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

German M. Traglia[a,1], Kori Place[b,1], Cristian Dotto[c], Jennifer S. Fernandez[b], Sabrina Montaña[c], Camila dos Santos Bahiense[b], Alfonso Soler-Bistue[d], Andres Iriarte[e], Federico Perez[f], Marcelo E. Tolmasky[b], Robert A. Bonomo[f], Roberto G. Melano[g], María Soledad Ramírez[b,*]

[a] *Laboratorio de Bacteriología Clínica, Departamento de Bioquímica Clínica, Hospital de Clínicas José de San Martín, Facultad de Farmacia y Bioquímica, Buenos Aires, Argentina*
[b] *Center for Applied Biotechnology Studies, Department of Biological Science, College of Natural Sciences and Mathematics, California State University Fullerton, Fullerton, California, USA*
[c] *Instituto de Microbiología y Parasitología Médica (IMPaM, UBA-CONICET), Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina*
[d] *Instituto de Investigaciones Biotecnológicas 'Dr Rodolfo A. Ugalde', Instituto Tecnológico de Chascomús, CONICET, Universidad Nacional de San Martín, Buenos Aires, Argentina*
[e] *Laboratorio de Biología Computacional, Dpto. de Desarrollo Biotecnológico, Instituto de Higiene, Facultad de Medicina, Universidad de la República, Montevideo, Uruguay*
[f] *Research Service, Louis Stokes Cleveland Department of Veterans Affairs Medical Center, Cleveland, Ohio, USA*
[g] *Public Health Ontario Laboratories, Toronto, Ontario, Canada*

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ABSTRACT

The human pathogen *Acinetobacter baumannii* possesses high genetic plasticity and frequently acquires antimicrobial resistance genes. Here we investigated the role of natural transformation in these processes. Genomic DNA from different sources, including from carbapenem-resistant *Klebsiella pneumoniae* strains, was mixed with *A. baumannii* A118 cells. Selected transformants were analysed by whole-genome sequencing. In addition, bioinformatics analyses and in silico gene flow prediction were also performed to support the experimental results. Transformant strains included some that became resistant to carbapenems or changed their antimicrobial susceptibility profile. Foreign DNA acquisition was confirmed by whole-genome analysis. The acquired DNA most frequently identified corresponded to mobile genetic elements, antimicrobial resistance genes and operons involved in metabolism. Bioinformatics 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 multidrug-resistant strains.

1. Introduction

In contrast to Eukarya where genetic duplication plays a major 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 have received far less attention. In particular, transformation by natural competence is a HGT mechanism in which bacteria take up DNA directly from the environment and incorporate it into their genomic repertoire [4]. Increasing evidence indicates that it is an under-recognised mechanism for the acquisition of antimicrobial resistance genes [4].

The recent rise in multidrug-resistant (MDR) bacterial strains in clinics alarms both scientists and government agencies [5,6]. *Acinetobacter baumannii*, a pathogen associated with high-mortality MDR infections [7,8], easily acquires antimicrobial resistance determinants and is highly adaptable to unfavourable conditions. These properties may be due to extreme genome plasticity combined with mechanisms of HGT such as conjugation, transformation or the recently described predation [9]. Previous studies have shown high variability in genome organisation among *Acinetobacter* strains

* Corresponding author. Present address: M.S. Ramírez, Department of Biological Science, California State University Fullerton, 800 N. State College Blvd., Fullerton, CA 92831, USA.
E-mail address: msramirez@fullerton.edu (M.S. Ramírez).
[1] These two authors contributed equally to this work.

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as well as foreign DNA in their genomes, suggesting exogenous acquisition of genetic traits [10,11]. Recent reports that members of the Enterobacteriaceae harbour resistance determinants associated with *Acinetobacter* support an active exchange of DNA between these bacteria [12–16].

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

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 *Klebsiella pneumoniae* (CRKp) strains, namely VA360 (bla_{TEM-1}, bla_{KPC-2}, bla_{SHV-11} and bla_{SHV-12}) [18] and Kb18 (bla_{KPC} and bla_{OXA-23}) were also used. The 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 methicillin-resistant *Staphylococcus aureus* 'Cordobes' clone (SAC) (*mecA*) [19,20]. A total of 22 CRKp 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 for IS*Kpn1*, respectively.

