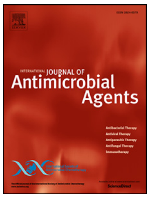
International Journal of Antimicrobial Agents 53 (2019) 876–877

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: www.elsevier.com/locate/ijantimicag



Letter to the Editor

Novel *vanA*-carrying plasmid in a clinical isolate of *Enterococcus avium*

Sir,

Since its first isolation in 1986, vancomycin-resistant enterococci (VRE) have been increasingly reported and now represent a global public-health concern. This is mainly due to their ongoing spread in nosocomial settings along with the limited number of available effective antimicrobial treatment options [1,2]. Among the eight types responsible for acquired vancomycin resistance, the VanA phenotype (defined by high-level vancomycin and teicoplanin resistance) is the best characterised and accounts for the majority of glycopeptide resistance in clinical settings [1,3].

The main feature responsible for the ongoing spread of vancomycin resistance in clinical isolates of *Enterococcus* spp. is the plastic antimicrobial resistance transposon Tn*1546*, a 10 851-bp derivative of Tn*3* originally described in *Enterococcus faecium*. This element is easily able to transpose into diverse conjugative plasmid backbones and carries the *vanA* operon responsible for the vancomycin resistance phenotype [3,4]. Tn*1546* also shows a typical high degree of heterogeneity, illustrated by tens of mutational derivatives of the *A1* transposon prototype. These are distinguished by numerous point mutations, deletions and insertion sequence (IS) elements (e.g. IS*1216*) in different positions and orientations, likely acquired by independent recombination events [1]. However, although frequently described in *E. faecium* and *Enterococcus faecalis*, the *vanA* determinant has been rarely described in *Enterococcus avium* (Supplementary Table S1). In particular, only one 80-kb plasmid carrying a Tn*1546* transposon has been deposited so far in GenBank [5].

Here we describe the first whole genome of an *E. avium* clinical isolate with a VanA phenotype harbouring a novel plasmid backbone and carrying a variant of Tn*1546* previously reported only in *E. faecium*.

The *E. avium* isolate (LC0559/18) was recovered from a 26-year-old male patient hospitalised in the intensive care unit (ICU) of A. Manzoni Hospital (Lecco, Italy) in February 2018 for respiratory distress syndrome. The patient was transferred from a neurological rehabilitation unit and was already receiving treatment with ceftriaxone (1 g twice daily). On ICU admission, therapy was switched to meropenem (2 g three times daily), vancomycin (2 g once daily) and oseltamivir (150 mg loading dose, followed by 75 mg twice daily) for a 9-day period. Five days after admission the patient was investigated by rectal swab for the presence of VRE as part of a routine screening programme. Culture on selective plates (chromID® VRE; bioMérieux, Marcy-l'Étoile, France) was positive for enterococci able to grow in the presence of vancomycin. Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) (VITEK® MS; bioMérieux) allowed the identification of *E. avium*. The resistance phenotype was assessed by VITEK®2 (bioMérieux) and revealed the following resistance phenotype according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) v.8.0 (2018) criteria: ampicillin [minimum inhibitory concentration (MIC) $\leq$2 mg/L]; levofloxacin (MIC = 0.25 mg/L); vancomycin (MIC $>$ 16 mg/L); teicoplanin (MIC = 16 mg/L); and linezolid (MIC = 2 mg/L).

Hybrid assembly of whole-genome sequencing (WGS) data was performed by merging data of both HiSeq (Illumina Inc., San Diego, CA) and MinION (Oxford Nanopore Technologies, Oxford, UK) platforms. Briefly, MinION reads underwent trimming with Porechop and filtering with Filtlong software, followed by assembly and contig circularisation using Canu and Circlator pipelines, respectively. Adapters of HiSeq reads were trimmed using Trimmomatic and were then further aligned to assembled MinION contigs using Bowtie 2 aligner. Final genome polishing and annotation were performed using Pilon and Prokka software. Corrected FASTA sequences (GenBank accession no. RYZS00000000) were uploaded to the Center for Genomic Epidemiology online database (http://www.genomicepidemiology.org/) to obtain data on resistance genes, major plasmid replicon types and virulence factors. Species identity was confirmed by nucleotide comparison with the 16S rRNA gene of *E. avium* strain ATCC 14025 (GenBank accession no. DQ411811).

