NATIONAL REFERENCE CENTRE FOR ENTEROCOCCI

REPORT 2012-2024

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LIST OF ABBREVIATIONS

cgMLST	Core genome multi-locus sequence typing
CSF	Cerebrospinal fluid
EARS-BE	European Antimicrobial Resistance Surveillance for Belgium
EARS-Net	European Antimicrobial Resistance Surveillance Network
LRE	Linezolid-resistant *Enterococcus*
LVRE	Linezolid-and vancomycin-resistant *Enterococcus*
MDRO	Multidrug-resistant organisms
MIC	Minimum inhibitory concentration
MGE	Mobile genetic element
NRC	National Reference Centre
PTE	Putative transmission event
SKA	Split k-mer analysis
ST	Sequence type
VRE	Vancomycin-resistant *Enterococcus*

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1. INTRODUCTION

This report decribes the activities performed by the National Reference Centre (NRC) for enterococci between 2012 and 2024, including species identification via MALDI-TOF MS, phenotypic susceptibility testing for vancomycin, linezolid and eravacycline via gradient strip and broth microdilution, genotypic resistance determination (PCR for glycopeptide and linezolid resistance genes or whole genome sequencing (WGS)) and typing of strains using WGS, mostly in outbreak settings. Currently, Belgian laboratories are not obligated to submit their vancomycin- or linezolid-resistant strains to the NRC.

2. STRAIN IDENTIFICATION, PATIENT DEMOGRAPHICS AND SAMPLE TYPES

The strains received by the NRC for enterococci are sent mainly for confirmation of glycopeptide resistance. Less common but on the rise are requests for confirmation of linezolid resistance and outbreak investigation of vancomycin-resistant enterococci (VRE).

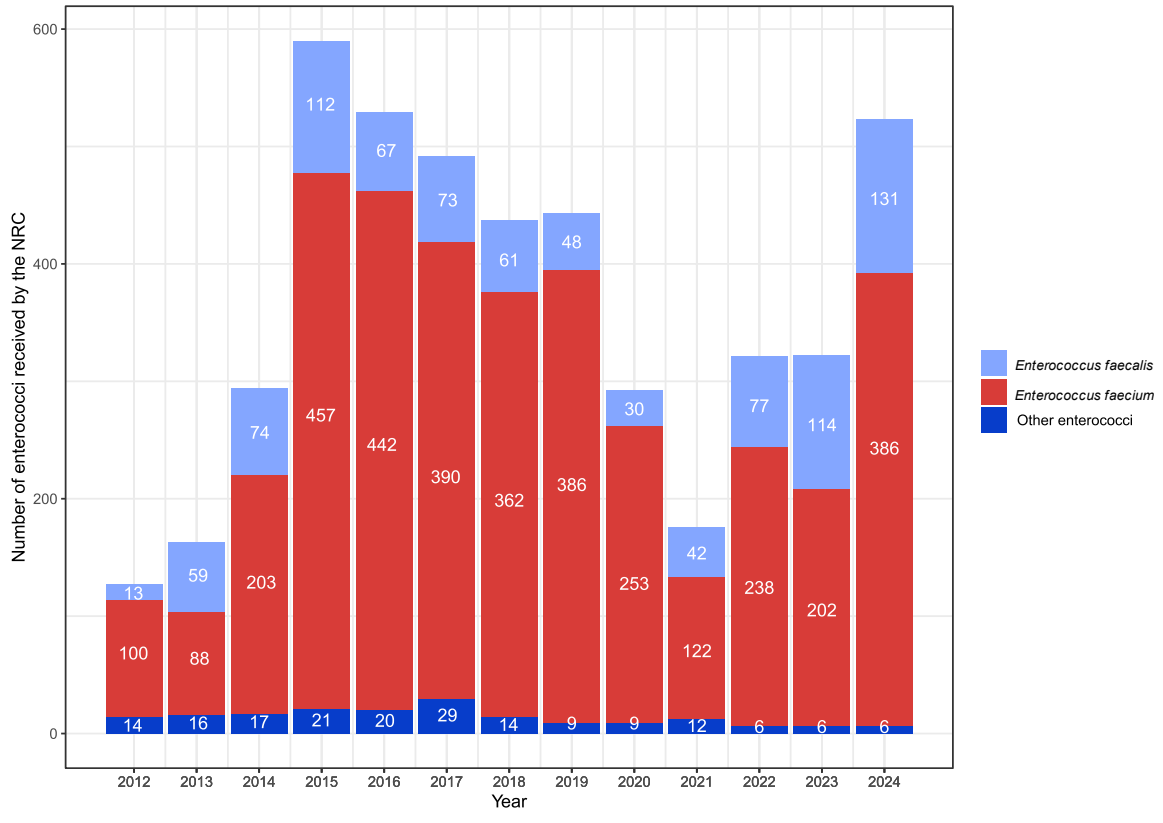


Figure 1: **Number of *Enterococcus* isolates received per year by the National Reference Centre.** Colours indicate *Enterococcus* species with differentiated between *E. faecalis, E. faecium* and other enterococci (*E. avium, E. casseliflavus, E. durans, E. gallinarum, E. gilvus, E. mundtii, E. raffinosus*). NRC: National Reference Centre.

In 2024, 523 *Enterococcus* strains were sent to the NRC. The NRC receives mainly *E. faecalis* and *E. faecium* isolates and only a small portion of other enterococci (*E. avium, E. casseliflavus, E. durans, E. gallinarum, E. gilvus, E. mundtii* and *E. raffinosus*) (**Figure 1**). *E. faecium* predominates the enterococcal strains received by the NRC (55% to 87% of the strains received per year). The proportion of *E. faecalis* fluctuates over the years ranging from 10 to 35%. Other *Enterococcus* species make up 1-11% of the received enterococci.

The total number of *Enterococcus* isolates received varied per year with an increasing number of strains received every year until 2015 and a slow decline thereafter until 2021. According to the European Antimicrobial Resistance Surveillance Network (EARS-Net) data from Belgium, no increasing trend in the number of invasive vancomycin-resistant *E. faecium* was noticed between 2017 and 2021 in Belgium [1]. During the COVID-19 pandemic years (2020-2021), a remarkable lower number of strains was received. The decline could be due to both a lower prevalence of VRE as a consequence of control measures, although not observed in all sending centres, or the inability of laboratories to send strains during the COVID-19 pandemic. The number of laboratories submitting isolates to the NRC was lower in 2020 (n=56) and in 2021 (n=45) compared to pre-COVID (67 in 2019) and post-COVID years (70 in 2022, 67 in 2023 and 71 in 2024). In 2022 and 2023, the number of received strains increased again, however, remained lower compared to the pre-COVID-19 years. This could be due to the lower prevalence of VRE or decreased need of laboratories to confirm resistance or typing in case of outbreaks since sending strains to the NRC is not mandatory. In 2024, the number of received strains increased to levels comparable to pre-pandemic years.
Most enterococci (78%) were collected from patients over 60 years old (**Figure 2**).

