Primer informe de Aislados de *Enterobacterales* Productores de OXA-181 en América Latina

First Report of OXA-181-Producing *Enterobacterales* Isolates in Latin America

TESIS EN LA MODALIDAD DE ARTÍCULO CIENTÍFICO PARA OPTAR POR EL TÍTULO PROFESIONAL DE LICENCIADO EN TECNOLOGÍA MÉDICA EN LA ESPECIALIDAD DE LABORATORIO CLÍNICO Y ANATOMÍA PATOLÓGICA

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En memoria y recuerdo de mis abuelos: Octavio Cuicapuza Ilizarbe, Nicolás Arteaga Cuellar, Rosa Espinoza Guillén y Dilma Beltran Roncal

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Los autores declaran no tener conflictos de interés.

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RESUMEN

Antecedentes: Las infecciones por *Enterobacterales* productoras de carbapenemasas (CPE) son una amenaza creciente para la salud publica. Aunque las enzimas carbapenemasas KPC, NDM e IMP son las más frecuentes en todo el mundo, las β-lactamasas tipo OXA-48 (oxacilinasas) están en aumento. Las enzimas *bla*$_{\text{OXA-181}}$ (una variante de OXA-48) muestran un alto nivel de actividad hidrolítica contra las penicilinas y un bajo nivel de hidrólisis de los carbapenémicos, con una fuerte preferencia por el imipenem, lo cual es un desafío para el diagnóstico de laboratorio. **Objetivo:** Caracterizar fenotípicamente y genotípicamente cinco aislamientos clínicos de CPE de dos establecimientos de salud en Lima, Perú. **Métodos y Materiales:** Estudio transversal, las pruebas de identificación y susceptibilidad se realizaron en el equipo Vitek2(bioMérieux, Francia) y el secuenciamiento de genoma completo se realizó mediante el instrumento Illumina MiSeq(Illumina, EE. UU.). **Resultados:** Los aislamientos fueron identificados como *Klebsiella pneumoniae* (n=3), *Citrobacter portucalensis* y *Escherichia coli*, todos presentaron fenotipos multirresistentes(MDR). El análisis bioinformático reveló la presencia del gen *bla*$_{\text{OXA-181}}$ como la única carbapenemasa en todos los aislamientos. También se encontraron genes asociados con la resistencia a aminoglucósidos, tetraciclinas y trimetoprima. El plásmido IncX3 se identificó en todos los genomas en un transposón Tn6361 truncado flanqueado por secuencias de inserción ΔIS26. El gen qnrS1 se encontró aguas abajo de *bla*$_{\text{OXA-181}}$, lo que confirió resistencia a las fluoroquinolonas a todos los aislados. **Conclusión:** Reportamos el primer informe de *Enterobacterales* productores de MDR y OXA-181 de Latinoamérica, en asociación con el gen qnrS1 en plásmidos de tipo IncX3.

Palabras claves: Enterobacterales productores de carbapenemasas, blaOXA-181, IncX3 plasmid, Peru

ABSTRACT

Background: Carbapenemase-producing Enterobacteriaceae (CPE) infections are a growing threat to public health. Although carbapenemase enzymes KPC, NDM and IMP are the most prevalent enzymes worldwide, β-lactamases of the OXA-48 (oxacillinase) type are on the rise. The *bla*$_{\text{OXA-181}}$ enzymes (a variant of OXA-48) show a high level of hydrolytic activity against penicillins and a low level of hydrolysis of carbapenems, with a strong preference for imipenem, which is a challenge for laboratory diagnosis. **Objective:** To phenotypically and genotypically characterize five clinical isolates of CPE from two health facilities in Lima, Peru. **Methods and Materials:** Cross-sectional study, identification and susceptibility tests were performed on the Vitek2 equipment (bioMérieux, France) and whole genome sequencing was performed using the Illumina MiSeq instrument (Illumina, USA). **Results:** Isolates were identified as Klebsiella pneumoniae (n=3), Citrobacter portucalensis and Escherichia coli, all presenting multidrug resistant (MDR) phenotypes. Bioinformatic analysis revealed the presence of the *bla*$_{\text{OXA-181}}$ gene as the only carbapenemase in all isolates. Genes associated with resistance to aminoglycosides, tetracyclines and trimethoprim were also found. The IncX3 plasmid was identified in all genomes in a truncated Tn6361 transposon flanked by ΔIS26 insertion sequences. The qnrS1 gene was found downstream of *bla*$_{\text{OXA-181}}$, which conferred fluoroquinolone resistance to all isolates. **Conclusion:** We report the first report of MDR OXA-181-producing Enterobacteriaceae from Latin America in association with the qnrS1 gene in IncX3-type plasmids.

