Diverse mobilized class 1 integrons are common in the chromosome of pathogenic *Pseudomonas aeruginosa* clinical isolates

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Running Title: Class 1 integrons in the *P. aeruginosa* chromosome

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Abstract

Eleven clinical class 1 integron-containing *Pseudomonas aeruginosa* isolates from Australia and Uruguay were investigated for the genomic location of these elements. Several novel class 1 integron/transposons were found in at least four distinct locations in the chromosome including genomic islands. These elements seem to be undergoing successful dispersal by lateral gene transfer since integrons were identified across several lineages and more than one clonal line.

TEXT

Clinically, *Pseudomonas aeruginosa* is one of the most important nosocomial and opportunistic pathogens (4, 13). The acquisition of virulence factors and antibiotic resistance genes in recent years by this species has seen the evolution of pathogenic strains that are difficult to treat with antibiotics (2, 17). This acquired accessory genome, includes integrons carrying a variety of gene cassettes, transposons, and genomic islands (GIs). Class 1 integrons are commonly highly mobilized being found in plasmids and conjugation is a major mechanism by which resistance genes are spread between cells and across Genera. However, class 1 resistance integrons can also be found in chromosomes, frequently in GIs of pathogenic bacteria. Probably the best example of this is *Salmonella* genomic island 1 (SGI1) found in multiple serovars of *Salmonella enterica* (11). Although the association between class 1 integrons and GIs has been reported in other bacteria (5), including *P. aeruginosa* (8), the extent of the association in the latter is less clear. Recently we identified a two class 1 integron-containing transposon, Tn*6060* (Fig 1), in a genomic island (here referred to as GI1) of the *P. aeruginosa* clinical isolate 37308 (21). The cassette arrays in Tn*6060* have been commonly reported elsewhere although the genetic context is, in most cases, not known (19, 20, 24). To investigate whether Tn*6060* or relatives are dispersed in *P. aeruginosa*, *intI1*-positive clinical isolates derived from Australia and Uruguay were examined. We identified novel class 1 integrons/transposons in multiple chromosomal locations in several distinct clonal lines suggesting that non-plasmid lateral exchange of resistance regions may be common in *P. aeruginosa.*

P. aeruginosa isolates were recovered from three hospitals, two in Sydney, Australia (2010) and one in Montevideo, Uruguay (2008). Eleven *intI1* positive isolates (nine from Sydney and two from Montevideo) known to carry class 1 integrons were screened for the presence

of the transposition module of Tn*6060* and for the insertion of it in GI1 as reported previously (21). Two isolates positive from this screen, C79 from Sydney and U09 from Montevideo, were selected to investigate the context of class 1 integrons via the construction of fosmid genomic libraries (21). These two isolates were epidemiologically unrelated and comprised two different genetic clones based on PFGE analysis (Table 1). Both clonal types were different to the strain 37308 in which Tn*6060* was identified. Sequencing revealed that both C79 and U09 possessed two class 1 integrons and all were contained within mercury resistance transposons. A class 1 integron was present in both strains that, like Tn*6060,* are linked to a Tn*1403* transposition module (Fig 1). The sequence of the class 1 integron/transposons in each of C79 and U09 were identical despite their geographical and clonal origins thus implying lateral transfer. This transposon was designated Tn*6162* (Fig 1). Also, Tn*6162* in both C79 and U09 were inserted into GI1 at the same location as Tn*6060* in 37308 from Sydney (21). In both C79 and U09, PCR with appropriate primers (Table S1) and sequencing confirmed that the GI in these strains is located in the same position in the chromosome as reported in the cystic fibrosis strain PACS171b in which this GI (without an integron) was first found (7) and in the Tn*6060*-containing strain 37308. The cassette array common to Tn*6162* in C79 and U09, *aadA6*-gcuD (formerly orfD), is different to either of the two cassette arrays in Tn*6060* (21) (Fig 1). This array has been recovered in a *P. aeruginosa* isolate from France in 1998 (17) although the sequence context in which the array was found was not reported.