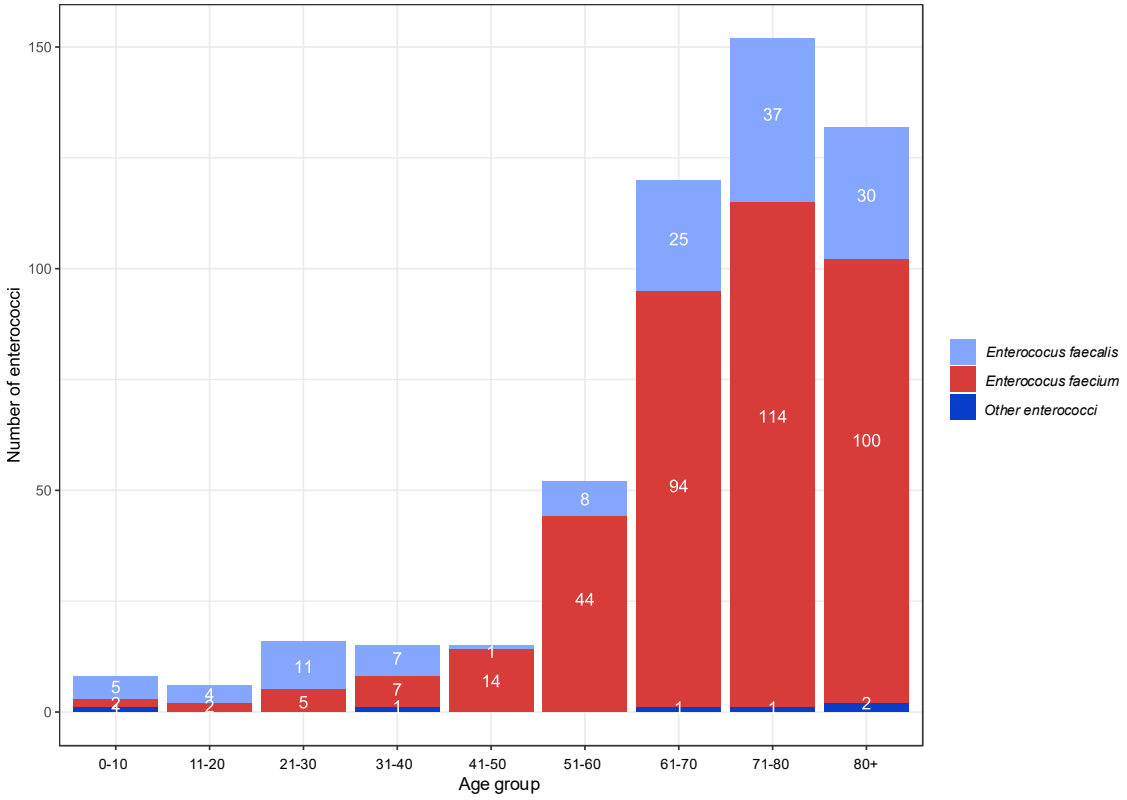
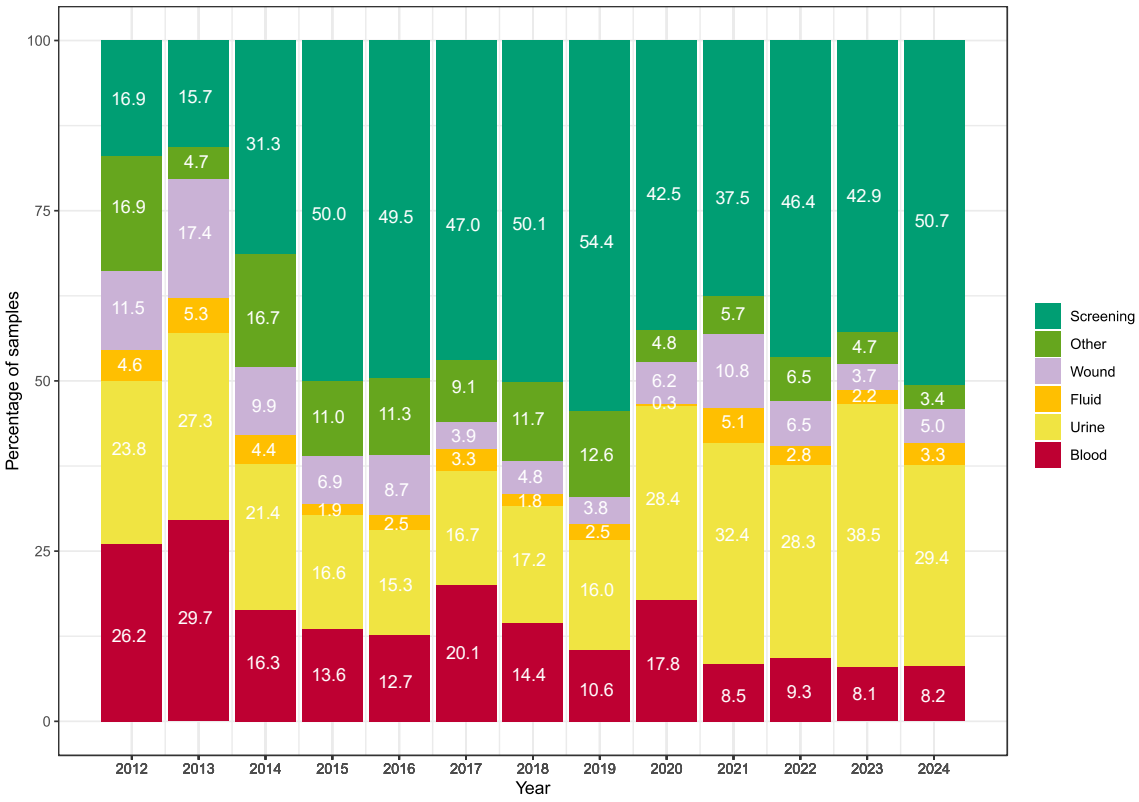


Figure 2: Number of *Enterococcus* isolates per age group in 2024. Colours indicate *Enterococcus* species with differentiated between *E. faecalis, E. faecium* and other enterococci (*E. avium, E. casseliflavus, E. durans, E. gallinarum, E. gilvus, E. mundtii* and *E. raffinosus*).

Enterococci can be isolated from screening and clinical samples (**Figure 3**). In 2024, 49.3% of the isolates were isolated from clinical samples, 50.7% were from screening samples. Clinical isolates originate mostly from urine, followed by blood, wounds, fluids and other sample types. The 'fluid' category includes peritoneal, pleural and ascitic fluid. Other samples consist mainly of respiratory samples, tissue and samples of unknown origin. Screening samples originate mainly from rectal/perianal swabs and faeces. Since 2020, the proportion of enterococci from clinical samples was higher compared to screening samples. Important to note is that screening for VRE in Belgian healthcare facilities is not mandatory [2]. Over the years, the proportion of enterococci isolated from blood decreased, while the proportion of enterococci isolated from urine increased (**Figure 3A**). In 2024, enterococci isolated from urine were sent more frequently to the NRC (n=154) compared to those isolated from blood (n=43) (**Figure 3B**). On the contrary, according to the EARS-NET data, the estimated EU incidence of vancomycin-resistant *E. faecium* bloodstream infections increased with a significantly increasing trend over the period of 2019-2023 [3]. In 2024, the number of screening samples increased again to pre-pandemic levels, probably reflecting putative or confirmed outbreaks.

(A)



(B)

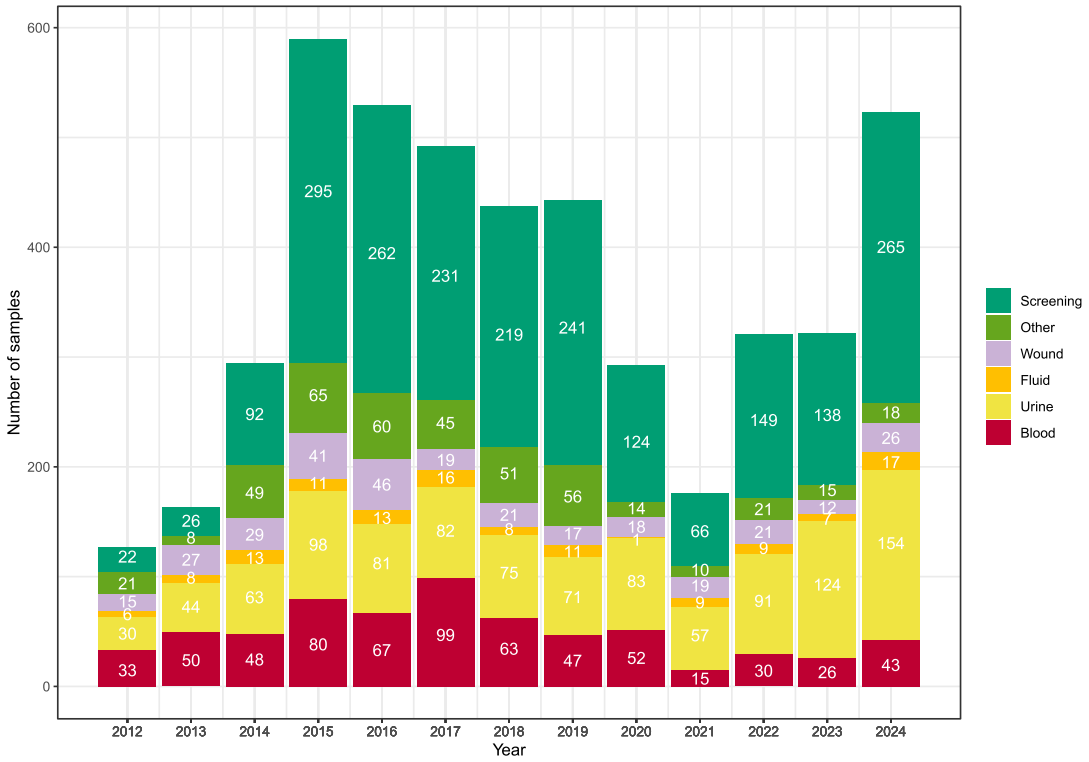


Figure 3: Sample types from which enterococci were isolated per year. (A) Numbers indicate the percentages of enterococci isolated from each sample type. (B) Numbers indicate the absolute number of enterococci isolated from each sample type. The 'fluid' category includes peritoneal, pleural and ascites fluid. Other samples consist mainly of respiratory samples, tissue and samples of unknown origin.

3. GEOGRAPHICAL DISTRIBUTION

The geographical distribution of strains received in 2024 based on the patient's zip codes shows the correlation between number of strains received (**Figure 4A**), the population densities in Belgium (**Figure 4B**) and the number of laboratories sending strains to the NRC (**Figure 4C**). Similar to previous years, the southern part of Belgium is less represented most likely related to the lower number of inhabitants in that area and to the differences in the number of laboratories sending strains.