2.2. Standard molecular biology techniques

DNA extraction was performed using commercial kits [Wizard® Genomic DNA Purification Kit (Promega Corp., Madison, WI) or MasterPure™ DNA Purification Kit (Epicentre Biotechnologies, Madison, WI)]. PCR was performed using Zymo*Taq*™ PreMix (Zymo Research Corp., Irvine, CA).

2.3. Natural transformation assays

Standard natural transformation assays were performed as previously described [5,17]. Briefly, 200 ng of DNA was added to a mix containing 50 µL of fresh Luria–Bertani (LB) broth (Thermo Fisher Scientific, Fair Lawn, NJ) and 50 µL of *A. baumannii* A118 culture in stationary phase. The mix was incubated at 37°C for 1 h and was then plated on selective agar plates containing adequate antibiotic; 1 µg/mL meropenem (MEM), 10 µg/mL cefotaxime (CTX) or 200 µg/mL ampicillin (AMP) were used for selection of transformant clones. Negative controls were included in each assay. To score transformation events, MEM-, CTX- or AMP-resistant colonies were counted and were confirmed by antimicrobial susceptibility testing by disk diffusion assay and minimum inhibitory concentration (MIC) determination. CFUs was assessed by plating serial dilutions on LB agar. Clinical and Laboratory Standards Institute (CLSI) guidelines were followed [21]. MICs were determined by the gradient diffusion (Etest) method with commercial strips (bioMérieux, Marcy-l'Étoile, France) as recommended by the manufacturer.

2.4. Whole-genome sequencing (WGS) of Acinetobacter baumannii A118 clinical strain and A118 transformant cells

Shotgun WGS was performed using an Illumina MiSeq I System (Illumina Inc., San Diego, CA) with Nextera XT libraries for sample preparation. De novo assembly was performed with SPAdes Assembler v.3.1.0 [22] using a pre-assembly approach with Velvet [23]. The RAST (Rapid Annotation using Subsystem Technology) server was used to predict and annotate open reading frames [24], and BLAST v.2.0 software (http://www.ncbi.nlm.nih.gov/BLAST/) was utilised 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 *A. baumannii* 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 v.2.0 software. The result was sorted using R project software with a 30% minimum identity, 70% minimum coverage and 1e-5 minimum of E-value. The non-coding sequences inserted into strain A118 were validated with InterProScan. These analyses were done by comparison with protein domains or motifs in the InterProScan database. The genomic schemes were performed using Circos (http://circos.ca/) and Easyfig software (http://mjsull.github.io/Easyfig/). ARG-ANNOT, ISfinder and PHAST were also used [27]. BLAST was used to identify resistance genes in *A. baumannii* or *K. pneumoniae* sequences deposited in GenBank.

2.6. In silico prediction of horizontal gene transfer by tree reconciliation

The explicit phylogenetic method was used to analyse potential HGT from one genus to another. The search was based on analysis of topology differences between the phylogenetic trees of gene (protein) clusters and the corresponding phylogenetic species trees (tree reconciliation analysis). To further validate the observation of a HGT event detected by explicit phylogenetic methods, information in the HGTree database and the NCBI Smart BLAST tool with a parallel BLASTp search were used to find the closest matches to high-quality sequences.

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

2.7. Growth curve of recipient cell and transformant cells

Strains A118, A118::VA360 and A118::Kb18 were grown to stationary phase in LB broth with 200 rpm agitation at 37°C. Growth rate curves were generated using a Synergy™ 2 Multi-Mode Microplate Reader (BioTek, Winooski, VT) and Gen5™ Microplate Reader Software (BioTek), which measured and recorded the optical density at 600 nm (OD_{600}) every 20 min. Each condition was tested in triplicate over 24 h with light agitation at 37°C. The mean of triplicates from a single trial was used to report the growth rate curve.

2.8. Killing assay

Strains A118 and VA360 were used to perform killing assays as described previously [28]. Two other *Acinetobacter* strains (*A. baumannii* A42 strain and non-*baumannii* A47 strain) were used in parallel.

3. Results and discussion

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

Natural transformation assays were performed using *A. baumannii* A118 strain as recipient [17] with 200 ng of *K. pneumoniae* VA360 gDNA (GenBank accession no. NZ_ANGI00000000.2).