Based on the information obtained from C79 and U09, the remaining nine isolates were examined for the presence of Tn*6162* by sequencing or PCR analysis (Table 1). All nine possessed Tn*6162* in GI1. The second Uruguay isolate was the same PFGE clonal type as U09. Seven of the eight remaining Sydney isolates were the same PFGE clonal type as C79

81 with one comprising another distinct grouping based on PFGE, again implying lateral
82 movement of this GI and associated resistance region between strains.
83
84 The second class 1 integron and surrounding sequence in each of the strains C79 and U09
85 were recovered from appropriate fosmid clones and sequenced. In C79, this second integron
86 was not in GI1 but, rather, a second GI, here designated GI2. A GI closely related to GI2,
87 LESGI-3, has previously been reported in LESB58, an epidemic strain in United Kingdom
88 and is located in the chromosome (26). The second integron in C79 (Fig 2 and Table 1)
89 contained a four cassette array that included $bla_{\text{GES-5}}$. The integron had acquired a number of
90 IS elements including a variant of the *attC* targeting IS*Pa21* (23), here named IS*Pa21e,* as
91 well as IS*6100* and an IS*4*-like element (18). This integron was embedded in a mercury
92 resistance transposon Tn*4380*. This transposon, without an integron, has a small number of
93 precedents in the databases, including in the plasmid pMOL30, present in the environmental
94 soil bacterium *Cupriavidus metallidurans* strain CH34 (Accession number CP000354.2).
95 This implied mobility is consistent with a report (9), that showed that GIs in *P. aeruginosa*
96 can move between unrelated Genera. In C79 the transposon, here designated Tn*6163* (Fig 2),
97 was flanked by direct repeats implying insertion into GI2 by transposition. Tn*6163* was not
98 present in the two Uruguay isolates but was present in six of the remaining eight Sydney
99 isolates. All isolates with Tn*6163* had indistinguishable PFGE profiles and may thus
100 represent clonal spread within the Sydney region. The two Sydney isolates lacking Tn*6163*
101 also lacked GI2 based on PCR analysis.
102
103 In U09, the insertion point of the second class 1 integron/transposon was within the
104 chromosomal gene *oprD* (Fig 3A). Insertion into *oprD* is noteworthy since loss of this gene
105 is associated with increased levels of resistance to carbapenems (6, 12, 25). Sequence beyond

IRt revealed a Tn*21*-like *mer* operon beyond which was sequence of a GI similar to PAGI-2C (10). The integron in U09 possessed a five cassette array that included the $bla_{\text{OXA-129}}$ gene cassette. This cassette has only been seen once previously in a *Salmonella enterica* serovar Bredeney isolate from a pig in Brazil (14). PCR analysis revealed that U61, even though it has an indistinguishable PFGE profile to U09, has a complete and uninterrupted *oprD* gene. Extensive sequencing revealed that the second integron in U61 (Fig 3B) was identical to that of U09 with respect to IS*26* and IS*26*-linked sequence (that is, sequence to the left of IS*26* as shown in Fig 3). In U61 the sequence immediately beyond IS*26* consisted of about 4kb that was identical to sequence from another GI that is located in the chromosome of the betaproteobacterium *Herminiimonas arsenicoxydans* (16). *H. arsenicoxydans* is a recently characterized bacterium associated with arsenic contaminated water and sediments. In U61 this 4kb region is followed by a $tRNA_{gly}$ gene located in the *P. aeruginosa* chromosome (Fig 3B). The integrons associated with IS*26* in strains U09 and U61 are likely to be moving in a way that is mediated by this IS since the insertion point in each of the two strains is different. This IS*26*-associated mobilized region includes the integron, associated *mer* region and adjacent PAGI-2c like GI. It has been hypothesized that IS*26* can initiate non standard transposition that results in only a single copy appearing in the transposed product (3) and it is possible such an event is responsible for the mobilization seen here.

We have identified several multi drug resistant class 1 integron/transposons within the chromosomes of *P. aeruginosa* pathogenic isolates. Class 1 integron/transposons were found in at least four chromosomal locations and in different clonal lines based on PFGE analysis (Table 1). This locus variability is partly region specific. It is also noteworthy that lateral gene transfer is probably occurring between clinical isolates and bacteria from food production animals and the general environment given that a $bla_{OXA\text{-}129}$ containing IS*26*-

linked element is present in the former (14) and U61 has sequence identical to that found in the latter (16).

The chromosome may be an important platform in the dispersal of complex resistance regions in *P. aeruginosa.* All 20 integrons across the 11 isolates examined were linked, based on sequencing or PCR analysis, to either a core region of the *P. aeruginosa* chromosome or to a GI that is only known to be present in the chromosome. U09 is also noteworthy in that the insertion of the integron into the *oprD* gene is in itself extending the antibiotic resistance profile of this strain despite the fact that inactivation of this gene is likely to reduce fitness outside an infection context (1). In contrast, in a clinical context insertion at *oprD* may be selected for and we note that recently, a *P. aeruginosa* isolate from Japan was found to have a class 1 integron linked to IS*26* inserted at *oprD* (15). In this strain, the insertion point was different to that seen in U09 as was the cassette array. We predict that the chromosomal spread of diverse complex multidrug resistant regions is likely to be a common theme globally in *P. aeruginosa* pathogenic isolates.

