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Changing epidemiology of vancomycin-resistant *Enterococcus faecium*: Results of a genome-based study at a regional neurological acute hospital with intensive care and early rehabilitation treatment

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SUMMARY

Background: Vancomycin-resistant *Enterococcus faecium* (VREfm) are an emerging threat worldwide. In Germany, a VRE-belt with higher VREfm prevalences transversing its central east-west axis and including the state of Hesse was previously described. Recently, we detected a predominant VREfm clone in hospitals throughout the Rhine-Main metropolitan area of Hesse.
Aim: Here we expanded our study on VREfm to a regional neurological acute hospital outside of the metropolitan area with patient referrals from throughout Hesse and the neighboring federal state of Rhineland-Palatinate.
Material/Methods: VREfm isolates obtained between 2016-2018 from a regional neurological acute hospital with intensive care and early rehabilitation units were investigated (n=55). Patient data was collected and analyzed together with whole-genome sequencing data to investigate antibiotic resistance and virulence determinants of the VREfm. The population structure of VREfm was investigated using the Core genome-based multilocus sequence typing (cgMLST).

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Findings: The average age of the patients was 67.1 years. For 96% of the patients, a previous hospital stay was reported. 64% of the patients were treated with antibiotics. All VREfm harbored the *vanB* vancomycin resistance gene. The multilocus sequence types (STs) detected changed abruptly from four different STs in 2016 to a predominant ST in 2017 and 2018 (ST117). Most of the ST117 isolates were members of the cgMLST type CT71.

Conclusion: The results indicate a sudden shift of the VREfm population structure from a semi-heterogeneous population to a pre-dominant clone within an interval of two years. Further investigations are warranted to understand the epidemiology and emergence of this clone.

Introduction

Vancomycin-resistant Enterococci (VRE) are an increasing problem in hospitals in many European countries, as well as in Germany [1—6]. In 2017, WHO has listed vancomycin-resistant *Enterococcus faecium* (VREfm) as a pathogen with high priority in its global priority list of antibiotic-resistant bacteria, emphasizing the paucity of available and effective treatment options. The clinical outcome of patients with VRE infections is significantly poorer [7,8] when compared to infections with non-resistant enterococci, with increased costs for treatment of hospitalized infections [9].

The population structure of VRE-associated infections has changed in recent years in Germany. The German National Reference Center for Staphylococci and Enterococci reported a shift in the predominance of the VRE species from *Enterococcus faecalis* to *E. faecium* and from *vanA* to *vanB*. In addition, the rate of *E. faecium* isolates resistant to vancomycin (VREfm) is increasing [10—14]. This increase has been particularly "dominant" in the central states of Germany, designated the "VRE-belt" [15]. Such an emergence of VREfm could coincide with an expansion of a specific lineage.

This hypothesis has been addressed by studies from some areas within the VRE-belt. In a study at an acute care hospital Weber *et al.* reported recent emergence of the VREfm multilocus sequence type ST117 almost entirely associated with the cgMLST type CT71 [16]. A genome-based analysis of entry screening VREfm specimens collected from 17 hospitals in 2017/2018 and covering a catchment area of 5,000 km^2 and three million inhabitants in the Rhine-Main metropolitan region, also revealed near ubiquitous presence of clonal ST117/CT71 isolates [17]. A predominance of this VREfm type had not been reported until that time.

In the present manuscript, we have extended our studies to include data from a regional referral neurological hospital with units for intensive care and early rehabilitation. Such institutions receive severely debilitated patients with neurological symptoms and provide continued intensive and intermediate care treatment, for in-house patients, and those patients from other acute-care hospitals who no longer require specialized interventions. Clinical specimens were collected from admission-based- and routine-screening from patients admitted from throughout the state of Hesse. Isolates from between 2016 to 2018 were examined to enable a study within a broader geographical range and time.

Materials and methods

Sample inclusion

The regional neurological hospital is located in Weilmünster (50.4310° N, 8.3784° E), in central Hesse, outside of the Rhine-Main metropolitan region. The recruiting area of patient referrals covers the entire State (size: 21.115 km^2) and the neighboring federal state of Rhineland-Palatinate.

