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Genetic characterisation of antibiotic resistance transposons Tn*6608* and Tn*6609* isolated from clinical *Pseudomonas* strains in Cyprus

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ABSTRACT

Objectives: Antibiotic therapy for *Pseudomonas* infections is becoming increasingly difficult. In this study, the transposons from two multidrug-resistant (MDR) clinical *Pseudomonas* strains containing related transposons responsible for giving rise to resistance determinants were characterised.

Methods: Two MDR clinical *Pseudomonas* isolates were obtained from a medical facility in Cyprus. The strains were identified as *Pseudomonas putida* C54 and *Pseudomonas aeruginosa* C69. DNA was extracted from both strains and was sequenced. Transposons were identified, annotated and compared with DNA sequences in GenBank.

Results: Two related nested transposons, here named Tn*6608* (from *P. putida* C54) and Tn*6609* (from *P. aeruginosa* C69), were characterised. The transposons are built on an ancestral Tn*1403* base element (here named Tn*1403A*) that contains only the transposition module (*tnpA* and *tnpR*) and the associated cargo gene module (*orfA, orfB, orfC* and *orfD*) flanked by a 38-bp inverted repeat. The nested transposons identified in this study have evolved via acquisition of multiple transposons, adding multiple resistance genes to an ancestral transposon that originally lacked any resistance determinants.

Conclusion: Transposons related to Tn*6608* and Tn*6609* have evolved and are globally disseminated. Of particular interest is that most of these nested transposons are located within the same site in a genomic island, providing alternative avenues for dissemination.

1. Introduction

Multi-antibiotic-resistant *Pseudomonas* spp., particularly *Pseudomonas aeruginosa*, are opportunistic pathogens that poses serious health concerns in acute and chronic infections [1]. *Pseudomonas aeruginosa* belongs to the 'ESKAPE' pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, P. aeruginosa* and *Enterobacter* spp.), emphasising their ability to resist antibiotics and the urgency for discovery of new antimicrobials. As such, *P. aeruginosa* infections will continue to contribute to increased rates of mortality until new effective treatment option becomes available. *Pseudomonas aeruginosa* is widespread and non-clonal, and a diverse range of high-risk groups are recognised based on multilocus sequence typing (MLST), i.e. ST235, ST111 and ST175 [2].

Pseudomonas aeruginosa continues to gain attention due to widespread β-lactam resistance giving rise to carbapenem resistance, most of which occurs due to the presence of a gene cassette captured in a class 1 integron [3,4]. Class 1 integrons are gene capture and expression systems that contain a variable region flanked by two conserved segments, i.e. 5′-CS and 3′-CS. The 5′-CS is composed of an integrase-encoding *intI1* gene, an *attI* recombination site and a Pc promoter responsible for expression of the captured gene cassette. The 3′-CS is composed of a *qacEΔ1* gene fused to a complete *sul1* gene giving rise to quaternary ammonium compound and sulfonamide resistance, respectively [5]. The 3′-CS can also be divided into different lineages owing to the presence of ad-

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ditional genes of unknown function (i.e. *orf5* and *orf6*) and insertion sequence (IS) elements (e.g. IS*6100* and IS*1326*) [6]. The IS elements are thought to have generated deletions of genes that make up the transposition module of Tn*402* as they retain the associated inverted repeat (IR) borders [7].

Tn*402* is a member of the *res* site hunter family of transposons and has been shown to display target and orientational specificity for *res* sites within the *par* locus of plasmid RP1 and other *res* sites within some transposons [8]. The *res* site hunter transposons are flanked by 25-bp imperfect IRs and contain a four-gene transposition module (*tniA, tniB, tniQ* and *tniR*) that relies on an external resolvase and targets its cognate *res* site [9]. Depending on the accessory module, *res* site hunter transposons are further divided into subgroups: Tn*402* subgroup comprises a class 1 integron (described above); and Tn*5053* subgroup possess a mercury (II) resistance operon [9–11]. The defective transposition module of the Tn*402*-like class 1 integrons often contain a remnant that includes *tniA* and a partial *tniB* [6,7]. For example, In*0* and In*2* contain an 852-bp *tniB* remnant and In*5* contains a 450-bp *tniB* remnant and a complete *tniA*. Other integrons have lost all genes involved in transposition but retain their IRs and have been shown to translocate with *in trans*-assisted functions [12]. As such, some class 1 integrons reported in *P. aeruginosa* are part of larger mobile genetic elements such as transposons and genomic islands (GIs). Tn*1403* and Tn*6060* are two such examples of nested transposons containing a class 1 integron and are present within a GI [2,13]. These nested transposons give rise to multiple antibiotic resistance and can use different avenues to disseminate their resistance determinants.