Nucleotide sequence Accession numbers.

The sequence for Tn*6162*, Tn*6163* and the IS*26* linked regions in U09 and U61 have been submitted to GenBank under accession numbers. JF826498, JF826499, JF826500 and JN559393 respectively.

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255
256

Table 1. Features of class 1 integron-containing *P. aeruginosa* isolates

Isolate	Source	Date	Origin	Element/Location	Element/Location	PFGE[a]	*oprD*[b]	MIC (µg/ml)					
								IPM	MEM	ATM	CAZ	FEP	GEN
C79	S	04/2010	Urine	Tn*6162*/GI1	Tn*6163*/GI2	B	+	128	256	<8	64	<8	>256
S491	S	03/2010	Wound	Tn*6162*/GI1	Tn*6163*/GI2	B	+	32	128	<8	32	<8	>256
n=5	S	2010		Tn*6162*/GI1	Tn*6163*/GI2	B	+						
n=2	S	2010		Tn*6162*/GI1	NSI	B,D	+						
U09	M	05/2008	Catheter	Tn*6162*/GI1	IS*26*-like/*oprD*	C	-	8	16	<8	4	<8	>256
U61	M	07/2008	Urine	Tn*6162*/GI1	IS*26*-like/$tRNA_{gly}$	C	+	<0.5	2	<8	8	<8	>256

[a]Letters define different PFGE profiles (>7 band differences) as defined by Tenover et. al. (22) Profile in strain 37308 (21) is defined as ”A”. Multiple letters indicate examples of more than one profile in the group.

[b]Indicates a functional (+) or interrupted (-) *oprD* gene.

Abbreviations. S:Sydney, M:Montevideo, NSI: No second integron, IPM: imipenem, MEM: Meropenem, ATM: Aztreonam, CAZ: Ceftazidime, FEP: Cefepime, GEN: Gentamicin

Figure Legends

Figure 1. Structure of Tn*6162* in comparison to Tn*6060*.

Numbered horizontal lines indicate regions common to Tn*6060* and Tn*6162*. **A.** Tn*6060*. This is a modified version of Figure 1 from Roy Chowdhury et. al. (21). See also Accession number GQ161847. Top line depicts the transposon backbone in which the integron (bottom line) is inserted. Vertical arrow indicates the point of insertion. The filled vertical rectangles indicate inverted repeats (IRs) as shown. Filled horizontal arrows represent genes or operons and direction of transcription. Filled diamond is the *attI1* site and the filled ovals *attC* sites. Gene designations are as described in the text. **B.** Tn*6162*. The general organization is as for Tn*6060*

Figure 2. Structure of Tn*6163* and its genetic context.

