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Title: Class 1 integrons in *Acinetobacter baumannii*: a weak expression of gene cassettes to counterbalance the lack of LexA-driven integrase repression

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Running head : Lower expression of integron gene cassettes in *A. baumannii*

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Abstract

Integrans are able to recruit resistance genes through integrase-driven recombination events that are regulated by the bacterial SOS response and require the repressor LexA. Class 1 integrans genes are expressed from a common promoter, Pc, of which at least 5 predominant variants, classified from weak to strong, have been described. In *Escherichia coli*, there is an intertwined regulation between gene cassette expression and integrase activity: the stronger the promoter is, the weaker the integrase is. Class 1 integrans have been frequently described in *Acinetobacter baumannii*. However, *Acinetobacter* spp. lack the LexA repressor suggesting that the integrase is constitutively expressed. We characterized the integron content of 83 clinical and environmental *A. baumannii* strains. We found a predominance of Pc variants described as strong in *E. coli*. The Pc expression level was 2 to 4-fold lower in *A. baumannii* than in *E. coli*, and the diversity of the gene cassette array was low. In *A. baumannii* integrans with a PcS promoter might have been selected to allow a sufficient resistance level while avoiding the toxicity of a highly active integrase. Furthermore, a transcriptional interference between PcS and PintI1 (as shown in *E. coli*) may limit the expression of the integrase and thus counterbalance the lack of LexA-driven integrase repression to prevent the cost of the integrase.

Key words: *Acinetobacter baumannii*, integron, cassette promoter, PcS, integrase, regulation

Background

The genus *Acinetobacter*, and principally *Acinetobacter baumannii-calcoaceticus* complex, has emerged over the past two decades as a cause of both nosocomial and community-acquired infections. The WHO lists *A. baumannii* among critical antibiotic-resistant "priority pathogens" (<http://www.who.int/mediacentre/news/releases/2017/bacteria-antibiotics-needed/en/>). Treatment of *Acinetobacter* infections is increasingly difficult, as these bacteria are intrinsically resistant to multiple antimicrobial agents and have a remarkable ability to adapt rapidly to environmental selection pressure, acquiring new resistance determinants through multiple mechanisms [1]. *A. baumannii* isolates resistant to all commonly used antimicrobials cause near-intractable infections [2]. Most clinical isolates belong to two main clones, named GC1 and GC2, which are distributed worldwide [3,4]. Clones GC1 and GC2 are often multidrug-resistant [3,5]. They carry genes that encode resistance to antibiotics or heavy metals and are located in genomic resistance islands [6] named AbaR in GC1 and AbGRI1, AbGRI2 and AbGRI3 in GC2. AbaR and AbGRI1 are inserted in the *comM* gene encoding an ATPase [7]. AbGRI1, AbGRI2 and AbGRI3 have different structures and contain different resistance genes than AbaR. AbGRI2 and AbGRI3 are bounded by two copies of IS26 [8]. AbaR contains a 16.3-kb backbone transposon (Tn6019) interrupted by a large composite transposon that contains a variable region comprising antibiotic resistance genes, including a class 1 integron, bounded by copies of Tn6018 [9].

Integrations play a major role in the dissemination of multidrug resistance among Gram-negative bacteria, through their ability to capture and express resistance genes embedded within cassettes [10]. A wide variety of gene cassettes encoding resistance to nearly all antibiotic families have been described [11]. Integration and excision of gene cassettes are driven by the integron's integrase, through site-specific recombination (Figure 1). Moreover, the IntI1 integrase is regulated by the SOS response, which is a regulatory network controlled by the transcriptional repressor LexA [12].

Several classes of integron have been described, based on the integrase amino acid sequence. Class 1 integrons predominate in clinical settings. Gene cassettes are composed of an open reading frame and a recombination site *attC*. They are usually promoterless and are expressed from a common promoter, Pc, located within the integrase gene sequence. Several Pc promoter variants of different strengths have been identified in class 1 integrons. The five most frequent are the strong variants PcS, PcH2 and PcW_{TGN-10}, and the weak variants PcH1 and PcW [13]. A predominance of weak Pc variants has been reported in clinical *Escherichia coli* isolates [14,15], poultry-meat *E. coli* strains [16] and wastewater *Enterobacteriaceae* [17].

Multidrug resistance in *A. baumannii* has been associated with class 1 integrons [18–21] but no data have been available on the Pc distribution in this species. Furthermore, *Acinetobacter* lacks the LexA protein [22,23] consequently LexA repression system, suggesting that IntI1 might be constitutively expressed.

We thus examined the distribution and strength of class 1 integron Pc promoters in class 1 integron-containing *A. baumannii*, based on *in silico* and experimental analysis of clinical and environmental isolates. We characterized the integron content of the isolates and found a predominance of strong Pc variants. Interestingly, we showed that the Pc expression level was 2 to 4 times lower in *A. baumannii* than in *E. coli*, and that the diversity of the gene cassette array was low.

2. Materials and methods

2.1 Strains

We studied a collection of 83 class 1 integron-containing *A. baumannii* isolates of clinical (n=75) or environmental (n=8) origin (Table 1). We had access either to the strains (from French hospitals and

Cameroonian hospital effluents) or to total DNA (bioMerieux DNA collection, 3 hospital strains and 3 hospital effluent strains).

2.2 Plasmid construction

To study the strength of Pc variants in *A. baumannii*, we constructed plasmids derived from those used by Jové *et al.* [13], into which we cloned an origin of replication allowing plasmid replication in both *A. baumannii* and *E. coli* (Table 1). We used the reporter plasmid pSU38 Δ totlacZ and three derived plasmids in which the Pc promoter was inserted in transcriptional fusion with the reporter gene *lacZ*. We inserted the replicon RSF1010, obtained by *EcoR*I restriction of the pAL4000 vector [24], in the *EcoR*I site of the different plasmids containing Pc variants, namely pPcS, pPcW and pPcW_{TGN-10}, to obtain pSU38 Δ totlacZRSF1010, pPcSRSF1010, pPcWRSF1010 and pPcW_{TGN-10}RSF1010 (Table 1). *A. baumannii* clinical strain CM148 and *E. coli* MG1656 were transformed with the recombinant plasmid.