All patients, newly admitted to either a neurological intensive care- or early rehabilitation-unit, were screened for VRE on admission ("screening <48h after admission"). Pre-selection of patients for <48h screening based on risk factors was not performed. Additional screening was performed, whenever patients were transferred between wards and when the initial screening had either not been carried out, was not recent, or when a patient had been previously housed in a room with other patients ("screening >48h after admission"). Data on gender, age, major underlying diseases, place of residence, previous hospital stays, and previous antimicrobial therapies were derived from patient's health records.

Isolate characterization

The collection of VRE isolates was performed from patients in the early neurological rehabilitation unit as well as the intensive care unit between January 2016 and December 2018. Colonies were identified using selective media and species determination made using MALDI/TOF. A random selection of these VRE isolates (n=55) was subjected to whole genome sequencing as reported before [17]. Sequencing was performed on an Illumina NextSeq to obtain an average read length of 125 nt and an average coverage of 206x. Quality control and assembly of the raw sequencing data was performed using ASA^{3}P [18]. Resistance gene prediction and multilocus sequence typing was performed applying goseqit (https://www.goseqit.com/). Further differentiation of the ST117 isolates was performed using a core-genome MLST (cgMLST) typing (Ridom SeqSphere+ v. 5.1.0., Ridom GmbH, Münster, Germany; *Enterococcus faecium* scheme developed by de Been *et al.* [19]). Comparative analysis with a representative isolate of the previous study on VREfm was performed using the isolate VRE-11-02-s and the Ridom software [17].

The raw sequencing data is available in the SRA archive, under the project number PRJNA631114.

Statistical analysis

Statistical analysis was performed using the Fisher's exact test.

Results

Patients' characteristics

Within the study period (January 2016—December 2018), patient screening for the presence of VRE on admission (<48h hospital stay) or upon ward transfer (>48h hospital stay) was performed.

The average age of the 55 patients included was 67.1 years with a range of 33—87 years. Fifty-five percent of the patients were male. Sixty-four percent of the participants lived in the region of the MDRO network Rhine-Main (Frankfurt am Main included). The remaining patients lived either outside the Rhine-Main-region of Hesse (n = 17) or in the neighboring federal state of Rhineland-Palatinate (n = 3, Table I, Table A1). Ninety-six percent of the patients had previously been treated at other hospitals including those within the city of Frankfurt/Main (n = 33). Nine of the 55 patients were treated in the Rhine-Main region excluding the city of Frankfurt/Main, while ten patients were from outside the Rhine-Main-region, and one patient from Rhineland-Palatinate. For 4% of the participants, no previous hospital stay was reported. For 64% of the participants, previous antimicrobial therapy had been reported, most often with Piperacillin/Tazobactam (35%), followed by Carbapenems (29%), and Vancomycin (27%) (Table I).

Isolate characteristics

A random selection of the collected VREfm isolates was sequenced (n=55) to enable a genome-based epidemiological analysis of these VRE. Fifty-five *E. faecium* VRE isolates from 2016-2018 were analyzed (2016: n=11; 2017: n=34; 2018: n=10).

All isolates were derived from screening samples (combined nasal/rectal swabs, n=51; tracheal secretion, n=1, urine, n=2, unknown: n=1). Thirty-four isolates derived from screenings performed <48h hours after admission, while 21 isolates derived from screenings >48h after admission. All these isolates encoded the vancomycin resistance determinant *vanB*.

Table I
Participants' and isolate data

		2016—2018	2016	2017	2018
Year of collection	Patients per year	55	11	34	10
Sex	male	30	7	16	7
	female	25	4	18	3
Age (year)	X ± sdev	67.1 ± 12.8	71.3 ± 13.5	65.9 ± 12.9	66.0 ± 10.6
	min - max	33—87	39—86	33—87	46—82
Place of residence	Frankfurt am Main (Region 1)	14	2	10	2
	Rhine-Main-region without Frankfurt (Region 2)	21	5	12	4
	Hessen without Rhine-Main and Frankfurt (Region 3)	17	2	12	3
	Rhineland-Palatinate (Region 4)	3	2	0	1
Place of previous hospital stay	Frankfurt am Main (Region 1)	33	6	20	7
	Rhine-Main-region without Frankfurt (Region 2)	9	4	4	1
	Hessen without Rhine-Main and Frankfurt (Region 3)	10	0	9	1
	Rhineland-Palatinate (Region 4)	1	1		
	no previous hospital stays reported	2	0	1	1
Previous antimicrobial therapy	Vancomycin	15	2	8	5
	Piperacillin/Tazobactam	19	3	10	6
	Carbapenems	16	3	8	5
	other	19	7	11	1
	not reported	20	4	15	1
Sequence type	ST117	51	7	34	10
	NEW	1	1	0	0
	ST18	2	2	0	0
	ST262	1	1	0	0
Sequence type/cgMLST combination	ST NEW, CT NEW	1	1		
	ST117, CT NEW	3	1	1	1
	ST117, CT36	3	3		
	ST117, CT71	45	3	33	9
	ST18, CT NEW	2	2		
	ST262, CT1815	1	1		

