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EPIDEMIOLOGY AND SURVEILLANCE

OXA-48-Like-Producing *Klebsiella pneumoniae* in Southern Spain in 2014–2015

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ABSTRACT The aim of this study was to characterize the population structure of 56 OXA-48-like-producing *Klebsiella pneumoniae* isolates, as well as extended-spectrum β-lactamase (ESBL) and carbapenemase genes, recovered in 2014 and 2015 from 16 hospitals in southern Spain. XbaI pulsed-field gel electrophoresis and multilocus sequence typing were performed to assess clonal relatedness. Representative isolates belonging to OXA-48-like-producing and CTX-M-15-coproducing pulsotypes were selected for characterization of $bla_{\text{OXA-48-like}}$- and $bla_{\text{CTX-M-15}}$-carrying plasmids by PCR-based replicon typing, IncF subtyping, whole-genome sequencing analysis, and typing of Tn*1999* structures. Forty-three OXA-48-producing isolates (77%) were recovered from clinical samples and 13 from rectal swabs. All isolates showed ertapenem MIC values of ≥1 mg/liter, although 70% remained susceptible to imipenem and meropenem. Forty-nine isolates (88%) produced OXA-48, 5 produced OXA-245, and 2 produced OXA-181. Twenty-eight different pulsotypes (5 detected in more than 1 hospital) and 16 sequence types (STs) were found. The most prevalent clones were ST15 (29 isolates [52%]) and ST11 (7 isolates [13%]). Forty-five (80%) isolates were also $bla_{\text{CTX-M-15}}$ carriers. The $bla_{\text{CTX-M-15}}$ gene was mostly (82%) located on IncR plasmids, although ST15 and ST11 isolates also carried this gene on IncF plasmids. The composite transposon variant Tn*1999.2*-like was the most frequent. Among ST15 and ST11 isolates, different transposon variants were observed. The $bla_{\text{OXA-48}}$ gene was mainly located on IncL plasmids, although IncM plasmids were also observed. The spread of OXA-48-like-producing *K. pneumoniae* in southern Spain is mainly due to ST15 and ST11 clones. Variation within clonal lineages could indicate different acquisition events for both ESBL and carbapenemase traits.

KEYWORDS *Klebsiella pneumoniae*, OXA-48, southern Spain

The most important carbapenemases in *Enterobacteriaceae* are the KPC (class A), NDM, IMP, VIM (class B), and OXA-48 (class D) types (1). Among carbapenemase-producing microorganisms, the increasing number of OXA-48-producing *Enterobacteriaceae*, mainly *Klebsiella pneumoniae*, is becoming a serious threat, particularly in Europe (2). OXA-48 was first observed in a clinical isolate of *K. pneumoniae* in Turkey in 2001 (3). Since that time, it has spread across North Africa and Europe (1). OXA-48-type carbapenemases hydrolyze penicillins and carbapenems but show weak activity against extended-spectrum cephalosporins (3). Isolates harboring $bla_{\text{OXA-48}}$ also carry other β-lactamase genes, such as $bla_{\text{CTX-M-15}}$ and $bla_{\text{OXA-1}}$, resulting in resistance to cepha-

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losporins and amoxicillin/clavulanate (4). In Spain, increasing numbers of cases of carbapenemase-producing *Enterobacteriaceae* (CPE) have been reported in recent years. In a Spanish multicenter study performed in 2012, the most common CPE type detected as a cause of nosocomial outbreaks and individual infections was OXA-48-producing *K. pneumoniae* (5).

Dissemination of OXA-48 is principally related to the spread of a highly transferable IncL/M plasmid carrying only $bla_{\text{OXA-48-like}}$ genes as resistance determinants (6). The $bla_{\text{OXA-48}}$ gene is carried by the Tn*1999*.1 transposon or its variants Tn*1999.2* and Tn*1999.3* (with 1 or 2 copies, respectively, of IS*1R* inserted into IS*1999*) (7). Two new variants of Tn*1999.2* have recently been reported, namely, Tn*1999.4* and Tn*1999.5*, in which the *lysR* gene is truncated by Tn*2015* and IS*Kpn19*, respectively (8, 9). Despite the growing prevalence of OXA-48 producers, little is known regarding the prevalence or genetic environment of $bla_{\text{OXA-48}}$ and its accompanying resistance determinants in Spain.