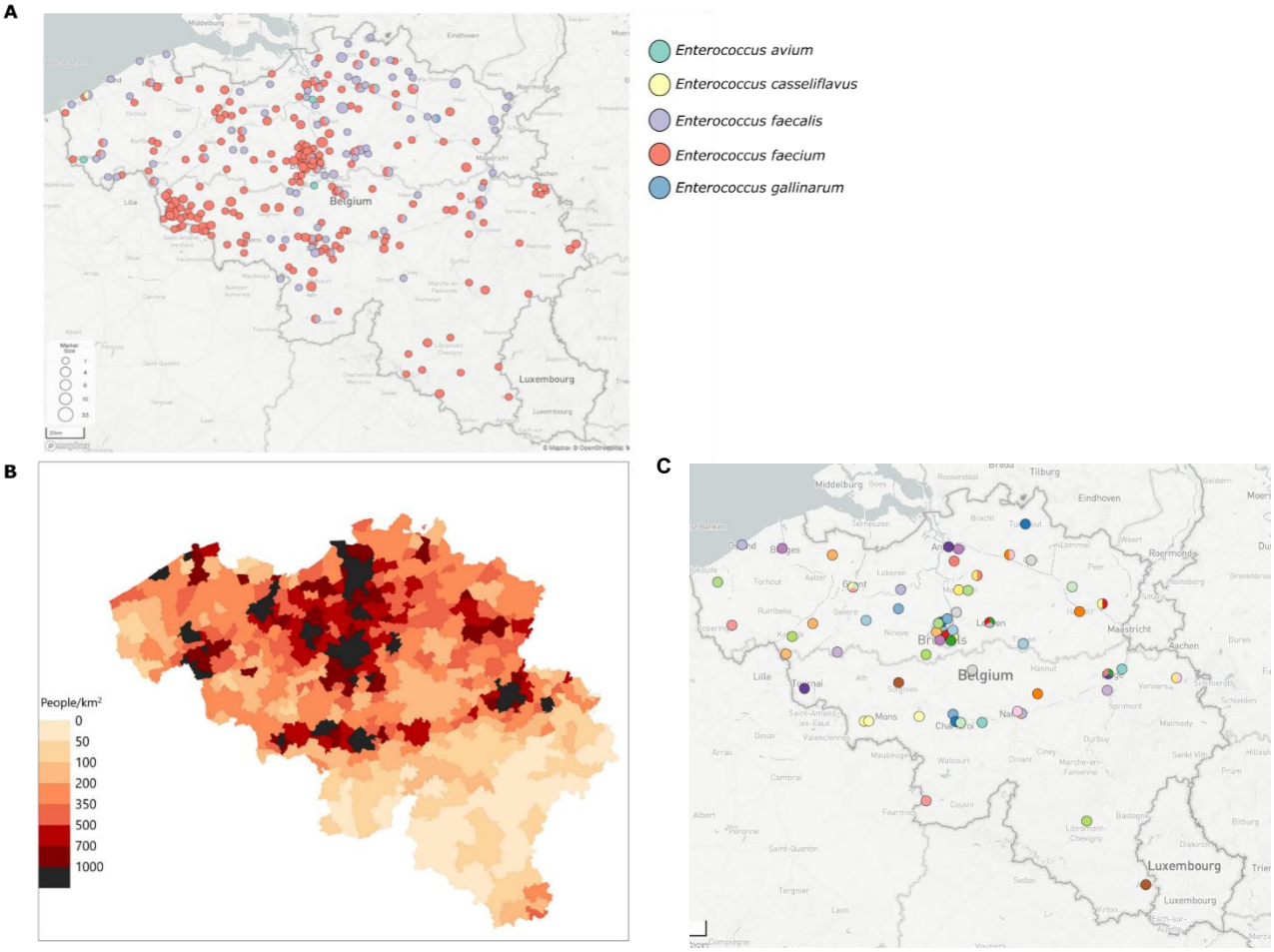


Figure 4: **Geographical distribution of strains received by the National Reference Centre in 2024** based on the patient's residence zip codes (A), the population density in Belgium (B) and the laboratories sending isolates (C). Each dot represents a number of strains as defined by the size of the circle. The distribution maps were generated using Microreact [4]. Population density map was downloaded from: https://nwegeo.com/belgium-map-population-density/.

4. ANTIBIOTIC RESISTANCE IN ENTEROCOCCI

A major challenge in the treatment of enterococcal infections is the intrinsic and acquired antibiotic resistance. Enterococci are intrinsically resistant to cephalosporins, penicillinase-resistant penicillins and low-level resistance to aminoglycosides [5]. In addition, enterococcal genomes show a remarkable adaptability and have the capacity to acquire antimicrobial resistance genes. Acquired resistance to glycopeptides (vancomycin) and linezolid play a pivotal role in these opportunistic pathogens and limit therapeutic options. Enterococci acquiring resistance to vancomycin are important multidrug resistant organisms (MDRO) among gram-positive bacteria. Both clonal spread and exchange of mobile genetic elements (MGEs) play an important role in the dissemination of VRE [6]. According to EARS-Net surveillance data, the occurrence of VRE in Europe increased in the last decade [3]. This increasing trend was not observed by the Belgian NRC nor by the EARS-BE surveillance investigating resistance in a collection of *E. faecium* and *E. faecalis* isolates from blood/cerebrospinal fluid (CSF) and urine [7]. Since 2016, a decreasing trend in the number of VRE received by the NRC was observed (**Figure 5**). On the contrary, an increase in the absolute numbers of linezolid-resistant enterococci (LRE) was shown in the last few years, although this observation might be biased due to increased susceptibility testing for linezolid and increased awareness. The EARS-BE surveillance reports indicate still very low (<2%) resistance to linezolid in clinical *E. faecalis* and *E. faecium* isolates from blood/CSF and urine [8]. To closely monitor linezolid resistance in enterococci, a call for submission of linezolid-resistant enterococci to the NRC was launched on the Sciensano website in September 2022. Linezolid is considered a last resort antibiotic by the World Health Organization (WHO) (AWaRe database [9]). NRCs of the Czech Republic, France and Germany have reported increased linezolid resistance rates in enterococci when analysing pre-selected submitted strains [10-13], while other surveillance studies have not reported increasing trends [14-16]. Yearly, few strains (<10/year) showed resistance to both linezolid and vancomycin (linezolid-and vancomycin-resistant enterococci, LVRE). Combined resistance to linezolid and vancomycin was exclusively observed in *E. faecium* strains.

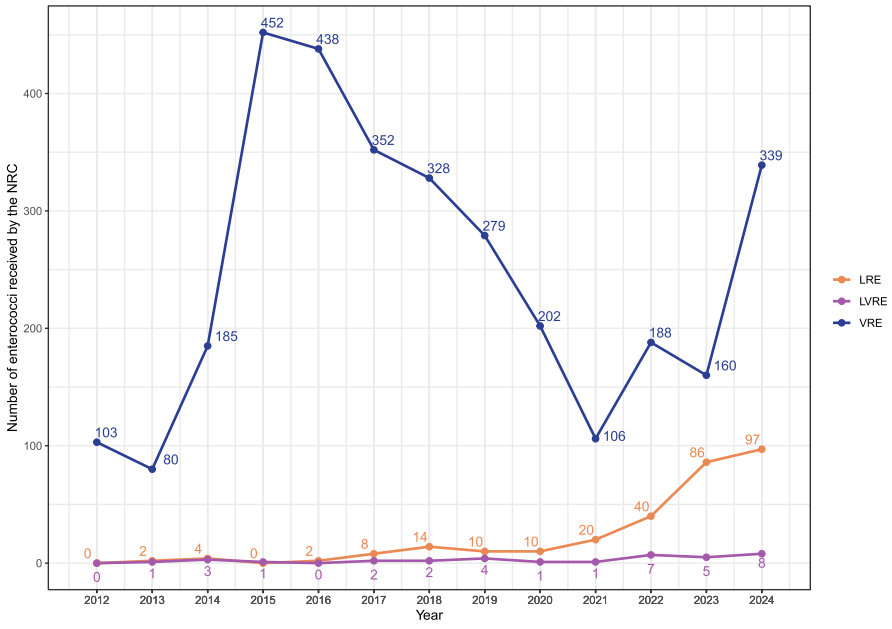


Figure 5: The number of enterococci received by the National Reference Centre between 2012 and 2024 differentiated based on resistance pattern. LRE: linezolid-resistant enterococci, LVRE: linezolid-and vancomycin-resistant enterococci, VRE: vancomycin-resistant enterococci, NRC: national reference centre.

4.1. Glycopeptide resistance

A total of 3247 isolates received by the NRC (2012-2024) were vancomycin resistant. Although vancomycin resistance occurs in *E. faecalis* and other *Enterococcus* species, infections with VRE are mainly attributed to *E. faecium* (**Figure 6**). The number of vancomycin-resistant *E. faecium* increased in 2024 to pre-pandemic levels.

