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*Published in:*
Journal of Medical Microbiology

*DOI:*
10.1099/jmm.0.001731

*Publication date:*
2023

*Document version:*
Final published version

JOURNAL OF MEDICAL MICROBIOLOGY

RESEARCH ARTICLE

Hansen *et al.*, *Journal of Medical Microbiology* 2023;72:001731

DOI 10.1099/jmm.0.001731

MICROBIOLOGY SOCIETY

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# Vancomycin-sensitive *Enterococcus faecium* bacteraemia – hospital transmission and mortality in a Danish University Hospital

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**Abstract**

**Introduction.** The emergence of vancomycin-resistant *Enterococcus faecium* (VREfm) has left the vancomycin-sensitive *E. faecium* (VSEfm) strains almost unnoticed.

**Hypothesis.** Molecular characteristics, hospital transmission patterns and clinical impact of VSEfm have changed, and VSEfm is a predictor of VREfm introduction.

**Aim.** We wanted to do a molecular characterization of VSEfm to identify hospital transmissions and links between VSEfm and VREfm, and to investigate the demographics, treatment and impact on mortality of VSEfm bacteraemia.

**Methodology.** VSEfm and VREfm blood culture isolates from Odense University Hospital, Denmark, from 2015 to 2019 were characterized using whole-genome sequencing and core-genome multilocus sequence typing (cgMLST). Clonal shifts and diversity of the VREfm isolates were compared to the VSEfm isolates. Hospital records were used for clinical data and transmission investigation of VSEfm cases.

**Results.** Six-hundred and thirty VSEfm isolates from 599 patients belonged to 42 sequence types (STs) and 131 complex types (CTs) in several clusters. Multiple types were involved in putative transmission, occurring over the entire period. Twenty-seven VREfm bacteraemia cases were included. No correlation between the VSEfm and VREfm clones was identified. The 30 day mortality was 40%, but only in 6.3% of the cases, VSEfm bacteraemia was the likely cause of death.

**Conclusion.** The molecular types of VSEfm bacteraemia isolates are changing and diverse. No direct correlation between VSEfm and the introduction of VREfm was found, but widespread hospital transmission indicates a presence of risk factors that could facilitate transmission of other micro-organisms as well. VSEfm bacteraemia is rarely the cause of death, indicating that 30 day mortality does not reflect the cause of death.

## INTRODUCTION

*Enterococcus faecium* is a Gram-positive bacterium that comprises a small amount of the human microbiota in the gut [1, 2]. It is found in hospitals all over the world, where it thrives very well in the environment, belonging to the group of hospital-adapted

Received 06 February 2023; Accepted 20 June 2023; Published 12 July 2023

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**Keywords:** bacteraemia; *Enterococcus faecium*; infection control ; mortality; transmission; VSE.

**Abbreviations:** ACVC, arterial or central venous catheter; CC, clonal complex; cgMLST, core-genome MLST; CT, complex type; ICU, Intensive Care Unit; MADS, Microbiology Departments Data System; MLST, multilocus sequence typing; OUH, Odense University Hospital; rMLST, ribosomal MLST; SLC, single-linkage clustering; SNP, single nucleotide polymorphism; ST, sequence type; VREfm, vancomycin-resistant *Enterococcus faecium*; VSEfm, vancomycin-sensitive *Enterococcus faecium*; VVEfm, vancomycin-variable *Enterococcus faecium*; WGS, whole-genome sequencing.

The GenBank/EMBL/DDBJ BioProject accession numbers for the sequences of the *Enterococcus faecium* are PRJEB63070, PRJEB63096 and PRJEB38219.

One supplementary table and other supplementary material are available with the online version of this article.

bacteria with the acronym ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* species) and causing hospital-associated infections [3–5].

Decades ago, *E. faecium* was investigated extensively due to its acquisition of ampicillin-resistance. However, after the widespread introduction of vancomycin resistance, both the ampicillin-resistant and the vancomycin-sensitive *E. faecium* (VSEfm) were almost forgotten [6–12]. In Denmark, only a few per cent of the *E. faecium* isolates have remained susceptible to ampicillin, and the subdivisions of the species are today referred to as vancomycin-sensitive *E. faecium* (VSEfm) and vancomycin-resistant *E. faecium* (VREfm) [13].

By use of multilocus sequence typing (MLST), clonal relatedness of *E. faecium* clones was found to split into clades. In one branch (clade A), the isolates containing ampicillin-resistance were genetically related, and associated with epidemic hospital strains (clade A1) or sporadic human infection strains (clade A2) [12]. In the other branch (clade B), the ampicillin-susceptible isolates represented the commensals, having a high clonal diversity, and a low prevalence in hospitals [10, 12, 14, 15]. Based on molecular investigations, a recent study suggests that all the isolates in clade B should be reclassified as *Enterococcus lactis* [16]. Nowadays, whole-genome sequencing (WGS) is the gold standard for bacterial strain typing supplemented with core-genome MLST (cgMLST) or single nucleotide polymorphism (SNP) analysis. In 2015, de Been *et al*. developed a cgMLST scheme for *E. faecium*, thereby transferring the SNP diversity into a standardized allele system that could overcome the inter-laboratory surveillance exchange [17]. The scheme has been used worldwide, and although mostly for VREfm, it also creates an opportunity to gain new and more detailed information about VSEfm [18].

Furthermore, studies have suggested that VREfm emerge from the circulating VSEfm by transposon gain events [19, 20]. At Odense University Hospital (OUH), Denmark, we detected the first cases of VREfm infection in 2014, and until mid-2018 only sporadic findings were detected. The dominating types were ST80, ST117 and ST203, all harbouring a *vanA* gene. In 2016, the vancomycin-variable *E. faecium* (VVEfm) clone ST1421-CT1134 was detected for the first time in Denmark. This VVEfm was characterized by its containing the *vanA-vanX* gene complex but being phenotypically susceptible to vancomycin [21, 22]. This VVEfm was introduced at OUH in 2018 and caused transmission on a larger scale in the hospital during the following years [13, 23]. Since enterococci thrive in the environment, and transmission has been described to follow the same pathways in hospitals regardless of the susceptibility, it is also of interest to investigate whether VSEfm can be used as an indicator of risk factors that contributes to the spread of VREfm [4, 5, 24, 25]. If so, VREfm transmission can be prevented at an earlier stage by use of infection control measures.

