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Short Communication

Using core genome multilocus sequence typing (cgMLST) for vancomycin-resistant *Enterococcus faecium* isolates to guide infection control interventions and end an outbreak☆

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ABSTRACT

Objectives: Until July 2016, vancomycin-resistant*Enterococcus faecium* (VREfm) was sporadically detected in Odense University Hospital, Denmark. After July 2016, the number of VREfm cases increased. This study aimed to apply a core genome multilocus sequence typing (cgMLST) scheme for *E. faecium* to type and analyse VREfm isolates collected at a single Danish hospital and to compare the results with cgMLST data from other regions of Denmark to trace transmission.

Methods: A total of 38 VREfm clinical isolates from inpatients at the hospital in the period January 2014 through June 2017 were included in the study and analysed using whole-genome sequencing. Use of SeqSphere + software was initiated from the beginning of June 2017 to obtain MLST, cgMLST and epi curves. Admission histories were incorporated and national surveillance data on cgMLST were used to identify transmission routes.

Results: Six different sequence types (STs) were identified, the most frequent being ST80, ST117 and ST203. cgMLST subdivided the 38 isolates into 18 different complex types (CTs) with 13 isolates (34%) belonging to ST80-CT993. Epi curves indicated transmission of ST80-CT993 in several departments. Transmission from patients transferred from other hospitals was not identifiable. Infection control interventions launched in one department ended the outbreak.

Conclusion: The high resolution of cgMLST allowed for detailed interpretation with evidence of nosocomial transmission of specific CTs. cgMLST made it easy to compare our local isolates with national findings, thereby clarifying transmission routes. Supplemented with admission histories, cgMLST targeted the epidemiological investigation and delineated the expensive and time-consuming infection control interventions.

1. Introduction

Vancomycin resistance in *Enterococcus faecium* has been known since the end of the 1980s, and in human clinical isolates it is primarily due to *vanA* or *vanB* genes [1,2]. Vancomycin-resistant *E. faecium* (VREfm) thrive very well in the hospital environment and may cause hospital-acquired infections. VREfm have for the last decade been rising in number worldwide, and infections caused by these isolates are difficult to treat owing to their inherent resistance to many antimicrobials [3,4].

For many years, the prevalence of clinical VREfm found in blood cultures and spinal fluids in Denmark was ~1%, but in 2012 large outbreaks in two of the five Danish regions began. More than 1000 patients were infected or colonised during the period 2012–2015 and the transmission is still ongoing, leaving the Danish prevalence of these clinical VREfm above 10% in 2018 [5]. Multilocus sequence

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typing (MLST) of clinical VREfm from all kind of materials revealed that several sequence types (STs) were involved in the outbreaks, in particular ST80, ST117 and ST203. In 73% of the VREfm isolates, resistance to vancomycin was due to the *vanA* gene [5–7]. Initially these outbreaks did not affect hospitals in the Region of Southern Denmark despite transfer of patients from affected hospitals. At Odense University Hospital in Region of Southern Denmark, five to six patients were identified with VREfm each year in this period. However, in 2016 the number of patients with VREfm increased, and in the first half of 2017 an outbreak was suspected [5].

The traditional method used for outbreak investigation in Denmark had for many years been based on *Sma*I macrorestriction analysis through pulsed-field gel electrophoresis (PFGE). More recently, single nucleotide polymorphism (SNP)-based mapping of short-read (Illumina) data against a reference genome has been applied for outbreak investigations, but this method has now been substituted with core genome MLST (cgMLST). Typing based on cgMLST, with the designation of a complex type (CT), has a high discriminatory power and studies have found the method concordant with the SNP-based mapping mentioned above in evaluating the relatedness of bacteria [8–11]. Due to the availability of easy-to-use software solutions, we implemented the cgMLST method in the Department of Clinical Microbiology at Odense University Hospital in June 2017 and used it for investigation of the local rise of VREfm.

The aim of this study was to apply a cgMLST scheme for *E. faecium* implemented in SeqSphere + software to type and analyse the VREfm isolates collected at a single Danish hospital over a period of 4 years, to compare the results with cgMLST data of VREfm isolates identified in other regions of Denmark as a part of the national VREfm surveillance, and to use these data to trace transmission.

