Spread of OXA-48 and NDM-1-Producing *Klebsiella pneumoniae* ST48 and ST101 in Chicken Meat in Western Algeria

Nadia Chaalal,^{1–3} Abdelaziz Touati,¹ Sofiane Bakour,⁴ Mohamed Amine Aissa,² Albert Sotto,⁵ Jean-Philippe Lavigne,³ and Alix Pantel³

Aim: We investigated the prevalence of carbapenemase-producing Enterobacteriaceae (CPE) in chicken meat in Western Algeria in 2017.

Results: From February to July 2017, samples of chicken meat from three poultry farms in Western Algeria were screened for the presence of CPE. Strains were characterized with regard to antibiotic resistance, β -lactamase content, Plasmid-mediated quinolone resistance, sulfonamide resistance genes, clonality (repetitive sequence-based profiles and multilocus sequence typing) and virulence traits. Of 181 samples analyzed, 29 (16.0%) carbapenemase-producing *Klebsiella pneumoniae* were detected. Twenty-three OXA-48-producers (79.3%) and six (20.7%) New Delhi metallo (NDM)-1-producers were observed. Clonality analysis showed three distinct lineages and clonal expansions of the OXA-48-producing *K. pneumoniae* ST48 and the NDM-1-producing *K. pneumoniae* ST101. These isolates harbored *fimH*, *ureA*, *mrkD*, *entB*, *uge*, and *wabG*. Neither capsular serotype genes nor hypermucoviscous phenotype were detected. Plasmid analysis confirmed that all these isolates harbored the transferable IncL and IncFIIK plasmids.

Conclusions: This study reports the spread of OXA-48 and NDM-1-producing *K. pneumoniae* ST48 and ST101 in chicken meat in Western Algeria and demonstrates that food represents a reservoir of the carbapenemases encoding genes.

Keywords: *Klebsiella pneumoniae*, OXA-48, NDM-1, ST101, ST48, chicken meat

Introduction

ENTEROBACTERIACEAE, NORMALLY INHABITING Gram-negative bacteria of intestinal microbiota, are responsible for several infections associated with endemic outbreaks in many countries and a high rate of mortality in both the medical and veterinary clinic.^{1,2} Due to their broad distribution, these organisms can spread easily between humans and animals through the consumption of contaminated food or water.^{1,3,4} They also have a high capacity to acquire genetic material through horizontal gene transfer (plasmids and transposons) conferring resistance to a large panel of antibiotics and contributing to therapeutic failure.¹ Due to the widespread use of β -lactam antibiotic for the treatment of Enterobacteriaceae infections, resistance to this family of antibiotic has emerged through produc-

tion of β -lactamases.^{1,2} Carbapenems represent the latest β -lactams having a broad activity against third-generation cephalosporin-resistant Enterobacteriaceae, leading to their increasing use for the treatment of hospital-acquired human infections.¹ The consequence has been the emergence and diffusion of carbapenem-resistant Enterobacteriaceae (CRE) among humans, now representing a worldwide problem. Carbapenems are not approved for veterinary use,² however, resistance to these antimicrobial agents has emerged in animals in recent years because of successful transmission of Enterobacteriaceae clonal groups and frequent horizontal gene transfer of carbapenemase-expressing plasmids among Gram-negative bacilli.^{3,5}

Public health officials sounded the alarm when a carbapenemase-producing Enterobacteriaceae (CPE) was isolated for the first time in German swine farms in 2012⁶

¹Laboratoire d'Ecologie Microbienne, FSNV, Université de Bejaia, Bejaia, Algeria.

²Laboratory of Microbiology, National Institute of Veterinarian Sciences, Tiaret, Algeria.

Departments of ³Microbiology and Hospital Hygiene and ⁵Infectious Diseases, VBMI, INSERM U1047, CHU Nîmes, University of Montpellier, Nîmes, France.

⁴Aix Marseille Univ, IRD, APHM, MEPHI, IHU-Méditerranée Infection, Marseille, France.

