Characterization of Carbapenemase-Producing Klebsiella oxytoca in Spain, 2016-2017

María Pérez-Vazquez , Jesús Oteo-Iglesias, Pedro J Sola-Campoy, Hugo Carrizo-Manzoni , Verónica Bautista, Noelia Lara, Belén Aracil , Almudena Alhambra, Luis Martínez-Martínez, José Campos, Spanish Antibiotic Resistance Surveillance Program Collaborating Group

Antimicrob Agents Chemother. 2019 May 24;63(6):e02529-18.

https://doi.org/10.1128/AAC.02529-18

Characterization of carbapenemase-producing *Klebsiella oxytoca* in Spain, 2016-2017

María Pérez-Vazquez[1,2,a], Jesús Oteo-Iglesias[1,2,a]*, Pedro J. Sola-Campoy[1], Hugo Carrizo-Manzoni[1,3], Verónica Bautista[1,2], Noelia Lara[1,2], Belén Aracil[1,2], Almudena Alhambra[4], Luis Martínez-Martínez[2,5,6,7], José Campos[1,2], AND the Spanish Antibiotic Resistance Surveillance Program collaborating Group†.

[a]María Pérez-Vázquez and Jesús Oteo-Iglesias contributed equally to this work.

[1]Laboratorio de Referencia e Investigación en Resistencia a Antibióticos e Infecciones Relacionadas con la Asistencia Sanitaria, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain.

[2]Spanish Network for Research in Infectious Diseases (REIPI RD16/0016), Instituto de Salud Carlos III, Madrid, Spain.

[3]Laboratorio de Bacteriología, Micología y Parasitología, Centro de Educación Médica e Investigaciones Clínicas "Norberto Quirno", Buenos Aires, Argentina.

[4]Servicio de Microbiología laboratorio Abacid, Hospital Universitario HM Sanchinarro, Madrid, Spain.

[5]Unidad de Microbiología, Hospital Universitario Reina Sofía, Córdoba, Spain.

[6]Departamento de Microbiología, Universidad de Córdoba, Spain

[7]Instituto Maimonides de Investigación Biomédica de Córdoba (IMIBIC), Spain.

Running title: Carbapenemase-producing *Klebsiella oxytoca.*

Keywords: carbapenemases, whole genome sequencing, *Klebsiella oxytoca*, plasmids, cgMLST

*Corresponding author. Centro Nacional de Microbiología, Instituto de Salud Carlos III, Carretera Pozuelo a Majadahonda, 28220 Majadahonda, Madrid,

Spain. Tel: +34–918223650; Fax: +34-915097966; E-mail: jesus.oteo@isciii.es

Abstract

There is little information about carbapenemase-producing **(**CP**)** *Klebsiella oxytoca*, an important nosocomial pathogen**.** We characterized CP *K. oxytoca* isolates collected from different Spanish hospitals between January 2016 and October 2017.

During the study period, 139 unduplicated CP K. oxytoca isolates were identified; of which 80 were studied in detail. Carbapenemase and ESBL genes were identified by PCR and sequencing. Genetic relatedness was studied by PFGE. Whole genome sequencing (WGS) carried out on 12 representative isolates was used to identify the resistome, to elucidate the phylogeny and to determine the plasmids encoding carbapenemase genes.

Forty-eight (60%) isolates produced VIM-1, 30 (37.5%) OXA-48, 3 (3.7%) KPC-2, 2 (2.5%) KPC-3 and one (1.2%) NDM-1; four isolates co-produced two carbapenemases. By PFGE, 69 patterns were obtained from the 80 CP *K. oxytoca*, and four well-defined clusters were detected: Cluster 1 consisted of 11 OXA-48-producing isolates, the other three clusters included VIM-1-producing isolates (five, three and three isolates, respectively). In the 12 sequenced isolates, the average number of acquired resistance genes was significantly higher in VIM-1-producing isolates (10.8) than in OXA-48-producing isolates (2.3). All 12 isolates had chromosomally-encoded genes of the *bla*$_{\text{OXY-2}}$ genotype and by MLST most belonged to ST2. Carbapenemase genes were encoded on IncL, IncHI2, IncFII, IncN, IncC and IncP6 plasmid types.

The emergence of CP *K. oxytoca* was principally due to the ~~clonal and polyclonal~~ spread of VIM-1- and OXA-48-producing isolates encoded on IncL, IncHI2, IncFII and IncN plasmids. The sequence type ST2 and the genotype *bla*$_{\text{OXY-2}}$ predominated among the 12 sequenced isolates.

INTRODUCTION

The prevalence of multidrug-resistant carbapenemase-producing Enterobacteriaceae (CPE), a major public health threat, continues to increase on a global level and is associated with significant morbidity and mortality (1). Frequently, patients with CPE infections cannot be treated with effective antibiotics because of the dearth of alternative drugs (2). Currently, the epidemiological situation regarding CPE in Spain is considered “endemic”, mainly due to carbapenemase-producing *Klebsiella pneumoniae.* However, carbapenemase production is also increasing in other Enterobacteriaceae species such as *Escherichia coli*, *Citrobacter* spp. *Enterobacter* spp. and *Serratia marcescens* (3-5).

Klebsiella oxytoca has been recognised as an opportunistic pathogen causing healthcare-associated infections such as urinary and respiratory tract infections, and sepsis, primarily in immunocompromised and debilitated patients admitted to ICUs. However, little information is available about carbapenemase-producing (CP) *K. oxytoca*, since only a few individual cases or nosocomial outbreaks have been reported (6-9).

The aim of this study was to gain insight into the microbiological features and molecular epidemiology of CP *K. oxytoca* submitted to the Spanish National Reference Laboratory for Antibiotic Resistance.

RESULTS AND DISCUSSION

Bacterial isolates and carbapenemase types.

Between January 2016 and September 2017, 139 unduplicated CP *K. oxytoca* isolates, submitted from 22 hospitals located in 9 different Spanish provinces, were identified (5.2% of the 2673 CPE identified by the reference laboratory during the same

period of time). Of them, 80 (57.5%) isolates were selected for further studies (Table S1); this selection excluded 59 isolates obtained from rectal samples collected in three hospitals with nosocomial outbreaks.

Sixty-one isolates produced clinical infections as follows: 28 (45.9%) urinary tract infections, 11 bacteraemia (18%), 10 (16.4%) respiratory tract infections, 6 (9.8%) wound infections and 6 (9.8%) abscesses. The patients infected with CP *K. oxytoca* were mainly males (65.6%) aged 18 to 65 years (59%).

Of the 80 CP *K. oxytoca* isolates, 48 (60%) produced VIM-1; 30 (37.5%) OXA-48; 3 (3.7%) KPC-2; 2 (2.5%) KPC-3, and one (1.2%) NDM-1; four isolates co-produced two different carbapenemases: two VIM-1 plus KPC-2, one VIM-1 plus OXA-48, and one VIM-1 plus KPC-3. The CarbaNP tests yielded positive results for all 80 CP isolates.