Another important topic to address in relation to the above is the clinical impact of *E. faecium* bacteraemia. Several studies have reported a high 30 day mortality of 24–66% of *E. faecium* bacteraemia [26, 27]. The reported 30 day mortality is different for VREfm (40–56%) and VSEfm (29–32%) bacteraemia, but both are correlated to severe underlying illness [28–30]. This points to these patients having a poor state of health before the onset of infection, which raises the questions of: it is the underlying disease and extensive use of antibiotics that facilitates the growth of *E. faecium*, and whether the patient dies of or with the *E. faecium* bacteraemia. Therefore, we need to investigate the demographics, treatment and impact on mortality of VSEfm bacteraemia, to determine whether VSEfm bacteraemia is an indicator of severe disease rather than the cause of death.

Therefore, we conducted a descriptive study to analyse VSEfm isolated from patients with bloodstream infections at OUH in Denmark, in the period 2015 to 2019, by using cgMLST and hospital records, in order to do: (i) a molecular characterization of the isolates, (ii) an investigation of transmission, (iii) an investigation of prevalence, types and diversity of VSEfm as a predictor for VREfm introduction, and (iv) an investigation of the demographics, treatment and impact on mortality.

## METHODS

### Bacterial isolates

All VSEfm and VREfm isolates detected from blood cultures at the Department of Clinical Microbiology, OUH, Denmark, from January 2015 through December 2019, were included in the study. Isolates were stored at −80 °C and identified by data harvest in the laboratory information system, Microbiology Departments Data System (MADS) (www.madsonline.dk). Consecutive isolates from the same patient were included if there was more than 1 month between the collection dates, in accordance with the case definition of a new bacteraemia episode in the national database, Healthcare-Associated Infections Database (HAIBA) (https://miba.ssi.dk/overvaagningssystemer/haiba/casedefinitioner/bakteriaemi).

Each isolate was cultured on a 5% blood agar plate (SSI Diagnostica) for 48 h at 35 °C. From this agar plate, one colony was chosen and re-cultured on a new 5% blood agar plate and afterwards used for bacterial identification and WGS. Bacterial identification was performed with matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF/MS) (Microflex LT; Bruker Daltonik).

## Susceptibility testing

Susceptibility to vancomycin was tested according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST; www.eucast.org) guidelines. In cases of uncertainty, in-house PCR was applied for detecting the presence of the vancomycin-resistance genes *vanA*, *vanB* and *vanX* [23]. Successively, presence of vancomycin-resistance genes was crosschecked against the WGS data (see below) and for VSEfm used for discarding isolates found false susceptible by the EUCAST susceptibility test or PCR.

## WGS analysis

Genomic DNA was extracted using a MagNA Pure 96 DNA and Viral NA kit (Roche) or Chemagic 360 CMG-1091 (PerkinElmer), and library preparation was performed using a Nextera XT kit (Illumina), according to the manufacturer's instructions. Paired-end fragments of at least 2×150 bp were sequenced on a NextSeq system (Illumina), and quality control, genome assembly (SKESA v. 2.2), detection of resistance genes, as well as species identification, were carried out using the Bifrost pipeline (https://github.com/ssi-dk/bifrost). All the included VSEfm and VREfm isolates were submitted to GenBank (https://www.ncbi.nlm.nih.gov/genbank/). In case of doubt regarding species identification or questionable read-quality parameters, PubMLST – rMLST (ribosomal MLST) (https://pubmlst.org/species-id) was used to define the species.

Clonal complexes (CCs), sequence types (STs) and complex types (CTs) were determined using SeqSphere⁺ software (Ridom) (version 8.3.1,) [31]. Core-genome distance matrices were submitted anonymously to cgMLST.org for isolates with an unknown CT for generating and retrieving the new CT numbers. In case of an unknown ST, the WGS result was submitted to the *Enterococcus faecium* Typing Database (https://pubmlst.org/bigsdb?db=pubmlst_efaecium_seqdef).

Cluster groups of CTs were generated by using a maximum distance threshold at 20 alleles between the nearest isolates with different CTs and named by the lowest ST-CT in the group. Minimum spanning trees and epicurves of CT cluster groups were visualized by use of SeqSphere⁺ software. Minimum spanning trees were created by using the parameter 'pairwise ignoring missing values' and SNP-allele distance matrices with a maximum distance threshold at 20 alleles for the core genome [17].

Local single-linkage clustering (SLC) was calculated in SeqSphere⁺ in order to enhance our study on putative transmissions. We defined the maximum allelic distance for the SLC clusters by investigating the maximum allelic distances in each of the two largest putative transmission episodes from our dataset.

## Clinical impact and transmission

The number of all cause hospitalizations was reported by the Data Section at OUH, and the total number of patients having a blood culture during admission was extracted from MADS. The number of VSEfm bacteraemia episodes was extracted from MADS. Demographic and clinical data were gathered for all patients with VSEfm bacteraemia, with each patient included with the first VSEfm isolate. If the patient had more than one bacteraemia episode, the latest isolate was used for the investigation of correlation between the molecular characteristics and 30 day mortality and cause of death. Gender, age, collection date, requisition ward and data on intravenous or arterial catheters were extracted from MADS, as well as the hospital records (Cambio COSMIC – https://www.cambiogroup.com).

The antibiotic treatment for the individual patient, and the date of death were extracted from the hospital records in June 2022. Because removal or change of arterial or central venous catheters (ACVCs) with a single dose of vancomycin is a treatment strategy at OUH, we extracted these events from the hospital records as well.

Cause of death due to VSEfm for patients who died within 30 days was divided into the groups 'likely', 'possible', 'unlikely' and 'unknown', based on an algorithm developed by the authors. The algorithm required access to the hospital records and can be found in the supplementary material (available with the online version of this article). All cases were investigated by a medical doctor who was a specialist in clinical microbiology. The cases allocated in the possible group were reviewed by a further algorithm by a second medical doctor who was a specialist in clinical microbiology (see the supplementary material). If there were any discrepancies between the doctors' assessments, the worst-case scenario was selected.

Putative transmission was determined by combining the SeqSphere⁺ data with the date of requisition and ward for each patient included in the study. At least two patients with the same CT cluster group and related to the same ward within a month from the VSEfm detection had to be present to register a possible transmission.