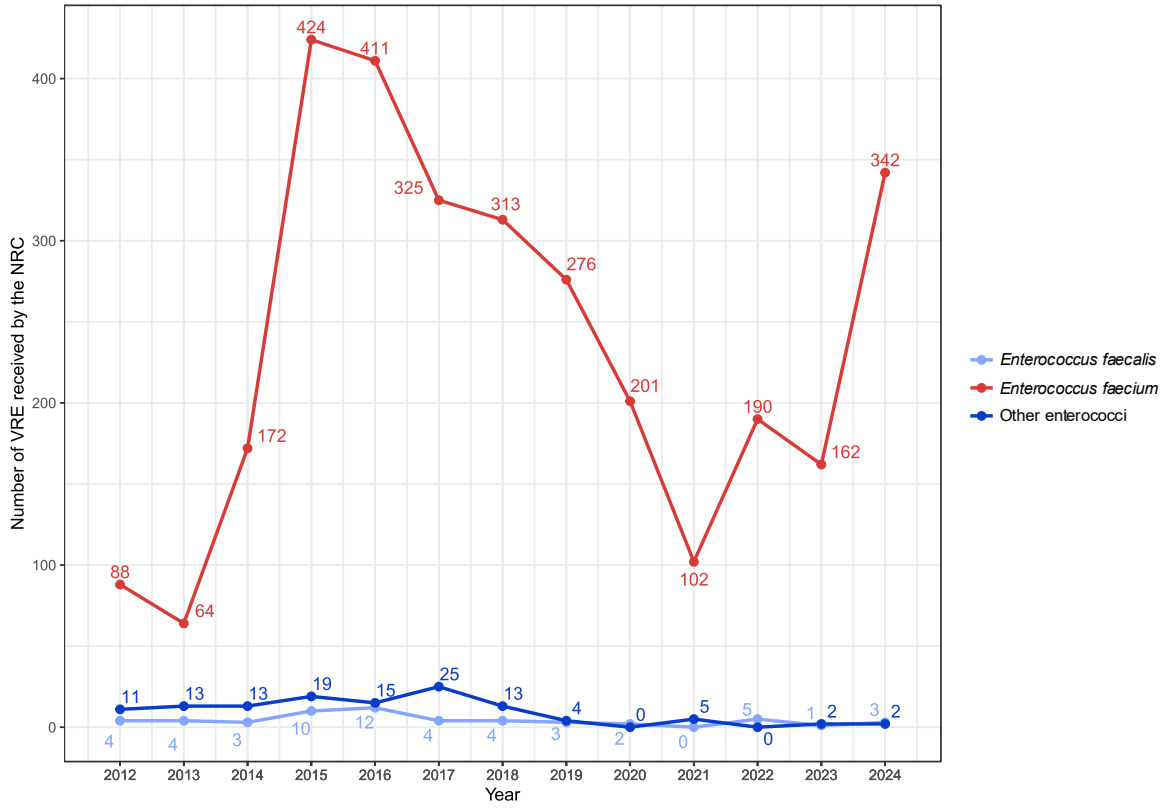


Figure 6: The number of vancomycin-resistant *Enterococcus* received per year by the National Reference Centre differentiated based on identification. Other enterococci include *E. avium, E. gallinarum, E. casseliflavus* and *E. raffinosus*.

Glycopeptide (vancomycin) resistance in enterococci is confirmed by the detection of glycopeptide resistance genes (*van* operons). Firstly, a PCR for the detection of *vanA* and *vanB* is performed. In absence of these genes, phenotypic glycopeptide resistance is confirmed using broth microdilution. In case of confirmation of resistance, WGS is performed to detect other, less common *van* genes (e.g. *vanD, vanE, vanG, vanL, vanM, vanN*). For intrinsically resistant enterococci, such as *E. gallinarum* and *E. casseliflavus* harbouring a *vanC* gene, additional resistance genes are determined in case of MIC values higher than their usual ranges.

The most commonly detected vancomycin resistance gene is *vanA*, both in screening (86% *vanA* in 2024) and clinical samples (80% *vanA* in 2024) (**Figure 7**). Since 2017, the proportion of *vanB* positive strains increased to more than 20% with a peak in 2019-2021 to more than 35%. After which the proportion of *vanB* decreased again to 15% in 2024. *vanC* was detected exclusively in the intrinsically resistant *E. gallinarum* (n=66) and *E. casseliflavus* (n=37). *vanD* was detected in 12 *E. faecium* isolates since 2012 and all originated from screening samples. Other *van* genes, such as *vanP* (n=1, *E. faecium* from urine sample) [17] and *vanG* (n=1, *E. faecium* from screening sample), were rarely detected. Few *Enterocccus* isolates harboured multiple *van* genes (*vanA* combined with *vanB* in 8 *E. faecium*, *vanA* together with the naturally carried *vanC gene* in 1 *E. gallinarum*, *vanB* and intrinsically present *vanC* in 2 *E. gallinarum*).

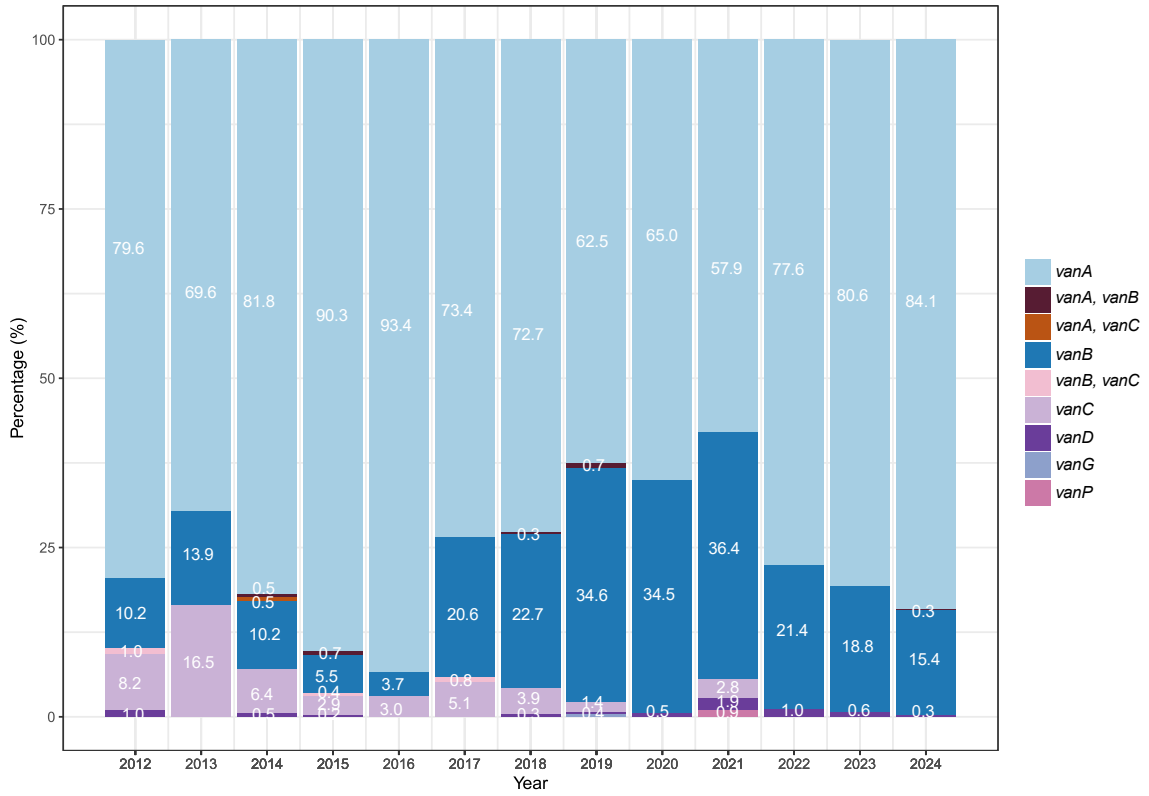


Figure 7: Percentages of vancomycin resistance genes detected in vancomycin-resistant *Enterococcus*.

vanA and *vanB* in *E. faecalis* are associated with vancomycin resistance phenotypes (MIC: 8-≥16 mg/L). Other *van* genes were not detected in *E. faecalis*. In *E. faecium*, the presence of *vanD* was associated with high vancomycin MIC values (≥16 mg/L). *vanP* and *vanG* were associated with vancomycin MICs of 4 mg/L and >16 mg/L, respectively. *vanE, vanF* and *vanH* genes were not detected. Variable vancomycin MIC values were observed for *E. faecium* harbouring *vanA* and *vanB* genes (**Figure 8**). Low-level expression of *vanB* may complicate routine diagnostics of *vanB*-harbouring isolates and may lead to an underestimation of the prevalence of these strains. Additionally, few *vanA*-positive isolates showed a vancomycin-susceptible phenotype possibly representing vancomycin-variable enterococci (VVE). The majority of vancomycin-resistant *E. faecium* isolates carries the *vanA* gene cluster. The gene clusters consist of nine genes with specific functions: transposition (*orf1* and *orf2*), signal transduction controlled by a regulatory two-component system (*vanR* and *vanS*), vancomycin resistance (*vanH*, *vanA*, *vanX*, *vanY*) and teicoplanin resistance (*vanZ*). An intact *vanH/vanA/vanX* cassette is necessary for a vancomycin-resistant phenotype. Presence of mutliple *van* genes (presence of both *vanA* and *vanB* present as confirmed using WGS) was detected in few *E. faecium* isolates (n=6) and were associated with vancomycin MIC of 128 to >256 mg/L.

