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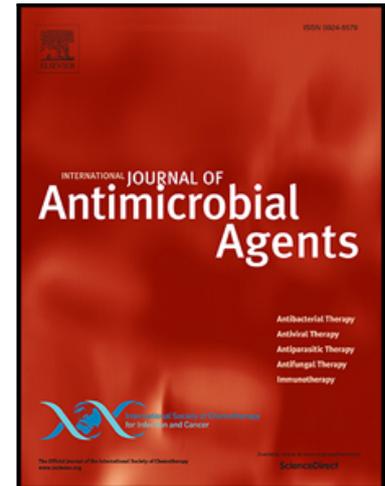
Interspecies DNA acquisition by a naturally competent Acinetobacter baumannii strain

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Highlights

- *A. baumannii* A118 was able to acquire genomic DNA (gDNA) of two carbapenem-resistant *Klebsiella pneumoniae* (CRKp) clinical isolates

- The whole genome sequence of two transformants cells was obtained confirming the acquisition of foreign genomic DNA. The *K. pneumoniae* DNA segments that were acquired, includes mobile elements, resistance determinants, and genes involved in metabolism.

- Additional assays showed that *A. baumannii* A118 was also able to incorporate gDNA of *Providencia rettgeri* and *Staphylococcus aureus,* two phylogenetically distant species.

- This study showed that *A. baumannii* can acquire foreign DNA from different species and that transformation may play a fundamental role in the increasing frequency of emergence of MDR strains

Interspecies DNA acquisition by a naturally competent *Acinetobacter baumannii* strain

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Abstract

The human pathogen *Acinetobacter baumannii* possesses high genetic plasticity

and frequently acquires antibiotic resistance genes. Here, we investigate the role

of natural transformation in these processes.

Genomic DNA (gDNA) from different sources including that from carbapenem-

resistant *Klebsiella pneumoniae* (CRKp) strains was mixed with *A. baumannii*

A118 cells. Selected transformants were analyzed by whole genome sequencing.

In addition, bioinformatic analyses and *in silico* gene flow prediction were also

performed to support our experimental results.

Transformant strains included some that became resistant to carbapenem or

changed the antibiotic susceptibility profile. Foreign DNA acquisition was

confirmed by whole genome analysis. The acquired DNA that was most

frequently identified corresponded to mobile elements, antibiotic resistance

genes, and operons involved in metabolism. Bioinformatic analyses and *in silico*

gene flow prediction showed continued exchange of genetic material between *A. baumannii* and *K. pneumoniae* when they share the same habitat.

Natural transformation plays an important role in the plasticity of A. *baumannii* and concomitantly in the emergence of MDR strains.

1. Introduction

In contrast to Eukarya where genetic duplication plays a main role, the most important force driving prokaryotic evolution is Horizontal Gene Transfer (HGT) [1]. In Eubacteria, this process occurs mainly by conjugation, transduction and transformation by natural competence. Since conjugation was historically far more studied [2, 3], the two latter had received far less attention. In particular, transformation by natural competence is an HGT mechanism in which bacteria uptake DNA directly from the environment and incorporate it into their genomic repertoire[4]. Increasing evidence indicates that it is an underscored mechanism for the acquisition of antibiotic resistance genes[4].

The recent rise in multidrug resistant strains in clinics alarms both scientists and government agencies [5, 6]. *Acinetobacter baumannii*, a pathogen associated with high-mortality multi-drug-resistant (MDR) infections [7, 8], easily acquires antibiotic resistance determinants and is highly adaptable to unfavorable conditions. These properties may be due to an extreme genome plasticity combined with mechanisms of HGT like conjugation, transformation, or the recently described predation [9] . Previous studies have shown high variability in genome organization among *Acinetobacter* strains and foreign DNA in their genomes, suggesting exogenous acquisition of genetic traits [10, 11]. The recent

reports that members of *Enterobacteriaceae* harbor resistance determinants associated with *Acinetobacter* support and active exchange of DNA between these bacteria [12-16].

To gain insights into the role of natural transformation in *A. baumannii*, we carried out transformation assays with different sources of genomic DNA (gDNA) and analyzed the acquisition of foreign gDNA.

2. Material and Methods

2.1 Bacterial strains

The naturally competent carbapenem-susceptible clinical strain *A. baumannii* A118 was used in the transformation assays [5, 17]. Two carbapenem-resistant *K. pneumoniae* (CR*Kp*) strains, VA360 (*bla*$_{TEM-1}$, *bla*$_{KPC-2}$, *bla*$_{SHV-11}$, and *bla*$_{SHV-12}$) [18] and Kb18 (*bla*$_{KPC}$ and *bla*$_{OXA-23}$). This latter strain was isolated in 2014 from an intensive care unit in Buenos Aires, Argentina. Other DNA sources used for transformation were DNA from *Providencia rettgeri* strain M15758 (*bla*$_{NDM-1}$) and *Staphylococcus aureus* "Cordobes" clone (SAC) (*mecA*) [19, 20]. Twenty-two CRKp *K. pneumoniae* and 23 *A. baumannii* strains randomly selected from our collection were used to search for the presence of *bla*$_{OXA-23}$, IS*Aba125,* IS*Aba1* and *IS*CR2; and IS*Kpn1*, respectively.