Table 1
Antimicrobial susceptibility testing by the disk diffusion method of *Acinetobacter baumannii* A118 and A118 cells transformed with DNA of *Klebsiella pneumoniae* strains VA360 and Kp18 (A118::VA360 and A118::Kb18)

Strain	Zone 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
Antimicrobial susceptibility testing by Etest of *Acinetobacter baumannii* A118 and A118 cells transformed with DNA of *Klebsiella pneumoniae* strains VA360 and Kb18 (A118::VA360 and A118::Kp18)

Strain	MIC (μg/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

MIC, minimum inhibitory concentration; GEN, gentamicin; CAZ, ceftazidime; CTX, cefotaxime; IPM, imipenem; MEM, meropenem.

Table 3
General features of *Acinetobacter baumannii* strain A118 and A118 cells transformed with DNA of *Klebsiella pneumoniae* strains VA360 and Kb18 (A118::VA360 and A118::Kp18)

Strain	A118	A118::VA360	A118::Kb18
Size (bp)	3 853 242	3 885 049	4 058 089
G+C contents (%)	38.69	38.81	38.65
ORFs	3589	3819	4058
tRNA	60	49	72

ORF, open reading frame.

Transformant isolates were recovered at a mean ± standard deviation transformation frequency of $8.38 \times 10^{-7} \pm 5.64 \times 10^{-7}$ transformants/CFU on LB agar plates containing 1 μg/mL MEM. The number of CFU/mL was $1.06 \times 10^{9} \pm 6.84 \times 10^{8}$ 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 (Table 1). Changes in antimicrobial susceptibility were confirmed by MIC determination. The MICs of MEM and imipenem increased 128-fold and 6-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 summarised in Table 3. The A118::VA360 genome includes 47 DNA fragments (31 807 bp) that are not present in the A118 genome. These DNA fragments had a mean size of 676 bp, with 2812 bp and 75 bp being the largest and smallest fragments (Fig. 1).

Next, the sequences of these 47 DNA fragments were compared with 5432 *A. baumannii* genome sequences from GenBank (excluding the A118 genome). Of the 47 DNA fragments, 12 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*], antimicrobial resistance genes such as *sul1, qacEΔ1* and *aac(6′)-Ib*-cr and six genes encoding efflux pumps, and 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* encoding a dihydroorotate dehydrogenase [29,30], *fieF* encoding a ferrous-iron efflux system [31,32] and a fragment of *hpaX*, a gene that encodes 4-hydroxyphenylacetate permease, an enzyme associated with 4-hydroxyphenylacetate metabolism.

It was recently shown that bacterial predation plays a role in DNA acquisition by *Acinetobacter baylyi* [33]. To test bacterial predation in *A. baumannii*, we determined whether *A. baumannii* A118 cells can lyse *K. pneumoniae* VA360 and *Escherichia 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 Fig. S1). We are presently performing experiments to determine whether the released DNA is incorporated into the predator's genome.

3.2. DNA acquisition and genomic analysis of Acinetobacter baumannii A118 after transformation with DNA from carbapenem-resistant Klebsiella pneumoniae Kb18, a strain harbouring bla_{KPC} and blaOXA-23

Since bla_{KPC} was not detected among the genes acquired by *A. baumannii* A118 after transformation with VA360 gDNA, another experiment was carried out using gDNA from CRKp strain Kb18, which harbours bla_{KPC} and bla_{OXA-23}. Transformation assays resulted in a frequency $7.17 \times 10^{-7} \pm 1.89 \times 10^{-7}$ CFU/mL on LB agar plates containing 1 μg/mL MEM. After an initial susceptibility screening, one colony (A118::Kb18) that showed elevated levels of resistance to all β-lactams was selected for further studies (Tables 1 and 2). The general features of the A118::Kb18 draft genome are summarised in Table 3.