2. Materials and methods

2.1. Demographic data

Denmark is divided into five regions (NUTs, level 2), of which the Region of Southern Denmark (RSD) covers approximately one-fifth (1.2 million) of the Danish population [12]. The biggest hospital in RSD is Odense University Hospital (OUH), with approximately 1000 beds.

All samples collected from patients admitted to OUH are analysed in the Department of Clinical Microbiology of the hospital.

2.2. Bacterial isolates

Consecutive VREfm isolates from clinical samples (non-screening) from hospitalised patients in the period 1 January 2014 to 1 July 2017 were analysed. Only the first isolate from each patient was included in this study. Isolates from the start of June 2017 onwards were analysed in real time, while the rest were sequenced and analysed retrospectively.

Bacterial identification was performed by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF/MS) (Microflex LT; Bruker Daltonik GmbH, Bremen, Germany).

Resistance to vancomycin was detected by agar disk diffusion susceptibility testing according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines using 4-mm Mueller–Hinton agar plates and 5 mg Vancomycin Neo-Sensitabs™ (Rosco, Taastrup, Denmark).

All isolates were tested for the presence of the *vanA* and *vanB* genes using an in-house real-time PCR. The *vanA* primer and probe sequences were based on the study of Fang et al. [13]. The *vanB* primer and probe sequences were designed using Primer Express 3.0 (Thermo Scientific) and consisted of the following: forward primer GGRAACGAGGATGATTTGATTG; reverse primer CGTGGCTCARCCGGATT; and probe VIC-CGG CGAAGTGGATC-MGB. Detection was performed in a 25 μL reaction volume containing 12.5 μL of *Taq*Man™ FAST Universal PCR Master Mix (Applied Biosystems), 1000 nM of each primer, 200 nM of the probes and 5 μL of template DNA using a 7500 Fast Real-Time PCR System (Applied Biosystems) with the following cycling parameters: 95 °C for 20 s; and 45 cycles of 95 °C for 3 s and 60 °C for 30 s.

VREfm screening was based on culturing a single rectal swab from each patient. Culturing was carried out on a 5% blood agar plate [Statens Serum Institut (SSI), Denmark] read after 24 h and 48 h of incubation at 35 °C, followed by visual inspection for enterococcal growth, bacterial identification with MALDI-TOF/MS, and vancomycin susceptibility testing as described above. If the isolate was found to be resistant to vancomycin or single colonies were found in the clear zone near the vancomycin tablet, the colonies in the zone or a scrape from the edge of the growth nearest the vancomycin tablet was tested for the presence of *vanA* and *vanB* using the in-house method described above.

2.3. Whole-genome sequencing (WGS) data analysis

WGS was carried out on a MiSeq platform (Illumina Inc., San Diego, CA, USA) according to the manufacturer's instructions to obtain paired-end reads of 2 × 150 bp in length.

Draft genomes were de novo assembled using SKESA v.2.2 in the Bifrost pipeline at SSI. A quality control has been performed on the raw reads using the Bifrost pipeline (https://github.com/ssi-dk/bifrost) with accepted avg. coverage >30.

The draft genome sequences of all isolates were analysed using SeqSphere + software v.5.0 (Ridom GmbH, Germany) (*E. faecium* cgMLST scheme v.1.1;1423 loci) by which sequence types (STs), complex types (CTs) and cluster arrangement were obtained. The parameter 'pairwise ignoring missing values' was activated and the cluster distance threshold for the core genome was ≤20 allele differences [10].

2.4. Epidemiology and relatedness to other regions in Denmark

Admission history within a period of 1 year from the VREfm finding of the individual patient was extracted from the patient journal system Cambio COSMIC (https://www.cambiogroup.com/our-solutions/cambio-cosmic/). Collection date and sample material were extracted from the Danish microbiological department database system 'MADS' (www.madsonline.dk). Epidemic curves (epi curves) of the cgMLST results were constructed using SeqSphere + software. All cases were examined for prior VREfm findings.

The local data were compared with the national data on VREfm from clinical samples, which have undergone WGS since 2015 [5]. In cases where the patient had been transferred from another region, the local Department of Clinical Microbiology in the region in question was contacted for cgMLST and/or MLST results.

