranean countries (6, 9, 21, 22). These carbapenemases have been increasingly recovered in Spain, and OXA-48 isolates are currently the most prevalent ones (3, 6). In our study, the prevalence of carbapenemase in our institution was 0.4%, and the most prevalent carbapenemase was OXA-48 (50.3%). The carbapenemase situation in our institution, which started with VIM producers in 2005 and KPC producers in 2009 (1, 12, 13), can be observed in Fig. S2. In this article, we characterize the early isolates of OXA-48-producing *Enterobacterales* collected in our hospital; currently, the situation in

our institution is one of OXA-48 infection endemicity (11). Due to the difficulties in the detection of OXA-48 carbapenemase producers by the use of carbapenem susceptibility results as a screening test, we implemented measurement of increases in temocillin MIC as a surrogate marker to detect its presence in CarbaNP test-positive isolates with a negative result for MBL or KPC enzymes (23, 24). Nevertheless, we recovered isolates with decreased susceptibility to carbapenemase to perform specific PCR for OXA-48, and results were negative (data not shown).

Isolation of OXA-48-producing *Enterobacterales* in our hospital started in the urology ward in 2012 (Fig. S2). In that ward, most of the *K. pneumoniae* isolates belonged to the same clone (KP-A) which corresponded to ST11. Interestingly, this ST was previously found in our institution in 2010 in a patient admitted to general surgery who was infected with a KPC-3-producing *K. pneumoniae* strain and in 2011 in 2 patients admitted to the neurosurgery ICU who were colonized with a VIM-1-producing *K. pneumoniae* strain. These isolates belonged to the same ST and showed closely related PFGE patterns, suggesting that the circulation of a clonal local pool in our institution plays an important role in the emergence and spread of new resistant variants, as happened with the OXA-48-producing clone. Later, the same clone was detected in other wards in addition to ST405 *K. pneumoniae*. The latter was first identified at the nearby Hospital La Paz (500 m from our hospital) where such infections had become endemic (2). However, in spite of the close proximity of the two hospitals, these ST405 isolates were found to have different PFGE patterns (data not shown), suggesting independent introduction events. In a study published in 2017 by the national reference laboratory that included data representing *K. pneumoniae* isolates collected in different Spanish areas, both sequence types were found among the most prevalent ones, highlighting their persistence over time in our country (25).

We detected the presence of *bla*_{CTX-M-15} in most OXA-48-producing isolates. It is of note that we previously found CTX-M-15 in ST11 *K. pneumoniae* isolates recovered from rectal swabs (data not published), denoting possible acquisition of *bla*_{OXA-48} determinants in circulating clones in our institution. Coproduction of OXA-48 and CTX-M-15 in *Enterobacterales* has been widely described previously (26, 27). However, in pRYC-OXA-48, *bla*_{CTX-M-15} was associated with *ISEcp1*, but without interrupting the *Tn1999.2* which contains *bla*_{OXA-48} (Fig. 2), a configuration also previously described (28–30).

It should be noted that 7 isolates (4 *K. pneumoniae* and 3 *E. coli*) coproduced VIM-1 and that 6 of them (4 *K. pneumoniae* and 2 *E. coli*) also coproduced CTX-M-15. The presence of more than one *bla* gene in these isolates can be explained by the carbapenemase endemic situation involving *bla*_{VIM-1}, *bla*_{KPC} and *bla*_{CTX-M-15} genes in our hospital (1, 11, 13). The presence of both *bla*_{VIM-1} and *bla*_{OXA-48} enzymes in the same isolate enhances the difficulty of phenotypically detection of OXA-48 producers. We believe that in these cases, the double-disk synergy test performed with EDTA is not entirely reliable to identify VIM-1 producers as in our study we had five out of seven false-negative results.

To our knowledge, there have been only limited previously published descriptions of the coexistence of OXA-48 and VIM-1 (31–34), but the availability of such reports might increase in the future. Interestingly, we observed that *bla*_{OXA-48} and *bla*_{VIM-1} genes, mostly associated with *K. pneumoniae* ST11, were located on the same plasmid. The plasmids detected in our study (among different PFGE patterns) showed a high degree of similarity (>99%) with previously described OXA-48 plasmids, even with those also encoding *bla*_{VIM-1} and *bla*_{CTX-M-15}. This indicates that the spread of genes encoding this carbapenemase, which are often linked with other beta-lactamase genes, is associated not only with the appearance of highly adapted clones in the hospital but also with the dissemination of highly transmissible plasmids. The presence of these plasmids in the hospital setting allows acquisition and rapid spread of new resistance determinants such as *bla*_{OXA-48}. The characterized IncL/M plasmid is currently present in our institution (31).