## Statistical analysis

The data is described by median, mean and proportions. For each year, the prevalence of the specific CTs and STs was calculated and directly compared. The diversity was calculated for each year as the total number of specific CTs, and directly compared between the years. Chi-square test for contingency tables and Fisher's exact test were used for calculation of statistical significance [32].

**Table 1.** Number of patients admitted to the hospital, blood cultures and VSEfm blood isolate distribution in patients, STs and CTs during the period 2015–2019, at OUH, Denmark

| Characteristic | Year | | | | | |
|---|---|---|---|---|---|---|
| | **2015 (*n*)** | **2016 (*n*)** | **2017 (*n*)** | **2018 (*n*)** | **2019 (*n*)** | **Total (*n*)** |
| No. of hospital admissions | 113560 | 97519 | 95737 | 93322 | 91030 | 491168 |
| No. of admitted patients with a blood culture | 13356 (11.8%) | 13382 (13.7%) | 13958 (14.6%) | 14790 (15.8%) | 14804 (16.3%) | 70290 (14.3%) |
| No. of blood cultured patients with VSE bacteraemia | 133 (1.0%) | 122 (0.9%) | 118 (0.85%) | 117 (0.79%) | 109 (0.74%) | 599 (0.85%) |
| No. of VSE isolates | 137 | 130 | 125 | 123 | 115 | 630 |
| VSE singletons | 10 | 16 | 18 | 21 | 20 | 85 |
| No. of different VSE STs | 11 | 14 | 12 | 15 | 16 | 42 |
| No. of different VSE CTs | 25 | 39 | 38 | 44 | 44 | 129 |
| New VSE STs compared to previous years | – | 7 | 6 | 10 | 8 | – |
| New VSE CTs compared to previous years | – | 28 | 25 | 32 | 19 | – |
| New VSE CT cluster groups compared to previous years | – | 20 | 20 | 30 | 18 | – |
| Most prevalent VSE type (*n*=%) | ST117-CT24 (77=56%) | ST117-CT24 (42=32%) | ST80-CT1160 (43=34%) | ST117-CT1180 (32=26%) | ST117-CT1180 (31=27%) | – |
| Second most prevalent VSE type(s) (*n*=%) | ST192-CT46 (14=10%) | ST80-CT1160 (25=19%) | ST117-CT1180 (17=14%) | ST80-CT1160 (17=14%) | ST80-CT1160 (7) and ST203-CT1513 (7=6%) | – |

# RESULTS

## Isolates

A total of 630 VSEfm isolates from 599 patients in the period 2015 to 2019 was included in the study. The number of VSEfm isolates was stable with 115 to 137 isolates and 109 to 133 patients per year; also when compared to the number of hospital admissions and the number of blood-cultured patients (Table 1). Twenty-six patients were included with more than one isolate, and their isolates were equally distributed in time.

## Molecular characterization

Of the 630 VSEfm isolates, 28 were identified with rMLST as *E. lactis* (Fig. 1). Dividing the isolates into CC groups, 591 (94%) of the isolates belonged to CC17, 14 (2%) to CC94, and for 25 isolates a CC was not identified. All 14 isolates belonging to CC94 and 14 of the isolates without a CC were *E. lactis*. Thirty-three (5.2%) of the VSEfm isolates were susceptible to ampicillin, leaving 94.8% resistant. Four of the *E. lactis* isolates were resistant to ampicillin.

Of the 26 patients with more than one VSEfm isolate, 14 patients had the same ST-CT combination, 3 patients had VSEfm isolates belonging to the same ST but different CT, 1 patient had isolates with a different ST but the same CT, and the 8 remaining patients had isolates with different ST and CT combinations. Forty-two different STs were found with the most frequent being ST80 and ST117, accounting for 76% of the isolates. The isolates were subdivided into 131 CTs of which 70 were singletons (Table S1).

Application of CT cluster groups consisting of five or more isolates resulted in 20 groups, of which 8 consisted of more than one CT, and included a total of 45 CTs (Table 2). Two of the large CT cluster groups (ST117-CT24 and ST117-CT1180) should have been combined according to the method, but we chose to separate them, because the ST-CTs only were connected with a single isolate and a distance of 19 alleles. A diversity with a mean of 14 different STs, 38 CTs and 33 cluster groups each year was found. The mean of new types each year was 7.75 for STs, 26 for CTs and 22 for CT cluster groups (Table 1). The most prevalent types during the whole period were ST117-CT24 (*n*=139), ST80-CT1160 (*n*=94) and ST117-CT1180 (*n*=81). All the dominating types were typically replaced by a new type after 2 to 3 years (Table 2, Fig. 2).

## Transmission

Isolates belonging to at least 7 of the 42 STs and more than 40 of the 131 CTs were involved in putative transmission and occurred during the entire period. Formerly reported STs involved in outbreaks, such as ST17, ST18 and ST192, were retrieved, and involved putative transmissions consisting of 5 to 29 patients (Fig. 1, Table 2).