As previously described in other Enterobacteriaceae species such as *Citrobacter freundii*, *Enterobacter* spp, and *Serratia marcescens* (3-5), VIM-1 was the most frequent carbapenemase produced by CP *K. oxytoca* in this study. However, in *K. pneumoniae* and *Escherichia coli* the most frequent carbapenemases detected in Europe are KPC, OXA-48 and NDM (2). Recently, the clonal spread of an IMP-8-producing *K. oxytoca* clone was described in a Spanish hospital (9).

Five (6.25%) CP *K. oxytoca* also co-produced ESBLs as follows: two VIM-1 producing isolates (2.5%) also produced CTX-M-9 plus SHV-12; one VIM-1 and OXA-48- and one OXA-48-producing isolates (2.5%) also produced CTX-M-9, and one VIM-1 producing isolate (1.25%) also produced CTX-M-14. The NDM-1-producing *K. oxytoca* co-produced the p-AmpC CMY-4.

Antibiotic susceptibility.

Antibiotic susceptibility of all CP *K. oxytoca* isolates is detailed in Table 1 and Table S2. In comparison with VIM-1 producing isolates, the OXA-48 producing isolates were significantly more susceptible to gentamicin, tobramycin, cotrimoxazole, cefotaxime and ceftazidime ($P<0.05$) (Table 1).

Susceptibity to carbapenems was 36.2% to meropenem, 11.2% to imipenem and 8.7% to ertapenem (Table 1); all isolates were non-susceptible to at least one carbapenem antibiotic. These data suggest that ertapenem has the highest sensitivity for the detection of CP *K. oxytoca*, although specificity could not be determined. However, susceptibility to carbapenems highly varied in relation to the carbapenemase types as follows: ertapenem, imipenem and meropenem susceptibility was 0%, 24.1% and 62.1%, respectively, in OXA-48 producers; and 15.9%, 4.5% and 25%, respectively, in VIM-1 producers. These differences between carbapenemases have been previously described in other species such as *Citrobacter freundii* and *Escherichia coli* (3,4).

Of the 28 OXA-48-producing isolates that were negative for other carbapanemases, ESBLs or p-AmpCs, 11 were cefotaxime susceptible but 17 (60.7%) were cefotaxime resistant. Overproduction of chromosomic OXY enzymes can result in reduced susceptibility or resistance to penicillin/inhibitor combinations, cefuroxime, cefotaxime and aztreonam, and has been classically observed in 10-20% of clinical isolates of *K. oxytoca* (10).

Forty nine isolates (61.3%) were resistant to ceftazidime/avibactam, but all of them produced metallo-β-lactamases. Only two isolates were colistin-resistant but were negative for *mcr* genes. The most frequently described chromosomal resistance mechanisms to colistin in *K. pneumoniae* are mutations caused by insertional inactivation or deletion of the *mgrB* gene, upregulated transcription of *phoP*, *phoQ*, and *pmrK* (which is part of the *pmrHFIJKLM* operon) (11)

The percentages of susceptibility to ertapenem, imipenem and meropenem, according to EUCAST breakpoints (12), significantly varied between VIM-1-producing isolates (15.9%, 4.5% and 25%, respectively) and OXA-48-producing isolates (0%, 24.1% and 62.1%, respectively) ($P<0.05$) (Table 1). However, all isolates had meropenem MICs >0.25 mg/L, and therefore would be suspected of producing carbapenemases according to the screening cut-off values proposed by EUCAST (13).

Molecular epidemiology.

PFGE analysis revealed a high degree of genetic diversity as 69 different PFGE patterns were obtained from the 80 CP *K. oxytoca* analyzed (simple diversity index: 86.2%). However, four well-defined clusters with more than two isolates each were detected considering a genetic linkage ≥85% (Figure S1). The largest cluster (Cluster 1) consisted of 11 OXA-48-producing isolates submitted from two hospitals (that sent nine and two isolates, respectively) located in the same province. The other three clusters consisted of five (Cluster 2), three (Cluster 3) and three (Cluster 4) VIM-1-producing isolates, each of these clusters came from individual hospitals. In addition, five clusters of two isolates each were also detected (Figure S1).

These PFGE results suggest polyclonal dissemination, since 46 of the 80 CP *K. oxytoca* isolates showed non-related PFGE patterns. However, specific clonal disseminations of CP *K. oxytoca* either within the same hospital or between different close hospitals were detected.

Resistome of CP K. oxytoca as determined by WGS analysis.

WGS was performed on 12 selected isolates representing: i) the four main clusters detected by PFGE (five isolates), ii) the clusters formed by pairs of isolates (four isolates), and iii) three singular isolates: one producing NDM-1, one producing VIM-1 plus KPC-2, and one producing VIM-1 plus KPC-3. According to PFGE results,

these 12 isolates are representative of 33 of the 80 isolates studied (11 isolates of Cluster 1, 5 of Cluster 2, 3 of Cluster 3 and 3 of Cluster 4, four couples and three individual isolates). The sequences are available in the ENA web with the accession number PRJEB30102.

In the 12 sequenced isolates, the average number of acquired resistance genes was 9.2 (range 1-16), being much higher in the 5 VIM-1 isolates (average 10.8, range 6-15) than in the three OXA-48 isolates (average 2.3, range 1-3). A summary of the main antibiotic resistance genes detected is listed in Table 2. The isolate with the highest number of acquired resistance genes (n=16) was the NDM-1-producing *K. oxytoca* which had several genes encoding resistance to β-lactams, aminoglycosides, sulfonamides, quinolones, chloramphenicol, macrolides, rifampicin, tetracycline and trimethoprim; in addition, this isolate encodes the methyl transferase gene *arm*A, which is of special relevance since it confers high resistance levels to all clinically important aminoglycosides (14). ArmA has been associated with *K. oxytoca* in Japan and China (15,16); however only sporadic cases of ArmA have been described in Enterobacteriaceae in Spain, mainly obtained from animals (17,18), but none of them from *K. oxytoca.* Association of $bla_{\text{NDM-1}}$ and *arm*A genes in the same plasmid has been previously described (19).

In addition to carbapenemase genes, the most predominant acquired resistance genes were: *aad*A1 (91.7%), *aac(6')-Ib* (75%), *sul*1 (75%) and *cat*B2 (66.7%).

Concerning the chromosomal bla_{OXY} genes, all 12 isolates had the $bla_{\text{OXY-2}}$ genotype, with three variants detected: $bla_{\text{OXY-2-8}}$ (n=5), $bla_{\text{OXY-2-2}}$ (n=4), and $bla_{\text{OXY-2-5}}$ (n= 3) (Table 2). Eight different types of OXY enzymes have been described (10,20). Of the 68 isolates demonstrating non-susceptibility to 3rd generation cephalosporins obtained from 14 hospitals across Europe (10), 42 (61.8%) belonged to phylogroup

KoII, which includes isolates of the OXY-2 type (20). Interestingly, in this study KoII isolates were frequently OXY hyperproducers.

