

REPORT 2012 - 2022

NATIONAL REFERENCE CENTRE FOR ENTEROCOCCI

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This report describes the activities performed by the National Reference Centre for Enterococci during the period 2012 – 2022, including species identification via MALDI-TOF MS, phenotypic susceptibility determination via gradient strip and microdilution, genotypic resistance determination (PCR for glycopeptide and linezolid resistance genes or WGS) and typing of strains (via PFGE and later WGS, mostly in outbreak settings).

1. STRAIN IDENTIFICATION

The strains received at the National Reference Centre for Enterococci are sent for various reasons, but mainly for confirmation of glycopeptide resistance. Less common requests are for confirmation of linezolid resistance (linezolid resistant enterococci, LRE) and for vancomycin resistant enterococcus (VRE) outbreak investigation. The total number of strains received per year is shown in Figure 1 with a differentiation between *E. faecalis*, *E. faecium* and other enterococcus species (*E. avium*, *E. casseliflavus*, *E. gallinarum*, *E. malodoratus* and *E. raffinosus*). As visualised in figure 1 *E. faecium* predominates the enterococcal strains received at the NRC. The proportion of *E. faecalis* fluctuates over the years ranging from 9-25%.

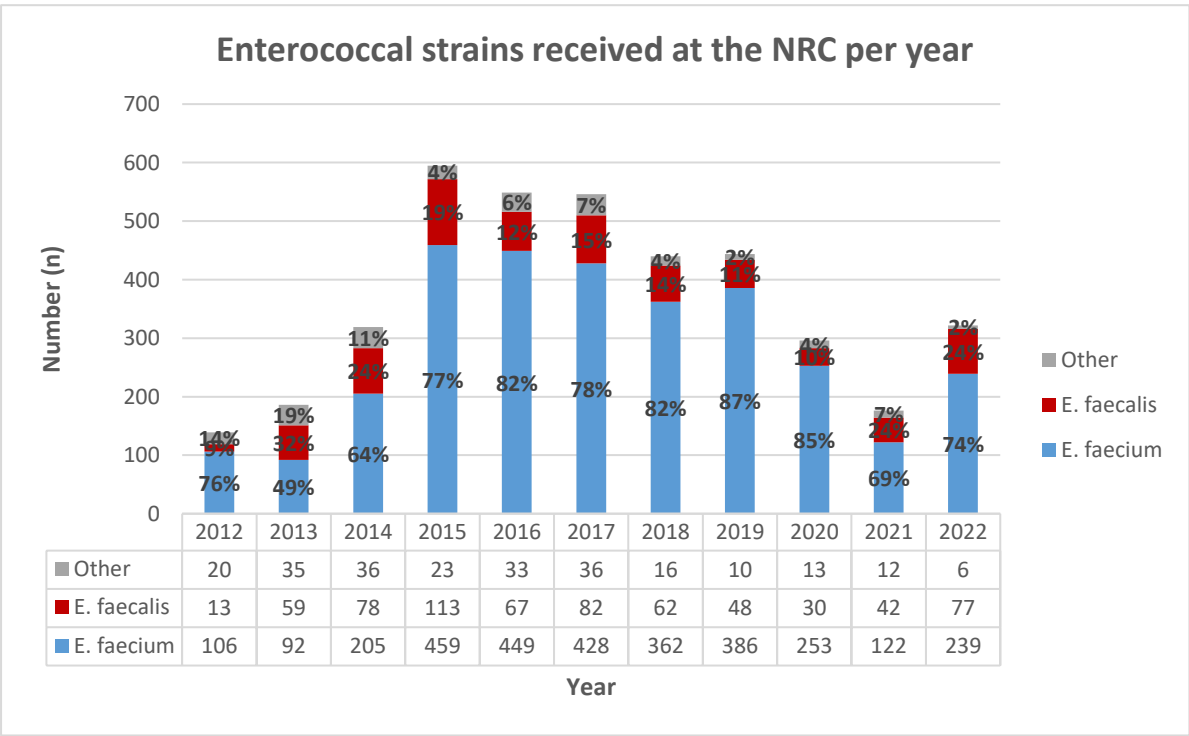


Figure 1: Strains received per year with differentiation between *E. faecium*, *E. faecalis* and other species (*E. avium*, *E. gallinarum*, *E. casseliflavus*, *E. malodoratus*, *E. raffinosus*)