2.2 Standard molecular biology techniques

DNA extractions were carried out using commercial kits (Promega, Madison, WI or MasterPure DNA Purification kit, Epicentre Biotechnologies). PCR reactions were performed using the Zymo Taq™ PreMix (Irvine, CA, USA).

2.3 Natural transformation assays

Standard natural transformation assays were performed as previously described [5, 17]. Briefly, we added 200 ng of DNA to a mix containing 50 µl of fresh LB broth and 50 µl of A118 culture in stationary phase. This mix was incubated at 37°C for 1 hour and then plated on selective agar plates containing the adequate antibiotic. One µg/ml of meropenem (MEM), 10 µg/ml of cefotaxime or 200 µg/ml of ampicillin were used for selection of transformant clones. Negative controls were included in each assay. To score transformation events, MEM[R], CTX[R] or AMP[R] colonies were counted and were confirmed by susceptibility testing by disk diffusion assays and minimal inhibitory concentrations (MICs). CFUs was assessed by plating serial dilutions on LB agar. Clinical Laboratory Standards Institute (CLSI) guidelines were followed [21]. MICs were determined by the gradient diffusion method (E-test method) with commercial strips (Biomerieux) as recommended by the supplier (Table 2).

2.4 Whole-genome sequence of A118 clinical strain and A118 transformant cells

"Shotgun" whole-genome sequencing (WGS) was performed using Illumina MiSeq- I, with Nextera XT libraries for sample preparation. De novo assembly was performed with SPADES assembler version 3.1.0 [22], using a pre-assembly approach with Velvet [23]. RAST server was used to predict and annotate open

reading frames [24] and BLAST (version 2.0) software was utilized to confirm the predictions. tRNAscan-SE was used to predict tRNA genes [25]. Contig sets were ordered and oriented with the Mauve Contig Mover, using the ATCC 17978 genome as reference. Genomes were concatenated clone-wise to generate virtual genomes [26]. Sequencing reads were deposited at a local server (http://www.higiene.edu.uy/ddbp/Andres/gtraglia_et_al_2018_data.html)

2.5 Genomic analysis

Sequence analysis was carried out using BLAST (version 2.0) software (http://www.ncbi.nlm.nih.gov/BLAST/). The result was sorted by using the R project software, with a 30% minimum identity, 70% minimum coverage and 1e-5 minimum of E-value. The non--coding sequences inserted into A118 strain were validated with InterProScan. These analyses were done by comparison with protein domains or motifs in the InterProScan database. The genomic schemes were performed by using Circos (http://circos.ca/) and EasyFig softwares (http://mjsull.github.io/Easyfig/). ARG-ANNOT, ISfinder and PHAST softwares were used [27]. BLAST was used to identify resistance genes in *A. baumannii* or *K. pneumoniae* sequences deposited in the GenBank.

2.6 *In-silico* prediction of horizontal gene transfer (HGT) by trees reconciliation

The explicit phylogenetic method was used to analyze potential HGT from one genus to another. Search was based on analysis of topology difference between the phylogenetic trees of gene (protein) clusters and the corresponding phylogenetic species trees (tree reconciliation analysis). To further validate the

observation of HGT event detected by explicit phylogenetic methods, we used the information of HGTree database and the NCBI smart blast tool with a parallel BLASTp search to find the closest matches to high-quality sequences.

The number of genomes for each analysis were selected per the number of genomes into the HGTree framework available.

2.7 Growth curve of recipient cell and transformant cells

A118, A118::VA360, and A118::Kb18 were grown to stationary phase in Luria-Bertani (LB) broth (Fisher BioReagents, Fair Lawn, NJ, USA) with 200 rpm agitation at 37°C. Growth rate curves were generated using a Synergy 2 multi-mode plate reader (BioTek, Winooski, VT, USA) and Gen5 microplate reader software (BioTek), which measured and recorded OD_{600} every 20 minutes. Each condition was tested in triplicates over 24 hours with light agitation at 37°C. Averages of the triplicates from a single trial were used to report the growth rate curve.

2.8 Killing Assay

A118 and VA360 were used to perform the killing assays as described by Weber et al 2015 [28]. In parallel, two other *Acinetobacter* strains were used (*A. baumannii* A42 strain and non-*baumanniii* A47 strain).

