



# Complete Genome Sequencing of *Acinetobacter baumannii* Strain K50 Discloses the Large Conjugative Plasmid pK50a Encoding Carbapenemase OXA-23 and Extended-Spectrum $\beta$ -Lactamase GES-11

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**ABSTRACT** Multidrug-resistant (MDR) *Acinetobacter baumannii* strains appeared as serious emerging nosocomial pathogens in clinical environments and especially in intensive care units (ICUs). *A. baumannii* strain K50, recovered from a hospitalized patient in Kuwait, exhibited resistance to carbapenems and additionally to ciprofloxacin, chloramphenicol, sulfonamides, amikacin, and gentamicin. Genome sequencing revealed that the strain possesses two plasmids, pK50a (79.6 kb) and pK50b (9.5 kb), and a 3.75-Mb chromosome. *A. baumannii* K50 exhibits an average nucleotide identity (ANI) of 99.98% to the previously reported Iraqi clinical isolate AA-014, even though the latter strain lacked plasmid pK50a. Strain K50 belongs to sequence type 158 (ST158) (Pasteur scheme) and ST499 (Oxford scheme). Plasmid pK50a is a member of the AcI6 (replication group 6 [RG6]) group of *Acinetobacter* plasmids and carries a conjugative transfer module and two antibiotic resistance gene regions. The transposon Tn2008 carries the carbapenemase gene *bla*<sub>OXA-23</sub>, whereas a class 1 integron harbors the resistance genes *bla*<sub>GES-11</sub>, *aacA4*, *dfrA7*, *qac $\Delta$ 1*, and *sul1*, conferring resistance to all  $\beta$ -lactams and reduced susceptibility to carbapenems and resistance to aminoglycosides, trimethoprim, quaternary ammonium compounds, and sulfamethoxazole, respectively. The class 1 integron is flanked by MITEs (miniature inverted-repeat transposable elements) delimiting the element at its insertion site.

**KEYWORDS** antibiotic resistance,  $\beta$ -lactamase, carbapenemase, conjugative transfer, *Acinetobacter baumannii*

In recent years, infections caused by *Acinetobacter baumannii*, a Gram-negative, nonfermenting, oxidase-negative bacterium, have increasingly occurred and now represent one of the most frequent life-threatening nosocomial infections in immunocompromised patients (1, 2). The opportunistic pathogen *A. baumannii* tolerates unfavorable environmental conditions and features very few specific nutritional requirements. Many accessory modules specifying adaptive traits were incorporated into the *A. baumannii* bacterial chromosome as well as into plasmids if present (3). Due to this

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flexible and adaptable genome, *A. baumannii* strains are particularly prone to accumulating antibiotic resistance determinants through horizontal gene transfer (HGT) mechanisms involving mobile genetic elements (MGEs) (4).

Carbapenem resistance in *Acinetobacter* spp. is most frequently caused by the production of class D  $\beta$ -lactamases featuring carbapenemase activity (OXA type). In this context, the most common OXA subtype is OXA-23 (5). The corresponding genes are often carried by plasmids and can be associated with IS*Aba1* elements that play an important role in the dissemination and expression of these genes (5, 6). The occurrence of the extended-spectrum  $\beta$ -lactamase (ESBL) GES-11 was first reported for an *A. baumannii* clinical isolate originating from a hospital in Nancy, France (7). The *bla*<sub>GES-11</sub> gene was located on a gene cassette embedded in a plasmid-borne class 1 integron containing further resistance gene cassettes. GES-11 mediates resistance to  $\beta$ -lactam antibiotics, including aztreonam, and reduced susceptibility to carbapenems (7). Later, GES-positive *A. baumannii* strains were identified in Belgian hospitals (8). The variants GES-11, GES-12, and GES-14 were found to be integron associated and introduced into hospitals by patients from Middle Eastern countries. The Middle East and Northeastern African regions appeared to be reservoirs for *A. baumannii* derivatives producing GES-11 and GES-14 enzymes, because corresponding isolates were reported in Kuwait (9), Turkey (10), Tunisia (11, 12), and Saudi Arabia (13). It was recently shown that the sequence type 499 (ST499) clone (ST158 in the Pasteur system), identified as a source of an outbreak in a Tunisian neonatal unit, corresponded to one predominant GES-producing clone in the Middle East (14). In Turkey, a series of GES-11-, GES-11- and OXA-23-, and GES-22- and OXA-23-producing *A. baumannii* isolates belonging to either ST2 or ST158 was identified (10, 15). Overall, when looking at the literature, *bla*<sub>GES-11</sub>-positive strains also carry the *bla*<sub>OXA-23</sub> carbapenemase gene (10, 11), and some studies highlighted that both genes might be colocated on the same plasmid (10).

We previously identified a multidrug-resistant *A. baumannii* clone coproducing a GES-type  $\beta$ -lactamase and an OXA-23  $\beta$ -lactamase, which was found to be involved in a nosocomial outbreak (9). In order to gain insights into the pathogenic potential and antibiotic resistance determinants of this clone, the genome of one representative strain was fully sequenced.

## RESULTS AND DISCUSSION

**Origin of the clinical isolate *A. baumannii* strain K50.** *A. baumannii* strain K50 was recovered from the urine of a patient hospitalized in Kuwait in 2008. This isolate showed resistance to all  $\beta$ -lactams, including carbapenems, and was additionally resistant to amikacin, gentamicin, chloramphenicol, sulfonamides, and ciprofloxacin. It was shown to be responsible for a nosocomial outbreak at the Al Jahra Hospital, Kuwait (9).