Since the start of the NRC the number of strains received increased every year with a peak in 2015. Thereafter a slow decline was observed up until 2021 in which a remarkably lower number of strains was received. This decline could be due both to a lower prevalence of VRE, as a consequence of control measures although not observed in all centers, and also the inability of laboratories to send strains during the COVID-19 pandemic [1], [2]. In 2022 the number of received strains again increased yet to a level that remains lower than in pre-COVID-19 years. Such decrease most likely no longer reflects the high workload during the pandemic, but could be due to a lower

prevalence or decreased need of laboratories for confirmation of resistance or typing in case of outbreaks since sending strains to the NRC is not mandatory. The percentage of invasive VRE isolates in Belgium presented by European Centre for Disease Prevention and Control (EARS-NET) shows no statistically significant trend over the period 2017 – 2021 [3]. Data of invasive VRE strains of 2022 is not published yet.

Figure 2 shows the geographical distribution of strains received in 2022 based on the patient’s zip codes. The strains received originate from all over Belgium with the highest numbers from urban areas, which correlates with the population density of these regions. The south-eastern part of Belgium is underrepresented, similar to previous years.

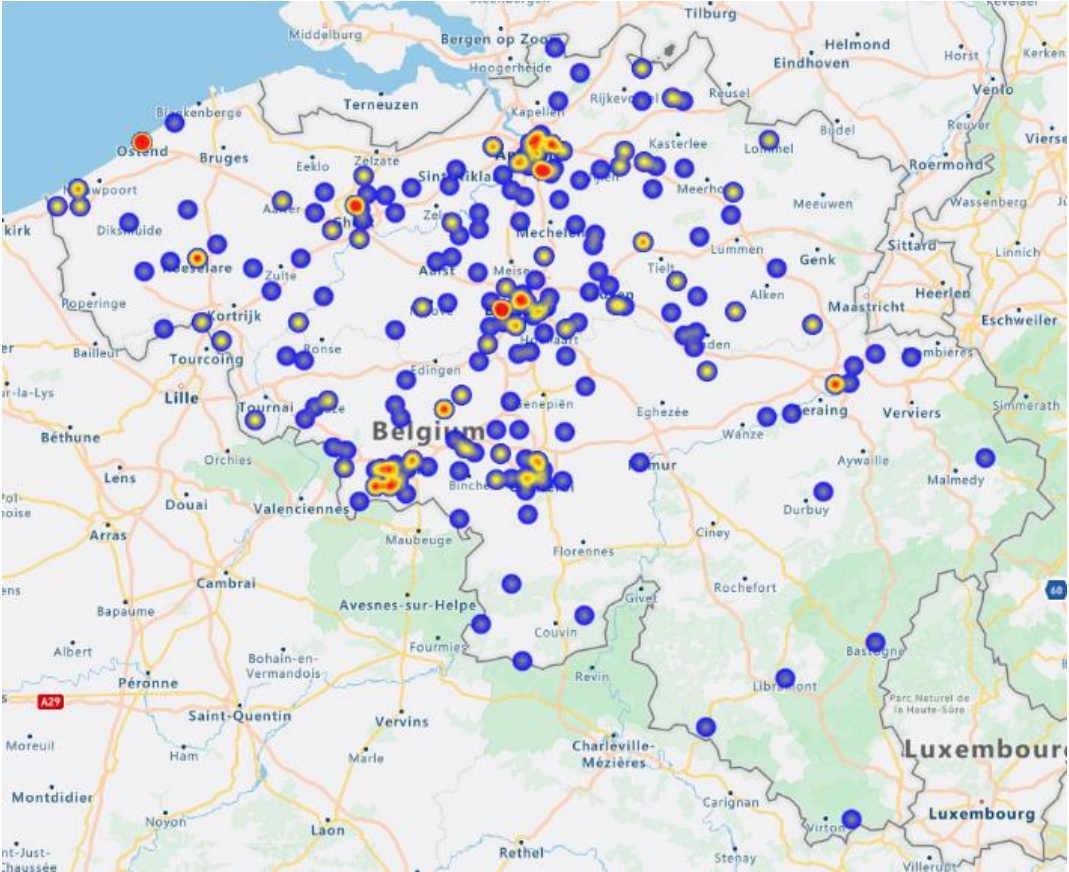


Figure 2: Geographical distribution of strains received in 2022 based on patient’s residence zip codes. Blue shows one received strain, yellow two to five received strains and red more than five received strains

