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Complete genome sequencing of *Acinetobacter baumannii* str. K50 disclosed the large conjugative plasmid pK50a encoding the carbapenemase OXA-23 and the extended-spectrum β–lactamase GES-11

Daniel Wibberg[1#], Ileana P. Salto[2#], Felix G. Eikmeyer[7], Irena Maus[1], Anika Winkler[1], Patrice Nordmann[3,4,5,6], Alfred Pühler[1], Laurent Poirel[3,4,5], Andreas Schlüter[1]

[1] *Genome Research of Industrial Microorganisms, Center for Biotechnology (CeBiTec), Bielefeld University, D-33594 Bielefeld, Germany*

[2] *IBBM (Instituto de Biotecnología y Biología Molecular), CCT-CONICET-La Plata, Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Calles 47 y 115 (1900) La Plata, Argentina*

[3] *Emerging Antibiotic Resistance Unit, Medical and Molecular Microbiology, Department of Medicine, Faculty of Science, University of Fribourg, Switzerland*

[4] *INSERM European Unit (LEA Paris, France), University of Fribourg, Switzerland*

[5] *Swiss National Reference Center for Emerging Antibiotic Resistance (NARA), University of Fribourg, Switzerland*

[6] *HFR - Hôpital Cantonal, Fribourg, Switzerland*

[7] *von Laer Stiftung, Detmolder Straße 68, D-33604 Bielefeld*

[#] DW and IPS contributed equally to this work

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Corresponding author: Andreas Schlüter, aschluet@cebitec.uni-bielefeld.de

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) value of 99.98% to the previously reported Iraqi clinical isolate AA-014, even though that the latter strain lacked plasmid pK50a. Strain K50 belongs to the Sequence Type ST158 (Pasteur scheme) and ST499 according to the Oxford scheme. Plasmid pK50a is a member of the Aci6 (RG6) group of *Acinetobacter* plasmids, and encodes a conjugative transfer module and two antibiotic resistance gene regions comprising the transposon Tn*2008*. The transposon carries the carbapenemase gene $bla_{\text{OXA-23}}$ and a class 1 integron harboring the resistance genes $bla_{\text{GES-11}}$, *aacA4*, *dfrA7*, *qacEΔ1* and *sul1* conferring resistance to all β-lactams and reduced susceptibility to carbapenems, resistance to aminoglycosides, trimethoprim, quaternary ammonium compounds and sulfamethoxazole, respectively. The class 1 integron is flanked by MITEs (Miniature Inverted repeat Transposable Element) delimiting the element at its insertion site.

Introduction

In recent years, infections caused by *Acinetobacter baumannii*, a Gram-negative, non-fermenting, oxidase-negative bacterium, 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 little 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 flexible and adaptable genome, *A. baumannii* strains are particularly prone to accumulate 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 production of class D β-lactamases featuring carbapenemase activity (OXA-type). In this context, the most common OXA-subtype is OXA-23 (5). Corresponding genes are often plasmid-encoded and can be associated to IS*Aba1* elements that play an important role in dissemination and expression of those genes (5, 6). Occurrence of the Extended Spectrum β-lactamase (ESBL) GES-11 was first reported for an *A. baumannii* clinical isolate originating from a hospital in Nancy, France (7). The $bla_{\text{GES-11}}$ 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 β-lactam antibiotics including aztreonam and reduced susceptibility to carbapenems (7). Later on, 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 the hospital 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 from Kuwait (9), Turkey (10), Tunisia (11, 12) and Saudi Arabia (13). It was recently shown that clone ST499 (ST158 in 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 + OXA-23, and GES-22 + OXA-23-producing *A. baumannii* isolates were identified, either belonging to ST2 or ST158 (10, 15). Overall, when looking at the literature, $bla_{\text{GES-11}}$-positive strains also carry the $bla_{\text{OXA-23}}$ carbapenemase gene (10, 11), and some studies highlighted that both genes might be co-located onto the same plasmid (10).

We previously identified a multidrug-resistant *A. baumannii* clone co-producing a GES-type and an OXA-23 β-lactamase that 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* str. K50. *A. baumannii* strain K50 was recovered from urine of a patient hospitalized in Kuwait in 2008. This isolate showed resistance to all β-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, phylogenetic and comparative analyses of the *A. baumannii* str. K50 genome. To analyze the multidrug resistance phenotype of the *A. baumannii* str. 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* str. 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 Supplementary Figure 1, Figure 1 and Figure 2, respectively.