Phylogenetic analysis of carbapenem-producing Klebsiella oxytoca by MLST and core genome MLST

The 12 sequenced isolates belonged to the following MLST types: ST2 (n=5), ST36 (n=3), ST176 (n=2), ST20 (n=1), and ST141 (n=1) (Table 2, Figure 1). Of the 12 isolates, eight (66.7%) had STs belonging to the clonal complex 2 (CC2): ST2, ST176 (DLV of ST2) and ST141 (DLV of ST2). CC2 has been previously described as the most frequent clonal complex in *K. oxytoca* (10,21).

Genome assemblies of the 12 *K. oxytoca* isolates were analyzed using a gene-by-gene approach (22), and the allelic distance from cgMLST was visualized in a minimum spanning tree (Figure 1). Additionally, study of single nucleotide polymorphism (SNPs) was performed as described in methodology (Figure S2). ST2 isolates differed by an average of 56 alleles (range 15 to 88) and 288 SNPs (range 35 to 448); in ST36 isolates these differences were 139 alleles (range 48 to 188) and 1013 SNPs (range 120 to 1495), and in ST176 isolates were 134 alleles and 403 SNPs. The most prevalent cgMLST clusters ST2, ST136 and ST176 included isolates producing different carbapenemase types (Figure 1).

Characterization of plasmids harbouring carbapenemases genes

In 11 of the 12 *K. oxytoca* isolates (Table 2), the PlasmidID mapping tool was used for the identification and reconstruction of 13 plasmids harbouring carbapenemase genes (seven carrying $bla_{\text{VIM-1}}$, three $bla_{\text{OXA-48}}$, two bla_{KPC} and one $bla_{\text{NDM-1}}$). In the remaining isolate plasmids reconstruction failed.

In the three isolates producing class D carbapenemases, $bla_{\text{OXA-48}}$ was encoded in IncL plasmids, one of ~63 kb (two isolates), almost identical to plasmid pOXA-48

NZ_CP018342.1 (average identity > 95% and coverage percentage of 99.8%) (Figure S3), and another of ~48 kb (one isolate), highly similar to plasmid NZ_CP018694.1 (average identity > 95% and coverage percentage of 94.3%), reconstructed with a low number of contigs (8, 7 and 3 respectively) (Table 2). In all three isolates, *bla*$_{\text{OXA-48}}$ was flanked by IS*1999* sequences. In the two isolates within Cluster 1/ST2 (Figure 1), *bla*$_{\text{OXA-48}}$ was located in a *Tn1999.2* (Figure S3), and in the ST141 isolate *bla*$_{\text{OXA-48}}$ was located in a *Tn1999.3*.

In VIM-1-producing isolates, four different plasmid types encoding *bla*$_{\text{VIM-1}}$ were identified as follows (Table 2): one IncL plasmid of ~70 kb identical to NZ_CP023419.1 in three isolates (Figure S4); one IncHI2 plasmid of ~250 kb in two isolates, very similar to plasmid NZ_CP026661.1 (average identity > 95% and coverage percentage of 81.8%) (Figure S5), one IncN plasmid of ~50 kb in one isolate, very similar to plasmid NC_014208.1 (average identity > 95% and coverage percentage of 90.96%); and one IncFII plasmid of ~70 kb in one isolate, similar to plasmid NZ_CP023419.1 (average identity > 95% and coverage percentage of 86.4%).

In Greece, a multiclonal epidemic of VIM-1-producing *K. pneumoniae* mainly due to the spread IncN plasmids between 50-70 kb was described (23). A conjugative IncHI2 plasmid of ~300 kb has previously been associated with the dissemination of *bla*$_{\text{VIM-1}}$ among genetically diverse *Enterobacter cloacae* isolates (24). We identified two different genetic environments for *bla*$_{\text{VIM-1}}$; in IncN, IncL and IncFII plasmids, *bla*$_{\text{VIM-1}}$ was located in the In624 class 1 integron (24,25) (Figure S4); in IncHI2 plasmids, VIM-1 was linked to another similar class 1 integron that was previously described (26) (Figure S5). In Spain, In624 has been described in VIM-producing *E. cloacae* (24) and *C. freundii* (25), suggesting an important role in the interspecies transfer of *bla*$_{\text{VIM-1}}$.

Two plasmids encoding *bla*$_{KPC}$ were identified (Table 2). One of them was an IncP6 plasmid of ~39 kb identical to NZ_CP026224.1 with *bla*$_{KPC-2}$ as the sole antimicrobial resistance gene in the plasmid (Figure S6). The genetic environment of the *bla*$_{KPC-2}$ gene included an ISKpn27 and a truncated *bla*$_{TEM-1}$ (Figure S6), and was the same as previously detected in the first IncP6 plasmid carrying *bla*$_{KPC-2}$ in *K. oxytoca* (6). The other one was an IncFIB plasmid of ~103 kb, similar to plasmid NZ_CP015395.1 (average identity > 95% and coverage percentage of 88.36% bp), that harbours *bla*$_{KPC-3}$ in the classical transposon Tn*4401* isoform (27,28), *bla*$_{TEM-1A}$ and *bla*$_{OXA-9}$ (Figure S7).

In the NDM-1-producing isolate, *bla*$_{NDM-1}$ was encoded in an IncC plasmid of ~154 kb, similar to plasmid NZ_CP029118.1 (average identity > 95% and coverage percentage of 82.18%) (Table 2, Figure 2); the genetic environment of *bla*$_{NDM-1}$ included the IS*Aba125* composite transposon Tn*125* (Figure 2), previously described in pM214_A/C2 (29). Additionally, a class 1 integron located adjacent to Tn*125* contained several genes associated with resistance to trimethoprim (*dfrA14*), rifamycins (*arr*-2), chloramphenicol (*clm*A5 codifying efflux MFS transporter), β-lactams (*bla*$_{OXA-10}$), aminoglycosides (*aad*A1), and sulphonamides (*sul*1) (Table 2, Figure 2). This IncC plasmid also contained the *armA*, *bla*$_{CMY-4}$ and *msr*E/*mph*E genes coding resistance to aminoglycosides, β-lactams and macrolides, respectively. The *bla*$_{CMY-4}$ gene was included in an *ISEcp1* transposition unit (Figure 2), which is usually found in A/C plasmids, and thought to be responsible for the dissemination of the *bla*$_{CMY}$ cephalosporinase genes (30).

