NARA
Nationales Referenzlaboratorium zur Früherkennung
neuer Antibiotikaresistenzen und Resistenzmechanismen

13/02/2024

Vancomycin-resistant *Enterococcus faecium* with decreased susceptibility to daptomycin

Warning; we would like to draw your attention on the increasing identification of vancomycin-resistant *Enterococcus faecium* exhibiting decreased susceptibility to daptomycin (VRE-DAP) from several parts of Switzerland

VRE strains usually remain susceptible to several antibiotics such as daptomycin, linezolid, quinopristin/dalfopristin. Daptomycin (DAP) is a cyclic lipopeptide (Figure 1, A) but working only on Gram-positive bacteria. DAP complexes with calcium to form small micelles, and subsequent membrane insertion is dependent on both the presence of calcium and phosphatidylglycerol. Once inserted, DAP oligomerizes and transitions to the inner membrane leaflet. It works by disrupting membrane function and causing leakage of essential potassium ions, ultimately leading to loss of membrane potential and cell death.

(A) (B)

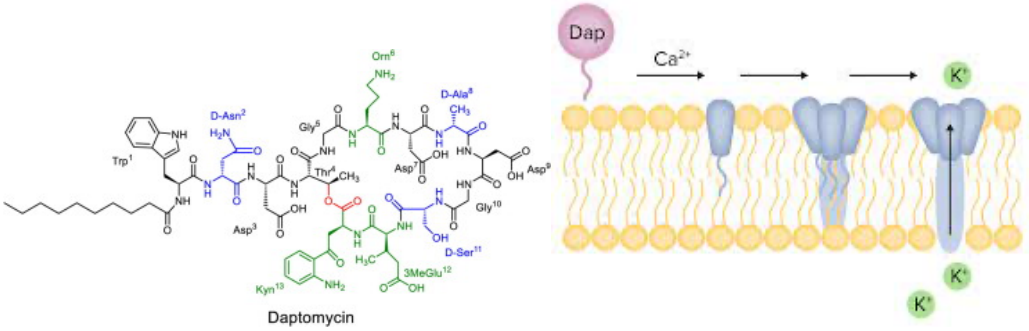

Figure 1. *Structrue (A) and mechanism of action of DAP (B)*

A pioneer study has been performed recently by Dr Peter Keller, from the University hospital of Basel. He identified 47 VER-DAP Swiss isolates over the past 5 years that were of the same MLST type, namely ST612. Genomic typing (core genome MLST) showed limited diversification among these isolates, suggesting a clonal spread. By investigating genomic public databases, closely-related isolates were also identified from earlier investigations performed in Switzerland, Denmark, and Germany (Figure 2). Importantly, two mutations involved in reduced susceptibility to daptomycin were identified.