3. Results

A total of 38 VREfm isolates from 38 patients were included in the study. A comprehensive list of characteristics of the isolates, including sample type, site of isolation, collection date, *vanA*/*B*, sequencing results and admission histories, is given in Table 1.

3.1. Phylogenetic analysis and resistance genes

The 38 isolates belonged to six different STs, with the most frequent being ST80, ST117 and ST203.

Table 1
Characterisation of the vancomycin-resistant *Enterococcus faecium* (VREfm) isolates at Odense University Hospital (OUH), Region of Southern Denmark, in the period 1 January 2014 to 1 July 2017 (n = 38) and appertaining epidemiological data in a 12-month period prior to the sample date.

Number	Sample date (month/year)	MLST	cgMLST	*van* gene	Sample	Department number at OUH where sample was collected	Prior admissions in a 12-month period before sample date at OUH	Admissions in other regions of Denmark and other countries	Prior VREfm type
01	03/2014	ST80	CT14	*vanA*	Urine	1			
02	03/2014	ST80	CT2499	*vanA*	Urine	2	6		
03	04/2014	ST117	CT24	*vanA*	Urine from catheter	2	3		
04	05/2014	ST192	CT17	*vanA*	Blood culture	3		CR	ST192-CT17
05	05/2014	ST117	CT24	*vanA*	Swab from abscess	3	2		
06	06/2014	ST18	CT864	*vanA*	Liquid from abdominal drain	4	6, 2		
07	05/2015	ST80	CT866	*vanA*	Urine	5		CR	
08	06/2015	ST78	CT1438	*vanA*	Liquid from abdominal drain	6		RZ	
09	08/2015	ST80	CT880	*vanB*	Blood culture	7	13		
10	09/2015	ST117	CT1834	*vanA*	Blood culture	3			
11	10/2015	ST80	CT14	*vanA*	Urine	1	6		
12	02/2016	ST80	CT14	*vanA*	Ascites	3			
13	05/2016	ST203	CT859	*vanA*	Urine from catheter	2	8	CR	
14	05/2016	ST80	CT993	*vanA*	Urine	7	1		
15	06/2016	ST80	CT880	*vanB*	Urine	8			
16	08/2016	ST80	CT993	*vanA*	Blood culture	1	2, 14		
17	10/2016	ST80	CT993	*vanA*	Urine	9	15		
18	11/2016	ST80	CT993	*vanA*	Urine from catheter	7	16, 17		
19	11/2016	ST80	CT993	*vanA*	Liquid from abdominal drain	2	9		
20	11/2016	ST80	CT993	*vanA*	Urine	10	6		
21	12/2016	ST80	CT993	*vanA*	Urine	2	9		
22	01/2017	ST203	CT1144	*vanA*	Urine from catheter	11	18	RZ	
23	02/2017	ST203	CT1143	*vanA*	Urine	1	18		
24	02/2017	ST80	CT993	*vanA*	Abdominal wound swab	2	3, 6, 19		
25	02/2017	ST80	CT993	*vanA*	Urine from catheter	1			
26	02/2017	ST203	CT859	*vanA*	Urine	7	13, 1		
27	02/2017	ST80	CT993	*vanA*	Urine	9			
28	03/2017	ST203	CT859	*vanA*	Urine	7	13		
29	03/2017	ST80	CT993	*vanA*	Urine from catheter	2	9		
30	03/2017	ST80	CT1839	*vanA*	Urine from catheter	12		CRD	ST80-CT997
31	04/2017	ST117	CT1182	*vanA*	Urine from catheter	2	9		
32	05/2017	ST80	CT32	*vanB*	Urine from catheter	2		Germany	Unknown
33	05/2017	ST80	CT866	*vanA*	Urine	7	20	CR	Unknown
34	05/2017	ST80	CT1508	*vanA*	Urine from catheter	8			
35	05/2017	ST80	CT993	*vanA*	Sputum	9	2		
36	06/2017	ST80	CT1508	*vanA*	Urine	8			
37	06/2017	ST80	CT1545	*vanA*	Blood culture, catheter	6	1, 2, 3, 11, 12		
38	06/2017	ST80	CT993	*vanA*	Tissue, ischial tuberosity	9	8		

MLST, multilocus sequence typing; cgMLST, core genome multilocus sequence typing; ST, sequence type; CT, complex type; CR, Capital Region of Denmark; RZ, Region Zealand; CRD, Central Denmark Region.