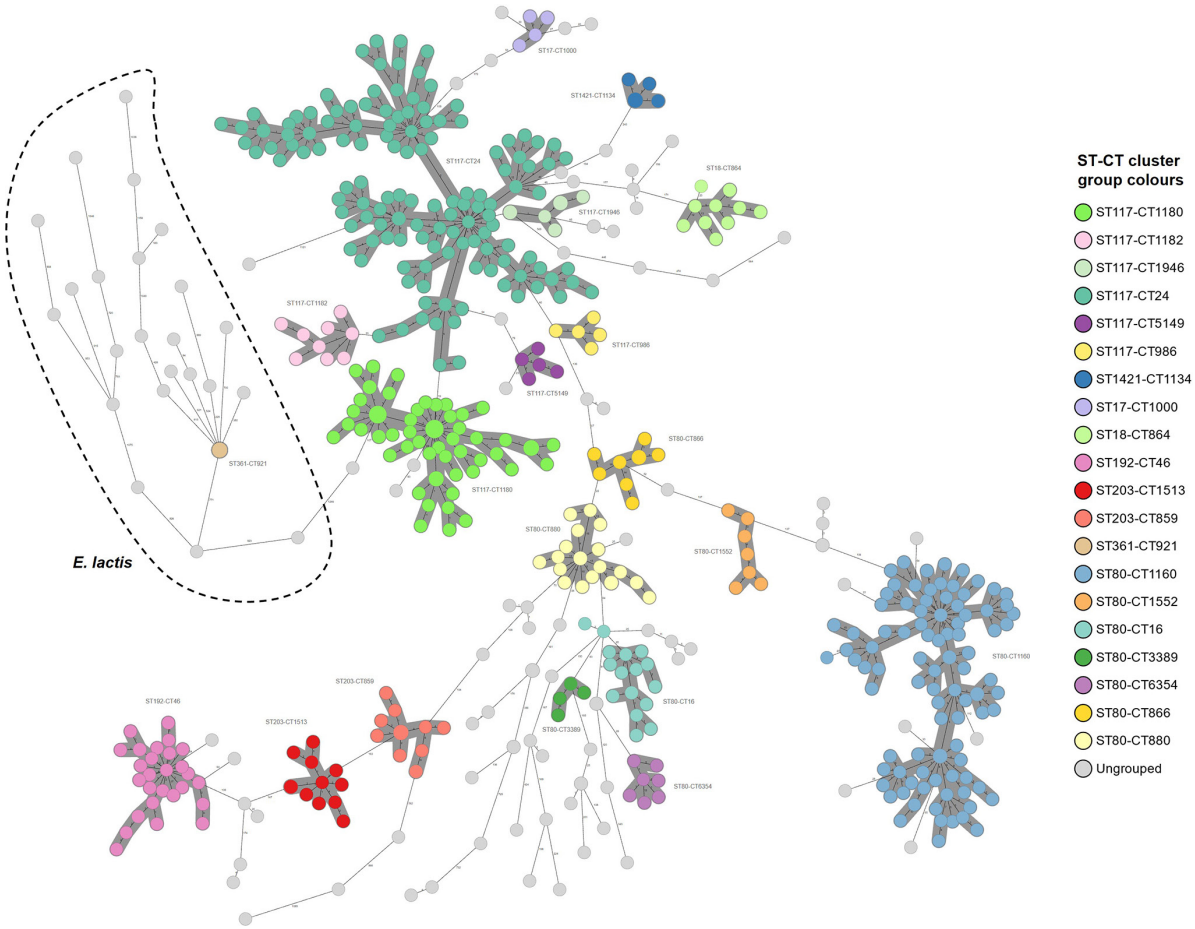


**Fig. 1.** Minimum-spanning tree of VSEfm blood isolates (*n*=630), detected in the period 2015–2019, at OUH, Denmark. The isolates are coloured by ST-CT cluster groups consisting of five or more isolates each.

Putative transmission was most frequent in the Intensive Care Unit (ICU) and the Department of Haematology, OUH, Denmark. The largest putative transmission episode concerned cluster group ST117-CT24 *E. faecium* and involved 150 patients, of which 59 had been in the ICU and 30 at the Department of Haematology. At the ICU, nine putative transmission episodes were registered according to the definition, with the largest involving 21 patients. At the Department of Haematology, five putative transmission episodes were registered, with the largest involving seven patients.

Investigating the maximum allelic distances between any two isolates in each of the two largest putative transmission episodes, we observed a maximum allelic distance at 11, which was applied in SeqSphere⁺ for determining SLC clusters. This resulted in 44 clusters, of which 14 did not belong to a ST-CT cluster. Seven ST-CT clusters contained more than one SLC cluster, and included 17 SLC clusters all together (Table 2).

In 10 of the 44 SLC clusters, two or less departments were involved, and the ICU was represented in 34 of the 44 SLC clusters. In seven cases, the SLC clusters provided a more specific epidemiologic information than by use of the ST-CT cluster information.

## VSEfm and VREfm relatedness

A total of 27 VREfm blood isolates from 27 patients was identified in the period 2015 to 2019. The number of VREfm isolates was distributed with 1 to 5 isolates each year in the period 2015–2018, while 15 isolates were identified in 2019 (Table 3).

All 27 isolates were ampicillin resistant and belonged to CC17. Five different STs and eight CTs were found, with the most frequent being the VVEfm clone ST1421-CT1134 (*n*=15) and 12 of these isolates were detected in 2019.

There was no correlation between the total prevalence, the diversity in STs, CTs or cluster groups of VSEfm during the years with the introduction of VREfm (Table 3). The first VSEfm ST1421-CT1134 blood isolate was detected more than 4 months after

**Table 2.** Distribution of VSEfm-bacteraemia CT cluster groups consisting of five or more isolates, and stratified by STs, CTs and departments during the period 2015–2019, at OUH, Denmark (*n*=630)

Departments: A, ICU1; B, Haematology; C, Gastroenterology; D, Abdominal surgery 1; E, ICU2; F, Infectious diseases; G, Urinary tract diseases; H, Nephrology; I, Abdominal surgery 2; J, Oncology; K, Geriatric diseases.