The primers used in this study are listed in Table S1 (supplementary data).

2.3 DNA extraction

DNA was extracted for PCR and Sanger sequencing by using the QIAamp® DNA Mini Kit (QIAGEN), following the manufacturer's instructions. DNA for next-generation sequencing (NGS) was extracted by using the SaMag™ Bacterial DNA extraction kit and the SaMag™-12 system (Sacace biothechnologies, Como, Italy).

2.4 Plasmid copy number quantification

We used real-time PCR quantification to determine the number of plasmid copies per strain directly from a bacteria suspension. For each sample, we quantified the *lacZ* target gene and the 16S rRNA gene. Real-time PCR was performed on a Mx3005P (Stratagene) device in a final volume of 25 μ L, using PerfeCTa® SYBR® Green SuperMix (Quanta Biosciences) and suitable primers (for *lacZ*:

lacZ LC4 / lacZ LC5 at a final concentration of 0.4 μ M, for 16S rRNA: 518R and 331F at a final concentration of 0.2 μ M). The mixture was subjected to denaturation for 10 min at 95 ° C, followed by 40 cycles: 30 sec at 95 ° C and 1 min at 60 ° C. The Data Analysis software automatically calculated the concentrations of the *lacZ* target gene and the 16S rRNA reference gene. We deduced the plasmid copy number from the target gene / 16S rRNA gene ratio.

2.5 Amplification methods

PCRs for Pc variant detection, gene cassette characterization and plasmid construction were performed using One Taq® Quick-load (Biolabs) in a final volume of 25 μ L, with a 0.9 μ M final concentration of each primer, 12.5 μ L of One Taq buffer and 1 μ L of DNA extract. Amplification took place in the following conditions: 30 s at 95°C for initial denaturation, 30 cycles of 30 s at 95°C for denaturation, 30 s at 55°C for annealing, 30 s per amplified 500 bp at 68°C for extension, and a final 7-min extension step at 68°C.

2.6 Purification of PCR products

PCR products were analyzed by electrophoresis in 0.8% agarose gel in 0.5 \times TBE-containing Midori Green Advance DNA stain® (Nippon Europe Genetics GmbH, Düren, Germany). A UVP gel documentation system was used for DNA band visualisation. Fragments of interest were purified using Wizard® SV Gel and the PCR Clean Up System (Promega, Lyon, France).

2.7 *AbaR* detection

First, the *resX* gene belonging to the *AbaR* genomic island was sought by amplifying a specific 425-bp fragment with ResX-F and ResX-R. Then, to identify the location of the integron within *AbaR*, the junction between the integron and *resX* was sought by using a forward primer located in the last integron gene cassette and ResX-R. To confirm the *AbaR* location of the integron, we performed an

amplification using this forward primer and Tn6018R, a reverse primer located in Tn6018, downstream of the integron (Figure S1) [7].

2.8 Sequencing

Sanger sequencing PCRs were done using BigDyeTM sequencing buffer (Applied Biosystems, Foster City, California, United States), according to the manufacturer's recommendations. PCR products were purified by gel filtration on Sephadex® G50 Superfine (Sigma-Aldrich). Sanger sequencing was performed on an ABI PRISM 3100 (Applied Biosystems). The sequences thus obtained were read with Chromas Lite software. Gene cassette sequences were compared to available GenBank sequences by using BLAST (<http://blast.ncbi.nlm.nih.gov/>). The Pc variant was identified by comparing the obtained sequences to reference sequences reported by Jové *et al.* [13].

NGS was performed with Ion ProtonTM technology (Thermo Fisher Scientific, Villebon sur Yvette, France) according to the manufacturer's instructions. Reads were assembled with MIRA (mimicking intelligent read assembly) and SPAdes-3.5.0, using raw reads and reads after removal of human reads. Then, the set of contigs was queried using blastn (version 2.5.0+) for the presence of known 5' and 3' conserved sequences. The extremities of these elements were then used as queries (blastn, version 2.5.0+) against custom databases (sequences were retrieved from the NCBI nucleotide database with the Entrez search engine): *A. baumannii* chromosome, *A. baumannii* plasmid. Blastn results were filtered and compiled with a custom script (blastn2spreadsheet.py).

2.9 Strain typing

The strains were compared by means of random amplified polymorphic DNA (RAPD) using primers VL1 and AP4 (Table S1). PCRs were carried out in 25- μ L reaction mixes comprising 12.5 μ L of One Taq® Quick-load (Biolabs), 0.9 μ M RAPD primer, and 2 μ L of DNA extract. The RAPD-PCR configuration was as follows: (i) initial denaturation (94 C for 5 min), followed by initial annealing

for 1 min at 32°C for VL1 and 40°C for AP4, (ii) 36 cycles of extension (68 C for 3 min), denaturation (94 C for 1 min), and annealing (temperature adapted to each primer pair, for 1 min), and (iii) a final extension step (68 C for 1 min). The RAPD-PCR reactions were performed in a 2720 thermal cycler (Applied Biosystems, Foster City, California, United States). Bands were interpreted visually.

MLST typing was performed using the Pasteur scheme as described in <http://pubmlst.org/abaumannii/>. Each strain whole genome sequencing was uploaded to <http://pubmlst.org/abaumannii/> to identify the MLST alleles and the sequence types (ST). The clonal complex was then determined using the ST type.

2.10 β -galactosidase assay

β -galactosidase assay was performed with 0.5-ml aliquots of exponential-phase cultures (optical density at 600 nm [OD₆₀₀] 0.6 to 0.8) as previously described [22]. Experiments were done at least five times for each strain. One-way ANOVA followed by *post hoc* Tukey HSD statistics were used to determine the significance of differences in expression levels ($p < 0.01$).

