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Interregional dissemination of vancomycin-resistant *Enterococcus faecium* (VREfm) vanA ST612 in Switzerland

The emergence and rapid spread of the VREfm clone ST612 were noted earlier this year (P. Keller, USB, Basel) with a particular concern regarding its non-susceptibility to daptomycin due to two mutations involved in resistance, but not sufficient to decrease the susceptibility to daptomycin.

The Swissnoso national center for infection control and the NARA issued an alert in February. In order to evaluate the significance of this clone in Switzerland, two actions were proposed:

1. **Evaluation of the prevalence of clone ST612 in 2024 in Switzerland.** All labs identifying any VREfm (during the months of February and March 2024) were asked to either send the whole genome sequences (if already performed) or to send the isolates to the NARA.
2. **Retrospective evaluation of ST612 in Switzerland.** All labs performing whole genome sequencing were asked to send VREfm ST612 genome sequences of isolates recovered before February 2024.

We would like to thank all laboratories for their contribution by sending us isolates and sequences.

Material and methods

Susceptibility testing to daptomcin was performed using a microdilution method in liquid medium (Sensititre).

A preselection of isolates possibly belonging to ST612 was performed by sequencing the *gyd* allele from the MLST scheme. Allele #5 at this locus is highly specific to ST612 (sensitivity of 100% and the specificity was expected to be higher than 90%). Thus, only isolates with the *gyd* allele#5 were analyzed by whole genome sequencing (WGS).

WGS was performed on the Illumina MiSeq platform at the Genomics Unit of the Institute of Microbiology at Lausanne. The sequence reads were analyzed in Bionumerics V. 8.1. MLST was determined from the MLST database (http://pubmlst.org/MLST). The cgMLST scheme used is that of Ridom (http://www.ridom.com/). The isolates resistome was characterized using AMRFinderPlus version 3.12.8 with default parameters.

Results

Evaluation of the prevalence of clone ST612 (February and March 2024)

A total of 93 VREfm isolates were received at the NARA laboratory. Twelve were recovered outside the period of February to March (10 before and 2 after).

The 82 isolates recovered between February and March 2014 originated from 17 different cantons (Figure 1).

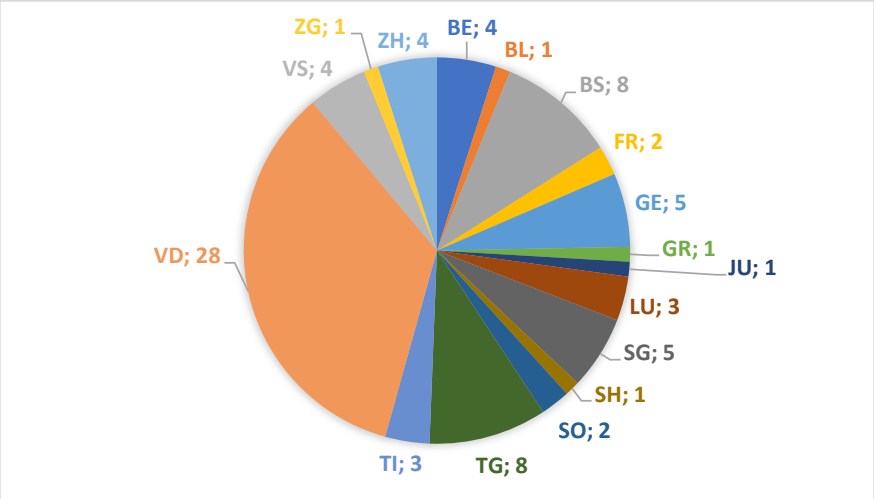

Figure 1. Canton of origin of VREfm isolated in Switzerland betwen February and March 2024.

Among these isolates, 15 were already sequenced by laboratories and the *gyd* gene was sequenced in the remaining 67 isolates. Fifty-two had allele #12 (possibly belonging to ST80) or #1, and 15 had allele #5. WGS was performed on these 15 isolates: 13 belonged to ST612, whereas two belonged to ST18 (Table 1). These 13 isolates originated from 7 cantons (BL, BS, JU, LU, SO, VS and ZH). Results of genomic analysis of these ST612 isolates are shown in the next section.

