IncH-Type Plasmid Harboring *bla*_{CTX-M-15}, *bla*_{DHA-1}, and *qnrB4* Genes Recovered from Animal Isolates

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The whole sequence of plasmid pENVA carrying the extended-spectrum β -lactamase gene *bla*_{CTX-M-15} was determined. It was identified from a series of clonally related *Klebsiella pneumoniae* sequence type 274 strains recovered from companion animals. This plasmid was 253,984 bp in size and harbored, in addition to *bla*_{CTX-M-15}, a large array of genes encoding resistance to many antibiotic molecules, including β -lactams (*bla*_{TEM-1}, *bla*_{DHA-1}), aminoglycosides (*aacA2*, *aadA1*), tetracycline (*tetA*), quinolones (*qnrB4*), trimethoprim (*dfrA15*), and sulfonamides (two copies of *sul1*). In addition, genes encoding resistance to mercury, tellurium, nickel, and quaternary compounds were identified. It also carried genes encoding DNA damage protection and mutagenesis repair and a locus for a CRISPR system, which corresponds to an immune system involved in protection against bacteriophages and plasmids. Comparative analysis of the plasmid scaffold showed that it possessed a structure similar to that of only a single plasmid, which was pNDM-MAR encoding the carbapenemase NDM-1 and identified from human *K. pneumoniae* isolates. Both plasmids possessed two replicons, namely, those of IncFIB-like and IncHIB-like plasmids, which were significantly different from those previously characterized. The *bla*_{CTX-M-15} gene, together with the other antibiotic resistance genes, was part of a large module likely acquired through a transposition process. We characterized here a new plasmid type carrying the *bla*_{CTX-M-15} gene identified in a *K. pneumoniae* isolate of animal origin. The extent to which this plasmid type may spread efficiently and possibly further enhance the dissemination of *bla*_{CTX-M-15} among animal and human isolates remains to be determined.

Antibiotic resistance within animal microbiomes (food-producing or companion animals) is now recognized as an emerging public health threat (1, 2). Resistance to broad-spectrum β -lactams in the *Enterobacteriaceae* is mainly caused by extended-spectrum β -lactamases (ESBLs) and plasmid-encoded AmpC-type cephalosporinases (3). During the past decade, ESBLs of the CTX-M type have been recognized to be of growing importance worldwide, frequently being widely reported among enterobacterial isolates recovered from human specimens (4) either from the community (mainly *Escherichia coli*) or from hospitals (mainly *Klebsiella pneumoniae*). Furthermore, CTX-M-producing *E. coli* isolates have been identified among a wide range of animal species, including pets (5), poultry (6), cattle (7, 8), and wild animals (9, 10), and even from retail meat (11, 12). In addition, some plasmid-encoded AmpC-type β -lactamase genes, such as *bla*_{DHA} and *bla*_{CMY}, have been reported worldwide from either human or animal isolates (13, 14). Overall, these data have raised some concerns about the transfer of ESBL and AmpC genes between human and animal bacterial strains.

Plasmids are often involved in the dissemination of broad-spectrum β -lactamase-encoding genes. Six main plasmid families have been shown to mediate antimicrobial resistance dissemination among enterobacterial species, namely, IncF, IncA/C, IncL/M, IncN, IncI, and IncHI2 (14–17).

IncH-type plasmids are frequently involved in the acquisition of antibiotic resistance in both human and animal bacterial isolates (5, 18). A large number of IncHI2 plasmids (usually with sizes larger than 250 kb) harboring *bla*_{CTX-M} (with the exception of *bla*_{CTX-M-15}), *bla*_{SHV}, *bla*_{IMP}, *bla*_{VIM}, *armA*, *qnrA1*, *qnrS1*, and

qnrB2 genes have been identified in many different enterobacterial species (5, 19).

CTX-M-15 is currently the CTX-M variant most commonly identified in enterobacterial species of human origin worldwide. In contrast, the most prevalent acquired ESBL among animal isolates is CTX-M-1, with CTX-M-15 being rarely identified from animal isolates (4, 20, 21). The *bla*_{CTX-M-15} gene has been identified on plasmids belonging to unrelated incompatibility groups, such as IncA/C, IncL/M, and IncN; however, the most frequently reported are IncI1 and IncF (14, 22).

Recently, we identified phylogenetically related CTX-M-15-producing *K. pneumoniae* isolates recovered from companion animals in France (23). In those isolates, the *bla*_{CTX-M-15} gene was located on a plasmid of ca. 250 kb in size that could not be classified in any of the known incompatibility groups and that was self-transferable by conjugation to *E. coli* (23). The aim of this study was to determine the entire sequence of this plasmid in order to better evaluate its genetic relationship with those plasmids known to disseminate *bla*_{CTX-M-15} among human isolates.