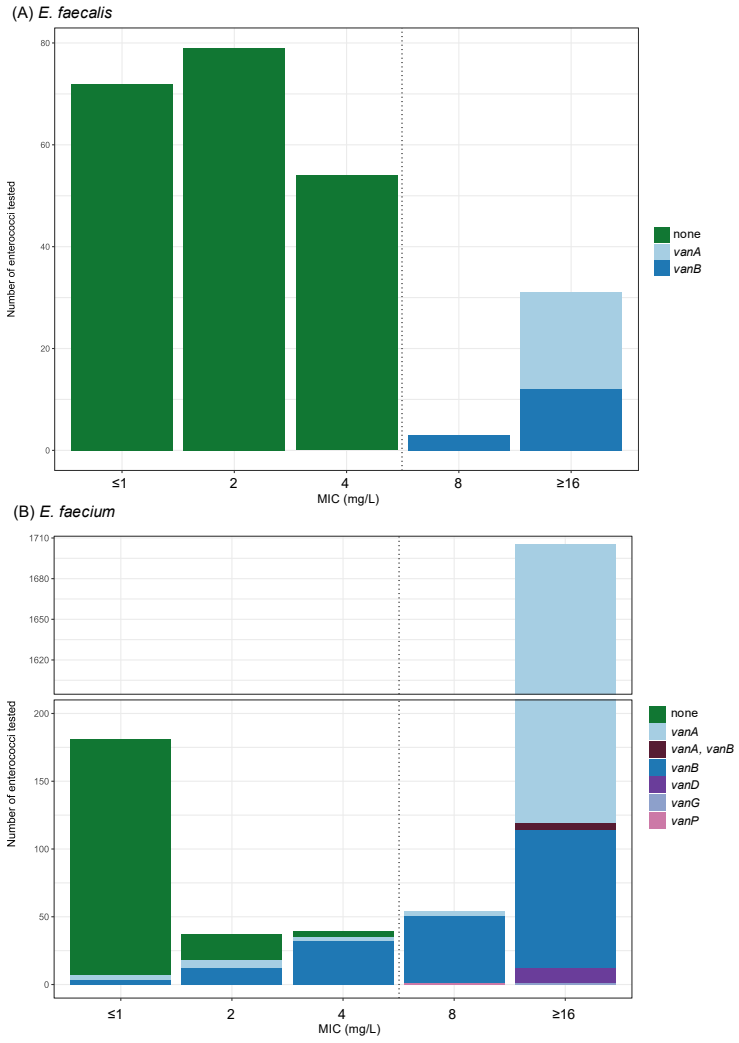


Figure 8: Vancomycin resistance genes and their associated vancomycin minimum inhibitory concentrations (mg/L) in *E. faecalis* (A) and *E. faecium* (B). The dotted vertical line indicates the EUCAST breakpoint for vancomycin.

4.2. Linezolid resistance

To closely monitor linezolid resistance in enterococci, a call for submission of linezolid-resistant enterococci to the NRC was launched on the Sciensano website in September 2022.

Of the 3274 enterococci received by the Belgian NRC between 2013 and 2024 that were phenotypically or genotypically tested, 328 (10%) were linezolid resistant. The majority of LRE isolates were *E. faecalis* (281/328 (86%)), whereas *E. faecium* accounted for 47/328 (14%).

Linezolid resistance is mediated by chromosomal point mutations in the domain V of 23S rRNA alleles or by acquisition of transferable resistance genes (rRNA methylases (*cfr* and its variants) or ABC-F proteins for ribosome protection (*optrA*, *poxtA*) [18].

Linezolid resistance was mediated by mobile resistance genes in *E. faecalis* (e.g. *optrA* (96%) and *poxtA* (4%)), while resistance was mainly encoded by chromosomal mutations in the 23S rDNA alleles (45%) in *E. faecium*. Other linezolid-resistant *E. faecium* harbour *cfr(B)* (7%), *optrA* (16%), *poxtA* (20%) or *poxtA-Ef* (11%).

The concordance between linezolid resistance and the presence of one or more resistance determinants is shown in **Figure 9**.

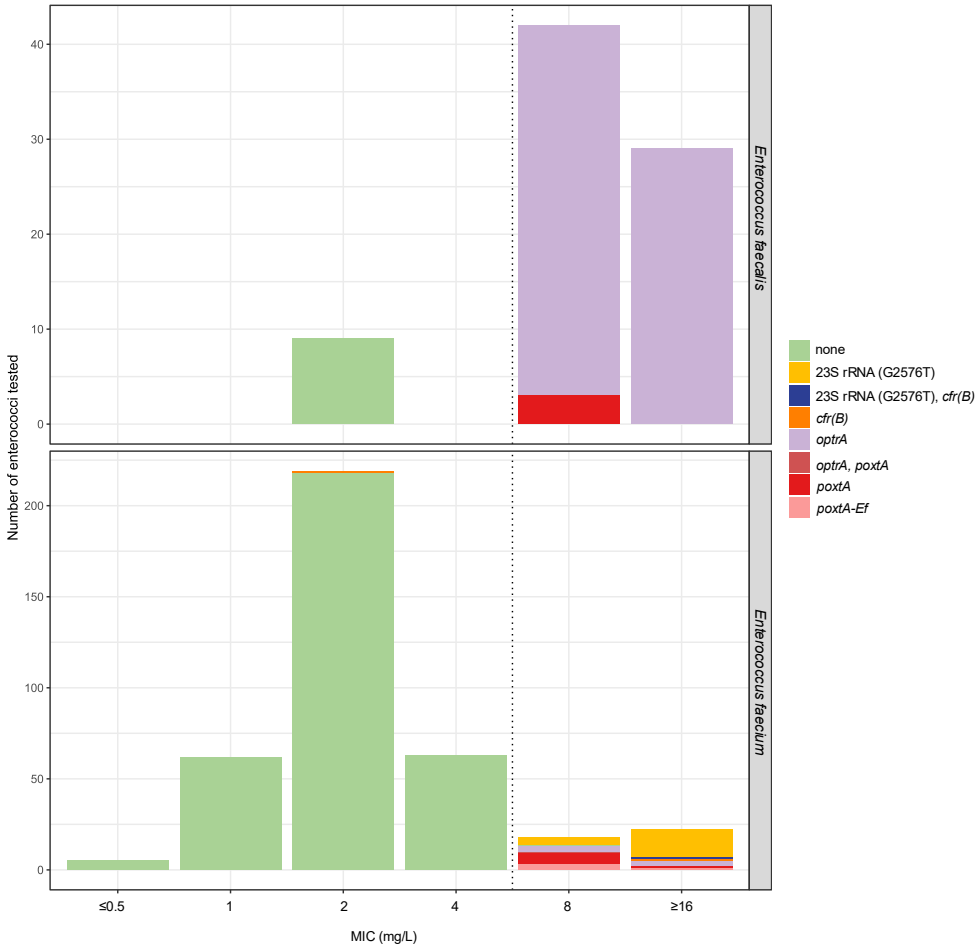


Figure 9: Association between linezolid minimum inhibitory concentration (MIC, mg/L) and resistance determinants. The dotted vertical line indicates the EUCAST breakpoint for linezolid.

4.3. Eravacycline resistance

Infections caused by multidrug-resistant enterococci are a significant clinical challenge. A new antibiotic, Eravacycline, was introduced in Belgium in 2024. Eravacycline is a novel, broad-spectrum synthetic tetracycline class antibiotic suitable for the treatment of complicated intra-abdominal infections in which enterococci might be involved. Eravacycline is active against bacteria harbouring common tetracycline-specific acquired resistance mechanisms (ribosomal protection by *tet(M),* and *tet(Q)* and efflux mediated by *tet(A), tet(B)* and *tet(K)*). Eravacycline binds to the 30S ribosomal subunits, disrupting bacterial protein synthesis [19]. Eravacycline MICs of 45 *E. faecalis* and 159 *E. faecium* were determined using ETEST® (bioMérieux). The majority of the tested isolates were resistant to vancomycin (n=129), linezolid (n=43) or both (n=32). Most enterococci (194/204, 95%) were sensitive to eravacycline. MIC50 was 0.032 mg/L for *E. faecalis* and 0.064 mg/L for *E. faecium*. MIC90 was 0.064 mg/L for *E. faecalis* and 0.25 mg/L for *E. faecium*. One linezolid-resistant *E. faecalis* isolate was borderline resistant with an eravacycline MIC of 0.5 mg/L. Nine vancomycin-resistant *E. faecium* isolates (5.6%) were resistant to eravacycline (MIC=0.5 mg/L (n=8) and MIC=32 mg/L (n=1) (**Figure 10**). In summary, eravacycline is an effective antbiotic against most (multidrug-resistant) enterococci including LVRE, however, eravacycline resistance has been found in a low number of clinical isolates before any clinical usage of this drug and should therefore be monitored closely.