Table 1. MSLT sequence type or *gyd* allele and origin (cantons) of VREfm isolated in Switzerland between February and March 2024.

MLST ST /*gyd* allele	Number	Cantons
ST612	13	SO, VS, ZH, JU, BS, LU
ST117	3	BE, LU
ST18	2	TG
ST80	11	VD, TI, SG
STnew	1	VD
gyd #12 (ST80?)	39	BE, BS, FR, GE, SG, TG, TI, VD, ZH
gyd #1	13	BE, GR, SG, SH, TG, VD, ZH

Prospective and retrospective evaluation of ST612 in Switzerland

ST612 isolates in Switzerland originated from:

- Sequencing database from the University hospital of Basel (P. Keller)
- Sequencing database from the University hospital of Lausanne (D. Blanc), including previous isolates and those from the NARA investigation of February to March 2024.

A total of 117 sequences of ST612 of *E. faecium* were recorded. The phylogenetic relationship between these ST612 isolates was evaluated with the cgMLST analysis (Figure 2).

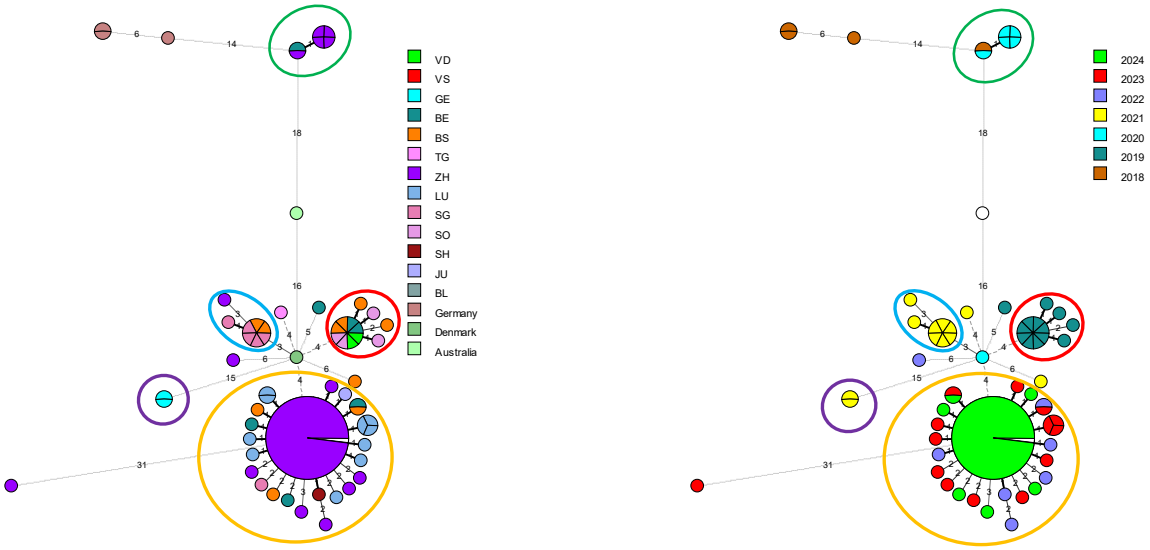

Figure 2. cgMLST minimum spanning tree (MST) of 117 VREfm Swiss isolates and four international references of ST612. Each circle represents one or several isolates. Number between circles are the number of loci differences between these isolates.

Based on the MST (cgMLST) and spaciotemporal data, five clusters of genetically highly related isolates were identified. The monthly incidence of these isolates according to their canton of origin or grouping in clusters are presented in Figure 3.

