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OXA-48-producing Enterobacterales in different ecological niches in Algeria: clonal expansion, plasmid characteristics and virulence traits

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Objectives: To investigate the prevalence and molecular characteristics of OXA-48-carbapenemase-producing Enterobacterales strains recovered from various ecological niches in Algeria.

Methods: In total, 3309 samples were collected from different ecological niches (human carriage, animal farms, wild animals, pets, food products, aquatic environment and wastewater treatment plants) distributed among six provinces in Algeria between December 2015 and April 2017. The potential presence of OXA-48-producing Enterobacterales isolates was screened on selective medium. Resistance and virulence profiles were characterized by PCR and sequencing. The clonal relatedness of the different isolates was studied using Rep-PCR and MLST. Conjugation was performed for all OXA-48-producing isolates. The plasmids were analysed by PCR-based replicon typing and WGS.

Results: A total of 78 OXA-48-producing Enterobacterales isolates were detected from 3309 samples (2.4%). OXA producers were observed in all the screened sources. The $bla_{CTX\text{-}M\text{-}15}$ gene was only observed in two isolates. Clonality analysis revealed distinct lineages of the isolates and a clonal expansion of *Klebsiella pneumoniae* ST13. *K. pneumoniae* and *Escherichia coli* had few virulence factors. Plasmid analysis confirmed that all the isolates harboured a very similar transferable plasmid (belonging to IncL) with a similar structure to the pOXA-48a plasmid carried by *K. pneumoniae* strain Kp11978.

Conclusions: This study suggests a global dissemination of OXA-48-producing Enterobacterales in different niches due mainly to the spread of an epidemic plasmid. Furthermore, it clearly shows that *K. pneumoniae* and commensal *E. coli* can be reservoirs of the $bla_{OXA\text{-}48}$ gene, contributing to the dissemination and transfer of this gene to diverse bacteria among different sources.

Introduction

Carbapenems represent the last-line therapeutics to treat infections due to MDR Gram-negative bacilli. Their use has increased in clinical practice as a result of the spread of ESBL- and/or AmpC β-lactamase-producing Enterobacterales.[1,2] Nevertheless, during recent years the prevalence of resistance to carbapenems has increased worldwide and has become a major public health issue. Carbapenem resistance in Enterobacterales is due mainly to carbapenemase production. These enzymes are diverse, belonging to three classes: Ambler class A (mostly KPC-type enzymes), class B (i.e. MBLs) or class D (OXA-48-like enzymes).[3,4] The OXA-48-type carbapenemase was initially identified in a *Klebsiella pneumoniae* strain isolated in Turkey in 2004.[5] In Algeria, its first report was in 2012 from an *Escherichia coli* clinical isolate.[6] Since then, OXA-48-producing bacteria have been endemic in this country and have been reported in cases of nosocomial, community-acquired and foodborne infections, animals and environmental compartments.[7–11] However, their prevalence, relationship and plasmid dissemination in different ecological niches have not been investigated, probably due to the difficulty in detecting OXA-48-

producing isolates in developing countries.[12] The aim of this study was to investigate the prevalence and molecular characteristics of OXA-48-carbapenemase-producing Enterobacterales recovered in various samples collected from different ecological niches distributed among six provinces of Algeria. Isolates that carried bla_{OXA-48} were characterized for their clonal expansion, plasmids, transferability, genetic environment and virulence potential, to understand the diffusion of this resistance mechanism.

Materials and methods

Sampling and microbiological procedures

From December 2015 to April 2017, different randomly selected niches were enrolled, each in the six provinces in Algeria (Bejaia, Tizi Ouzou, Bouira, Jijel, Setif and Bordj Bou Arreridj). A total of 3309 samples were randomly collected, including fresh faecal samples from humans [hospital (n=100) and community (n=50)], farm animals (n=1251), wild animals (n=822) and pets (n=112) and fresh samples from food products (n=874) and the water environment (n=100) (Tables S1 and S2, available as Supplementary data at *JAC* Online). Rectal swabs were taken to screen humans and animal species. For wild birds, and wild fish and bees, fresh faecal droppings and intestinal content samples were taken, respectively. Food products were randomly obtained from different markets, stores and farms in the six studied regions. Samples from the natural environment were collected from seawater (Mediterranean Sea, n=16), spring water (n=10), rivers (n=23), lakes (n=4), dam water (n=3) and fountains (n=14). Wastewater samples were collected from five wastewater treatment plants (i.e. effluent, aeration tank and activated sludge) (n=30). One hundred millilitre aliquots of milk and water samples were taken in sterile flasks.