Key words: Carbapenemase-producing Enterobacteriaceae, blaOXA-181, IncX3 plasmid, Peru

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OBSERVATION

First Report of OXA-181-Producing *Enterobacterales* Isolates in Latin America

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ABSTRACT We characterized five carbapenemase-producing *Enterobacterales* (CPE) isolates from two health care institutions in Lima, Peru. The isolates were identified as *Klebsiella pneumoniae* (n = 3), *Citrobacter portucalensis* (n = 1), and *Escherichia coli* (n = 1). All were identified as bla_{OXA-48}-like gene carriers using conventional PCR. Whole-genome sequencing found the presence of the $bla_{OXA-181}$ gene as the only carbapenemase gene in all isolates. Genes associated with resistance to aminoglycosides, quinolones, amphenicols, fosfomycins, macrolides, tetracyclines, sulfonamides, and trimethoprim were also found. The plasmid incompatibility group IncX3 was identified in all genomes in a truncated Tn*6361* transposon flanked by ΔIS*26* insertion sequences. The *qnrS1* gene was also found downstream of $bla_{OXA-181}$, conferring fluoroquinolone resistance to all isolates. CPE isolates harboring bla_{OXA}-like genes are an increasing public health problem in health care settings worldwide. The IncX3 plasmid is involved in the worldwide dissemination of $bla_{OXA-181}$, and its presence in these CPE isolates suggests the wide dissemination of $bla_{OXA-181}$ in Peru.

IMPORTANCE Reports of carbapenemase-producing *Enterobacterales* (CPE) isolates are increasing worldwide. Accurate detection of the β-lactamase OXA-181 (a variant of OXA-48) is important to initiate therapy and preventive measures in the clinic. OXA-181 has been described in CPE isolates in many countries, often associated with nosocomial outbreaks. However, the circulation of this carbapenemase has yet to be reported in Peru. Here, we report the detection of five multidrug-resistant CPE clinical isolates harboring $bla_{OXA-181}$ in the IncX3-type plasmid, a potential driver of dissemination in Peru.

KEYWORDS $bla_{OXA-181}$, carbapenemase-producing *Enterobacterales*, IncX3 plasmid, Peru, carbapenemase-producing *Enterobacteriaceae*

Members of the family *Enterobacterales* are commensals in the intestinal tract and are considered opportunistic pathogens of humans and animals (1). More than 60% of all antibiotics used to treat enterobacterial infections are β-lactams (2). Several resistance mechanisms to β-lactams have been reported, with β-lactamase production being the most common (3). Carbapenems are β-lactams that are highly resistant to degradation by β-lactamases (4). Various carbapenemase-producing *Enterobacterales* (CPE) isolates have been identified, and their spread represents a public health risk worldwide (5).

The KPC, NDM, IMP, VIM, and OXA enzymes are the most common carbapenemases worldwide (6). OXA β-lactamases (class D β-lactamases or oxacillinases) are divided into

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four groups; OXA-48-like enzymes are included in group III, can hydrolyze carbapenems, and are poorly inhibited by clavulanic acid, tazobactam, and sulbactam (7–9). They show high-level hydrolytic activity against penicillins and low-level hydrolysis of carbapenems with a strong preference for imipenem (10). Previous studies have reported OXA-48-like carbapenemase-producing *Enterobacterales* isolates in humans, animals, food products, and environmental sources (11). Forty-eight OXA-48-like variants have been described (https://www.ncbi.nlm.nih.gov/pathogens/refgene/#oxa-48), with OXA-181 being the second most common (12). It contains four substitutions (the Thr-to-Ala change at position 104 [Thr104Ala], Asn110Asp, Glu168Gln, Ser171Ala) compared to $bla_{\text{OXA-48}}$ and was first identified in a *Shewanella xiamenensis* isolate in India (13).