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MATERIALS AND METHODS

Susceptibility testing. Susceptibility testing was performed by disk diffusion assay (Sanofi-Diagnostic Pasteur, Marnes-la-Coquette, France), and MICs were determined by Etest (bioMérieux) on Mueller-Hinton agar plates at 37°C and interpreted according to CLSI guidelines (24).

Sequencing, assembly, and annotation of plasmid pENVA. A whole-genome shotgun library was generated using 500 ng of plasmid DNA and sequenced on a Genome Sequencer FLX system (Roche Diagnostics, Mannheim, Germany) by applying Titanium chemistry in one quarter of a PicoTiterPlate. In the course of Rapid Library preparation according to the manufacturer's protocol, the DNA was tagged with a multiplex identifier (GS Rapid Library MID adaptor kit). Prior to emulsion PCR (emPCR), the pENVA plasmid library was combined with other MID-tagged libraries and subsequently sequenced. Sequencing reads were sorted on the basis of their MID tags and assembled by means of the GS *De Novo* Assembler (version 2.6) by application of default settings (Roche Diagnostics). Ordering of contigs was done as described previously (25, 26), using the reference plasmid pNDM-MAR (GenBank accession no. JN420336) for mapping (27) and the computer program r2cat (28). Subsequently, 68 contigs showing homology to the reference plasmid were extracted, ordered according to the mapping results, and stored in a new project. Reads protruding contig ends were used to identify contigs that flank a certain source contig. Sequence gaps between contigs were closed by primer walking on plasmid template DNA. Moreover, contig graph information calculated by the GS *De Novo* Assembler was exploited to verify joining of contigs. Sequence finishing and polishing were accomplished using the CONSED software package (29). The complete sequence of plasmid pENVA was annotated by means of the GenDB genome annotation system (version 2.0) (30). After automatic annotation, sequence information was refined manually as described previously (25, 26, 31). The plasmid genome plot was drawn and labeled as described previously (31, 32).

Phylogenetic analyses. For phylogenetic classification of plasmid pENVA, the *repA* replication initiation marker gene was used, since plasmids are commonly classified on the basis of the amino acid sequence similarity of their replication initiator proteins (33). The nucleotide sequences of different related *repA* genes were extracted from corresponding database entries and aligned using the multiple-sequence-alignment program ClustalW (34). A phylogenetic tree was calculated by applying the MEGA (version 5) program (35) and the neighbor-joining algorithm (36). A bootstrap analysis using 1,000 repetitions was carried out.

PCR-based replicon typing. PCR-based replicon typing aiming at identification of the main plasmid incompatibility groups reported for enterobacterial isolates was performed as described previously (37).

Comparative genome analyses. To analyze the similarity of the sequenced plasmid pENVA and the reference plasmid pNDM-MAR, a comparative analysis was performed as described previously (31, 32, 38) by applying the tool M-GCAT (39).

Nucleotide sequence accession number. The annotated nucleotide sequence of plasmid pENVA was submitted to the GenBank database and is accessible under accession number [HG918041](#).

RESULTS AND DISCUSSION

Characterization of the plasmid bearing the *bla*_{CTX-M-15} gene. Fourteen sequence type 274, clonally related, and CTX-M-15-producing *K. pneumoniae* isolates harbored a ca. 250-kb untypeable plasmid carrying the *bla*_{CTX-M-15} gene. They had been recovered from either dogs, cats, sheep, or a hedgehog (23). Once it was transferred by conjugation, this plasmid conferred to the *E. coli* recipient strain resistance to broad-spectrum β -lactams, tetracycline, gentamicin, sulfonamides, and trimethoprim and reduced susceptibility to nalidixic acid (23). Mating-out assays were performed with *K. pneumoniae* Kp15 recovered from the urine of a

cat, and the corresponding plasmid, named pENVA, was further studied.

Sequencing and general features of the antibiotic resistance plasmid pENVA. Plasmid pENVA was fully sequenced, yielding 43,358 sequence reads that were assembled into 1,258 large (>500 bp) and 354 small (<500 bp) contigs. Reads and contigs representing contamination with the chromosome of the host bacterium *E. coli* were discarded. After filtering, plasmid pENVA consisted of 108 contigs, which were finally assembled after a PCR-based polishing approach. The complete plasmid pENVA has a size of 253,984 bp with an average GC content of 46.8%. The plasmid sequence revealed 300 predicted coding sequences that could be assigned to 16 different functional modules (Fig. 1).