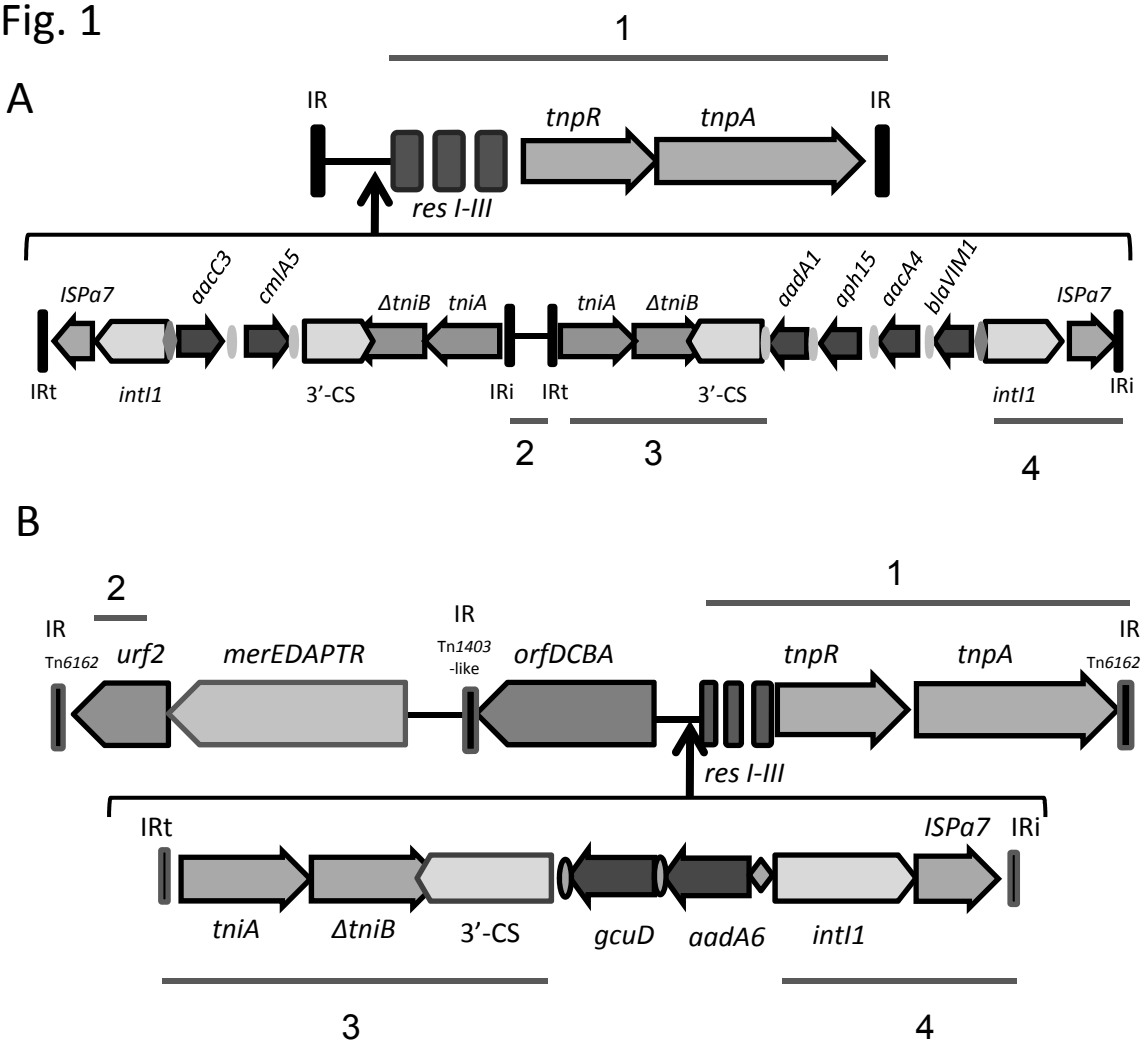
The general organization and symbols are as for Figure 1. Tn*6163*: IS4-like defines an element with 62% protein to identity to accession number NC_007336.1. *aacA4*-like gene cassette with 25 nucleotides replacing nucleotides 1-24 of the standard cassette. GI2 refers to genomic island 2. PAO2583 is a gene located in the core *P. aeruginosa* genome (Accession number AE004091) and is the inferred position of GI2 based on the known location of LESGI-3 (26) to which GI2 is highly similar.

Figure 3. Structure and location of IS*26*-associated integrons.

The general organization and symbols are as for Figure 1. **A.** Strain U09. *oprDΔ* is an insertionally inactiveated *oprD* gene. PAO958 is a gene located in the core *P. aeruginosa* genome (Accession number AE004091) **B.** HEAR2053 refers to a conserved hypothetical protein (Accession number CAL62196.1) and HEAR2054 refers to a DNA integrase (non-

298 integron) protein (CAL62197.1) located in the genome of a *Herminiimonas arsenicoxydans*
299 strain. The corresponding encoded proteins found here match most closely to these. PAO 2820 is
300 a gene located in the core *P. aeruginosa* genome (Accession number AE004091)
301
302

Fig. 1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aadA1
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ISPa7
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3'-CS
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Fig. 2