From June 2019 to September 2021, five carbapenem-resistant enterobacterial isolates were obtained from different patients at two health care institutions in Lima, Peru. KP1137, KP1139, and EC1141 were isolated from urine; KP1138 and CP1140 were isolated from blood and bronchial secretions, respectively. Identification and susceptibility testing were performed on the Vitek2 compact system (bioMérieux, France). The results were analyzed using criteria from the Clinical and Laboratory Standards Institute (CLSI) (14). Phenotypic detection of carbapenemases was performed using the Triton-Hodge test (15). Detection of OXA-48-like carbapenemase production was performed using the RESIST-4 OKNV (OXA-48-like, KPC, NDM, VIM) immunochromatographic lateral flow assay (Coris BioConcept, Belgium) following the manufacturer's instructions. Molecular confirmation of OXA-48-like genes was performed by conventional PCR using the primers 5′-ATGCGTGTATTAGCCTTATCGG-3′ (forward) and 5′-TGAGCACTTCTTTTGTGATG-3′ (reverse), yielding a 775-bp amplicon (16).

DNA was extracted from overnight cultures in LB broth using the GeneJET DNA purification kit (Thermo Fisher Scientific, USA), according to the manufacturer's instructions. The DNA concentration was measured using a Qubit 4 fluorometer (Life Technologies, USA). Genomic libraries were prepared using the Nextera XT DNA library preparation kit (Illumina, USA) and sequenced on an Illumina MiSeq instrument, generating 2 × 250-nucleotide (nt) reads. The read quality was assessed using FastQC v0.11.5 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Adapters and low-quality reads were removed using Trimmomatic v0.39 (17), and *de novo* assembly was performed using SPAdes v3.15.2 (18). The assembled contigs were polished by paired read mapping using Pilon v1.24 (19), and genome assembly metrics were generated using QUAST v5.0.2 (https://github.com/ablab/quast). Genomic multilocus sequence type (MLST), virulence, and plasmid profiles were obtained using MLST v2.22.0 (https://github.com/tseemann/mlst) and the associated PlasmidFinder and VFDB databases (http://www.mgc.ac.cn/VFs/). Contigs were screened for antimicrobial resistance genes (ARGs) using ABRicate v1.0.1 (https://github.com/tseemann/abricate) by querying the NCBI and ResFinder databases. *Klebsiella* locus profile species were determined using Kleborate v0.2.0 (20). Serotype profiling for *Escherichia coli* isolates was defined using SerotypeFinder v2.0 and SeqSero v1.0. *In silico* typing of *E. coli* was performed using ClermonTyping v1.3.0 (21). The genetic context of $bla_{\text{OXA-181}}$ was determined by extracting the contigs that carried this gene and annotating them using Prokka v1.14.6 (22) with a BLASTP/BLASTN combination. Transposable genetic elements (Tn) were investigated using Integrall (http://integrall.bio.ua.pt/), Mobile Element Finder v1.0.1 (https://cge.food.dtu.dk/services/MobileElementFinder/), and ISfinder (https://isfinder.biotoul.fr/) and were finally identified in the Transposon Registry (https://transposon.lstmed.ac.uk/tn-registry) (23). The Prokka GBK annotation files were parsed for Easyfig image output (https://mjsull.github.io/Easyfig/).

The isolates were identified as *Klebsiella pneumoniae* ($n = 3$), *Citrobacter portucalensis* ($n = 1$), and *Escherichia coli* ($n = 1$). *K. pneumoniae* isolates KP1137 and KP1138 were resistant to ertapenem and susceptible to second-, third-, and fourth-generation cephalosporins. KP1139 was resistant to all β-lactams, including ertapenem, imipenem, and meropenem. It was also resistant to fluoroquinolones, tetracyclines, and sulfamethoxazole/trimethoprim, only showing susceptibility to aminoglycosides. The isolates *E. coli* EC1141 and *C. portucalensis* CP1140 were resistant to ertapenem (see Table S1 in the supplemental material).