Only four MLST sequence types (STs) were detected, with a significant year-wise trend in both screening settings (<48h, >48h) (Figure 1). In 2016, four different STs were detected with a predominance of ST117 (n=7) followed by ST18 isolates (n=2), together with two individual isolates, one exhibiting ST262 and the other a new MLST type (Figures 1 and 2). In 2017 and 2018, only ST117 isolates were detected (2017: n=34; 2018: n=10). Among the ST117 isolates, a significant year-wise trend to the cgMLST type CT71 was detected: In 2016, CT36 and CT71 were found in equal numbers (n=3 each), while in 2017 and 2018 CT36 disappeared and CT71 was detected predominantly (Figure 2).

All of the ST117 isolates (n=51) analyzed harbored a chromosomal insertion of the *vanB* operon. The location of the insertion was dependent on the cgMLST type. In CT71 isolates, the insertion was in an hypothetical gene HMPREF0351_10592, as reported earlier [20], while the CT36 isolates carried an insertion into the *ara*A locus, encoding a putative L-arabinose isomerase (Table II). Forty-nine ST117 isolates (96%) harbored the virulence genes *hylEfm*, *efaAfm* and *acm* (Table II).

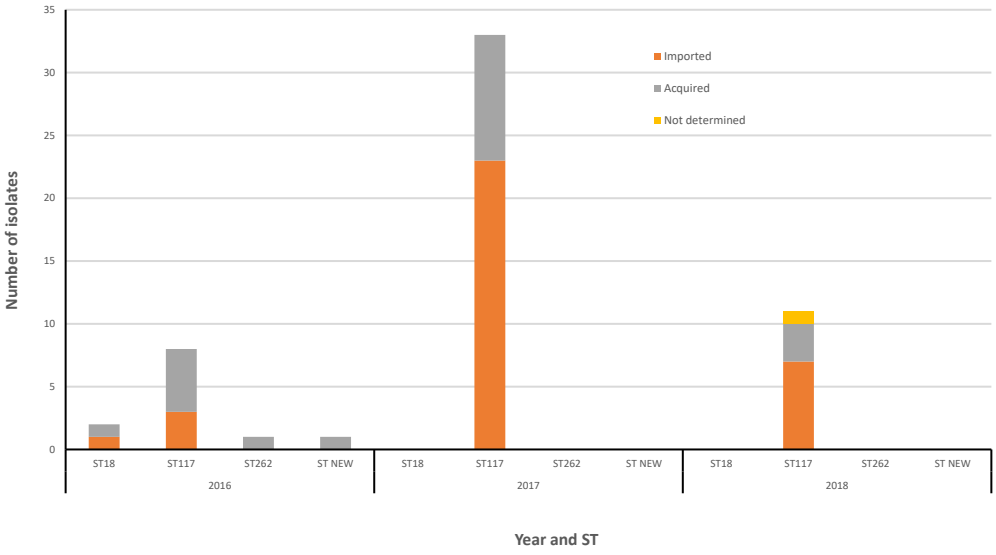

Figure 1. Year-wise depiction of detected STs.