Backbone and accessory functions of plasmid pENVA. Plasmid pENVA encoded two different replicon types, one belonging to the IncFIB family and the second corresponding to an IncHIB-type module. The first RepA protein (IncFIB-like) shared 40% amino acid sequence identity with the canonical RepA of IncFIB (GenBank accession no. [YP_788007.1](#)). The second RepA (IncHIB-like) shared less than 60% amino acid sequence identity with other reported IncHIB-type replication initiation proteins. However, it shared a perfect identity with a recently identified replicase, the gene of which was identified on plasmid pNDM-MAR, recovered from a clinical *K. pneumoniae* human isolate, harboring the *bla*_{NDM-1}, *bla*_{CTX-M-15}, and *qnrB1* antibiotic resistance genes (27). Hence, plasmid pENVA *repA* sequences are only distantly related to the corresponding sequences from reference plasmids, explaining the lack of amplification when using the PBRT primers. Therefore, plasmid pENVA appeared to be a hybrid between two plasmid scaffolds, retaining their replication modules.

Plasmid pENVA harbored an *umuC-umuD* locus known to code for a mutagenesis repair system conferring resistance to UV light and related DNA damage (Fig. 2A). A locus of five genes encoding a putative type III CRISPR (clustered regularly interspaced short palindromic repeat)-like system was also identified. Interestingly, CRISPR systems were shown to correspond to primitive immune systems involved in the protection of host bacteria against bacteriophages and plasmids (40). Plasmid pENVA harbored a functional partitioning system (*parA parB*) and a *higB*-type toxin/antitoxin system. It also encoded a *flaC*-like gene that was predicted to play a role in the biosynthesis of flagella and that therefore likely constituted a virulence factor.

Plasmid pENVA harbors a large multidrug resistance module comprising 13 resistance genes encoding resistance to six different antibiotic classes. Nine antibiotic resistance genes were identified on plasmid pENVA, namely, *aacC2* and *aadA1* (conferring resistance to aminoglycosides, including kanamycin, netilmicin, gentamicin, and tobramycin), *bla*_{TEM-1} (conferring resistance to penicillins), *bla*_{CTX-M-15} and *bla*_{DHA-1} (conferring resistance to broad-spectrum β -lactams), *tetA* (conferring resistance to tetracycline), *sulI* (two copies; conferring resistance to sulfonamides), *qnrB4* (conferring resistance to quinolones), and *dfrA15* (conferring resistance to trimethoprim). In addition, genes encoding resistance to mercury, tellurium, nickel, and quaternary ammonium compounds were identified. This array of genes encoding multidrug resistance was located in a large module that was bracketed by two copies of insertion sequence (IS) IS4321 (IS110 family) inserted in opposite orientations to each other. Direct repeats representing possible transposition signatures were not identified

FIG 1 Genetic map of the fully sequenced plasmid pENVA. The circles (from the innermost to the outermost) represent (i) GC skew, (ii) GC content, (iii) annotated coding sequences as arrows, and (iv) plasmid modules. These modules are colored depending on their functional assignments, as shown by the labeling.

either at the extremities of each of these IS4321 elements or at the extremities of the potential composite transposon formed by the two copies of IS4321 flanking the multidrug resistance locus. This is in accordance with what has been observed for other members of the IS110 family that do not generate target site duplications upon transposition (41). However, it may be speculated that the two copies of IS4321 indeed form a composite transposon carrying a large ca. 50-kb array of resistance genes. The latter putative composite transposon includes an internal resistance module that is bracketed by two IS26 elements and that encompasses the ISEcp1-made transposon (Tn2012) at the origin of *bla*_{CTX-M-15} acquisition (Fig. 2A). As expected, a 5-bp target site duplication (TATGA) was identified at each extremity of Tn2012, as described previously (42, 43). The module flanked by IS26 elements also harbored the *tetR* and *tetA* genes, encoding inducible resistance to tetracycline (Fig. 2B), together with the AmpC β -lactamase gene *bla*_{DHA-1}, which was associated with the LysR-type regulatory gene *ampR* (Fig. 2B). Moreover, the *qnrB4* gene was identified in association with genes encoding five putative phage-related shock proteins. This gene arrangement was similar to that of a gene cluster identified on plasmid pKP048 from China (44). A noteworthy finding was that the promoter sequences (−35 [TTGGAC] and

−10 [TACCAT]) upstream of the *qnrB4* gene were not part of the phage-related structures. The *lexA*-binding site, which was shown to be involved in regulation of a SOS response, was found in the vicinity of the *qnrB4* start codon, as previously described (45).