CONCLUSIONS

We observed the progressive emergence of CP *K. oxytoca* in Spain. Our data reveal several remarkable findings of concern that deserve active surveillance: (i) clonal and mainly polyclonal spread of CP *K. oxytoca* across different geographical areas and hospitals; (ii) production of five different carbapenemase types, mainly VIM-1 and OXA-48, but also KPC and NDM; (iii) identification of a NDM-1-producing isolate presenting a high load of antibiotic resistance genes including *amr*A; iv) predominance of ST2 and OXY-2 among the studied isolates; v) detection of the plasmid types IncL, IncHI2, IncFIB, IncN, IncC, and IncP6, likely responsible of the dissemination of carbapenemase genes in *K. oxytoca* in Spain.

MATERIAL AND METHODS

Study design and bacterial isolates.

This study was performed by the unrestricted and non-mandatory national Spanish Antibiotic Resistance Surveillance Programme operated by our official public health Institute (Instituto de Salud Carlos III) (3,4). The number of carbapenemase-producing *K. oxytoca* isolates referred to this program experienced a constant increase from 2012 (n = 6) (31) to 2015 (n = 35), although its proportion with respect to the total number of carbapenemase-producing Enterobacteriaceae isolates remained stable around 2-3%. The observation of an unexpected increase in this proportion in 2016 (5.2%) prompted the present investigation. In this study, we included all CP *K. oxytoca* isolates submitted to our reference laboratory between January 2016 and October 2017; during this period a total of 119 public hospitals (about 35% of Spanish public hospitals) voluntarily participated in the surveillance program.

Only the first isolate per patient was analyzed. Bacterial isolate identification was performed using API20E (BioMérieux) and matrix-assisted laser

desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) (Bruker Daltonik GmbH, Leipzig, Germany).

Antibiotic susceptibility of CP K. oxytoca and phenotypic characterization of carbapenemase production.

Antibiotic susceptibility testing was performed by microdilution (DKMGN panel, Thermo Fisher Scientific, East Grinstead, UK) and interpreted according to EUCAST breakpoints (12,13). Susceptibility to colistin was carried out by the in-house microdilution method following the recommendations of the CLSI-EUCAST joint polymyxins working group (32). According to EUCAST (13), inhibition of carbapenemase activity was determined by using EDTA, phenyl-boronic acid and cloxacillin. In addition, all the isolates were tested by the Carba NP method (33).

Characterization of resistance mechanisms.

The presence of genes encoding carbapenemases (*bla*$_{\text{OXA-48}}$, *bla*$_{\text{KPC}}$, *bla*$_{\text{VIM}}$, *bla*$_{\text{IMP}}$ and *bla*$_{\text{NDM}}$), extended spectrum β-lactamases (*bla*$_{\text{CTX-M}}$, *bla*$_{\text{SHV}}$, and *bla*$_{\text{TEM}}$), plasmid mediated AmpC β-lactamases (p-AmpC) (*bla*$_{\text{CMY}}$, *bla*$_{\text{DHA}}$, *bla*$_{\text{MOX}}$, *bla*$_{\text{ACC}}$, *bla*$_{\text{EBC}}$ and *bla*$_{\text{FOX}}$) and *mcr* genes coding for colistin resistance were identified using PCR and DNA sequencing as previously described (31,34-36).

Pulsed-Field Gel Electrophoresis.

The genetic relationship between the CP *K. oxytoca* isolates was elucidated by PFGE after total chromosomal DNA digestion with *Xba*I (34). A simple diversity index (SDI) was calculated in order to determine the population diversity as follows: number of different PFGE profiles/total number of isolates×100 (37).

Whole genome sequencing (WGS), resistome and core genome MLST analysis.

300 WGS was performed on 12 isolates representative of the main CP *K. oxytoca*
301 clusters previously detected by PFGE, and also in two additional isolates coproducing
302 two carbapenemase types each.
303 DNA was extracted using the QIAamp® DNA Mini Kit (Qiagen®, Hilden,
304 Germany). Genomic DNA paired-end libraries were generated using the Nextera XT
305 DNA sample preparation kit (Illumina Inc, San Diego, CA, USA). The libraries were
306 sequenced using the Illumina NextSeq 500 sequencer system with 2 x 150-bp paired-
307 end reads (Illumina Inc).
308 The quality of the high-throughput sequence data was assessed by FastQC and
309 short reads were subsequently assembled de novo into contigs using SPAdes 3.9.0 (38)
310 testing 5 different kmers under parameters optimized to give the best assembly using
311 QUAST (http://quast.bioinf.spbau.ru/) in order to analyze the most relevant statistics
312 (largest contig, N50 and NG50). Scaffolding was performed with SSPACE (39) and
313 GapFiller was used to close sequence gaps (40). Automatic de novo annotation of draft
314 genomes was performed using Prokka version 1.12-beta (41).
315 Antimicrobial resistance genes were analyzed using ResFindertool (CGE server:
316 https://cge.cbs.dtu.dk) (June 2018, date last accessed) with an ID threshold of 98% with
317 the exception of β-lactamase variants, which were determined with 100% identity.
318 Additionally SRST2 (42) was used to detect resistance genes and alleles with ARGannot
319 database (43).
320 A core genome multilocus sequence typing (cgMLST) was applied; an ad hoc
321 scheme was created using the MLST+ target definer with the default parameters and a
322 reference sequence (*K. oxytoca* KONIH1, accession number NZ_CP008788.1). A total
323 of 13 NCBI RefSeq genomes were used as query genomes to validate in a pairwise
324 comparison using BLAST. The final cgMLST scheme consisted of 3201 target genes

and 2380 accessory genes. The percentage of good targets included for distance calculation was 94% (Table S3). A distance matrix among all isolates was calculated with 3013 target genes to analyze the homology within each cluster, genes with missing values were excluded (Table S4).

Curated Illumina sequence reads were mapped onto the reference sequence (*K. oxytoca* KONIH1, accession number NZ_CP008788.1) and putative SNPs were obtained using the Lasergene Genomics Suite Software (DNASTAR Inc., Madison, WI, USA) as described (44)

Characterization of plasmids carrying carbapenemases genes

In order to reconstruct the plasmids carrying the carbapenemase genes, an in house script (Plasmid ID, https://github.com/BU-ISCIII/plasmidID) was used to: 1) map reads over a plasmid curated database to find those with the higher coverage and de novo assembly of these reads, 2) make local alignments to localize resistance and replicative genes, and 3) make a graphic representation of the plasmids identified (45)

Statistical analysis.

Differences in the prevalence of antibiotic susceptibility of OXA-48- and VIM-1-producing isolates were assessed using Fisher's exact test. The null hypothesis was rejected when a *P* values ≤ 0.05 was calculated. Statistical analysis was performed using the GraphPad Prism software, version 3.02 (GraphPad Software, Inc., San Diego, CA, USA).

ACKNOWLEDGMENTS

We thank the Genomics Unit of the Centro Nacional de Microbiología for carrying out the DNA sequencing.

We thank Michael McConnell for the revision of the English style and grammar.