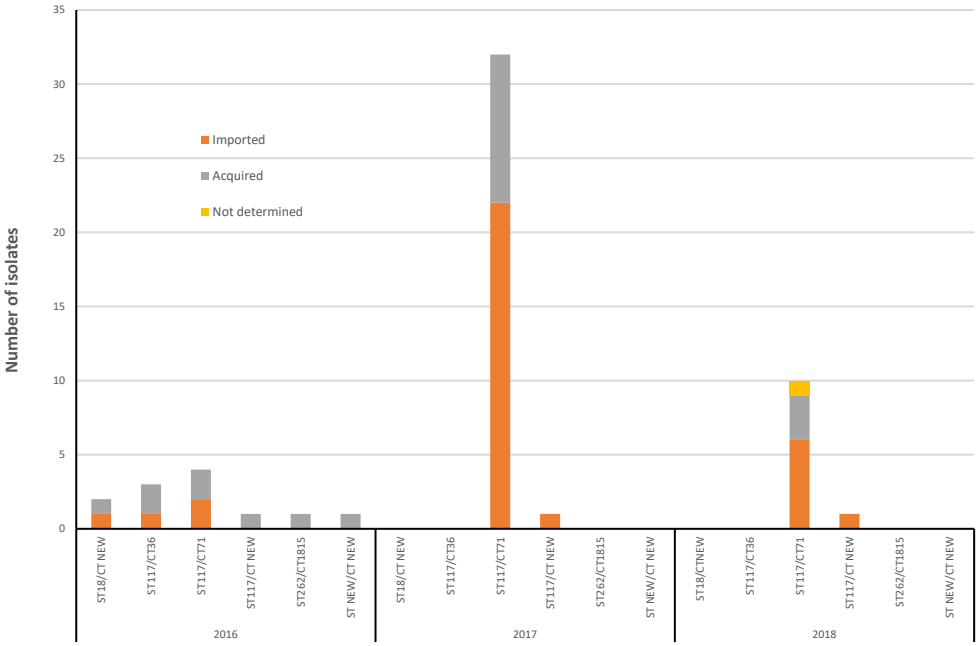

Figure 2. Year-wise depiction of ST/CT combinations detected.

Table II
Isolate characteristics. High relatedness to the Rhine-Main clone indicates a cgMLST allele difference of ≤10 cgMLST alleles