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and carbapenemase-producing *Acinetobacter* spp. isolates from livestock (dairy farm) in France in 2010.⁷ They warned about the possibility of the transmission of these multidrug-resistant (MDR) bacteria through oral consumption in the community and/or hospitals.⁸ Following this report, several worldwide investigations have reported CPE in food, livestock, companion animals, and wildlife.^{3,5} Altogether, these observations led to the One Health initiative, a movement to forge a multidisciplinary network between physicians, veterinarians, and other scientific health and environment-related disciplines. In Algeria, the presence of CPE in nonhuman samples has been extensively reported. Carbapenemase-encoding genes were detected in companion animals,^{9–11} livestock,^{11,12} wild animals,^{11,13–16} and the environment.^{11,17,18} Very few data are available concerning food products, although the New Delhi metallo (NDM)-carbapenemase genes (*bla*_{NDM-5} and *bla*_{NDM-1}) have been detected in cows' milk.^{12,19} To better characterize the diffusion of CPE in food animals, the aim of this study was to evaluate the presence and the molecular characteristics of these MDR bacteria in chicken meat in Western Algeria.

Materials and Methods

Sampling and microbiological procedures

From February to July 2017, we randomly selected 181 samples from chicken meat from the three main poultry farms localized in three cities in Western Algeria (Oran, Tiaret, and Saïda) (Supplementary Table S1 and Fig. 1). Each farm had

around 4,000 chickens originating from Oran. The samples were collected from 6-week-old chickens and immediately transported at +4°C to the laboratory of Microbiology at the National Institute of Veterinarian Sciences (Tiaret, Algeria) for analysis and processed within 24 hr. After slaughter, the leg meat was crushed and then 25 g of each sample was cultured in 225 mL of Luria Bertani (LB) broth (Pasteur Institute, Algiers) supplemented with 32 mg/L of vancomycin and amphotericin B (2 mg/L) and incubated for 18 hr at 37°C.¹¹ Following incubation, 200 µL aliquot was plated onto MacConkey broth (Fluka, St. Louis) containing 0.5 mg/L of ertapenem (0.5 mg/L) and incubated for 18–24 hr at 37°C.²⁰ For selection of CRE isolates, 200 µL of each culture was then plated on two selective chromogenic media: ChromID® Carba and ChromID OXA-48 (bioMérieux, Marcy l'Etoile, France). The resulting colonies were isolated and identified by the Vitek2® and Vitek MS system (bioMérieux). Further confirmation of carbapenemase producers was investigated using the KPC/MBL and OXA-48 Confirm Kit (Rosco Diagnostica A/S, Taastrup, Denmark) according to the manufacturer's instructions.²¹

Antibiotic susceptibility testing

For each isolate, susceptibility to antimicrobial agents was tested by the disk diffusion method (Bio-Rad, Marnes La Coquette, France) on Mueller Hinton agar plates according to recommendations of the European Committee on Antimicrobial Susceptibility Testing EUCAST 2018.

FIG. 1. Area sampling and distribution of carbapenemase-producing *Klebsiella pneumoniae* clones in chicken meat in different cities of Western Algeria.

Ertapenem, imipenem, meropenem, and colistin minimal inhibition concentrations were determined by Etest method (bioMérieux) and microbroth dilution (Umic®; Biocentric, Bandol, France). Susceptibility patterns were interpreted according to the EUCAST 2018 guidelines.

Molecular characterization of the antibiotic resistance genes

Total DNA was extracted using the EZ1 DNA Tissue Kit on the BioRobot EZ1 extraction platform (Qiagen, Courtaboeuf, France). Genes encoding the most clinically prevalent carbapenemases (*bla*_{KPC}, *bla*_{OXA-48-like}, *bla*_{VIM}, *bla*_{IMP}, and *bla*_{NDM}) and extended-spectrum β -lactamases (*bla*_{TEM}, *bla*_{SHV}, and *bla*_{CTX-M}) were detected by PCR using specific primers and confirmed by sequencing, as described previously.^{22,23} The presence of genes encoding the sulfonamide resistance genes (*sul1*, *sul2*, *sul3*), and the plasmid-mediated quinolone resistance (PMQR) [*qnrA*, *qnrB*, *qnrS*, *qepA*, *oqxAB*, and *aac(6')-Ib/Ib-cr*] were screened as previously described.^{24–28}

Virulence profiles

A panel of 18 known virulence factors (VFs) associated genes was assessed by different PCR assays^{29,30}: (i) Fimbriae and/or adhesins: *fimH* (type 1 fimbriae), *mrKD* (adhesion type 3 fimbriae), and *cf29a* (adhesion CF29K); (ii) toxins: *ureA* (urease synthesis); (iii) iron uptake: *entB* (siderophore), *ybtS* (yersiniabactin), *kfu* (iron transport and phosphotransferase function), and *iutA* (hydroxamate siderophore); (iv) protectins: *magA* (capsular serotype K1 and hypermucoviscosity phenotype), *rmpA* (regulator of mucoid phenotype A), *kpsMII* (group2 capsule), and the genes of capsular serotypes K5, K57, K54, and K20; and (v) others: *allS* (associated with allantoin metabolism); *uge* (uridine disphosphate galacturonate 4-eimerase) and *wabG* (core LPS biosynthesis).