A total of 62 new DNA fragments with a mean size of 4331 bp and maximum and minimum fragment sizes of 36 369 bp and 1042 bp, respectively, were identified (Fig. 1). These fragments included seven antimicrobial resistance genes (bla_{TEM-1}, bla_{OXA-23}, *strA, strB, aadA1, sat2* and *dfrA1*), four transposons including Tn*7* and Tn*3*, and eight insertion sequences, 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 and led us to perform a retrospective surveillance to identify the presence of bla_{OXA-23}, IS*Aba125* and IS*Aba1* in a collection of 22 CRKp isolates. Three CRKp strains (Kp16, Kp8 and 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 insertions sequences was observed in *K. pneumoniae* sequences deposited in the GenBank database (Supplementary Tables 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}

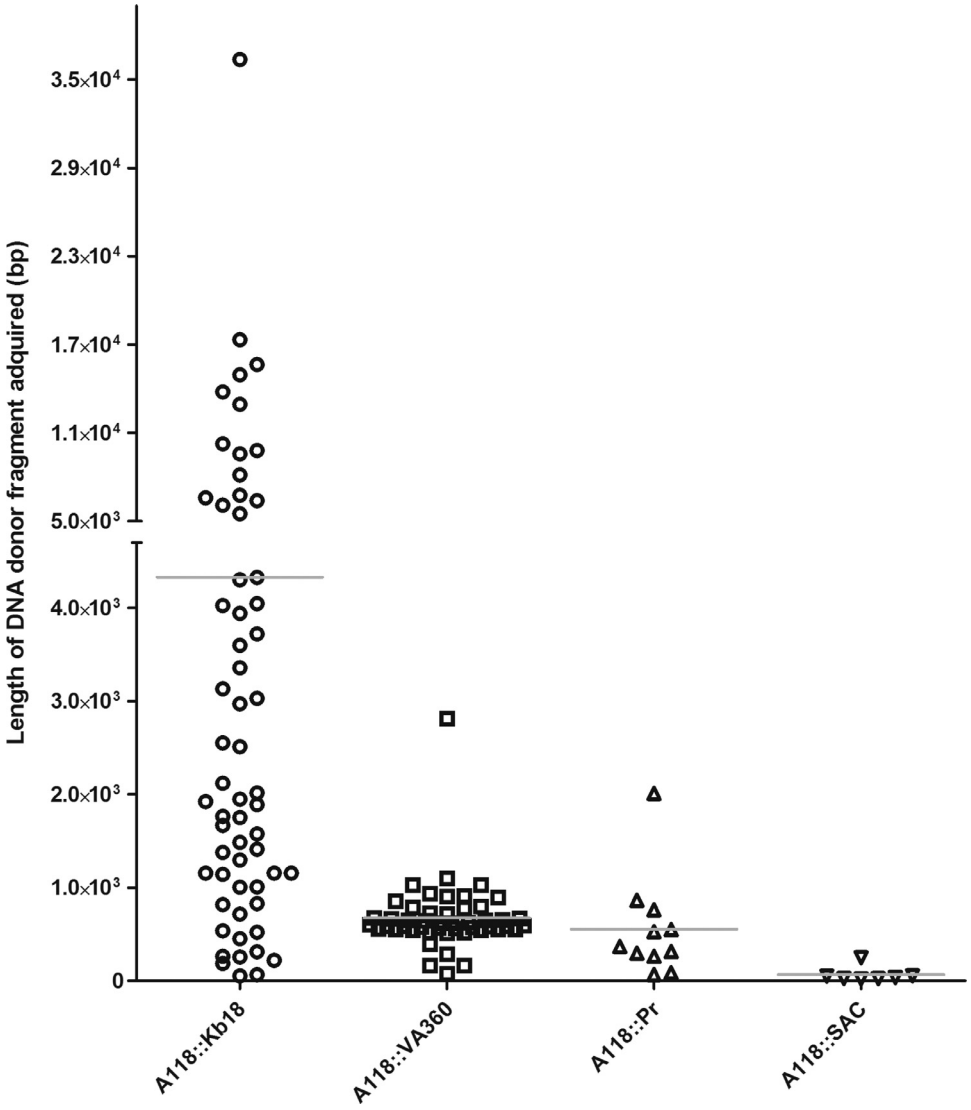

Fig. 1. Dot plot representing the distribution of DNA-acquired fragment lengths in *Acinetobacter baumannii* A118 transformant isolates (A118::Kb18, A118::VA360, A118::Pr and A118::SAC). The mean is indicated by a line. Circles represent Kb18 acquired-DNA, squares represent VA360 acquired-DNA, triangles represent Pr acquired-DNA, and inverted-triangles represent SAC acquired-DNA. Kb18, carbapenem-resistant *Klebsiella pneumoniae* (CRKp) strain (bla_{KPC} and bla_{OXA-23}); VA360 CRKp strain (bla_{TEM-1}, bla_{KPC-2}, bla_{SHV-11} and bla_{SHV-12}); Pr, *Providencia rettgeri* strain M15758 (bla_{NDM-1}); SAC, methicillin-resistant *Staphylococcus aureus* 'Cordobes' clone (*mecA*).

