to that of the widely spread Tn*1548*-bearing plasmid pMDR-ZJ06 (Rep3 family), with the gene cassette *aac(3')-II* being replaced by *aacA4* and *catB8* (accession no. CP001938). The *K. pneumoniae* strain belongs to MLST sequence type 307 (ST307) and harboured a 20 kb plasmid (IncF) that was not investigated further.

This study demonstrates that pan-aminoglycoside resistance may be acquired by *P. aeruginosa* following the transfer of Tn*1548* on *Pseudomonas*-specific plasmids such as pOZ176. Whether the Tn*1548* module was transmitted *in vivo* from *K. pneumoniae* or *A. baumannii* is unknown. However, the observation that a patient may be colonized with several phylogenetically different ArmA-producing bacteria highlights the diffusion potentials of Tn*1548* among Gram-negative species and reinforces the need to screen for 16S rRNA methylases not only in *Acinetobacter* and Enterobacteriaceae species, but also in *P. aeruginosa*. A high resistance to arbekacin (MIC > 256 mg/L) may serve as an indicator of methylase production prior to the use of molecular biology methods.

Acknowledgements

We thank Fabrice Poncet and Marie-José Nasone for excellent laboratory assistance.

Funding

The French National Reference Centre for Antibiotic Resistance is funded by the French Ministry of Health through the InVS agency.

Transparency declarations

None to declare.

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J Antimicrob Chemother 2017
doi:10.1093/jac/dkw471
Advance Access publication 20 December 2016

Genomic islands 1 and 2 carry multiple antibiotic resistance genes in *Pseudomonas aeruginosa* ST235, ST253, ST111 and ST175 and are globally dispersed

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Sir,

Studies of MDR or XDR *Pseudomonas aeruginosa* often describe an inability to transfer antimicrobial resistance loci into recipient cells, suggesting that plasmids do not play a prominent role in dissemination of MDR loci in this species.[1–6] While two recent studies have highlighted the important role of genomic islands (GIs) in the carriage and transfer of multiple antimicrobial resistance in *P. aeruginosa*,[7,8] much of the hypothesis was based on earlier studies.[4,7,9,10] Here we present a pilot bioinformatics analysis (strategy detailed in Figure S1, available as Supplementary data at *JAC* Online) on 22 complete and 252 draft *P. aeruginosa* (Taxa ID: 287, resistance profiles unknown) genome sequences in the NCBI-Microbial-BLAST database (on 11 April 2016) as evidence of the presence of GI1, GI2 and associated transposons on other globally dispersed clonal lineages.

Excluding our Australian isolates in Table S1, 11% (31/274) of *P. aeruginosa* genomes in the database carry GI1 while 14% (38/274) carry GI2 or variants of them. It is notable that none of the non-Australian genomes contains both GI1 and GI2. The 31 *P. aeruginosa* strains that contained GI1 were from the USA, Spain,

France, Germany, Japan, Mexico, Argentina and Israel and belonged to different *P. aeruginosa* STs, including ST235 (19 strains), ST253 (5 strains), ST348 (3 strains), ST179 (2 strains) and ST463 (1 strain). GI2 was identified in 38 *P. aeruginosa* strains from various European countries, including Germany, Romania, France, Spain, the Netherlands, Belgium, Croatia, Italy, Portugal and Greece as well as the USA, Columbia and India. While most belonged to ST111 (19 isolates), ST175 (10 isolates) or ST235 (7 isolates), one strain of *P. aeruginosa* typed as ST395 and another as ST823. *P. aeruginosa* ST235 appears to play a significant role in the carriage of GI1, although some ST235 strains harbour GI2. GI2 associates mostly with *P. aeruginosa* ST111 and ST175 (Table S1). We also sequenced four additional ST235 isolates collected between 2007 and 2011 from various sources in Sydney (GenBank accession numbers LVEC01000000, LVED01000000, LVEF01000000 and LWGS01000000) and found versions of GI1 and GI2 that were identical to the islands described in strain RNS_PA1 from 2006.