All samples were immediately transported at 4°C to the Microbiological Ecology Laboratory at the University of Bejaia (Algeria) for analysis. They were processed within the day after sampling. The samples were cultured on Trypticase Soy Broth (TSB) (Fluka, St Louis, USA) supplemented with ertapenem (0.5 mg/L), vancomycin (32 mg/L) and amphotericin B (2 mg/L) and incubated for 18 h at 37°C. For food products, 25 g (except a volume of 25 mL for milk) of each food item was cultured in 225 mL of TSB supplemented with the same antibiotics as described above and incubated for 18 h at 37°C. Following incubation, a 200 µL aliquot was plated onto MacConkey agar (Fluka, St Louis, USA) containing 0.5 mg/L ertapenem and incubated for 18–24 h at 37°C.[11,13] For water and wastewater, the procedure of isolation consisted of inoculation of 200 µL aliquots of 1:10 diluted samples on MacConkey agar containing 0.5 mg/L ertapenem.

The resulting colonies isolated from the different screening agar plates were then isolated and identified by API®20E and the Vitek® MS system (bioMérieux, Marcy-l'Étoile, France). Further confirmation of carbapenemase producers was investigated using the modified Carba NP test[14] and the KPC/MBL & OXA-48 Confirm Kit (Rosco Diagnostica A/S, Taastrup, Denmark).[15]

Antibiotic susceptibility testing

Susceptibility to antimicrobial agents was tested by the disc diffusion method (Bio-Rad, Marnes La Coquette, France) on Mueller–Hinton agar according to recommendations of EUCAST 2018 (www.eucast.org). The MICs of carbapenems (ertapenem, imipenem and meropenem) and colistin were determined by the Etest method (bioMérieux) and microbroth dilution (Umic®, Biocentric, Bandol, France), respectively. Susceptibility patterns were interpreted according to the EUCAST 2018 guidelines.

Molecular characterization of 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 ESBLs (bla_{TEM}, bla_{SHV} and bla_{CTX-M}) were detected by PCR using specific primers and confirmed by sequencing the PCR products, as described previously.[16,17] A multiplex PCR was used for the detection of plasmidic bla_{AmpC} genes.[18] Plasmid-mediated quinolone resistance (PMQR)-encoding genes [*qnrA*, *qnrB*, *qnrS*, *qepA*, *oqxAB* and *aac(6′)-Ib-cr*] were screened for as previously described.[19–21]

Phylogenetic groups and virulence genes

For *E. coli* isolates, PCRs were performed to determine the phylogenetic groups (A, B1, B2, C, D, E, F and clade I) as previously described.[22] The *E. coli* isolates were tested by PCR for the presence of a panel of 19 genes encoding known virulence factors (VFs):[23–25] (i) fimbriae and/or adhesins: *fimH* (D-mannose-specific adhesin, type 1 fimbriae), *papG1*, *papG2* and *papG3* [Gal(α1-4)Gal-specific pilus tip adhesin molecule], *papAH* (major structural subunit of P fimbrial shaft; defines F antigen), *papC* (pilus assembly; central region of pap operon), *papEF*, *sfaS* and *focG* (S fimbriae and F1C fimbriae) and *afa/draBC* (Dr family adhesin); (ii) toxins: *cnf1* (cytotoxic necrotizing factor-1) and *hlyA* (haemolysin); (iii) iron uptake: *iutA* [ferric aerobactin receptor (iron uptake: transport)] and *iroN* [catecholate (salmochelin) siderophore receptor]; (iv) protectins: *kpsMTI* and *kpsMTII* (capsule synthesis) and *traT* [surface exclusion, serum survival (outer membrane protein)]; and (v) others: *malX* (a marker for pathogenicity-associated island marker from archetypal uropathogenic strain CFT073) and *irp2* (yersiniabactin).

For *K. pneumoniae* isolates, the presence of VF-associated genes was assessed by PCR assays:[26,27] (i) fimbriae and/or adhesins: *fimH* (type 1 fimbriae), *mrkD* (adhesin type 3 fimbriae) and *cf29a* (adhesin 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* (group 2 capsule) and the genes of the capsular serotypes K5, K57, K54 and K20; and (v) others: *alls* (associated with allantoin metabolism), *uge* (uridine diphosphate galacturonate 4-epimerase) and *wabG* (core LPS biosynthesis).