3. Results

3.1 *In silico* Pc variant distribution

Among 3747 class 1 integron sequences we recovered from NCBI on 13 October 2016, 1604 displayed a complete gene cassette array with an available Pc variant sequence. As previously shown by Jové *et al.* [13], the overall distribution was similar for the main Pc variants (PcS, PcH2, PcW_{TGN-10}, PcH1 and PcW). When sorted according to the bacterial genus, we noted that integrons from *Acinetobacter* mainly harbored Pc known as "strong" in *E. coli*, notably PcS and PcW_{TGN-10} (Figure 2). The rare integrons with other Pc variants were present in non *baumannii* *Acinetobacter*.

As the class 1 integrase is regulated by the SOS response which is controlled by the transcriptional repressor LexA, we analysed LexA homologs and IntI1 sequences in the sequences recovered from NCBI. No LexA homologs were found in *Acinetobacter* genus, confirming the lack of that protein in this genus [22,23]. Interestingly, *Acinetobacter* presented the highest proportion, 43.3%, of truncated IntI1 after *Escherichia* (35.7%) (Table 3).

3.2 *In vivo* Pc variant distribution

We used a collection of 83 class 1 integron-containing clinical or environmental isolates from various geographic regions. We obtained 52 RAPD patterns, reflecting the genetic diversity of the isolates (Table 2). The diversity of the environmental isolates (8 profiles for 8 isolates) was higher than that of the clinical isolates (45 profiles for 75 isolates). All 83 strains had previously been screened for integrons with a qPCR targeting class 1, 2 and 3 integrases, as described previously [25]. All strains carry at least one class 1 integron (personal data). We characterized the entire integron content of each isolate by PCR mapping with primer pair 5'CS/3'CS. We detected 108 class 1 integrons based on the sizes of the PCR products, representing between 1 and 3 integrons per isolate. Four isolates contained 3 integrons, 17 harbored 2 integrons, and 59 strains harbored 1 integron (Table 2). Three isolates (see Table 2) yielded no amplification product, suggesting that they harbored variations in the 3'-conserved region [26] or in the 5'-conserved region, although less common.

The Pc variant distribution among the 108 class 1 integrons was analysed by PCR and sequencing of the Pc promoter region. Amplification was performed with primers Int4b and DORF11 for the isolates containing 1 integron and with Int4b and a primer located in the first cassette of the network for the isolates with 2 or 3 integrons. We found that 98.2% of the integrons (106 of 108) contained a

strong Pc variant, namely PcS (85.2%, n=92), PcW_{TGN-10} (7.4%, n=8), PcH2_{TGN-10} (4.6%, n=5) or PcH2 (0.9%, n=1), and only 1.8% (n=2) of the integrons contained the weak PcW. The proportion of “strong” Pc variants in *A. baumannii* was even higher *in vivo* (98.2%) than *in silico* (73.3%). However, the predominance of these Pc variants was not linked to predominant clones (Table 2).

3.3 Evaluation of Pc strength in *A. baumannii*

The predominance of PcS variants, known as strong promoter variants in *E. coli*, led us to evaluate their strength in *A. baumannii*. We constructed 4 plasmids able to replicate in both *E. coli* and *A. baumannii*: 3 with different Pc variants in transcriptional fusion with the reporter gene *lacZ*, and a control plasmid without the Pc region (Table 1). Surprisingly, we found that the expression levels of all the Pc variants were 2 to 4 times weaker in *A. baumannii* than in *E. coli* ($p < 0.01$; Figure 3). This difference in expression levels was not due to difference in growth rates or plasmid copy numbers. Indeed, we found a similar plasmid copy number in the two species, without significant differences, whatever the plasmid construct (Table 4). The so-called “strong” Pc variants are thus weakly expressed in *A. baumannii*.

3.4 Gene cassette contents

Sequencing of the PCR products obtained with primer pair 5'CS/3'CS showed that all the gene cassettes we detected had been already described in Genbank and were composed of 11 different resistance genes and 18 different gene cassette arrays (Table 2). The majority (90.5%) of the gene cassettes coded for aminoglycoside resistance, and at least 1 or 2 of these gene cassettes were found in 15 (83.3%) of the 18 arrays. The arrays often comprised 2 gene cassettes encoding proteins of unknown function (*gcuP* and *gcuQ*). The most frequent arrays were *aacC1+orfP+orfQ+aadA1* and *aacC1*. We identified 5 arrays that had not previously been described in *A. baumannii* (*aacA4+aadA1*, *aacA4+catB8+orfP+orfQ+aadA1*, *aacA4+orfP+orfQ+aadA1*, *aadA12*, *dfrA1*).

The array *aacA4+aadA1* had previously been described in *Pseudomonas aeruginosa* (Genbank accession number FN823039). The *aadA12* gene cassette had previously been found alone in an integron of *E. coli* (Genbank accession number FJ381668), *Yersinia enterocolitica* (Genbank accession number AY940491), and *Salmonella typhimurium* (Genbank accession number FJ460240). The *dfrA1* gene cassette had previously been found alone in *A. baumannii*, but only in a class 2 integron (Genbank accession number FJ785526). The gene cassette arrays *aacA4+catB8+orfP+orfQ+aadA1* and *aacA4+orfP+orfQ+aadA1* had not previously been described. Eight combinations of gene cassettes were identified only once (Table 2).

3.5 Genetic environment of the integrons

As multidrug resistance in *A. baumannii* is often linked to chromosomal multiresistance genomic islands frequently containing class 1 integrons, we characterized the genetic environment of the class 1 integrons. We focused on one strain per RAPD profile, except for profile B, for which we selected two isolates as we found two different gene cassette array combinations. A total of 53 isolates and 66 integrons were analyzed. With an amplification strategy targeting the *resX* gene (belonging to the AbaR genomic island) and the junction sequence (see Materials and Methods), we found that 27 integrons from 27 strains were embedded within an AbaR island (Table 2), indicating that these strains belonged to the clone GC1.