Analysis of clonality

The clonal relatedness of the CPE isolates was performed using repetitive sequence-based PCR (rep-PCR) and multi-locus sequence typing (MLST). The rep-PCR was performed on the Diversilab® System (bioMérieux) following the manufacturer's instructions. Isolates with identical strain patterns were considered indistinguishable if the similarity percentage was $\geq 95\%$. MLST analysis was performed using the Pasteur Institute's MLST software (bigsd.bweb.pasteur.fr).

Plasmid characterization

Plasmid DNA was extracted from the isolates and the transconjugant using two different methods: the Plasmid Miniprep Kit (QIAGEN) and the method previously published by Kado and Liu.³¹ The two methods were used to improve the isolation of the plasmids. The transferability of the OXA-48/NDM-1-carrying plasmids was performed by conjugation³² between OXA-48- ($n=23$) or NDM-1-producing isolates ($n=6$) as donor and *Escherichia coli* J53 rifampicin resistant as recipient. Transconjugants were selected on LB agar plates supplemented with rifampicin (300 mg/L) and ampicillin (64 mg/L) for 24 hr at 37°C. Successful conjugation was confirmed by antimicrobial susceptibility testing and the PCR detection of the corresponding carbapenemase-encoding genes.

Plasmid incompatibility types of the isolates and the transconjugants were identified on the extracted plasmids by a PCR-based replicon typing (PBRT) method as described previously.³³ Another PBRT was used to discriminate IncL and IncM groups using the methodology described by Carattoli *et al.*³⁴

Results

Characteristics of bacterial isolates

During the 6-month period of the study, 74 isolates from 74 samples on 181 analyzed (40.9%) were identified to be resistant to carbapenem: 32 (43.2%) *Pseudomonas* spp., 13 (17.6%) *Aeromonas* spp., and 29 (39.2%) Enterobacteriaceae. All the Enterobacteriaceae isolates were *Klebsiella pneumoniae*. Phenotyping analysis showed that these isolates were carbapenemase producers, giving an overall prevalence of 16.0% (29/181) (Fig. 1 and Supplementary Table S1). Total resistance to amoxicillin, amoxicillin/clavulanic acid, piperacillin, piperacillin/tazobactam, ticarcillin, ticarcillin/clavulanic acid, cefoxitine, cefixime, ceftazidime, cefotaxime, and ertapenem was detected. Variable rates of resistance were noted for the other molecules: imipenem (28, 96.6%), fluoroquinolones (27, 93.1%), meropenem (17, 58.6%), cotrimoxazole, and aminoglycosides (13 and 44.8%, respectively). All isolates were susceptible to colistin (Table 1).

Characterization of antibiotic resistance genes

Using molecular analysis, we confirmed that only *K. pneumoniae* were carbapenemase producers: 23 (79.3% of the carbapenemase-producing *K. pneumoniae* [CPKP]) isolates harbored *bla*_{OXA-48} gene and 6 (20.7%) *bla*_{NDM-1}. In addition, 26 strains out of 29 (89.7%) also harbored *bla*_{SHV-11}.

Different PMQR were identified: *oqxAB* (24, 82.8%), *aac(6')-Ib-cr* (13, 44.8%), and *qepA* (6, 20.7%) (Table 1). Ten isolates (34.5%) harbored a *qnr* gene. Four (4/23) and six (6/6) *qnrB* were identified in OXA-48- and NDM-1-producing *K. pneumoniae*, respectively. Twelve isolates (41.8%) harbored *sul1* gene. Finally, we noted association between *qnrB* and *aac(6')-Ib-cr* in 9 isolates and *oqxAB*, *qnrB*, and *aac(6')-Ib-cr* in all the NDM-1-producing *K. pneumoniae*.