**Sequencing and phylogenetic and comparative analyses of the *A. baumannii* strain K50 genome.** To analyze the multidrug resistance phenotype of *A. baumannii* strain K50 on a molecular basis, its whole genome was sequenced to identify antibiotic resistance and virulence genes to determine its relationship to other sequenced clinical and multiresistant *A. baumannii* isolates, also considering epidemiological aspects.

The genome of *A. baumannii* strain K50 consists of two closed plasmids, namely, pK50a (79.6 kb) and pK50b (9.5 kb), and a draft chromosomal sequence (3.75 Mb) containing two gaps in repetitive regions. Genome features of the three K50 replicons are summarized in Table 1. Genome plots of the K50 chromosome, pK50a, and pK50b are shown in Fig. S1 in the supplemental material and in Fig. 1 and 2, respectively.

Phylogenetic classification of *A. baumannii* strain K50 was performed by applying the EDGAR comparative genomics tool (16) based on all *A. baumannii* core genes considering finished genome sequences. The resulting phylogenetic tree (Fig. 3) is based on 1,877 concatenated core genes of 21 *A. baumannii* strains. It comprises the two known clonal complexes (CCs), CC1 and CC2, as described previously (17, 18), as well as three further clades. Two of the latter clades are represented by *A. baumannii* strain K50 and *A. baumannii* AA-014 (GenBank accession no. [GCA\\_000335595.1](https://www.ncbi.nlm.nih.gov/nuccore/GCA_000335595.1)) as well as *A. baumannii* SDF (GenBank accession no. [GCA\\_000069205.1](https://www.ncbi.nlm.nih.gov/nuccore/GCA_000069205.1)), respectively. Hence, *A.*

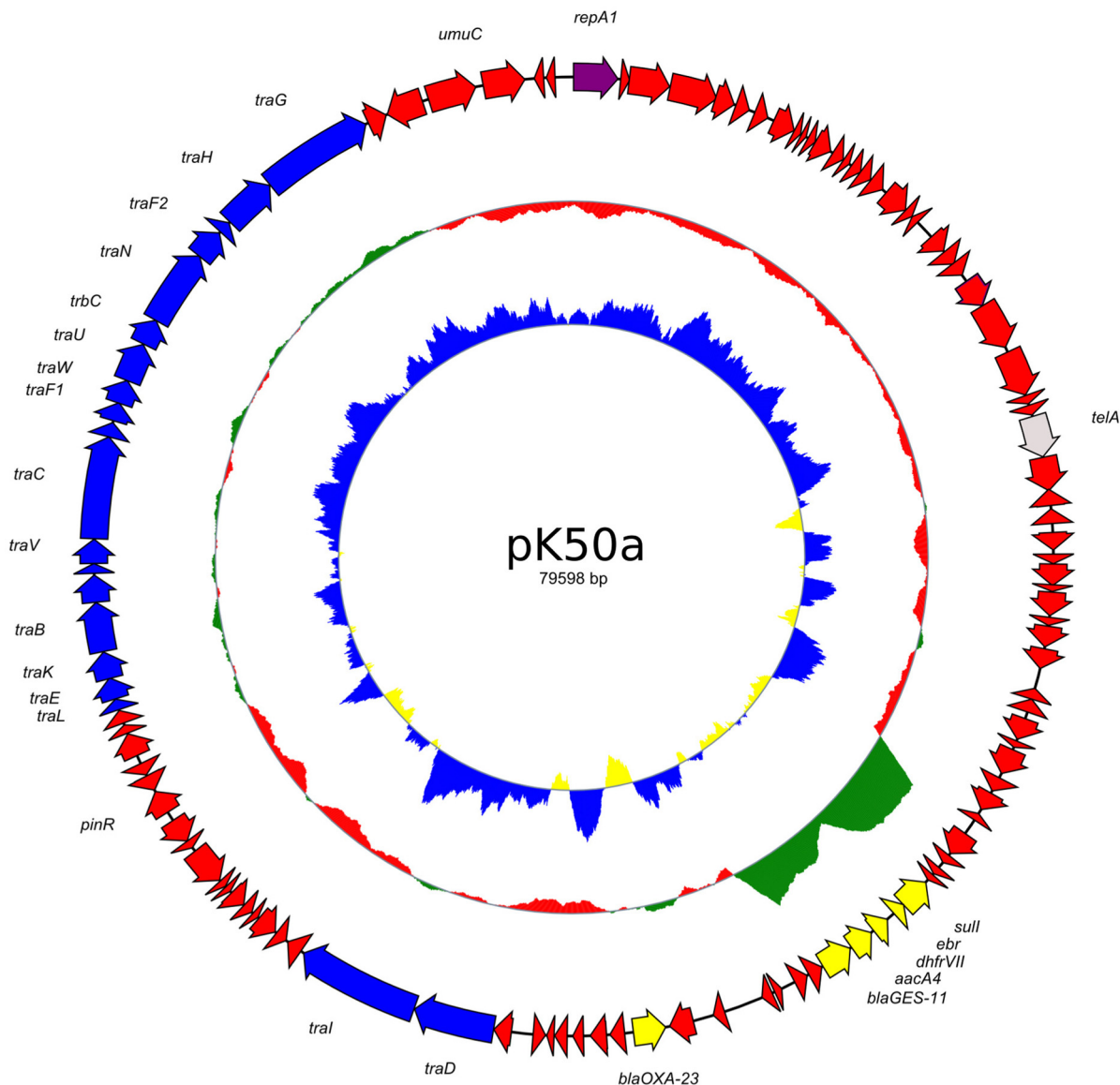
**TABLE 1** Genome features of *A. baumannii* strain K50

