that assesses gene function and approves new allele designations. To allow the database to reflect the content of the live repositories to within a useful time frame, any regular automated updates of the reference database from live repositories could label new alleles with ‘provisional’ designations prior to committee approval. As a method for identifying resistance genes from WGS data the use of a reference pseudomolecule/database may yet be superseded, but the above model is attractive for use against highly clinically relevant factors including metallo-carbapenemases and other antimicrobial resistance factors.

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Transparency declarations
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Supplementary data
Tables S1 and S2 and Figure S1 are available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References

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Amikacin resistance plasmids in extensively antibiotic-resistant GC2 Acinetobacter baumannii from two Australian hospitals

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Sir,

Previously, we studied a collection of extensively antibiotic-resistant Acinetobacter baumannii belonging to global clone 2 (GC2) from Australian hospitals in Sydney, Brisbane and Canberra.1 These 61 closely related carbapenem-resistant isolates shared a common set of resistance genes, but were subdivided into five different groups based on their aminoglycoside resistance phenotypes. One group harboured aacC1 (gentamicin resistance), aphA1 in Tn6020 (kanamycin and neomycin resistance) and aphA6 in TnaphA6 (amikacin, kanamycin and neomycin resistance). We subsequently showed that aacC1 and aphA1 were located in a chromosomal resistance island, AbGR12-1, in two representatives of this collection that did not carry TnaphA6.2

The aphA6 gene was originally recovered from an A. baumannii isolate on a 63 kb plasmid that was transferrable to other Acinetobacter spp.3 However, this plasmid was not sequenced. Recently, TnaphA6 was found on a completely sequenced 70 kb conjugative plasmid, pAb-G7-2 (GenBank accession number KF669606),4 which was in a GC1 isolate from Melbourne, pAb-G7-2 belongs to the repAc16 plasmid family.5 The archetype of the repAc16 family,6 pAC1CU2, was originally reported to be cryptic.6 However, it was recently shown to carry TnaphA6 in the same position as pAb-G7-2 and to transfer amikacin resistance.7

Here, we have examined whether the TnaphA6 in the GC2 isolates, D72 and C20, which also harbour aacC1 and aphA1, may also be on a conjugative repAc16 family plasmid. While D72 and C20 had the same resistance phenotype and gene content,8 they were isolated from different Sydney hospitals 8 years apart, C20 in 2002 and D72 in 2010.

To determine whether C20 and D72 harboured pAb-G7-2, the PCR mapping strategy described previously was used.6 For C20, all of the PCRs produced amplicons identical to those in pAb-G7-2, indicating that C20 contained a plasmid with TnaphA6 in the same position. A draft genome sequence for C20 was

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subsequently generated at the Wellcome Trust Sanger Institute using Illumina HiSeq. A standalone BLAST search using pAb-G7-2 as a query retrieved a 68 kb contig that matched pAb-G7-2 and a contig containing most of TnaphA6. The assembled repAci6 plasmid in C20 was identical to pAb-G7-2.

In D72, the plasmid backbone was present, but was not interrupted at the TnaphA6 position in pAb-G7-2, and this was confirmed using the draft genome sequence of D72 (Figure 1). However, two plasmid backbone contigs ended with a fragment of ISAba125, which flanks aphA6 in TnaphA6. Primers adjacent to the ISAba125 pieces, RH1319 (TTCCATTGCATCGTTTGAGA) and RH1320 (TTTACGATAATTGCATGGATGT), amplified a 3.3 kb product, indicating that TnaphA6 was at a different position relative to that in pAb-G7-2 (Figure 1). PCRs linking RH1319 and RH1320 to aphA6 produced amplicons of the size predicted if TnaphA6 was present at this second location.

The complete sequence of a 70102 bp plasmid in D72 was assembled as described previously for pAb-G7-2 (Figure 1) and was deposited into GenBank under the accession number KM051846. This plasmid was named pD72-2.

The backbones of pD72-2 and pAb-G7-2 shared 99.9% sequence identity. There were 61 nucleotide differences, four single-base gaps and one two-base gap in pD72-2. There was a single nucleotide difference between the repAci6 genes that resulted in a single amino acid change (L344I) and a single additional base in the third of the three previously defined repeat regions (3 in Figure 1). Comparison of pD72-2 with pABTJ2 did not fit within one of the previously defined A. baumannii rep families, and its Rep protein of 373 amino acids shared only 41% amino acid identity with a 218 amino acid segment of RepAc12 (316 amino acids).