Analysis of clonality

The clonal relatedness of the OXA-48-producing isolates was assessed using repetitive sequence-based PCR (Rep-PCR) and MLST. Rep-PCR was performed on the DiversiLab® system (bioMérieux) for species where more than three isolates were detected, following the manufacturer's instructions. Isolates with identical strain patterns were considered indistinguishable if the similarity percentage was ≥95. MLST analysis was performed using the Pasteur Institute's MLST scheme (bigsdb.web.pasteur.fr). Furthermore, *E. coli* strains were characterized by Achtman MLST (https://enterobase.warwick.ac.uk/species/ecoli/allele_st_search), according to the protocol described previously.[28]

Plasmid characterization

The transferability of the OXA-48-carrying plasmids was assessed by conjugation.[29] Conjugation was performed between OXA-48-producing isolates (n=78) as donor and *E. coli* J53 (rifampicin resistant) as recipient. Transconjugants were selected on LB agar plates supplemented with rifampicin (300 mg/L) and ampicillin (64 mg/L). Transformation was carried out for strains for which no transconjugant could be obtained by the hot alkaline method using electrocompetent *E. coli* TOP10.[30] Selection of transformants was done on LB agar plates with 64 mg/L ampicillin. Successful conjugation and transformation were confirmed by antimicrobial susceptibility and PCR detection of the bla_{OXA-48} gene. Replicon typing of the isolated plasmid DNA was done by a PCR-based replicon-typing (PBRT) method as

described previously.[31] Another PBRT was used to discriminate IncL and IncM groups according to Carattoli *et al.*[32]

The sequences of the plasmids carrying the bla_{OXA-48} gene were obtained on the Illumina Genome Analyzer IIX system by GenoScreen SA (Lille, France). The quality and potential defects of tested sequences were estimated using Fastqc Software (version 0.11.7). Before analysis, non-matching primer sequences (adapters) obtained from high-throughput sequencing at the beginning of the investigated sequences were removed using cutadapt software (version 1.16). To merge the overlapping reads, Paired-End Read Merger was used (version 0.9.10).

To generate comparisons with other fully sequenced plasmids carrying bla_{OXA-48}, we used the NCBI nucleotide database (www.ncbi.nlm.nih.gov/nucleotide) (Table S3). Results were filtered to exclude partial plasmid sequences, those that did not contain bla_{OXA-48} or belong to the IncL/M group and those that represented duplicates of the same plasmid sequence. Complete sequence alignments were then undertaken using CLUSTALW on the CLUSTAL Omega website of the EMBL-EBI (www.ebi.ac.uk/Tools/service/web) for pOXA-48 (as the earliest available sequence) and the respective studied plasmids and to compare publicly available plasmids. The neighbour-joining method[33] was used to construct a phylogenetic tree for the nucleotide sequences, using the program MEGA X software.[34] The evolutionary distances were computed using the maximum composite likelihood method.[35]

GenBank accession numbers

The complete sequences of the different plasmids identified in this study have been deposited at DDBJ/EMBL/GenBank under the accession numbers MK121443 to MK121456 and MK249855 to MK249861.

Results

Isolates

During the 18 month period of the study, 78 isolates from 76 samples, two of which carried two strains (E44A/E44B and E78/E78B), were identified to be carbapenemase producers by phenotypic methods, giving an overall prevalence of 2.4% (78/3309). The isolates were recovered from humans (*n*=4, 2.6%), farm animals (*n*=16, 1.2%), pets (*n*=1, 0.8%), wild animals (*n*=34, 4.1%), food products (*n*=10, 1.1%) and water (*n*=13, 13%) (Table S2). These isolates were identified as *K. pneumoniae* (*n*=59), *E. coli* (*n*=7), *Raoultella ornithinolytica* (*n*=4), *Enterobacter cloacae* (*n*=2), *Cronobacter malonaticus* (*n*=2), *Klebsiella oxytoca* (*n*=1), *Raoultella planticola* (*n*=1), *Citrobacter werkmanii* (*n*=1) and *Pluralibacter gergoviae* (*n*=1). Among these carbapenemase-producing Enterobacterales isolates, total resistance to amoxicillin, amoxicillin/clavulanic acid, piperacillin, piperacillin/tazobactam, ticarcillin, ticarcillin/clavulanic acid and ertapenem was noted (100%), followed by variable resistance levels to imipenem (46.1%), meropenem (25.6%), co-trimoxazole (24.3%), fosfomycin and fluoroquinolones (17.9%), cefotaxime (8.9%), ceftazidime (6.4%) and aminoglycosides (7.6%) (Table S4). All isolates were susceptible to colistin.