Feature	Value		
	Chromosome	pK50a	pK50b
Size (bp)	3,751,333	79,598	9,539
GC content (%)	38.91	35.56	33.68
Total no. of genes	3,556	114	13
No. of rRNA operons	6	0	0
No. of tRNAs	60	0	0
No. of protein-coding genes (CDSs)	3,478	114	13
No. of genes with a predicted function	2,481	45	5

*baumannii* strain K50 appeared to be located outside the CC1 and CC2 clusters but is actually closely related to *A. baumannii* strain AA-014. To determine the ST of strain K50, multilocus sequence typing (MLST) revealed that strain K50 belongs to sequence type 158 according to the Pasteur scheme and ST499 according to the Oxford scheme. *A. baumannii* strain AA-014 also is a member of ST158 (Pasteur scheme) and ST499 (Oxford scheme). The latter strain was sequenced in the frame of the Genomic Sequencing of a Diversity of US Military *Acinetobacter baumannii*/*A. calcoaceticus* Complex Isolates project. It was isolated from a soldier's wound in Iraq (2008), and its draft genome sequence consists of 61 contigs. Both strains (K50 and AA-014) featured an average nucleotide identity (ANI) value of 99.98%. Comparison of the K50 and AA-014 genome sequences by means of r2cat (19) revealed no larger inserted or deleted regions in any of the genomes. Strain AA-014 lacks a plasmid that is related to plasmid pK50a. A counterpart of plasmid pK50b was also found to be present in strain AA-014. Both strains share most of their genes, featuring a core genome of 3,426 genes. Strain K50 possesses 176 singletons, whereas strain AA-014 harbors 186 singletons. In total, 114 singletons of strain K50 are carried on plasmid pK50a (see below), and 62 are located in the chromosome. Many of the chromosomal singletons were annotated as "hypothetical" or encode transposases (54 of 62 genes). The functionally annotated unique genes encode housekeeping functions, e.g., a magnesium transport system, 3-oxoadipate CoA transferase subunit B,  $\beta$ -ketoacyl-CoA thiolase, NADP-dependent fatty aldehyde dehydrogenase, and alkyl hydroperoxide reductase subunit F.

#### Chromosomal virulence and resistant determinants of *A. baumannii* strain K50.

In total, 20 putative antimicrobial resistance genes were identified in the chromosome of *A. baumannii* strain K50. Interestingly, none of them is linked to mobile elements, such as insertion sequences (IS), transposons, or integrons. The identified antibiotic resistance genes include class A, C, and D  $\beta$ -lactamases. In addition, the chromosome harbors the *aadA*, *tetA*, and *cat* genes, encoding resistance to streptomycin, tetracycline, and chloramphenicol, respectively. Moreover, multiple genes for multidrug efflux pumps and ABC transporters potentially mediate resistance to fluoroquinolones and macrolides, among other compounds. These and other resistance genes in the chromosome of strain K50 are listed in Table S1 in the supplemental material. Genes potentially involved in virulence were identified by comparisons to the Virulence Factor Database (VFDB) (20). It appeared that *A. baumannii* strain K50 possesses different virulence genes featuring predicted functions in adherence, biofilm formation, immune evasion, and iron uptake, among others (Table S2). *A. baumannii* commonly synthesizes capsular polysaccharides and lipooligosaccharides (LOSs) that are involved in virulence and pathogenesis (21). The corresponding genes are clustered in two loci. Capsule biosynthesis and transport genes are located in the pathogenicity region known as the K locus, encoding enzymes involved in the synthesis and assembly of capsular oligosaccharide repeating units (K antigen). The K locus is inserted between the *fkpA* and *lldP* genes of two conserved modules responsible for capsule transport and the synthesis of UDP-sugar precursors. The K locus of *A. baumannii* strain K50 was found to be 98% identical to the capsule biosynthesis loci of *A. baumannii* strains RBH4 (22) and ABNIH3 (23), which have been classified as KL6 (Table S3). Although capsular units specified by the KL6 gene cluster have been disclosed (22), it remains unknown whether this locus