Given that GI1 or GI2 were identified in 69/274 (25%) of the *P. aeruginosa* genome sequences deposited in the database, we examined the frequency of carriage of transposons Tn*6060*, Tn*6162*, Tn*6163* and Tn*6249* within the selected cohort (Table S1, part C). Nineteen of the 274 (7%) genomes carried a Tn*6060*-family transposon, and in 74% (14/19) of the genomes it was inserted at an identical location in GI1. Of these 14 strains, 6 belonged to ST235, 5 were ST253 and the remaining 3 strains were ST179, ST463 and an unknown ST, from different countries. Irrespective of ST, 11 of the 14 strains carrying GI1 also contained the cassette array (*aadA6-gcuD*) found in Tn*6162*. Strains NCGM1900 and NCGM1984 from Japan had an extra cassette (*aacA7*) inserted in the array, while the cassette array in strain U2504 was different.

Of 274 genomes in the database 30 (11%) harboured a Tn*6163* backbone. Fourteen of these (47%), with ST111, ST175 and ST235, were in GI2 (Table S1, part C). In the remaining 16 strains, Tn*6163* was not integrated into GI2 and was found in strains with various STs. None of the *P. aeruginosa* strains with GI2 carried the Ambler class A carbapenemase gene bla_{GES-5} that is seen in XDR strains from Sydney.[7] ST111 strains from different European countries, all of which carried a Tn*6163*-like transposon in GI2, contained an *aacA4-blaP1b-aadA2* cassette array encoding resistance to gentamicin/tobramycin (*aacA4*), carbenicillin (*blaP1b*) and streptomycin/spectinomycin (*aadA2*). Thus, it is evident that while GI1 and GI2 are hotspots for the insertion of Tn*6060*-family transposons and Tn*6163*, respectively, these transposons can also integrate at alternative sites in the *P. aeruginosa* genome.

While these data are consistent with ST235 being a globally dispersed clone, the acquisition (or loss) of the GIs and the transposons the GIs harbour are likely to influence their resistome. Since all of the *P. aeruginosa* strains in our study carry one or more class 1 integrons in their genomes, opportunities exist to gain or lose resistance gene cassettes or evolve complex antibiotic resistance loci via homologous recombination, as seen in Tn*6060* and Tn*6249*.

Data presented in the current study provide a snapshot of a global scenario that implicates GI1 and GI2 in the mobilization of MDR loci not only within ST235 but in other globally dominant clones of *P. aeruginosa*, including ST111 and ST175. All ST235 strains from Sydney that carried both GI1 and GI2 clustered within ST235 strains that contain either GI1 or GI2 (Figure S2), suggesting the ST235 clonal lineage dominant in Sydney is distinct and may have arisen either by transfer of GI1 into a strain of *P. aeruginosa* that carried GI2 or by the phage-mediated transfer of GI2 into a strain that carried GI1, followed by clonal expansion.

Acknowledgements

We wish to thank colleagues at the Microbiology Laboratory of Royal North Shore Hospital (RNSH) in Sydney for identifying the bacteria and making this project possible.

Funding

This work was funded by the Australian Centre for Genomic Epidemiological Microbiology (AUSGEM) partnership and by a University of Technology Sydney (UTS) small grant to P. R. C.

Transparency declarations

None to declare.

Author contributions

P. R. C. initiated the study, designed and performed BLAST searches and compiled the tables and figures (with assistance from M. J. S.). S. P. D. and P. R. C. wrote the manuscript.

Supplementary data

Figures S1 and S2 and Table S1 are available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

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J Antimicrob Chemother 2017
doi:10.1093/jac/dkw451
Advance Access publication 17 November 2016

Comparative *in vitro* activity of oritavancin and other agents against vancomycin-susceptible and -resistant enterococci

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Sir,

Antibiotic research has focused on discovering agents with activity against MDR pathogens (e.g. ESKAPE pathogens[1]), including VRE, which along with *Staphylococcus aureus* are commonly isolated from healthcare-associated infections.[2] Several new agents have been approved for the treatment of skin and skin structure infections caused by MRSA and enterococci, including oxazolidinones (linezolid and tedizolid), lipoglycopeptides (oritavancin, dalbavancin and telavancin), a cyclic lipopeptide (daptomycin), a glycylcycline (tigecycline) and an anti-MRSA cephalosporin (ceftaroline). Lipoglycopeptides, though active against vancomycin-susceptible enterococci (VSE), have variable activity against VRE, with oritavancin being the sole agent maintaining potent activity against VanA-type VRE.[3]