Of the carbapenemase producers, all the isolates harboured the bla_{OXA-48} gene. No other carbapenemase producers were detected. In addition, two *K. pneumoniae* isolates (E103 and E104) carried the ESBL $bla_{CTX-M-15}$ gene. No other β-lactamase-encoding genes were detected. Different PMQR genes were identified: *aac(6)-Ib-cr* and *qnrB* simultaneously in three *K. pneumoniae* isolates (E78B, E103 and E104). The *qnrA* gene was detected in two *E. coli* isolates (E46 and E77); the *E. coli* E77 isolate also carried a *qnrS* gene (Table S4).

Virulence profiles

Virulence gene profiles of the 59 studied *K. pneumoniae* isolates showed that these isolates possessed different patterns of VFs with five to nine genes per isolate of the 18 screened. Interestingly, all the isolates harboured *ureA*, *fimH* and *mrkD* genes. The *ugeF* gene was also highly prevalent (*n*=57, 97%) (Figure S1). Neither capsular serotype genes nor hypermucoviscous phenotype was detected.

Analysis of *E. coli* phylogroups showed that all the isolates had a commensal origin, with three isolates belonging to phylogroup B1 (E78A, E90 and E92), two to phylogroup A (E73 and E77) and two to phylogroup E (E44B and E46) (Figure S2). Thus, the majority of the isolates had a low prevalence of VFs except for the *fimH* gene, which was present in all the isolates.

Clonality analysis

The Rep-PCR results revealed distinct lineages among the 59 OXA-producing *K. pneumoniae*, with a total of 19 different patterns detected (Figure S1). Two profiles included a large number of isolates: patterns A (*n*=15) and G (*n*=13). They were recovered from different origins for pattern A and from different wild fishes for pattern G. The residual patterns contained six (one Rep-PCR pattern), three (two Rep-PCR patterns), two (five Rep-PCR patterns) or single isolates (nine Rep-PCR patterns). The MLST also showed the clonal expansion of the *K. pneumoniae* isolates with eight STs detected, including ST13 (*n*=50), ST35 (*n*=2), ST323 (*n*=1), ST405 (*n*=1), ST441 (*n*=1), ST1393 (*n*=2), ST1517 (*n*=1) and ST1878 (*n*=1) (Figure 1 and Figure S1).

The Rep-PCR results also showed different STs of the *E. coli* isolates. Two isolates were clonal (E78A and E44B), recovered from wild birds (*Pagophilae burnea*) in the same region of Bejaia. A total of six Pasteur STs (PSTs) were observed and one was identified for the first time: PST5, PST8, PST19, PST88, PST638 and PST716 using the Pasteur Institute's MLST scheme and ST34, ST38, ST443, ST540, ST4652 and a non-referenced ST using Achtman MLST (Figure 1 and Figure S2).

Finally, the MLST results revealed that *E. cloacae* isolates E67B and E105 belonged to ST527 and ST931, respectively (Figure 1).

Plasmid analysis experiments

The PBRT performed on the 78 OXA-48-producing Enterobacterales isolates showed that all the bla_{OXA-48} genes carried were in the IncL group plasmid. Additional plasmids are harboured by five strains, including IncFIIK and IncColE in three *K. pneumoniae* isolates (E78B, E103 and E104) and IncL1 and IncX2 plasmids in two *E. coli* isolates (E46 and E77, respectively). These other plasmids carried the non-carbapenemase co-resistant genes. The different attempts at conjugation failed to transfer these specific plasmids. However, the IncL plasmids carrying the bla_{OXA-48} gene were transferred by conjugation (*n*=76) or transformation (*n*=2; Tr49 and Tr78B) from all the OXA-48-producing Enterobacterales isolates. All recombinants exhibited similar resistance patterns, the presence of the bla_{OXA-48} gene and only one type of plasmid assigned to the IncL group. From these results,