Of the 38 isolates, 26 (68%) belonged to ST80, 4 belonged to ST117 and 5 belonged to ST203, while the remaining three STs (ST18, ST78 and ST192) comprised 1 isolate each.

cgMLST analysis of the 38 isolates revealed 18 different CTs (Table 1). The ST80 group was subdivided into nine different clusters, with ST80-CT993 being the dominant type.

The ST80-CT993 isolates had an allele difference of 0–25 among the isolates. The second largest ST80 group was CT14, which consisted of three isolates with 7–24 allele differences among the isolates, while the remaining six ST80 clusters consisted of only one or two isolates each.

Using cgMLST, two of the four ST117 isolates showed no allele differences and belonged to CT24. Likewise, for ST203 three of the five isolates appeared related and belonged to CT859, with allele differences of 2–3. The remaining CTs were all singletons (Table 1).

Vancomycin resistance in 35 of the 38 isolates was found to be related to the *vanA* gene, while only 3 isolates contained *vanB*.

3.2. Epidemiological data and transmission

Use of cgMLST data for an epi curve illustrated some small clusters at the beginning of 2014, the end of the investigation period in 2017, and a larger cluster during the last year of the period (Fig. 1).

Before the actual rise of VREfm in mid-2016, only two small clusters of ST117-CT24 and ST80-CT14 were detected. Patient admission histories for the two patients with ST117-CT24 isolates revealed that transmission was likely to have occurred in Department 1 or 2 (Table 1). Two of the three patients with ST80-CT14 had been admitted to the same ward, but with 1.5 years in between, and the last patient had not been on this ward at all. Isolates from patients admitted to the same ward had an allele difference of 24, and taking the admission dates into account, transmission was rejected. Two of the patients had isolates differing by 7 alleles, but no connection regarding their hospitalisation was found.

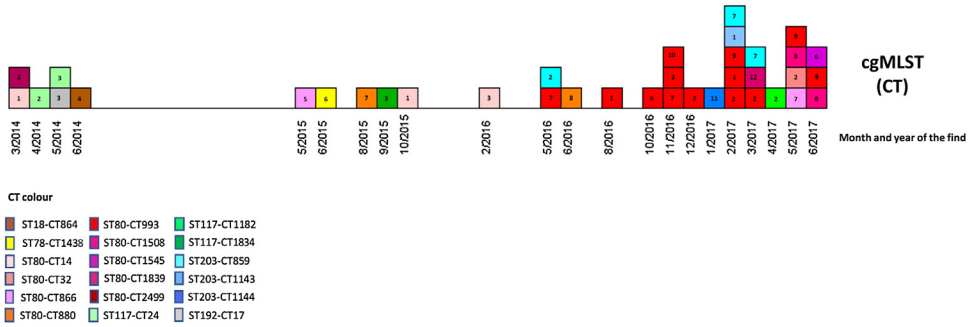

Fig. 1. Epidemic curves (epi curves) for vancomycin-resistant *Enterococcus faecium* isolates detected at Odense University Hospital, Region of Southern Denmark, during 1 January 2014 to 1 July 2017. The epi curves were based on multilocus sequence typing (MLST) with creation of sequence types (STs), and core genome MLST (cgMLST) with creation of complex types (CTs). Each box represents one specific isolate (1–38). Colours correspond to the ST-CT number. The number in the box correlates to the department number where the sample was collected.

Two isolates of ST80-CT866 and ST80-CT880, respectively, were found in the period 2015–2017. The patients with ST80-CT880 had not been admitted to the same ward, and the ST80-CT866 isolates were found with >1 year apart. The allele difference in both cases was <20, but due to the distance in time and admission histories, transmission was rejected. Three isolates of ST203-CT859 were found in mid-2016 and the beginning of 2017. The isolates from the two patients detected in 2017 had an allele difference at 2. The patients had been admitted to the same ward and transmission was found to be most likely.