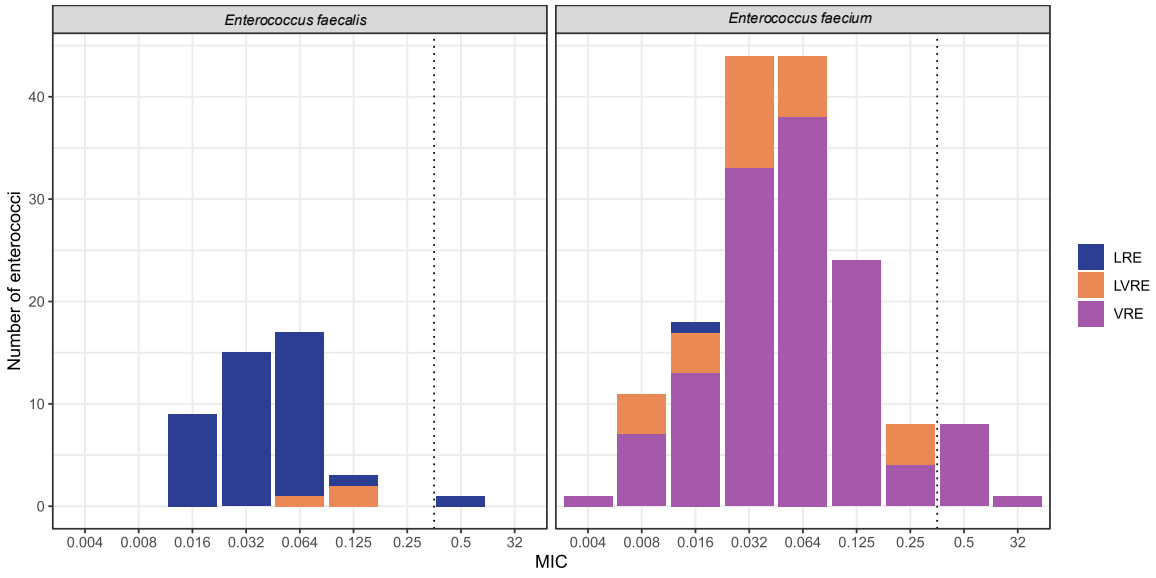


Figure 10: Eravacycline minimum inhibitory concentration (MIC) distributions of enterococci in Belgium, categorized based on linezolid and vancomycin. The EUCAST Eravacycline clinical breakpoint (v15.0) is indicated with a dashed vertical line. LRE: linezolid-resistant *Enterococcus,* LVRE: linezolid-and vancomycin-resistant *Enterococcus*, VRE: vancomycin-resistant *Enterococcus*.

5. STRAIN TYPING AND OUTBREAK INVESTIGATION

The NRC supports Belgian hospitals with VRE outbreak investigation using WGS since 2017. Outbreaks with *E. faecalis* are rare, while outbreaks with *E. faecium* are frequently reported to the NRC. **Figure 11** shows the sequence types (ST) of *E. faecalis* and *E. faecium* from 2013 until 2024. Currently, 2853 *E. faecium* ST and 2040 *E. faecalis* ST are known according to the pubMLST database (July 1, 2025) [20]. However, only a small number of these ST were detected among the hospital-associated strains sent to the NRC, indicating that a few dominant ST reside in the hospital environment in Belgium. For *E. faecalis*, ST480 was dominant between 2016 and 2021. From 2022 onwards, the variety of *E. faecalis* ST increased. For *E. faecium*, dominant ST were ST80, ST612, ST17 and ST117 both before (2017-2019) and after the COVID-19 pandemic (2022-2023). In 2024, 1 *E. faecalis* isolate with ST963 was detected and 14 different STs for 153 *E. faecium* isolates, predominantly ST80 (n=113), ST117 (n=19), ST17 (n=9). We detected 3 novel STs in 2024: ST2789, ST2819 and ST2824. The geographical distribution of *E. faecium* ST detected in 2024 are shown in **Figure 12**. In particular, ST80 was present nationwide in 2024.

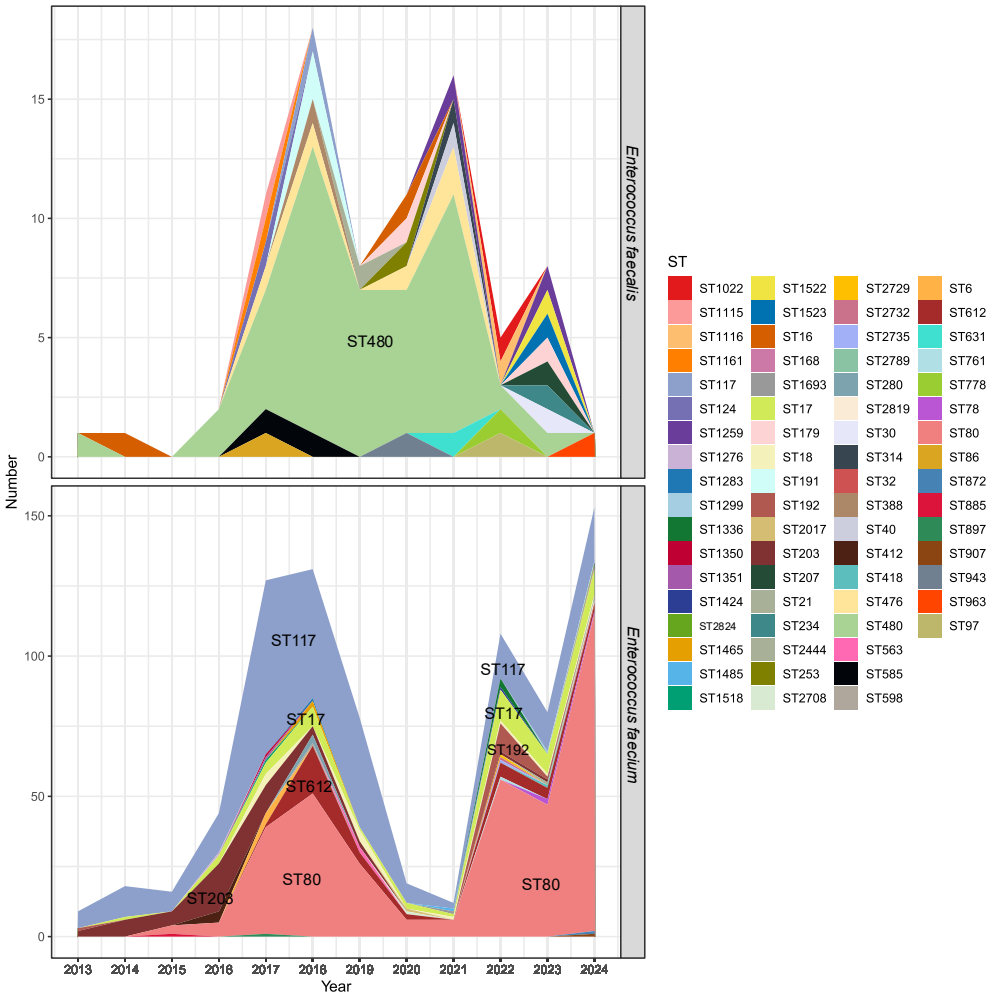


Figure 11: **Occurrence of sequence types (ST) of *E. faecalis* and *E. faecium* from 2013 to 2024 in Belgium.** Numbers indicate the most prevalent sequence types.

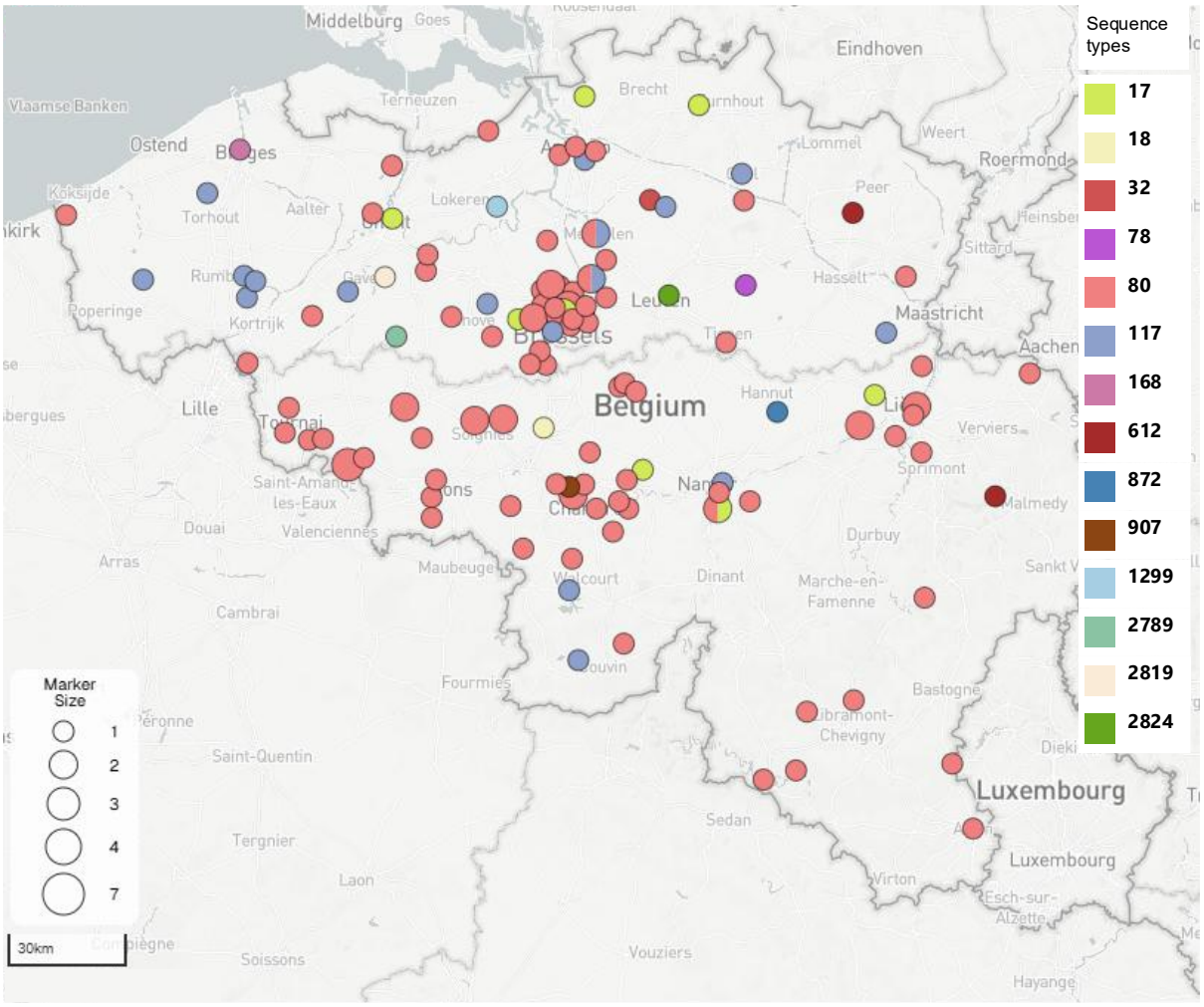


Figure 12: Geographical distribution of sequence types of *E. faecium* in 2024 in Belgium. Each dot represents a number of strains as defined by the size of the circle.