3. Results and Discussion

3.1 Natural transformation, genomic analysis and distinctive features of A118::VA360 transformant.

We performed natural transformation assays using the *A. baumannii* A118 strain as acceptor [17] with 200 ng of *K. pneumoniae* VA360 gDNA (accession number NZ_ANGI00000000.2). Transformant isolates were recovered at a transformation frequency of 8.38 X 10^{-7} transformants/CFU (SD± 5.64 x 10-7 transformants/CFU) in LB agar plates containing 1 µg/ml of meropenem (MEM). Colony forming-unit (CFU/ml) was 1.06 x 10^9 CFU/ml (SD± 6.84 x 10^8 CFU/ml). Disk diffusion was used as the screening method to explore additional changes in the resistance phenotype of one of the transformant colonies (A118::VA360). Susceptibility was modified for the β-lactam antibiotics tested, including MEM and imipenem (IPM) (Table 1). Changes in antibiotic susceptibility were confirmed by MIC determination. MEM and IPM MIC values increased 100-fold and 5-fold, respectively in A118::VA360 (Table 2). The whole genome sequences of A118::VA360 and the wild type A118 were obtained and compared. The general features of these draft genomes are summarized in Table 3. The A118::VA360 genome includes 47 DNA fragments (31,807 bp) that are not present in A118 genome. These DNA fragments had a 676 bp size average, with 2,812 bp and 75 bp being the largest and smallest fragments (Figure 1).

We next compared the sequence of these 47 DNA fragments to 5,432 *A. baumannii* genome sequences from GenBank (excluding A118 genome). Twelve out of the 47 DNA fragments were previously identified in *A. baumannii* genomes. Analysis of the fragments acquired in strain A118::VA360 identified mobile genetic elements (IS*Aba14*, IS*Kpn26*, IS*CR1* (IS*91* family), IS*26*, IS*1R* and Tn*3*), antibiotic resistance genes such as *sul1 qacEΔ1* and *aac(6')-Ib-cr* and six genes encoding efflux pumps, seven genes associated with metabolic pathways, hypothetical proteins, and intergenic regions from *K. pneumoniae* VA360 strain (Supplementary table S1). Among the genes associated with metabolic functions were *cutA*, which encodes for a dihydroorotate dehydrogenase [29, 30], *fieF*, which encodes for a ferrous iron efflux system [31, 32], and a fragment *hpaX*, a gene that encodes 4-hydroxyphenylacetate permease, an enzyme associated with 4-hydroxyphenylacetate (4-HPA) metabolism.

It was recently shown that bacterial predation plays a role in DNA acquisition by *A. baylyi* [33]. To test bacterial predation in *A. baumannii*, we determined if A118 cells can lyse *K. pneumoniae* VA360 and *E. coli* MG1655-Rif. Killing assays [28, 34] using A118 as predator and *K. pneumoniae* or *E. coli* as prey showed that *A. baumannii* can kill both prey bacteria (Supplementary figure S1A and B). We are presently performing experiments to determine if the released DNA is incorporated into the predator's genome.

3.2 DNA acquisition and genomic analysis of A118 after transformation with DNA from CRKp Kb18, a strain harboring *bla*$_{KPC}$ *and bla*$_{OXA-23}$

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Since we did not detect bla_{KPC} among the genes acquired by *A. baumannii* A118 after transformation with VA360 gDNA, we carried out another experiment using gDNA from the CRKp strain Kb18, which harbors bla_{KPC} and bla_{OXA-23}. Transformation assays resulted in a frequency 7.17 X 10^{-7} (SD± 1.89 x 10^{-7}) CFU/ml in LB agar plates containing 1 µg/ml of MEM. After an initial susceptibility screening, one colony (A118::Kb18) that showed elevated levels of resistance to all ß-lactams was selected for further studies (Table 1 and 2). The general features of the A118::Kb18 draft genome are summarized in Table 3. Sixty-two new DNA fragments with an average size of 4,331 bp and maximum and minimum fragment sizes of 36,369 bp and 1,042 bp, respectively were identified (Figure 1). These fragments included six antibiotic resistance genes, (bla_{TEM-1}, bla_{OXA-23}, *strA*, *strB,* *aadA1*, *sat2*, and *dfrA1*), four transposons including Tn*7* and Tn*3*, and eight ISs, one of which was identified as IS*Aba125* (Supplementary table S1).

The presence of IS*Aba125 and bla*$_{OXA-23}$ in the gDNA source (Kb18) and in the selected transformant cell, called our attention. This led us to perform a retrospective surveillance to identify the presence of $bla_{OXA-23,}$ IS*Aba125,* and IS*Aba1* in a collection of twenty-two CRKp isolates. Three CRKp strains (Kp16, Kp8, Kp21) were positive for bla_{OXA-23} by PCR and Sanger sequencing. Moreover, bioinformatics analyses searching for shreds of evidence of the presence of $bla_{OXA-23,}$ $bla_{OXA-24,}$ $bla_{OXA-51,}$ $bla_{OXA-58,}$ IS*Aba125,* and IS*Aba1* were performed. With the exception of bla_{OXA-58}, the presence of all the aforementioned genes and IS was observed in *K. pneumoniae* sequences