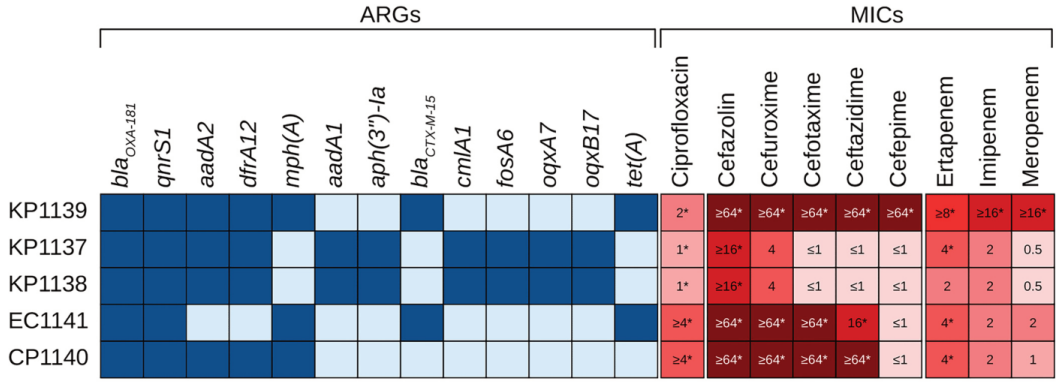

FIG 1 Antimicrobial resistance genes and MIC values of our *Enterobacteriaceae* isolates. ARG, antimicrobial resistance gene; KP, *Klebsiella pneumoniae*; EC, *Escherichia coli*; CP, *Citrobacter portucalensis*. The asterisks (*) indicate the MIC values for each antibiotic.

All isolates were identified as carbapenemase producers using the Triton-Hodge test. Moreover, all were positive for the RESIST-4 OKNV immunochromatography test and bla_{OXA-48}-like genes using conventional PCR. All isolates had multidrug-resistant (MDR) phenotypes. MDR bla_{OXA} producers are a rising health care issue that lengthens hospital stays and comorbidities because of their resistance to the antibiotics used to treat CPE infections (24).

Whole-genome sequencing revealed that *E. coli* isolate EC1141 belongs to sequence type 131 (ST131), serotype O50:H4, and Clermont phylogroup B2. *K. pneumoniae* isolates KP1137 and KP1138 were identified as ST25, with capsular type wzi72, capsular locus KL2, and antigen locus O1. *K. pneumoniae* KP1139 was identified as ST1174, with capsular type wzi275, KL38, and O12. The *C. portucalensis* isolate was identified as ST129. Thirty-one ARG hits were identified across all isolates, with a mean of 11.8 ARGs per genome. The $bla_{OXA-181}$ gene was identified in all genomes, being the only carbapenemase gene in the data set. Additionally, *K. pneumoniae* KP1139 and *E. coli* EC1141 exhibited the extended spectrum β-lactamase (ESBL) gene $bla_{CTX-M15}$ (Fig. 1). ARGs associated with resistance to aminoglycosides, quinolones, amphenicols, fosfomycins, macrolides, tetracyclines, sulfonamides, and trimethoprim were also identified (Table S2).

The $bla_{OXA-181}$ gene has been reported in *K. pneumoniae* (13), *E. coli* (25), and *Citrobacter* sp. (26) isolates. The plasmid incompatibility group IncX3 was identified in all our isolates, in line with previous studies in CPE (27). This plasmid group is involved in the worldwide dissemination of $bla_{OXA-181}$ (28), and its presence in these CPE isolates suggests extended dissemination of $bla_{OXA-181}$ in Peru. The $bla_{OXA-181}$ gene was located in a truncated transposon Tn*6361*, flanked by two Δ*IS26* insertion sequences. The *qnrS1* gene was detected downstream of $bla_{OXA-181}$, conferring fluoroquinolone resistance in all isolates. The $bla_{OXA-181}$ gene was flanked upstream by the Tn*3*-like Δ*IS3000* truncated insertion sequence, followed by the truncated IS*Ecp1* gene, and downstream by the Δ*lysR* (transcriptional regulator)-Δ*ere* (erythromycin esterase)-Δ*repA* (Col replicase) gene cluster (Fig. 2). This fragment is followed by the IS*Kpn19-tnpR-qnrS1*-IS*2*-like gene set, whose structure is similar to Tn*6292*. This region was also compared to pRIVM_C011701_1 (GenBank accession number CP068340; 100% coverage and 100% sequence similarity) and pKP709-OXA181 (MN227183; 100% coverage and 100% sequence similarity).

In summary, we present the first report of MDR OXA-181-producing *Enterobacterales* isolates from Peru and highlight the use of WGS to monitor the dissemination of CPE isolates. Our results suggest the emergence and wide distribution of $bla_{OXA-181}$ in association with the *qnrS1* gene on IncX3-type plasmids, which could represent the primary vector for the spread of $bla_{OXA-181}$ in Latin America.