5.1. Genetic relatedness among *Enterococcus* isolates in Belgium in 2024

No outbreaks with vancomycin-resistant *E. faecalis* isolates were reported to the NCR between 2012 and 2024. On the other hand, outbreaks with vancomycin-resistant *E. faecium* isolates are frequently reported. Genetically, *E. faecium* can be subdivided into distinct clades: clade A associated with hospitalized patients and clade B associated with carriage in the community. Upon admission, patients often acquire the predominant hospital clade A [21]. ST17 is regarded as the first globally dispersed hospital-adapted clonal lineage and is the founder of clonal complex 17 (CC17), also known as clonal lineage A1. Many of the dominant *E. faecium* sequence types associated with hospital-associated infections are derived from CC17. Examples of the STs associated with CC17 are ST17, ST18, ST80 and ST203 [22, 23].

This adaptation to the hospital environment of certain STs is also reflected by the dominant STs involved in hospital outbreaks in Belgium (**Figure 12** and **Table 1**). For outbreak analysis, VRE isolates differing in less than 15 core genes were considered closely related [24]. Over the years, the most prevalent *E. faecium* STs in hospital outbreaks were ST80 (*vanA/vanB/vanD*), ST117 (*vanA/vanB*), ST17 (*vanA/vanB*), ST18 (*vanA/vanB*) and ST612 (*vanA*) corresponding to important hospital-associated STs.

During the COVID-19 pandemic in 2020 and 2021, sequencing of outbreak isolates was limited to 12 isolates within 6 different clusters. In 2023, 69 outbreak isolates belonging to 10 clusters were sequenced. In 2024, 58 strains were sent for outbreak analysis, additionally 83 strains were sequenced. Altogether, 13 clusters of related isolates based on cgMLST were detected in 2024 (**Figure 13**). To identify putative transmission events, split k-mer analysis and a threshold of 6 pairwise SNPs were used (**Figure 14**). In 2024, *E. faecium* ST80, ST17 and ST117 were involved in putative transmission events based on cgMLST and SNP analysis. These were the same sequence types that were involved in outbreaks in 2023. The epidemiological landscape of VREfm in Belgian hospitals is dominated by clonal complex 17, particularly *vanA*-harbouring VREfm ST80. Putative transmission events (PTE) mainly occurred within one hospital (14/20 PTE, namely PTE2-3,6,8-11,13-17,19). However, transmission was also probable between hospitals in 7/20 events (PTE1,4-5,7,12,18,20) (**Figure 14**). Transmission events of related isolates among different hospitals highlight possible inter-hospital transmission or community introduction.

In summary, the control of vancomycin-resistant *E. faecium* remains a challenge. Surveillance of outbreaks by using WGS can assist in the control of enterococcal infections and guide infection control interventions. Lack of reporting patient MDRO status across hospitals and mandatory sending of outbreak strains to the NRC possibly hinders VREfm control in Belgium. Broader epidemiological surveillance and enhanced inter-hospital collaboration are essential for effective prevention strategies.

Table 1: Clusters of related *E. faecium* isolates in Belgium in 2024. Clusters indicate genetically related isolates with ≤15 alllelic differences based on a cgMLST scheme with 1312 loci (Figure 13). The SNP distance was determined using split k-mer analysis (SKA). Putative transmission events were defined by ≤6 pairwise SNP differences between isolates. Isolates and hospitals involved in putative transmission events (PTE) can be observed in Figure 14. Probable transmission events involve clusters of closely related isolates, although the isolates have a genetic distance above the threshold of 6 pairwise SNPs.

cgMLST cluster	Nr isolates	Nr hospitals	Collection date first isolate	Collection date last isolate	MLST	*van* gene	Allelic difference [min-max]	SNP distance [min-max]	Putative transmission events (PTE)
1	2	2	13/02/2024	13/09/2024	ST80	*vanA*	3	279	No
2	9	5	30/1/2024	01/08/2024	ST80	*vanB*	0-11	0-24	PTE1-2
3	4	1	21/05/2024	25/06/2024	ST80	*vanA*	0	0-1	PTE3
4	2	2	08/04/2024	26/11/2024	ST80	*vanA*	8	12	Probable
5	6	2	21/05/2024	25/06/2024	ST80	*vanA*	0-2	1-7	PTE4
6	46	15	03/01/2024	27/12/2024	ST80	*vanA*	0-18	0-2182	PTE5-14
7	6	2	08/04/2024	23/04/2024	ST80	*vanB*	1-9	1-30	PTE15
8	2	2	04/06/2024	27/06/2024	ST117	*vanB*	3	7	Probable
9	8	5	10/01/2024	15/10/2024	ST117	*vanA/ vanB*	0-17	1-213	PTE16
10	5	3	03/01/2024	19/03/2024	ST17	*vanA/ vanB/ vanD*	0-8	0-33	PTE17
11	17	5	08/02/2024	15/10/2024	ST80	*vanA*	0-4	0-19	PTE18-19
12	2	2	11/01/2024	17/05/2024	ST17	*vanA*	2	5	PTE20
13	2	2	28/03/2024	08/11/2024	ST612	*vanA*	3	8	Probable

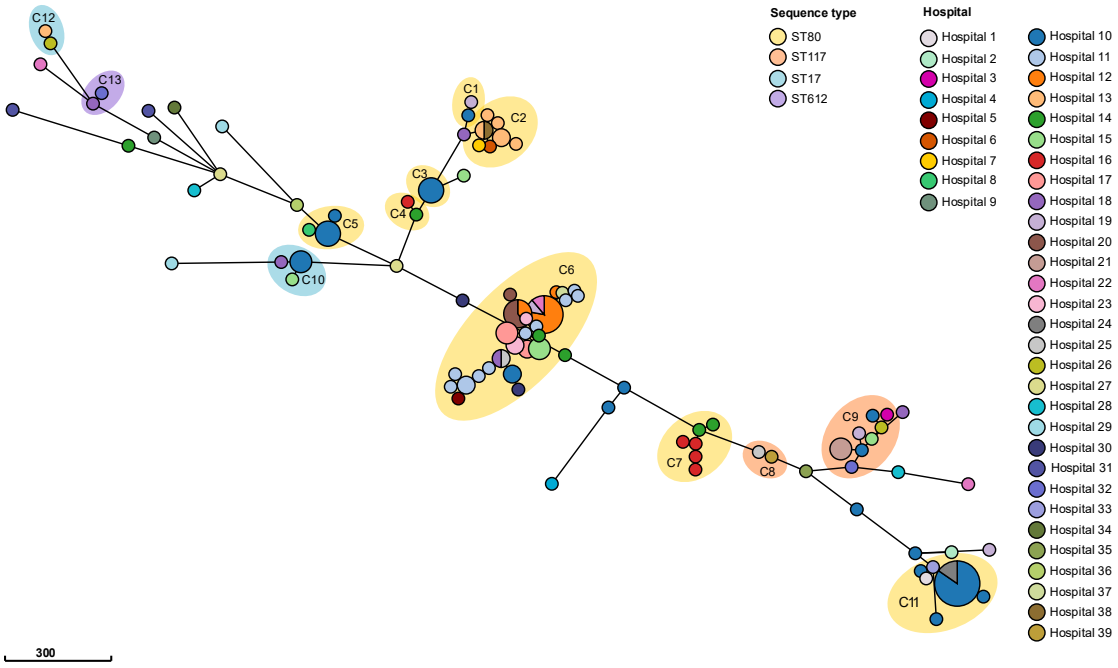


Figure 13: Minimum spanning tree of *E. faecium* isolates sequenced by the Belgian National Reference Centre in 2024. Minimum spanning trees based on allelic distances of cgMLST profile data (1315 loci). Branch lengths indicate the allelic distance as indicated by the tree scale. Collapsed nodes indicate core-genome genetically identical isolates. Clusters (C1-C13) indicate genetically related isolates with ≤15 alllelic differences. The sequence type is indicated for each cluster of related isolates. The hospital of isolation is shown as colored nodes for each isolate. Figure was generated using Grapetree [25].