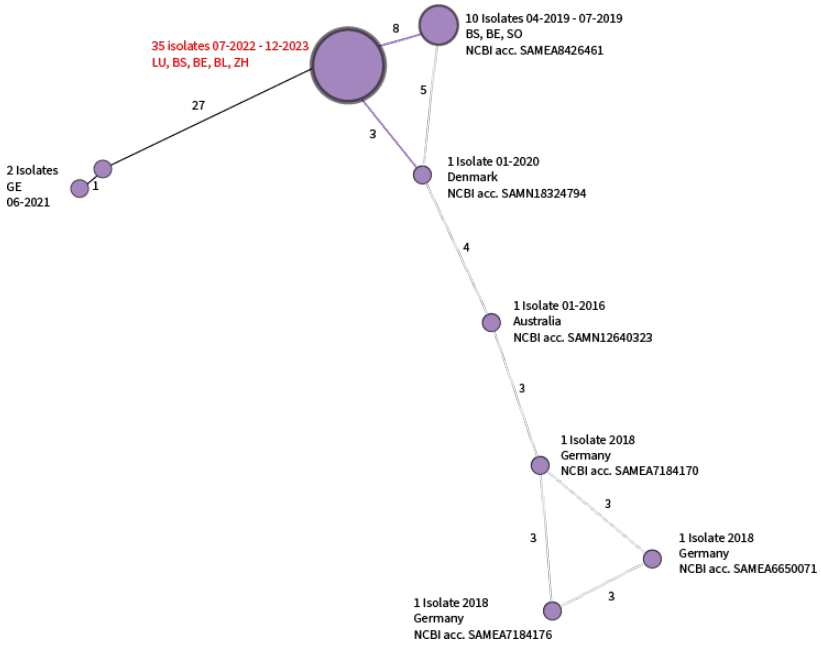

Figure 2. *cgMLST tree with isolates of the current ST612 vanA VRE outbreak.*

By further analyzing a collection of 590 sequenced VRE isolates at the NARA site of Lausanne, 11 isolates recovered from three patients and exhibiting the same phenotype/genotype were identified. Two patients had been hospitalized in the canton of Vaud (VD) and one in the canton of Thurgovie (TG). The same cgMLST comparison was performed and these isolates together with representative VRE ST612 sequences (302574-23 and 607166-22) from the University Hospital Basel (Figure 3) (Figure 3). No obvious epidemiological links could be identified between VD and TG patients. For one VD patient, 9 isolates were available at 0, 6 and 12 months of carriage. These isolates showed 0 to 3 loci differences, which is an indicator of the cgMLST diversification over time.

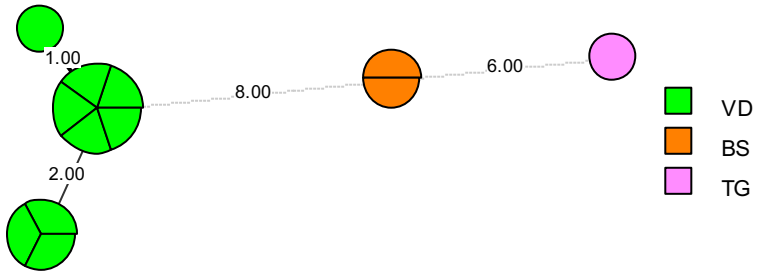

Figure 3*. Minimum spanning tree of cgMLST of ST612 isolates in the Lausanne WGS database compared with representative VREfm ST612 outbreak isolates from the University Hospital Basel (BS).*

Susceptibility testing was performed for representative strains of Peter Keller collection (strains 5021, 5022, Table 1). Criteria for categorization of susceptibility/resistance were those from the EUCAST for all antibiotics except for DAP which is not available. Hence, CLSI criteria were retained for DAP. Non-susceptibility to DAP is defined when MIC value is > 4 mg/L. Of note, the only validated technique for DAP is broth microdilution. This is partly due to the fact that a determined concentration of Ca^{++} is needed for DAP activity (see above).

The five isolates (two for P. Keller, 2 from VD and one from TG) of the ST612 clone remained susceptible to DAP (MIC of 4 mg/L) but were therefore at the limit of susceptibility.

Table 1. Susceptibility pattern of two representative ST612 isolates. MIC was performed in broth microdilution (Sensititre EUVEN).

	5021		**5022**		**Control**		**EUCAST 14.0**	
	302574-23		**607166-22**		**ATCC 29212**		**MIC breakpoints**	
Antibiotic	**RIS**	**MIC**	**RIS**	**MIC**	**Measured**	**Expected**	**S≤**	**R>**
Vancomycin	R	>128	R	>128	4	1 - 4	4	4
Teicoplanin	R	32	R	32	≤0.5	0.12 - 0.5	2	2
Quinupristin/dalfopristin	S	1	S	1	8	2 - 8	1	1
Tetracycline		128		64	32	8 - 32	--	--
Daptomycin		**4**		**4**	2	1 - 4	--	--
Ciprofloxacin	R	>16	R	>16	1	0.25 - 2	4	4
Erythromycin		>128		>128	≤1	1 - 4	--	--
Tigecycline	S	0.12	S	0.25	0.25	0.03 - 0.12	0.25	0.25
Linezolid	S	2	S	2	2	1 - 4	4	4
Gentamicin	HLR	>1024	HLR	>1024	≤8	4 - 16		>128
Ampicillin	R	>64	R	>64	1	0.5 - 2	4	8
Chloramphenicol		8		8	8	4 - 16	--	--
Fosfomycin		>1024		192	NA			

RIS : Resistant, Susceptible increased exposure, Susceptible. HLR, High level resistance

Mechanism of resistance to daptomycin

Specific mutations in the LiaFSR three-component regulatory cell envelope stress response pathway has been reported to result in reduced binding of daptomycin to the cell surface in *E. faecium*, and eventually lead to failures during daptomycin therapy with a subsequent mutation, most commonly in the *cls* gene encoding the phospholipid biosynthesis enzymes cardiolipin synthase. Among the mutations identified, substitutions in LiaR (W73C), LiaS (T120A) and Cls (H215R and R218Q) are among the most frequently observed.

These mutations were screened in all genomes. The two mutations W73C in LiaR and T120A in LiaS were observed in the ST612 isolates and no mutation was observed in Cls.