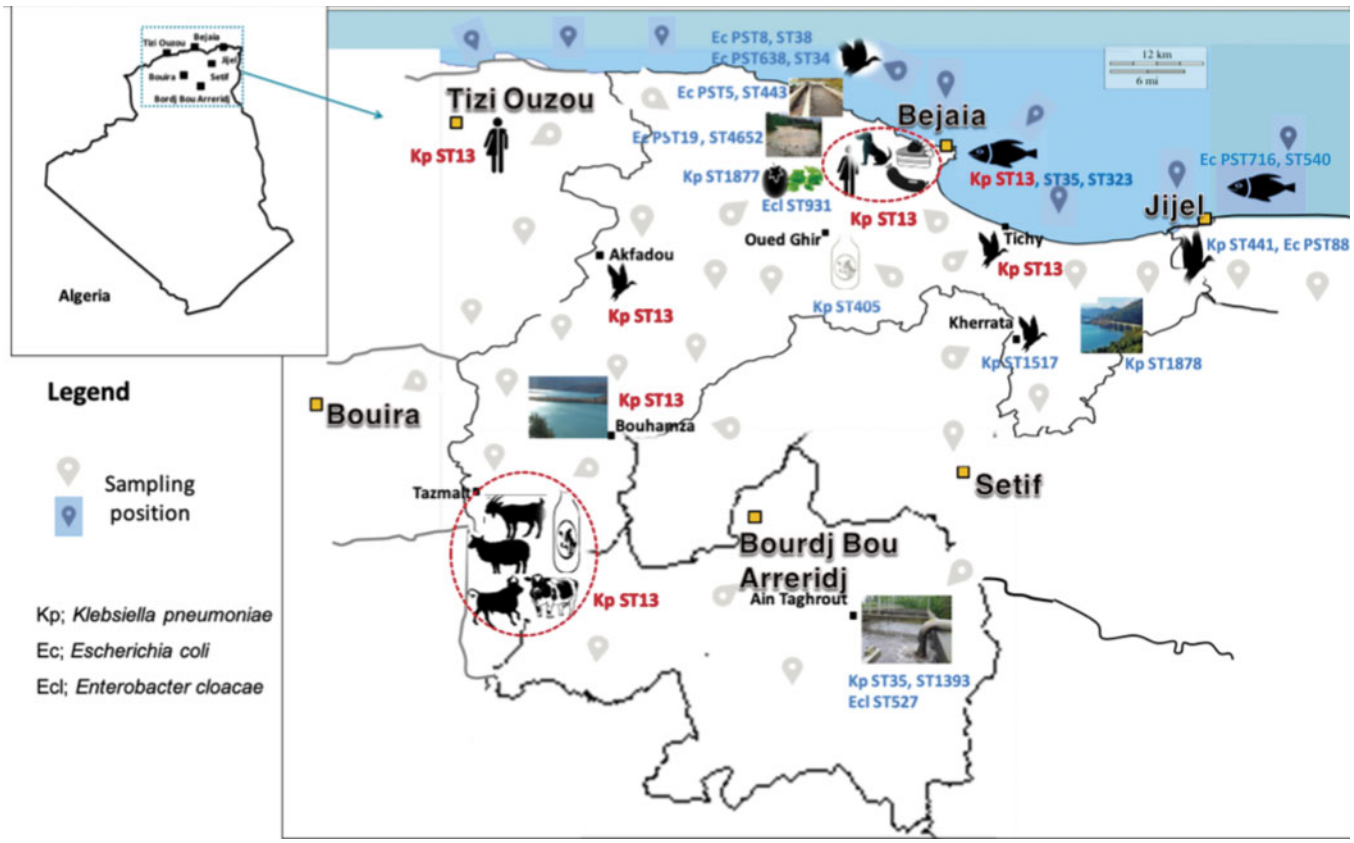

Figure 1. Sampling locations and distribution of the OXA-48-producing Enterobacterales clones circulating in different ecological niches in Algeria.

a selection of 21 plasmids was made according to the Rep-PCR profiles, the variability of niches and the diversity of sampling sites from which they were obtained (Table S5).