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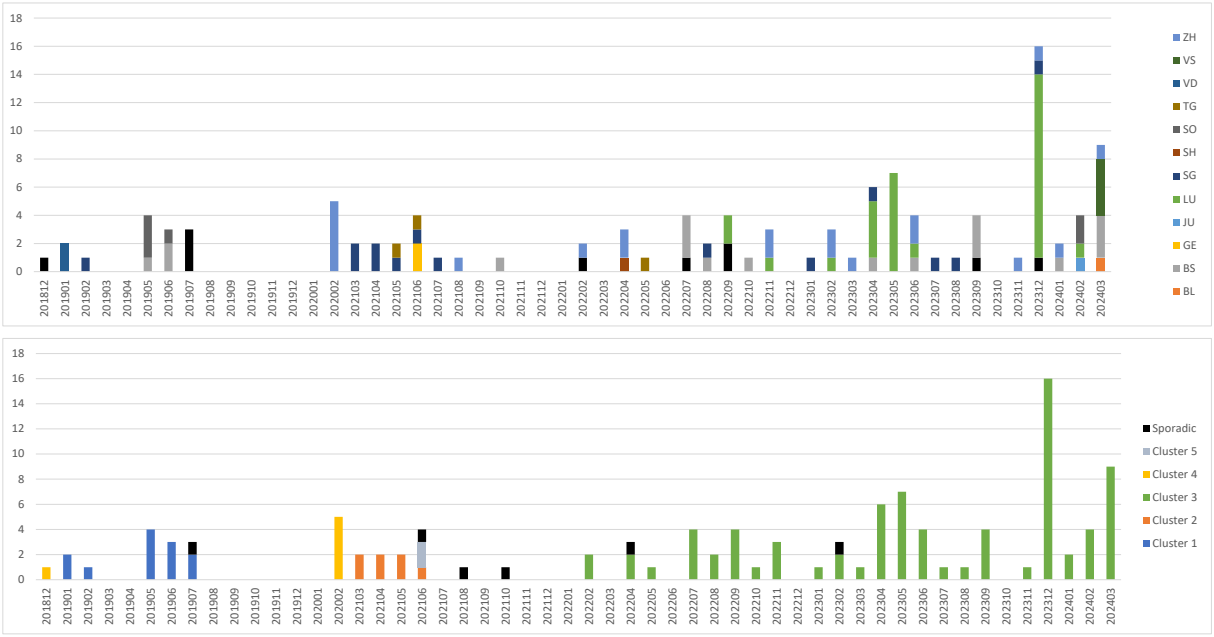

Figure 3. Monthly incidence of ST612 cases according to their canton of origin (up) or belonging to genomic clusters (down).

Cluster 1 includes 12 isolates recovered in 2019 in four cantons (BE, BS, SO and VD).

Cluster 2 includes 8 isolates recovered in 2021 (March to August) in three cantons (BS, SG and ZH).

Cluster 3 is the larger one with 78 isolates recovered from February 2022 to March 2024. It has been found in 11 different cantons (Table 2, Figure 4)

Table 2. Number of isolates, number of institutions and periods of recovering ST612 cluster 3.

Canton	No isolates	No institutions	Period
BE	6	3	02.2022-12.2023
BL	1	1	03.2024
BS	14	3	07.2022-03.2024
JU	1	1	02.2024
LU	30	3	09.2022-02.2024
SG	6	1	08.2022-12.2023
SH	1	1	04.2022
SO	2	1	02.2024
TG	1	1	05.2022
VS	4	2	03.2024
ZH	12	2	02.2022-03.2024

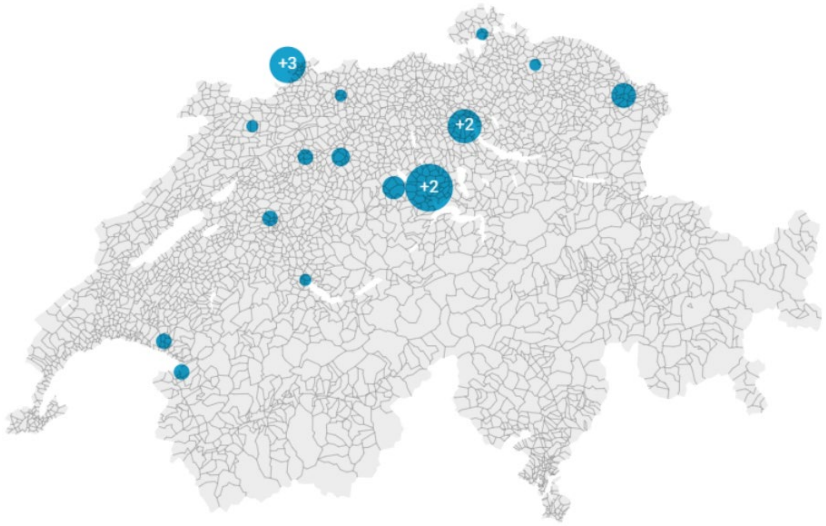

Figure 4. Geographic distribution of cluster 3 isolates of VREfm ST612 (postal code of the institution)