In this study, we characterised two evolutionarily-related nested transposons found in multidrug-resistant (MDR) clinical *Pseudomonas* isolates. We uncover an additional 21 related transposons located within the same site in a GI in *P. aeruginosa*. These transposons are globally disseminated within clinical *P. aeruginosa* strains, contributing to antibiotic-resistant infections. This study demonstrates how the acquisition of integrons and other transposons has increased the resistance determinants located within this transposon. Interestingly, one lineage of these transposons contains two integrons, generating an integron platform within the nested structure and increasing the chances of capturing gene cassettes.

2. Materials and methods

2.1. Bacterial strains, PCR analysis and 16S rRNA gene sequencing

Pseudomonas putida C54 and *P. aeruginosa* C69 were identified as infectious agents and sourced from the Pathology Department at Near East University Hospital (Nicosia, Cyprus). Strains were grown on nutrient agar (Oxoid), nutrient broth (Oxoid) or Diagnostic sensitivity (DST) agar (Oxoid). Antibiotics were used at the following concentrations in nutrient agar; chloramphenicol, 30 μg/mL; kanamycin, 350 μg/mL; mercuric chloride, 10 μg/mL; streptomycin, 100 μg/mL; and DST sulfonamide, 400 μg/mL.

PCR amplification of the 16S rRNA gene involved the use of Bio-Rad iProof™ High Fidelity DNA Polymerase (Bio-Rad, Gladesville, NSW, Australia). Universal primers 27F (AGAGTTTGATCCTGGCTCAG) and 1492R (GGTTACCTTGTTACGACTT) were used. Reaction mixtures were placed in a thermal cycler for 30 cycles under the following conditions: 98°C for 2 min (first cycle only); 98°C for 30 s, 55°C for 30 s and 72°C for 45 s; and 72°C for 10 min (last cycle only). PCR products were detected using agarose gel electrophoresis and staining with SYBR® Safe (Thermo Fisher Scientific, Scoresby, VIC, Australia). PCR products were sequenced at the Australian Genome Research Facility using an ABI3730 DNA Sequencer.

2.2. DNA extraction, next-generation sequencing and annotations

DNA from *P. putida* C54 and *P. aeruginosa* C69 was obtained using a Wizard® Genomic Extraction Kit (Promega, Alexandria, NSW, Australia) according to the manufacturer's instructions. DNA libraries were prepared for sequencing using a Nextera XT v.2 DNA Library Preparation Kit (Illumina Inc., San Diego, CA, USA) according to the manufacturer's instructions. DNA sequencing was performed using an Illumina MiSeq V3 Reagent Kit (300 cycles) (Illumina Inc.). De novo assembly of sequencing reads was conducted using Unicycler [14], and gene annotation was performed manually and confirmed using BLAST NCBI. MLST of the strains was performed using MLST software (Galaxy v.2.16.1; https://github.com/tseemann/mlst).

2.3. Nucleotide accession numbers

Sequences of Tn*6608* and Tn*6609* were deposited in GenBank under the accession numbers **MZ361366** and **MZ361367**, respectively.