Phylogenetic classification of *A. baumannii* str. K50 was performed by applying the comparative genomics tool EDGAR (16) based on all *A. baumannii* core genes considering finished genome sequences. The resulting phylogenetic tree (Figure 3) is based on 1,877 concatenated core genes of 21 *A. baumannii* strains. It comprises the two known Clonal Complexes (CC) 1 and 2 as described previously (17, 18) as well as three further clades.

Two of the latter clades are represented by *A. baumannii* str. K50 and *A. baumannii* AA-014 (GenBank assembly accession No.: GCA_000335595.1) and *A. baumannii* SDF (GenBank assembly accession No.: GCA_000069205.1), respectively. Hence, isolate *A. baumannii* str. K50 appeared to be located outside of the CC1 and CC2 clusters, but is actually closely related to *A. baumannii* strain AA-014. To determine the sequence type (ST) of strain K50, a Multilocus sequence typing (MLST) revealed that strain K50 belongs to the sequence type 158 of the Pasteur scheme and ST499 according to the Oxford scheme. The *A. baumannii* strain AA-014 also is a ST158 (Pasteur scheme) and ST499 (Oxford scheme) member. 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 3426 genes. Strain K50 possesses 176 singletons whereas strain AA-014 harbors 186 singletons. In total, 114 singletons of strain K50 are encoded on the 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, β-ketoadipyl-CoA

thiolase, NADP-dependent fatty aldehyde dehydrogenase, and alkyl hydroperoxide reductase subunit F.

Chromosomal virulence and resistant determinants of *A. baumannii* str. K50. In total, 20 putative antimicrobial resistance genes were identified in the chromosome of *A. baumannii* str. K50. Interestingly, none of them is linked to mobile elements such as Insertion Sequences (IS), transposons or integrons. Identified antibiotic resistance genes include class A, C and D β-lactamases. In addition, the chromosome harbors the genes *aadA*, *tetA* and *cat* encoding resistance to streptomycin, tetracycline and chloramphenicol, respectively. Moreover, multiple genes for multidrug efflux pumps and ABC transporters potentially mediate resistances to fluoroquinolones and macrolides among other compounds. These and other resistance genes in the chromosome of strain K50 are listed in the Supplementary Table S1. Genes potentially involved in virulence were identified by comparisons to the Virulence Factor Database (VFDB) (20). It appeared that *A. baumannii* str. 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 (LOS) that are involved in virulence and pathogenesis (21). Corresponding genes are clustered in two loci. Capsule biosynthesis and transport genes are located in the pathogenicity region known as K-locus encoding enzymes involved in the synthesis and assembly of capsular oligosaccharide repeating units (K antigen). The K-locus is inserted between the genes *fkpA* and *lldP* of two conserved modules responsible for capsule transport and synthesis of UDP-sugar precursors. The K-locus of *A. baumannii* str. K50 was found to be 98% identical to

the capsule biosynthesis loci of the *A. baumannii* strains RBH4 (22) and ABNIH3 (23), which have been classified as KL6 (see Supplemental Table S3). Although capsular units specified by the KL6 gene cluster have been disclosed (22), it remains unknown whether this locus is associated with enhanced virulence when compared to other K-locus arrangements. The second cluster, named OC-locus, encodes genes involved in synthesis of Outer Core components of LOS, and is located between the genes *ilvE* and *aspS*. Analysis of the OC-locus from the strain K50 revealed that it is 98% identical to the OC-locus from *A. baumannii* str. 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). 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.