Cluster 4 includes one isolate recovered in 2018 in the canton of Bern and 5 isolates recovered in February 2020 in Zürich.

Cluster 5 includes two isolates recovered in June 2021 in the canton of Geneva.

Four isolates were considered as unique showing 6 to 31 loci differences with other isolates from clusters.

Susceptibility to daptomycin

The MIC distribution of daptomycin for the 93 isolates received at the NARA is similar to reported results for *E. faecium*, where most of the isolates have a MIC value of daptomycin being between 2 and 4 (Turnidge 2020, doi.org/10.1016/j.cmi.2020.04.027).

Table 1. Distribution of MIC of daptomycin for the 94 *E. faecium* received isolates.

MIC	≤0.25	0.5	1	2	4	8
No isolates	0	0	5	32	56	1

The resistome of the 117 sequences of ST612 was analyzed. All have the mutations LLiaR W73C & liaS T120A.

The isolate with an MIC=8 mg/L was also sequenced in order to determine the mechanism of resistance. It belonged to ST2664 and only the mutations liaR W73C & liaS T120A were observed, no other known genetic factor responsible for the resistance was identified, highlighting the fact that the mechanism of resistance is not yet completely understood.

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Discussion

The CLSI guidelines define daptomycin non-susceptible *Enterococcus faecium* (DNSEfm) when the MIC is > 4 mg/L. The EUCAST guidelines do not provide information on this antibiotic. The first case of DNSEfm has been reported in USA in 2005. Since then, case reports in Germany, Spain and Switzerland in recent years suggest that DNSEfm may be emerging globally. (Douglas et al. 2019). The mechanisms of daptomycin resistance in VREfm isolates remains to be fully elucidated. Among the mutations identified to reduce the susceptibility to daptomycin, substitutions in LiaR (W73C), LiaS (T120A) and Cls (H215R and R218Q) are among the most frequently observed, although mutations in either gene alone are not sufficient to confer a resistant phenotype in enterococci. (Wang et al. 2018, Douglas et al. 2019, Coll et al. 2024). All ST618 Swiss isolates harbored the mutations LiaR (W73C) and LiaS (T120A), but none exhibit the Cls mutation and no tested isolate showed a MIC >4 mg/L.

By combining spaciotemporal data and genomic relationships of isolates, we grouped the Swiss ST612 isolates into 5 different clusters. Considering the genetic distances with international strains (Germany, Denmark and Australia) we can speculate with good probability that the ST612 clone was introduce at least on four occasions (≥15 loci differences). Due to the lower genetic distance, it is not clear if the clusters 1, 2 and 3 evolved successively in Switzerland or if they were introduced on three occasions. It should be noted that the ST612 clone represent 10% of VRE reported at the national center in France (Zouari, 2023, BEH 22-23).

It is highly probable that transmissions occurred within hospitals as highly similar isolates were recovered in the same institution over a short period of time (few weeks, data not shown). Inter-hospital and inter-cantonal transmissions are more difficult to assess as the diversification rate of VREfm is known to be relatively low with cgMLST (only 0 to 3 loci differences were observed between isolates of cluster 3 recovered over a two year-period; identical isolates in cluster 4 are found 14 months apart…). However, as isolates of clusters 1, 2 and 3 were recovered from many institutions in different cantons, inter-institution transmissions are more probable than independent importation from a common foreign region.

In conclusion, the VREfm clone ST612 is still present in early 2024 in many cantons of Switzerland. However, despite they carried the LiaR-LiaS mutations, none showed a resistance pattern to daptomycin.

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