A cryptic *vanB2* operon carried on a Tn*1549*-like element in *Clostridium difficile*

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Summary: The emergence of vancomycin resistance in *Clostridium difficile* would have dramatic clinical consequences. We describe a phenotypically silent *vanB2* operon in a strain of *C. difficile* isolated from an Australian veal calf at slaughter, carried on a ~42k mobile genetic element showing significant homology and synteny to Tn*1549*, a conjugative transposon linked with the emergence and global dissemination of vancomycin resistant enterococci.

MATERIALS AND METHODS

C. difficile strain (AI0499, PCR RT 033) was recovered from the carcass of a neonatal calf (aged less than 7 days) at an abattoir in Victoria, Australia in 2013 [1].

The genome of strain AI0499 was sequenced in two independent runs using illumina chemistry (Miseq/ HiSeq) and investigated using short-read sequence typing (SRST2), *de novo* assembly and comparative genomic analysis [2-5]. *In vitro* susceptibility to vancomycin was determined using agar and Etest methodologies [6].

RESULTS

De novo assembly of the AI0499 genome revealed a chromosome of 4,095,918 bp and 28.75% GC with a N50 of 58,582 bp and overall coverage of ~130x.

Annotation identified 3960 unique coding sequences (CDS) and 3 predicted intact phage sequences corresponding to ΦMMP02, ΦCD119 and CDMH1.

SRST2 identified 7 vancomycin resistance genes with >99% sequence identity to *vanXB*, *vanB*, *vanHB*, *vanW*, *vanYB*, *vanSB* and *vanRB*. AI0499 was negative for $vanG_{Cd}$.

These genes comprised a *vanB2* operon and were carried on a ~42k mobile genetic element showing significant homology and synteny to Tn*1549*, a conjugative transposon linked with the emergence and global dissemination of vancomycin resistant enterococci (**Fig. 1**).

Notably, AI0499 did not show any reduced susceptibility to vancomycin (MIC 1 mg/L).

AI0499 was characterised as ST 11 within MLST clade 5, and having an S-layer cassette type 3. It contained a complete binary toxin locus (CdtLoc) and an uncommon Pathogenicity Locus (PaLoc) identified as toxinotype XI [4] (**Fig. 2**).