The complete sequences of the 21 pOXA-48 plasmids were analysed and revealed that all plasmids were ~61.9 kb in size. They were compared using the neighbour-joining algorithm and revealed maximum score (1.135×10^5), coverage (100%) and identity (99.9%) with the pOXA-48a plasmid carried by the *K. pneumoniae* strain Kp11978, recovered in Turkey in 2001 and then reported worldwide (Figure 2).[36] The studied plasmids were divided into two main clusters (Figure S3). One cluster (Cluster I) contained three identical plasmids (Tr103, Tr49 and Tr46) and the reference strain Kp11978 which differed by 36 nt. The second cluster (Cluster II) contained the other 18 plasmids: 3 (Tr76, Tr91 and Tr94) and 5 (Tr77, Tr98, Tr105, Tr78B and Tr9) were identical and differed from the pOXA-48a reference strain by 129 and 131 nt, respectively; the 10 others differed from the pOXA-48a reference strain by 128 nt (Tr48) to 133 nt (Tr73) (Figure 2).

When aligned by CLUSTALW, there were differences between the 21 pOXA-48 plasmids analysed and the pOXA-48a reference strain (Table S6). All the modifications corresponded to nucleotide substitutions and were located in different genes and regions, including non-coding regions (n=65), *orf19* (n=5), *orf25* (n=46), *orf33* (n=1), *orf34* (n=4), *nuc* (n=7), *ssb* (n=3), *dnaG* (n=4), *traN* (n=5), *traW* (n=1) and *trb* (n=1). In the *orf25* gene, the two substitutions encoded a nonsense codon (positions Lys8179Stop and Lys8246Stop) and formed a truncated protein.

Discussion

OXA-48-producing Enterobacterales isolates have been reported from hospital and extra-hospital reservoirs. Most of the studies on this subject have been conducted in developed countries, but the major epicentres of OXA-48-producing Enterobacterales are located in Mediterranean countries such as Algeria, where this resistance mechanism is endemic.[10] Recently, the 'One Health' concept was proposed to organize a worldwide strategy for expanding interdisciplinary collaborations and communications in all aspects of healthcare for humans, animals and the environment. A recent systematic review of all the studies on the spread of carbapenemase-producing bacteria in livestock, food and companion animals showed a dissemination of these bacteria with prevalence varying between 0.6% and 26% (0.6%–13.4% in wild boars, 1.5%–4% in companion animals, 6% in milk samples and 26% in chickens) in Algeria.[37] Those authors encouraged prospective prevalence studies to better define the dissemination of these multidrug resistant bacteria. Due to the importance of this problem, we decided to describe the prevalence, geographic distribution and clonal expansion of OXA-48-producing Enterobacterales in different ecological niches (humans, food, animals and water) in six Algerian provinces. Knowledge of the host specificity and geographic distribution of these bacteria are important parameters for understanding the ecology, epidemiology and spread of this resistance gene. Therefore, our study has established, for the first time to our knowledge, a valuable database of OXA-48-producing