In 2014 and 2015, 16 hospital laboratories in Andalusia (southern Spain) submitted 276 *K. pneumoniae* isolates of non-wild-type carbapenem susceptibility (10), both single and clustered in time, to the reference laboratory of the PIRASOA program (Hospital Universitario Virgen Macarena, Seville, Spain). A total of 177 isolates (64%) were confirmed to be carbapenemase producers; 56 isolates (32%) were OXA-48-like producers and the remainder were KPC-3 producers. The resistance determinants ($bla_{\text{OXA-48-like}}$ and $bla_{\text{CTX-M-15}}$ genes), plasmids, Tn*1999*-type composite transposons, and population structure of OXA-48-like-producing *K. pneumoniae* isolates were characterized.

RESULTS AND DISCUSSION

Patients and isolates. Forty-three OXA-48-like-producing *K. pneumoniae* isolates (77%) came from clinical samples, distributed as follows: urine, 23 isolates (41%); blood, 8 isolates (14%); wounds, 6 isolates (11%); sputum, 2 isolates (4%); bile, 2 isolates (4%); catheter tip, 1 isolate (2%); ascitic fluid, 1 isolate (2%). Thirteen isolates (23%) came from rectal swabs (see Table S2 in the supplemental material). Two groups of isolates were recovered from outbreak investigations in hospitals (9 and 5 isolates from hospital H8), and the remainder were sporadic isolates.

Antibiotic susceptibility and antibiotic resistance genes. All isolates were nonsusceptible to amoxicillin-clavulanic acid, piperacillin-tazobactam, temocillin, and ertapenem. Thirty-nine isolates (70%) remained susceptible to both imipenem and meropenem. Susceptibility to other antibiotics was as follows: ceftazidime and aztreonam, 18%; cefotaxime and cefepime, 11%; ciprofloxacin, 12%; gentamicin, 73%; tobramycin, 23%; amikacin, 77%; fosfomycin, 54%; trimethoprim-sulfamethoxazole, 29% (Table S2). All isolates showed positive results for imipenem hydrolysis using the β-Carba assay.

Three OXA-48 variants were identified; $bla_{\text{OXA-48}}$ was identified in 49 (87.5%) of 56 isolates, $bla_{\text{OXA-245}}$ in 5 isolates (8.9%), and $bla_{\text{OXA-181}}$ in 2 isolates (3.6%) (Table 1; also see Table S2). OXA-48 predominated over other variants, which coincides with the results of other surveys in which OXA-48 was the most widespread (11, 12). OXA-181 was first identified in India and has been reported in many different countries (United Kingdom, Canada, Australia, and Japan) (1). To our knowledge, this is the first report of the $bla_{\text{OXA-181}}$ gene in Spain. The OXA-181-producing isolates came from Almeria and Cordoba. The $bla_{\text{OXA-245}}$ gene was first detected in Spain in a hospital outbreak in Malaga (13), and 4 of 5 OXA-245-producing isolates came from hospitals in Malaga.

Forty-five isolates (80%) in this study also coharbored $bla_{\text{CTX-M-15}}$ genes (Table 1), as in previous studies (4, 13). Only 2 isolates (4%) coproduced CMY-2, CTX-M-15, and OXA-48-like β-lactamases. Two OXA-48-producing isolates (4%) coproduced the extended-spectrum β-lactamase (ESBL) SHV-27. While there have been frequent reports worldwide of the coexistence of OXA-48-like and other class A and B carbapenemases in *K. pneumoniae* (1), none of our isolates coproduced other carbapenemases.