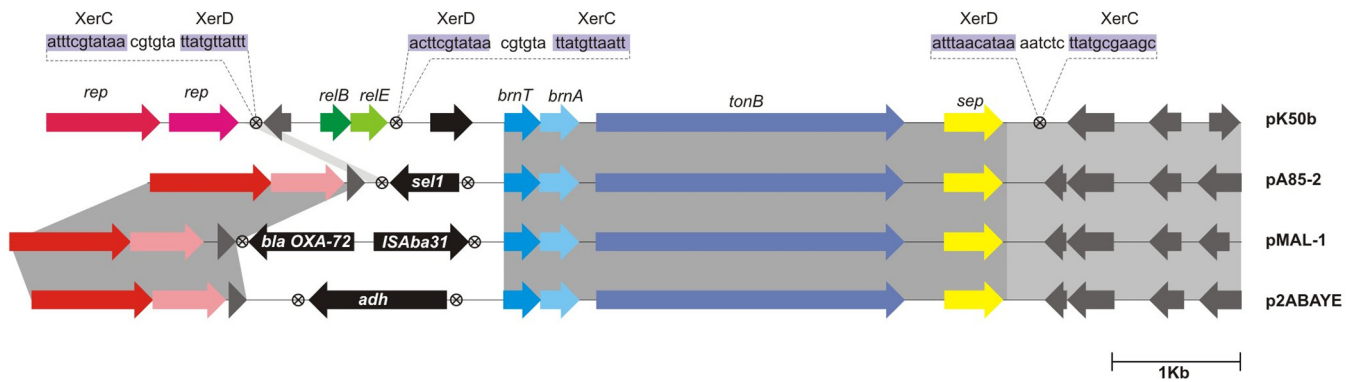


**FIG 1** Genetic map of *Acinetobacter baumannii* plasmid pK50a. The circles represent (from innermost to outermost) GC skew, GC content, and annotated coding sequences, marked as arrows. Arrows are colored to indicate a specific plasmid module, e.g., replication initiation (purple), antibiotic resistance regions (yellow), plasmid transfer (blue), and heavy metal resistance (gray).

is associated with enhanced virulence compared to other K locus arrangements. The second cluster, named the OC locus, harbors genes involved in the synthesis of outer core components of LOS and is located between the *ilvE* and *asp5* genes. Analysis of the OC locus of strain K50 revealed that it is 98% identical to the OC locus of *A. baumannii* strain RBH4, classified as OCL1 (Table S4). This OC arrangement is widely distributed in CC1 and CC2 isolates and has been identified in several *A. baumannii* strains belonging to various sequence types, including ST158 (24).

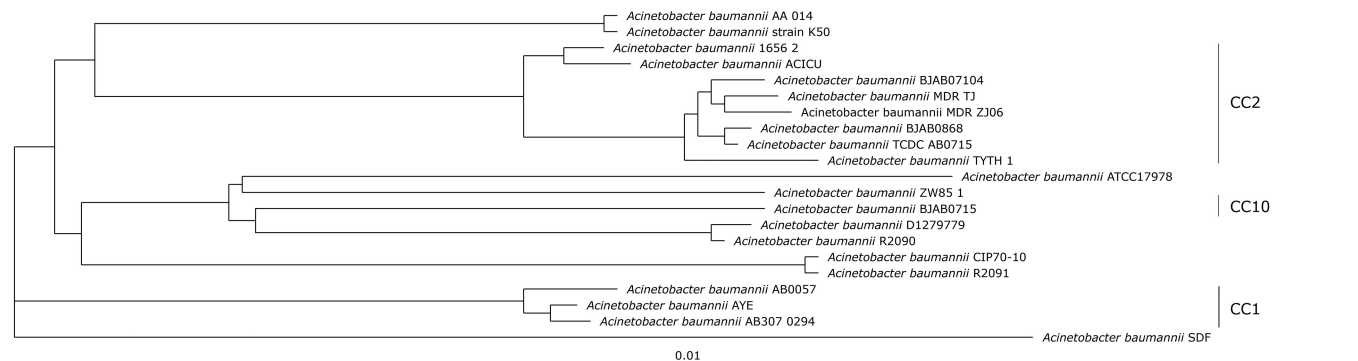
Although many of the antibiotic resistance determinants in *A. baumannii* are commonly organized within resistance islands (AbaR [*A. baumannii* resistance island]), none of the previously described AbaR regions was identified in the chromosome of strain K50.

**Genomic islands and prophage sequences within the *A. baumannii* K50 chromosome.** The prediction of genomic islands (GIs) by the application of Island Viewer 4 (25) led to the identification of nine putative GIs within the K50 chromosome that were tentatively designated GI-1 to GI-9 (see Table S5 in the supplemental material). Se-



**FIG 2** Genetic map and comparative analysis of plasmid pK50b with other *A. baumannii* virulence plasmids encoding a TonB-dependent receptor protein and septicolysin. Homologous regions are highlighted in gray. Annotated coding sequences are displayed as arrows. XerD/XerC recombination sites are marked as crossed circles within the plasmid backbone sequences. *rep*, replication initiation gene; *sep*, septicolysin gene; *sel1*, gene encoding a Sel1-like repeat protein; *adh*, alcohol dehydrogenase gene.

sequence analysis of each region revealed that six of them harbor phage-related coding DNA sequences (CDSs). The predicted GI-1 represents a region that is present in many other *Acinetobacter* species chromosomes and contains the *tol-pal* gene cluster (26). The encoded gene products are involved in the maintenance of outer membrane integrity and facilitate the uptake of group A colicins and DNA of infecting filamentous bacteriophages (26). It has been demonstrated that Pal is essential for bacterial survival and pathogenesis, although its molecular function has not been clearly defined (27, 28). The genetic context of this gene cluster varies considerably among different Gram-negative bacteria but seems to be conserved among strains belonging to the same genus (Fig. S2). Chromosomal regions corresponding to GI-3, GI-4, and GI-9 were also found in other *A. baumannii* genomes. However, these regions are not very frequently represented in other *Acinetobacter* species and are not present in other bacteria. In contrast, comparative nucleotide sequence analyses with other *Acinetobacter* sp. genomes disclosed that GI-2 and GI-8 feature unique genetic structures. GI-2 is 13,124 bp and contains 12 putative CDSs comprising mostly hypothetical genes, a *tetR* transcriptional regulator gene, a NADH oxidase gene, and a metal-dependent hydrolase gene. A similar structure is present in *Acinetobacter pittii* PHEA-2 (GenBank accession no. CP002177), isolated from industrial wastewater in China (65% coverage and 82% sequence identity), and in *Acinetobacter oleivorans* DR1 (GenBank accession no. CP002080), a diesel-degrading strain isolated from paddy rice soil from South Korea (27% coverage and 92% sequence identity). Alignments of these regions are shown in Fig. S2 in the supplemental material. GI-2-flanking regions seem to be conserved among different *A. baumannii* strains and less conserved than those of other *Acinetobacter* species. Flank-