Regarding the integrons that were not detected with the specific PCR, NGS analysis showed that 26 integrons were also located on the chromosome. However, a large number of repeated sequences limited the length of the contigs we obtained with Ion ProtonTM technology, and the short contigs were too small to determine the whole resistance island harboring these integrons. Nevertheless we could determine the strain MLST typing, 4 isolates were ST1 belonging to the GC1, 10 were ST2

belonging to GC2, and 1 was ST499. The low sequence quality of one isolate (A33 RAPD profile) did not allow to determine the ST type. For 13 integrons, as we had only DNA samples in insufficient quantities, we were unable to perform NGS. Overall, we found a chromosomal location for all the integrons we analysed (n=53/66). As the other strains belonged to the same RAPD profile and /or had the same integron, one can hypothesize that all integrons in our strain selection are located on the chromosome.

4. Discussion

Acinetobacter baumannii resistance has been previously linked to class 1 integrons [18–21]. However, when we began this study, no data were available on the Pc variant distribution in clinical and environmental strains. Interestingly, using both *in silico* and *in vivo* data, we found a large proportion of so-called strong Pc variants in the class 1 integrons of *A. baumannii*.

Specific features of PcS and *A. baumannii* could explain these findings. First, we found that all these Pc variants were weaker in *A. baumannii* than in *E. coli*. Integrons with PcS might have been selected to allow *A. baumannii* to express antibiotic resistance at a sufficient level. A lower expression has already been reported in *A. baumannii* (or *A. calcoaceticus*, according to the former taxonomy) compared to *E. coli*. Expression of the *catA* gene in under the control of a *lac* promoter, was two-fold lower in *A. calcoaceticus* than in *E. coli* [27]. Likewise, the *pca* gene cluster expression was 10- to 30-fold lower in *A. calcoaceticus* than in *E. coli* [28]. More recently, Girlich *et al.* reported that the transcription level of the *bla*_{KPC} gene (encoding resistance to beta-lactams) was 10 to 100 times lower in *A. baumannii* than in *E. coli* but, interestingly, the resistance phenotypes did not correlate with the transcription level [29]. There is some evidence that inter-species differences in the Pc expression level could be due to differences in the transcriptional machinery.

The *A. baumannii* RNA polymerase has a similar structure [30] and 50 to 70% amino acid sequence homology when compared to its *E. coli* counterpart, with good conservation of functional regions [23]. Despite this, the two RNA polymerases behave differently *in vitro*, the *A. baumannii* enzyme being less efficient than its *E. coli* counterpart [30]. Moreover, the *A. baumannii* and *E. coli* transcriptional σ^{70} factor amino acid sequences are very similar, with good conservation of functional regions [23,31]. Nevertheless, alternative σ factors are missing in *A. baumannii*, suggesting different regulation of transcription relative to *E. coli* [23]. Furthermore, to our knowledge, there is no -35 and -10 sequence consensus of *A. baumannii* but some data suggest variations from the promoter sequence consensus of *E. coli*, notably in the spacer region [32,33]. Such a different promoter consensus could influence both the transcription level and the resistance phenotype, as reported by Girlich *et al.* [29].

Second, the selection of PcS in *A. baumannii* could be associated with a lower biological cost to avoid integrase toxicity. In *E. coli*, the class 1 integron integrase is regulated via the SOS response, which involves the LexA repressor, via a LexA binding site overlapping the *intI1* promoter *PintI1* [12]. Moreover, the Pc promoter being located within the *intI1* sequence in *E. coli*, different Pc variants actually correlate with amino acid changes in the *IntI1* sequence and the integrase variant *IntI1*_{R32_N39} corresponding to the PcS promoter has the lowest efficiency [13]. We recently showed in *E. coli* that the repression of the SOS regulon prevents the expression of costly integrases whose cost is activity dependent. The lowest cost was observed with the integron encoding *IntI1*_{R32_N39} integrase (associated with PcS) [34]. It has been shown in *Acinetobacter baylyi* harboring an integron with a PcS variant, that frameshift mutations rapidly emerge to inactivate the *IntI1* protein and to restore host fitness [35]. We found *in silico* a higher proportion (43%) of truncated integrases in *A. baumannii* compared to other integron hosts (Table 3). This suggests that the selection of PcS in this species could only partially suppress integrase

activity and therefore inactivation could be associated to prevent its toxicity. However these in silico results on published genome are not epidemiological data and should be confirmed in human, animal and environmental strains of different bacterial integron hosts.

Third, another mechanism regulating integrase expression in *E. coli* depends on transcriptional interference between PcS and PintI1, PcS preventing *intI1* expression [36]. *Acinetobacter* spp. lack the LexA repressor [22,23], suggesting that the integrase is constitutively expressed. If this transcriptional interference also occurs in *A. baumannii*, the observed predominance of PcS may limit the expression of the integrase via this transcriptional interference and thus counterbalance the lack of LexA-driven integrase repression to prevent the cost of the integrase.

The predominance of a poorly efficient integrase in *A. baumannii* (IntI1_{R32_N39} associated with PcS) would lead to limited movement of gene cassettes and thus confer higher stability of gene cassettes within the array. This is in line with what we observed in the integron gene cassette arrays of our *A. baumannii* collection. Indeed, we found a redundancy of gene cassette arrays, with 5 arrays representing 75.2% (n=79) of all characterized integrons (n=105). This redundancy of gene cassette arrays was not due to clonal distribution of our isolates, as we found no identical RAPD profiles for strains with identical arrays (Table 2). All the environmental isolates in our collection had different RAPD profiles, which were also all different from those of the clinical isolates. The environmental and clinical isolates shared the two most frequent gene cassette arrays (*aacC1+orfP+orfQ+aadA1* and *aacC1*), which accounted for 54.6% (n=6/11) of the environmental integrons and 60.6% (n=57/94) of the clinical integrons (Table 2). It should be noted that the environmental isolates were obtained from hospital wastewater and can thus be considered to have been influenced by antibiotic selection pressure. Aminoglycoside-modifying enzymes have been linked to class 1 integrons in *A. baumannii* [5,37]. Moreover, most of the gene cassette arrays (94.3%) in our study harbored at least one aminoglycoside resistance gene