Prediction of Genomic Islands (GIs) by 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 Supplemental Table S5). Sequence analysis of each region revealed that six of them harbor phage-related CDSs. The predicted GI-1 represents a region that is present in many other *Acinetobacter* spp. chromosomes, and contains the *tol-pal* gene cluster (26). Encoded gene products are involved in maintenance of outer membrane integrity and facilitate 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). Genetic context of this gene cluster varies considerably among different Gram-negative bacteria, but seem to be conserved among strains belonging to the same genus (Figure S2). Chromosomal regions corresponding to GI-3, GI-4 and GI-9 were also found in other *A. baumanni* 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 the genomic islands GI-2 and GI-8 feature unique genetic structures. GI-2 is 13,124 bp in size and contains 12 putative CDSs comprising mostly hypothetical genes, a *tetR* transcriptional regulator gene, a NADH oxidase and a metal-dependent hydrolase gene. A similar structure is present in *Acinetobacter pitti* PHEA-2 (CP002177), isolated from industrial wastewater in China (65% coverage and 82% sequence identity), and *Acinetobacter oleivorans* DR1 (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 Figure S2. GI-2 flanking regions seem to be conserved among different *A. baumannii* strains and less conserved compared with other *Acinetobacter* species. Flanking genes generally encode proteins involved in lipid metabolism and/or solventogenesis (not shown). GI-8 comprises 23,659 bp and 19 CDSs (Figure 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 the *A. baumannii* strain AB5075-UW (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 (2255 bp) and four CDSs encoding hypothetical proteins in strain AB5075-UW (4299bp) (Figure S2). Flanking regions of related GI-8 structures found in other *A.*

baumanni 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. Predicted GIs 5, 6 and 7 probably represent prophage structures as detailed below. Interestingly, none of the GIs predicted in the K50 chromosome is associated with antimicrobial resistance or virulence determinants.

Prophage sequences were identified applying the PHASTER server (29) (Supplemental 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. Comparison of prophage regions with the predicted GIs described above revealed 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 (NZ_LT594095). However, similarity between these sequences is mostly due to hypothetical genes rather than phage-related genes.

The small putative virulence plasmid pK50b. Plasmid pK50b (9.5 kb) harbors thirteen 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 (PF1051), as well as two type II toxin/antitoxin system genes, namely *relEB* 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) that 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 (Figure 2). Although, pK50b encodes genes that are associated with virulence and iron uptake, but its biological importance remains to be determined. Nevertheless, this MGE may facilitate acquisition of further accessory genetic information for example *via* XerC/XerD mediated recombination.

Modular structure of plasmid pK50a. Plasmid pK50a is 79,598 bp in size featuring 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 the replication group 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/ParB proteins represent a partitioning system and the RelB-RelE type II toxin-antitoxin system was predicted to be involved in post-segregational killing. The PinR

recombinase probably also enables 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 self-transfer of the plasmid. Mating experiments confirmed that plasmid pK50a was transferable among *A. baumannii* strains and corresponding transconjugants showed an MIC of imipenem at 16 µg/ml (compared to 0.25 µg/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 γ-*Proteobacteria* (40).

Plasmid pK50a harbors the Insertion Sequences IS*Aba1* and IS*Aba125*. Plasmid pK50a hosts three IS elements previously identified in *A. baumannii*, namely two IS*Aba1*-like elements and one IS*Aba125* copy. A copy of IS*Aba1* is located within the coding sequence for a hypothetical protein downstream of a tellurite resistance gene. A second copy of IS*Aba1* in plasmid pK50a is associated with transposon Tn*2008* (41). This element harbors the carbapenemase gene $bla_{\text{OXA-23}}$ along with an ATPase gene. Tn*2008* 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 IS*Aba125* inserted in a region downstream of the transfer genes *traD-traI* 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* (sulphonamides), *aacA4* (aminoglycosides) and bla_{GES-11} (extended-spectrum β-lactamase) (7) (Figure 4). Accordingly, resistances to these antimicrobial compounds were confirmed phenotypically. The class 1 integron is flanked by Miniature Inverted repeat Transposable Elements (MITEs) (45, 46), as already described for plasmid p1AB5075 (42) and recently in pAb8098 (14). The left and right ends of the pK50a integron, including the MITE elements 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 pK50a.