ID	MLST	CT	*van* allele	Acquired/ Imported	Integration site of Van*	virulence genes	Cluster	Highly related to Rhine-Main clone
ING-1	117	71	*vanB*	Imported	10592	*hylEfm, efaAfm, acm*	5	no
ING-10	117	71	*vanB*	Imported	10592	*hylEfm, efaAfm, acm*	none	no
ING-11	117	71	*vanB*	Imported	10592	*hylEfm, efaAfm, acm*	1	yes
ING-12	117	71	*vanB*	Imported	10592	*hylEfm, efaAfm, acm*	5	no
ING-13	117	71	*vanB*	Acquired	10592	*hylEfm, efaAfm, acm*	1	yes
ING-14	117	71	*vanB*	Acquired	10592	*hylEfm, efaAfm, acm*	3	no
ING-15	117	71	*vanB*	Imported	10592	*hylEfm, efaAfm, acm*	2	no
ING-16	117	71	*vanB*	Imported	10592	*hylEfm, efaAfm, acm*	1	yes
ING-17	117	71	*vanB*	Imported	10592	*hylEfm, efaAfm, acm*	1	yes
ING-18	117	71	*vanB*	Imported	10592	*hylEfm, efaAfm, acm*	none	no
ING-19	117	71	*vanB*	Acquired	10592	*hylEfm, efaAfm, acm*	1	yes
ING-2	117	71	*vanB*	Imported	10592	*hylEfm, efaAfm, acm*	1	yes
ING-20	117	71	*vanB*	Imported	10592	*hylEfm, efaAfm, acm*	none	no
ING-22	117	71	*vanB*	Imported	10592	*hylEfm, efaAfm, acm*	none	no
ING-23	117	71	*vanB*	Acquired	10592	*hylEfm, efaAfm, acm*	1	yes
ING-24	117	71	*vanB*	Imported	10592	*hylEfm, efaAfm, acm*	1	yes
ING-25	117	71	*vanB*	Imported	10592	*hylEfm, efaAfm, acm*	2	no
ING-26	117	71	*vanB*	Acquired	10592	*hylEfm, efaAfm, acm*	1	yes
ING-27	117	71	*vanB*	Imported	10592	*hylEfm, efaAfm, acm*	1	yes
ING-28	117	71	*vanB*	Imported	10592	*hylEfm, efaAfm, acm*	1	yes
ING-29	117	71	*vanB*	Imported	10592	*hylEfm, efaAfm, acm*	1	yes
ING-3	117	71	*vanB*	Acquired	10592	*hylEfm, efaAfm, acm*	1	yes
ING-30	117	71	*vanB*	Imported	10592	*hylEfm, efaAfm, acm*	2	no
ING-31	117	71	*vanB*	Acquired	10592	*hylEfm, efaAfm, acm*	1	yes
ING-32	117	71	*vanB*	Acquired	10592	*hylEfm, efaAfm, acm*	1	yes
ING-34	117	71	*vanB*	Acquired	10592	*hylEfm, efaAfm, acm*	2	no
ING-35	117	71	*vanB*	Imported	10592	*hylEfm, efaAfm, acm*	none	no
ING-36	117	71	*vanB*	Acquired	10592	*hylEfm, efaAfm, acm*	none	no
ING-37	117	71	*vanB*	Imported	10592	*hylEfm, efaAfm, acm*	none	no
ING-38	18	NEW	*vanB*	Imported	ND	*hylEfm, efaAfm, acm*	NA	NA
ING-39	262	1815	*vanB*	Acquired	10592	*efaAfm, acm*	NA	NA
ING-4	117	71	*vanB*	Imported	10592	*hylEfm, efaAfm, acm*	none	no
ING-40	117	71	*vanB*	Acquired	10592	*efaAfm, acm*	1	yes
ING-41	117	NEW	*vanB*	Acquired	*araA*	*hylEfm, efaAfm, acm*	NA	NA
ING-42	18	NEW	*vanB*	Acquired	ND	*hylEfm, efaAfm, acm*	NA	NA
ING-43	117	36	*vanB*	Acquired	*araA*	*hylEfm, efaAfm, acm*	NA	NA
ING-44	NEW	NEW	*vanB*	Imported	*araA*	*hylEfm, efaAfm, acm*	NA	NA
ING-45	117	36	*vanB*	Imported	*araA*	*efaAfm, acm*	NA	NA
ING-46	117	71	*vanB*	Imported	10592	*hylEfm, efaAfm, acm*	none	no
ING-47	117	36	*vanB*	Acquired	*araA*	*efaAfm, acm*	NA	NA
ING-48	117	71	*vanB*	Acquired	10592	*hylEfm, efaAfm, acm*	none	no
ING-49	117	71	*vanB*	Imported	10592	*hylEfm, efaAfm, acm*	4	no
ING-5	117	71	*vanB*	Imported	10592	*hylEfm, efaAfm, acm*	none	no
ING-50	117	NEW	*vanB*	Imported	10592	*hylEfm, efaAfm, acm*	NA	NA
ING-51	117	71	*vanB*	Acquired	10592	*hylEfm, efaAfm, acm*	none	no
ING-52	117	71	*vanB*	Imported	10592	*efaAfm, acm*	none	no
ING-53	117	71	*vanB*	Imported	10592	*hylEfm, efaAfm, acm*	3	no
ING-54	117	71	*vanB*	Acquired	10592	*hylEfm, efaAfm, acm*	2	no
ING-55	117	71	*vanB*	Imported	10592	*hylEfm, efaAfm, acm*	none	no
ING-56	117	71	*vanB*	Acquired	10592	*hylEfm, acm, efaAfm*	4	no
ING-57	117	71	*vanB*	not determined	10592	*hylEfm, efaAfm, acm*	2	no
ING-6	117	71	*vanB*	Imported	10592	*hylEfm, efaAfm, acm*	3	no
ING-7	117	71	*vanB*	Imported	10592	*hylEfm, efaAfm, acm*	1	yes
ING-8	117	71	*vanB*	Imported	10592	*hylEfm, efaAfm, acm*	3	no
ING-9	117	NEW	*vanB*	Imported	10592	*hylEfm, efaAfm, acm*	NA	NA

Statistical analysis on the ST117/CT71 isolates was performed to determine possible correlations of this clone with the place of residence or the year of isolation. It was shown that the presence of ST117/CT71 isolates was dependent on the year of isolation (P-value < 0.001), and not (for example) dependent on the patients' place of residence (P-value $= 0.75$).

Comparative analysis with the common Rhine-Main ST117/CT71 clone

As ST117/CT71/*vanB* isolates were detected in the Rhine-Main area in an earlier study [17], a comparative analysis with a representative isolate from this study was performed. Among the ST117/CT71 isolates, five clusters with <10 cgMLST differences were detected (Figure 3). "Cluster 1" comprises of 15 isolates predominantly isolated in 2017 together with a representative isolate from the earlier Rhine-Main study isolated in 2018 (Figure 3). The cluster harbored isolates from all three regions of Hesse.