We report here a direct comparison of the *in vitro* activity of lipoglycopeptides and other skin agents against vancomycin-susceptible and -resistant *Enterococcus faecalis* (VSEfa and VREfa) and *Enterococcus faecium* (VSEfm and VREfm). Comparative evaluations included MIC and MBC determinations and time–kill kinetics. Since variation in inoculum density has been shown to impact the activity of several of these agents against *S. aureus*,[4,5] time–kill kinetics were assessed at both standard and high inoculum densities.

The evaluated isolates consisted of 74 random non-duplicate clinical isolates of VSEfa, VREfa, VSEfm and VREfm from the Micromyx repository (Kalamazoo, MI, USA) and The Medicines Company (Ville Saint Laurent, Quebec, Canada). VanA-phenotype (vancomycin and teicoplanin resistant) and VanB-phenotype (vancomycin resistant and teicoplanin susceptible) VRE were selected based on prior glycopeptide susceptibility test history. Agents were handled per CLSI (formerly NCCLS) guidelines and had results within CLSI quality control ranges during testing.[6] Evaluations of lipoglycopeptides incorporated polysorbate 80 at a final concentration of 0.002% (v/v). MIC and MBC values were determined in accordance with standard CLSI methods.[6–8] The time–kill kinetics of select isolates (one per phenotype evaluated) at standard inoculum ($\sim 5 \times 10^5$ cfu/mL) and high inoculum ($\sim 5 \times 10^7$ cfu/mL) were determined as described by Arhin *et al.*[9] using a method derived from the CLSI[8] for agents at their fC_{max} (calculated from the respective prescribing information as 16 mg/L for oritavancin, dalbavancin, linezolid, vancomycin and ceftaroline, 8 mg/L for telavancin, 4 mg/L for daptomycin and 1 mg/L for tedizolid).

The activity of the tested agents against enterococci is summarized by species and phenotype in Table 1. Among *E. faecalis*, VSEfa were susceptible to all agents with the lipoglycopeptides having the most potent activity by MIC_{90}. Ceftaroline and oritavancin were the only consistently bactericidal agents against VSEfa based on the proportion of isolates with MBC:MIC ratios of $\leq$4. Based on MIC_{90}, oritavancin, daptomycin, linezolid, tedizolid and ceftaroline maintained potent activity against VanA VREfa. Ceftaroline maintained bactericidal activity against VanA VREfa, while the other agents typically had MBC:MIC ratios >4.

Against VSEfm and VREfm (VanA and VanB phenotypes), oritavancin was the most potent agent evaluated based on MIC_{90}. All agents, excluding ceftaroline, which was largely inactive against *E. faecium*, had potent activity against VSEfm by MIC_{90}. Oritavancin, daptomycin, linezolid and tedizolid maintained potent activity against VREfm. Oritavancin was $\geq$16-fold more potent by MIC_{90} than the comparator lipoglycopeptides against VREfm. Against VSEfm, oritavancin and daptomycin were the only consistently bactericidal agents based on the proportion of isolates with MBC:MIC ratios of $\leq$4. Daptomycin maintained bactericidal activity by MBC:MIC ratio against VanA and VanB VREfm.

Consistent trends in bactericidal activity were apparent for each agent by time–kill at fC_{max} across the evaluated *E. faecalis* (one isolate each of VSEfa and VanA VREfa) and *E. faecium* (one isolate each of VSEfm, VanA VREfm and VanB VREfm) isolates. At the standard inoculum density, oritavancin and daptomycin were rapidly bactericidal with 3 log killing typically achieved within 0.25 and 4 h, respectively, with singular exceptions (4 h for oritavancin and 1 h for daptomycin against the VanA VREfa isolate). Telavancin was typically bactericidal, with 3 log killing observed at 24 h at the standard inoculum density with the exception of the VanB VREfm isolate, as was ceftaroline for *E. faecalis* but not *E. faecium*. Vancomycin, dalbavancin, tedizolid and linezolid did not achieve 3 log killing at the standard inoculum density for any of the