Comparative analyses of *A. baumannii* pK50a with 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), p2ABTCDC0715 (GenBank Accession No. CP002524) (48), and pAb8098 (GenBank Accession No. KY022424.1) (14) (Table S7). To evaluate their genomic relationships, a comparative analysis was performed as described previously (49, 50). The results of this analysis are shown in Figure 5. All plasmids share a common 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, p2ABTCDC0715, pAb8098 and pK50a share

58 genes, namely the replication initiation gene, five stable maintenance genes, 19 conjugative transfer genes, 29 hypothetical genes and four 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). Average Nucleotide Identity (ANI) analyses revealed that plasmids pK50a, p1AB5075, pAb8098, and pAbG7-2 form a sub-group 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 p2ABTCDC0715 display ANI values of 98.31 to 98.8% to plasmids of the latter group. The host strains of plasmids pABKp1 and p2ABTCDC0715 were classified as CC2 members, whereas p1AB5075 and pAb-G7-2 originate from CC1 strains. The host of plasmid pK50a does belong neither to CC1 nor to CC2 but to a so far uncharacterized clade outside of the known CCs. Interestingly, plasmid pAb8098 originates from an *A. baumanii* 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 ST499 and ST158 member. Hence, clones belonging to this sequence type have now been identified in countries such as Kuwait, Iraq, Saudi Arabia (51), Egypt (52), Tunisa (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 Miniature Inverted-repeat Transposable Elements (MITEs) as illustrated in Figure 4 and 5. Transposon Tn*2008*, located upstream of the transfer genes *traDI* of plasmid pK50a, is absent on plasmid p1AB5075 (see Figure 5). The latter plasmid harbors two copies of IS*Aba125* flanking the aminoglycoside-resistance gene *aphA6*,

constituting the composite transposon Tn*aphA6* encoding resistance to amikacin (42, 47). Tn*aphA6* is missing on plasmid pK50a but is part of the conjugative plasmid pAb-G7-2 from an Australian CC1 *A. baumannii* clone. Plasmid p1AB5075 is 99.9% identical to plasmid pAb8098 (14) and therefore, for clarity reasons, was not included in Figure 5. Plasmid pAb8098 can formally be regarded as p1AB5075 deletion derivative lacking an approx. 940 bp fragment carrying part of IS*Aba125*.

Conclusions

Phylogenetic and MLST analysis of the 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 African (Egypt, Tunisia) and the Middle East countries Saudi Arabia, and Kuwait. This suggests dissemination of this clonal lineage in the Gulf region and spreading to North Africa. Recently, appearance of a ST499 (Oxford scheme) clone harboring the $bla_{\text{GES-11}}$ gene was also reported from the Odense University Hospital (Denmark, Europe) (53) confirming transfer of this variant to Europe. In contrast to strain AA-014, *A. baumannii* str. K50 harbored the large conjugative plasmid pK50a containing several resistance determinants which recently may have been acquired by horizontal gene transfer events. Hence, plasmid pK50a and related RG6 (Aci6) replicons facilitate dissemination of genetic information among different *A. baumannii* clonal complexes (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 for 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 severeness.

Comparative analyses of pK50a and pK50b to related plasmids showed that they possess a conserved backbone specifying plasmid functions. Differences among these replicons are mainly attributed to accessory modules associated with mobile genetic elements or recombination loci indicating that plasmids maintain efficient modular structures ensuring 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 rDNA gene sequencing. Upon 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 agar. MICs were determined by using E-test strips (AB bioMérieux, La Balme-les-Grottes, France) and interpreted according to the CLSI guidelines (CLSI). Mating-out assays were performed as described (9) using *A; baumannii* BM4547 (rifampin resistant) as recipient strain. 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 using *A. baumannii* CIP70.10 as recipient. For mating experiments, selection of recipient strains was done on ticarcillin (50 µg/ml) and sodium azide (100 µg/ml), or rifampicin (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 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 using the GS *de novo* Assembler software (version 2.8, Roche). To facilitate gap closure and assembly validation, a combination of a PCR-based and *in silico* based strategy was applied (59). The three final K50 replicons (the bacterial chromosome and two plasmids) were annotated by means of the rapid prokaryotic genome annotation tool Prokka (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 the COG annotation with default settings (E-value threshold of 1 x 10^{-20}) (62). Moreover, all genes that were discussed in this study were manually annotated with the help of BLAST and other tools as described recently (49, 59, 63). The genome sequence for *A. baumannii* str. K50 is accessible under the EMBL/GenBank project identification No. PRJEB22063.

Comparative analysis, phylogenetic classification and multilocus sequence typing. Comparative analyses and phylogenetic classification of *A. baumannii* strain K50 were performed using EDGAR 2.0 (16) as recently described (57). The sequence type (ST) of *A.*

baumannii str. K50 was determined by applying the *A. baumannii* multilocus sequence typing (MLST) database according to the Pasteur scheme (*cpn60*, *fusA*, *gltA*, *pyrG*, *recA*, *rplB*, 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 for pK50a and the related plasmids p1AB5075, ABKp1, pAb-G7-2 and p2ABTCD0715, an Average Nucleotide Identity analysis (ANI) was performed as described previously (64, 65).