cassette (*aacC1*, 66.7%, *aadA1*, 50.5% and *aacA4*, 14.3%) (Table 2). The array containing two aminoglycoside resistance genes (*aacC1-orfP-(orfP-Q)orfQ-aadA1*) is frequently found in *A. baumannii* class 1 integrons [5,9,38]. Many strains contain either *aacC1* or *aacA4* associated with the *aadA1* gene cassette, encoding the resistance to 4-6 deoxystreptamine aminoglycosides (mainly used in clinical settings) and streptomycin. This high frequency of aminoglycoside-resistance gene cassettes has been previously described in *A. baumannii* [6]. However, predominance of gene cassettes encoding resistance to aminoglycosides it is not specific to *Acinetobacter* as these gene cassettes are the most frequently found in class 1 integrons, whatever the host [11]. In, *A. baumannii* integrons are often found on the chromosome, usually within resistance islands [8,37,39]. Consistently, all the studied integrons in our collection were found on the chromosome too and at least 27 in a *AbaR* island.

5. Conclusion

Our results highlight that gene cassettes PcS variants are predominant in *A. baumannii*. Integrons with a PcS promoter might have been selected to allow a sufficient resistance level while avoiding the toxicity of a highly active integrase. Altogether, our data obtained in *E. coli* [13,15,37] and *Acinetobacter* suggest that integrons need to be tightly regulated for an adaptive response designed to lower their cost and thereby to favour their maintenance.

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Declarations

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Ethical Approval: Not required

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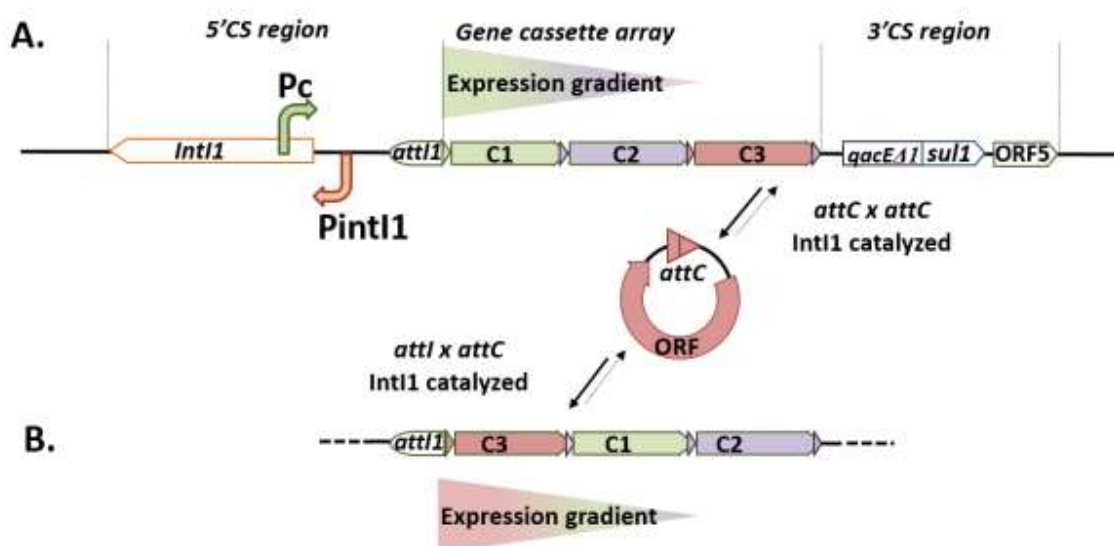


Figure 1: **Organization of class 1 integrons.** **A.** Structure of a class 1 integron, the gene cassette array consists of expressed cassette with a gradient of expression from the *Pc*, indicated by the fading fill color. Cassettes are an open reading frame (ORF) generally without promoter and flanked by two *attC* recombination sites. Cassettes can be excised through *attC* × *attC* recombination from any position in the array, catalyzed by the integrase. *Pc*, cassette promoter; *Pint*, integrase promoter; *intI1*, class 1 integron integrase gene; *attI1*, *attC_n* and *attC_{n-1}*, recombination sites; C1, C2 and C3, gene cassettes. The integron functional platform, 5'-conserved region, contains 3 key elements i) the *intI* gene, encoding an integrase, ii) a specific recombination site, *attI*, and iii) a promoter, *Pc*, driving gene cassette expression. Most clinical class 1 integrons comprise a 3'-conserved region that contains the genes *qacEΔ1* and *sul1* conferring resistance to quaternary ammonium compounds and sulfonamides, respectively (40). **B.** The excision intermediate can thus be integrated by the integrase, preferentially at the recombination site *attI*. Exogenous intermediates can also be integrated, owing to the low specificity of the integrase activity, rendering the system prone to horizontal transfer.