In conclusion, our data showed rapid penetration and spread of *bla*_{OXA-48} genes in multiresistant clones of *Enterobacterales* after its emergence in our institution. The

globally spread ST11 *K. pneumoniae* clone, previously associated in our hospital with CTX-M-15, VIM-1, and KPC-3 enzymes, is now also present coproducing OXA-48 (11). This scenario reinforces the idea of the contribution of high-risk clones in the dissemination and persistence of antibiotic resistance genes. Moreover, emergence of multi-resistant ST11 among nonhospitalized patients highlights the difficulties in preventing dissemination of such strains into the community.

MATERIALS AND METHODS

Bacterial isolates. Over a 21-month study period (March 2012 to December 2013), all isolates ($n = 71$) recovered from clinical specimens (27 urine samples, 9 wounds, 8 sterile fluids, 5 respiratory samples, 2 blood cultures) and surveillance cultures (20 rectal swabs) suspected of carbapenemase production but negative for the phenotypic expression of classes A and B carbapenemases (see below) were investigated for OXA-48-like-producing enzymes. Patients' demographic characteristics were also reviewed. The study was approved by the ethical committee of our institution (reference 251-13). For clonal comparison, we also included in the analysis the CPE clinical isolates associated with contemporary outbreaks due to strains producing MBL or KPC in our institution (1, 12, 13) and to OXA-48 producers collected in a nearby hospital (Hospital La Paz, 500 m from our hospital) (2).

Bacterial identification, susceptibility testing, and phenotypic assays. Species identification and antibiotic susceptibility testing were performed using matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) (Bruker Daltonics, Leipzig, Germany) and the MicroScan automated system (Beckman Coulter, Brea, CA), respectively. MICs were interpreted using EUCAST criteria (www.eucast.org). Screening for the presence of carbapenemase production was performed with CarbaNP (23). MBL production was investigated by a double-disk synergy test using EDTA plus meropenem and ceftazidime disks as previously described (1, 35). KPC production was inferred by an increase of the inhibition zone of an unsupplemented meropenem disk compared with that of a meropenem disk supplemented with 0.3 mg of boronic acid (36). In addition, OXA-48 production was phenotypically inferred by the lack of the inhibition zone of the temocillin disk (37).

Characterization of *bla* genes and clonal relatedness. The presence of genes encoding carbapenemase (*bla*_{OXA-48}, *bla*_{VIM}, and *bla*_{KPC}) and ESBLs (*bla*_{TEM}, *bla*_{SHV}, and *bla*_{CTX-M}) classes was screened by PCR as previously described (1, 38). Clonal relatedness was established by pulsed-field gel electrophoresis (PFGE) analysis of XbaI (New England Biolabs, Inc., England)-digested genomic DNA (39). Multilocus sequencing typing (MLST) was performed for *K. pneumoniae* isolates (<https://bigsdbs.pasteur.fr/klebsiella/klebsiella.html>) and *E. coli* isolates (<http://mlst.warwick.ac.uk/mlst/dbs/Ecoli>).

Transferability and location of *bla* carbapenemase genes and plasmid typing. Mating experiments were performed using *E. coli* strain BM21 (nalidixic acid and rifampin resistant, lactose fermentation positive, and plasmid free) as the recipient (1). The plasmid content (number and size) of each strain was analyzed by S1-digested genomic DNA PFGE, the *bla* gene location being detected by standard hybridization procedures (40, 41). Transconjugants were selected on Luria-Bertani agar plates containing imipenem (0.5 µg/ml) and rifampin (100 µg/ml) and were incubated at 37°C for 24 h. Plasmid incompatibility groups were inferred by PCR typing schemes based on the presence of genes encoding replication initiator proteins (RIP) (18, 19). Further characterization of OXA-48 gene-carrying plasmids was performed for all *K. pneumoniae* PFGE types by comparison of restriction fragment length polymorphism (RFLP) patterns of DraI-digested plasmid DNA. To further characterize the OXA-48 IncL/M plasmids, the genes associated with replication (*repA*), conjugation (*traU*), and maintenance (*parA*) were amplified and further sequenced (20).