In May 2017, two isolates of ST80-CT1508 appeared. These two patients had been admitted to the same ward within 1 month, suggesting transmission (Table 1). The allele difference was 34, leaving the connection weaker.

From May 2016 through June 2017, a total of 13 isolates of ST80-CT993 were continuously detected and matched the rise in number of VREfm detected at OUH. For each specific isolate, an allele difference at 0–5 to the nearest neighbour was found. At the time of sampling, the patients were distributed in 5 departments but with prior admissions in a total of 13 departments in the past 12 months. Seven of the patients had been admitted to Department 9 and four of these patients had also stayed in Department 2, where a further two patients with ST80-CT993 had been admitted. Three of the four patients found in Department 2 had been previously admitted to Department 9, while only one patient prior to admission in Department 9 had been in Department 2, and was in addition found late in the investigation period.

3.3. Relatedness to other regions in Denmark

In 7 of the 38 VREfm cases, patients were transferred from Danish hospitals located in high-incident regions. Three of these seven patients had prior to admission to OUH been found harbouring VREfm, with two of them being singletons and one with no sequencing data available (Table 1).

The other four patients were not known to be colonised before admission to OUH, but according to our results one of these four patients was found to harbour an ST203-CT859 *vanA E. faecium* and one patient an ST80-CT866 *vanA E. faecium*. These types were highly prevalent in the Capital Region and the Central Denmark Region, from where the patients were transferred, and the patients might have been colonised during hospitalisation in these regions [5–7].

The remaining two patients were colonised with ST203-CT1144 and ST78-CT1438 strains (Table 1). Both types had been rarely found in Denmark—in our own region (RSD) and in the Capital Region [5].

A single patient was transferred from a German hospital and was colonised with a ST80-CT32 *vanB*-positive isolate, which to our knowledge has never been detected in Denmark [5].

On a national level, 11 (~3%) of 422 collected clinical VREfm isolates in 2016 belonged to ST80-CT993 *vanA E. faecium* [5]. Of these 11 patients, we found that 7 patients had been at OUH. Three patients had been hospitalised in the same region as OUH but at a minor hospital, and one patient had been found in the Central Denmark Region. One of the patients from the minor hospital and the patient from the Central Denmark Region were detected with an ST80-CT993 *vanA E. faecium* strain before this type appeared at OUH. None of the patients with a ST80-CT993 that were diagnosed at OUH in 2016–2017 had been hospitalised outside the Region of Southern Denmark (Table 1). No connection was found between the index patient at OUH and other hospitals inside the region.

3.4. Infection control interventions

Based on cgMLST analysis and the admission histories, an epidemiological link was established among 7 of the 13 patients with ST80-CT993. Transmission had most likely happened in Department 2 or 9. Due to the highest number of cases in Department 9, and since three of the four patients carrying ST80-CT993 in Department 2 also had been admitted to Department 9, infection control interventions were launched in the latter department at the end of June 2017.

To reveal whether there was a large ongoing outbreak in Department 9, we initiated screening samples from all currently hospitalised patients in this department on 30 June 2017. No further screening criteria besides being hospitalised in Department 9 on the screening day was used. A total of 18 patients were included and all samples were negative using culture-based screening.

An audit in Department 9 revealed the need for improved compliance with standard precautions and suggested transmission through environmental surfaces. A tidying up of the entire department, including rinsing, storage and staff rooms, took place before cleaning the environment. This was followed by non-touch automated disinfection with hydrogen peroxide or manual disinfection with chlorine.

After completion of the infection control interventions in Department 9, only one additional case of ST80-CT993 colonisation occurred in the following 6 months and therefore no further infection control interventions or screening tests were initiated in the hospital departments.

4. Discussion

Analysis of cgMLST data indicated that multiple clones of VREfm were introduced at OUH during the period 1 January 2014 to 1 July 2017 and that transmission occurred between patients within the hospital.

Use of cgMLST on VREfm allowed for a detailed interpretation of the diversity, thereby indicating transmission of only certain complex types and not all isolates with an identical sequence type. This confirms the results from other clinical studies with other micro-organisms in this field [11,14,15].

cgMLST in combination with allele differences and admission histories revealed transmissions of three minor (ST203-CT859, ST117-CT24 and ST80-CT1508) and a single larger clone of ST80-CT993, representing the first outbreak of VREfm in the Region of Southern Denmark.

