Letter to the Editor

Vancomycin-resistant *Enterococcus faecium* high-resolution typing by core genome multilocus sequence typing

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Dear Editor,

In recent decades, the harmless commensal *Enterococcus faecium* (*E. faecium*) has become an important nosocomial pathogen worldwide [1]. This is mainly due to high genome plasticity, which allows *E. faecium* to acquire, by horizontal gene transfer, virulence genes and clusters, as well as antibiotic resistance determinants. Notably, the acquisition of the vancomycin resistance operons gives rise to the more worrisome vancomycin-resistant *E. faecium* (VREf), which is able to prompt wounds, urinary tract infections, and bacteraemia, especially in severely ill and immune-compromised patients [2], increasing length of patients' hospital stays, mortality, and healthcare costs [3].

Currently, nosocomial *E. faecium* clones are identified by the Multi Locus Sequence Typing (MLST) method [4], which allows the identification of specific sequence types (STs) included in the hospital-associated clonal complex 17 (CC17) [5]. However, due to the high recombination rate in *E. faecium* and to the small number of MLST genes, the resolution of traditional MLST is limited.

In the past year, whole-genome analysis based on next-generation sequencing technologies has become a good alternative to the classical typing methods for nosocomial bacterial pathogens [6,7].

Thanks to the increase of *E. faecium* whole-genome sequencing (WGS) data, the correct ancestral origin of CC17, previously incorrectly thought to be the sequence type ST17, now includes also ST18 and ST78 clones [8]. ST17 and ST18 represented the first wave of hospital-acquired infections in the 1980s in United States [9], while ST78, the successful hospital-associated lineage circulating since 2005 in Europe [10] thought to have evolved from farm or pet animals [11], is now being isolated at increasing rates in hospitals in Europe, Asia, and South America [1].

Recently, a core genome MLST (cgMLST) scheme for *E. faecium* strains, consisting of 1,423 target genes, was developed by de Been *et al*., and compared by the authors to a single-nucleotide polymorphism (SNP)-based approach since it allows a high-resolution tracing of *E. faecium* clones [12].

Here, the *E. faecium* cgMLST scheme within the SeqSphere+2.3 platform (Ridom GmbH, 115 Münster, Germany; http://www.ridom.de/seqsphere/), was applied to contigs obtained by WGS of 4 selected VREf clones (strains SAU1, SAU16, SAU27, and SAU28) spreading in a Saudi Arabian hospital [13], and of 17 *E. faecium* whole-genome sequences publicly available in GenBank (ftp://ftp.ncbi.nih.gov/genomes/) (Table 1). A total of 17 different STs were represented. cgMLST was applied with 767 targets out of the 1,423 identified in the de Been scheme, due to the poor quality of 656 targets in draft genomes.

VREf isolates obtained from the King Faisal Specialist Hospital and Research Centre (Riyadh, Saudi Arabia) were characterized in our previous studies [13,14]. Three isolates (SAU1, SAU16, and SAU28) were VanB type, of which the last showed a VanA phenotype, while isolate SAU27 was VanA. Additionally, the isolates were multidrug resistant and carried the IS*16* element [13], which is related to an

enhanced potential of *E. faecium* for nosocomial transmission and is considered a molecular screening marker for hospital-associated strains. By MLST, VREf isolates were assigned to sequence types ST117, ST795, ST80, and ST546, all belonging to CC17. Moreover, the comparative eBURST analysis with the STs of the entire *E. faecium* MLST database showed that both ST795 and ST80 derived from ST117, and that ST546 derived from ST17 [13]. Here, the cgMLST analysis confirmed the genetic relatedness of isolates SAU1 (ST117) and its related mutant (SLV) SAU28 (ST795) (Figure 1). It also revealed the correct origin of the epidemic clone ST80 (SAU27) grouped here with its DLV ST18 (Figure 1) and not with its SLV ST117, as previously improperly predicted by the traditional MLST coupled with eBURSTv3 analysis [13]. Moreover, the UPGMA phylogenetic three showed that the ST546 clone, like ST80, clustered with ST18, suggesting the latter and not ST17 was the ancestor for both sequence types, and that ST546 probably evolved from ST80 during hospital adaptation.

Comparative genome analysis revealed that the Saudi Arabian VREf isolates were virulent and dangerous. They possessed a high level of homology with virulence genes and gene clusters recognized to be specific for hospital-associated *E. faecium* (Table 2), including the genomic island GI. This provides a competitive advantage over the indigenous *E. faecium*

microbiota [15], as previously demonstrated for isolate SAU28 [13].

Furthermore, isolates SAU1 and SAU16, belonging to different STs, carried the same VanB$_2$ operon with 99.8% homology with the Tn*1549* of strain TSGH1 (AF310956), suggesting lateral exchange of this genetic element among the isolates. The VanA type strain (SAU27) carried the Tn*1546* that showed 100% sequence homology with the transposon carried by the *E. faecium* Efm32007 strain (KR349520).