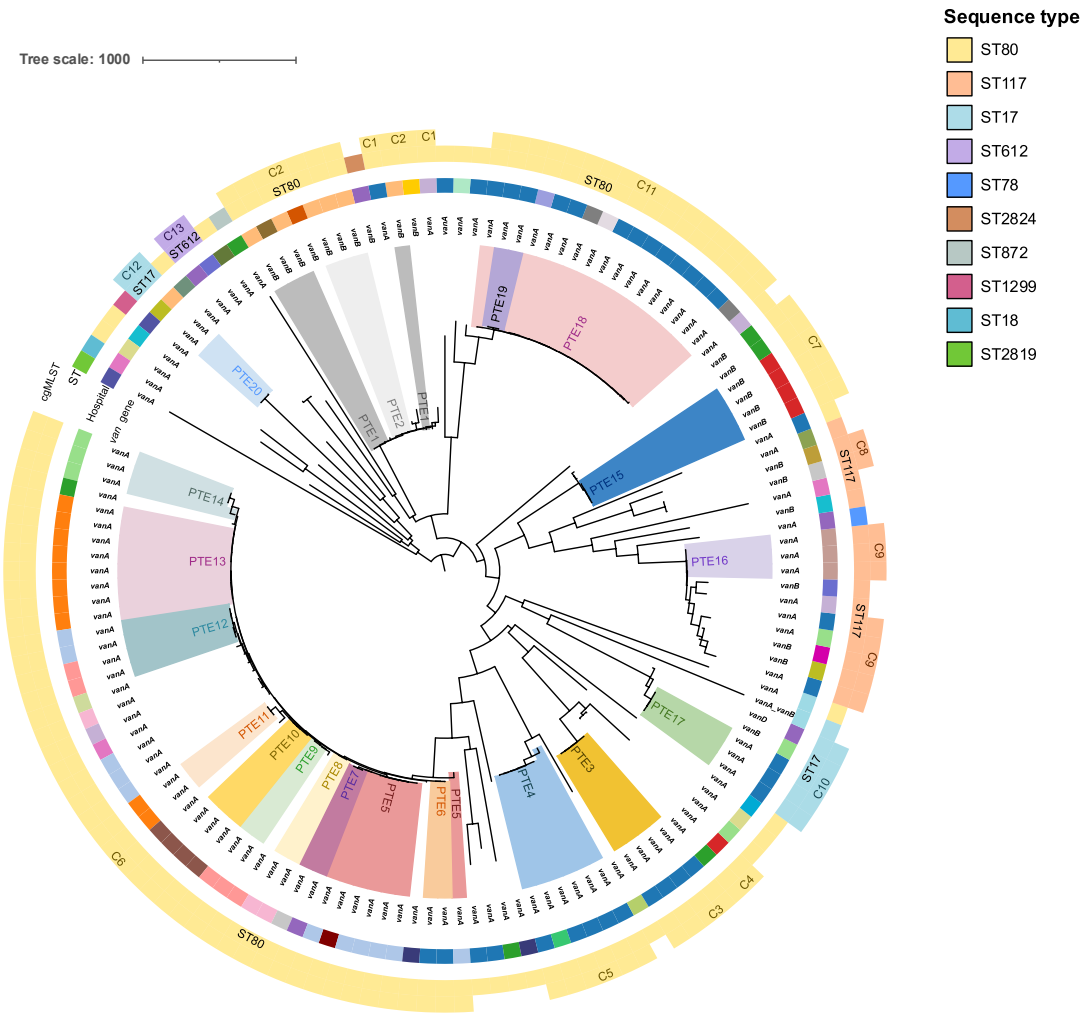


Figure 14: Neighbor joining tree of *E. faecium* isolates sequenced by the Belgian National Reference Centre in 2024. Midpoint rooted neighbour joining tree for 141 *E. faecium* isolates based on split k-mer analysis. Metadata is shown in circles around the tree. The inner ring shows the vancomycin resistance gene. The middle ring show the hospital of isolation. The outer ring indicates the sequence type based on the general pubMLST scheme and the cgMLST clusters (≤15 alllelic differences based on a scheme of 1315 loci). Putative transmission events (PTE1-PTE20) were identified by split k-mer analysis (pairwise SNP distance ≤6 SNPs and are show as colored ranges. The figure was annotated using iTOL [26].

6. SUMMARY

This report presents the data of the **NRC for enterococci in Belgium** from 2012 until 2024. In 2024, a total of 523 enterococci were received. The **dominant species** was *E. faecium* followed by *E. faecalis*. Most enterococci (78%) were collected from patients over 60 years old. A total of 49.7% of the isolates were isolated from clinical samples, 50.3% were from screening samples. Clinical isolates originated mostly from **urine**, followed by blood, wounds, fluids and other sample types.

Acquired resistance to glycopeptides (vancomycin-resistant enterococci, VRE) and linezolid (linezolid-resistant enterococci, LRE) limit the therapeutic options to treat enterococcal infections. Since 2016, we observed a declining trend in the number of VRE isolates received, however, this trend reversed with an increase noted in 2024. An increase in the absolute numbers of linezolid-resistant enterococci (LRE) was observed in the last few years, although a bias is possibly due to increased diagnostic testing and increased awareness. Few strains are resistant to both vancomycin and linezolid (LVRE). In 2024, 339 VRE, 97 LRE and 8 LVRE were sent to the NRC.
Vancomycin resistance is mediated by acquired *van* genes, mainly *vanA* (84.1% of VRE in 2024), followed by *vanB* (15.4% of VRE in 2024).
In Belgium, 10% (n=328) of the enterococci were resistant to linezolid. **Linezolid resistance** was detected mainly in *E. faecalis* (281/759) rather than *E. faecium* (47/2397). The molecular mechanisms linked to resistance differed between both species. Linezolid resistance in *E. faecalis* was mediated by mobile resistance genes (*optrA* (96%) and *poxtA* (4%)), while resistance was mainly encoded by chromosomal mutations in the 23S rDNA alleles (45%) in *E. faecium* and is to a lesser extend mediated by *cfr(B)* (7%), *optrA* (16%), *poxtA* (20%) or *poxtA-Ef* (11%).
A selection of LRE, VRE and LVRE were tested for eravacycline using Etest. Most enterococci (194/204, 95%) were sensitive to eravacycline. **Eravacycline resistance** was detected in one linezolid-resistant *E. faecalis* isolate with an eravacycline MIC of 0.5 mg/L. Nine vancomycin-resistant *E. faecium* isolates (5.6%) were resistant to eravacycline (MIC=0.5 mg/L (n=8) and MIC=32 mg/L (n=1). In summary, eravacycline is an effective antibiotic against most (multidrug-resistant) enterococci, including LVRE. However, eravacycline resistance has been found in a low number of clinical isolates before its introduction in the clinical and will therefore need close monitoring.

Hospital outbreaks are dominated by hospital-associated vancomycin-resistant *E. faecium* strains. The most prevalent *E. faecium* sequence types in hospital outbreaks were ST80 (*vanA/vanB/vanD*), ST117 (*vanA/vanB*), and ST17 (*vanA/vanB*). In summary, the risk of outbreaks with vancomycin-resistant *E. faecium* remains present. Adherence to recommendations for preventing the spread of vancomycin-resistant isolates and hospital infection control practices are of utmost importance. Surveillance of outbreaks by using WGS can assist in the control of enterococcal infections and guide infection control interventions.

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8. RECENT NRC PUBLICATIONS

Oral/ePoster/Poster presentations:

1. **Loens K., Xavier Basil B., Coppens J., Matheeussen V., Malhotra-Kumar S., Goossens H.**. Clonal spread of vancomycin resistant *E. faecium* ST80, ST117 and ST203 across Belgian hospitals. 29th ECCMID, 13-16/04/2019, Amsterdam, The Netherlands, oral presentation.
2. **Matheeussen V**. VRE detection: results of 6 consecutive years of QCMD external quality assessment. QCMD International Advisory Board, 23-24/10/2019, Glasgow, Schotland, oral presentation.
3. **van Kleef-van Koeveringe MS**, **Vande Sande F, Mermans I, Jansens H, Goossens H, Matheeussen V**, Performance of gradient strip tests for detection of vancomycin resistance in Enterococci. 2022. Sciensano meeting, Brussels, Belgium, poster presentation.
4. **De Koster S, van Kleef-van Koeveringe MS**, **Mermans I, Matheeussen V**, The molecular mechanisms of eravacycline resistance in linezolid-and vancomycin-resistant enterococci in Belgium. 2024. EMMD, Noorwijk, the Netherlands, poster presentation.
5. **De Koster S, van Kleef-van Koeveringe MS, Jansens H, Vandamme S, Demuyser T, Vanden Driessche M, Glupczynski Y, Malhotra-Kumar S, Matheeussen V,** Molecular epidemiology and transmission dynamics of vancomycin-resistant Enterococcus faecium in Belgian hospitals (2022-2023). 2025. ESCMID Global, Vienna, Austria, eposter flash session presentation.

Peer reviewed papers:

1. **Mortelé O., van Kleef-van Koeveringe S., Vandamme S., Jansens H.**, **Goossens H., Matheeussen V.** Epidemiology, resistance mechanisms and genetic diversity of linezolid resistant enterococci isolates in Belgium from 2013 to 2021. J Glob Antimicrob Resist. 38:21-26. (2024). doi: 10.1016/j.jgar.2024.04.010.
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