Class 1 integron-associated spread of resistance regions in *Pseudomonas aeruginosa*: plasmid or chromosomal platforms?

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Sir,

Multidrug-resistant *Pseudomonas aeruginosa* infections are a growing clinical problem. Of particular concern is the range of β-lactamase genes associated with this species. If the spread of resistance is to be controlled, it is critical that researchers have a good understanding of the mechanisms by which resistance genes are spread. In the Enterobacteriaceae, the role of plasmids in the lateral gene transfer (LGT) of resistance is extensive. However, many clinical isolates of Gram-negative bacteria also commonly carry additional syntenic blocks of DNA as part of the chromosome that are lineage specific within a species and are known as genomic islands. 1 Many of these genomic islands can be large, comprising up to tens of thousands of base pairs of DNA. In the Enterobacteriaceae, some of these genomic islands include class 1 integrons and resistance genes and are known to be mobilizable between strains. 2 In *P. aeruginosa*, the spread of resistance via conjugative plasmids is known, but we believe that the role of chromosomally located elements such as genomic islands is greatly underappreciated.

Many studies investigating the resistance gene content of pathogenic Gram-negative bacteria take advantage of the fact that such genes are commonly associated with class 1 integrons. In clinical isolates the structure of this integron class often includes conserved regions flanking the resistance gene array. Taking advantage of this, a PCR with primers targeting conserved regions can allow amplification of the array even when the gene content is unknown. 3 This is a useful epidemiological tool for investigating the prevalence of resistance genes. It can also rapidly detect new resistance genes when they arrive in clinical isolates. However, since class 1 integrons cannot laterally transfer by themselves, this type of PCR screening is not helpful in understanding many of the forces that drive the spread of resistance genes through pathogens.

In a survey of the GenBank nucleotide database using the search terms ‘*Pseudomonas aeruginosa*’ (Organism) AND ‘integron’ (All Fields), we identified 450 entries relevant to integron arrays. Of these, only 18 confirmed the genomic location. Twelve of these sequences were part of plasmids and six were on the chromosome. Of the 12 plasmid sequences available, several of these were known to be promiscuous plasmids and were selected for sequencing for that reason. Two of the 12 were duplicate entries of the same plasmid. Most entries—432—comprised ‘partial’ sequences. Most of these were derived from the method described above. Where context sequence was available, it was insufficient to ascribe a genomic location. In many peer-reviewed publications, inferences about location place an emphasis on plasmids that, while possibly appropriate for the Enterobacteriaceae, are, in our opinion, misleading in the context of *P. aeruginosa*. There are a number of examples of this in the literature. In Rieber et al., 4 despite looking for the presence of plasmids harbouring GIM-1 genes in conjugation and transformation assays and finding no transconjugants/transformants, they nonetheless imply the genes are present on a non-conjugative plasmid on the basis of extrapolation from a previous study. Like others, they have also found that the same integron-associated resistance gene cassette array can be found in different clonal lines, suggesting some mechanism of LGT.

There are numerous reports showing that class 1 integrons are present in diverse *P. aeruginosa* clonal lines and that these display no obvious evidence of plasmids. For example, Siarkou et al. 5 show a cohort of strains that carry blaVIM-17 meeting this criterion. Because of the lack of evidence for plasmids, they conclude that the integron is located within the chromosome and suggest association with a transposon to explain LGT. These latter elements, however, cannot on their own explain mobilization between different clonal lines. Other studies often, after testing for plasmids and finding none, explain LGT on the basis that plasmids were present but were not detected for technical reasons. 6

In a recent study we investigated the context in which integrons and their associated cassettes are found in *P. aeruginosa* clinical isolates. 7 In the 11 class 1 integron-containing isolates examined from two continents, all the integrons present were found to be in the chromosome. Collectively these integrons were in at least four distinct locations and in several different clonal lines. Overall, it was clear that integrons located on the chromosome have the capability to spread by LGT, and this has obvious ramifications for the clinical management of resistant variants of this species. Notably, many of the cassette arrays we found are present in a number of the database entries described above. We thus believe that our cohort does not comprise a biased sample. Rather, dispersal of resistance from class 1 integrons located on the chromosome is likely to be a major form of global spread in *P. aeruginosa*. The precise mechanism of LGT remains unclear. Some integrons are embedded in genomic islands, although it remains to be shown if these are capable of horizontal transmission.

With the decreasing cost of genomic sequencing it will progressively become easier to identify the context in which complex multidrug-resistant regions are found, a point highlighted by the finding of a class 1 integron in the chromosome of a sequenced genome of a Japanese *P. aeruginosa* clinical isolate. 8 In the meantime, we believe it is important that researchers more critically analyse data when drawing conclusions about chromosomal versus plasmid origins in *P. aeruginosa*.
In the last 25 years, highly active antiretroviral therapy has been continuously improving the life expectation and quality of HIV-infected people. As new drugs were more effective and adverse events less frequent and severe, HIV infection turned into a chronic disease. Recently licensed drugs, such as raltegravir and darunavir, are characterized by high efficacy and tolerability. However, on 2 November 2011, updates to the Isentress (raltegravir) package insert were approved by the FDA to include a new warning, subsequent to the post-marketing experience. Severe, potentially life-threatening and fatal skin reactions have been reported during raltegravir treatment.

Several classes of antiretroviral drugs have been associated with cutaneous adverse events, in particular non-nucleoside reverse transcriptase inhibitors and protease inhibitors. Some of these events may lead to treatment discontinuation; their early detection is clinically relevant in order to prevent severe reactions.

In the literature, information about raltegravir and skin reactions is still scanty. A recent paper3 stating the efficacy and safety of raltegravir in association with etravirine and darunavir/ritonavir reported one case of recurrent epidermal necrolysis leading to raltegravir discontinuation, out of 100 multiexperienced patients (1.0%). Antiretroviral drugs associated with adverse cutaneous reactions were reviewed by Borrás-Blasco et al.,4 who reported that in Phase II and III studies raltegravir was mainly associated with skin rash of mild to moderate intensity, not leading to discontinuation. Long-term safety data from clinical trials5 showed that rash frequency during raltegravir treatment was higher compared with placebo, but lower in comparison with efavirenz.

To add information on this issue, we report the results from the SCOLTA (Surveillance COhort Long-term Toxicity Antiretrovirals) project. This is an online reporting system for adverse reactions to antiretroviral drugs, designed by the CISAI (Coordinamento Italiano per lo Studio Allergia e Infeczione HIV; Italian Coordination for the Study of Allergy and HIV Infection) group. It originated as a pharmacovigilance system for newly introduced drugs and as a sentinel scheme for unexpected or late adverse reactions arising during any antiretroviral treatment. It works through the internet site www.cisai.info and currently involves 18 Italian infectious disease centres. Cohorts of patients are established for each new drug as it comes onto the market, and these patients are followed

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9 Antimicrob Agents Chemother 2012
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