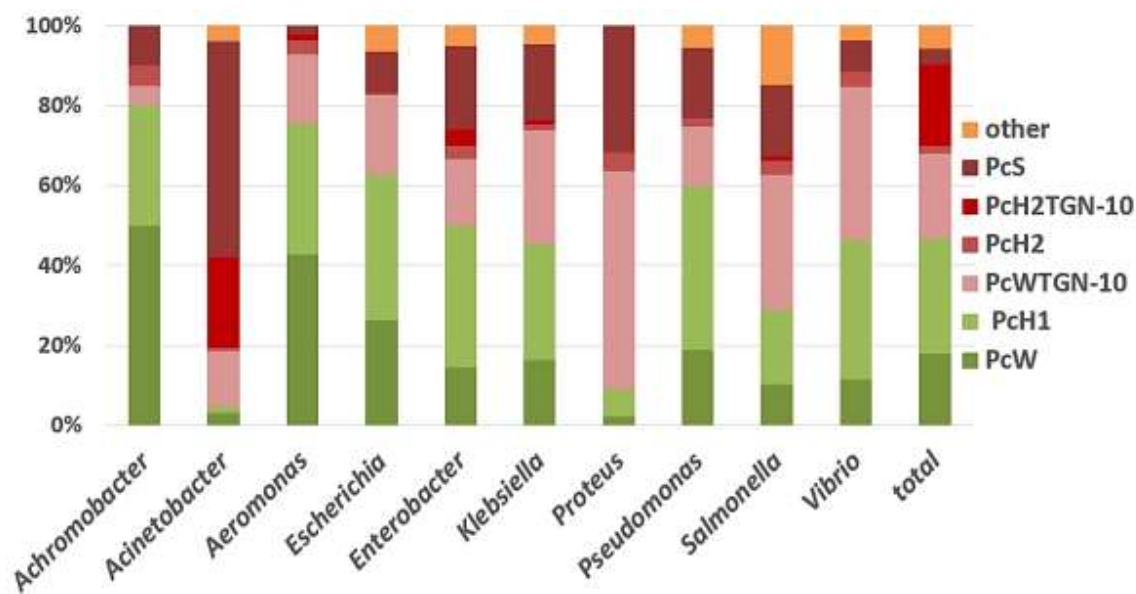


Figure 2: **A. Distribution of the Pc variants according to the host.** Each bar represents the Pc variant distribution in the different class 1 integrons with a Pc sequence available, (analysis performed on the 13th of October 2016), in the 10 most frequent mobile class 1 integron hosts: total of the different integron sequences (n=1604), *Acinetobacter* (n=124), *Pseudomonas* (n=340), *Escherichia* (n=435), *Klebsiella* (n=263), *Salmonella* (n=174), *Enterobacter* (n=96), *Aeromonas* (n=82), *Achromobacter* (n=20), *Proteus* (n=44) and *Vibrio* (n=26).

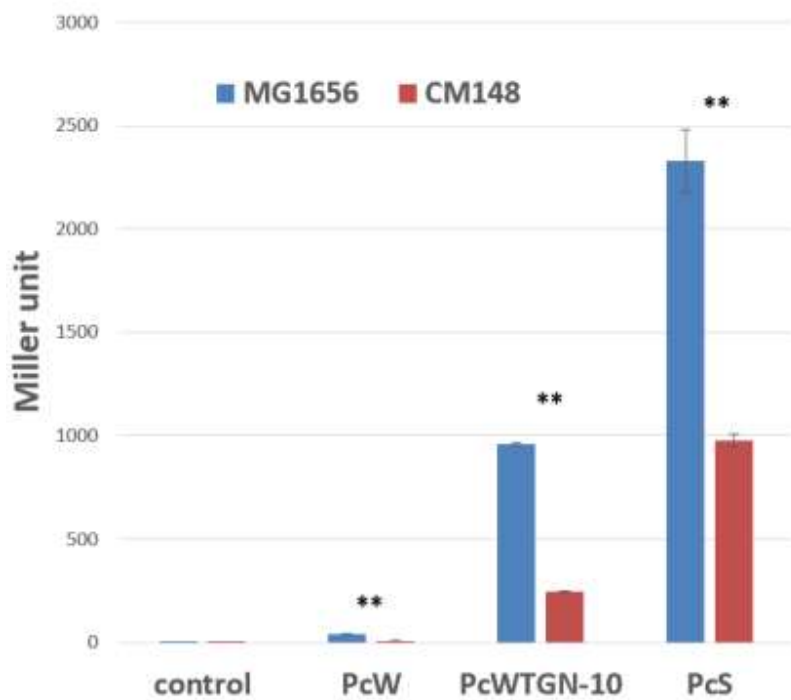


Figure 3: **Expression of the Pc variants.** β -galactosidase activity was measured in *E. coli* MG1656 (in blue) and *A. baumannii* CM148 (in red). Control: pSU38 Δ totlacZRSF1010, PcW: pPcWRSF1010, PcWTGN-10: pPcW_{TGN-10}RSF1010 and PcS: pPcSRSF1010.

** indicates significant statistical difference with $p < 0.01$.

Table 1: Bacterial strains and plasmids used in the study

Strain or plasmid	Description	Reference or source
Strains		
<i>A. baumannii</i> Collection	75 clinical strains and 8 environmental strains	This study See table 2
MG1656	Derivative of <i>E. coli</i> K12 MG1655 with <i>lacZ</i> deletion	[12]
CM148	Clinical competent <i>A. baumannii</i> strain	Kind gift from Thierry Lambert
Plasmids		
pAL4000	Contains an RSF1010 replicon active in <i>E. coli</i> and <i>Acinetobacter</i>	Greener et al. 1992
pSU38 Δ totlacZ	pSU38 derivate carrying <i>lacZ</i> coding sequence with no translation initiation region or promoter	[13]
pPcW	pSU38 Δ totlacZ derivate carrying PcW in transcriptional fusion with <i>lacZ</i>	[13]
pPcS	pSU38 Δ totlacZ derivate carrying PcS in transcriptional fusion with <i>lacZ</i>	[13]
pPcW _{TGN-10}	pSU38 Δ totlacZ derivate carrying PcW _{TGN-10} in transcriptional fusion with <i>lacZ</i>	[13]
pSU38 Δ totlacZR _{RSF1010}	Insertion of RSF1010 in <i>EcoR1</i> in pSU38 Δ totlacZ	This study
pPcW _{RSF1010}	Insertion of RSF1010 in <i>EcoR1</i> in pPcW	This study
pPcS _{RSF1010}	Insertion of RSF1010 in <i>EcoR1</i> in pPcS	This study
pPcW _{TGN-10} _{RSF1010}	Insertion of RSF1010 in <i>EcoR1</i> in pPcW _{TGN-10}	This study

Table 2: **Summary of our study results.** ^a PCR of the gene cassette array were realized using 5'CS/3'CS primers pair, « - » means negative PCR; ^b Localization was determined by specific PCR (AbaR) or NGS (Chromosomal), « - » means that AbaR specific PCR was negative but we had not enough DNA to perform the NGS analysis.