Plasmid pENVA harbors heavy metal resistance genes. Plasmid pENVA possessed two loci corresponding to putative heavy metal resistance genes. The first one was a complete mercury resistance operon (*merDACPTR*) encoding a system known to transport mercury-derived compounds out of the bacterial cell (46). The second operon encoded resistance to tellurite-derived compounds. The corresponding *ter*-type genes are markers characteristic of all IncHI2-type plasmids (except for R476b). This operon has also been shown to be responsible for the control of resistance to infection by various bacteriophages (known as phage inhibition) and resistance to pore-forming colicins (47).

Plasmid pENVA harbors additional insertion sequences. Among the different mobile elements identified within plasmid pENVA, insertion sequence ISKpn21 was identified. It belongs to the ISNCY family and has recently been identified on different *K. pneumoniae* plasmids encoding the NDM-1 carbapenemase, including the IncH-type plasmid pNDM-MAR (27, 48). The re-

FIG 2 (A) Major structural features of pENVA and comparison with the reference plasmid pNDM-MAR. White boxes indicate plasmid backbone regions that commonly occur in plasmids. The *tra* locus (conjugative transfer) is indicated by white boxes with capital letters, which indicate the respective *tra* genes. Resistance genes are indicated by orange boxes, except for the β -lactamase genes, which are indicated by red boxes. Transposon-specific genes (*trpA*, *trpR*, *trpM*) and insertion sequences are indicated by green boxes, and class 1 integrase genes are indicated by dark gray boxes. Other genes are indicated by colored boxes, as follows: violet, replicase genes; light gray, partitioning systems and DNA methylase genes; blue, heavy metal resistance clusters. (B) Schematic representation of the multidrug resistance module of plasmid pENVA. The genes and their annotations are indicated by arrows and colored according to their functions.

cently identified *ISKpn20* element belonging to the IS3 family was also identified. It was flanked by a 4-bp direct repeat (ACTT), which was the likely signature of the insertion event. *ISKpn20* was recently identified on a plasmid encoding the KPC-2 carbapenemase in Greece (49). A novel IS that is related to *ISRaql* (50) identified in *Rahnella aquatilis* (an OrfB transposase sharing 90% amino acid identity) and that therefore belongs to the IS3 family was also identified with a target site duplication of 4 bp (GAAT) on each extremity. Considering that those ISs were likely inserted as single elements and therefore not associated with other mobilized DNA sequences, it is likely that they had a minor impact on the evolution of plasmid pENVA.

Plasmid pENVA is related to the IncH-type *bla*_{NDM-1}-positive plasmid pNDM-MAR identified in *K. pneumoniae*. To estimate the genetic relationship of plasmid pENVA to other plasmids, a phylogenetic tree based on the *repA* marker gene was computed by applying the tool MEGA5 (35). The neighbor-joining algorithm (36, 51) comprising selected reference sequences was used (see Table S1 in the supplemental material). This approach resulted in a phylogenetic tree which was divided into four different groups. Plasmid pENVA clustered together with the se-

quence originating from the IncH-type plasmid pNDM-MAR, encoding NDM-1 from *K. pneumoniae* (27). Not only were the *repA* gene products of both plasmids identical, as mentioned above, but comparative analysis of both plasmids revealed that large regions of the sequence of plasmid pENVA were nearly identical to large regions of the pNDM-MAR sequence (see Fig. S1 in the supplemental material). Alignment of the homologous regions indicated that there were only 2.6% nucleotide mismatches over a length of 211,770 bp. In comparison to plasmid pNDM-MAR, pENVA harbored additional modules accounting for a total of 42,214 bp. Differences were mostly identified in the antibiotic resistance regions of both plasmids (see Fig. S1 in the supplemental material).

Regarding the resistance genes, the *bla*_{CTX-M-15} gene was the sole gene identified on both plasmids, whereas all other antibiotic resistance genes were specific to each plasmid. Differences in the resistance modules of the plasmids resulted from insertions of different mobile genetic elements, such as insertion sequences and transposons harboring antibiotic resistance genes. However, it is noteworthy that both plasmids harbored distinct resistance modules flanked by IS4321 and IS26 elements, therefore highlighting

the involvement of those IS elements in the genetic plasticity of that plasmid scaffold.

Apart from these resistance modules, which contain different accessory genes, both plasmids possessed very similar backbone modules and other accessory genes. However, one large segment encompassing the *tra* locus (conjugative transfer) and also the mercury and tellurite resistance loci was found to be in opposite orientations in the two plasmids, as highlighted in Fig. 2B.