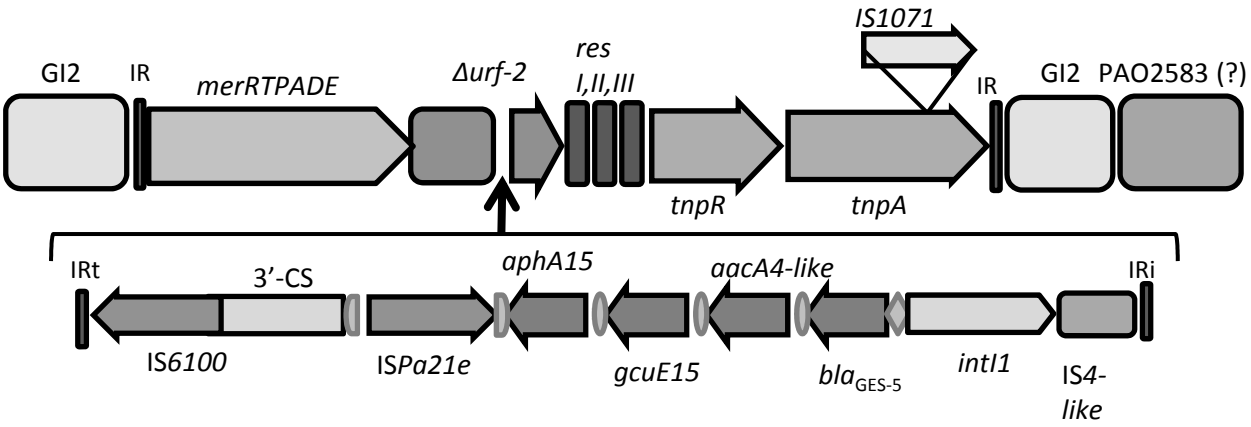
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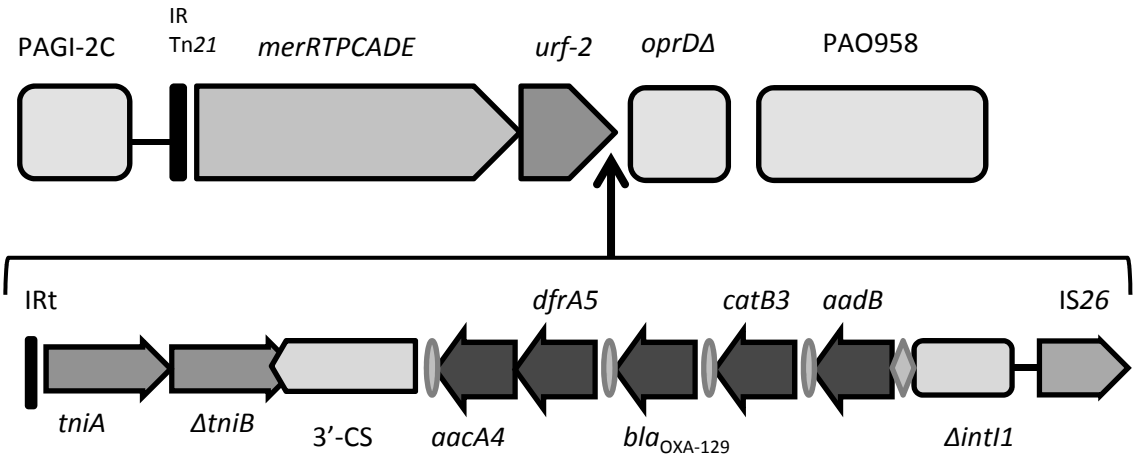
Fig. 3

A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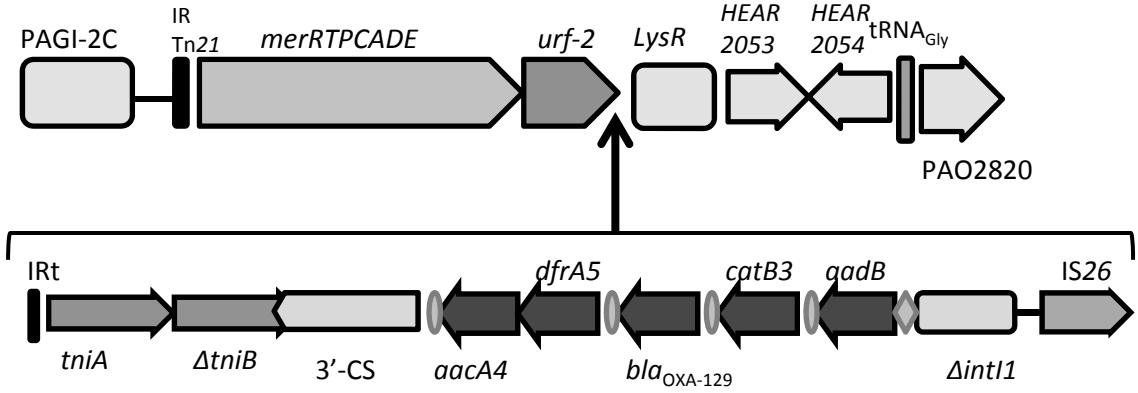
B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ΔintI1