Molecular epidemiology. Pulsed-field gel electrophoresis (PFGE) analysis of XbaI digests revealed 28 well-defined pulsotypes (1 to 12 isolates/pulsotype), and multilocus

TABLE 1 Molecular epidemiology and antibiotic resistance gene characterization for OXA-48-like-producing *K. pneumoniae* isolates

ST	Pulsotype	No. of isolates	Geographical area[a]	Hospital	$bla_{OXA-48-like}$	$bla_{OXA-48-like}$ plasmid type	Tn*1999* variant[b]	$bla_{CTX-M-15}$	$bla_{CTX-M-15}$ plasmid type[c]	pAmpC (bla_{CMY-2})
ST15	P01	1	Seville	H16	bla_{OXA-48}	IncL	2, 4, or 5	Yes	IncR	No
	P02	12	Malaga (n = 9), Seville (n = 3)	H13 (n = 9), H16 (n = 3)	bla_{OXA-48}	IncL	2, 4, or 5	Yes (n = 9)	IncR	No
	P03	7	Malaga (n = 4), Seville (n = 2), Granada (n = 1)	H5 (n = 1), H8 (n = 2), H13 (n = 2), H14 (n = 1), H16 (n = 1)	bla_{OXA-48}	IncL	1	Yes (n = 6)	IncR	No
	P04	1	Cordoba	H6	bla_{OXA-48}	IncL	2, 4, or 5	Yes	IncR	No
	P05	5	Malaga	H8	bla_{OXA-48}	IncL	1	Yes	IncR	No
	P06	1	Seville	H16	bla_{OXA-48}	IncL	2, 4, or 5	Yes	FIIK7:A−:B−	No
	P07	2	Seville (n = 1), Malaga (n = 1)	H11 (n = 1), H13 (n = 1)	bla_{OXA-48}	IncL	1	Yes	IncR	No
ST11	P08	2	Malaga	H3 (n = 1), H13 (n = 1)	bla_{OXA-48}	IncL	2, 4, or 5	Yes	IncR	No
	P09	1	Malaga	H3	$bla_{OXA-245}$	IncL	1	Yes	IncR	No
	P10	2	Malaga	H13	bla_{OXA-48}	IncL	2, 4, or 5	Yes	IncR	No
	P11	2	Jaen (n = 1), Cadiz (n = 1)	H4 (n = 1), H12 (n = 1)	bla_{OXA-48}	No TF	2, 4, or 5	Yes	FIIK1:A−:B−	No
ST1	P12	1	Malaga	H13	$bla_{OXA-245}$	IncL	2, 4, or 5	Yes	No TF	Yes
	P13	1	Malaga	H13	bla_{OXA-48}	IncL	2, 4, or 5	Yes	IncR	Yes
	P14	1	Jaen	H1	$bla_{OXA-245}$	IncL	2, 4, or 5	Yes	No TF	No
ST147	P15	1	Cordoba	H9	$bla_{OXA-181}$	No TF	ND	Yes	No TF	No
ST392	P16	2	Seville	H16	bla_{OXA-48}	IncM	1	No		No
	P17	1	Seville	H16	bla_{OXA-48}	IncM	2, 4, or 5	No		No
	P18	2	Seville	H15	bla_{OXA-48}	No TF	1	Yes	FIIK7:A−:B−	No
ST17	P19	2	Seville	H16	bla_{OXA-48}	IncL	2, 4, or 5	Yes (n = 1)	FIIK7:A−:B−	No
ST225	P20	1	Jaen	H4	bla_{OXA-48}	IncL	2, 4, or 5	No		No
ST251	P21	1	Malaga	H3	$bla_{OXA-245}$	IncL	1	No		No
ST307	P22	1	Seville	H10	bla_{OXA-48}	IncL	2, 4, or 5	Yes	FIIK7:A−:B−	No
ST391	P23	1	Seville	H16	bla_{OXA-48}	IncL	2, 4, or 5	Yes	IncR	No
ST405	P24	1	Malaga	H3	$bla_{OXA-245}$	IncL	2, 4, or 5	Yes	FIIK7:A−:B−	No
ST431	P03	1	Malaga	H3	bla_{OXA-48}	IncL	2, 4, or 5	Yes	No TF	No
ST753	P25	1	Cadiz	H2	bla_{OXA-48}	IncL	2, 4, or 5	No		No
ST836	P26	1	Almeria	H7	$bla_{OXA-181}$	No TF	ND	Yes	No TF	No
ST899	P27	1	Malaga	H8	bla_{OXA-48}	IncL	2, 4, or 5	No		No
ST974	P28	1	Seville	H5	bla_{OXA-48}	IncL	2, 4, or 5	No		No