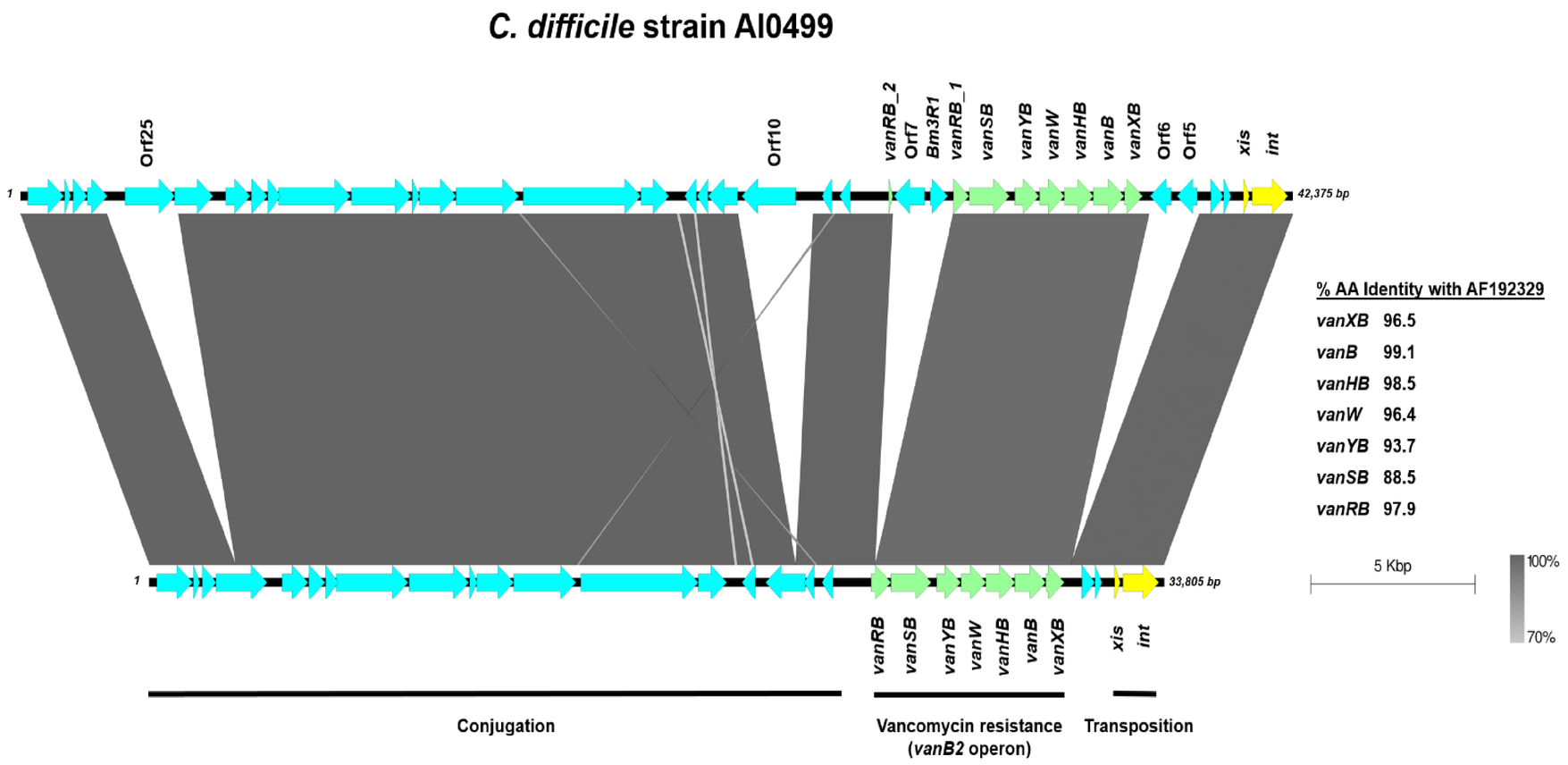

Fig. 1 Comparative genomic analysis of Tn*1549*-like element in *C. difficile* strain AI0499 and *Tn*1549 of E. faecalis (AF192329)
Arrows indicate open reading frames (ORFs) and direction of transcription. Excisionase (*xis*) and integrase (*int*) genes are shown in yellow, genes comprising the *vanB2* operon (*vanXB*, *vanB*, *vanHB*, *vanW*, *vanYB*, *vanSB* and *vanRB*) are shown in light green, with the remaining ORFs shown in light blue. Figure prepared using Easyfig v2.2.2 [5].

DISCUSSION AND CONCLUSION

The absence of phenotypic vancomycin resistance in strain AI0499 is not unexpected. Despite an inducible and functionally active $vanG_{Cd}$ operon which is found in the genome of some strains, and significant amounts of non-native D-alanine-D-serine containing peptidoglycan precursors, *C. difficile* strains retain susceptibility to vancomycin [7,8].

It is possible that in strain AI0499 *vanB* mediated resistance is induced by vancomycin but a yet-to-be identified mechanism prevents any modifications to the peptidoglycan structure [8]. Equally, it is likely that the presence of *Bm3R1* and orf7 within the *vanRB* gene result in a loss of function and subsequent lack of transcription of vancomycin resistance genes. Further studies are underway to investigate these hypotheses.

In conclusion, the isolation of a Tn*1549*-like element from *C. difficile* is alarming and vancomycin resistance expressed *in vivo* could have dramatic consequences. These data further confirm that anaerobes, particularly those of the animal gut microbiota, represent a reservoir of clinically important *vanB*-like resistance operons.

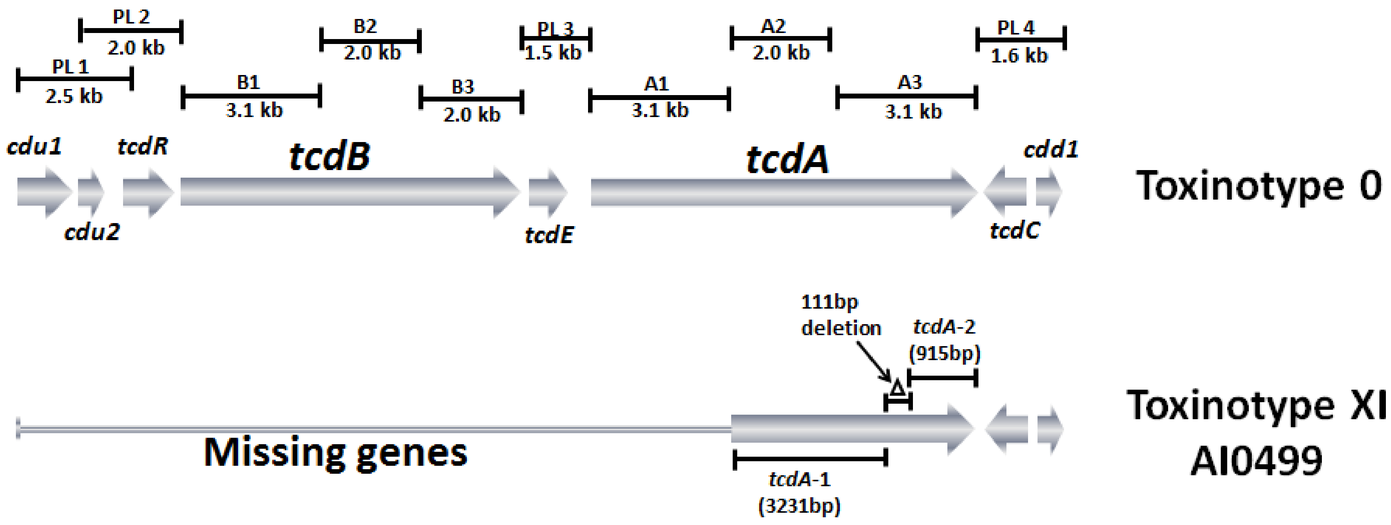

Fig. 2 Uncommon PaLoc of strain AI0499 compared to toxinotype 0
Strain AI0499 contains a deletion within the PaLoc as shown above; a trait exhibited by toxinotype XI strains [9]. The remaining fragments of the *tcdA* gene are 3231bp and 915bp in size and correspond to the A2 and A3 fragments of the *C. difficile* 630 *tcdA* gene, respectively.

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