Concluding remarks. Plasmid pENVA, encompassing two replicons and a large set of antibiotic resistance genes, was identified from an animal isolate. Interestingly, a similar plasmid backbone has recently been identified from a *K. pneumoniae* clinical isolate recovered in Morocco (27, 52). In the latter case, the plasmid also harbored a series of clinically relevant resistance genes and, in particular, the *bla*_{NDM-1} carbapenemase gene. It remains to be evaluated whether this plasmid type might therefore play a significant role in the dissemination of the *bla*_{CTX-M-15} gene. Primers FIB-M FW and FIB-M RV, whose sequences have been reported by Villa et al. (27), are adequate for such screening approaches.

Plasmid pENVA additionally harbored genes encoding resistance to other clinically relevant antibiotics which are prescribed in either human or veterinary medicine. These antibiotics are quinolones, trimethoprim, aminoglycosides, sulfonamides, and tetracycline. It is noteworthy that this plasmid identified in animal isolates might have been selected from the environment, given the high number of heavy metal resistance genes. This hypothesis is reinforced by the fact that the plasmid harbors genes encoding resistance not only to heavy metal derivatives, such as mercury or tellurite compounds, but also to UV radiation.

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REFERENCES

- Liebana E, Carattoli A, Coque TM, Hasman H, Magiorakos AP, Mevius D, Peixe L, Poirel L, Schuepbach-Regula G, Torneke K, Torren-Edo J, Torres C, Threlfall J. 2013. Public health risks of enterobacterial isolates producing extended-spectrum β -lactamases or AmpC β -lactamases in food and food-producing animals: an EU perspective of epidemiology, analytical methods, risk factors, and control options. Clin. Infect. Dis. 56:1030–1037. <http://dx.doi.org/10.1093/cid/cis1043>.
- Lloyd DH. 2007. Reservoirs of antimicrobial resistance in pet animals. Clin. Infect. Dis. 45(Suppl 2):S148–S152. <http://dx.doi.org/10.1086/519254>.
- Paterson DL, Bonomo RA. 2005. Extended-spectrum β -lactamases: a clinical update. Clin. Microbiol. Rev. 18:657–686. <http://dx.doi.org/10.1128/CMR.18.4.657-686.2005>.
- Poirel L, Bonnin RA, Nordmann P. 2012. Genetic support and diversity of acquired extended-spectrum β -lactamases in Gram-negative rods. Infect. Genet. Evol. 12:883–893. <http://dx.doi.org/10.1016/j.meegid.2012.02.008>.
- Carattoli A. 2008. Animal reservoirs for extended spectrum β -lactamase producers. Clin. Microbiol. Infect. 14(Suppl 1):117–123. <http://dx.doi.org/10.1111/j.1469-0691.2007.01851.x>.
- Girlich D, Poirel L, Carattoli A, Kempf I, Lartigue MF, Bertini A, Nordmann P. 2007. Extended-spectrum β -lactamase CTX-M-1 in *Escherichia coli* isolates from healthy poultry in France. Appl. Environ. Microbiol. 73:4681–4685. <http://dx.doi.org/10.1128/AEM.02491-06>.
- Shiraki Y, Shibata N, Doi Y, Arakawa Y. 2004. *Escherichia coli* producing CTX-M-2 β -lactamase in cattle, Japan. Emerg. Infect. Dis. 10:69–75. <http://dx.doi.org/10.3201/eid1001.030219>.
- Wittum TE, Mollenkopf DF, Daniels JB, Parkinson AE, Mathews JL, Fry PR, Abley MJ, Gebreyes WA. 2010. CTX-M-type extended-spectrum β -lactamases present in *Escherichia coli* from the feces of cattle in Ohio, United States. Foodborne Pathog. Dis. 7:1575–1579. <http://dx.doi.org/10.1089/fpd.2010.0615>.
- Costa D, Poeta P, Sáenz Y, Vinué L, Coelho AC, Matos M, Rojo-Bezares B, Rodrigues J, Torres C. 2008. Mechanisms of antibiotic resistance in *Escherichia coli* isolates recovered from wild animals. Microb. Drug Resist. 14:71–77. <http://dx.doi.org/10.1089/mdr.2008.0795>.