Discussion

The first case of VRE-DAP has been reported in USA in 2005. Since then, case reports in Germany, Spain and Switzerland reported during recent years suggest that daptomycin-non-susceptible VREfm might be emerging globally (Douglas et al. 2019).

The mechanisms of DAP 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 any of the corresponding genes alone are not sufficient to confer a resistant phenotype in enterococci (Wang 2018, Douglas 2019, Coll 2024).

Comparative genomic analysis (cgMLST) revealed limited core genome diversity amongst the VREfm ST612 *vanA*-positive isolates. Considering the data available so far, the first isolate this clone ST612 in Switzerland was in January 2019 (VD). Then, a first wave was observed with 10 isolates from April to July 2019 in BS, BE and SO. In 2021 there were one isolate from TG and 2 from GE. In 2022-2023 a second wave was observed with 35 isolates from LU, BS, BE, BL and ZH. Interestingly, isolates within each of these episodes had only 0-1 loci differences. Inter-cantonal transmissions are to be suspected within each of these two waves and should be confronted with epidemiological data. On the other hand, the diversity of isolates between episodes showed 6 to 27 loci differences, and only few loci (3-7) differentiate these Swiss isolates with other international isolates. This suggests a multiple introduction of the clone in Switzerland.

The ST612 clone is present in other European countries. It represents 10% of French isolates (Zouari, 2023), 5% in Ireland (Egan, 2022), and were also reported in Sweden (Gideskog, 2022). However, decreased susceptiblity/resistance to daptomycin was not reported/examined.

At least at the Swiss level, this clone constitutively harbored both mutations LiaR (W73C) and LiaS (T120A). Such clone has already been reported (Wang 2018) and the presence of both mutations in the LiaRS raise concern as subsequent mutation in the *cls* gene seems to be sufficient to confer daptomycin resistance.

Conclusion

This study therefore highlights that ;

- Clonally-related VRE with decreased susceptibility to DAP are circulating in Switzerland.
- The circulating clone possesses already two mutations in genes that explain decreased susceptibility to DAP. An additional mutation might therefore be prompt to confer clinical resistance to DAP upon selective pressure.
- Identification of susceptibility to DAP must rely on microdilution and not on techniques using agar culture medium.

Recommendations

1 - As a consequence of this observation, we therefore recommend to perform/confirm daptomycin susceptibility using broth microdilution technique (for example EUVENC Sensititre plate, Thermofischer). We also remind that screening procedures for identification of VRE should be performed with chromogenic media and an enrichment step (rectal swab/stools/urines in broth added with 1 mg/L vancomycin, grown overnight before plating will improve the recovery rate of VRE (Sadek et al, 2020) (https://www.unifr.ch/med/nara/fr/assets/public/files/guidelines/NARA_VRE-guidelines_V5_201907.pdf).

Screening plates for VRE identification are commercialized by several furnishers :

Furnisher	Ref	*Medium brand*
BioMérieux	43004	CHROMID® VRE
ChroMagar	VR952	CHROMagar™ VRE
Thermo Scientific	PO1175A	Thermo Scientific™ Brillliance™ VRE
Bio-Rad	63751	VRESelect Agar
Liofilchem	11621	Chromatic VRE

2- **Evaluation of the prevalence of clone ST612 in 2024**. In order to have a rough picture of the prevalence of this clone in Switzerland, it shall be proposed that each lab identifying any VREfm (clinical strains and screening strains) during the months of February and March 2024 send either the whole genome sequences (if already performed) to Dominique.Blanc@chuv.ch, or, if not sequenced, the isolates to the NARA (sequencing of isolates will be performed without fees). In both cases, thanks to use the NARA form to report demographic and clinical data (https://www.unifr.ch/med/nara/fr/prestations/mail-sample.html). For labs that cannot perform MICs to daptomycin, this will be done by Dominique Blanc.

3. **Retrospective evaluation of ST612 in Switzerland**. In order to gain a more complete picture about potential relatedness of other VREfm ST612 already identified in the past, we would appreciate to receive all ST612 genome sequences from labs that already have analyzed VREfm by whole genome sequencing before 2024, ideally including demographic and clinical data requested for in the NARA form (https://www.unifr.ch/med/nara/fr/prestations/mail-sample.html). Thanks to send these files to Dominique.Blanc@chuv.ch.

For clinicians and infection prevention and control teams, there will be a separate communication with further guidance issued by Swissnoso and partners (release of information planned for Feb 14, 2024).

Dr. D.S. Blanc

Dr P. Keller
University Hospital Basel

Dr. L. Poirel

Prof P. Nordmann

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