The patients with ST80-CT993 VREfm clone had connections to a total of 13 departments, but transmission had most likely happened in 1 or 2 departments. Infection control interventions were launched in Department 9, which stopped the outbreak.

Initial transmission from outside the region or from a regional hospital into OUH could not be established. Transmission could however have occurred through an unknown carrier, and it is most likely that transmission occurred between hospitals in Southern Denmark owing to the higher number of patient transfers. Screening samples from patients in Department 9 were all found negative using culturing. This may be explained by the large turnover of patients in the department, with the risk that we did not test the right patients, or due to an inefficient screening method for colonisation with a low number of VREfm, by culturing. Today, this may be solved by using a molecular diagnostic method and enterococcus selective growth media for detecting colonisation.

It has not been possible to find any descriptions of the ST80-CT993 *vanA* clone from outside Denmark by searching www.pubmed.gov and www.cgMLST.org.

Transmission inside OUH with clones from patients transferred from regions with ongoing outbreaks was not identified. This was a surprise, especially for the ST203-CT859 *vanA* VREfm, which had a high prevalence in the other regions, accounting for 51–61% of the Danish VREfm in 2015–2017 [5]. The ST80-CT14 and ST80-CT866 were both frequent clones in 2015 but were reduced to ~4% in 2017. An analysis of which factors prevented these transmissions could prove valuable.

A single patient was transferred from a German hospital and harboured an ST80-CT32 *vanB* VREfm. This clone has been detected several times in Germany [16]. ST80, together with ST117 and ST203, are the most frequent sequence types both in Germany and Denmark, but in contrast to the Danish isolates the vancomycin resistance in the German clones is often mediated by *vanB* [17].

cgMLST has been described for outbreak investigation but, as far as we know, there is only a limited number of outbreak descriptions where cgMLST has been used during the investigation to target hygiene interventions [14,18]. In this present study, we demonstrated the ability of cgMLST to identify outbreak strains, to assess whether VREfm was introduced from outside the region, and to help decrease the number of departments where infection control interventions were introduced to terminate transmission. We experienced that cgMLST results were easy to use for non-bioinformaticians, but it is a field that should be investigated further in order to achieve an enhanced use of the system in the frontline.

In conclusion, we found that cgMLST was useful in local characterisation of VREfm, distinguishing sporadic clones from outbreak strains. Use of cgMLST made it easy to compare our local isolates with the national findings, thereby clarifying transmission routes. In combination with admission histories, cgMLST targeted possible outbreaks and located the specific wards involved. This limited the outbreak and saved us from expensive and time-consuming infection control interventions.

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None.

Competing interests

None declared.

Ethical approval

The Danish Patient Safety Authority has approved the collection from all the databases used in this paper [Ref. number: 3-3013-2554/1].