(Supplementary Table S3). IS*Aba1* and IS*Aba125* were also found among Enterobacteriaceae genomes (Supplementary Table S3). IS*Aba1* was found in *K. pneumoniae, E. coli, Salmonella enterica* and *Shigella flexneri*. IS*Aba125* was more frequently observed in more diverse bacterial species (Supplementary Table S3).

Analysing the genome of 1777 extended-spectrum β-lactamase (ESBL)-positive *K. pneumoniae* strains, Long et al. observed the presence of OXA genes (bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-48} and bla_{OXA-83}) in 23 strains [35]. Five strains contained bla_{OXA-23} and five were positive for bla_{OXA-24}. Collectively, the results of the current study and the present lines of evidence highlight the importance of investigating genes that are frequently reported in certain species found in hospitals. Furthermore, a genetic platform that contains several insertion sequences, 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* (Fig. 2).The presence of IS*CR2* was also examined in the collection of 22 CRKp, of which 6 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 (Fig. 3; Supplementary Tables S3 and S4). In agreement with our observations, Ramírez et al. described the ability of *A. baumannii* strain A118 to gain and maintain a plasmid harbouring the $bla_{CTX-M-2}$ 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, the insertion of a 36 369-bp putative prophage within a gene that codes for a hypothetical protein upstream of the *tonB* gene cluster was identified, which could interfere with normal gene expression.

Analysis of the A118::Kb18 genome also showed a large number of DNA fragments totalling 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 of 87 374 bp have identity with genes with unknown functions or sequences that were identified as intergenic regions of *K. pneumoniae*

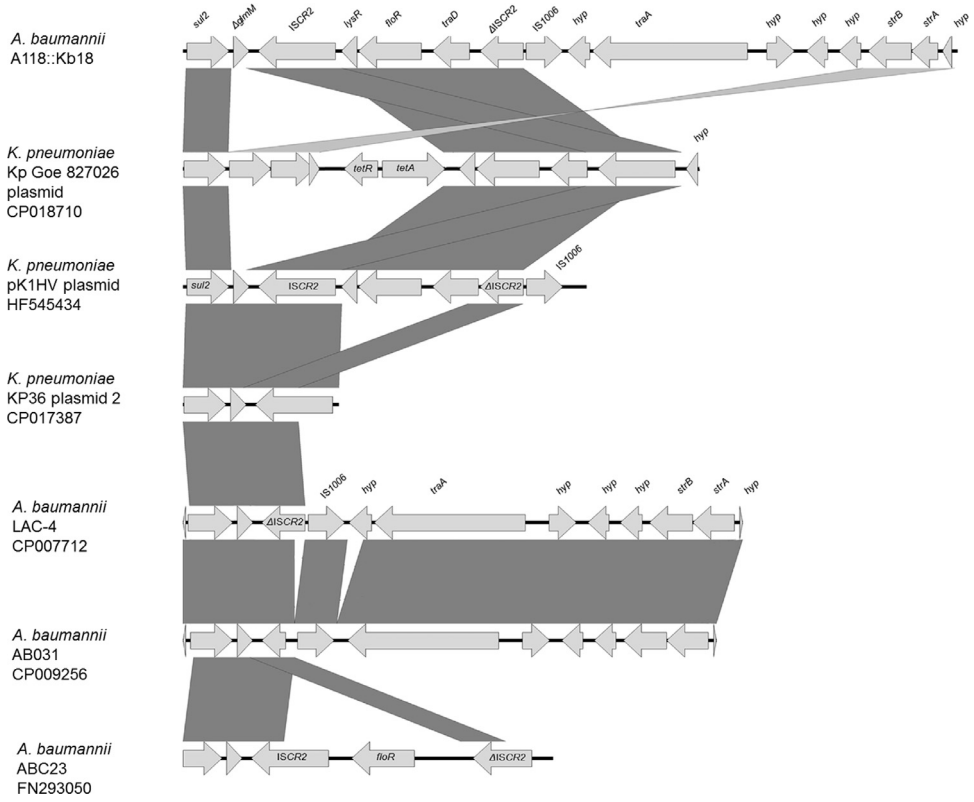

Fig. 2. Genetic structure and comparison of IS*CR2* and its associated antimicrobial resistance genes. The genetic structure of transformant A118::Kb18 was compared with similar genetic structures from *Klebsiella pneumoniae*. The graphical representation was made using Easyfig v.2.2.0.