WGS analysis indicated that LC0559/18 carried two plasmids (pLC0559/18-1 of 40 456 bp in size and pLC0559/18-2 of 52 156 bp). pLC0559/18-1 was of replicon type rep17 and harboured the antimicrobial resistance genes (ARGs) *vanA* (on transposon Tn*1546 B2*-type), *aph(3′)-III* and *ant(6)-Ia*, whilst pLC0559/18-2 was of replicon type repUS7 and did not carry any ARGs. Virulence factors were not present.

Local alignment of the complete sequence of pLC0559/18-1 with NCBI BLAST indicated that pLC0559/18-1 was a novel plasmid. However, the genetic environment of *vanA*, spanning from 1909 bp to 10 041 bp (Fig. 1), showed 99% nucleotide identity with 20 deposited *E. faecium* sequences, 9 of which covered up to 17 kb of its sequence. Part of this region constitutes a variant of the widely reported transposon Tn*1546 B2*-type whose characteristics are the presence of transposase IS*1216* (belonging to IS*6* family) upstream and in opposite orientation with respect to *vanR* as well as the consequent absence of transposase ORF1 and resolvase ORF2 [1,3]. The present transposon variant (reported to date only in *E. faecium*) is characterised by an additional IS*1215*-like element inserted upstream of *vanS* (Fig. 1). The two further additional resistance genes [*aph(3′)-III* and *ant(6)-Ia*] were located 1246 bp and 3146 bp upstream of the accessory gene *vanR*, respectively.

This report reveals detailed characteristics of the *vanA* gene cassette in rarely reported vancomycin-resistant *E. avium*, thereby highlighting variability of Tn*1546* as well as host range expansion for the VanA phenotype. Moreover, it implicates the presence of an