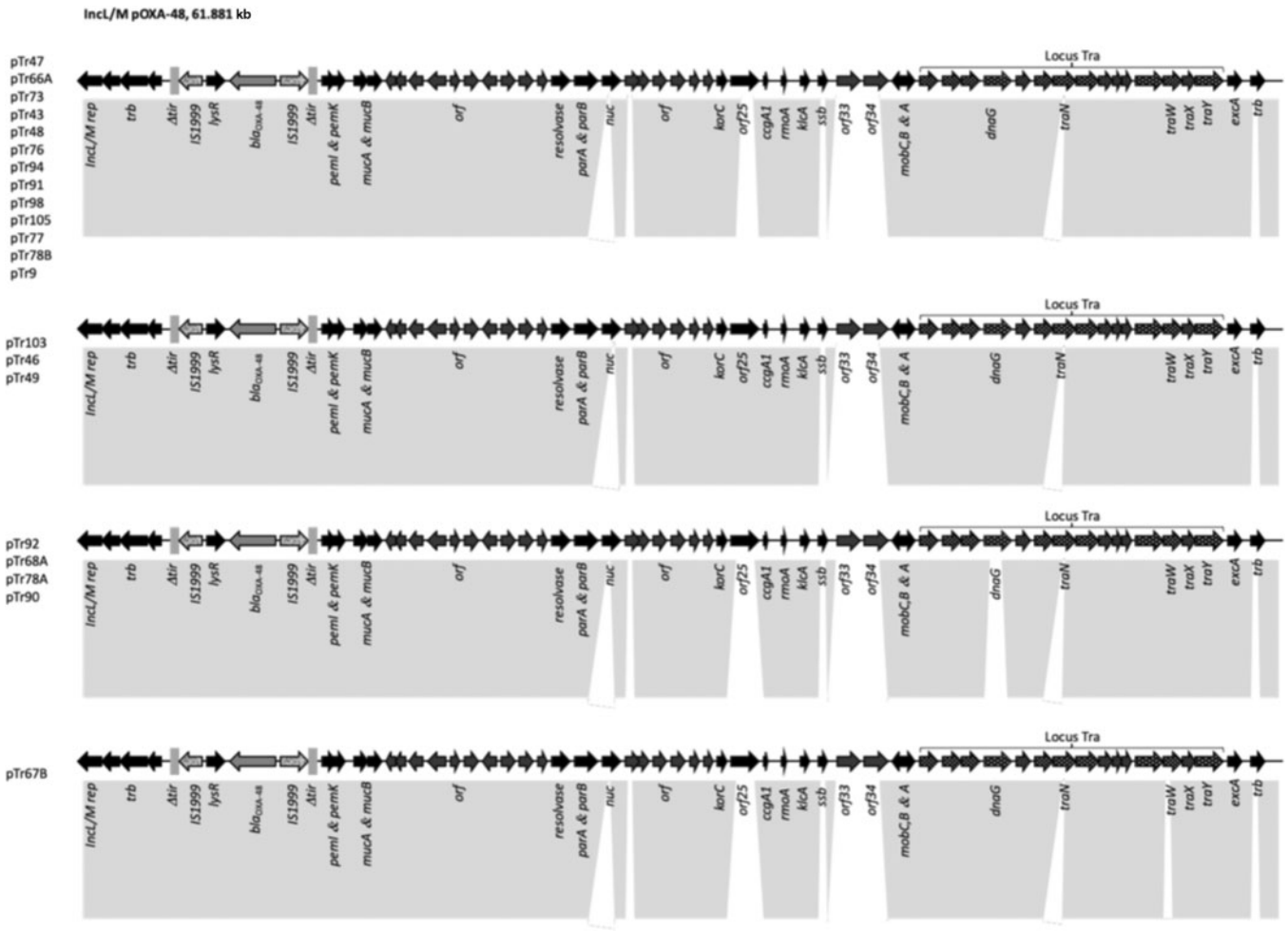

Figure 2. Plasmid structures of the 21 bla_{OXA-48}-harbouring IncL plasmids characterized in this study in comparison with pOXA-48a (JN626286). Grey shading indicates regions of homology, whereas white shading indicates a mutation in the region. ORFs are indicated by arrows. pTr92, MK121443; pTr77, MK121444; pTr47, MK121445; pTr66A, MK121446; pTr68A, MK121447; pTr73, MK121448; pTr78A, MK121449; pTr90, MK121450; pTr67B, MK121451; pTr103, MK121452; pTr43, MK121453; pTr48, MK121454; pTr76, MK121455; pTr94, MK121456; pTr105, MK249855; pTr91, MK249856; pTr98, MK249857; pTr46, MK249858; pTr9, MK249859; pTr49, MK249860; and pTr78B, MK249861.

Enterobacterales recovered from different sources and their molecular characteristics. Interestingly, the Bejaia region was particularly affected by the spread of these isolates, certainly due to the fact that this province has a high-density population, the majority of farms are concentrated in this area, and it is near the Mediterranean Sea.

Among a wide range of different reservoirs screened, it is interesting to note that the bla_{OXA-48} gene was the only carbapenemase gene found in our study, representing 2.4% of all the samples screened. This prevalence was particularly high if we note that these OXA-48 producers were isolated from all sources (human, animal, food and water), demonstrating a wide dissemination of this resistance mechanism in Algeria. This wide dissemination is also accentuated by the presence of the bla_{OXA-48} gene in a large panel of enterobacterial species, including for the first time *P. gergoviae* and *C. malonaticus*. In addition, some of the studied niches provided the first report of OXA-48-producing Enterobacterales isolates including wild fish, farm animals, food (except vegetables) and wild birds other than storks.[10] All these points highlight the worrisome situation of pan-resistance in this country.