[a]Numbers of isolates are indicated for geographical areas, hospitals, and CTX-M-15 coproducers.
[b]ND, not determined.
[c]No TF, no transformants were obtained.

sequence typing (MLST) analysis found 16 sequence types (STs) (Table 1; also see Table S2 and Fig. S1). ST15 (29 isolates [51.8%]) and ST11 (7 isolates [12.5%]) accounted for more than one-half of the isolates. ST15 isolates were detected in 7 hospitals in different geographical areas. Among the ST15 isolates, 7 different pulsotypes (pulsotypes P01 to P07) were observed, 1 of which (pulsotype P03 [7 isolates]) was detected in 5 hospitals, i.e., 2 isolates each in 2 hospitals with high patient flows in the same province and 3 isolates in 3 hospitals, one each, with no epidemilogical link. Two pulsotypes were detected in 2 hospitals (pulsotype P02, 12 isolates; pulsotype P07, 2 isolates) in different geographical areas (Table 1). Pulsotype P07 clustered with the other ST15 pulsotypes with less than 60% similarity, and the other 6 pulsotypes shared more than 80% similarity. ST11 isolates were detected in 4 hospitals and showed 4 different pulsotypes (pulsotypes P08 to P11), 2 of which (pulsotype P08, 2 isolates; pulsotype P11, 2 isolates) were detected in different hospitals with no known epidemiological link. According to pulsotype, 3 ST15 pulsotypes (pulsotypes P02, P03, and P07) and 2 ST11 pulsotypes (pulsotypes P08 and P11) were detected in more than 1 hospital (26 isolates).

The ST15 clone carrying ESBLs and carbapenemases is distributed worldwide, notably in Europe (14). OXA-48-like-producing *K. pneumoniae* isolates belonging to ST15 were detected previously in Spain, France, Bulgaria, and other countries (15–17), although this ST has frequently been associated with KPC- and VIM-producing *K. pneumoniae* isolates (1). In a previous multicenter survey of hospitals in all parts of

Spain in 2012 and 2013, ST11 and ST405 were the most prevalent STs detected among OXA-48-producing *K. pneumoniae* isolates (16). In the present study in the south of Spain, in contrast, ST15 was more prevalent than ST11 and also was more widely distributed (7 hospitals versus 4 hospitals). The main immigrant population in the south of Spain comes from the Maghreb. OXA-48-producing *K. pneumoniae* isolates belonging to ST15 and ST11 were detected in a hematology unit in Tunisia (18), although the most important clones associated with the dissemination of OXA-48 in these countries are different (e.g., ST147, ST307, and ST395) (19, 20). It was thought that OXA-48 producers caused mainly sporadic cases, with less interhospital transmission than other carbapenemase producers, although several examples of the interhospital spread of $bla_{\text{OXA-48}}$ were observed with ST395 and ST101 in Hungary (21, 22). Concurrent with transmission of ST15 and ST11 in our area, a single pulsotype was detected in 10 hospitals and more than 1 pulsotype was detected in 6 hospitals. The number of isolates belonging to the ST15 clone in our area decreased from 22 (69%) in 2014 to 7 (29%) in 2015.

Only 1 isolate belonging to ST405 was detected. This clone was previously responsible for a major nosocomial outbreak of OXA-48-producing *K. pneumoniae* in a hospital in Madrid (center of Spain), as well as in other regions in Spain (23). In Andalusia, the same clone was responsible for an outbreak due to CTX-M-15-producing *K. pneumoniae* lacking the $bla_{\text{OXA-48}}$ gene (24).