Collaborating Group for the Spanish Antibiotic Resistance Surveillance Program: Isabel Sánchez-Romero, Beatriz Orden and Rocío Martínez-Ruiz (Hospital Universitario Puerta de Hierro-Majadahonda, Majadahonda, Madrid); Esteban Aznar (Laboratorio BrSalud, San Sebastián de los Reyes, Madrid); Emilia Cercenado (Hospital General Universitario Gregorio Marañón, Madrid); Pedro de la Iglesia (Hospital de Cabueñes, Gijón, Asturias); Luis López-Urrutia (Hospital Universitario Río Hortega, Valladolid); Santiago Salso (Hospital HM Montepríncipe, Boadilla del Monte, Madrid); José Vicente Saz (Hospital Universitario Príncipe de Asturias); Sagrario Reyes (Hospital Severo Ochoa, Leganés, Madrid), José Cobos (Megalab, Madrid); Luisa García-Picazo (Hospital "El Escorial", San Lorenzo del Escorial, Madrid); María Ortega-Lafont and Gregoria Megías-Lobón (Complejo Asistencial Universitario de Burgos, Burgos); Noelia Arenal Andrés and Elisa Rodríguez Tarazona (Hospital Santos Reyes, Aranda de Duero, Burgos); Patricia Álvarez-García (Complejo Hospitalario de Pontevedra, Pontevedra); Dionisia Fontanals (Hospital Parc Taulí de Sabadell, Barcelona); Rafael Carranza (Hospital La Mancha Centro, Ciudad Real); Susana Hernando (Hospital General de Segovia, Segovia); Mª Fe Brezmes (Complejo Asistencial de Zamora, Zamora); Luis Moisés Ruiz-Velasco (Hospital Central de la Cruz Roja San José y Santa Adela, Madrid); Paloma Cascales (Hospital de Elda Virgen de la Salud, Alicante); Carmen Guerrero (Hospital JM Morales Meseguer); Yolanda Gil (Hospital de Móstoles, Móstoles, Madrid); Irene Rodríguez-Conde (Hospital Povisa, Pontevedra); Alicia Saez (Hospital Universitario Santa Cristina, Madrid)

FUNDING

This work was supported by Plan Nacional de I+D+i 2013-2016 and Instituto de Salud Carlos III, Subdirección General de Redes y Centros de Investigación Cooperativa,

Ministerio de Ciencia, Innovación y Universidades, Spanish Network for Research in Infectious Diseases (REIPI RD16CIII/0004/0002, RD16/0016/0008) - co-financed by European Development Regional Fund ERDF “A way to achieve Europe”, Operative program Intelligent Growth 2014-2020.

This work was also supported by a grant from the Instituto de Salud Carlos III (grant number MPY 1135/16), and by the Antibiotic Resistance Surveillance Program of the Centro Nacional de Microbiología (Instituto de Salud Carlos III, Ministerio de Economía y Competitividad) of Spain.

TRANSPARENCY DECLARATIONS

None to declare.

References

1. Logan LK, Weinstein RA. 2017. The epidemiology of carbapenem-resistant Enterobacteriaceae: The impact and evolution of a global menace. J Infect Dis 215 (suppl_1): S28-S36.

2. 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.

3. Arana DM, Ortega A, González-Barberá E, Lara N, Bautista V, Gómez-Ruíz 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.

4. Ortega A, Sáez D, Bautista V, Fernández-Romero S, Lara N, Aracil B, Pérez-Vázquez M, Campos J, Oteo J; Spanish Collaborating Group for the Antibiotic Resistance Surveillance Programme. 2016. Carbapenemase-producing *Escherichia coli* is becoming more prevalent in Spain mainly because of the polyclonal dissemination of OXA-48. J Antimicrob Chemother 71: 2131-2138.

5. 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 reveals high in vitro susceptibility to colistin and meropenem. Antimicrob Agents Chemother 59: 3406-3412.

6. Wang J, Yuan M, Chen H, Chen X, Jia Y, Zhu X, Bai L, Bai X, Fanning S, Lu J, Li J. 2017. First report of *Klebsiella oxytoca* strain simultaneously producing NDM-1, IMP-4, and KPC-2 carbapenemases. Antimicrob Agents Chemother 61. pii: e00877-17.

7. De Belder D, Lucero C, Rapoport M, Rosato A, Faccone D, Petroni A, Pasteran F, Albornoz E, Corso A, Gomez SA. 2018. Genetic diversity of KPC-producing *Escherichia coli*, *Klebsiella oxytoca*, *Serratia marcescens*, and *Citrobacter freundii* isolates from Argentina. Microb Drug Resist 24: 958-965.

8. Simon M, Melzl H, Hiergeist A, Richert K, Falgenhauer L, Pfeifer Y, GerlachRG, Fuchs K, Reischl U, Gessner A, Jantsch J. 2017. Colistin- and carbapenem-resistant *Klebsiella oxytoca* harboring $bla_{\text{VIM-2}}$ and an insertion in the *mgr*B gene isolated from blood culture. Int J Med Microbiol 307: 113-115.

9. Vergara-López S, Domínguez MC, Conejo MC, Pascual Á, Rodríguez-Baño J. 2015. Lessons from an outbreak of metallo-β-lactamase-producing *Klebsiella oxytoca* in an intensive care unit: the importance of time at risk and combination therapy. J Hosp Infect 89: 123-131.

10. Izdebski R, Fiett J, Urbanowicz P, Baraniak A, Derde LP, Bonten MJ, Carmeli Y, Goossens H, Hryniewicz W, Brun-Buisson C, Brisse S, Gniadkowski M; MOSAR WP2, WP3 and WP5 Study Groups. 2015. Phylogenetic lineages, clones and β-lactamases in an international collection of *Klebsiella oxytoca* isolates non-susceptible to expanded-spectrum cephalosporins. J Antimicrob Chemother 70: 3230-3237.

11. Cannatelli A, Giani T, D'Andrea MM, Di Pilato V, Arena F, Conte V, Tryfinopoulou K, Vatopoulos A, Rossolini GM; COLGRIT Study Group. 2014. MgrB inactivation is a common mechanism of colistin resistance in KPC-producing *Klebsiella pneumoniae* of clinical origin. Antimicrob Agents Chemother 58:5696-5703.

12. The European Committee on antimicrobial susceptibility testing. Break points tables for interpretation of MICs and zone diameters. Version 9.0. *European Society of Clinical Microbiology and Infectious Diseases.* Available in: http://www.eucast.org/clinical_breakpoints/.

13. EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Version 1.0. *The European Society of Clinical Microbiology and Infectious Diseases.* Available in*: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Resistance_mechanisms/EUCAST_detection_of_resistance_mechanisms_v1.0_20131211.pdf*

14. Galimand M, Courvalin P, Lambert T. 2003. Plasmid-mediated high-level resistance to aminoglycosides in Enterobacteriaceae due to 16S rRNA methylation. Antimicrob Agents Chemother 7: 2565-2571.

