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The nature of bel-1 attC stabilizes its genetic environment and contributes to antibiotic resistance spreading

The rapid emergence of antibiotic resistance is a worldwide crisis, endangering the efficacy of antibiotic treatment. Multidrug resistance in Gram negatives is now recognized as an issue of worldwide interest. Those bacteria possess various resistance mechanisms compromising the efficacy of several classes of antibiotics such as beta-lactams.

In this study, we investigated the blaBEL-1 gene which encodes an extended-spectrum β-lactamase, BEL-1, that is present at the second position of the variable region of class 1 integrons identified in *Pseudomonas aeruginosa*. Class 1 integrons are genetic elements that can acquire and rearrange gene cassettes, including genes carrying antibiotic/disinfectant resistance genes, therefore participating in the evolution toward multidrug resistance. The bel-1 cassettes are associated with aacA4 and aadA5 gene cassettes, coding for an aminoglycoside-modifying enzyme, and also with the smr cassette, encoding resistance to antiseptics.

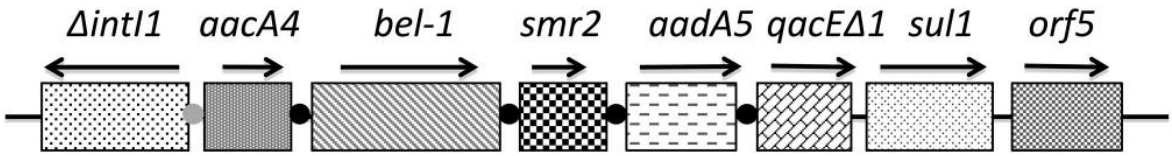

Fig. 1. Schematic representation of integrons containing the *bla*BEL-1 gene found in clinical strain *P. aeruginosa* 51170. Arrows, orientation of gene transcription; black and gray circles, *attC* and *attI1* sites, respectively.

Integrons are bracketed by two segments at their 5' and 3' ends. The 5' includes intI1, a gene encoding a site-specific recombinase of the DNA integrase family, with attI being the cassette integration site and the promoter Pc driving the expression of the cassettes. The gene cassettes are independent units each consisting of a gene bracketed by copies of a recombination site named attC. attC sites are involved in site-specific recombination catalyzed by the integrase IntI1 leading to cassette integration or excision.

We investigated here the putative mobility of the bel-1 gene cassette and the role of several antibiotic molecules in the putative induction of its mobility. Interestingly, we found after 10 days of *P. aeruginosa* culture with sub-inhibitory concentrations of antibiotics, that the bel-1 cassette remained at the second position in the integron, highlighting its stability in *P. aeruginosa*.

Therefore, the effect of IntI1 integrase overproduction was investigated. The cointegration frequencies depend on the recombination efficiency of the *attC* site available for recombination in the donor integron. We showed that the *bel-1 attC* site was likely inefficient for recombination with the *attI1* site or an *attC* site in a receiving integron In3. The *smr2 attC* site was thus more efficient for recombination than the *bel-1 attC* site itself.

The excision/mobilization experiments showed that the *attC* site of the *bel-1* gene cassette was inefficient and that this gene cassette was not mobilizable independently. This might likely be explained by the sequence of the *bel-1 attC* site itself, which does not correspond to the ones better recognized by the Int1 integrase. Here

the extrahelical bases constituting the *bel-1 attC* bottom strand are distantly related to those well recognized by Int1. We also showed that the *smr2 attC* site is enhanced over the *bel-1 attC* site, as it is involved in almost all recombination events when both gene cassettes are present on a plasmid. *smr2* always remained associated with the *bel-1* gene cassette.

Overall, our work provides some insights into the organization of *bla*BEL-1-containing integrons. It is likely that those later evolved from a common ancestor carrying an early association between the *bel-1* and *smr2* gene cassettes. It is also possible that *smr2* was responsible for *bel-1* gene cassette recruitment and for the comobilization of *bel-1–smr2* into class 1 integrons. Although *bla*BEL-1-containing integrons are subject to gene cassette rearrangements, we propose that the nature of *bel-1 attC* stabilizes its genetic environment, probably by impairing recombination events that could lead to its loss and thus maintaining antibiotic resistance.

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