Interestingly, the MLST analysis showed different lineages of isolates harbouring bla_{OXA-48} rather than a clonal expansion of an isolate. However, in *K. pneumoniae*, the most reported enterobacteria in our study (59/78, 76% of the isolates), ST13 was the predominant ST (50/59) recovered in different sources, particularly in the province of Bejaia (Figure 1). This ST has been previously described in this geographic zone in wild boars, newborns and mothers.[38,39] We suggest that ST13 is now endemic in this province. Among *E. coli* isolates, we also observed different STs. ST38 was isolated from a seagull in our study and was previously detected in white storks in Algeria[40] and in water from Soummam River.[41] As the samples were taken in the same period and in a zone near the site of isolation (~30 km), we suggest that flying seagulls could constitute a vector facilitating the spread of OXA-48 determinants. Finally, the *E. cloacae* ST527 isolate detected in our

study from wastewater had previously been reported in pets in Algeria.[11]

As Enterobacterales distributed over a wide area harboured the bla_{OXA-48} gene, we investigated the plasmids carrying this gene to better understand the diffusion of OXA-48 producers in Algeria. Thus, we observed that the bla_{OXA-48} gene is carried by an IncL plasmid type in all isolates. The complete sequences of the different plasmids (MK121443 to MK121456 and MK249855 to MK249861) characterized in this study possessed a similar architecture to that of the pOXA-48a previously sequenced and low similarity to other plasmids publicly available. In detail, BLASTn analysis showed that the studied plasmids had an overall identity (99%) with query overages of 100% with pOXA-48 (JN626286.1) carried by the *K. pneumoniae* strain Kp11978.[36] Conjugation analysis confirmed that the bla_{OXA-48} gene in pOXA-48-like was easily transferable (76 of the 78 plasmids analysed), demonstrating the high potential for horizontal transfer of this kind of plasmid and the large host spectrum. Our work also suggests that the IncL plasmid can be considered to be an endemic plasmid with a high capacity for transfer and considerable ability to disseminate in different Enterobacterales species. To date, IncL-type plasmids are globally reported to be associated with bla_{OXA-48}.[42] This successful dissemination of this plasmid is supported by *Tn*1999, which carries the gene inserts in the *tir* gene encoding transfer inhibition protein, as observed in this work (Figure 2).[43] If the plasmids isolated in our study are certainly derived from pOXA-48, some substitutions have been detected in the 21 studied plasmids. However, these mutations are mainly located in non-coding regions (n=65) or in accessory genes (n=77) (Table S6). A majority of these mutations had no impact. Only in the *orf25* gene did two substitutions involve the formation of a nonsense codon (positions Lys8179Stop and Lys8246Stop) that formed a truncated protein. However, these mutations have no role in the dissemination of the plasmid, while these plasmids were easily transferred by conjugation. The absence of environmental barriers between humans and animals contributes to the spread of pOXA-48 in various interconnected ecological niches. pOXA-48, once it has emerged, is not confined to the ecological niche where it primarily appeared.

Finally, the study of virulence potential of the suspected pathogenic bacteria isolated in our study showed that *K. pneumoniae* and *E. coli* had a commensal origin. All *K. pneumoniae* isolates studied were negative for the *K1* and *K2* genes and did not present a hypermucoviscous phenotype. Moreover, the different *E. coli* isolates belonged to the commensal phylogroups A, B1 and E. These results support the idea that commensal bacteria could be a good reservoir of the bla_{OXA-48} gene, contributing to the dissemination and transfer of this gene to diverse bacteria among different sources.

In conclusion, this study suggests a high prevalence and wide dissemination of OXA-48-producing Enterobacterales among different ecological niches. This dissemination was mainly due to the success of the IncL pOXA-48-like plasmid type among diverse enterobacterial species and a clonal dissemination of a *K. pneumoniae* isolate (ST13). This wide dissemination in different niches other than the clinical setting may constitute a reservoir of resistant strains that can be responsible for transmission from humans to their cohabitants and in veterinary hospitals. Its ability to spread in wild animals and the environment suggests that although international travel represents a large risk factor for cross-transmission and spread of OXA-48-producing bacteria, in an endemic setting (like Algeria), the 'environment' is also a factor that needs to be taken into account.

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Transparency declarations

None to declare.

Supplementary data

Tables S1 to S6 and Figures S1 to S3 are available as Supplementary data at *JAC* Online.

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