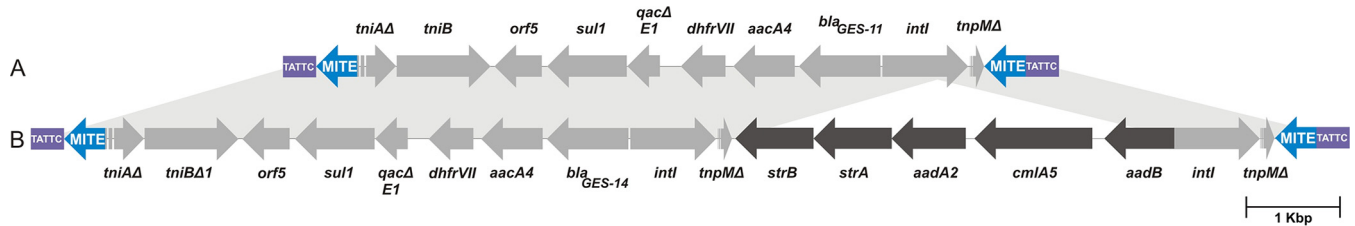
**FIG 3** Phylogenetic tree of completely sequenced *A. baumannii* strains based on 1,877 core gene products (alignments at the amino acid level) for all isolates, calculated by applying the EDGAR comparative genomics tool (16) with standard settings. CC1, CC2, and CC10, according to previous reports (17, 67, 68), are marked. *A. baumannii* strain K50 clusters together with strain AA-014 in a so-far-undefined clade. The bar denotes phylogenetic distance.

ing genes generally encode proteins involved in lipid metabolism and/or solventogenesis (not shown). GI-8 comprises 23,659 bp and 19 CDSs (Fig. S2). In this region, two gene clusters were identified, one containing mostly hypothetical genes and a second cluster encoding hypothetical proteins probably originating from bacteriophages. Database searches showed that a similar structure is present in *A. baumannii* strain AB5075-UW (GenBank accession no. [NZ\\_CP008706](#)) (87% coverage and 99% identity). Differences between these regions are restricted to a small segment comprising two CDSs related to phage genes in strain K50 (2,255 bp) and four CDSs encoding hypothetical proteins in strain AB5075-UW (4,299bp) (Fig. S2). Flanking regions of related GI-8 structures found in other *A. baumannii* chromosomes are conserved and correspond to genes involved in general carbon/nitrogen metabolism. Genomic structures of GI-3, GI-4, and GI-9 were found in multiple *A. baumannii* strains from all around the globe and partially in other *Acinetobacter* species. The predicted GIs 5, 6, and 7 probably represent prophage structures, as detailed below. Interestingly, none of the GIs predicted for the K50 chromosome is associated with antimicrobial resistance or virulence determinants.

Prophage sequences were identified by application of the PHASTER server (29) (Table S6). Region 1, consisting of 70 CDSs, was considered to represent an intact prophage. Region 2 harbors 68 CDSs and is ambiguous regarding its completeness. A comparison of prophage regions with the predicted GIs described above revealed the association of GI-5 with region 1, comprising a complete prophage, whereas GI-6 and GI-7 are located in close proximity to prophage region 2. Nucleotide sequence comparison of the latter cluster with other genomes deposited in databases revealed that it is unique but similar to two regions of the *A. baumannii* BAL062 chromosome (GenBank accession no. [NZ\\_LT594095](#)). However, the similarity between these sequences is due mostly to hypothetical genes rather than phage-related genes.

**The small putative virulence plasmid pK50b.** Plasmid pK50b (9.5 kb) harbors 13 predicted genes. Five of these genes were annotated as “hypothetical.” Among the genes with predicted functions, two replication initiation genes (*rep*), one encoding a Rep\_3 superfamily protein ([PF01051](#)), as well as two type II toxin-antitoxin system genes, namely, *relBE* and *brnTA*, were identified. Moreover, a gene encoding a cholesterol-dependent cytolysin named septicolysin and a TonB-dependent outer membrane receptor gene are present on the plasmid. Septicolysin is a pore-forming toxin (30), and the TonB-dependent receptor protein was predicted to be involved in iron acquisition and virulence (31). Hence, plasmid pK50b harbors two putative virulence determinants. Database searches revealed that plasmid pK50b is highly similar to other *A. baumannii* plasmids, such as pA85-2 (32) and pAB0057 (33), which were also isolated from multiresistant strains. However, there are two major differences between pK50b and other related plasmids: (i) the presence of XerD-XerC recombination sites flanking three CDSs, namely, the toxin-antitoxin genes *relBE* and one hypothetical gene, and (ii) the presence of two *rep* genes whose sequences are not closely related to those found in similar plasmids (Fig. 2). Although pK50b carries genes that are associated with virulence and iron uptake, its biological importance remains to be determined. Nevertheless, this MGE may facilitate the acquisition of further accessory genetic information, for example, via XerC/XerD-mediated recombination.