- Simões RR, Poirel L, Da Costa PM, Nordmann P. 2010. Seagulls and beaches as reservoirs for multidrug-resistant *Escherichia coli*. Emerg. Infect. Dis. 16:110–112. <http://dx.doi.org/10.3201/eid1601.090896>.
- Silva N, Costa L, Gonçalves A, Sousa M, Radhouani H, Brito F, Igrejas G, Poeta P. 2012. Genetic characterisation of extended-spectrum β -lactamases in *Escherichia coli* isolated from retail chicken products including CTX-M-9 containing isolates: a food safety risk factor. Br. Poult. Sci. 53:747–755. <http://dx.doi.org/10.1080/00071668.2012.740554>.
- Agersø Y, Aarestrup FM, Pedersen K, Seyfarth AM, Struve T, Hasman H. 2012. Prevalence of extended-spectrum cephalosporinase (ESC)-producing *Escherichia coli* in Danish slaughter pigs and retail meat identified by selective enrichment and association with cephalosporin usage. J. Antimicrob. Chemother. 67:582–588. <http://dx.doi.org/10.1093/jac/dkr507>.
- Jacoby GA. 2009. AmpC β -lactamases. Clin. Microbiol. Rev. 22:161–182. <http://dx.doi.org/10.1128/CMR.00036-08>.
- Carattoli A. 2009. Resistance plasmid families in Enterobacteriaceae. Antimicrob. Agents Chemother. 53:2227–2238. <http://dx.doi.org/10.1128/AAC.01707-08>.
- Carattoli A, Villa L, Poirel L, Bonnin RA, Nordmann P. 2012. Evolution of IncA/C *bla*_{CMY-2}-carrying plasmids by acquisition of the *bla*_{NDM-1} carbapenemase gene. Antimicrob. Agents Chemother. 56:783–786. <http://dx.doi.org/10.1128/AAC.05116-11>.
- Bonnin RA, Nordmann P, Carattoli A, Poirel L. 2013. Comparative genomics of IncL/M-type plasmids: evolution by acquisition of resistance genes and insertion sequences. Antimicrob. Agents Chemother. 57:674–676. <http://dx.doi.org/10.1128/AAC.01086-12>.
- Cain AK, Hall RM. 2012. Evolution of IncHI2 plasmids via acquisition of transposons carrying antibiotic resistance determinants. J. Antimicrob. Chemother. 67:1121–1127. <http://dx.doi.org/10.1093/jac/dks004>.
- Cain AK, Hall RM. 2013. Evolution of IncHI1 plasmids: two distinct lineages. Plasmid 70:201–208. <http://dx.doi.org/10.1016/j.plasmid.2013.03.005>.
- García Fernández A, Cloeckert A, Bertini A, Praud K, Doublet B, Weill FX, Carattoli A. 2007. Comparative analysis of IncHI2 plasmids carrying *bla*_{CTX-M-2} or *bla*_{CTX-M-9} from *Escherichia coli* and *Salmonella enterica* strains isolated from poultry and humans. Antimicrob. Agents Chemother. 51:4177–4180. <http://dx.doi.org/10.1128/AAC.00603-07>.
- Dahmen S, Haenni M, Châtre P, Madec JY. 2013. Characterization of *bla*_{CTX-M} IncFII plasmids and clones of *Escherichia coli* from pets in France. J. Antimicrob. Chemother. 68:2797–2801. <http://dx.doi.org/10.1093/jac/dkt291>.
- Dahmen S, Haenni M, Madec JY. 2012. IncI1/ST3 plasmids contribute to the dissemination of the *bla*_{CTX-M-1} gene in *Escherichia coli* from several animal species in France. J. Antimicrob. Chemother. 67:3011–3012. <http://dx.doi.org/10.1093/jac/dks308>.
- Madec JY, Poirel L, Saras E, Gourguechon A, Girlich D, Nordmann P, Haenni M. 2012. Non-ST131 *Escherichia coli* from cattle harbouring human-like *bla*_{CTX-M-15}-carrying plasmids. J. Antimicrob. Chemother. 67:578–581. <http://dx.doi.org/10.1093/jac/dkr542>.
- Poirel L, Nordmann P, Ducroz S, Boulouis HJ, Arné P, Millemann Y. 2013. Extended-spectrum β -lactamase CTX-M-15-producing *Klebsiella pneumoniae* of sequence type ST274 in companion animals. Antimicrob. Agents Chemother. 57:2372–2375. <http://dx.doi.org/10.1128/AAC.02622-12>.
- Clinical and Laboratory Standards Institute. 2013. Performance standards for antimicrobial susceptibility testing; 23rd informational supplement, M100-S23. Clinical and Laboratory Standards Institute, Wayne, PA.
- Heinl S, Wibberg D, Eikmeyer F, Szczepanowski R, Blom J, Linke B, Goemann A, Grabherr R, Schwab H, Pühler A, Schlüter A. 2012.