ST11 whole-genome sequencing and plasmid characterization. *K. pneumoniae* ST11 strains associated with production of either VIM-1 (31) or KPC (13) have been prevalent in our hospital since their first detection in 2005. Due to the high transmissibility and persistence of this clone (11, 42), we sequenced one ST11 *K. pneumoniae* isolate, which was selected based on its ESBL-positive phenotype (this isolate coproduced both OXA-48 and CTX-M-15). Whole-genome sequencing (WGS) was performed using Illumina (280× coverage), and DNA fragments were subjected to *de novo* assembly with Velvet and Spades (43). In addition, the first ST11–OXA-48 isolate found in our hospital (ryc_12106801) was also sequenced and assembled using PacBio and RS_HGAP Assembly 2, respectively. Combining the sequences obtained by Illumina and PacBio, and using SPAdes, the plasmid of this isolate was closed (44–47). The *in silico* plasmid typing was carried out using the PlasmidFinder tool (48). This plasmid was compared with other indexed plasmids at the NCBI database (GenBank accession numbers [JN626286.1](https://ncbi.nlm.nih.gov/nuccore/JN626286.1), [KC335143.1](https://ncbi.nlm.nih.gov/nuccore/KC335143.1), [KC354801.1](https://ncbi.nlm.nih.gov/nuccore/KC354801.1), and [NC_021502.1](https://ncbi.nlm.nih.gov/nuccore/NC_021502.1)) using BRIG (49) (<http://brig.sourceforge.net/>) and MAUVE (<http://darlinglab.org/mauve/mauve.html>).

Data availability. Data representing this Whole Genomes Shotgun project have been deposited at DDBJ/ENA/GenBank under accession number [VILG0000000](https://ncbi.nlm.nih.gov/nuccore/VILG0000000). The version described in this paper is version [VILG01000000](https://ncbi.nlm.nih.gov/nuccore/VILG01000000).

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

FIG S1, TIF file, 2.1 MB.

FIG S2, TIF file, 1.1 MB.

ACKNOWLEDGMENTS

We thank Mary Harper for correction of the English used in the manuscript, Rosa Gómez-Gil (Hospital La Paz, Madrid, Spain) for lending us the strains from her hospital for comparison with our isolates, and Marta Hernandez-García for technical assistance.

This study was funded by the European Commission, Seven Framework Program (grants R-GNOSIS-FP7-HEALTH-F3-2011-282512); by Centro para el Desarrollo Tecnológico Industrial (CDTI), Instituto de Salud Carlos III of Spain, CDTI project NEXTMI-CRO (grant IDI-20120242); and by Plan Estatal DE I+D+I 2013-2016 (REIPI RD12/0015/0004 and RD16/0016/0011; Spanish Network for Research in Infectious Diseases) and was cofinanced by the European Development Regional Fund, “A Way to Achieve Europe” (FEDER). D.G. was funded through a research contract of Instituto de Salud Carlos III of Spain (CM12/00146). A.V. was funded by a postdoctoral fellowship, “Juan de la Cierva” (JCI-2011-10863) from Ministerio de Economía y Competitividad of Spain (2012-2014). A.P.T. and I.R. were funded by EvoTAR (EvoTAR-282004).

We declare that we have no conflicts of interest.