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deposited in the GenBank database (Supplementary table S2 and S3). The presence of bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-51}, and bla_{OXA-58}, was also investigated in *Enterobacteriaceae* genomes deposited in the GenBank database. In addition to *K. pneumoniae*, we found that only one sequence of *E. coli* and two sequences of *Proteus mirabilis* possessed bla_{OXA-23} (Supplementary table S3). IS*Aba1* and IS*Aba125* were also found among *Enterobacteriaceae* genomes (Supplementary table S3). We found IS*Aba1* in *K. pneumoniae, E. coli, Salmonella enterica* and *Shigella flexneri*. IS*Aba125* was more frequently observed in more diverse bacterial species (Supplementary table S3). Analyzing the genome of 1,777 ESBLs positive *K. pneumoniae* strains, Long et al. observed the presence of OXA genes (OXA-23, OXA-24, OXA-48, and OXA-83) in 23 strains [35]. Five contained OXA-23, and five were positive for OXA-24. Collectively, our results and the present lines of evidence highlight the importance of investigating genes that are frequently reported in certain species that are found in hospitals. Furthermore, a genetic platform that contains several ISs, such as IS*CR2*, ΔIS*CR2,* and IS*1006*, and the genes *strA*, *strB,* and *floR* was also acquired by A118::Kb18. Different genetic structures sharing some of the elements found in A118::Kb18 were also described previously in *K. pneumoniae* (Figure 2).The presence of IS*CR2* was also examined in the collection of our twenty-two CRKp, and six isolates were positive.

A search for the presence of genes and elements usually reported in *Enterobacteriaceae* showed that bla_{KPC}, bla_{TEM-1}, bla_{SHV-2}, $bla_{CTX-M-9}$, $bla_{CTX-M-2}$, $bla_{CTX-M-1}$, IS*CR1* and IS*Ecp1* where all present in at least one *A. baumannii*

genome (Figure 3 and Supplementary table S3-4). In agreement with our observations, Ramirez et al, described the ability of *A. baumannii* strain A118 to gain and maintain a plasmid harboring *bla*$_{CTX-M-2}$ gene, which was previously found in several *P. mirabilis* isolates [36].

Also, four prophages detected in A118::Kb18 were acquired when A118 was transformed with Kb18 gDNA. Interestingly, we identified the insertion of a 36,369 bp putative prophage within a gene that codes for a hypothetical protein upstream of the *tonB* gene cluster, which could interfere with normal gene expression.

The analysis of the A118::Kb18 genome also showed a large number of DNA fragments totaling 87,374 bp that did not show significant homology to any known element. These DNA fragments might be inserted into the genome by homologous or illegitimate recombination. Their in-depth analysis showed that 46,365 out of 87,374 bp have identity to genes with unknown functions or sequences that were identified as intergenic regions of *K. pneumoniae* genomes. However, we have also observed some fragments (n=9) containing genes or cluster of genes related to virulence traits or metabolic processes (Supplementary table S1).

A region of 12,946 bp contained genes related to 4-hydroxyproline uptake and utilization. A comparative assay showed that the A118::Kb18 strain utilized 4-hydroxyproline more efficiently than the wild type A118 (Figure 4).

Additionally, genes encoding unknown proteins were also incorporated, increasing the potential for success of *A. baumannii* as a pathogen.

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3.3 Growth of *A. baumannii* A118 transformant isolates is not impaired.

All three strains A118, A118::VA360, and A118::Kb18 were identical
(Supplementary figure S2) suggesting that unless a DNA fragment is inserted in
certain specific locations, acquisition of long DNA fragments is not detrimental for
growth.

3.4 Supporting evidence of genetic exchange between *A. baumannii* and *K. pneumoniae* by HGT

To further support and validate the gene flow and the interplay between these
two bacterial species, predictive analysis was performed using 15 *A. baumannii*
and 8 *K. pneumoniae* complete genomes from the Genbank (Supplementary
table S5). We determined HGT-acquired genes by phylogenetic tree
reconstruction and reconciliation analysis to predict HGT events. To make this
analysis more stringent we excluded mobile gene elements (such as ISs),
integrons, pseudogenes, phage related sequence and intergenic regions.
We observed an average of 14 (11-18) horizontally transferred genes per
genome from *K. pneumoniae* to *A. baumannii*. For example, the genome of *A. baumannii* strain ZW85-1 showed the presence of 18 horizontally transferred
genes; in comparison, strain AB0057 contained 11 genes that were transferred
(Supplementary table S3).
Considered individually, our *in-silico* analysis predicted that an average of 11 (9-
14) genes with known function (e.g. *rpoN*, *rhtC*, *lpxB*) and two (1-4) genes with
unknown function would be transferred per genome (Supplementary table S3).