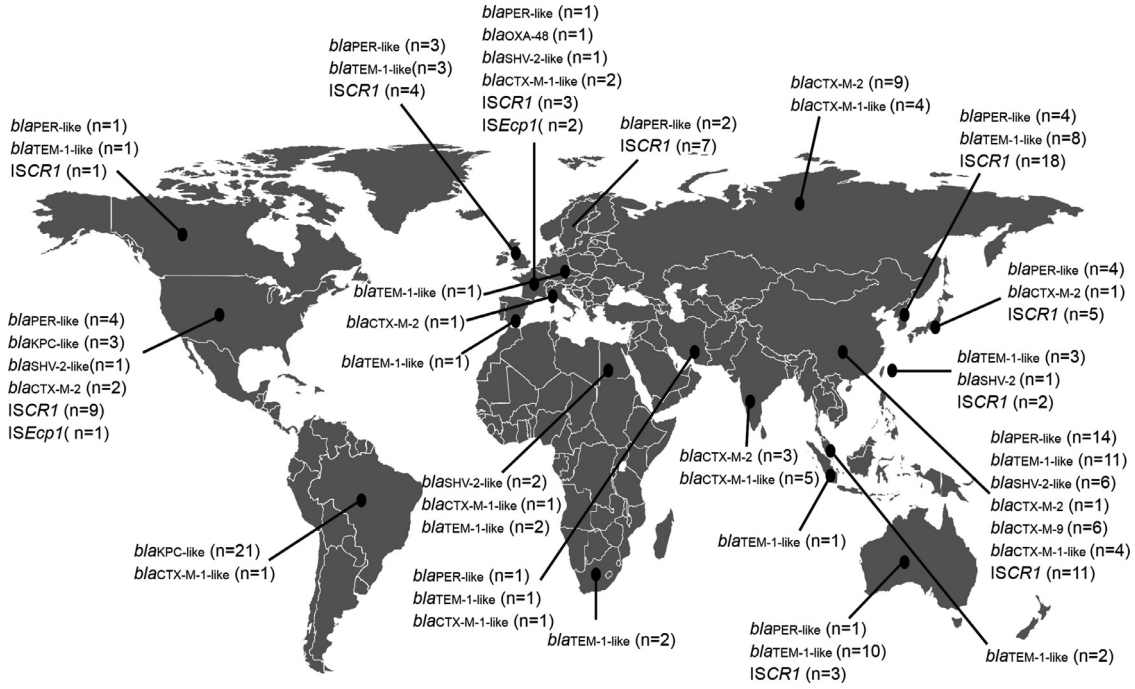

Fig. 3. Global distribution of Enterobacteriaceae-associated genes in *Acinetobacter baumannii* genomes.

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 utilisation. A comparative assay showed that A118::Kb18 strain utilised 4-hydroxyproline more efficiently than the wild-type A118 (Fig. 4).

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

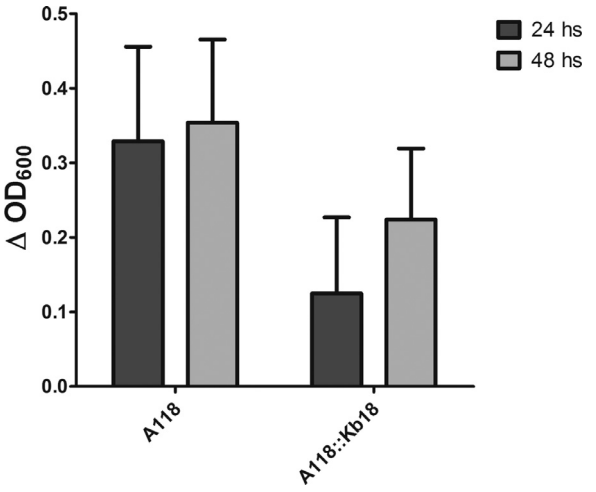

Fig. 4. Utilisation of 4-hydroxyproline of transformant A118::Kb18 compared with the *Acinetobacter baumannii* A118 wild-type strain. OD_{600}, optical density at 600nm.