Virulence profiles

The distribution of the 18 virulence-associated genes tested in the 29 studied *K. pneumoniae* isolates showed different patterns of VFs, with 6–9 genes detected per isolate. Interestingly, all the isolates harbored *fimH*, *ureA*, *mrKD*, *entB*, *uge*, and *wabG*. Genes implicated in iron metabolism, *kfu* (9, 31%), *ybtS* (4, 13.8%), and *iutA* (1, 3.4%) were sparsely detected. Neither capsular serotype genes nor hypermucoviscous phenotype were detected (Supplementary Table S2).

Clonality analysis

The rep-PCR results revealed the existence of three distinct lineages among the 29 CPKP (Fig. 2). OXA-48-producers were present in two patterns: patterns I ($n=20$) in which isolates originated from Oran ($n=15$) and Tiaret ($n=5$); and patterns II ($n=3$) from which isolates were recovered only from Saida. The last pattern (patterns III) gathered the NDM-1-producers ($n=6$), which contained isolates from Oran ($n=4$) and Tiaret ($n=2$). The MLST

TABLE 1. CHARACTERISTICS OF CARBAPENEMASE-PRODUCING *KLEBSIELLA PNEUMONIAE* ISOLATES RECOVERED FROM CHICKEN MEAT IN WESTERN ALGERIA