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We compared the candidate genes predicted by the *in-silico* tree reconciliation analysis to our experimental analysis of A118::VA360 and A118::Kb18 genomes and found similarities between both of them (some of the genes transferred into A118::VA360 and A118::Kb18 were also present in our *in-silico* analysis). For example, *hpa* was predicted to be transferred into *A. baumannii* ZW85-1 genome. The putative threonine efflux protein (*rhtC*) and the lipid A disaccharide synthetase (*lpxB*) from *K. pneumoniae* was predicted to be transferred into all 15 *A. baumannii* genomes from GenBank. In agreement, *rhtC* and *lpxB* were transferred into the A118::Kb18 transformant. Similarly, the transfer of one putative transcriptional regulator belonging to the MerR family from *K. pneumoniae* was predicted into 12 *A. baumannii* genomes. As expected, MerR was found in the A118::Kb18 transformant.

Next, we assessed the occurrence of reciprocal gene flow between *A. baumannii* to *K. pneumoniae*. Notably, we observed an average of 30 genes (27-34) transferred from *A. baumannii* to *K. pneumoniae*. Among them, an average of 28 genes (25-35) have known function (e.g. *aroB*, *dapE*, *dprA*, *gpml*) and, on average, a range of 1 to 4 genes possess unknown function (hypothetical protein) per genome (Table S5). Considering our gene flow in-silico analysis, gene transfer is bidirectional albeit, *K. pneumoniae* is more prone to acquire genes from *A. baumannii* than vice-versa.

Our results expose the dynamic and frequent exchange of genetic material between two species of Gamma-proteobacteria. Therefore, exchange of genetic

material could be a consequence of the continuous interplay between *A. baumannii* and *K. pneumoniae* in a clinical setting.

3.5 Further evidences of A118 DNA acquisition using other DNA sources

We performed *A. baumannii*'s transformation assays using other DNA sources like *Providencia rettgeri* strain M15758 (harboring bla_{NDM-1}) and the methicillin-resistant *Staphylococcus aureus* "Cordobes" clone (SAC) strain CD. Two colonies resistant to 10 µg/ml of cefotaxime and 200 µg/ml of ampicillin, strains named A118::Pr and A118::SAC, respectively, were selected for whole genome sequencing.

Global analysis confirmed the acquisition of foreign DNA from both DNA sources. The average size of DNA fragments acquired by natural transformation in A118::Pr was 556 bp, ranging from 67 bp to 2,011 bp. We observed DNA acquisition from non-coding sequences (n=3) integrated in intergenic regions of A118 genome, as well as the acquisition of several DNA fragments containing genes related with metabolic pathways (acetyl-CoA acetyltransferase) or oxidative stress (alkyl-hydroperixode reductase) (Supplementary table S1). Strikingly, we found *pilJ* and *pilK* genes from *Salmonella enterica*, both associated with type IV pilus biogenesis in this species[37]. In addition, we found the acquisition of an aminoglycoside resistance gene, *aadB*, preceded by the class 1 integron integrase.

Only four DNA transfer events were observed in the A118::SAC transformant cell. All four events corresponded to non-coding sequences that were found in the intergenic region in the A118 genome (Supplementary table S1). These

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results suggest that a low homology sequence and a great phylogenetic distance between two species plays an important role in DNA acquisition into the *A. baumannii* genome.

As previously performed, tree reconciliation analysis [38] were used to explore the occurrence of HGT events between *A. baumannii* and *S. aureus* using available genomes in Genbank. For this purpose, eight *A. baumannii* genomes and 37 *S. aureus* genomes were used (Supplementary table S5).

We observed the presence of one to two horizontally transferred genes per genome from *S. aureus* to *A. baumannii* (Supplementary table S5). All the predicted transferred genes obtained by *in-silico* analysis possessed a known function (Supplementary table S5).

The occurrence of reciprocal gene flow from *A. baumannii* to *S. aureus* showed an average of two horizontally transferred genes (1-3 genes) from *A. baumannii* to *S. aureus*. Among these genes, all have a known function, such as topoisomerases, phosphopantothenoylcysteine decarboxylases, ligases, and genes involved in capsular polysaccharide synthesis, among others (Table S5). Accordingly, a few HGT events were observed in the experimental assay and through the *in-silico* genome-wide analysis, suggesting infrequent gene flow between *A. baumannii* and *S. aureus*.