REFERENCES

- Tato M, Coque TM, Ruiz-Garbajosa P, Pintado V, Cobo J, Sader HS, Jones RN, Baquero F, Cantón R. 2007. Complex clonal and plasmid epidemiology in the first outbreak of *Enterobacteriaceae* infection involving VIM-1 metallo-beta-lactamase in Spain: toward endemicity? *Clin Infect Dis* 45:1171–1178. <https://doi.org/10.1086/522288>.
- Paño-Pardo JR, Ruiz-Carrascoso G, Navarro-San Francisco C, Gómez-Gil R, Mora-Rillo M, Romero-Gómez MP, Fernández-Romero N, García-Rodríguez J, Pérez-Blanco V, Moreno-Ramos F, Mingorance J. 2013. Infections caused by OXA-48-producing *Klebsiella pneumoniae* in a tertiary hospital in Spain in the setting of a prolonged, hospital-wide outbreak. *J Antimicrob Chemother* 68:89–96. <https://doi.org/10.1093/jac/dks364>.
- Oteo J, Ortega A, Bartolomé R, Bou G, Conejo C, Fernández-Martínez M, González-López JJ, Martínez-García L, Martínez-Martínez L, Merino M, Miró E, Mora M, Navarro F, Oliver A, Pascual Á, Rodríguez-Baño J, Ruiz-Carrascoso G, Ruiz-Garbajosa P, Zamorano L, Bautista V, Pérez-Vázquez M, Campos J; GEIH-GEMARA (SEIMC) and REIPI. 2015. Prospective multicenter study of carbapenemase producing *Enterobacteriaceae* from 83 hospitals in Spain: high in vitro susceptibility to colistin and meropenem. *Antimicrob Agents Chemother* 59:3406–3412. <https://doi.org/10.1128/AAC.00086-15>.
- Cantón R, Akóva M, Carmeli Y, Giske CG, Glupczynski Y, Gniadkowski M, Livermore DM, Miriagou V, Naas T, Rossolini GM, Samuelsen Ø, Seifert H, Woodford N, Nordmann P, Poirel L, Bogaerts P, Navon-Venezia S, Cornaglia G; European Network on Carbapenemases. 2012. Rapid evolution and spread of carbapenemases among *Enterobacteriaceae* in Europe. *Clin Microbiol Infect* 18:413–431. <https://doi.org/10.1111/j.1469-0691.2012.03821.x>.
- Glasner C, Albiger B, Buist G, Tambić Andrasević A, Canton R, Carmeli Y, Friedrich AW, Giske CG, Glupczynski Y, Gniadkowski M, Livermore DM, Nordmann P, Poirel L, Rossolini GM, Seifert H, Vatopoulos A, Walsh T, Woodford N, Donker T, Monnet DL, Grundmann H; European Survey on Carbapenemase-Producing Enterobacteriaceae (EuSCAPE) Working Group. 11 July 2013, posting date. Carbapenemase-producing *Enterobacteriaceae* in Europe: a survey among national experts from 39 countries, February 2013. *Euro Surveill* <https://www.eurosurveillance.org/content/10.2807/1560-7917.ES2013.18.28.20525>.
- Grundmann H, Glasner C, Albiger B, Aanensen DM, Tomlinson CT, Andrasević AT, Cantón R, Carmeli Y, Friedrich AW, Giske CG, Glupczynski Y, Gniadkowski M, Livermore DM, Nordmann P, Poirel L, Rossolini GM, Seifert H, Vatopoulos A, Walsh T, Woodford N, Monnet DL; European Survey of Carbapenemase-Producing Enterobacteriaceae (EuSCAPE) Working Group. 2017. Occurrence of carbapenemase-producing *Klebsiella pneumoniae* and *Escherichia coli* in the European survey of carbapenemase-producing *Enterobacteriaceae* (EuSCAPE): a prospective, multinational study. *Lancet Infect Dis* 17:153–163. [https://doi.org/10.1016/S1473-3099\(16\)30257-2](https://doi.org/10.1016/S1473-3099(16)30257-2).
- Poirel L, Héritier C, Tolün V, Nordmann P. 2004. Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 48:15–22. <https://doi.org/10.1128/aac.48.1.15-22.2004>.