15. Uechi K, Tada T, Shimada K, Nakasone I, Sonozaki T, Kirikae T, Fujita J. 2018. Emergence of ArmA, a 16S rRNA methylase in highly aminoglycoside-resistant clinical isolates of *Klebsiella pneumoniae* and *Klebsiella oxytoca* in Okinawa, Japan. J Infect Chemother 24: 68-70.

16. Zhang Y, Zhou H, Shen XQ, Shen P, Yu YS, Li LJ. 2008. Plasmid-borne *arm*A methylase gene, together with $bla_{\text{CTX-M-15}}$ and $bla_{\text{TEM-1}}$, in a *Klebsiella oxytoca* isolate from China. J Med Microbiol 57: 1273-1276.

17. González-Zorn B, Teshager T, Casas M, Porrero MC, Moreno MA, Courvalin P, Domínguez L. 2005. *arm*A and aminoglycoside resistance in *Escherichia coli*. Emerg Infect Dis 11: 954-956.

18. Hidalgo L, Gutierrez B, Ovejero CM, Carrilero L, Matrat S, Saba CK, Santos-Lopez A, Thomas-Lopez D, Hoefer A, Suarez M, Santurde G, Martin-Espada C, Gonzalez-Zorn B. 2013. *Klebsiella pneumoniae* sequence type 11 from companion animals bearing ArmA methyltransferase, DHA-1 β-lactamase, and QnrB4. Antimicrob Agents Chemother 57: 4532-4534.

19. Ho PL, Lo WU, Yeung MK, Lin CH, Chow KH, Ang I, Tong AH, Bao JY, Lok S, Lo JY. 2011. Complete sequencing of pNDM-HK encoding NDM-1 carbapenemase from a multidrug-resistant *Escherichia coli* strain isolated in Hong Kong. PLoS One 6: e17989.

20. Fevre C, Jbel M, Passet V, Weill FX, Grimont PA, Brisse S. 2005. Six groups of the OXY β-lactamase evolved over millions of years in *Klebsiella oxytoca*. Antimicrob Agents Chemother 49: 3453–3462.

21. Herzog KA, Schneditz G, Leitner E, Feierl G, Hoffmann KM, Zollner-Schwetz I, Krause R, Gorkiewicz G, Zechner EL, Högenauer C. 2014. Genotypes of *Klebsiella oxytoca* isolates from patients with nosocomial pneumonia are distinct from those of isolates from patients with antibiotic-associated hemorrhagic colitis. J Clin Microbiol 52: 1607-1616.

22. Holt KE, Baker S, Weill FX, Holmes EC, Kitchen A, Yu J, Sangal V, Brown DJ, Coia JE, Kim DW, Choi SY, Kim SH, da Silveira WD, Pickard DJ, Farrar JJ, Parkhill J, Dougan G, Thomson NR. 2012. *Shigella sonnei* genome sequencing and phylogenetic analysis indicate recent global dissemination from Europe. Nat Genet 44:1056-1059.

23. Psichogiou M, Tassios PT, Avlamis A, Stefanou I, Kosmidis C, Platsouka E, Paniara O, Xanthaki A, Toutouza M, Daikos GL, Tzouvelekis LS. 2008. Ongoing

epidemic of *bla*$_{VIM-1}$-positive *Klebsiella pneumoniae* in Athens, Greece: a prospective survey. J Antimicrob Chemother 61: 59-63.

24. Villa J, Viedma E, Brañas P, Orellana MA, Otero JR, Chaves F. 2014. Multiclonal spread of VIM-1-producing *Enterobacter cloacae* isolates associated with In624 and In488 integrons located in an IncHI2 plasmid. Int J Antimicrob Agents 43: 451-455.

25. Villa J, Arana DM, Viedma E, Perez-Montarelo D, Chaves F. 2017. Characterization of mobile genetic elements carrying VIM-1 and KPC-2 carbapenemases in *Citrobacter freundii* isolates in Madrid. Int J Med Microbiol 307: 340-345.

26. Giakkoupi P, Xanthaki A, Kanelopoulou M, Vlahaki A, Miriagou V, Kontou S, Papafraggas E, Malamou-Lada H, Tzouvelekis LS, Legakis NJ, Vatopoulos AC. 2003. VIM-1 metallo-beta-lactamase-producing *Klebsiella pneumoniae* strains in Greek hospitals. J Clin Microbiol 41: 3893-3896.

27. Leavitt A, Chmelnitsky I, Carmeli Y, Navon-Venezia S. 2010. Complete nucleotide sequence of KPC-3-encoding plasmid pKpQIL in the epidemic *Klebsiella pneumoniae* sequence type 258. Antimicrob Agents Chemother 54: 4493-4496.

28. 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 Program Collaborating Group V. 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.

29. Sugawara Y, Akeda Y, Sakamoto N, Takeuchi D, Motooka D, Nakamura S, Hagiya H, Yamamoto N, Nishi I, Yoshida H, Okada K, Zin KN, Aye MM, Tomono K, Hamada S. 2017. Genetic characterization of *bla*$_{NDM}$-harboring plasmids in carbapenem-resistant *Escherichia coli* from Myanmar. *PLoS One* 12: e0184720.

30. Papagiannitsis CC, Kutilova I, Medvecky M, Hrabak J, Dolejska M. 2017. Characterization of the complete nucleotide sequences of IncA/C(2) plasmids carrying In809-like integrons from Enterobacteriaceae isolates of wildlife origin. Antimicrob Agents Chemother 24; 61. pii: e01093-17.

31. 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.

32. Recommendations of the CLSI-EUCAST joint polymyxins working group. Available in: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/General_documents/Recommendations_for_MIC_determination_of_colistin_March_2016.pdf.

33. Dortet L, Bréchard L, Cuzon G, Poirel L, Nordmann P. 2014. Strategy for rapid detection of carbapenemase-producing Enterobacteriaceae. Antimicrob Agents Chemother 58: 2441-2445.

34. Oteo J, Navarro C, Cercenado E, Delgado-Iribarren A, Wilhelmi I, Orden B, García C, Miguelañez S, Pérez-Vázquez M, García-Cobos S, Aracil B, Bautista V, Campos J. 2006. Spread of *Escherichia coli* strains with high-level cefotaxime and ceftazidime resistance between the community, long-term care facilities, and hospital institutions. J Clin Microbiol 44: 2359-2366.

35. Lima Barbieri N, Nielsen DW, Wannemuehler Y, Cavender T, Hussein A, Yan SG, Nolan LK, Logue CM. 2017. *mcr*-1 identified in avian pathogenic *Escherichia coli* (APEC). PLoS One 12: e0172997.

36. Pérez-Pérez FJ, Hanson ND. 2002. Detection of plasmid-mediated AmpC β-lactamase genes in clinical isolates by using multiplex PCR. J Clin Microbiol 40: 2153-2162.