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References

[1] Cetinkaya Y, Falk P, Mayhall CG. Vancomycin-resistant enterococci. Clin Microbiol Rev 2000;13:686–707, doi:http://dx.doi.org/10.1128/CMR.13.4.686-707.2000.
[2] Gorrie C, Higgs C, Carter G, Stinear TP, Howden B. Genomics of vancomycin-resistant *Enterococcus faecium*. Microb Genomics 2019;5:e000283, doi:http://dx.doi.org/10.1099/mgen.0.000283.
[3] Wagenvoort JHT, De Brauwer EIGB, Penders RJR, Willems RJ, Top J, Bonten MJ. Environmental survival of vancomycin-resistant *Enterococcus faecium*. J Hosp Infect 2011;77:282–3, doi:http://dx.doi.org/10.1016/j.jhin.2010.11.008.
[4] Kramer A, Schwebke I, Kampf G. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. BMC Infect Dis 2006;6:130, doi:http://dx.doi.org/10.1186/1471-2334-6-130.
[5] DANMAP. *Reports*. https://www.danmap.org/reports. [Accessed 8 July 2020].
[6] Hammerum AM, Baig S, Kamel Y, Roer L, Pinholt M, Gumpert H, et al. Emergence of *vanA Enterococcus faecium* in Denmark, 2005–15. J Antimicrob Chemother 2017;72:2184–90, doi:http://dx.doi.org/10.1093/jac/dkx138.
[7] Pinholt M. Hospital-acquired vancomycin-resistant Enterococcus faecium in Denmark [PhD thesis]. Copenhagen, Denmark: University of Copenhagen, Faculty of Health and Medical Science; 2016.
[8] Quainoo S, Coolen JPM, van Hijum SAFT, Huynen MA, Melchers WJG, van Schaik W, et al. Whole-genome sequencing of bacterial pathogens: the future of nosocomial outbreak analysis. Clin Microbiol Rev 2017;30:1015–63, doi:http://dx.doi.org/10.1128/CMR.00016-17.
[9] Mellmann A, Andersen PS, Bletz S, Friedrich AW, Kohl TA, Lilje B, et al. High interlaboratory reproducibility and accuracy of next-generation-sequencing-based bacterial genotyping in a ring trial. J Clin Microbiol 2017;55:908–13, doi:http://dx.doi.org/10.1128/JCM.02242-16.
[10] De Been M, Pinholt M, Top J, Bletz S, Mellmann A, Van Schaik W, et al. Core genome multilocus sequence typing scheme for high-resolution typing of *Enterococcus faecium*. J Clin Microbiol 2015;53:3788–97, doi:http://dx.doi.org/10.1128/JCM.01946-15.
[11] Pearce ME, Alikhan NF, Dallman TJ, Zhou Z, Grant K, Maiden MCJ. Comparative analysis of core genome MLST and SNP typing within a European *Salmonella* serovar Enteritidis outbreak. Int J Food Microbiol 2018;274:1–11, doi:http://dx.doi.org/10.1016/j.ijfoodmicro.2018.02.023.
[12] Hammerum AM, Justesen US, Pinholt M, Roer L, Kaya H, Worning P, et al. Surveillance of vancomycin-resistant enterococci reveals shift in dominating clones and national spread of a vancomycin-variable *vanA Enterococcus faecium* ST1421-CT1134 clone, Denmark, 2015 to March 2019. Euro Surveill 2019;24:1900503, doi:http://dx.doi.org/10.2807/1560-7917.ES.2019.24.34.1900503.
[13] Fang H, Ohlsson AK, Jiang GX, Ullberg M. Screening for vancomycin-resistant enterococci: an efficient and economical laboratory-developed test. Eur J Clin Microbiol Infect Dis 2012;31:261–5, doi:http://dx.doi.org/10.1007/s10096-011-1304-0.
[14] Woksepp H, Ryberg A, Berglind L, Schön T, Söderman J. Epidemiological characterization of a nosocomial outbreak of extended spectrum β-lactamase *Escherichia coli* ST-131 confirms the clinical value of core genome multilocus sequence typing. APMIS 2017;125:1117–24, doi:http://dx.doi.org/10.1111/apm.12753.
[15] Van Walle I, Björkman JT, Cormican M, Dallman T, Mossong J, Moura A, et al. Retrospective validation of whole genome sequencing-enhanced surveillance of listeriosis in Europe, 2010 to 2015. Euro Surveill 2018;23:1700798, doi:http://dx.doi.org/10.2807/1560-7917.ES.2018.23.33.1700798.
[16] *Enterococcus faecium* cgMLST. Search strains. https://www.cgmlst.org/ncs/schema/991893/searchstrain/?f=ct&v=32. [Accessed 23 August 2020].

[17] Eichel V, Klein S, Bootsveld C, Frank U, Heeg K, Boutin S, et al. Challenges in interpretation of WGS and epidemiological data to investigate nosocomial transmission of vancomycin-resistant *Enterococcus faecium* in an endemic region: incorporation of patient movement network and admission screening. J Antimicrob Chemother 2020;75:1716–21, doi:http://dx.doi.org/10.1093/jac/dkaa122.

[18] Mellmann A, Bletz S, Böking T, Kipp F, Becker K, Schultes A, et al. Real-time genome sequencing of resistant bacteria provides precision infection control in an institutional setting. J Clin Microbiol 2016;54:2874–81, doi:http://dx.doi.org/10.1128/JCM.00790-16.