3. Results and discussion

Two MDR clinical *Pseudomonas* isolates responsible for a urinary tract infection and a wound infection were obtained from a medical facility in Cyprus. The strains were identified as *P. putida* C54 and *P. aeruginosa* C69, respectively, using matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) analysis and 16S rRNA gene sequencing. DNA was extracted from both strains and was sequenced on an Illumina MiSeq platform, confirming the identification of the strains and further revealing their MLST grouping as ST15 for *P. putida* and ST253 for *P. aeruginosa*. The sequence revealed two related nested transposons, here named Tn*6608* (from *P. putida* C54) and Tn*6609* (from *P. aeruginosa* C69). The transposons are built on an ancestral Tn*1403* base element (here named Tn*1403A*) that contains only the transposition module (*tnpA* and *tnpR*) and the associated cargo gene module (*orfA, orfB, orfC* and *orfD*) flanked by a 38-bp IR (Fig. 1).

Although Tn*6608* and Tn*6609* are derived from the same Tn*1403A* base element, they differ in their structural components. Tn*6608* contains the *mer* operon (*merRTPADE*) most closely related to Tn*AO22* [15] disrupting the transposase gene of Tn*1403A* (Fig. 1). The *urf2* gene within the *mer* module is disrupted by the insertion of an intact Tn*5393c*, a streptomycin resistance transposon and is flanked by 5-bp direct repeats (DR) (AGCTT) [16]. The transposition module of Tn*AO22* is also missing a portion of the Tn*1403A* transposition module, i.e. *tnpR* and the 5′ region of *tnpA*. The cargo genes of Tn*1403A* have also been disrupted by the insertion of a class 1 integron, here named In*1708*. The integron has an In*4* backbone structure comprised of 3′-CS followed by the insertion of IS*6100* (Fig. 1) [6]. The integron has a single gene cassette, *cmlA1*, giving rise to chloramphenicol resistance. The entire structure is flanked by the 25-bp IR and 5-bp DR sequences (TCGTC), suggesting its placement here was due to transposition. The insertion site of the integron occurs within *orfD* of the accessory module and is not in a *res* site as shown in Tn*1403* [13] (Fig. 1). Based on the sequence analysis, we conclude that Tn*1403A* first inherited Tn*AO22*, which may have already occupied Tn*5393c* or was acquired later. A subsequent deletion event has resulted in deletion of the Tn*AO22* transposition module as well as a portion of the transposition module in Tn*1403A* (Fig. 1). Acquisition of the transposable elements within the nested structure of Tn*6608* has provided this element with additional genes giving rise to mercury, streptomycin, chloramphenicol and sulfonamide resistance.

Tn*6609* is structurally more complex than Tn*6608*. The entire transposition module is present and the *res* site is disrupted by the