Assembly of contigs in the draft genomes of C20 and D72 using PCR showed that they both contained AbGRI2. C20 harboured an island identical to AbGRI2-1, which has aacC1 and aphA1, while D72 had a variant of AbGRI2-1 with at least one additional copy of aphA1. Both C20 and D72 also had a chromosomal resistance island in comM that was identical to Tn6167, herein renamed AbGR1-2. The blaoxa-23 gene, conferring resistance to carbapenems, is within Tn6167 in AbGR1-2.

This study shows that TnaphA6 has been acquired by almost identical repAci6 plasmids on at least two separate occasions. Screening for the position of TnaphA6 in these plasmids should be a valuable epidemiological tool and aid in tracking the spread of these conjugative resistance plasmids.

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References
Molecular characterization of NDM-1-producing Acinetobacter pittii isolated from Turkey in 2006

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Sir,

In Europe, blaNDM-1 has been found in the chromosome of a few Acinetobacter baumannii isolates typically linked with patient travel to North Africa or the Balkans, while in south Asia and China in particular, NDM is frequently found in different Acinetobacter spp. and it is usually plasmid encoded.1

In this study, we report the earliest occurrence of NDM-1 in Acinetobacter pittii. The isolate was recovered from a female patient in her late teens from a small city in eastern Turkey, who was transferred to Hacettepe University with a presumptive diagnosis of peritoneal and meningeal carcinomatosis. The patient underwent surgery for metastatic carcinoma (Krukenberg tumour) in early 2006 and had no history of travel outside of her home town except for travel to Ankara for her current medical treatment during the preceding month. During the subsequent 4 months of hospitalization, the patient experienced several febrile neutropenic episodes due to chemo- and radiotherapy, for which she was treated with pipercillin/tazobactam, imipenem, meropenem, metronidazole, teicoplanin, vancomycin and amphotericin B lipid complex at different times.

By the end of the fourth month, a blood culture was positive for Acinetobacter, initially reported as A. baumannii, but later identified as A. pittii by MALDI-TOF MS and amplified ribosomal DNA restriction analysis.2,3 Antibiotic susceptibility testing performed by disc diffusion or Etest and interpreted according to EUCAST guidelines (version 4.0, 2014; http://www.eucast.org) showed resistance to carbapenems and amikacin and susceptibility to gentamicin, tobramycin, netilmicin, ciprofloxacin and colistin (Table 1). She was given cefoperazone/sulbactam (1/1 g twice daily) and netilmicin (130 mg once daily) before the susceptibility results were available. The patient rapidly defervesced and this treatment was continued for 14 days. A follow-up culture on the third day of therapy did not yield any microbial growth. Upon completion of therapy, the patient was discharged.

The A. pittii isolate (designated as JV AP02) yielded positive results when using either the cloverleaf test (modified Hodge test) or imipenem-EDTA Etest strips to screen for carbapenemase/metallo-β-lactamase production. PCR screening for β-lactamase genes followed by DNA sequencing identified the presence of blaNDM-1, but was negative for blaOXA-51, blaOXA-23, blaOXA-24, blaOXA-58, blaVIM or blaIMP.

MLST performed according to the MLST database (http://www.pasteur.fr/recherche/genopole/PF8/MLST/) identified four new alleles (cprn60:66, pyrG:35, recA:72 and rpoB:62) and strain JVAP02 was assigned a novel sequence type, ST457, by the Pasteur curators.

S1-PFGE and Southern hybridization with a digoxigenin-labelled probe against blaNDM-1 demonstrated the genetic location of blaNDM-1 within a plasmid of ~45 kb, which could not be assigned to any plasmid replicon group using the PCR-based replicon typing scheme devised for A. baumannii.6

Characterization of the genetic structures surrounding the blaNDM-1 gene was performed by inverse PCR and PCR mapping revealing that blaNDM-1 was flanked by two ISAba125 forming a canonical Tn125 composite transposon identical to that of the pNDM-BJ01 plasmid from Acinetobacter lwoffi.7 Of note, the downstream ISAba125 was inserted within the delta-pac gene at the same location where alternative ISSs have been described in other NDM-producing non-baumannii Acinetobacter isolates,6–8 suggesting this might be a hotspot for other ISSs.


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