Genetic context of OXA-48-like genes and characterization of OXA-48 and CTX-M-15 plasmids. OXA-48-like enzymes are frequently carried on the epidemic IncL-type plasmid, which is responsible for the worldwide dissemination of $bla_{\text{OXA-48-like}}$ genes. The $bla_{\text{OXA-181}}$ gene has been located on IncT and IncX3 plasmids (25). In order to gain some insight into the type of transmission of $bla_{\text{OXA-48}}$ and $bla_{\text{CTX-M-15}}$ genes, plasmids recovered from transformants were analyzed. *Escherichia coli* DH10B transformants producing OXA-48-like carbapenemases were obtained from 24 (79%) of 28 selected isolates, which were representative of the pulsotypes detected. No transformants were obtained from OXA-181 producers. Using the PCR-based replicon typing (PBRT) scheme, 22 pulsotypes (48 isolates) carrying $bla_{\text{OXA-48-like}}$ plasmids belonged to the IncL compatibility group (Table 1; also see Table S2), which is consistent with the recognized IncL plasmid pOXA-48, responsible for the international dissemination of OXA-48 (26). In 2 pulsotypes (pulsotypes P16 and P17), the $bla_{\text{OXA-48}}$ gene was located on an IncM plasmid, a result that was confirmed by whole-genome sequencing (WGS), indicating a new *inc*RNAI region with respect to M1 and M2, M3. To our knowledge, this is the first description of the $bla_{\text{OXA-48}}$ gene located on a new IncM3 plasmid. These 2 pulsotypes belonged to ST392.

Twenty-six representative isolates of every pulsotype were selected for characterization of transposon Tn*1999*. OXA-181 producers were not included, because $bla_{\text{OXA-181}}$ is generally located on Tn*2013* (27). The composite transposon variant Tn*1999.2* (with the left IS*1999* truncated by IS*1R*) (28), or its variants Tn*1999.4* and Tn*1999.5*, was detected in 19 pulsotypes (65.5%) (Table 1). Sixteen and 3 pulsotypes carried OXA-48 and OXA-245, respectively. The Tn*1999.1* variant (with IS*1999* intact and IS*1R* absent) (29) was less prevalent and was detected in 8 pulsotypes (27.5%). This isoform was found in 6 pulsotypes linked to OXA-48 and in 2 pulsotypes associated with OXA-245. Tn*1999.3* (with both the upstream and downstream IS*1999* truncated by IS*1R*) (7) was not found. Different isoforms of Tn*1999* were detected in 3 clones, namely, ST15, ST11, and ST392. In the ST15 clone, Tn*1999.2*-like was found in 4 pulsotypes (15 isolates [52%]) and Tn*1999.1* was found in 3 pulsotypes (14 isolates [48%]). In clone ST11, the Tn*1999.1* variant was detected in only 1 pulsotype (1 isolate [14%]), while Tn*1999.2* (or its variants) was detected in 3 pulsotypes (6 isolates [86%]). In clone ST392, Tn*1999.1* was detected in 1 pulsotype and Tn*1999.2*-like was detected in the other pulsotype of this clone. These findings are consistent with different lineages of the same clone acquiring the $bla_{\text{OXA-48}}$ gene on separate occasions and other plasmids spreading this determinant.