Strains	Area	Sampling period	Antimicrobial resistance					MICs (mg/L)			AMR genes	Plasmid	ST
			IMP					ERT	MEM	CS			
30H OXA-48	Oran	July	AMX, AMC, TIC, TCC, PIL, PTZ, CXN, FOX, FIX, C2D, TEM, ERT, IMP, MEM, NAL, OFX, CIP	16	32	32	0.25	0.25	0.25	0.25	<i>bla</i> _{OXA-48} , <i>bla</i> _{SHV-11} , <i>oqxAB</i>	Incl	ST48
A70 OXA-48	Oran	July	AMX, AMC, TIC, TCC, PIL, PTZ, CXN, FOX, FIX, C2D, TEM, ERT, IMP, MEM, NAL, OFX, CIP	16	32	32	0.25	0.25	0.25	0.25	<i>bla</i> _{OXA-48} , <i>bla</i> _{SHV-11} , <i>qepA</i> , <i>oqxAB</i>	Incl	ST48
V19' OXA-48	Oran	July	AMX, AMC, TIC, TCC, PIL, PTZ, CXN, FOX, FIX, C2D, TEM, ERT, IMP, MEM, NAL, OFX, CIP	16	32	32	0.25	0.25	0.25	0.25	<i>bla</i> _{OXA-48} , <i>qepA</i>	Incl	ST48
A6C OXA-48	Oran	July	AMX, AMC, TIC, TCC, PIL, PTZ, CXN, FOX, FIX, C2D, TEM, ERT, IMP, MEM, NAL, OFX, CIP	16	32	1	0.25	0.25	0.25	0.25	<i>bla</i> _{OXA-48} , <i>bla</i> _{SHV-11} , <i>oqxAB</i> , <i>aac6'-Ib-cr</i>	Incl	ST48
A9C OXA-48	Oran	July	AMX, AMC, TIC, TCC, PIL, PTZ, CXN, FOX, FIX, C2D, TEM, ERT, IMP, MEM, NAL, OFX, CIP	16	2	1	0.25	0.25	0.25	0.25	<i>bla</i> _{OXA-48} , <i>bla</i> _{SHV-11}	Incl	ST48
A7C OXA-48	Oran	July	AMX, AMC, TIC, TCC, PIL, PTZ, CXN, FOX, FIX, C2D, TEM, ERT, IMP, MEM, NAL, OFX, CIP	16	1	0.25	0.25	0.25	0.25	0.25	<i>bla</i> _{OXA-48} , <i>bla</i> _{SHV-11} , <i>oqxAB</i>	Incl	ST48
V53C OXA-48	Oran	July	AMX, AMC, TIC, TCC, PIL, PTZ, CXN, FOX, FIX, C2D, TEM, ERT, IMP, MEM, NAL, OFX, CIP	16	32	0.38	0.25	0.25	0.25	0.25	<i>bla</i> _{OXA-48} , <i>bla</i> _{SHV-11} , <i>oqxAB</i>	Incl	ST48
A60 OXA-48	Oran	July	AMX, AMC, TIC, TCC, PIL, PTZ, CXN, FOX, FIX, C2D, TEM, ERT, IMP, MEM, NAL, OFX, CIP	16	32	3	0.25	0.25	0.25	0.25	<i>bla</i> _{OXA-48} , <i>bla</i> _{SHV-11} , <i>qepA</i> , <i>oqxAB</i>	Incl	ST48
V59C OXA-48	Oran	July	AMX, AMC, TIC, TCC, PIL, PTZ, CXN, FOX, FIX, C2D, TEM, ERT, IMP, MEM, NAL, OFX, CIP	16	32	0.5	0.25	0.25	0.25	0.25	<i>bla</i> _{OXA-48} , <i>bla</i> _{SHV-11} , <i>oqxAB</i>	Incl	ST48
V510 OXA-48	Oran	July	AMX, AMC, TIC, TCC, PIL, PTZ, CXN, FOX, FIX, C2D, TEM, ERT, IMP, MEM, NAL, OFX, CIP	6	32	0.5	0.25	0.25	0.25	0.25	<i>bla</i> _{OXA-48} , <i>bla</i> _{SHV-11} , <i>oqxAB</i>	Incl	ST48
V801 OXA-48	Oran	July	AMX, AMC, TIC, TCC, PIL, PTZ, CXN, FOX, FIX, C2D, TEM, ERT, IMP, MEM, NAL, OFX, CIP, SXT	16	32	0.75	0.25	0.25	0.25	0.25	<i>bla</i> _{OXA-48} , <i>bla</i> _{SHV-11} , <i>oqxAB</i>	Incl	ST48
A20C OXA-48	Oran	July	AMX, AMC, TIC, TCC, PIL, PTZ, CXN, FOX, FIX, C2D, TEM, ERT, IMP, MEM, NAL, OFX, CIP	16	4	0.38	0.25	0.25	0.25	0.25	<i>bla</i> _{OXA-48} , <i>bla</i> _{SHV-11} , <i>oqxAB</i> , <i>sulI</i>	Incl	ST48
A5 OXA-48	Oran	July	AMX, AMC, TIC, TCC, PIL, PTZ, CXN, FOX, FIX, C2D, TEM, ERT, IMP, MEM, NAL, OFX, CIP	16	4	0.38	0.25	0.25	0.25	0.25	<i>bla</i> _{OXA-48} , <i>bla</i> _{SHV-11} , <i>oqxAB</i>	Incl	ST48
46HC OXA-48	Oran	July	AMX, AMC, TIC, TCC, PIL, PTZ, CXN, FOX, FIX, C2D, TEM, ERT, IMP	16	0.75	0.75	0.25	0.25	0.25	0.25	<i>bla</i> _{OXA-48} , <i>bla</i> _{SHV-11}	Incl	ST48
A500 OXA-48	Oran	July	AMX, AMC, TIC, TCC, PIL, PTZ, CXN, FOX, FIX, C2D, TEM, ERT, NAL, OFX, CIP	1	2	0.38	0.25	0.25	0.25	0.25	<i>bla</i> _{OXA-48} , <i>bla</i> _{SHV-11} , <i>oqxAB</i>	Incl	ST48
A22C' NDM-1	Oran	July	AMX, AMC, TIC, TCC, PIL, PTZ, CXN, FOX, FIX, C2D, ERT, IMP, MEM, NAL, OFX, CIP, SXT	4	32	4	0.25	0.25	0.25	0.25	<i>bla</i> _{NDM-1} , <i>bla</i> _{SHV-11} , <i>oqxAB</i> , <i>qnrB</i> , <i>aac6'-Ib-cr</i> , <i>sulI</i>	IncFIHK	ST101
A13C NDM-1	Oran	July	AMX, AMC, TIC, TCC, PIL, PTZ, CXN, FOX, FIX, C2D, ERT, IMP, MEM, NAL, OFX, CIP, SXT	4	32	4	0.25	0.25	0.25	0.25	<i>bla</i> _{NDM-1} , <i>bla</i> _{SHV-11} , <i>oqxAB</i> , <i>qnrB</i> , <i>aac6'-Ib-cr</i> , <i>sul2</i>	IncFIHK	ST101
A3C NDM-1	Oran	July	AMX, AMC, TIC, TCC, PIL, PTZ, CXN, FOX, FIX, C2D, ERT, IMP, MEM, NAL, OFX, CIP, SXT	4	32	4	0.25	0.25	0.25	0.25	<i>bla</i> _{NDM-1} , <i>bla</i> _{SHV-11} , <i>oqxAB</i> , <i>qnrB</i> , <i>aac6'-Ib-cr</i> , <i>sul3</i>	IncFIHK	ST101
1A NDM-1	Oran	July	AMX, AMC, TIC, TCC, PIL, PTZ, CXN, FOX, FIX, C2D, ERT, IMP, MEM, NAL, OFX, CIP, SXT	6	32	4	0.25	0.25	0.25	0.25	<i>bla</i> _{NDM-1} , <i>bla</i> _{SHV-11} , <i>oqxAB</i> , <i>qepA</i> , <i>qnrB</i> , <i>aac6'-Ib-cr</i> , <i>sulI</i>	IncFIHK	ST101
A8' OXA-48	Tiaret	May	AMX, AMC, TIC, TCC, PIL, PTZ, CXN, FOX, FIX, C2D, TEM, ERT, IMP, MEM, NAL, OFX, CIP, SXT	16	32	4	0.25	0.25	0.25	0.25	<i>bla</i> _{OXA-48} , <i>bla</i> _{SHV-11} , <i>qnrB</i> , <i>oqxAB</i> , <i>aac6'-Ib-cr</i> , <i>sulI</i>	Incl, non TP	ST48