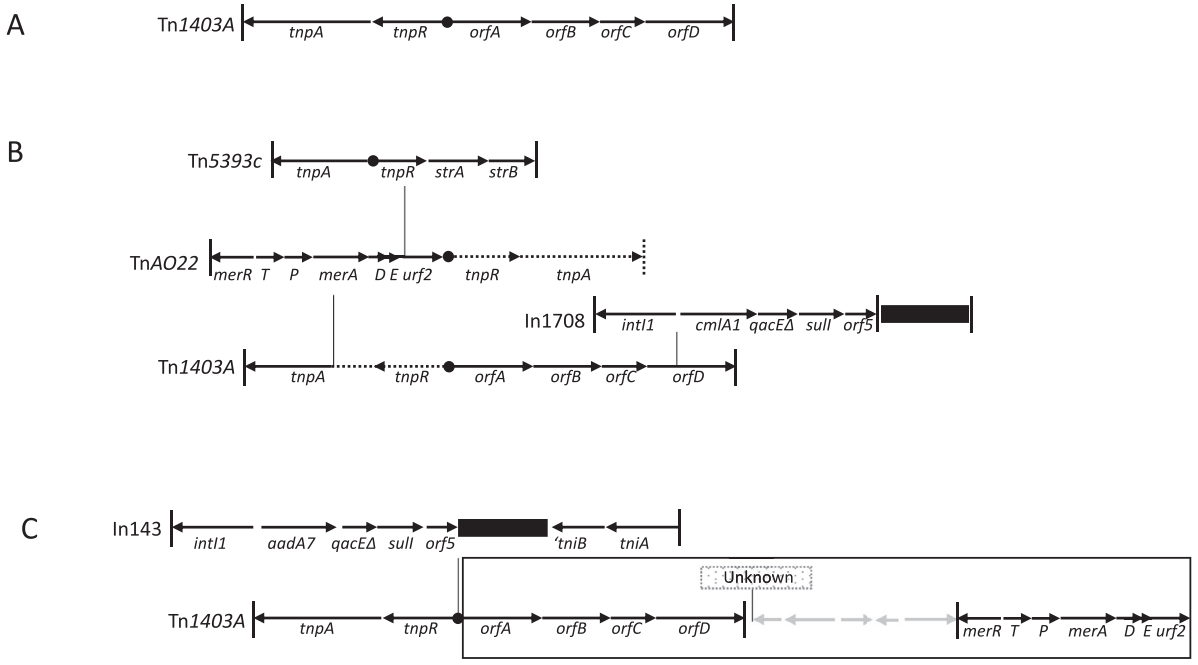

Fig. 1. Genetic map of (A) Tn*1403A* and genesis of (B) Tn*6608* and (C) Tn*6609*. (A) Tn*1403A* is the structure of the ancestral Tn*1403*. (B) The structure of Tn*6608* is derived from insertion of Tn*AO22*, Tn*5393c* and In*1708*. Dotted lines indicate deletion events that have resulted in the loss of these genes. (C) Tn*6609* is derived from the insertion of In*143* followed by a large inversion (indicated by the box). Gray genes are of unknown function, and the dotted insertion box indicates an unknown sequence that potentially contains insertion sequence (IS) elements (one end with IS*1162* and the other end with IS*UnCu4*). Open reading frames are represented as arrows, and gene names are indicated below the arrow. Vertical lines amongst the arrows indicate invert repeat (IR) sequences. Black boxes ff IS elements. Thin vertical lines indicate the point of insertion of the transposon.

insertion of a class 1 integron with a single gene cassette, *aadA7*, that encodes resistance to aminoglycosides, followed by the 3′-CS characteristic of class 1 integrons. Following the 3′-CS is IS*1326* flanking a partial sequence of *tniB* and a complete *tniA* (Fig. 1). This integron structure has been found in other *Escherichia coli* and *Salmonella enterica* plasmids previously and was named In*143* [17,18]. Adjacent to the integron is a mercury resistance module flanked by a 38-bp IR that appears to have replaced the cargo genes of the ancestral Tn*1403A* (Fig. 1). However, further upstream of the *mer* module, a cluster of hypothetical genes appear followed by the cargo module (*orfA, orfB, orfC* and *orfD*) flanked by a 38-bp IR. The cargo genes module is oriented in the opposite orientation compared with Tn*1403A* or Tn*6608*, suggesting a possible inversion (Fig. 1). Based on the sequence analysis, we conclude that Tn*1403A* inherited In*143* probably by an *in trans*-mediated event targeting the *res* site. The mercury resistance module together with the genes encoding hypothetical proteins and the *orf* cargo module may have undergone a DNA inversion event and minor deletions generating this structure (Fig. 1). Acquisition of the transposable elements within the nested structure of Tn*6609* has provided this element with additional genes giving rise to mercury, streptomycin and sulfonamide resistance.

Tn*6608* and Tn*6609* are nested transposons built on a base element of an ancestral Tn*1403* isolated from *Pseudomonas* spp. Tn*6608* appears to be defective due to the truncated transposition module, whereas Tn*6609* retains a complete transposition module suggesting that it is active. Acquisition of the DNA elements has converted the phenotypically cryptic Tn*1403A* into resistance transposons. Based on the predicted evolution of Tn*6608* and Tn*6609*, it is clear that they acquired similar elements but their evolutionary division occurred after the first acquisition. Tn*6608* appears to have inserted in the chromosomal gene *eutH* that encodes ethanolamine utilisation protein in *P. putida* and is flanked by a 5-bp DR (AAGTA). Tn*6609* is inserted within a GI similar to PA143/97 in *P. aeruginosa* and is flanked by a 5-bp DR (ATGAC). Of striking significance is that three previously described transposons, namely Tn*6060*, Tn*6249* and Tn*6162*, all appear to be inserted in the same site within a similar GI but have different configurations [3,19,20] (Fig. 2). That the elements are located within same site and share almost identical transposition genes (or remnants of) suggests that insertion, deletion and recombination events within a Tn*1403A* backbone have led to three different transposons structures.