- Insights into the completely annotated genome of *Lactobacillus buchneri* CD034, a strain isolated from stable grass silage. *J. Biotechnol.* 161:153–166. <http://dx.doi.org/10.1016/j.jbiotec.2012.03.007>.
26. Wibberg D, Blom J, Jaenicke S, Kollin F, Rupp O, Scharf B, Schneiker-Bekel S, Szczepanowski R, Goesmann A, Setubal JC, Schmitt R, Pühler A, Schlüter A. 2011. Complete genome sequencing of *Agrobacterium* sp. H13-3, the former *Rhizobium lupini* H13-3, reveals a tripartite genome consisting of a circular and a linear chromosome and an accessory plasmid but lacking a tumor-inducing Ti-plasmid. *J. Biotechnol.* 155:50–62. <http://dx.doi.org/10.1016/j.jbiotec.2011.01.010>.
 27. Villa L, Poirel L, Nordmann P, Carta C, Carattoli A. 2012. Complete sequencing of an IncH plasmid carrying the *bla*_{NDM-1}, *bla*_{CTX-M-15} and *qnrB1* genes. *J. Antimicrob. Chemother.* 67:1645–1650. <http://dx.doi.org/10.1093/jac/dks114>.
 28. Husemann P, Stoye J. 2010. r2cat: synteny plots and comparative assembly. *Bioinformatics* 26:570–571. <http://dx.doi.org/10.1093/bioinformatics/btp690>.
 29. Gordon D, Abajian C, Green P. 1998. Consed: a graphical tool for sequence finishing. *Genome Res.* 8:195–202. <http://dx.doi.org/10.1101/gr.8.3.195>.
 30. Meyer F, Goesmann A, McHardy AC, Bartels D, Bekel T, Clausen J, Kalinowski J, Linke B, Rupp O. 2003. GenDB—an open source genome annotation system for prokaryote genomes. *Nucleic Acids Res.* 31:2187–2195. <http://dx.doi.org/10.1093/nar/gkg312>.
 31. Eikmeyer F, Hadiati A, Szczepanowski R, Wibberg D, Schneiker-Bekel S, Rogers LM, Brown CJ, Top EM, Pühler A, Schlüter A. 2012. The complete genome sequences of four new IncN plasmids from wastewater treatment plant effluent provide new insights into IncN plasmid diversity and evolution. *Plasmid* 68:13–24. <http://dx.doi.org/10.1016/j.plasmid.2012.01.011>.
 32. Szczepanowski R, Eikmeyer F, Harfmann J, Blom J, Rogers LM, Top EM, Schlüter A. 2011. Sequencing and comparative analysis of IncP-1α antibiotic resistance plasmids reveal a highly conserved backbone and differences within accessory regions. *J. Biotechnol.* 155:95–103. <http://dx.doi.org/10.1016/j.jbiotec.2010.11.018>.
 33. Ilyina TV, Koonin EV. 1992. Conserved sequence motifs in the initiator proteins for rolling circle DNA replication encoded by diverse replicons from eubacteria, eucaryotes and archaeobacteria. *Nucleic Acids Res.* 20:3279–3285. <http://dx.doi.org/10.1093/nar/20.13.3279>.
 34. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG. 2007. Clustal W and Clustal X version 2.0. *Bioinformatics* 23:2947–2948. <http://dx.doi.org/10.1093/bioinformatics/btm404>.
 35. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol.* 113:1530–1534. <http://dx.doi.org/10.1093/molbev/msr121>.
 36. Saitou N, Nei M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4:406–425.
 37. Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ. 2005. Identification of plasmids by PCR-based replicon typing. *J. Microbiol. Methods* 63:219–228. <http://dx.doi.org/10.1016/j.mimet.2005.03.018>.
 38. Stolze Y, Eikmeyer F, Wibberg D, Brandis G, Karsten C, Krahn I, Schneiker-Bekel S, Viehöver P, Barsch A, Keck M, Top EM, Niehaus K, Schlüter A. 2012. IncP-1β plasmids of *Comamonas* sp. and *Delftia* sp. strains isolated from a wastewater treatment plant mediate resistance to and decolorization of the triphenylmethane dye crystal violet. *Microbiology* 158:2060–2072. <http://dx.doi.org/10.1099/mic.0.059220-0>.