type of isolates	material	RAPD profile	Pc variant	gene cassette array ^a	Localization ^b	Global clone	ST
clinical	strain	M	PcS PcS	<i>aacC1+orfP+orfP+orfQ+aadA1</i> <i>aacC1</i>			
clinical	strain	M	PcS PcS	<i>aacC1+orfP+orfP+orfQ+aadA1</i> <i>aacC1</i>	Chromosomal Chromosomal	GC2	ST2
clinical	strain	M	PcS PcS	<i>aacC1+orfP+orfP+orfQ+aadA1</i> <i>aacC1</i>			
clinical	strain	M	PcS PcS	<i>aacC1+orfP+orfP+orfQ+aadA1</i> <i>aacC1</i>			
clinical	strain	M	PcS PcS	<i>aacC1+orfP+orfP+orfQ+aadA1</i> <i>aacC1</i>			
clinical	total DNA	A05	PcS	<i>aacC1+orfP+orfP+orfQ+aadA1</i>	-		
clinical	total DNA	A06	PcS	<i>aacC1+orfP+orfQ+aadA1</i>	-		
clinical	total DNA	E	PcS	<i>aacC1+orfP+orfQ+aadA1</i>	AbaR	GC1	
clinical	total DNA	E	PcS	<i>aacC1+orfP+orfQ+aadA1</i>			
clinical	total DNA	E	PcS	<i>aacC1+orfP+orfQ+aadA1</i>			
clinical	total DNA	A07	PcS	<i>aadB+blaOXA-21</i>	AbaR	GC1	
clinical	total DNA	F	PcS	<i>aacC1+orfP+orfQ+aadA1</i>	AbaR	GC1	
clinical	total DNA	G	PcS	<i>aacC1+orfP+orfP+orfQ+aadA1</i>	AbaR	GC1	
clinical	total DNA	F	PcS	<i>aacC1+orfP+orfQ+aadA1</i>			
clinical	total DNA	G	PcS	<i>aacC1+orfP+orfP+orfQ+aadA1</i>			
clinical	total DNA	H	PcS	<i>aacC1+orfP+orfP+orfQ+aadA1</i>	AbaR	GC1	
clinical	total DNA	H	PcS	<i>aacC1+orfP+orfP+orfQ+aadA1</i>			
clinical	total DNA	A08	PcS	<i>aacC1+orfP+orfQ+aadA1</i>	AbaR	GC1	
clinical	total DNA	F	PcS	<i>aacC1+orfP+orfQ+aadA1</i>			
clinical	total DNA	F	PcS	<i>aacC1+orfP+orfQ+aadA1</i>			
clinical	total DNA	I	PcS	<i>aacA4</i>	AbaR	GC1	
clinical	total DNA	I	PcS	<i>aacA4</i>			
clinical	total DNA	I	PcS	<i>aacA4</i>			
clinical	total DNA	A40	PcS	<i>aadB</i>	AbaR	GC1	
clinical	total DNA	A09	PcS	<i>aacA4</i>	-		
clinical	total DNA	A10	PcS	<i>aacC1+orfP+orfQ+aadA1</i>	AbaR	GC1	
clinical	total DNA	J	PcS	<i>aadB</i>	AbaR	GC1	
clinical	total DNA	K	PcS	<i>aacC1+orfP+orfQ+aadA1</i>	AbaR	GC1	
clinical	total DNA	A11	PcS	<i>aacC1+orfP+orfQ+aadA1</i>	AbaR	GC1	
clinical	total DNA	J	PcS	<i>aadB</i>			
clinical	total DNA	J	PcS	<i>aadB</i>			
clinical	total DNA	J	PcS	<i>aadB</i>			
clinical	total DNA	J	PcS	<i>aadB</i>			
clinical	total DNA	A12	PcS	<i>aacC1+orfP+orfP+orfQ+aadA1</i>	AbaR	GC1	
clinical	total DNA	A13	PcS	<i>aacC1+orfP+orfQ+orfP+orfQ+aadA1</i>	AbaR	GC1	
clinical	total DNA	A14	PcS	<i>aacC1+orfP+orfP+orfQ+aadA1</i>	AbaR	GC1	
clinical	total DNA	A15	PcS	<i>aacA4</i>	AbaR	GC1	
clinical	total DNA	A16	PcS	<i>aacC1+orfP+orfP+orfQ+aadA1</i>	AbaR	GC1	
clinical	total DNA	A17	PcS	-	-		