TABLE 2 Antibiotic resistance genes located on sequenced IncR plasmids

			Gene[a]												
ST	Pulsotype	Isolate	*bla*$_{TEM-1B}$	*bla*$_{OXA-1}$	*bla*$_{SHV-28}$	*strA-strB*	*aac(6′)Ib-cr*	*aac(3)-IIa*	*sul2*	*catA2*	*catB4*	*dfrA14*	*qnrB1*	*qnrB66*	*tetA*
ST15	P01	2014081	+	−	−	+	−	−	+	−	−	−	−	−	−
ST15	P02	2014127	+	+	−	+	+	−	+	−	+	+	−	−	−
ST15	P03	2014028[b]	−	+	+	−	+	−	−	−	−	−	−	−	−
ST15	P04	2015254	+	+	−	+	+	−	+	−	+	+	−	−	−
ST15	P05	2014142	+	+	−	+	+	+	+	−	+	+	−	−	−
ST15	P07	2014090[b]	−	−	−	−	−	−	−	−	−	−	−	−	−
ST11	P08	2014126	−	+	−	−	+	−	−	+	+	−	+	−	−
ST11	P09	2015083	+	+	−	+	+	−	+	+	+	−	+	−	−
ST11	P10	2015013	+	+	−	+	+	−	+	+	+	−	+	−	−
ST1	P13	2015017	+	+	−	+	+	−	+	+	+	−	+	−	−
ST391	P23	2015141	+	−	−	+	−	−	−	−	−	+	−	+	+
ST836	P26	2014139	−	+	−	−	+	+	−	−	+	+	−	+	+

[a]All plasmids carried the *bla*$_{CTX-M-15}$ gene. +, presence; −, absence; *bla*$_{TEM-1B}$, gene encoding the β-lactamase TEM-1B; *bla*$_{OXA-1}$, gene encoding the β-lactamase OXA-1; *bla*$_{SHV-28}$, gene encoding the β-lactamase SHV-28; *strA-strB*, pair of genes encoding 3″-*O*-phosphotransferase type Ib and 6′-*O*-phosphotransferase type Id, respectively; *aac(6′)Ib-cr*, gene encoding 6′-*N*-aminoglycoside acetyltransferase type Ib-cr; *aac(3)-IIa*, gene encoding 3′-*N*-acetyltransferase type IIa; *sul2*, gene encoding dihydropteroate synthetase; *catA2*, gene encoding chloramphenicol acetyltransferase type A2; *catB4*, gene encoding chloramphenicol acetyltransferase type B4; *dfrA14*, gene encoding dihydrofolate reductase type II$_{14}$; *qnrB1*, gene encoding the Qnr B1 protein; *qnrB66*, gene encoding the Qnr B66 protein; *tetA*, gene encoding the efflux protein type A.

[b]A transformant could not be obtained from these 2 pulsotypes, and both IncR and resistance determinants were obtained from WGS of clinical isolates bearing only 1 plasmid.

Among the 28 pulsotypes detected in our set of isolates, only 21 included CTX-M-15-producing isolates. CTX-M-15-producing *E. coli* DH10B transformants were obtained from 18 pulsotypes (with none obtained from pulsotypes P12, P14, and P15). Only 6 plasmids could be typed using the PBRT scheme (Table 1) (30, 31), and all were IncFII-type; 5 pulsotypes yielded the FIIK7:A−:B− formula (including pulsotype P06, an ST15 pulsotype), and 1 pulsotype yielded the FIIK1:A−:B− formula. Because the PBRT scheme targets only replicons of the major plasmid families in *Enterobacteriaceae*, WGS was used to characterize the CTX-M-15 plasmid of the other 12 pulsotypes. Those 12 pulsotypes carried the *bla*$_{CTX-M-15}$ gene on IncR-type plasmids (Table 1), which is different from findings published previously in the literature, in which *bla*$_{CTX-M-15}$ was located principally on IncF plasmids (32). The association between the *bla*$_{CTX-M-15}$ gene and IncR plasmids was described previously for *K. pneumoniae* isolates belonging to successful clones (ST15, ST11, and ST147) in Europe (33, 34). In our study, most ST15 isolates coharboring *bla*$_{CTX-M-15-}$ and *bla*$_{OXA-48}$ (25 isolates from 6 pulsotypes) carried the *bla*$_{CTX-M-15}$ gene on an IncR plasmid; only the isolate with pulsotype P06 carried it on an IncFII-type plasmid. With respect to ST11 isolates, *bla*$_{CTX-M-15}$ was located on IncR plasmids in 3 pulsotypes (5 isolates) and on IncFII plasmids in 1 pulsotype (2 isolates). As was the case with *bla*$_{OXA-48}$ acquisition, the *bla*$_{CTX-M-15}$ gene was acquired by different lineages of each clone on separate occasions.