**Modular structure of plasmid pK50a.** Plasmid pK50a is 79,598 bp, features a GC content of 35.6%, which is clearly below the GC content of the chromosome (38.9%), and comprises 114 CDSs. Homology-based functional assignments to plasmid genes revealed that this replicon harbors different plasmid-specific modules for replication, transfer, and maintenance as well as accessory genetic information such as antibiotic resistance genes, consistent with the MDR phenotype of the strain. The replication initiation protein encoded on the plasmid is a member of replication group 6 (RG6) (Aci6) (34). Plasmids representing this group were previously identified in different *A. baumannii* strains and harbor conjugative transfer modules (9, 35–37). Stable maintenance proteins of the plasmid indicate that pK50a specifies different mechanisms to ensure plasmid survival and propagation: ParA/



**FIG 4** (A) Comparative analysis of the pK50a integron region flanked by MITEs. (B) Comparison of the pK50a integron to the corresponding region of *A. baumannii* plasmid p1AB5075. Homologous gene clusters between plasmids are highlighted in gray. Annotated coding sequences are displayed as gray (homologous) or black (unique) arrows. Miniature inverted-repeat transposable elements (MITEs) are marked as blue arrows. Target site duplications of MITEs are shown as green boxes.

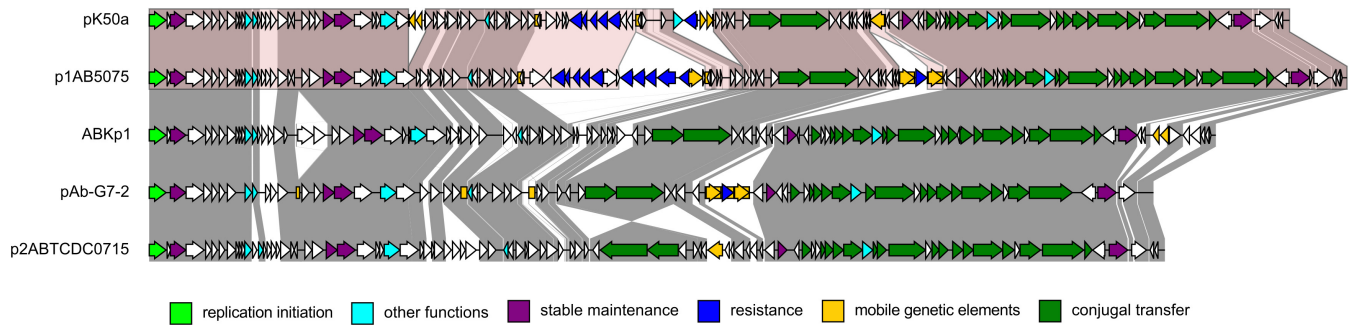
ParB proteins represent a partitioning system, and the RelB-RelE type II toxin-antitoxin system was predicted to be involved in postsegregational killing. The PinR recombinase probably also enables the equal distribution of plasmid DNA during bacterial cell division to daughter cells, whereas the UmuC DNA polymerase subunit additionally protects the plasmid DNA from damage (38).

Plasmid pK50a harbored a complete transfer (Tra) region (19 annotated conjugative transfer genes), encoding all protein components enabling the self-transfer of the plasmid. Mating experiments confirmed that plasmid pK50a was transferable among *A. baumannii* strains, and the corresponding transconjugants showed a MIC of imipenem of 16  $\mu\text{g/ml}$  (compared to 0.25  $\mu\text{g/ml}$  for the recipient strain). Phylogenetic analysis of the relaxase encoded within the Tra module revealed that this protein clusters within the MOB-F1 subfamily of relaxases (39). Members of this subfamily originate from IncN, IncF, IncP-9, and IncW plasmids of *Gammaproteobacteria* (40).

**Plasmid pK50a harbors the insertion sequences ISAba1 and ISAba125.** Plasmid pK50a hosts three IS elements previously identified in *A. baumannii*, namely, two ISAba1-like elements and one ISAba125 copy. A copy of ISAba1 is located within the coding sequence for a hypothetical protein downstream of a tellurite resistance gene. A second copy of ISAba1 in plasmid pK50a is associated with the transposon Tn2008 (41). This element harbors the carbapenemase gene *bla*<sub>OXA-23</sub> along with an ATPase gene. Tn2008 was also identified in the chromosome of *A. baumannii* strain AB5075-UW harboring plasmid p1AB5075 (see below) and containing the prophage phiOXA (42). The insertion sequence ISAba125 is inserted into a region downstream of the *traD-tral* transfer genes on plasmid pK50a.

**Plasmid pK50a harbors a class 1 integron flanked by MITEs.** Plasmid pK50a harbors a class 1 integron, including the typical 3'-conserved segment genes *sul1*, *qacEΔ1*, and *orf5* (43, 44) as well as the antibiotic resistance genes *dfrA7* (sulfonamides), *aacA4* (aminoglycosides), and *bla*<sub>GES-11</sub> (extended-spectrum  $\beta$ -lactamase) (7) (Fig. 4). Accordingly, resistance to these antimicrobial compounds was confirmed phenotypically. The class 1 integron is flanked by miniature inverted-repeat transposable elements (MITEs) (45, 46), as described previously for plasmid p1AB5075 (42) and as described recently for pAb8098 (14). The left and right ends of the pK50a integron, including the MITEs and the 5-bp target site duplication, are identical to the integron on plasmid p1AB5075. However, an additional 6-kb fragment on p1AB5075 containing another *int1* integrase gene and further antibiotic resistance genes, namely, *aadB*, *cmlA5*, *aadA2*, and *strAB*, is actually missing on plasmid pK50a.