clinical	total DNA	A18	PcS	<i>aacC1+orfP+orfQ+aadA1</i>	AbaR	GC1	
clinical	total DNA	A19	PcS	-	-		
clinical	total DNA	A20	PcW _{TGN-10}	<i>aacA4</i>	AbaR	GC1	
clinical	total DNA	A21	PcH2 _{TGN-10}	<i>aacA4+aadA1</i>	AbaR	GC1	
clinical	total DNA	A22	PcH2 _{TGN-10}	<i>aacA4+catB8+orfP+orfQ+aadA1</i>	AbaR	GC1	
clinical	total DNA	K	PcS	<i>aacC1+orfP+orfQ+aadA1</i>	AbaR	GC1	
clinical	total DNA	A23	PcS	-	-		
clinical	total DNA	A24	PcS	<i>aadA1</i>	-		
environmental	strain	A25	PcS PcH2	<i>aacC1+orfP+orfQ+aadA1</i> <i>aacA4+catB8+aadA1</i>	Chromosomal	GC2	ST2
environmental	strain	A26	PcW _{TGN-10}	<i>aacC1+orfP+orfQ+aadA1</i>	Chromosomal	GC2	ST2
environmental	total DNA	A27	PcW _{TGN-10}	<i>dfrA5</i>	-		
environmental	total DNA	A38	PcS	<i>aacC1+orfP+orfQ+aadA1</i>	-		
environmental	strain	A29	PcS	<i>aacC1+orfP+orfQ+aadA1</i>	AbaR	GC1	ST1
environmental	total DNA	A30	PcS	<i>aacC1+orfP+orfQ+aadA1</i>	-		
environmental	strain	A31	PcH2 _{TGN-10} PcH2 _{TGN-10} PcS	<i>aacA4+orfP+orfQ+aadA1</i> <i>aacA4+catB8+aadA1</i> <i>aacC1</i>	Chromosomal	GC2	ST2
environmental	strain	A32	PcW _{TGN-10}	<i>dfrA1</i>	Chromosomal		ST499
clinical	strain	B	PcS PcS	<i>aacC1+orfP+orfQ+orfQ+aadA1</i> <i>aacC1</i>	Chromosomal	GC2	ST2
clinical	strain	B	PcS PcS PcS	<i>aacC1+orfP+orfQ+orfQ+aadA1</i> <i>dfrA1-orfP</i> <i>aadA12</i>	Chromosomal	GC2	ST2
clinical	strain	B	PcS PcS	<i>aacC1+orfP+orfQ+orfQ+aadA1</i> <i>aacC1</i>	Chromosomal	GC2	ST2
clinical	strain	C	PcS PcS	<i>aacC1+orfP+orfQ+orfQ+aadA1</i> <i>aacC1</i>	Chromosomal	GC2	ST2
clinical	strain	D	PcS PcS	<i>aacC1+orfP+orfQ+orfQ+aadA1</i> <i>aacC1</i>	Chromosomal	GC2	ST2
clinical	strain	D	PcS PcS	<i>aacC1+orfP+orfQ+orfQ+aadA1</i> <i>aacC1</i>			
clinical	strain	D	PcS PcS	<i>aacC1+orfP+orfQ+orfQ+aadA1</i> <i>aacC1</i>			
clinical	strain	B	PcS PcS	<i>aacC1+orfP+orfQ+orfQ+aadA1</i> <i>aacC1</i>			
clinical	strain	C	PcS PcS	<i>aacC1+orfP+orfQ+orfQ+aadA1</i> <i>aacC1</i>			
clinical	strain	D	PcS PcS	<i>aacC1+orfP+orfQ+orfQ+aadA1</i> <i>aacC1</i>			
clinical	strain	C	PcS PcS	<i>aacC1+orfP+orfQ+orfQ+aadA1</i> <i>aacC1</i>			
clinical	strain	A01	PcS	<i>aacA4</i>	AbaR	GC1	
clinical	total DNA	A02	PcS	<i>aacC1+orfP+orfQ+orfQ+aadA1</i>	-		
clinical	total DNA	A03	PcW + P2	<i>blaIMP-10+aacA31+aadA1</i>	-		
clinical	total DNA	A04	PcW	<i>blaIMP-10+aacA31+aadA1</i>	-		
clinical	strain	A33	PcS	<i>aacC1</i>	Chromosomal	-	-
clinical	strain	A34	PcS	<i>aacA4</i>	AbaR	GC1	
clinical	strain	A35	PcS PcW _{TGN-10} PcS	<i>aadA1</i> <i>dfrA1+orfP</i> <i>aacC1</i>	Chromosomal	GC1	ST1
clinical	strain	A36	PcW _{TGN-10}	<i>dfrA1+orfP</i>	Chromosomal	GC1	ST1
clinical	strain	A37	PcS	<i>aacC1</i>	Chromosomal	GC2	ST2
clinical	strain	A38	PcS	<i>aacA4</i>	AbaR	GC1	
clinical	strain	A39	PcS	<i>aadA1</i>	Chromosomal	GC1	ST1

			PcW _{TGN-10}	<i>dfrA1+orfP</i>	AbaR		
			PcS	<i>aacC1</i>	Chromosomal		
clinical	strain	L	PcS	<i>aacC1</i>	Chromosomal	GC2	ST2
clinical	strain	L	PcS	<i>aacC1</i>			
clinical	strain	L	PcS	<i>aacC1</i>			
clinical	strain	L	PcS	<i>aacC1</i>			

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Table 3: **Proportion of truncated IntI1 and features of LexA homologue according to the host.** The number of truncated IntI1 was analyzed within available sequences (the 10th November 2018) with the complete sequence of its *intI1* gene. The LexA homologues were recovered on the same date using the NCBI BlastP alignment against the refseq_protein database.

Genus	Online sequences with a complete <i>intI1</i>			LexA homolog
	Total	Functional IntI1	Truncated IntI1 (%)	Accession Number (% identity/e-value ^a)
<i>Achromobacter</i>	14	10	4 (29)	WP_088596507 (60% / 7e-82)
<i>Acinetobacter</i>	90	51	39 (43)	None ^b
<i>Aeromonas</i>	28	23	5 (18)	WP_108537109 (73% / 5e-109)
<i>Escherichia</i>	224	144	80 (36)	NP_418467 (used as reference)
<i>Enterobacter</i>	78	66	12 (15)	WP_090464447 (94% / 1e-138)
<i>Klebsiella</i>	220	177	43 (20)	WP_110247500 (95% / 9e-140)
<i>Proteus</i>	40	35	5 (13)	WP_023583074 (87% / 5e-129)
<i>Pseudomonas</i>	174	138	36 (21)	WP_121784911 (84% / 1e-117)
<i>Salmonella</i>	140	96	44 (31)	WP_000646079 (97% / 3e-142)
<i>Vibrio</i>	13	12	1 (8)	WP_073583963 (76% / 8e-108)

^a e-value obtained from NCBI BlastP alignment against the refseq_protein database. The lower the e-value, the more significant the score and the alignment.

^b The closest match shares 38% identity (e-value = 2e-17).

	Plasmid copy number/bacterial genome	
	<i>E. coli</i> MG1656	<i>A. baumannii</i> CM148
	mean (std deviation of mean)	mean (std deviation of mean)
pSU38 Δ totlacZRSF1010	58.2 (\pm 15.3)	52.5 (\pm 8.8)
pPcWRSF1010	60.7 (\pm 21.8)	62.4 (\pm 11.1)
pPcW _{TGN-10} RSF1010	60.6 (\pm 15.3)	56.0 (\pm 8.1)
pPcSRSF1010	56.8 (\pm 18.5)	54.1 (\pm 16.9)

Table 4: Plasmid copy number in *E. coli* and *A. baumannii*