| CT cluster group | MLST | cgMLST | Single linkage clusters | Year, total no., departments ≥2 isolates (exact no.) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | ST | CT (*n*) | Count | 2015 | 2016 | 2017 | 2018 | 2019 | Total (*n*) |
| ST117-CT24 | ST117 | CT24 (139), CT1487 (1), CT1834 (1), CT2056 (1), CT6351 (2), CT6364 (1), CT6380 (1), CT6382 (3), CT6408 (1) | – | 78 | 43 | 16 | 8 | 5 | 150 |
| | | | 3 | A (35)<br>B (12)<br>C (5)<br>D (5)<br>E (3)<br>F (5) | A (18)<br>B (12)<br>C (5)<br>D (3) | A (4)<br>B (4)<br>D (2–) | B (2) | – | A (59)<br>B (30)<br>C (11)<br>D (10)<br>E (4)<br>F (5) |
| ST192-CT46 | ST192+ST2146 | CT46 (21), CT6389 (1), CT6394 (1), CT1838 (4) ST2146-CT1838 (2) | – | 15 | 11 | 3 | 0 | 0 | 29 |
| | | | 1 | A (8)<br>G (2) | A (8)<br>B (2) | – | – | – | A (16)<br>B (3)<br>C (2)<br>G (2) |
| ST18-CT864 | ST18 | CT864 (9), CT1835 (2), CT6373 (1) | – | 9 | 3 | 0 | 0 | 0 | 12 |
| | | | 2 | A (7) | H (2) | – | – | – | A (7)<br>H (2) |
| ST80-CT866 | ST80 | CT866 (10), CT6369 (2) | – | 6 | 5 | 1 | 0 | 0 | 12 |
| | | | 3 | A (5) | A (3) | – | – | – | A (9) |
| ST361-CT921 | ST361 | CT921 (5) | – | 3 | 2 | 0 | 0 | 0 | 5 |
| | | | 1 | – | A (2) | – | – | – | A (2) |
| ST80-CT16 | ST80 | CT16 (6), CT1840 (11) | – | 2 | 4 | 7 | 1 | 3 | 17 |
| | | | 2 | – | A (2) | A (2)<br>B (2) | – | – | A (5)<br>B (3) |
| ST80-CT880 | ST80 | CT880 (15), CT5907 (4), CT6350 (2), CT6376 (1), CT6384 (1) | – | 5 | 10 | 4 | 3 | 1 | 23 |
| | | | 2 | A (2) | A (2)<br>C (2) | – | A (3) | – | A (7)<br>C (5)<br>I (3) |
| ST17-CT1000 | ST17 | CT1000 (5) | – | 4 | 1 | 0 | 0 | 0 | 5 |
| | | | 1 | B (4) | – | – | – | – | B (4) |
| ST203-CT859 | ST203 | CT859 (15) | – | 1 | 1 | 2 | 6 | 5 | 15 |
| | | | 1 | – | – | A (2) | A (3) | A (3) | A (8)<br>C (2) |
| ST80-CT1160 | ST80 | CT1160 (94), CT2516 (1), CT6342 (1), CT6345 (1) CT6392 (1), CT6415 (1), | – | 2 | 25 | 44 | 18 | 10 | 99 |
| | | | 3 | A (2) | A (13)<br>B (2)<br>G (2) | A (18)<br>B (7)<br>C (5)<br>D (3)<br>E (3)<br>G (2) | A (6)<br>B (4)<br>C (3)<br>J (2) | A (4) | A (43)<br>B (13)<br>C (9)<br>D (5)<br>E (4)<br>G (4)<br>J (2) |
| ST80-CT1552 | ST80+ST2149 | CT1552 (6+1) | – | 0 | 1 | 3 | 2 | 1 | 7 |
| | | | 1 | – | – | – | – | – | K (2) |
| ST117-CT1180* | ST117 | CT1180 (81), CT6398 (2) | – | 0 | 1 | 17 | 34 | 31 | 83 |
| | | | 2 | – | – | A (8)<br>B (5) | A (15)<br>B (11)<br>E (2) | A (15)<br>B (3)<br>C (6)<br>J (3) | A (39)<br>B (19)<br>C (6)<br>E (4)<br>J (4) |

Continued

**Table 2.** Continued

| CT cluster group | MLST | cgMLST | Single linkage clusters | Year, total no., departments ≥2 isolates (exact no.) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | ST | CT (*n*) | Count | 2015 | 2016 | 2017 | 2018 | 2019 | Total (*n*) |
| ST117-CT1182 | ST117 | CT1182 (9) | – | 0 | 1 | 2 | 2 | 4 | 9 |
| | | | 1 | – | – | A (2) | A (2) | – | A (6) |
| ST80-CT6354 | ST80 | CT6354 (7) | – | 0 | 0 | 5 | 1 | 1 | 7 |
| | | | 1 | – | – | B (4) | – | – | A (2)<br>B (5) |
| ST117-CT1946 | ST117 | CT1946 (11) | – | 0 | 0 | 1 | 7 | 3 | 11 |
| | | | 1 | – | – | – | A (6) | A (3) | A (10) |
| ST117-CT986 | ST117 | CT986 (5) | – | 0 | 0 | 1 | 1 | 3 | 5 |
| | | | 1 | – | – | – | – | J (2) | A (2)<br>J (2) |
| ST203-CT1513 | ST203 | CT1513 (14) | – | 0 | 0 | 0 | 7 | 7 | 14 |
| | | | 1 | – | – | – | A (5) | A (3)<br>B (2) | A (8)<br>B (3)<br>I (2) |
| ST1421-CT1134 | ST1421 | CT1134 (9) | – | 0 | 0 | 0 | 3 | 6 | 9 |
| | | | 1 | – | – | – | B (2) | B (6) | B (8) |
| ST80-CT3389 | ST80 | CT3389 (5) | – | 0 | 0 | 0 | 2 | 3 | 5 |
| | | | 1 | – | – | – | A (2) | B (2) | A (3)<br>B (2) |
| ST117-CT5149 | ST117 | CT5149 (5) | – | 0 | 0 | 0 | 1 | 4 | 5 |
| | | | 1 | – | – | – | – | – | A (2) |

*ST117-CT1180 is separated from ST117-CT24 by only 19 alleles.

the first VREfm bacteraemia of the same type. There were too few VREfm blood isolates to investigate for correlation between exchanges of VREfm main types and the exchanges of VSEfm blood isolate types.

## Clinical impact

The included VSEfm bacteraemia patients were distributed equally in number and age each year. The youngest was <1 year and the oldest 99 years of age. The men/women ratio was 1.6, with a mean age of 67.7 years for women and 66.5 years for men, and a median of 69 years for women and 70 years for men. Of the 599 patients included in the study, 95% had one VSEfm bacteraemia episode in the investigation period, 4% had two episodes and 1% three or more. The number of departments with VSEfm bacteraemia patients was 25 out of 37 possible. The yearly affected number of departments was stable and ranged from 17 to 19 each year.

Of the 630 bacteraemia isolates, 297 (47%) were obtained from patients at the ICU, 105 (17%) from the Department of Haematology, 43 (7%) from the Department of Gastroenterology and 30 (5%) from the Department of Abdominal Surgery. The rest of the isolates were found in a variety of departments with less than 20 isolates for each place. The medical departments without the ICU accounted for around 36% of the findings. Of the strains susceptible to ampicillin, 39% were obtained from patients at the ICU, and the rest from a variety of departments.

Almost all STs, CTs or CT cluster groups were represented in patients hospitalized in the ICU or the Department of Haematology. Patients having an *E. lactis* isolate were in half of the cases admitted to the ICU, while the other half of the patients were from seven different wards.