We explored the GenBank database searching for transposition genes identical to that of Tn*6609* which appears to be part of a transposon and inserted in the same site in the GI PA143/97. Surprisingly, 21 transposons were detected that have inserted in the exact same site within the GI PA143/97 and all have almost identical transposition modules (Table 1). In addition, most strains were isolated from clinical sources and are disseminated worldwide (Table 1).

The structure of Tn*6609* appears most loosely related to Tn*6162* with the exception of a large inversion of the accessory module [19] (Fig. 2). In addition to the inversion, some minor differences occur within the centre of the region that includes genes of unknown function and an IS element (Fig. 1). The integron in Tn*6162* (i.e. In*51*) differs to In*143* in Tn*6609* as it contains a different gene cassettes array, lacks IS*1326* and is flanked by different DRs, suggesting independent insertion events. Another 11 transposon sequences detected in GenBank appear to have the same right-hand arm, but the left arm appears inverted to Tn*6609*, but similar to Tn*6162* except for four instances where the hypothetical modules were not inverted (Fig. 2). The integron in two isolates from Japan (GenBank accession nos. **AP014646** and **AP014622**) and one isolate from Toronto (GenBank accession no. **CP020603**) appear to have generated an intramolecular deletion within the *tnpA* of Tn*1403A* abolishing the DR sequences (Fig. 2). Interestingly, the Toronto isolate has also generated a deletion on the other side of the integron eliminating the left arm of the transposon. Other small unknown insertions have occurred in some of the