- Mairi A, Pantel A, Sotto A, Lavigne J-P, Touati A. 2018. OXA-48-like carbapenemases producing *Enterobacteriaceae* in different niches. *Eur J Clin Microbiol Infect Dis* 37:587–604. <https://doi.org/10.1007/s10096-017-3112-7>.
- Pitout JDD, Peirano G, Kock MM, Strydom K-A, Matsumura Y. 13 November 2019, posting date. The global ascendancy of OXA-48-type carbapenemases. *Clin Microbiol Rev* <https://doi.org/10.1128/CMR.00102-19>.
- Nordmann P, Naas T, Poirel L. 2011. Global spread of carbapenemase-producing *Enterobacteriaceae*. *Emerg Infect Dis* 17:1791–1798. <https://doi.org/10.3201/eid1710.110655>.
- Hernández-García M, Pérez-Viso B, Carmen Turrientes M, Díaz-Agero C, López-Fresneña N, Bonten M, Malhotra-Kumar S, Ruiz-Garbajosa P, Cantón R. 2018. Characterization of carbapenemase-producing *Enterobacteriaceae* from colonized patients in a university hospital in Madrid, Spain, during the R-GNOSIS project depicts increased clonal diversity over time with maintenance of high-risk clones. *J Antimicrob Chemother* 73:3039–3043. <https://doi.org/10.1093/jac/dky284>.
- Curiao T, Morosini MI, Ruiz-Garbajosa P, Robustillo A, Baquero F, Coque TM, Cantón R. 2010. Emergence of blaKPC-3-Tn4401a associated with a pKPN3/4-like plasmid within ST384 and ST388 *Klebsiella pneumoniae* clones in Spain. *J Antimicrob Chemother* 65:1608–1614. <https://doi.org/10.1093/jac/dkq174>.
- Ruiz-Garbajosa P, Curiao T, Tato M, Gijón D, Pintado V, Valverde A, Baquero F, Morosini MI, Coque TM, Cantón R. 2013. Multiclonal dispersal of KPC genes following the emergence of non-ST258 KPC-producing *Klebsiella pneumoniae* clones in Madrid, Spain. *J Antimicrob Chemother* 68:2487–2492. <https://doi.org/10.1093/jac/dkt237>.
- Pitart C, Solé M, Roca I, Fàbrega A, Vila J, Marco F. 2011. First outbreak of a plasmid-mediated carbapenem-hydrolyzing OXA-48 β -lactamase in *Klebsiella pneumoniae* in Spain. *Antimicrob Agents Chemother* 55:4398–4401. <https://doi.org/10.1128/AAC.00329-11>.
- Oteo J, Saez D, Bautista V, Fernández-Romero S, Hernández-Molina JM, Pérez-Vázquez M, Aracil B, Campos J; Spanish Collaborating Group for the Antibiotic Resistance Surveillance Program. 2013. Carbapenemase-producing *Enterobacteriaceae* in Spain in 2012. *Antimicrob Agents Chemother* 57:6344–6347. <https://doi.org/10.1128/AAC.01513-13>.
- Madueño A, González-García J, Alonso Socas MDM, Miguel Gómez MA, Lecuona M. 2018. Clinical features and outcomes of bacteraemia due to OXA-48-like carbapenemase-producing *Klebsiella pneumoniae* in a tertiary hospital. *Enferm Infecc Microbiol Clin* 36:498–501. <https://doi.org/10.1016/j.eimc.2017.09.008>.
- Brañas P, Gil M, Villa J, Orellana MÁ, Chaves F. 2018. Molecular epidemiology of carbapenemase-producing *Enterobacteriaceae* infection/colonisation in a hospital in Madrid. *Enferm Infecc Microbiol Clin* 36:100–103. <https://doi.org/10.1016/j.eimc.2016.10.004>.
- Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ. 2005. Identification of plasmids by PCR-based replicon typing. *63:219–228*.
- Villa L, García-Fernández A, Fortini D, Carattoli A. 2010. Replicon sequence typing of IncF plasmids carrying virulence and resistance determinants. *J Antimicrob Chemother* 65:2518–2529. <https://doi.org/10.1093/jac/dkq347>.