37. Gastmeier P, Schwab F, Bärwolff S, Rüden H, Grundmann H. 2006. Correlation between the genetic diversity of nosocomial pathogens and their survival time in intensive care units. J Hosp Infect 62: 181-186

38. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19: 455-477.

39. Boetzer M, Henkel CV, Jansen HJ, Butler D, Pirovano W. 2011. Scaffolding pre-assembled contigs using SSPACE. Bioinformatics 27: 578-579.

40. Nadalin F, Vezzi F, Policriti A. 2012. GapFiller: a de novo assembly approach to fill the gap within paired reads. BMC Bioinformatics 13 Suppl 14: S8.

41. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30: 2068-2069.

551 42. Inouye M, Dashnow H, Raven LA, Schultz MB, Pope BJ, Tomita T, Zobel J, Holt
552 KE. 2014. SRST2: Rapid genomic surveillance for public health and hospital
553 microbiology labs. Genome Med 6: 90.
554 43. Gupta SK, Padmanabhan BR, Diene SM, Lopez-Rojas R, Kempf M, Landraud L,
555 Rolain JM. 2014. ARG-ANNOT, a new bioinformatic tool to discover antibiotic
556 resistance genes in bacterial genomes. Antimicrob Agents Chemother 58: 212-220.
557 44. Rónai Z, Kreizinger Z, Dán Á, Drees K, Foster JT, Bányai K, Marton S, Szeredi L,
558 Jánosi S, Gyuranecz M. 2015. First isolation and characterization of *Brucella microti*
559 from wild boar. BMC Vet Res 11;11:147
560 45. Sola-Campoy PJ, Monzón S, Pérez-Vázquez M, et al. PlasmidID: a mapping based
561 tool for plasmid identication, annotation, typing and visualization. Abstract Nº 6606.
562 28th Congress of Clinical Microbiology and Infectious Diseases (2018), Madrid.
563
564

565 **Table 1**. Antibiotic susceptibility of 80 carbapenemase-producing *Klebsiella oxytoca*
566 isolates as determined by the microdilution method according to EUCAST clinical
567 breakpoints (reference 12)
568

Antibiotics	**S (%) Total isolates (n=80)**	**S (%) VIM-1 isolates (n=44)**	**S (%) OXA-48 isolates (n=29)**	**MIC_{50}[a] Total isolates**	**MIC_{90}[a] Total isolates**	**Range[a] Total isolates**
Colistin	97.5	95.5	100	0.5	1	0.5 - >8
Amikacin	86.2	81.8	96.5	4	16	4 - >32
Gentamicin	60	34.1	96.5	0.5	>8	≤2 - >8
Aztreonam	40	47.7	37.9	>32	>32	≤0.5->32
Cef/Avi	38.7	0	100	>16	>16	≤0.5->16
Tobramycin	38.7	4.5	89.6	8	>8	≤2 - >8
Meropenem	36.2	25	62.1	4	>16	0.5 - >16
Cotrimoxazole	35	6.8	79.3	>8	>8	≤1 - >8
Tigecycline	21.2	22.7	17.2	1	4	0.5 - 4
Ciprofloxacin	18.7	22.7	17.2	>2	>2	≤0.06 - >2
Imipenem	11.2	4.5	24.1	8	>16	1 - >16
Ceftazidime	11.25	0	31	>16	>16	≤1->16
Ertapenem	8.7	15.9	0	>2	>2	≤0.5->2
Cefotaxime	3.7	0	10.3	>8	>8	≤1->8
Pip/taz	0	0	0	>32/4	>32/4	>32/4
Amox/clav	0	0	0	>64/2	>64/2	>64/2->64/2

569
570 S: susceptibility. MIC: minimum inhibitory concentrations. Cef/Avi:
571 Ceftazidime/Avibactam. Pip/taz: Piperacillin/tazobactam. Amox/Clav:
572 Amoxicillin/clavulanic acid
573 [a] Expressed in mg/L.
574

Figure 1. Minimum spanning tree of 12 representative carbapenemase-producing *K. oxytoca* isolates (colored circles). Distance based on *an ad hoc cgMLST* of 3201 genes using the parameters "pairwise ignoring missing values" and all the *K. oxytoca* reference sequences available in NCBI. Each circle is named with the MLST type and colors indicate carbapenemase types; white circles represent control isolates available in NCBI (n=7) or representative of OXA-48-producing *K. oxytoca* outbreak from Tunisia (n=3, unpublished data*).