IncR plasmids carrying the *bla*$_{CTX-M-15}$ gene coharbored very diverse resistance genes (e.g., TEM-1 and OXA-1 β-lactamases, Qnr proteins B1 and B66, type Ib 3″-*O*-phosphotransferases, type Id 6′-*O*-phosphotransferases, type Ib-cr 6′-*N*-acetyltransferases, and type IIa 3′-*N*-acetyltransferases). IncR plasmids from pulsotypes P02 and P04 (both belonging to ST15) harbored the same resistance genes, and IncR plasmids from P10 and P13 similarly shared the same resistance determinants. The other 8 IncR plasmids analyzed carried different resistance genes (Table 2). These results support the idea that different resistance determinants were captured separately over time.

A major limitation of our study is that the collection studied was based on the estimated OXA-48-producing *K. pneumoniae* population in our region in 2014 and 2015. It was not obligatory to send isolates to the PIRASOA program laboratory (although in fact most isolates obtained from nosocomial outbreaks or clusters were sent). However, centralized analysis of isolates was able to demonstrate the spread of the same lineage of OXA-48 producers between hospitals in the same province and between different provinces in our region, which was not previously suspected even for sporadic cases. It should be borne in mind, however, that detection of the same lineage in different

centers could also be due to recent transmission between institutions or to unknown long-term transmission in the community. Another limitation of our study is that all isolates belonging to the same pulsotype were considered to share the same OXA-48 plasmid and genetic platform. The individual location of the gene was not studied for every isolate, but the genes were very close genetically (only 1 band of difference) and were found in the same area.

Conclusions. In summary, the spread of OXA-48-producing *K. pneumoniae* in the south of Spain is mainly due to interhospital dissemination of ST15 and ST11 clones. Our results suggest that lineages of the same clone captured $bla_{\text{OXA-48}}$, $bla_{\text{CTX-M-15}}$, and resistance genes on separate occasions. The greater diversity of the genetic platform of $bla_{\text{OXA-48}}$, compared with the CTX-M-15 plasmids, could indicate that successful clones acquired CTX-M-15 first and then OXA-48. Transmission of OXA-48 producers should lead to a review of the considerations for regional patient transfer, as well as local hospital measures, including both patient tracking and rapid detection of successful clones.

MATERIALS AND METHODS

Bacterial isolates. Between March 2014 and December 2015, 56 OXA-48-like-producing isolates were analyzed. The OXA-48-producing isolates came from 16 hospitals located in 7 of the 8 provinces of Andalusia (1 to 17 isolates/hospital); the hospitals were arbitrarily assigned numbered names of H1 to H16.

Bacterial identification and drug susceptibility testing. The isolates were identified using matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (MALDI-TOF Biotyper 3.1; Microflex Bruker, Madrid, Spain). Susceptibility testing was performed with commercial microdilution panels (MicroScan; Beckman Coulter, Madrid, Spain), using EUCAST breakpoints (35). Screening for ESBL and plasmid-encoded AmpC (pAmpC) production was performed with the double-disc synergy test on Mueller-Hinton (MH) agar and MH agar supplemented with 200 mg/liter cloxacillin. The β-Carba test (Bio-Rad, Madrid, Spain) was used to check for imipenem hydrolysis (36). Initial carbapenemase characterization was performed with combined-disk tests employing meropenem plus inhibitors and temocillin susceptibility disks, according to EUCAST guidelines (10).