Of the 599 patients, 438 (73%) had one or more arterial or central venous catheters, 160 patients (27%) did not have a catheter, and for 1 patient it was unknown whether a catheter was present or not. Presence of a catheter or not was equally distributed within the ampicillin-susceptible group, and there was no correlation to specific CCs. Of the 438 patients with a catheter, 93% had a blood culture drawn from the catheter, and in 91% of these cases, VSEfm was found in the catheter blood. No significant relation was found between specific CT cluster groups and the presence of a positive catheter blood culture ($P$>0.05).

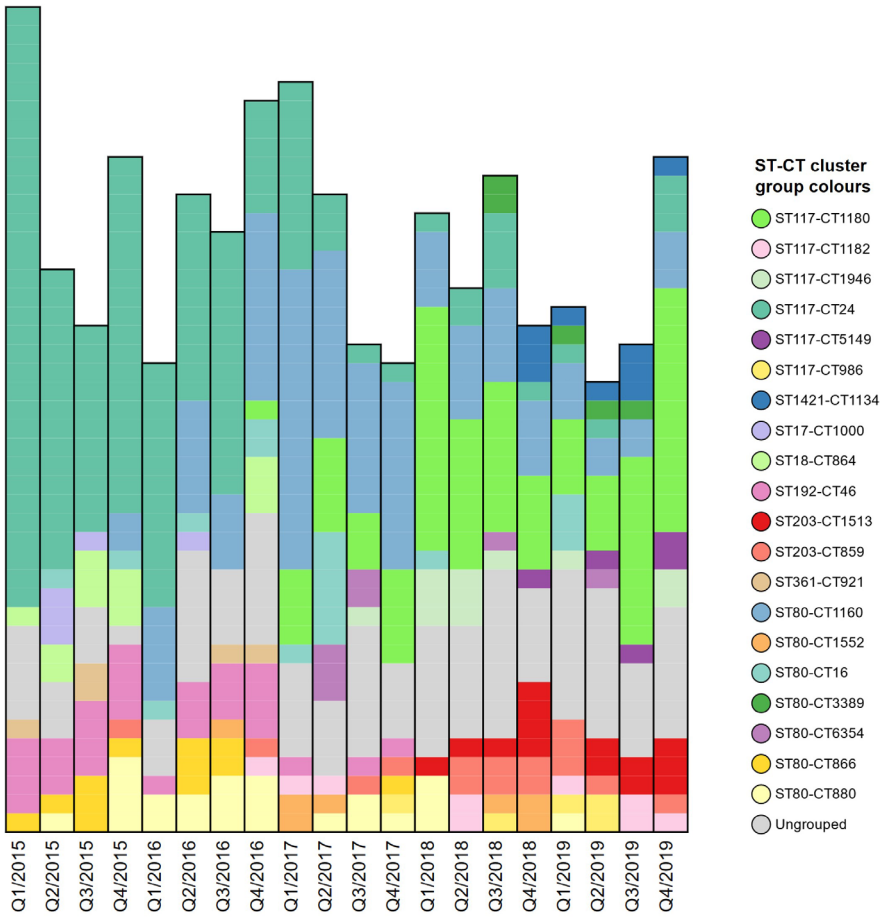


**Fig. 2.** Timeline distribution of VSEfm blood isolates (*n*=630), detected in the period 2015–2019, at OUH, Denmark. The isolates are stratified by ST-CT cluster groups consisting of five or more isolates each, and year of the collection.

Of the 160 patients without a catheter, 33 (21%) did not get any antibiotic treatment for the VSEfm bacteraemia. For patients with a catheter, this amounted to 15%, and a further 11% had the catheter removed or changed without any VSEfm active antibiotics (Table 4). Patients with a catheter, who did not receive antibiotics as a part of the treatment, had a significant ($P$<0.001) lower 30 day mortality if the catheter was changed or removed compared to not removing or changing it. Patients with a catheter who received antibiotics did not have a significant reduction in 30 day mortality if the catheter was removed or changed ($P$>0.5).

**Table 3.** Distribution of blood isolates of VREfm (*n*=27) and the corresponding VSEfm by CT cluster groups, during the period 2015–2019, at OUH, Denmark

| ST-CT cluster | Pathotype | Year | | | | | Total |
|---|---|---|---|---|---|---|---|
| | | 2015 | 2016 | 2017 | 2018 | 2019 | |
| ST80-CT880 | VSEfm/VREfm | 5/1 | 10/0 | 4/0 | 3/0 | 1/0 | 23/1 |
| ST203-CT859 | VSEfm/VSEfm | 1/0 | 1/1 | 2/0 | 6/1 | 5/1 | 15/3 |
| ST80-CT993 | VSEfm/VREfm | 0/0 | 0/2 | 0/0 | 0/1 | 0/0 | 0/3 |
| ST80-CT1545 | VSEfm/VREfm | 0/0 | 0/0 | 0/1 | 0/0 | 0/0 | 0/1 |
| ST1421-CT1134 | VSEfm/VREfm | 0/0 | 0/0 | 0/0 | 3/3 | 6/12 | 9/15 |
| ST80-CT1512 | VSEfm/VREfm | 0/0 | 0/1 | 0/0 | 0/0 | 0/0 | 0/1 |
| ST117-CT991 | VSEfm (CT1182)/VREfm (CT991) | 0/0 | 1/0 | 2/0 | 2/0 | 4/2 | 9/2 |
| ST18-CT1584 | VSEfm/VREfm | 0/0 | 0/0 | 0/1 | 0/0 | 0/0 | 0/1 |