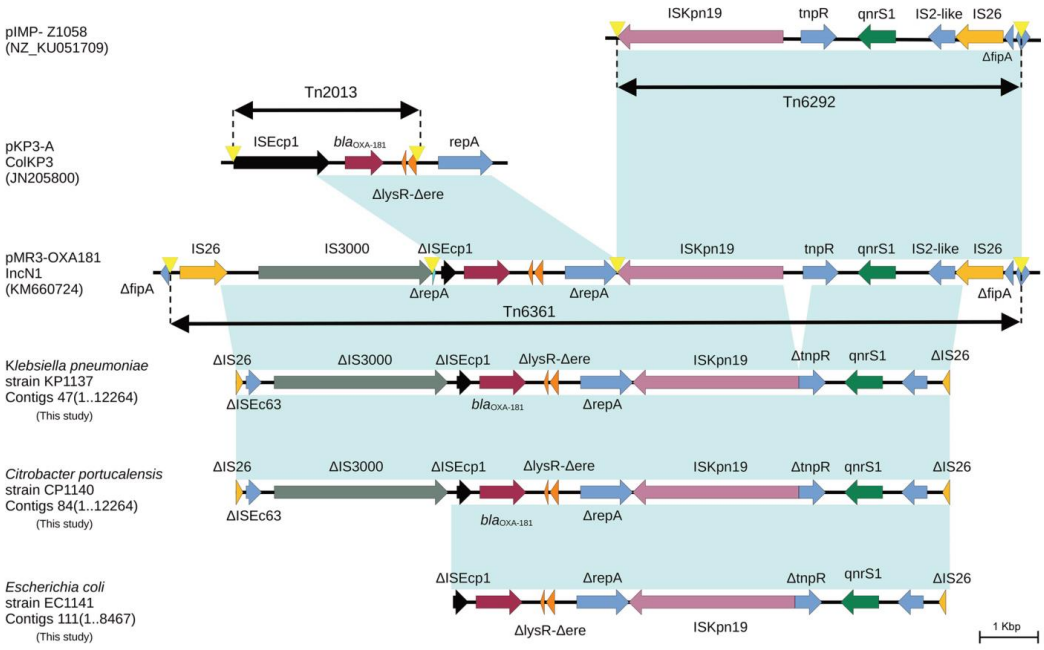

FIG 2 Genetic context of $bla_{OXA-181}$-carrying Tn*6361* (GenBank accession number KM660724) transposon structures in our *Enterobacterales* isolates. Transposons Tn*2013* (JN205800) and Tn*6292* (NZ_KU051709) were included for comparison. The same genetic environment of the $bla_{OXA-181}$-carrying Tn*6361* in *K. pneumoniae* KP1137 was found in strains KP1138 and KP1139. Shading indicates 100% sequence similarity.

Ethical approval. The study protocol was approved by the Institutional Ethics Committee of the Universidad Peruana Cayetano Heredia (SIDISI 207858). Patient information was anonymized and deidentified before analysis.

Data availability. The raw read files and assemblies for the five isolates are available at NCBI under BioProject accession number PRJNA860216.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, XLSX file, 0.01 MB.

SUPPLEMENTAL FILE 2, XLSX file, 0.01 MB.

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Anexos

Anexo 1

Tabla suplementaria 1. Valores de MIC de los aislados de *Enterobacterales* estudiados. R = resistente; I = intermedio, S = susceptible.