(continued)

TABLE 1. (CONTINUED)

Strains	Area	Sampling period	Antimicrobial resistance	MICs (mg/L)				AMR genes	Plasmid	ST
				IMP	ERT	MEM	CS			
V82' OXA-48	Tiaret	May	AMX, AMC, TIC, TCC, PIL, PTZ, CXN, FOX, FIX, CZD, TEM, ERT, IMP, MEM, NAL, OFX, CIP, SXT	2	32	4	0.25	<i>bla</i> _{OXA-48} , <i>bla</i> _{SHV-11} , <i>oqxAB</i> , <i>aac6'-Ib-cr</i> , <i>sulI</i>	Incl	ST48
V59' OXA-48	Tiaret	May	AMX, AMC, TIC, TCC, PIL, PTZ, CXN, FOX, FIX, CZD, TEM, ERT, IMP, MEM, NAL, OFX, CIP, SXT	16	32	32	0.25	<i>bla</i> _{OXA-48} , <i>bla</i> _{SHV-11} , <i>qnrB</i> , <i>oqxAB</i> , <i>aac6'-Ib-cr</i> , <i>sulI</i>	Incl, non TP	ST48
A24' OXA-48	Tiaret	May	AMX, AMC, TIC, TCC, PIL, PTZ, CXN, FOX, FIX, CZD, TEM, ERT, IMP, MEM, NAL, OFX, CIP, SXT	16	32	4	0.25	<i>bla</i> _{OXA-48} , <i>bla</i> _{SHV-11} , <i>oqxAB</i> , <i>aac6'-Ib-cr</i> , <i>sulI</i>	Incl	ST48
A221 OXA-48	Tiaret	May	AMX, AMC, TIC, TCC, PIL, PTZ, CXN, FOX, FIX, CZD, TEM, ERT, IMP, NAL, OFX, CIP, SXT	16	6	1	0.25	<i>bla</i> _{OXA-48} , <i>bla</i> _{SHV-11} , <i>qnrB</i> , <i>oqxAB</i> , <i>aac6'-Ib-cr</i> , <i>sulI</i>	Incl, non TP	ST48
A21C NDM-1	Tiaret	May	AMX, AMC, TIC, TCC, PIL, PTZ, CXN, FOX, FIX, CZD, ERT, IMP, MEM, NAL, OFX, CIP, SXT	6	32	4	0.25	<i>bla</i> _{NDM-1} , <i>bla</i> _{SHV-11} , <i>oqxAB</i> , <i>qepA</i> , <i>qnrB</i> , <i>aac6'-Ib-cr</i> , <i>sul2</i>	IncFIIK	ST101
A34 NDM-1	Tiaret	May	AMX, AMC, TIC, TCC, PIL, PTZ, CXN, FOX, FIX, CZD, ERT, IMP, MEM, NAL, OFX, CIP, SXT	6	32	4	0.25	<i>bla</i> _{NDM-1} , <i>bla</i> _{SHV-11} , <i>oqxAB</i> , <i>qepA</i> , <i>qnrB</i> , <i>aac6'-Ib-cr</i> , <i>sul3</i>	IncFIIK	ST101
A28 OXA-48	Saida	March	AMX, AMC, TIC, TCC, PIL, PTZ, CXN, FOX, FIX, CZD, TEM, ERT, IMP, MEM, NAL, OFX, CIP, SXT	16	32	32	0.25	<i>bla</i> _{OXA-48} , <i>aac6'-Ib-cr</i> , <i>qnrB</i>	Incl, non TP	ST48
A23C' OXA-48	Saida	March	AMX, AMC, TIC, TCC, PIL, PTZ, CXN, FOX, FIX, CZD, TEM, ERT, IMP, MEM, NAL, OFX, CIP	16	32	32	0.25	<i>bla</i> _{OXA-48}	Incl	ST48
A10C' OXA-48	Saida	March	AMX, AMC, TIC, TCC, PIL, PTZ, CXN, FOX, FIX, CZD, TEM, ERT, IMP, MEM	16	32	32	0.25	<i>bla</i> _{OXA-48} , <i>bla</i> _{SHV-11} , <i>oqxAB</i>	Incl	ST48