**Table 4.** Treatment, catheter intervention and death of VSEfm-bacteraemia patients in the period 2015–2019 at OUH, Denmark (*n*=599)

| Initial antibiotic treatment | Duration (days) | No. of patients (*n*=599) | Amp$^S$ (*n*=33) | Patients with ACVC (*n*=438) | ACVC changed or removed (*n*=318) | Enterococcal active secondary treatment (*n*=23) | Not dead in the investigation period (*n*=144) | No. of deaths >30 days after the latest VSE bacteraemia episode (*n*=216) | Cause of death for patients dead within 30 days after the latest VSE bacteraemia episode (*n*=238) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Total | Likely | Possible | Unlikely |
| No antibiotic | – | 149* | 8 | 116 | 50 | 0 | 31 | 48 | 69 | 2 | 3 | 64 |
| Unknown | – | 7 | 1 | 5 | 3 | 0 | 1 | 4 | 2 | 0 | 0 | 2 |
| Penicillins† | SD | 2 | 2 | 1 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 2 |
| | ≤1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 2–7 | 3 | 3 | 1 | 1 | 2 | 0 | 2 | 1 | 0 | 0 | 1 |
| | >7 | 4 | 4 | 0 | 0 | 1 | 1 | 3 | 0 | 0 | 0 | 0 |
| | Total | 9 | 9 | 2 | 1 | 3 | 1 | 5 | 3 | 0 | 0 | 3 |
| Vancomycin | SD | 115 | 5 | 111 | 108 | 0 | 32 | 38 | 45 | 1 | 0 | 44 |
| | ≤1 | 11 | 3 | 7 | 6 | 4‡ | 3 | 3 | 5 | 1 | 0 | 4 |
| | 2–7 | 129 | 5 | 87 | 68 | 8§ | 21 | 45 | 63 | 7 | 8 | 48 |
| | >7 | 178 | 2 | 109 | 82 | 8‖ | 55 | 73 | 50¶ | 4 | 7 | 38 |
| | Unknown# | 1 | 0 | 1 | – | – | 0 | 0 | 1 | 0 | 0 | 1 |
| | Total | 434 | 15 | 315 | 264 | 20 | 111 | 159 | 164¶ | 13 | 15 | 135 |
| Total | – | 599 | 33 | 438 | 318 | 23 | 144 | 216 | 238¶ | 15 | 18 | 204 |

Amp$^S$, Ampicillin susceptible; SD, single dose.
*Unknown whether one patient is dead or not.
†Penicillins=ampicillin and/or piperacillin/tazobactam.
‡Three patients were treated with piperacillin/tazobactam (all three isolates Amp$^S$) and one with linezolid.
§All eight was treated with linezolid.
‖Seven were treated with linezolid and one with daptomycin.
¶Unknown cause of death for one patient.
#Patients moved to another hospital.

Neither did we find a significant reduction for patients who had their catheter changed or removed if an antibiotic treatment was added ($P$>0.25).

The distribution of treatment and catheter intervention can be found in Table 4. The overall 30 day mortality was 40% and unrelated to the presence of a catheter, specific STs, CTs and cluster groups. Dividing the patients into age groups of each 10 years, the 30 day mortality rose from age of 40 with the highest mortality (85%) for patients in the group 90–99 years.

Only 15 (6.3%) of the patients died from the VSEfm bacteraemia within 30 days, i.e. VSEfm was the likely cause of death. In 18 (7.6%) of the cases, VSEfm bacteraemia was a possible cause of death, and in 86% cases, the VSEfm bacteraemia was unlikely to have caused death. All the patients with an *E. lactis* bacteraemia belonged to the unlikely group. Eight of the 15 patients with VSEfm as a likely cause of death, died with an isolate belonging to ST80, of which three were in CT cluster group ST80-CT880 and three in ST117-CT1180.

The 15 patients with VSEfm as a likely cause of death were distributed with seven patients in the ICU and the rest in each different department. Of the 18 cases with VSEfm as a possible cause of death, 50% of the patients were admitted in the ICU and 17% in the Department of Haematology. The distribution of 30 day mortality and cause of death can be found in Table 4.

## DISCUSSION

### Molecular characterization

Our molecular investigation found that most of the VSEfm isolates related to the hospital have remained ampicillin resistant and are designated as CC17. All the isolates designated as CC94 were identified as *E. lactis*, which supports the recent findings and explanation of the phylogenetic split into clades found in earlier studies [11, 12, 33]. However, not all the *E. lactis* isolates were found to be susceptible to ampicillin. Due to new classification, *E. lactis* has recently been found included in older studies as *E. faecium*, but clinical practices have been the same for these two species; therefore, the *E. lactis* isolates remained included in our study.

The most frequent STs of VSEfm were ST80 and ST117. Some of the formerly worldwide spread STs, e.g. ST17 and ST78, were also detected, but only in a few patients [2, 9, 11]. We found that a substitution of the dominating VSEfm STs seems to happen every second to third year, which almost applies to the changes in Danish VREfm isolates [22]. Using cgMLST as a typing tool, the isolates were found to be diverse, and with a high rate of CT exchange each year, which might be an indication of the rate of recombination in this species.

### Transmission

Many patients with several VSEfm CT clusters were found to have been involved in putative transmissions during the 5 year investigation period without our knowledge. By using the criteria for molecular relatedness of 20 alleles or less in difference, we found that some CTs were inadvertently grouped together [17]. Combining isolates in CT cluster groups may blur the number of mutations between the different CTs inside the group, raising the question whether all isolates can be assumed connected. For example, we found that two large cluster groups (ST117-CT24 and ST117-CT1180) had been combined according to the method. By using local SLC with a threshold at 11 alleles, we found an increased number of clusters than by using cgMLST, but only in a few putative transmission episodes this implied a more specific epidemiological information. There is a wide discrepancy in the chosen cluster thresholds between studies, and it has been suggested as tight as three alleles for hospital-outbreaks [34]. Our SLC threshold was based on investigating the two largest outbreaks according to our definition of putative transmission, but the allele threshold might have changed if the timespan in our definition was reduced. Reducing the allele threshold may increase the number of sub-clusters, but if the threshold gets too small there is a risk of missing linked patients.

Combining the WGS-based strain typing and analyses of clonal clusters with epidemiological data is necessary to enhance the probability of detecting true transmission, since cluster thresholds or SNP borders cannot be set by a reliable, single number – especially not with a highly recombinant micro-organism such as *E. faecium* [35]. The combination of the molecular and epidemiological results can be used to identify where the transmission might have taken place, saving time and costs in achieving infection control. However, no such system can be complete since ward-move data can be difficult to obtain, transmission might happen outside the ward, and links between patients might be missing [14].