In conclusion, the cgMLST scheme was useful in identifying the correct common origin of the micro-

Figure 1. UPGMA phylogenetic tree of 4 selected VREf clones and of 17 *E. faecium* whole-genome sequences publicly available in GenBank, generated by the SeqSphere+2.3 based on 767 targets.
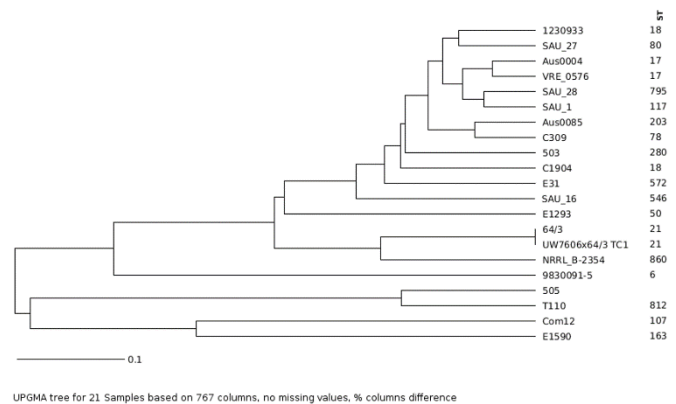

UPGMA tree for 21 Samples based on 767 columns, no missing values, % columns difference

Table 1. *E. faecium* strains used in cgMLST analysis.

Strain	ST	Chromosome/WGS Accession	Size (Mb)	Institution	Release Date
SAU1	ST117	This study	2,889,771	KFSHRC (Riyadh)	--
SAU16	ST546	This study	2,774,380	KFSHRC (Riyadh)	06/26/2016
SAU27	ST80	This study	2,922,922	KFSHRC (Riyadh)	06/26/2016
SAU28	ST795	This study	3,062,080	KFSHRC (Riyadh)	06/26/2016
Aus0004	ST17	NC_017022.1	3,019,779	University of Melbourne	03/01/2012
VRE0576	ST17	JAAK0000000	3,132,850	Swedish Institute for Communicable Disease Control	14/04/2014
Aus0085	ST203	NC_021994.1	3,239,279	University Of Melbourne	08/06/2013
T110	ST812	NZ_CP006030.1	2,737,960	SRM University	08/04/2014
1230933	ST18	ACAS01	3,140,779	Broad Institute	06/15/2009
503	ST280	AMBN01	2,746,730	Washington University	09/04/2012
C1904	ST18	AMBD01	2,855,420	Washington University	09/04/2012
EnGen0006/*E1293*	ST50	AHWU01	2,868,639	Broad Institute	05/03/2013
E31	ST572	JYCO01	3,048,470	Beijing Chao-Yang Hospital, Capital Medical Univ.	03/12/2015
C309	ST78	AJTW01	3,102,430	Shanghai Chinese Academy of Sciences	12/24/2012
64/3 VRE0576	ST21	CP012522.1	2,572,329	Robert Koch Institute	09/24/2015
UW7606x64/3 TC1	ST21	CP013009.1	2.753350019	Robert Koch Institute	10/26/2015
NRRL B-2354	ST860	NC_020207.1	2.849889994	University of California, Davis	02/04/2013
EnGen0138/9830091-5	ST6	AITC01	2.637219906	Broad Institute	05/03/2013
505	-	AMBL01	2.619859934	Washington University	09/04/2012
Com12	ST107	ACBC01	2.71374011	Broad Institute	05/20/2009
EnGen0003/E1590	ST163	AHXC01	3.021650076	Broad Institute	12/17/2012

Table 2. Homology of hospital-associated genes and gene clusters present in VREf. Percent of identity to the reference sequence is indicated for each gene and gene cluster.

Gene/gene clusters	Accession no.	SAU1	SAU16	SAU27	SAU28
Tn1546	KR349520	-	-	100	-
Tn1549	AF310956	99.8	99.8	-	99.5
efaAfm	FJ609170.1	94	94	94	94
sgrA adhesin	EFAU085 01549	100	99.9	100	99.9
acm	EFAU085 02356	99	100	100	100
esp	EFAU004 02750	100	99.6	100	>90
Genomic island (GI)	AAAK03000019	>90	>90	>90	>90
IS16/IS256	EFAU004 02683	100	100	100	100
Pilin 1 gene cluster	EU90969	100	99.5	91	99.1
Pilin 3 gene cluster	EU909696	100	100	100	100

epidemic of ST80 and ST546, undetected by traditional MLST coupled with eBURSTv3 analysis, and to highlight a high genetic recombination between CC17 clones spreading within the hospital. It was shown to be a suitable tool to carefully trace *E. faecium* clones and it would therefore be very helpful in hospital surveillance.

WGS and the cgMLST scheme allow for the exploration of virulence genes, antibiotic resistance mechanisms, and other important factors concordantly with molecular epidemiology investigation of bacterial outbreaks; they have a great ability to define clone relationships, thus significantly impacting the practice of infection control.

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