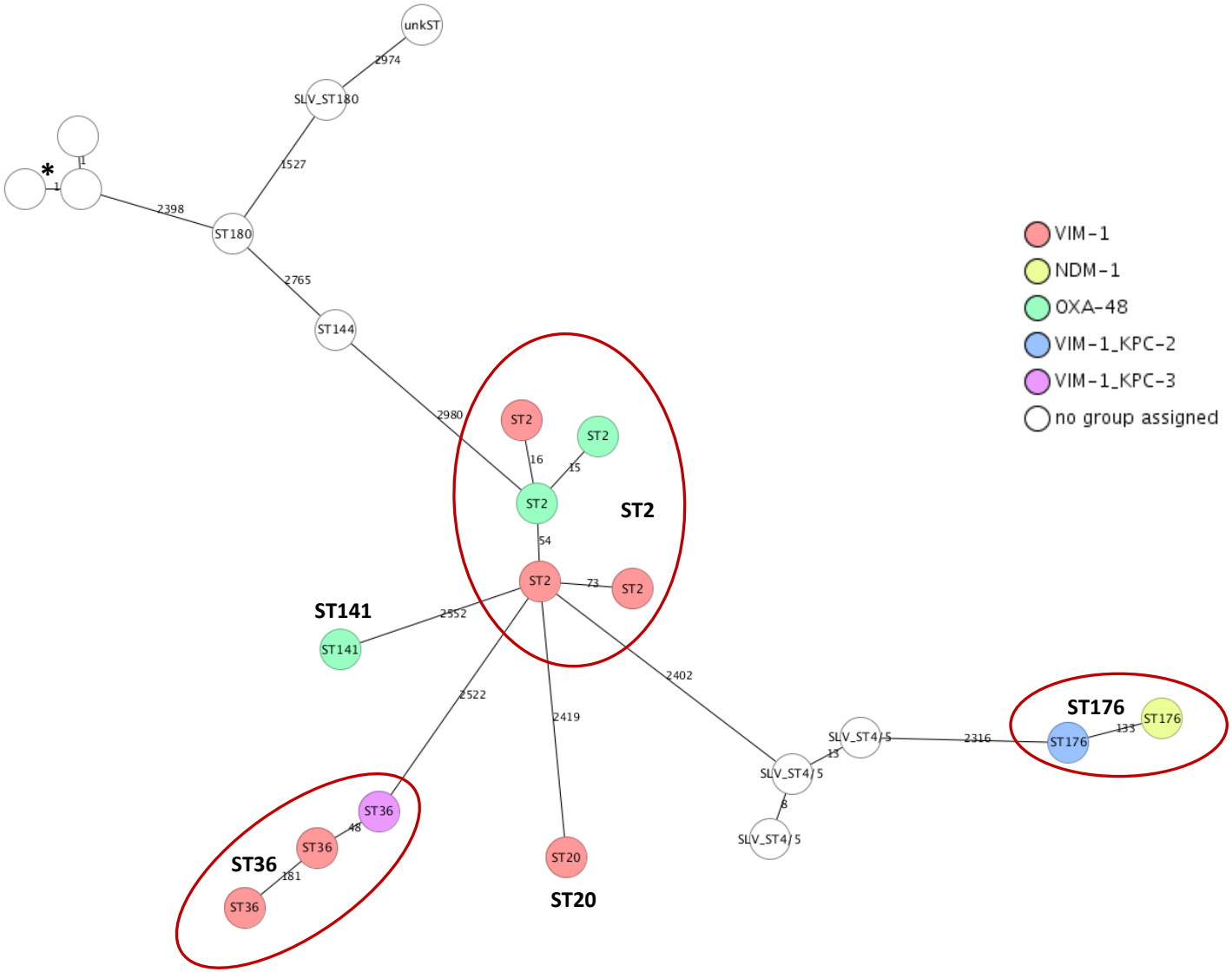

Table 2. Whole genome sequencing of 12 representative carbapenemase-producing *Klebsiella oxytoca* isolates: Main antibiotic resistance genes and other molecular markers detected.

Isolate	**PFGE/ ST**	**Carbapenemase genes**	**Plasmids carrying carbapenemases genes**	***bla*$_{OXY}$**	**Other *bla* genes**	***aad*A**	***aac(6')Ib***	***arm***	***ant(2'')-Ia***	***sul***	***dhfr***	***qnr***	***cat***	***tet***	***msr/mph***
K7533	C1/ST2	*bla*$_{OXA-48}$	IncL, ~ 63 kb	*bla*$_{OXY-2-8}$	N	*aad*A1	ND	N	N	ND	*dfr*A1	N	N	N	N
K0100	C1/ST2	*bla*$_{OXA-48}$	IncL, ~ 63 kb	*bla*$_{OXY-2-8}$	N	*aad*A1	ND	N	N	ND	*dfr*A1	N	N	N	N
K9103	C2/ST36	*bla*$_{VIM-1}$	IncHI2, ~ 250 kb	*bla*$_{OXY-2-2}$	N	*aad*A1	*aac(6')Ib*	N	N	*sul*1 *sul*2	*dfr*A1 *dfr*B1	N	*cat*A1 *cat*B2	N	*msr*E *mph*E
K9635	C3/ST2	*bla*$_{VIM-1}$	IncL, ~ 70 kb	*bla*$_{OXY-2-8}$	N	*aad*A1	*aac(6')Ib*	N	N	*sul*1	*dfr*A1 *dfr*B1	N	*cat*A1 *cat*B2	N	N
K9599	C4/ST36	*bla*$_{VIM-1}$	IncFII, ~70 kb	*bla*$_{OXY-2-2}$	*bla*$_{TEM-1A}$	*aad*A1	*aac(6')Ib*	N	N	*sul*1	*dfr*B1	N	*cat*B2	N	N
K8025	Couple/ST20	*bla*$_{VIM-1}$	IncL, ~ 70 kb	*bla*$_{OXY-2-5}$	N	*aad*A1	*aac(6')Ib*	N	N	*sul*1	*dfr*B1	N	*cat*A1 *cat*B2	N	*msr*E *mph*E
K8021	Couple/ST2	*bla*$_{VIM-1}$	IncHI2, ~ 250 kb	*bla*$_{OXY-2-8}$	*bla*$_{CTX-M-9}$ *bla*$_{SHV-12}$	*aad*A1 *aad*A2	*aac(6')Ib*	N	*ant(2'')-Ia*	*sul*1	*dfr*A1 *dfr*A16 *dfr*B1	*qnr*A1	*cat*A1 *cat*B2	*tet*A	N
K7929	Couple/ST141	*bla*$_{OXA-48}$	IncL, ~ 48 kb	*bla*$_{OXY-2-2}$	N	ND	ND	N	N	N	ND	N	N	N	*msr*E *mph*E
K8792	Couple/ST2	*bla*$_{VIM-1}$	ND	*bla*$_{OXY-2-8}$	N	*aad*A1	*aac(6')Ib*	N	N	*sul*1	*dfr*A1 *dfr*B1	N	*cat*B2	N	N
K9534	NR/ST36	*bla*$_{VIM-1}$ *bla*$_{KPC-3}$	IncN, ~50 kb IncFIB, ~103 kb	*bla*$_{OXY-2-2}$	*bla*$_{TEM-1A}$ *bla*$_{OXA-9}$	*aad*A1	*aac(6')Ib*	N	N	*sul*1	*dfr*A14 *dfr*B1	*qnr*S1	*cat*A1 *cat*B2	N	N
K9455	NR/ST176	*bla*$_{NDM-1}$	IncC, ~154 kb	*bla*$_{OXY-2-5}$	*bla*$_{OXA-10}$ *bla*$_{CMY-4}$	*aad*A1	*aac(6')Ib*	*arm*A	N	*sul*1 *sul*2	*dfr*A14	*qnr*B32	N	*tet*A	*msr*E *mph*E
K9682	NR/ST176	*bla*$_{VIM-1}$ *bla*$_{KPC-2}$	IncL, ~70 kb IncP6, ~39 kb	*bla*$_{OXY-2-5}$	*bla*$_{TEM-1A}$	*aad*A1	*aac(6')Ib*	N	N	*sul*1	*dfr*B1	N	*cat*A1 *cat*B2	N	N

C1, C2, C3, and C4: Representative isolates from PFGE Cluster 1, 2, 3, 4, respectively. Couple: Representative isolates of PFGE clusters of only two isolates. NR: Isolates Non-Related by PFGE to any other isolate.

ND: Non-determined. N: Negative.

Figure 2. Overview of the IncC plasmid harbouring *bla*$_{NDM-1}$ detected in *Klebsiella oxytoca* in this study. The figure represents the plasmid according to the homology with a highly similar one from database (blue outer ring). Graph represents the reads mapped against this reference with depth of coverage ranging from 0 (red) to 500, colored orange when values are 1-20 and green if higher than 200 reads. Gray boxes represent cds from automatic annotation, with dark and light color when they were found in forward or reverse strand respectively. Colored stripes represent a more detailed annotation that includes antibiotic resistance genes in red, IS in blue and Rep genes in yellow. Homology between reference plasmid and assembled contigs is represented in the inner ring, with each contig colored according to its number.