The large number of putative transmissions in our study may be due to the use of the official allele distance threshold for cgMLST clusters, and by using a smaller allele threshold on our data set, the number of patients involved in possible transmissions may be reduced. But still, putative transmissions of VSEfm are found during the entire period. This might be used as an indicator of the presence of risk factors, e.g. sub-optimal hand hygiene and cleaning procedures of utensils [36]. These risk factors could support transmission of VREfm and other micro-organisms as well, since many micro-organisms use the same transmission pathways. Achieving infection control in a hospital is not only a matter of preventing transmission of the most resistant micro-organisms. It should also prevent transmission of bacteria in general, regardless of the resistance profiles. If we prevent transmission of

antibiotic-susceptible clones, we also prevent transmission of the more resistant strains. In this study, only blood isolates were available, which is a limitation in detecting the extent of transmission, but due to the large amount of blood isolates, it is still an indicator of the enterococci flourishing in the hospital.

### VSEfm and VREfm relatedness

We found no correlation between the total prevalence, the prevalence of specific STs, CTs or CT clusters groups of VSEfm and the rise of VREfm or VVEfm in blood isolates (Table 4). The STs and CTs of the included VREfm and VVEfm in this study correspond, in general, to the findings in the rest of Denmark, and an introduction of VREfm and VVEfm into our hospital might be explained by hospital transfers of patients unaware of carrying them [13].

We used cgMLST for investigating relatedness between isolates, but there are a lot of other genetic material in the bacteria that could be relevant to study. Besides plasmids, other known possible transmission links could be horizontal transfer of mobile genetic elements, TN structures or transposons, and it could, therefore, be interesting to investigate for these elements in our isolates, to see whether we can find a connection between the VSEfm and the VREfm detected at OUH [37].

It has previously been discussed whether vancomycin resistance in *E. faecium* arises from an introduction of a resistance mechanism in many different receptive VSEfm types at the same time, or whether the resistance arises in a single clone that afterwards causes a clonal outbreak. A recent study from Ireland has found the *efm* gene to be a possible explanation of introduction of VREfm, supporting the first hypothesis. The study suggests that the spread of VREfm, besides the directly transfer of VREfm isolates between patients, mainly is due to genomic-related vancomycin-sensitive *efm* genes that transfer between *E. faecium* in patients, and afterwards acquire a vancomycin-resistant plasmid [38]. Unfortunately, the study does not describe whether this only concerns isolates found in certain human materials. We did not investigate for the presence of the *efm gene* in our isolates, but we found that ST1421-CT1134 VVEfm – the most dominating clone in Denmark during 2018 –2020 – did appear the same year as the first corresponding VSEfm was found in a blood culture [21]. The VVEfm though appeared months before the corresponding VSEfm ST1421-CT1134, and it was not possible to investigate whether this also applied to isolates from other materials. This could be due to an unknown VVEfm introduction followed by the VVEfm having lost the vancomycin resistance or due to the use of blood isolates only. Therefore, we call for studies investigating clinical isolates from other locations and faecal screening isolates, and the presence of the *efm* gene in those.

### Clinical impact

In our study, we found that the distribution of age and sex of the VSEfm bacteraemia patients corresponds to earlier findings from Denmark and Canada [26, 39]. We found that patients with VSEfm hospital-acquired bacteraemia were admitted to the ICU and medical departments in 47 and 36% of the cases, respectively. In a 10-year-old Danish study, patients with enterococcal hospital-acquired bacteraemia were admitted to the ICU and medical departments in 34.8 and 37.7% of the cases, respectively [39]. The difference in the ICU findings can be due to differences in local ward allocation plans and the bacterial environment of the hospital.

We also found that almost all the detected CTs were represented in the ICU or the Department of Haematology. This was not a surprise, because the patients in these departments often are critically ill and have received broad-spectrum antibiotic treatment for long periods, leaving an environment suitable for antibiotic-resistant micro-organisms. Other departments with a high prevalence were the departments taking care of abdominal diseases, which was expected due to the natural habitat of enterococci.

We found that 25% of the VSEfm bacteraemia patients did not receive any comprehensive enterococcal antibiotic treatment. Furthermore, we found a significant impact of catheter removal or change on reducing the 30 day mortality in patients not treated with antibiotics active against enterococci, which corresponds to results in other studies [40]. This may indicate the ability of *E. faecium* to colonize foreign materials, but the presence of a catheter together with the possibility to change or remove it may also indicate the patient's health condition. This is supported by our findings that the catheter removal or change had no significant impact on the 30 day mortality if the patient received antibiotics active against enterococci at the time of change or removal.

We found that the overall 30 day mortality of VSEfm bacteraemia was 40%, a result similar to another Danish–Dutch study, which found the 30 day mortality for VSEfm at 38% and VREfm at 48% [30]. This is a surprisingly high 30 day mortality compared to bacteraemia from *S. aureus* and *Escherichia coli* with levels for meticillin-sensitive *S. aureus* (MSSA) at 18%, and meticillin-resistant *S. aureus* (MRSA) at 25%, while *Escherichia coli* with an hospital-onset was found at 31% [41, 42]. By investigating the cause of death in detail, we revealed that in only 6.3% of the VSEfm bacteraemia cases was the VSEfm likely to have caused death. Most of the patients had underlying severe illness, which constituted a confounder and resulted in VSEfm appearing to have a greater impact on a fatal outcome than is the case. This divergence may also apply to other species and, therefore, it is important to investigate the actual cause of death including other diseases [43].

## Conclusion

In this study, we found a changing and diverse molecular pattern of VSEfm bacteraemia isolates during a 5 year period. Putative transmission of VSEfm occurred consistently in our hospital, possibly indicating the presence of risk factors, which could support transmission of other micro-organisms as well. The resistant isolates can be considered the tip of the iceberg, and maybe it is time to also have a look at microbes not having a significant resistance profile. We did not find any molecular patterns of VSEfm to predict the introduction of VREfm, which could be due to the use of blood isolates only. With this study, we also demonstrated that VSEfm bacteraemia rarely causes death, i.e. the 30 day mortality does not reflect the actual cause of death, indicating that the 30 day mortality must be interpreted with care.

Funding information
The authors received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Acknowledgements
Elisa Knudsen is thanked for her excellent technical assistance in the laboratory.

Conflicts of interest
The authors declare that there are no conflicts of interest

Ethical statement
The Danish Patients Safety Authority has approved the collection of data from the hospital records (reference no. 3-3013-2554/1).

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