**Comparative analyses of *A. baumannii* pK50a and closely related plasmids.** Nucleotide sequence analyses of plasmid pK50a revealed that it is closely related to other plasmids of clinical multiresistant *A. baumannii* strains, such as p1AB5075 (GenBank accession no. CP008707) (42), ABKp1 (GenBank accession no. CP001922), pAb-G7-2 (GenBank accession no. KF669606) (47), p2ABTDCDC0715 (GenBank accession no. CP002524) (48), and pAb8098 (GenBank accession no. KY022424.1) (14) (see Table S7 in the supplemental material). To evaluate their genomic relationships, a comparative analysis was performed, as described previously (49, 50). The results of this



**FIG 5** Comparative analysis of five *A. baumannii* plasmid genome sequences. Homologous gene clusters between plasmids pK50a, p1AB5075, ABKp1, pAb-G7-2, and p2ABTCD0715 were computed by means of M-GCAT (69) and are displayed as regions highlighted in gray. Homologous gene clusters of plasmids pK50a and p1AB5075 are highlighted in light red. Annotated coding sequences are displayed as arrows, and repetitive elements are displayed as rectangles. Coding sequences are colored based on their assigned gene functions.

analysis are shown in Fig. 5. All plasmids share a backbone composed of homologous regions. These regions account for 76% (plasmid p1AB5075) to 90% (plasmid pAb-G7-2) of the whole plasmid genome sequence. Plasmids p1AB5075, pABKp1, pAb-G7-2, p2ABTCD0715, pAb8098, and pK50a share 58 genes, namely, the replication initiation gene, 5 stable maintenance genes, 19 conjugative transfer genes, 29 hypothetical genes, and 4 genes encoding miscellaneous functions. The latter functions refer to a micrococcal nuclease (ACBK50\_pK50a\_12), a DNA binding protein (ACBK50\_pK50a\_13), a toxic anion or tellurite resistance protein (*telA*), and a protein disulfide isomerase (ACBK50\_pK50a\_95). ANI analyses revealed that plasmids pK50a, p1AB5075, pAb8098, and pAbG7-2 form a subgroup of very closely related plasmids, considering shared sequences (Table S8). These plasmids feature ANI values of 99.98 to 99.99%, whereas plasmids pABKp1 and p2ABTCD0715 display ANIs of 98.31 to 98.8% with plasmids of the latter group. The host strains of plasmids pABKp1 and p2ABTCD0715 were classified as members of CC2, whereas p1AB5075 and pAb-G7-2 originate from CC1 strains. The host of plasmid pK50a does not belong to either CC1 or CC2 but belongs to a so-far-uncharacterized clade outside the known CCs. Interestingly, plasmid pAb8098 originates from an *A. baumannii* strain belonging to the ST499 (Oxford scheme) and ST158 (Pasteur scheme) groups. It was isolated at the Rabta Hospital (Tunis, Tunisia). Likewise, strain K50 was classified as being a member of ST499 and ST158. Hence, clones belonging to this sequence type have now been identified in countries such as Kuwait, Iraq, Saudi Arabia (51), Egypt (52), and Tunisia (14) but also Denmark (53).

Sequences of the plasmids included in this comparative analysis differ with respect to insertions of mobile genetic elements that are characterized by transposase and integrase genes, (inverted) repeats, or MITEs, as illustrated in Fig. 4 and 5. The transposon Tn2008, located upstream of the *traDI* transfer genes of plasmid pK50a, is absent from plasmid p1AB5075 (Fig. 5). The latter plasmid harbors two copies of ISAbA125 flanking the aminoglycoside resistance gene *aphA6*, constituting the composite transposon TnaphA6, encoding resistance to amikacin (42, 47). TnaphA6 is missing from plasmid pK50a but is part of conjugative plasmid pAb-G7-2 from an Australian *A. baumannii* CC1 clone. Plasmid p1AB5075 is 99.9% identical to plasmid pAb8098 (14), and therefore, for clarity reasons, it was not included in Fig. 5. Plasmid pAb8098 can formally be regarded as a p1AB5075 deletion derivative lacking an ~940-bp fragment carrying part of ISAbA125.

**Conclusions.** Phylogenetic and MLST analyses of multidrug-resistant strain K50 showed that it is closely related to *A. baumannii* AA-014 isolated from Iraq and further ST499 (Oxford scheme) strains from North Africa (Egypt and Tunisia) and the Middle Eastern countries Saudi Arabia and Kuwait. This suggests the dissemination of this clonal lineage in the Gulf region and spread to North Africa. Recently, the appearance of a ST499 (Oxford scheme) clone harboring the *bla*<sub>GES-11</sub> gene was also reported from



the Odense University Hospital (Denmark) (53), confirming the transfer of this variant to Europe. In contrast to strain AA-014, *A. baumannii* strain K50 harbored the large conjugative plasmid pK50a, containing several resistance determinants, which may have been recently acquired by horizontal gene transfer events. Hence, plasmid pK50a and related RG6 (Aci6) replicons facilitate the dissemination of genetic information among different *A. baumannii* CCs, including so-far-uncharacterized CCs, since this plasmid type has been identified in different *A. baumannii* MDR lineages. Accordingly, RG6 (Aci6) plasmids are supposed to play an important role in the rapid adaptation of *A. baumannii* to clinical environments and antimicrobial treatment.