 39. Treangen TJ, Messegue X. 2006. M-GCAT: interactively and efficiently constructing large-scale multiple genome comparison frameworks in closely related species. *BMC Bioinformatics* 7:433. <http://dx.doi.org/10.1186/1471-2105-7-433>.
 40. Terns MP, Terns RM. 2011. CRISPR-based adaptive immune systems. *Curr. Opin. Microbiol.* 14:321–327. <http://dx.doi.org/10.1016/j.mib.2011.03.005>.
 41. Partridge SR, Hall RM. 2003. The IS1111 family members IS4321 and IS5075 have subterminal inverted repeats and target the terminal inverted repeats of Tn21 family transposons. *J. Bacteriol.* 185:6371–6384. <http://dx.doi.org/10.1128/JB.185.21.6371-6384.2003>.
 42. Potron A, Munoz-Price LS, Nordmann P, Cleary T, Poirel L. 2011. Genetic features of CTX-M-15-producing *Acinetobacter baumannii* from Haiti. *Antimicrob. Agents Chemother.* 55:5946–5948. <http://dx.doi.org/10.1128/AAC.05124-11>.
 43. Poirel L, Lartigue MF, Decousser JW, Nordmann P. 2005. ISEc1B-mediated transposition of *bla*_{CTX-M} in *Escherichia coli*. *Antimicrob. Agents Chemother.* 49:447–450. <http://dx.doi.org/10.1128/AAC.49.1.447-450.2005>.
 44. Jiang Y, Yu D, Wei Z, Shen P, Zhou Z, Yu Y. 2010. Complete nucleotide sequence of *Klebsiella pneumoniae* multidrug resistance plasmid pKP048, carrying *bla*_{KPC-2}, *bla*_{DHA-1}, *qnrB4*, and *armA*. *Antimicrob. Agents Chemother.* 54:3967–3969. <http://dx.doi.org/10.1128/AAC.00137-10>.
 45. Da Re S, Garnier F, Guérin E, Campoy S, Denis F, Ploy MC. 2009. The SOS response promotes *qnrB* quinolone-resistance determinant expression. *EMBO Rep.* 10:929–933. <http://dx.doi.org/10.1038/embor.2009.99>.
 46. Brown NL, Misra TK, Winnie JN, Schmidt A, Seiff M, Silver S. 1986. The nucleotide sequence of the mercuric resistance operons of plasmid R100 and transposon Tn501: further evidence for mer genes which enhance the activity of the mercuric ion detoxification system. *Mol. Gen. Genet.* 202:143–151. <http://dx.doi.org/10.1007/BF00330531>.
 47. Taylor A, Kedzierska S, Wawrzynów A. 1996. Bacteriophage lambda lysis gene product modified and inserted into *Escherichia coli* outer membrane: Rz1 lipoprotein. *Microb. Drug Resist.* 2:147–153. <http://dx.doi.org/10.1089/mdr.1996.2.147>.
 48. Huang TW, Chen TL, Chen YT, Lauderdale TL, Liao TL, Lee YT, Chen CP, Liu YM, Lin AC, Chang YH, Wu KM, Kirby R, Lai JF, Tan MC, Siu LK, Chang CM, Fung CP, Tsai SF. 2013. Copy number change of the NDM-1 sequence in a multidrug-resistant *Klebsiella pneumoniae* clinical isolate. *PLoS One* 8:e62774. <http://dx.doi.org/10.1371/journal.pone.0062774>.
 49. Papagiannitsis CC, Miriagou V, Giakkoupi P, Tzouveleklis LS, Vatopoulos AC. 2013. Characterization of pKP1433, a novel KPC-2-encoding plasmid from *Klebsiella pneumoniae* sequence type 340. *Antimicrob. Agents Chemother.* 57:3427–3429. <http://dx.doi.org/10.1128/AAC.00054-13>.
 50. Mejlhede N, Licznar P, Prère MF, Wills NM, Gesteland RF, Atkins JF, Fayet O. 2004. –1 frameshifting at a CGA AAG hexanucleotide site is required for transposition of insertion sequence IS1222. *J. Bacteriol.* 186:3274–3277. <http://dx.doi.org/10.1128/JB.186.10.3274-3277.2004>.
 51. Studier JA, Keppler KJ. 1988. A note on the neighbor-joining algorithm of Saitou and Nei. *Mol. Biol. Evol.* 5:729–731.
 52. Poirel L, Benouda A, Hays C, Nordmann P. 2011. Emergence of NDM-1-producing *Klebsiella pneumoniae* in Morocco. *J. Antimicrob. Chemother.* 66:2781–2783. <http://dx.doi.org/10.1093/jac/dkr384>.