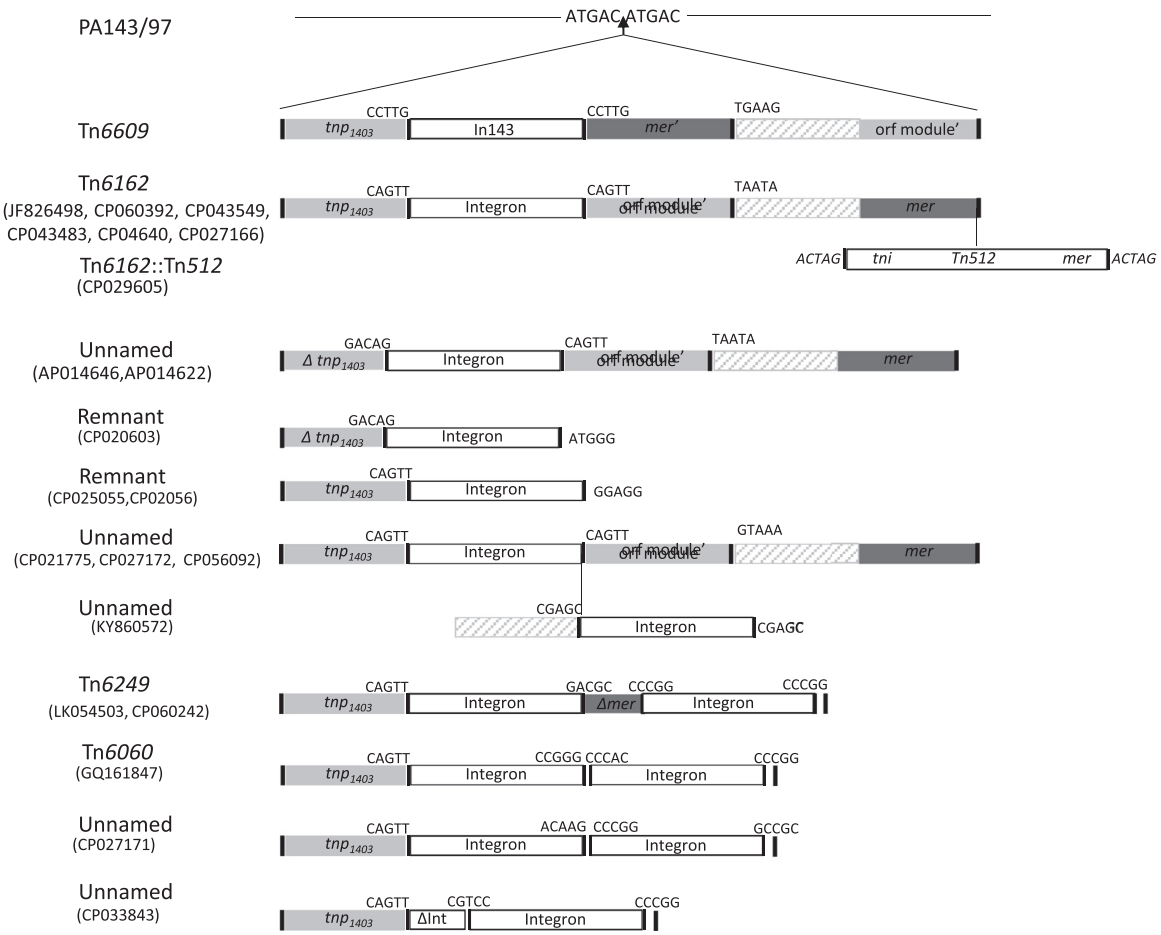

Fig. 2. Structures of transposons in the genomic island PA143/97. PA143/97 is represented by a straight line with the sequence of the direct repeats (DRs). The arrow represents the insertion site of all of the elements, and their structures or remnants are shown. Modules are shown in boxes shaded in different colours; light grey, tnp_{1403} and *orf* module; white, integron and Tn*512*; dark grey, *mer* module; and grey stripes, hypothetical module. Inverted repeats (IR) are indicated by black vertical lines, and DR sequences are listed closest to the IR. Integrons and hypothetical modules differ between the elements and are not always the same.

Table 1
Tn*1403A*-derived transposons in genomic island PA143/97 of *Pseudomonas aeruginosa*

GenBank accession no.	Strain	Source	Origin	Year
CP025056	*P. aeruginosa* PB367	Tracheal aspirate	USA	2016
CP025055	*P. aeruginosa* PB350	Sputum	USA	2016
CP043549	*P. aeruginosa* GIMC5002:PAT-169	Urine	Russia	2009
CP043483	*P. aeruginosa* GIMC5001:PAT-23	Human	Russia	2006
CP033843	*P. aeruginosa* FDAARGOS_501	Human	USA	2018 [a]
CP029605	*P. aeruginosa* 24Pae112	Blood	Colombia	2015
CP027166	*P. aeruginosa* AR_0357	Unknown	USA	2017 [a]
CP027172	*P. aeruginosa* AR_0353	Unknown	USA	2017 [a]
CP027171	*P. aeruginosa* AR_0354	Unknown	USA	2017 [a]
KY860572	*P. aeruginosa* 29785cz	Clinical	Czech Republic	2015
CP021775	*P. aeruginosa* Pa58	Bronchial washing	Mexico	2005
LK054503	*P. aeruginosa* genomic island VR-143/97	Clinical	Italy	1997
CP046402	*P. aeruginosa* SE5331	Respiratory	China	2018
CP060242	*P. aeruginosa* B-I-1	Urine	France	2016
CP056092	*P. aeruginosa* PABCH14	Endotracheal tube	USA	2013
GQ161847	*P. aeruginosa* 37308 genomic island	Clinical	Australia	2008
CP020603	*P. aeruginosa* E6130952	Sputum	Toronto	2014
AP014646	*P. aeruginosa* NCGM 1984	Clinical	Japan	2012
AP014622	*P. aeruginosa* NCGM 1900	N/D	Japan	2017 [a]
CP060392	*P. aeruginosa* 1903031130	Sputum	China	2019
JF826498	*P. aeruginosa* C79	Urine	Australia	2010

[a] Year sequence submitted to GenBank.

transposons, however one such example from the Czech Republic (**KY860572**) contains an additional integron generating the double integron platform (Fig. 2).