Antimicrobial agent	KP1137	KP1138	KP1139	CP1140	EC1141
	K.pneumoniae			*C.portucalensis*	*E.coli*
	MIC (µg/ml)				
Penicillins					
Ampicillin/Sulbactam	≥ 32 [R]	≥ 32 [R]	≥ 32 [R]	≥ 32 [R]	≥ 32 [R]
β-Lactam/β-Lactamase inhibitor					
Piperacillin/Tazobactam	≥ 128 [R]	≥ 128 [R]	≥ 128 [R]	≥ 128 [R]	≥ 128 [R]
Cephalosporins					
Cefazolin	≥ 16 [R]	≥ 16 [R]	≥ 64 [R]	≥ 64 [R]	≥ 64 [R]
Cefuroxime	4 [S]	4 [S]	≥ 64 [R]	≥ 64 [R]	≥ 64 [R]
Cefotaxime	≤ 1 [S]	≤ 1 [S]	≥ 64 [R]	≥ 64 [R]	≥ 64 [R]
Ceftazidime	≤ 1 [S]	≤ 1 [S]	≥ 64 [R]	≥ 64 [R]	16 [R]
Cefepime	≤ 1 [S]	≤ 1 [S]	≥ 64 [R]	≤ 1 [S]	≤ 1 [S]
Carbapenems					
Ertapenem	4 [R]	2 [R]	≥ 8 [R]	4 [R]	4 [R]
Imipenem	2 [I]	2 [I]	≥ 16 [R]	2 [I]	2 [I]
Meropenem	0.5 [S]	0.5 [S]	≥ 16 [R]	1 [S]	2 [I]
Aminoglycosides					
Amikacin	≤ 2 [S]	≤ 2 [S]	≤ 2 [S]	≤ 2 [S]	8 [S]
Gentamicin	≤ 1 [S]	≤ 1 [S]	≤ 1 [S]	≤ 1 [S]	≥ 16 [R]
Fluoroquinolones					
Ciprofloxacin	1 [R]	1 [R]	2 [R]	≥ 4 [R]	≥ 4 [R]
Tetracycline					
Tigecycline	≤ 0.5 [S]	≤ 0.5 [S]	4 [R]	≤ 0.5 [S]	1 [R]
Folate pathway inhibitors					
Trimethoprim/Sulfamethoxazole	≥ 320 [R]	≥ 320 [R]	≥ 320 [R]	≥ 320 [R]	≤ 20 [S]

MIC, Minimum Inhibitory Concentration (µg/ml); R, resistant; I, intermediate; S, susceptible.

Anexo 2

Tabla suplementaria 2. Genes de resistencia a los antimicrobianos, marcadores plasmídicos y estadísticas de secuenciación, ensamblaje y anotación de los aislados de *Enterobacterales* estudiados. CC = complejo clonal, ST = Secuenciotipo.

Statistics	KP1137	KP1138	KP1139	CP1140	EC1141
	K.pneumoniae			*C.portucalensis*	*E.coli*
Length (bp)	5462031	5455546	5472201	5192252	5384836
# Contigs	164	196	199	250	268
N50	179130	122951	146257	64866	73533
Coverage	49X	40X	36X	54X	50X
ST	ST25	ST25	ST1174	ST129	ST131
Resistance genotype					
β-lactams	***bla*** $_{OXA-181}$, *bla* $_{SHV-110}$	***bla*** $_{OXA-181}$, *bla* $_{SHV-110}$	***bla*** $_{OXA-181}$, *bla* $_{SHV-11}$, *bla* $_{CTX-M-15}$	***bla*** $_{OXA-181}$, *bla* $_{OXA-10,}$ *bla* $_{CMY-86}$	***bla*** $_{OXA-181}$, *bla* $_{CTX-M-15}$, *bla* $_{OXA-1}$, *bla* $_{EC-5}$
Aminoglycosides	aph(3')-Ia, aadA1, aadA2	aph(3')-Ia, aadA1, aadA2	aadA2	aph(3")-Ib, aph(6)-Id	aac(6')-Ib, aac(3)-IIe, aadA2
Quinolones	OqxA7, OqxB17, **qnrS1**	OqxA7, OqxB17, **qnrS1**	OqxB19, OqxA6, **qnrS1**	qnrB13, **qnrS1**	**qnrS1**
Amphenicols	cmlA1	cmlA1	-	cmlA5	-
Fosfomycin	fosA6	fosA6	fosA	-	-
Macrolides	-	-	mph(A)	mph(A)	mph(A)
Tetracyclines	-	-	tet(A)	tet(A)	-
Sulfonamides	sul3	sul3	sul1, sul2	sul1, sul2	-
Trimethoprim	dfrA12	dfrA12	dfrA12	dfrA21, dfrA12	-
# AMR genes	12	12	13	14	9
Plasmid type	IncX3, IncR, IncFIA(HI1)	IncX3, IncR, IncFIA(HI1)	IncX3, IncFIB(K)	IncX3, Col440II, ColRNAI, Col440I, IncFII(Yp), IncFIB(pB171)	IncX3, IncFIB, IncFIC(FII), IncFIA
Accession number	JANHEM000000000	NHEL0000000	NHEK0000000	ANHEJ00000000	NHEI0000000

CC, clonal complex; ST, sequence type