Detection of antibiotic resistance genes and pathogenicity determinants. Prediction of chromosomal antibiotic resistant genes was accomplished using the databases ARG-ANNOT (66) and GenBank applying default parameters. Identification of virulence and pathogenicity factors was carried out using the Virulence Factor Database 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. Prediction of chromosomal genomic islands was accomplished using IslandViewer 4 (25). Identification of prophage regions was carried out by the PHASTER software (29).

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Table 1: Genome features of *A. baumannii* str. K50

Feature	Chromosome	pK50a	pK50b
Size (bp)]	3,751,333	79,598	9,539
GC content (%)	38.91	35.56	33.68
Total number of genes	3556	114	13
rRNA operons	6	0	0
tRNAs	60	0	0
Protein coding genes (CDSs)	3478	114	13
Genes with a predicted function	2481	45	5

Figures

Figure 1:

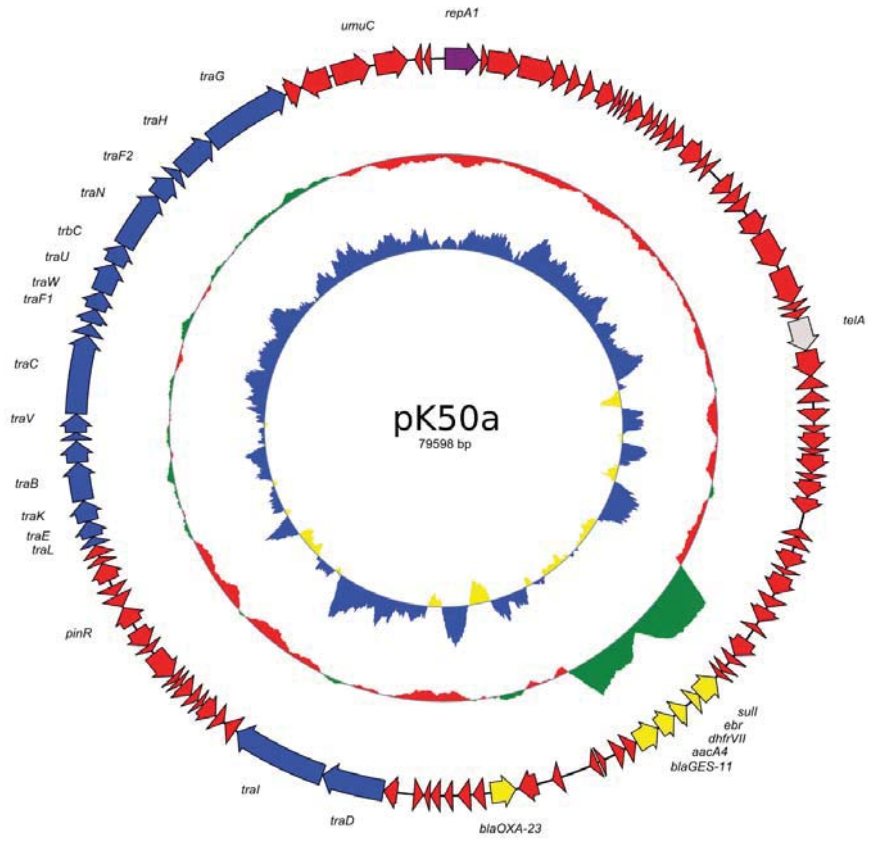

Figure 1.

Genetic map of the *Acinetobacter baumannii* plasmid pK50a. The circles represent (from inner to outer most): (i) GC-skew; (ii) GC-content; (iii) annotated coding sequences are 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 (grey).