The double integrons also appear in other examples of this family of transposons that are missing the cargo module and only retain the transposition module. These integron platforms appear to have been independently generated as the exact insertion site of the integrons differs (Fig. 2). The transposons appear to retain only the transposition module, two integrons that have presumably deleted the remainder of the genes within the Tn*1403A* transposon. Given that the integrons are not flanked by 5-bp DRs suggests that these elements may have undergone an intramolecular deletion resulting in the deletion of the cargo genes. Tn*402*-like transposons are shown to have insertional preference for the *res* sites of natural plasmids or transposons in the presence of a functional cognate resolvase gene [8] and possess orientation specificity, with the left IR (IRi) inserting closest to the target resolvase gene [12]. The class 1 integrons flanked by IRs have been shown to transpose when the transposition activity is provided *in trans* [12]. Here we identify double integrons, one located in the *res* site in the expected orientation and the other located in the opposite orientation. The mechanism of the second integron insertion adjacent to an already acquired integron has not been characterised.

In conclusion, Tn*6608* and Tn*6609* have evolved within a base element of Tn*1403A* via insertion, deletion and recombination events. These elements can serve as platforms to harbour class 1 integrons in *Pseudomonas* spp. and enable acquisition and dissemination of multiple antimicrobial resistance determinants. Tn*6609* is found within the GI PA143/97 and we identified 21 related elements in GenBank that were found within the same site of the GI in geographically dispersed *P. aeruginosa* strains. Some transposons identified in GenBank contain double integron platforms that could enable further incorporation of antimicrobial resistance determinants. This study reveals that the phenotypically cryptic transposon Tn*1403A* has acquired multiple antibiotic resistance genes providing its host with selective advantages. In addition, these elements are commonly encountered in *P. aeruginosa*, however the identification of Tn*6608* in *P. putida* provides evidence for dissemination in another species.

Funding

None.

Competing interest

None declared.

Ethical approval

Not required; this study was conducted on clinical isolates obtained from a hospital in Cyprus that were de-identified.