20. Poirel L, Bonnin RA, Nordmann P. 2012. Genetic features of the widespread plasmid coding for the carbapenemase OXA-48. *Antimicrob Agents Chemother* 56:559–562. <https://doi.org/10.1128/AAC.05289-11>.
21. Grundmann H, Livermore DM, Giske CG, Canton R, Rossolini GM, Campos J, Vatopoulos A, Gniadkowski M, Toth A, Pfeifer Y, Jarlier V, Carmeli Y. 18 November 2010, posting date. Carbapenem-non-susceptible *Enterobacteriaceae* in Europe: conclusions from a meeting of national experts. *Euro Surveill* <https://doi.org/10.2807/ese.15.46.19711-en>.
22. Queenan AM, Bush K. 2007. Carbapenemases: the versatile beta-lactamases. *Clin Microbiol Rev* 20:440–458. <https://doi.org/10.1128/CMR.00001-07>.
23. Nordmann P, Poirel L, Dortet L. 2012. Rapid detection of carbapenemase-producing *Enterobacteriaceae*. *Emerg Infect Dis* 18:1503–1507. <https://doi.org/10.3201/eid1809.120355>.
24. Glupczynski Y, Huang TD, Bouchahrouf W, Rezende De Castro R, Bauring C, Gérard M, Verbruggen AM, Deplano A, Denis O, Bogaerts P. 2012. Rapid emergence and spread of OXA-48-producing carbapenem-resistant *Enterobacteriaceae* isolates in Belgian hospitals. *Int J Antimicrob Agents* 39:168–172. <https://doi.org/10.1016/j.ijantimicag.2011.10.005>.
25. Esteban-Cantos A, Aracil B, Bautista V, Ortega A, Lara N, Saez D, Fernández-Romero S, Pérez-Vázquez M, Navarro F, Grundmann H, Campos J, Oteo J; EuSCAPE-Spain Network. 24 March 2017, posting date. The carbapenemase-producing *Klebsiella pneumoniae* population is distinct and more clonal than the carbapenem-susceptible population. *Antimicrob Agents Chemother* <https://doi.org/10.1128/AAC.02520-16>.
26. Potron A, Nordmann P, Rondinaud E, Jaureguy F, Poirel L. 2013. A mosaic transposon encoding OXA-48 and CTX-M-15: towards pan-resistance. *J Antimicrob Chemother* 68:476–477. <https://doi.org/10.1093/jac/dks397>.
27. Fernández J, Montero I, Martínez Ó, Fleites A, Poirel L, Nordmann P, Rodicio MR. 2015. Dissemination of multiresistant *Enterobacter cloacae* isolates producing OXA-48 and CTX-M-15 in a Spanish hospital. *Int J Antimicrob Agents* 46:469–474. <https://doi.org/10.1016/j.ijantimicag.2015.07.003>.
28. Carrer A, Poirel L, Yilmaz M, Akan OA, Feriha C, Cuzon G, Matar G, Honderlick P, Nordmann P. 2010. Spread of OXA-48-encoding plasmid in Turkey and beyond. *Antimicrob Agents Chemother* 54:1369–1373. <https://doi.org/10.1128/AAC.01312-09>.
29. Dimou V, Dhanji H, Pike R, Livermore DM, Woodford N. 2012. Characterization of *Enterobacteriaceae* producing OXA-48-like carbapenemases in the UK. *J Antimicrob Chemother* 67:1660–1665. <https://doi.org/10.1093/jac/dks124>.
30. Brañas P, Villa J, Viedma E, Mingorance J, Orellana MA, Chaves F. 2015. Molecular epidemiology of carbapenemase-producing *Klebsiella pneumoniae* in a hospital in Madrid: successful establishment of an OXA-48 ST11 clone. *Int J Antimicrob Agents* 46:111–116. <https://doi.org/10.1016/j.ijantimicag.2015.02.019>.
31. Hernández-García M, Pérez-Viso B, Navarro-San Francisco C, Baquero F, Morosini MI, Ruiz-Garbajosa P, Cantón R. 2019. Intestinal co-colonization with different carbapenemase-producing *Enterobacteriales* isolates is not a rare event in an OXA-48 endemic area. *EClinicalMedicine* 15:72–79. <https://doi.org/10.1016/j.eclinm.2019.09.005>.
32. Poirel L, Abdelaziz MO, Bernabeu S, Nordmann P. 2013. Occurrence of OXA-48 and VIM-1 carbapenemase-producing *Enterobacteriaceae* in Egypt. *Int J Antimicrob Agents* 41:90–91. <https://doi.org/10.1016/j.ijantimicag.2012.08.015>.
33. Arana DM, Ortega A, González-Barberá E, Lara N, Bautista V, Gómez-Ruiz D, Sáez D, Fernández-Romero S, Aracil B, Pérez-Vázquez M, Campos J, Oteo J; Spanish Antibiotic Resistance Surveillance Programme Collaborating Group. 2017. Carbapenem-resistant *Citrobacter* spp. isolated in Spain from 2013 to 2015 produced a variety of carbapenemases including VIM-1, OXA-48, KPC-2, NDM-1 and VIM-2. *J Antimicrob Chemother* 72:3283–3287. <https://doi.org/10.1093/jac/dkx325>.
34. Hajjeh Z, Gharsallah H, Najia H, Boutiba I, Labbene I, Ferjani M. 2016. Successful treatment of a carbapenem-resistant *Klebsiella pneumoniae* carrying *bla*_{OXA-48}, *bla*_{VIM-2}, *bla*_{CMY-2} and *bla*_{SHV} with high dose combination of imipenem and amikacin. *IDCases* 4:10–12. <https://doi.org/10.1016/j.idcr.2016.01.003>.
35. Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, Chong Y. 2002. Imipenem-EDTA disk method for differentiation of metallo-beta-lactamase-producing clinical isolates of *Pseudomonas* spp. and *Acinetobacter* spp. *J Clin Microbiol* 40:3798–3801. <https://doi.org/10.1128/jcm.40.10.3798-3801.2002>.
36. Giske CG, Gezelius L, Samuelsen Ø, Warner M, Sundsfjord A, Woodford N. 2011. A sensitive and specific phenotypic assay for detection of metallo-β-lactamases and KPC in *Klebsiella pneumoniae* with the use of meropenem disks supplemented with aminophenylboronic acid, dipicolinic acid and cloxacillin. *Clin Microbiol Infect* 17:552–556. <https://doi.org/10.1111/j.1469-0691.2010.03294.x>.
37. Huang T-D, Poirel L, Bogaerts P, Berhin C, Nordmann P, Glupczynski Y. 2014. Temocillin and piperacillin/tazobactam resistance by disc diffusion as antimicrobial surrogate markers for the detection of carbapenemase-producing *Enterobacteriaceae* in geographical areas with a high prevalence of OXA-48 producers. *J Antimicrob Chemother* 69:445–450. <https://doi.org/10.1093/jac/dkt367>.
38. Monstein HJ, Östholm-Balkhed Å, Nilsson MV, Nilsson M, Dornbusch K, Nilsson LE. 2007. Multiplex PCR amplification assay for the detection of *bla*_{SHV}, *bla*_{TEM} and *bla*_{CTX-M} genes in *Enterobacteriaceae*. *APMIS* 115: 1400–1408. <https://doi.org/10.1111/j.1600-0463.2007.00722.x>.
39. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, Swaminathan B. 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 33:2233–2239. <https://doi.org/10.1128/JCM.33.9.2233-2239.1995>.
40. Liu SL, Hesse A, Sanderson KE. 1993. Genomic mapping with I-Ceu I, an intron-encoded endonuclease specific for genes for ribosomal RNA, in *Salmonella* spp., *Escherichia coli*, and other bacteria. *Proc Natl Acad Sci U S A* 90:6874–6878. <https://doi.org/10.1073/pnas.90.14.6874>.
41. Sambrook J, Russell DW. 2001. *Molecular cloning: a laboratory manual*, 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
42. Oteo J, Pérez-Vázquez M, Bautista V, Ortega A, Zamarrón P, Saez D, Fernández-Romero S, Lara N, Ramiro R, Aracil B, Campos J; Spanish Antibiotic Resistance Surveillance Programme Collaborating Group. 2016. The spread of KPC-producing *Enterobacteriaceae* in Spain: WGS analysis of the emerging high-risk clones of *Klebsiella pneumoniae* ST11/KPC-2, ST101/KPC-2 and ST512/KPC-3. *J Antimicrob Chemother* 71:3392–3399. <https://doi.org/10.1093/jac/dkw321>.
43. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham SON, Pribelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev M, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *BMC Bioinformatics* 13:116–124. <https://doi.org/10.1186/1471-2109-13-116>.
44. Chin C-S, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korlach J. 2013. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. *Nat Methods* 10:563–569. <https://doi.org/10.1038/nmeth.2474>.
45. Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. *Bioinformatics* 29:1072–1075. <https://doi.org/10.1093/bioinformatics/btt086>.
46. Zerbino DR, Birney E. 2008. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. *Genome Res* 18:821–829. <https://doi.org/10.1101/gr.074492.107>.
47. Nurk S, Bankevich A, Antipov D, Gurevich AA, Korobeynikov A, Lapidus A, Pribelski AD, Pyshkin A, Sirotkin A, Sirotkin Y, Stepanauskas R, Clingenpeel SR, Woyke T, Mclean JS, Lasken R, Tesler G, Alekseyev MA, Pevzner PA. 2013. Assembling single-cell genomes and mini-metagenomes from chimeric MDA products. *J Comput Biol* 20:714–737. <https://doi.org/10.1089/cmb.2013.0084>.
48. Carattoli A, Zankari E, García-Fernández A, Larsen MV, Lund O, Villa L, Aarestrup FM, Hasman H. 2014. *In silico* detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrob Agents Chemother* 58:3895–3903. <https://doi.org/10.1128/AAC.02412-14>.
49. Alikhan N-F, Petty NK, Ben Zakour NL, Beatson SA. 2011. BLAST Ring Image Generator (BRIG): simple prokaryote genome comparisons. *BMC Genomics* 12:402. <https://doi.org/10.1186/1471-2164-12-402>.