AMX, amoxicillin; AMC, amoxicillin/clavulanic acid; TIC, ticarcillin; TCC, ticarcillin/clavulanic acid; PIL, piperacillin; PTZ, piperacillin/tazobactam; CXN, cefalexin; FOX, cefoxitin; FIX, cefixime; CZD, ceftazidime; TEM, temocillin; ERT, ertapenem; IMP, imipenem; MEM, meropenem; NAL, nalidixic acid; OFX, ofloxacin; CIP, ciprofloxacin; SXT, cotrimoxazole; CS, colistin; MIC, minimum inhibitory concentration; AMR, antimicrobial resistance; ST, sequence type; non TP, nontypable plasmid.

FIG. 2. Dendrogram illustrating the genetic relatedness (repetitive sequence-based PCR), sequence type and geographical distribution of 29 carbapenemase-producing *K. pneumoniae* strains isolated from chicken meat in Western Algeria.

showed the clonal expansion of the *K. pneumoniae* isolates with the diffusion of ST48 in OXA-48-producers and ST101 in NDM-1-producers (Figs. 1 and 2).

Plasmid analysis

The PBRT performed on the isolates showed that all the *bla*_{OXA-48} genes were carried in the IncL group plasmid, whereas all the *bla*_{NDM-1} genes were carried in the IncFIIK plasmid (Table 1). Four isolates (A221 OXA-48, A8' OXA-48, V59' OXA-48, and A28 OXA-48) carried a nontypable plasmid (absent in the tested panel). The plasmids were transferred by conjugation. This conjugation was successfully performed in all the isolates. After extraction, all the transconjugants exhibited similar resistance patterns (using the antimicrobial susceptibility testing), the presence of the *bla*_{OXA-48} and *bla*_{NDM-1} genes, and only one type of plasmid assigned to the IncL and the IncFIIK groups, respectively (Supplementary Table S3). The nontypable plasmid was not transferred. The conjugation rates were of 3.4×10^{-5} for IncL plasmid type and 1.3×10^{-7} for an IncFII plasmid type. The approximate sizes of the transferred plasmid were 60 kb (IncL) and 50 kb (IncFIIK).

Discussion

CRE and CPE are widely disseminated not only among humans but also in animal populations and the environment, making their control a major health concern worldwide.^{1,2,4} The absence of carbapenem use in the veterinarian setting has not prevented the appearance of CRE and CPE in animals.³ Studies have reported CRE emergence,^{5,11,14–16} and underlined the risk of their transmission from animals to humans through food-producing animals.^{3,5,11} In Algeria, the large-scale spread of CPE in human, livestock, and food (fresh vegetables and food-producing animals) suggests an endemic situation.^{4,11} There is still a lack of investigations that estimate potential food reservoirs of carbapenemase producers in terms of human contamination.³ The occurrence of NDM-5-producing *Escherichia coli* ST1284 isolated in crude milk in Bejaia city¹⁹ and NDM-1-producing *Acinetobacter baumannii* ST85 in raw milk in Tiaret city,¹² highlights this problem. Chicken meat has been proposed to constitute a source of CPE that colonizes and infects humans.³⁵ To date, two studies described the importance of CPE isolated from retail chicken meat in Egypt.^{36,37} Moreover, the prevalence of carbapenemases is high (26.1%) in poultry feces in this country.^{11,38} These data highlight the need for further studies to clarify the situation of poultry carriage and the possible risk of dissemination through alimentation.