We also observed a unique trend in DNA acquisition by *A. baumannii*. This could be explained by a secondary event of homologous recombination after DNA-uptake. A tendency to acquire non-coding DNA fragments over than coding sequences prevailed. Nevertheless, this suggests that the non-coding sequences

could generate a new target for additional recombination events within the *A. baumannii* population. Although, non-homologous recombination mediated by mobile element or illegitimate recombination occurs in *A. baumannii*, fact that was observed in the transformant isolates (A118::Kb18, A118:VA360 and A118:Pr). These results coincide with Dominguez et al. findings that natural transformation in *A. baylyi* ADP1 plays an essential role in the acquisition of mobile genetic elements [39]. Also, those results suggested that the DNA from Gram-negative bacteria served as a preferred source of genetic material than DNA from Gram-positive bacteria, that can ultimately contribute in the evolution of *A. baumannii.*

4. DISCUSSION

Our results show that *A. baumannii* A118 can acquire and integrate DNA from other species into its genome. This process can result in phenotypic modifications including acquisition of antibiotic resistance. Although the experiments described in this work were obtained *in vitro*, the elevated rate of transformation suggests that this mechanism of HGT can be an efficient alternative for adaptation and evolution of *A. baumannii*. *In silico* analysis supports these findings, indicating a "two-direction" genetic flux between Gram negative bacteria found in hospital settings.

Declarations

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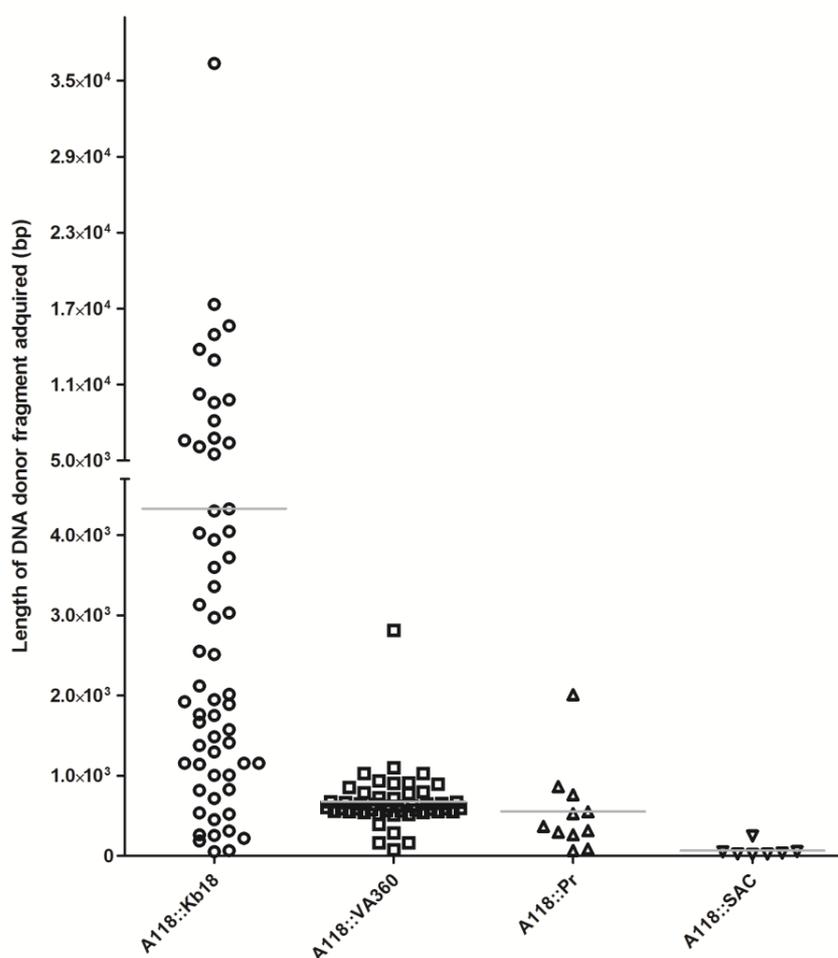
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LEGEND TO FIGURES