3.3. Growth of Acinetobacter baumannii A118 transformant isolates is not impaired

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

3.4. Supporting evidence of genetic exchange between Acinetobacter baumannii and Klebsiella pneumoniae by horizontal gene transfer

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 GenBank (Supplementary Table S5). HGT-acquired genes were determined by phylogenetic tree reconstruction and reconciliation analysis to predict HGT events. To make this analysis more stringent, mobile gene elements (such as insertion sequences), integrons, pseudogenes, phage-related sequences and intergenic regions, were excluded.

A mean of 14 (11–18) horizontally transferred genes per genome from *K. pneumoniae* to *A. baumannii* were observed. 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, the in silico analysis predicted that a mean of 11 (9–14) genes with known function (e.g. *rpoN, rhtC, lpxB*) and 2 (1–4) genes with unknown function would be transferred per genome (Supplementary Table S3).

The candidate genes predicted by in silico tree reconciliation analysis were compared with the experimental analysis of A118::VA360 and A118::Kb18 genomes and similarities were found between both of them (some of the genes transferred into A118::VA360 and A118::Kb18 were also present in the in silico analysis). For example, *hpa* was predicted to be transferred into the *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, transfer of one putative transcriptional regulator belonging to the MerR family from *K. pneumoniae* was predicted in 12 *A. baumannii* genomes. As expected, MerR was found in the A118::Kb18 transformant.

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

These results expose the dynamic and frequent exchange of genetic material between two species of Gammaproteobacteria. 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 evidence of Acinetobacter baumannii A118 DNA acquisition using other DNA sources

Transformation assays of *A. baumannii* were performed using other DNA sources such as *P. rettgeri* strain M15758 (harbouring bla_{NDM-1}) and the methicillin-resistant *S. aureus* 'Cordobes' clone (SAC). Two colonies resistant to 10 μg/mL CTX and 200 μg/mL AMP, strains named A118::Pr and A118::SAC, respectively, were selected for WGS.

Global analysis confirmed the acquisition of foreign DNA from both DNA sources. The mean size of DNA fragments acquired by natural transformation in A118::Pr was 556 bp, ranging from 67 bp to 2011 bp. DNA acquisition from non-coding sequences ($n = 3$) integrated in intergenic regions of the A118 genome, and the acquisition of several DNA fragments containing genes related to metabolic pathways (acetyl-CoA acetyltransferase) or oxidative stress (alkyl hydroperoxide reductase) was observed (Supplementary Table S1). Strikingly, *pilJ* and *pilK* genes from *S. enterica* were found, both associated with type IV pilus biogenesis in this species [37]. In addition, the acquisition of an aminoglycoside resistance gene (*aadB*) preceded by a class 1 integron integrase was found.

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 results suggest that a low sequence homology 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] was used to explore the occurrence of HGT events between *A. baumannii* and *S. aureus* using genomes available in GenBank. For this purpose, 8 *A. baumannii* genomes and 37 *S. aureus* genomes were used (Supplementary Table S5).

The presence of one to two horizontally transferred genes per genome from *S. aureus* to *A. baumannii* was observed (Supplementary Table S5). All of 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 a mean of 2 (1–3) horizontally transferred 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 (Supplementary 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*.

A unique trend in DNA acquisition by *A. baumannii* was also observed. This could be explained by a secondary event of homologous recombination after DNA uptake. A tendency to acquire non-coding DNA fragments rather 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 a mobile element or illegitimate recombination occurs in *A. baumannii*, a fact that was observed in the transformant isolates (A118::Kb18, A118:VA360 and A118:Pr). These results coincide with the findings of Domingues et al. that natural transformation in *A. baylyi* ADP1 plays an essential role in the acquisition of mobile genetic elements [39]. Also, those results suggested that DNA from Gram-negative bacteria served as a preferred source of genetic material than DNA from Gram-positive bacteria, which can ultimately contribute in the evolution of *A. baumannii.*

4. Discussion

These 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 antimicrobial resistance. Although the experiments described in this work were performed 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.

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Competing interests

None declared.

Ethical approval

Not required.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijantimicag.2018.12.013.

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