Among the lesser-related isolates, four clusters were detected. Cluster 2 (n = 7) and Cluster 3 included isolates from 2018 and 2017, while Cluster 4 included only isolates from 2018. Cluster 5 included two isolates from 2017. These findings indicate that VREfm ST117/CT71/*vanB* from Hesse are constantly evolving.

Discussion

Specialist regional neurological hospitals that have intensive care units and cater for early neurological and neuro-surgical rehabilitations form the bulwark of intermediate and intensive care treatment of patients in Germany. Recent studies have suggested that nearly 25% of all patients entering such facilities are either colonized or infected with multi-drug resistant bacteria [21].

Surveillance studies indicated an increase in VRE from screening- and clinical-samples in the regional neurological specialty hospital [21]. Detailed analysis of isolates using WGS indicated emergence of VREfm MLST type ST117, cgMLST type CT71 with the vancomycin resistance determinant *vanB* (Figure 3). As the neurological hospital with intensive care and early rehabilitation serves the entire federal state of Hesse and neighboring federal state of Rhineland-Palatinate, it was regarded as a representative sentinel point for statewide epidemiology.

Our results reflect the changing VREfm epidemiology in Germany. The national reference center for Staphylococci and

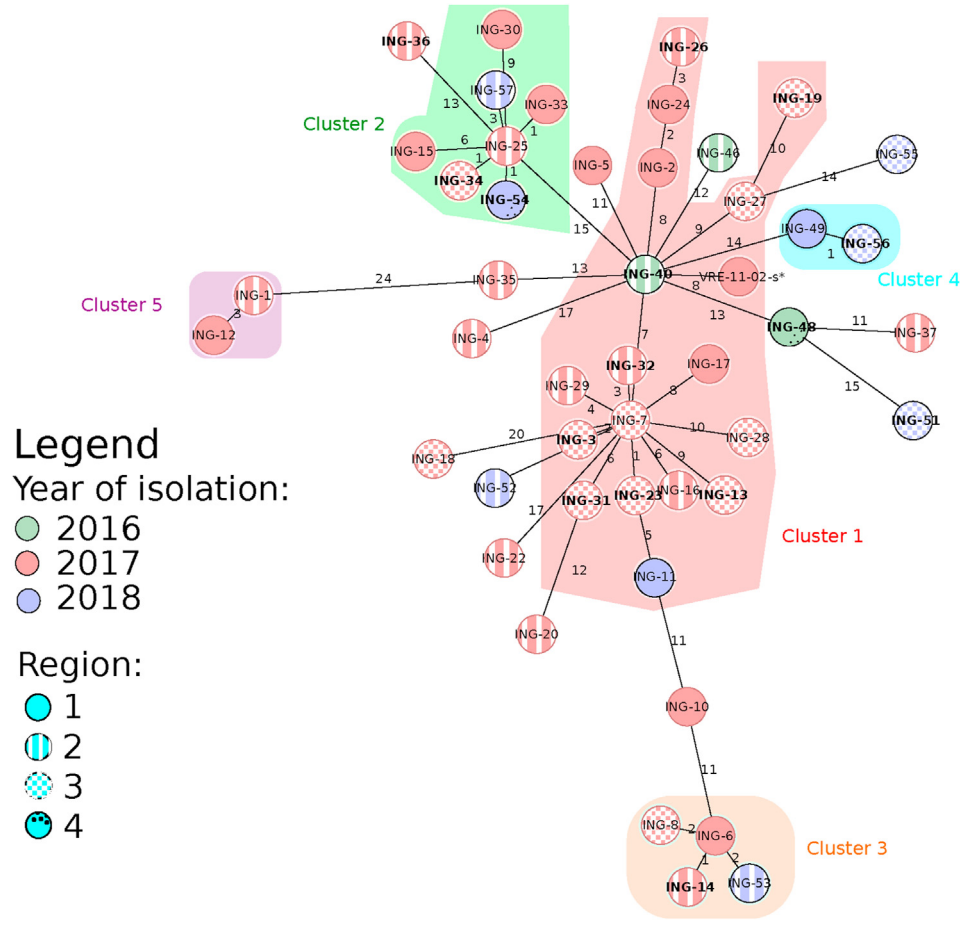

Figure 3. Comparative analysis of VREfm isolates with a representative isolate from the Rhine-Main study (marked with an asterisk). Clusters with a difference of ≤10 cgMLST alleles are marked with shading. Names of those isolates derived from screening performed >48h are marked with bold font.