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References

[1] Didelot X, Walker AS, Peto TE, Crook DW, Wilson DJ. Within-host evolution of bacterial pathogens. Nat Rev Microbiol 2016;14:150–62. doi:10.1038/nrmicro.2015.13.
[2] Roy Chowdhury P, Scott MJ, Djordjevic SP. Genomic islands 1 and 2 carry multiple antibiotic resistance genes in *Pseudomonas aeruginosa* ST235, ST253, ST111 and ST175 and are globally dispersed. J Antimicrob Chemother 2017;72:620–2. doi:10.1093/jac/dkw471.
[3] Roy Chowdhury P, Merlino J, Labbate M, Cheong EY, Gottlieb T. Stokes HW. Tn*6060*, a transposon from a genomic island in a *Pseudomonas aeruginosa* clinical isolate that includes two class 1 integrons. Antimicrob Agents Chemother 2009;53:5294–6. doi:10.1128/AAC.00687-09.
[4] Vézina G, Levesque RC. Molecular characterization of the class II multiresistance transposable element Tn*1403* from *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 1991;35:313–21. doi:10.1128/AAC.35.2.313.
[5] Hall RM, Brown HJ, Brookes DE, Stokes HW. Integrons found in different locations have identical 5′ ends but variable 3′ ends. J Bacteriol 1994;176:6286–94. doi:10.1128/jb.176.20.6286-6294.1994.
[6] Partridge SR, Brown HJ, Stokes HW, Hall RM. Transposons Tn*1696* and Tn*21* and their integrons In*4* and In*2* have independent origins. Antimicrob Agents Chemother 2001;45:1263–70. doi:10.1128/AAC.45.4.1263-1270.2001.
[7] Brown HJ, Stokes HW, Hall RM. The integrons In*0*, In*2*, and In*5* are defective transposon derivatives. J Bacteriol 1996;178:4429–37. doi:10.1128/jb.178.15.4429-4437.1996.
[8] Minakhina S, Kholodii G, Mindlin S, Yurieva O, Nikiforov V. Tn*5053* family transposons are *res* site hunters sensing plasmidal *res* sites occupied by cognate resolvases. Mol Microbiol 1999;33:1059–68. doi:10.1046/j.1365-2958.1999.01548.x.
[9] Kholodii GY, Mindlin SZ, Bass IA, Yurieva OV, Minakhina SV, Nikiforov VG. Four genes, two ends, and a *res* region are involved in transposition of Tn*5053*: a paradigm for a novel family of transposons carrying either a *mer* operon or an integron. Mol Microbiol 1995;17:1189–200. doi:10.1111/j.1365-2958.1995.mmi_17061189.x.
[10] Petrovski S, Blackmore DW, Jackson KL, Stanisich VA. Mercury(II)-resistance transposons Tn*502* and Tn*512*, from *Pseudomonas* clinical strains, are structurally different members of the Tn*5053* family. Plasmid 2011;65:58–64. doi:10.1016/j.plasmid.2010.08.003.
[11] Rajabal V, Stanisich VA, Petrovski S. Tn*6603*, a carrier of Tn*5053* family transposons, occurs in the chromosome and in a genomic island of *Pseudomonas aeruginosa* clinical strains. Microorganisms 2020;8:1997. doi:10.3390/microorganisms8121997.
[12] Petrovski S, Stanisich VA. Tn*502* and Tn*512* are *res* site hunters that provide evidence of resolvase-independent transposition to random sites. J Bacteriol 2010;192:1865–74. doi:10.1128/JB.01322-09.
[13] Stokes HW, Elbourne LD, Hall RM. Tn*1403*, a multiple-antibiotic resistance transposon made up of three distinct transposons. Antimicrob Agents Chemother 2007;51:1827–9. doi:10.1128/AAC.01279-06.
[14] Wick RR, Judd LM, Gorrie CL, Holt KE. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol 2017;13:e1005595. doi:10.1371/journal.pcbi.1005595.
[15] Ng SP, Palombo EA, Bhave M. The heavy metal tolerant soil bacterium *Achromobacter* sp. AO22 contains a unique copper homeostasis locus and two *mer* operons. J Microbiol Biotechnol 2012;22:742–53. doi:10.4014/jmb.1111.11042.
[16] L'Abée-Lund TM, Sørum H. Functional Tn*5393*-like transposon in the R plasmid pRAS2 from the fish pathogen *Aeromonas salmonicida* subspecies *salmonicida* isolated in Norway. Appl Environ Microbiol 2000;66:5533–5. doi:10.1128/AEM.66.12.5533-5535.2000.
[17] Cox KEL, Schildbach JF. Sequence of the R1 plasmid and comparison to F and R100. Plasmid 2017;91:53–60. doi:10.1016/j.plasmid.2017.03.007.
[18] Schuurmans JM, van Hijum SA, Piet JR, Händel N, Smelt J, Brul S, et al. Effect of growth rate and selection pressure on rates of transfer of an antibiotic resistance plasmid between *E. coli* strains. Plasmid 2014;72:1–8. doi:10.1016/j.plasmid.2014.01.002.
[19] Martinez E, Marquez C, Ingold A, Merlino J, Djordjevic SP, Stokes HW, et al. Diverse mobilized class 1 integrons are common in the chromosomes of pathogenic *Pseudomonas aeruginosa* clinical isolates. Antimicrob Agents Chemother 2012;56:2169–72. doi:10.1128/AAC.06048-11.
[20] Di Pilato V, Pollini S, Rossolini GM. Tn*6249*, a new Tn*6162* transposon derivative carrying a double-integron platform and involved with acquisition of the bla_{VIM-1} metallo-β-lactamase gene in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 2015;59:1583–7. doi:10.1128/AAC.04047-14.