Characterization of ESBL and carbapenemase genes and genetic platforms of OXA-48-like-encoding genes. The presence of genes encoding ESBLs (bla_{TEM}, bla_{SHV}, $bla_{\text{CTX-M-1 group}}$, and $bla_{\text{CTX-M-9 group}}$), pAmpC ($bla_{\text{MOX}}$, bla_{CIT}, bla_{CMY}, bla_{DHA}, bla_{FOX}, bla_{ACC}, and bla_{EBC}), and carbapenemases ($bla_{\text{OXA-48}}$, bla_{KPC}, bla_{IMP}, bla_{VIM}, and bla_{NDM}) was determined using group-specific PCR primers and further sequencing (37, 38). The Tn*1999* structure of $bla_{\text{OXA-48}}$ and $bla_{\text{OXA-245}}$ genes from selected isolates was determined by PCR using specific primers (see Table S1 in the supplemental material) and further sequencing. The sequences obtained were compared with those in the GenBank database. The primers used did not differentiate between Tn*1999.2*, Tn*1999.4*, and Tn*1999.5* variants.

Molecular epidemiology. PFGE analysis of XbaI-digested DNA (http://www.cdc.gov/pulsenet) was used to determine the degree of genetic relatedness between isolates. Isolates differing by 2 or more bands in XbaI PFGE assays were assigned to different pulsotypes. A dendrogram was created with Fingerprinting 3.0 software (Bio-Rad), using the Dice coefficient with position tolerance settings of 1% optimization and 1.2% band position tolerance. The Institut Pasteur MLST scheme was used to characterize a subset of isolates representing different PFGE clusters (http://bigsdb.pasteur.fr/klebsiella/klebsiella.html). All pulsotypes assigned to the same MLST were considered to belong to the same clone.

Transformation assays and characterization of OXA-48 and CTX-M-15 plasmids. A representative isolate of each pulsotype was selected for transformation assays. Plasmid DNA was extracted using the method described by Kieser (39) and was transformed by electroporation into *E. coli* DH10B (streptomycin-resistant). Transformants were selected on MacConkey agar (Becton, Dickinson France SA, Le Pont de Claix, France) supplemented with 0.125 mg/liter ertapenem or 2 mg/liter cefotaxime, to select transformants with $bla_{\text{OXA-48-like}}$ or $bla_{\text{CTX-M-15}}$ genes, respectively. The presence of $bla_{\text{OXA-48}}$-like in ertapenem-resistant transformants and, similarly, $bla_{\text{CTX-M-15}}$ in cefotaxime-resistant transformants was confirmed by phenotypic and genotypic methods, as described above, and the presence of only 1 plasmid was confirmed after an extraction using the Kieser method (39).

Plasmids from transformants were classified into incompatibility groups using a PBRT scheme (30). For IncF subtyping, replicon sequence typing was performed, following the nomenclature of the Plasmid MLST databases web site (http://www.pubmlst.org/plasmid). The new PBRT scheme was used for differentiation of IncL and IncM plasmids (26).

Whole-genome sequencing. WGS was used for analysis of genomes of transformants with plasmids that were not classified with the PBRT scheme and when only 1 plasmid was electroporated into *E. coli* DH10B, according to Kieser extraction. Genomic DNA was extracted from transformants and sequenced on the MiSeq platform (Illumina, San Diego, CA, USA), generating 300-bp paired-end reads. The library was prepared using the Nextera XT DNA library preparation kit (Illumina), involving DNA fragmentation, adaptor ligation, and amplification. Raw reads were quality filtered and assembled into contigs on CLC Genomics Workbench 10.0 (Qiagen, Madrid, Spain), using default software settings. An average coverage of 50× was observed. Plasmid replicons were then analyzed with the PlasmidFinder tool

(https://cge.cbs.dtu.dk/services/PlasmidFinder), using a 95% identity threshold (40). Acquired resistance genes were analyzed using the ResFinder tool (https://cge.cbs.dtu.dk/services/ResFinder), using a 90% identity threshold (41).

Accession number(s). The Fastq files corresponding to transformants or clinical isolates with CTX-M-15-bearing IncR plasmids and OXA-48-bearing IncM plasmids have been deposited in the GenBank database under accession no. PRJNA493254.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.01396-18.

SUPPLEMENTAL FILE 1, PDF file, 1.4 MB.

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We have no conflicts of interest to declare.

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