https://doi.org/10.1016/j.ijantimicag.2019.04.006

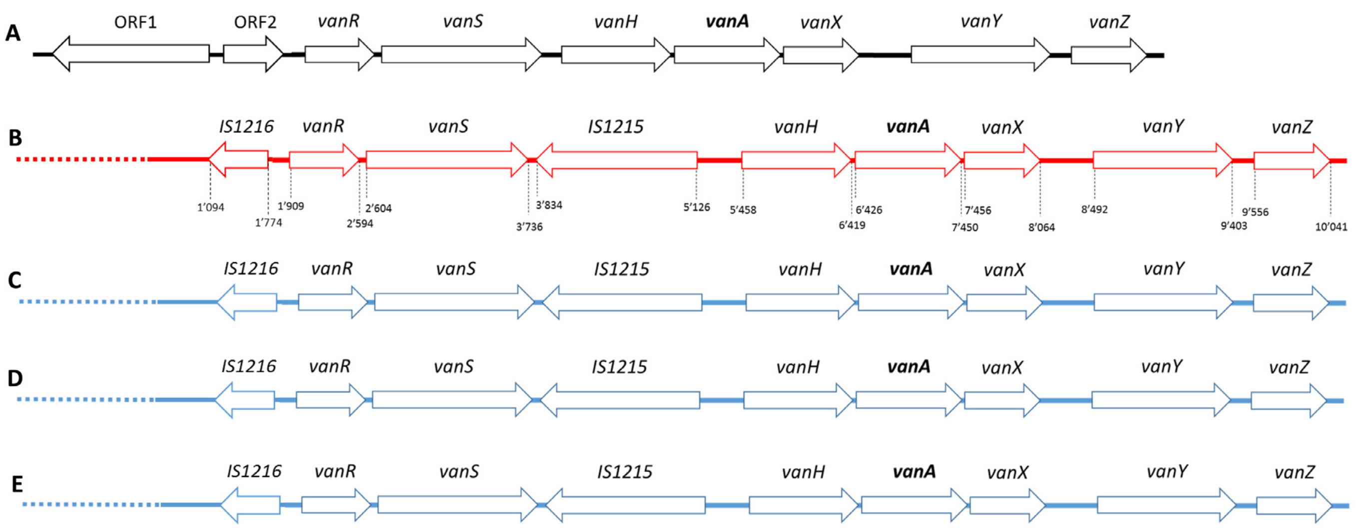


Fig. 1. Schematic representation of native and *B2*-type transposon Tn*1546* in plasmid pLC055/18-1 and in three other plasmids of *Enterococcus faecium* deposited in GenBank. Numbers indicate the start and end of depicted genes and are expressed in base pairs. Arrows indicate the direction of open reading frames (ORFs). ORF1, transposase; ORF2, resolvase; IS*1216*, transposase; *vanR*, vancomycin response regulator; *vanS*, sensor histidine kinase; IS*1215*-like transposase; *vanH*, pyruvate dehydrogenase; *vanA*, D-Ala-D-Lac ligase; *vanX*, D-Ala-D-Ala dipeptidase; *vanY*, D-Ala-D-Ala carboxypeptidase; *vanZ*, teicoplanin resistance protein. (A) *Enterococcus faecium* strain BM4147 plasmid pIP816 (accession no. NC_011140; deposited on 16 December 2014, France); (B) *Enterococcus avium* strain LC0559/18 plasmid pLC0559/18-1 in the present work (GenBank accession no. RYZS00000000); (C) *E. faecium* strain VREfm1 plasmid pPEC286 (accession no. KY595962.1; deposited on 24 May 2018, Hungary); (D) *E. faecium* strain ISMMS_VRE_10 plasmid ISMMS_VRE10_p3 (accession no. CP012474.1; deposited on 22 March 2017, USA); and (E) *E. faecium* strain Efm008 plasmid pJEG040 (accession no. KX810025.1; deposited on 19 November 2016, Australia).

emerging and additional bacterial player in the dissemination and evolution of vancomycin resistance in the nosocomial setting [1,3].

Funding

None.

Competing interests

None declared.

Ethical approval

Not required.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:doi:10.1016/j.ijantimicag.2019.04.006.

References

[1] Wardal E, Kuch A, Gawryszewska I, Zabicka D, Hryniewicz W, Sadowy E. Diversity of plasmids and Tn*1546*-type transposons among VanA *Enterococcus faecium* in Poland. Eur J Clin Microbiol Infect Dis 2017;36:313–28.
[2] Levitus M, Perera TB. Vancomycin-resistant enterococci (VRE). Treasure Island, FL: StatPearls; 2018.
[3] Novais C, Freitas AR, Sousa JC, Baquero F, Coque TM, Peixe LV. Diversity of Tn*1546* and its role in the dissemination of vancomycin-resistant enterococci in Portugal. Antimicrob Agents Chemother 2008;52:1001–8.
[4] Arthur M, Courvalin P. Genetics and mechanisms of glycopeptide resistance in enterococci. Antimicrob Agents Chemother 1993;37:1563–71.
[5] Sun L, Zhang P, Qu T, Chen Y, Hua X, Shi K, et al. Identification of novel conjugative plasmids with multiple copies of *fosB* that confer high-level fosfomycin resistance to vancomycin-resistant enterococci. Front Microbiol 2017;8:1541.

Odette J. Bernasconi
Institute for Infectious Diseases, University of Bern, Friedbühlstrasse 51, CH-3001 Bern, Switzerland

Luigi Principe, Valentina Viaggi, Francesco Luzzaro
Clinical Microbiology and Virology Unit, A. Manzoni Hospital, Lecco, Italy

Andrea Endimiani*
Institute for Infectious Diseases, University of Bern, Friedbühlstrasse 51, CH-3001 Bern, Switzerland

*Corresponding author. Tel.: +41 31 632 8 632; fax: +41 31 632 8 766.
E-mail address: andrea.endimiani@ifik.unibe.ch (A. Endimiani)

Received 10 January 2019
Accepted 6 April 2019