Figure 1. Dot plot representing the distribution of DNA-acquired fragment lengths in *A. baumannii* transformant isolates (A118::Kb18, A118::VA360, A118::Pr and A118::SAC). The arithmetic mean was indicated by a line. Circles represent Kb18 acquired-DNA, squares represent VA360 acquired-DNA, triangles represent Pr acquired-DNA, and inverted-triangle represents SAC acquired-DNA.
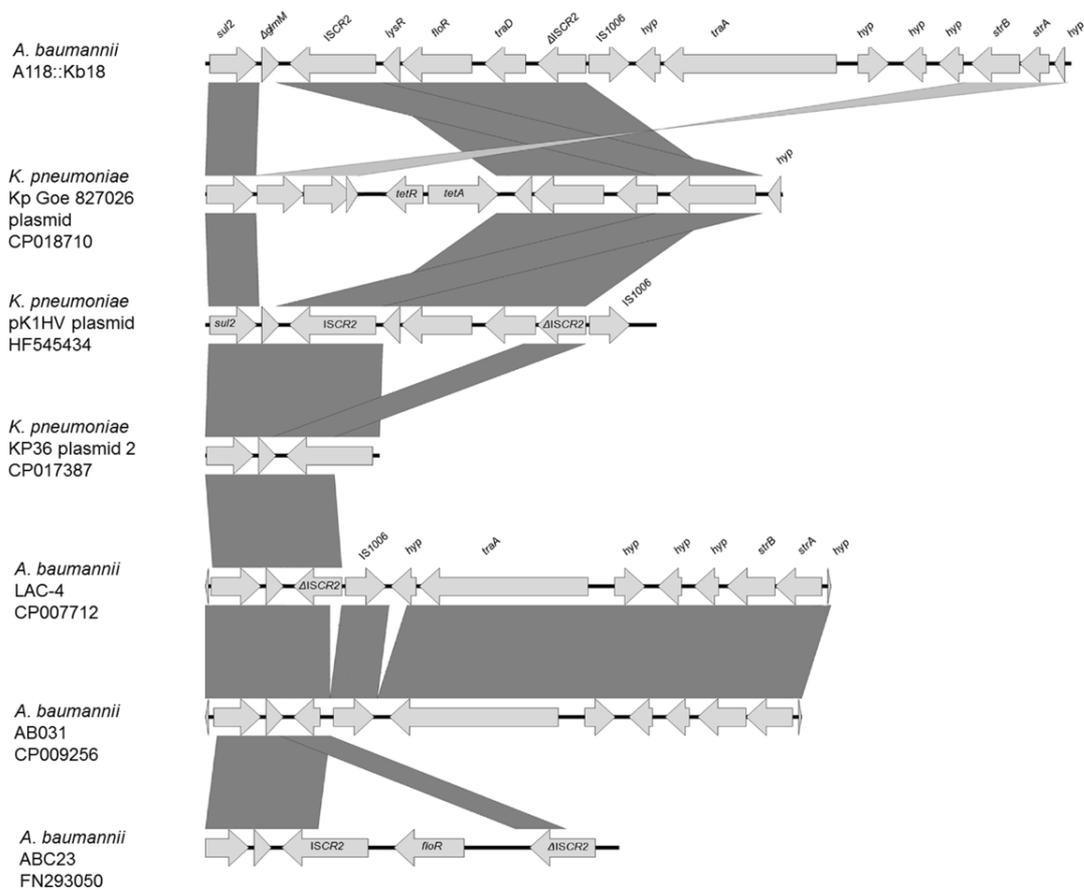
Figure 2. Genetic structure and comparison of IS*CR2* and its associated

antibiotic resistance genes. The genetic structure of A118::Kb18 was compared

with similar genetic structures from *K. pneumoniae*. The graphic representation
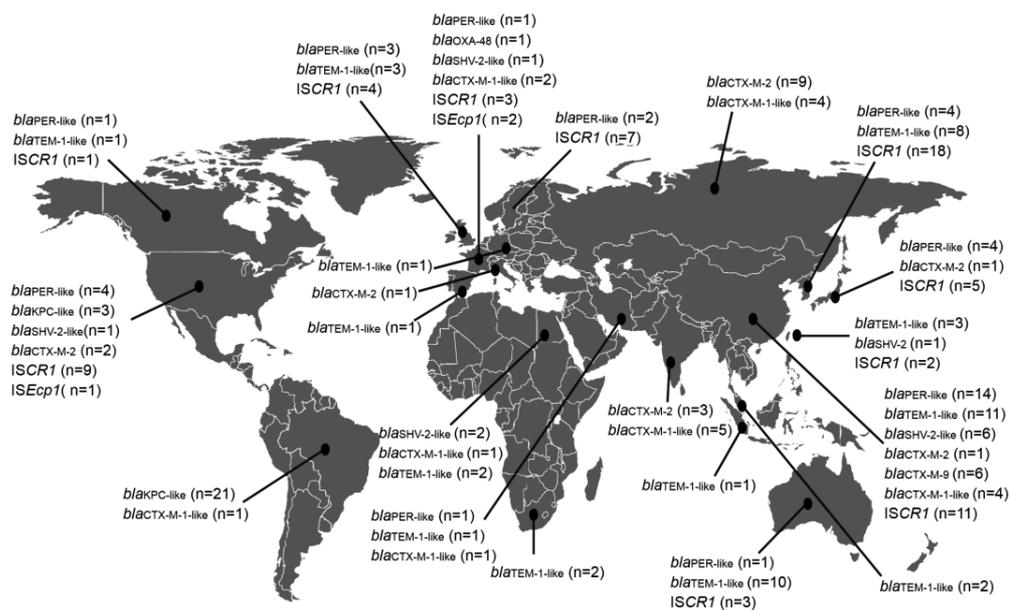
was made using the EasyFig version 2.2.0 software.