Figure 2:

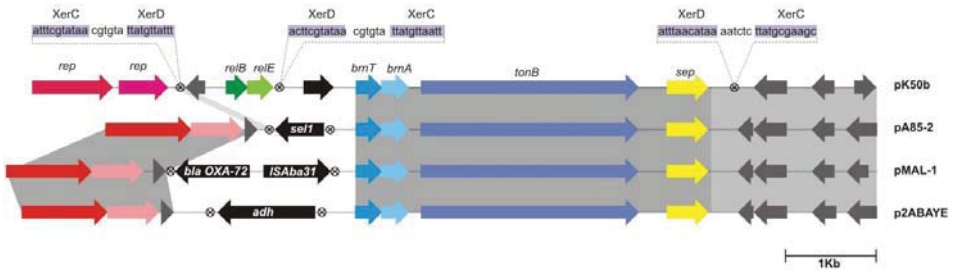

Figure 2.

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 grey. Annotated coding sequences are displayed as arrows. XerD/XerC recombination sites are marked as crossed circles within the plasmid backbone sequences. Abbreviations: *rep*, replication initiation gene; *sep*, septicolysin gene; *sel1*, gene encoding a Sel1-like repeat protein; *adh*, alcohol dehydrogenase gene.

Figure 3

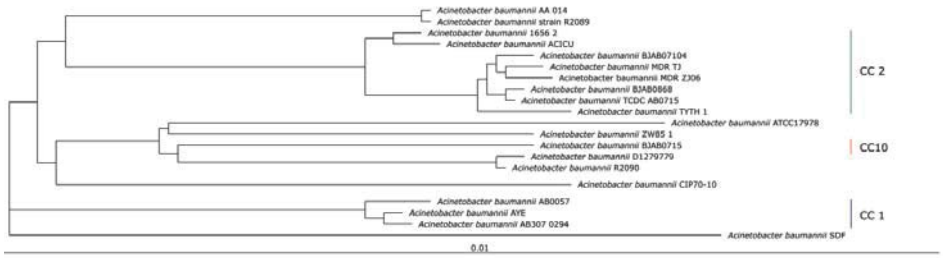

Figure 3.

Phylogenic tree of completely sequenced *A. baumannii* strains based on 1877 core gene products (alignments on amino acid level) for all isolates as calculated by applying the comparative genomics tool EDGAR (16) with standard settings. Clonal Complexes CC1, CC2 and CC10 according to previous publications (17, 67, 68) are marked. *A. baumannii* str. K50 clusters together with strain AA-014 in a so far non-defined clade. The scale bar denotes phylogenetic distance.

Figure 4:

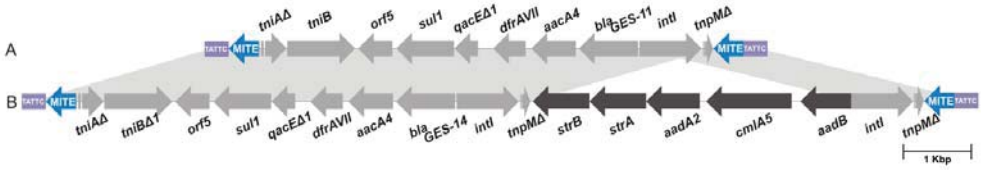

Figure 4.

Comparative analysis of the pK50a integron region flanked by MITEs (A). The pK50a integron is compared to the corresponding region of the *A. baumannii* plasmid p1AB5075 (B). Homologous gene clusters between plasmids are highlighted in grey. Annotated coding sequences are displayed as grey (homologous) or black (unique) arrows. Miniature Inverted-repeat Transposable Elements (MITEs) are marked as blue arrows. Target sites duplications of MITEs are shown as green boxes.

Figure 5:

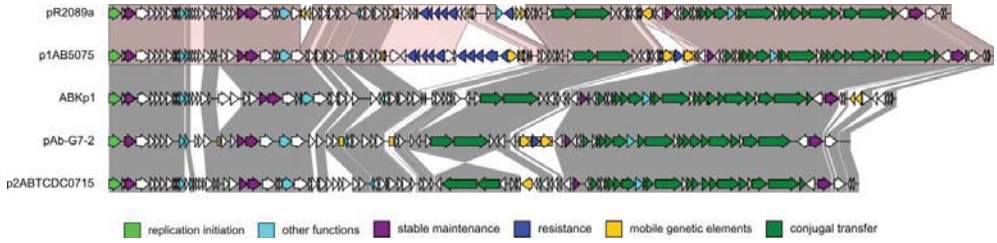

Figure 5.

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 as rectangles. Coding sequences are colored based on their assigned gene functions.

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