Enterococci has detected a supra-regional shift in VREfm MLST types, with the emergence of ST117. The total numbers of this ST type have doubled in 2018 as compared to 2016. ST117/CT71 has been detected in ten federal states in Germany [13]. A study performed at a large university hospital in Berlin comprising VREfm isolates from 2008, 2013, 2015 and 2018 [16] has reported a similar shift. From our study here, we document that this shift in the region studied occurred sometime between 2016 and 2017. Unlike the study from Berlin, a shift of *vanA* to *vanB* in the ST117/CT71 isolates was not detected. This could be the result from the shorter period during which the isolates were obtained here. Following our data, VREfm ST117/CT71 replaced other STs and was the predominant clone isolated from the neurological patients of this hospital. This sub-cluster was previously observed in other acute care clinics in the Rhine-Main-region from samples obtained in 2018, where ST117/CT71/*vanB* was detected in patients from intensive care, as well as from hematological and transplantation units [17]. Even though a shift to ST117 could have been expected from previous studies [13,16], the unique predominance of one VREfm sub-cluster type was unexpected.

The predominance of a single sub-cluster type in area with many hospitals is unusual, as VREfm outbreaks are generally oligo- or polyclonal in nature (e.g. Australia [22], UK [23], Germany [24,25]), and clonal outbreaks are only seldom reported (i.e. Turkey [26]). Whenever present, the analysis of the cgMLST types exhibit a broad range of variety of different ST/CT-clones, thus arguing against primary intra-hospital transmission, but rather for the import of new and emerging types [25]. Compared to this diversity, the detection of a single ST117/CT71/*vanB* clone in two independent studies, including in the over 40 isolates in this study which were from patients with geographically distinct places of residence and where previous hospital stays were in institutions from all over Hesse or even in cases without any known previous hospital stay [17], was unexpected and is remarkable. The ubiquitous presence of this clone from widely separated referral institutions clearly suggests inter-hospital spread but could also implicate other hitherto unrecognized vectoral components. It might be hypothesized that this clone has highly adaptive properties for the colonization of the gastrointestinal tract and for effective transmission and persistence stability within the hospital environment. Indeed, ST117/CT71/*vanB* VREfm isolates harbor elements associated with persistence and colonization, such as the collagen-binding gene *acm*, the enterococcal surface protein Esp, required for promoting biofilm formation and the PTS_{clin} phosphotransferase system associated with colonization potential of clinical isolates [27]. Further studies are warranted to understand the impact of these and hitherto undiscovered genes in the distribution and the emergence of the ST117/CT71/*vanB* clone.

Conclusions

The population structure of VREfm at a neurological hospital with intensive care and early rehabilitation changed dramatically within a short time-period. A predominant VREfm type (ST117/CT71/*vanB*) emerged and is currently present throughout Hesse as well as in many regions in Germany. Further studies are needed to understand the epidemiology and emergence of this specific VREfm clone, as well the contribution of other sources (e.g. water, food, animals) to its spread.

Limitations of the study

There are several limitations to our study. Only a representative collection of isolates were sequenced (n=55). The design of the study was retrospective, so that anamnestic data could only be derived from the prior patients' documentation and was not queried individually. While data for sex, age, place of residence and previous hospital stay are correct and complete, data on previous antibiotic therapies may have been less so. A further limitation of the study is that the overall number of isolates examined is relatively small.

Credit author statement

Conceptualization; CB, CI, DM, IP, UH, TC.
Data curation; IP, JF, LF, MF, UH.
Formal analysis; IP, JF, LF, UH, TC.
Funding acquisition; CI, UH, TC.
Investigation; IP, JF, LF, UH.
Methodology; All authors.
Project administration; CB, DM, IP, LF, UH, TC.
Resources; CB, CI, DM, UH, TC.
Software; CI, TC.
Supervision; CB, CI, DM, UH, TC.
Validation; IP, JF, LF, MF, UH.
Visualization; IP, JF, LF, UH.
Roles/Writing — original draft; All authors.
Writing — review & editing: All authors.

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Conflict of interest statement

The authors declare that they have no competing interest.

Ethics approval

The study was approved by the ethics committee of the medical faculty of the Justus Liebig University of Giessen (AZ: 179/16). All samples were taken as part of standard care procedures.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.infpip.2021.100138.

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