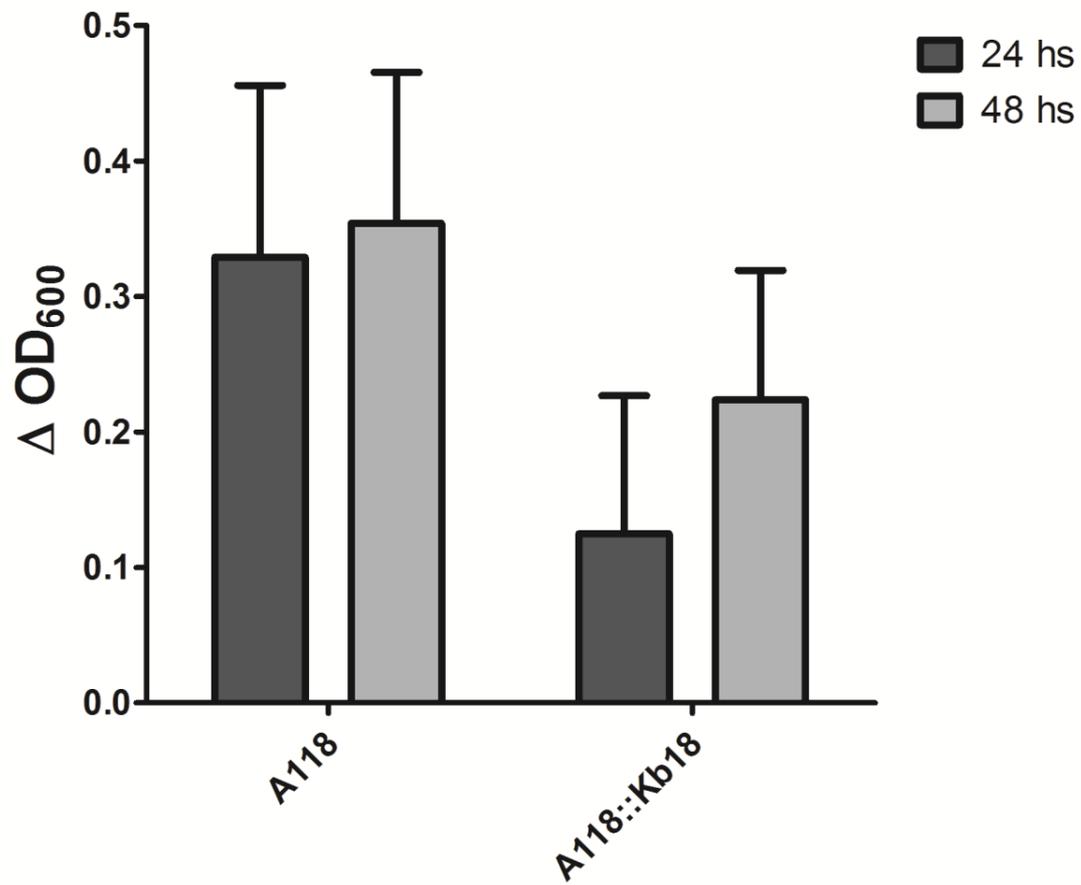**Figure 3.** Global distribution of *Enterobacteriaceae*-associated genes in *A. baumannii* genomes.

Figure 4. Utilization of 4-hydroxyproline of A118::Kb18 compared with the A118 WT strain.

Table 1. Antibiotic susceptibility test (disk diffusion) of A118 and A118 transformed cells with DNA of *K. pneumoniae* strain VA360 and Kp18 (A118::VA360 and A118::Kb18)

	DISK DIFFUSION (halo of inhibition, mm)													
	AMK	GEN	AMP	AMC	CEF	FEP	FOX	IPM	MEM	CIP	NAL	NOR	SXT	TET
A118	24	22	10	12	6	25	11	20	26	30	20	21	20	24
A118::VA360	22	25	8.5	10	6	25	8.5	11	12	26	16	22	11	25
A118::Kb18	24	24	6	6	6	13	6	12	7	26	20	20	18	24

AMK, amikacin, GEN, gentamicin, AMP, ampicillin, AMC, amoxicillin/clavulanic acid, CEF, cefalotin, FEP, cefepime, FOX, cefoxitin, IPM, imipenem, MEM, meropenem, CIP, ciprofloxacin, NAL, nalidixic acid, NOR, norfloxacin, SXT, trimethoprim/sulfamethoxazole, TET, tetracycline.

Table 2. Antibiotic susceptibility test of A118 and A118 transformed with DNA of *K. pneumoniae* strains VA360 and Kb18.

	MIC (ug/ml)				
	GEN	CAZ	CTX	IPM	MEM
A118	0.25	1.5	4	0.25	0.125
A118::VA360	0.5	2	8	1.5	16
A118::Kp18	0.25	8	16	16	16

GEN, gentamicin; CAZ, ceftazidime; CTX, cefotaxime; IPM, imipenem; MEM, meropenem.

Table 3. General features of A118, A118::VA60 and A118::Kb18

Strain	A118	A118::VA360	A118::Kb18
Size (bp[a])	3,853,242	3,885,049	4,058,089
G+C contents (%)	38.69	38,81	38.65
ORFs[b]	3,589	3,819	4,058
tRNA	60	49	72

[a]bp, base pairs.
[b]ORFs, Open reading frames.