In this study, we described for the first time, the occurrence of 16% of CPKP in chicken meat and the presence of the *bla*_{OXA-48} and *bla*_{NDM-1} genes among *K. pneumoniae* isolates, suggesting a widespread incidence of carbapenem resistance in foodstuff in Algeria. The predominance of OXA-48 producers is unsurprising due to its widespread description in previous investigations notably, very recently, in different ecological niches (human, animal, food, and environmental samples) in Central and Eastern Algeria.¹¹ Interestingly, NDM-1 producers were also described in the chicken meat specimens in this study. The emergence and diffusion of NDM-1-producing strains has been associated with Asia (particularly the Indian subcontinent) and the Balkans region, which represent the main reservoirs of such pathogens.³⁹ However, these enzymes were scarcely detected in livestock, mainly in chickens and pigs in

China and Italy.⁵ NDM-1 producers have also been diffused in Africa (around 10.8%) and notably in Algeria.⁴⁰ However, in this country, *bla*_{NDM-1} has mainly diffused in *A. baumannii*, notably in the Western region where the epidemiological studies on CPE are particularly scarce and where one report showed the emergence of NDM-1-producing *A. baumannii* ST85 transmitted by milk.¹² In the clinical setting, the presence of NDM-1-producing *K. pneumoniae* was exclusively noted in Annaba University Hospital (Eastern Algeria).⁴¹ This suggests that many parameters, at the global level, could play a significant role in spreading uncontrolled NDM-1-related resistance worldwide and deserve attention.

The potential spread of CPKP is linked to the dissemination of epidemic broad host-range plasmids. Thus, plasmid investigation showed that the *bla*_{OXA-48} gene is carried by an IncL plasmid in 79% of isolates. This gene was successfully transferred by conjugation, demonstrating the high potential of transfer of IncL plasmid between Enterobacteriaceae in Algeria as suggested by Mairi *et al.*¹¹ Several epidemiological investigations have shown a high diversity of plasmid backbones bearing the *bla*_{NDM-1} genes, which are responsible for their propagation among members of the Enterobacteriaceae.⁴² Overall, the *bla*_{NDM-1} gene has been more frequently reported on broad host range IncA/C-type plasmids, either in clinical or in environmental isolates.⁴³ In Algeria, the *bla*_{NDM-5} gene was carried by an IncX3 conjugative plasmid in *E. coli* isolates recovered from dairy cows in the area of Bejaia.¹⁶ The only clinical case of NDM-1-producing *K. pneumoniae* (described above) concerned an IncR plasmid with poor transferability.⁴¹ In this study, we described for the first time in Algeria the *bla*_{NDM-1} gene carried by IncFIIK plasmid. This plasmid belongs to a narrow host range family of plasmids, which are frequently identified among Enterobacteriaceae and notably involved in the worldwide dissemination of *bla*_{CTX-M-15} gene in the epidemic ST131 *E. coli*.⁴⁴ It is plausible that this epidemic plasmid will promote the diffusion of the *bla*_{NDM-1} gene.

While IncL has been involved in a widespread diffusion of *bla*_{OXA-48} gene in different niches,¹¹ MLST analysis showed that the clonal expansion of our strains isolated from chicken meat concerned ST48. This ST was particularly prevalent in Oran (15/23, 65.2%), but also found in the other two cities (Tiaret [5/23, 21.7%] and Saida [3/23, 13%]), showing its diffusion. We could also note that ST48 was previously detected in an urban wastewater treatment plant in Algeria.⁴⁵ Moreover, in our study, 20.7% of CPKP strains producing NDM-1 belonged to ST101, a widespread clone diffused in Algeria,^{46–48} but always associated with *bla*_{OXA-48}. The association between ST101 and NDM-1 producers was recently described in Tunisia,⁴⁹ Italy,⁵⁰ and Pakistan.⁵¹

In conclusion, this study reports for the first time the presence of CPKP in food-producing animals and specifically in chicken meat in Western Algeria. Our study supports implementation of a risk assessment for zoonotic CPE in the context of an endemic situation notably with the emergence of these bacteria in food-producing animals to prevent their occurrence and spread to humans and the environment.

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Disclaimer

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Supplementary Material

Supplementary Table S1
Supplementary Table S2
Supplementary Table S3

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Address correspondence to:

Jean-Philippe Lavigne, MD, PhD

Department of Microbiology and Hospital Hygiene

VBMI, INSERM U1047

CHU Nîmes

University of Montpellier

Place of Professor Robert Debre

Nîmes Cedex 09, 30029

France

E-mail: jean.philippe.lavigne@chu-nimes.fr