Strikingly, the chromosome of strain K50 does not harbor any resistance islands (AbaR) that are frequently found in *A. baumannii* multidrug-resistant strains (3, 54, 55). The K50 chromosomal resistance genes were dispersed and not associated with any specific genetic element. In addition to its MDR phenotype, strain K50 possessed several virulence and pathogenicity determinants that may have an impact on infection severity.

Comparative analyses of pK50a and pK50b with related plasmids showed that they possess a conserved backbone specifying plasmid functions. Differences among these replicons are attributed mainly to accessory modules associated with mobile genetic elements or recombination loci, indicating that plasmids maintain efficient modular structures to ensure their stable inheritance, propagation, and mobility (where applicable).

## MATERIALS AND METHODS

**Bacterial isolate, antibiotic susceptibility testing, and mating-out assays.** Isolate K50 was identified by using the API20NE system (bioMérieux, Marcy l'Etoile, France) and 16S rRNA gene sequencing. Upon the completion of the strain's genome sequence, it was also classified based on its genomic features. The antibiotic susceptibility of the isolate was determined by the disc diffusion technique on Mueller-Hinton (MH) agar. MICs were determined by using Etest strips (AB bioMérieux, La Balme-les-Grottes, France) and interpreted according to CLSI guidelines (<https://clsi.org/standards/>). Mating-out assays were performed as described previously (9), using *A. baumannii* BM4547 (rifampin resistant) as the recipient strain. The selection of the transconjugants was performed by supplementing MH agar plates with imipenem (4 µg/ml) and rifampin (200 µg/ml).

**Isolation and transfer of plasmid pK50a.** Plasmid DNA was extracted by using the Kieser method (56), and electroporation was performed by using *A. baumannii* CIP70.10 as a recipient. For mating experiments, the selection of recipient strains was done on ticarcillin (50 µg/ml) and sodium azide (100 µg/ml) or rifampin (100 µg/ml), depending on the recipient strain used.

**Sequencing and annotation of the *A. baumannii* K50 genome.** The genome of *A. baumannii* K50 was sequenced by using the Illumina MiSeq system followed by bioinformatic analyses, as previously described for *A. baumannii* strains R2090, R2091, and CIP70.10 (57, 58). In brief, an 8-kb mate pair sequencing library was constructed for strain K50, sequenced, and assembled by using *GS de novo* Assembler software (version 2.8; Roche). To facilitate gap closure and assembly validation, a combination of PCR-based and *in silico*-based strategies was applied (59). The three final K50 replicons (the bacterial chromosome and two plasmids) were annotated by means of the Prokka rapid prokaryotic genome annotation tool (60) and the GenDB annotation platform (61), based on the annotation of the reference strain *A. baumannii* AB307-0294 (GenBank accession no. CP001172), (33). In addition, WebMGA was applied for COG annotation, with default settings (E value threshold of  $1 \times 10^{-20}$ ) (62). Moreover, all genes that are discussed in this study were manually annotated with the help of BLAST and other tools, as described recently (49, 59, 63).

**Comparative analysis, phylogenetic classification, and multilocus sequence typing.** Comparative analyses and phylogenetic classification of *A. baumannii* strain K50 were performed by using EDGAR 2.0 (16), as recently described (57). The ST of *A. baumannii* strain K50 was determined by applying data in the *A. baumannii* MLST database according to the Pasteur scheme (*cpn60*, *fusA*, *gltA*, *pyrG*, *recA*, *rpIB*, and *rpoB*) and the Oxford scheme (*gltA*, *gyrB*, *gdhB*, *recA*, *cpn60*, *gpi*, and *rpoD*) (<https://pubmlst.org/abaumannii/>). To determine the similarity between *A. baumannii* strain K50 and *A. baumannii* strain AA-014 as well as between pK50a and the related plasmids p1AB5075, ABKp1, pAb-G7-2, and p2ABTCD0715, an ANI analysis was performed, as described previously (64, 65).

**Detection of antibiotic resistance genes and pathogenicity determinants.** The prediction of chromosomal antibiotic resistance genes was accomplished by using the ARG-ANNOT (66) and GenBank databases, applying default parameters. The identification of virulence and pathogenicity factors was carried out by using VFDB (20). Corresponding annotations were assigned when alignments resulted in identity values of at least 30%, minimally covering 90% of the gene.

**Detection of genomic islands and prophage sequences.** The prediction of chromosomal genomic islands was accomplished by using IslandViewer 4 (25). The identification of prophage regions was carried out by using PHASTER software (29).

**Accession number(s).** The genome sequence for *A. baumannii* strain K50 is accessible under the European Nucleotide Archive ENA accession no. [PRJEB22063](https://doi.org/10.1093/nar/nwz003). The accession no. for the chromosomal contigs are [OHJL01000001](https://doi.org/10.1093/nar/nwz003) to [OHJL01000004](https://doi.org/10.1093/nar/nwz003). The plasmids pK50a and pK50b are under accession no. [LT984690](https://doi.org/10.1093/nar/nwz003) and [LT984691](https://doi.org/10.1093/nar/nwz003), respectively.

## SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/AAC.00212-18>.

**SUPPLEMENTAL FILE 1**, PDF file, 1.0 MB.

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