2. GLYCOPEPTIDE RESISTANCE

Glycopeptide resistance of the enterococci sent to the NRC is confirmed by detection of glycopeptide resistance genes via PCR. Firstly, a PCR for *vanA* and *vanB* is performed. If none of these 2 genes are detected, a confirmation of the phenotypic glycopeptide resistance is performed via microdilution. If the strain is indeed glycopeptide resistant, WGS is performed to detect other *van* genes like *vanD*, *vanE*, *vanG*, *vanL*, *vanM* or *vanN* that are less frequently involved in glycopeptide resistance [4]. For the intrinsically resistant enterococci, *E. gallinarum* and *E. casseliflavus*, which harbour a *vanC* gene, determining additional resistance genes for glycopeptides is only performed if high MIC values are obtained. Figure 3 shows the confirmed VRE strains received in 2022 by age group. As expected, the highest incidence of VRE strains is observed in adults of 55+ (84%) and increases with age with the 80+ elderly representing one fifth of all strains (21%). This age distribution is comparable with previous years.

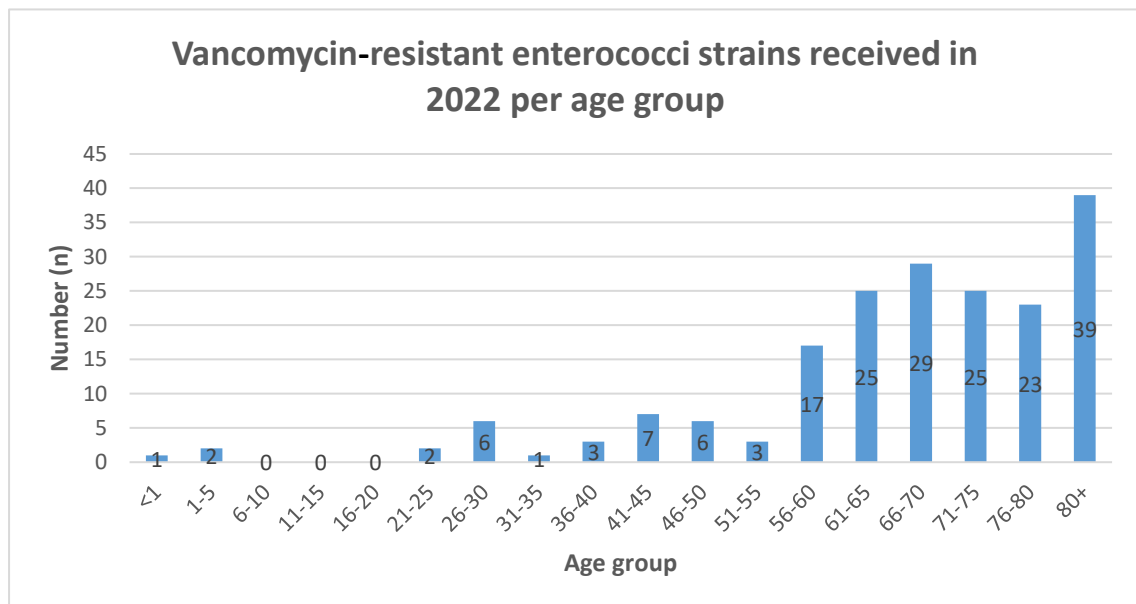


Figure 3: Number of vancomycin resistant enterococci strains received in 2022 per age group

VRE can be isolated from either screening or clinical samples (Figure 4). The screening samples slightly dominated in the years before COVID-19. However, 2022 is the first year where the proportion of screening samples over clinical samples increased to over 60%.

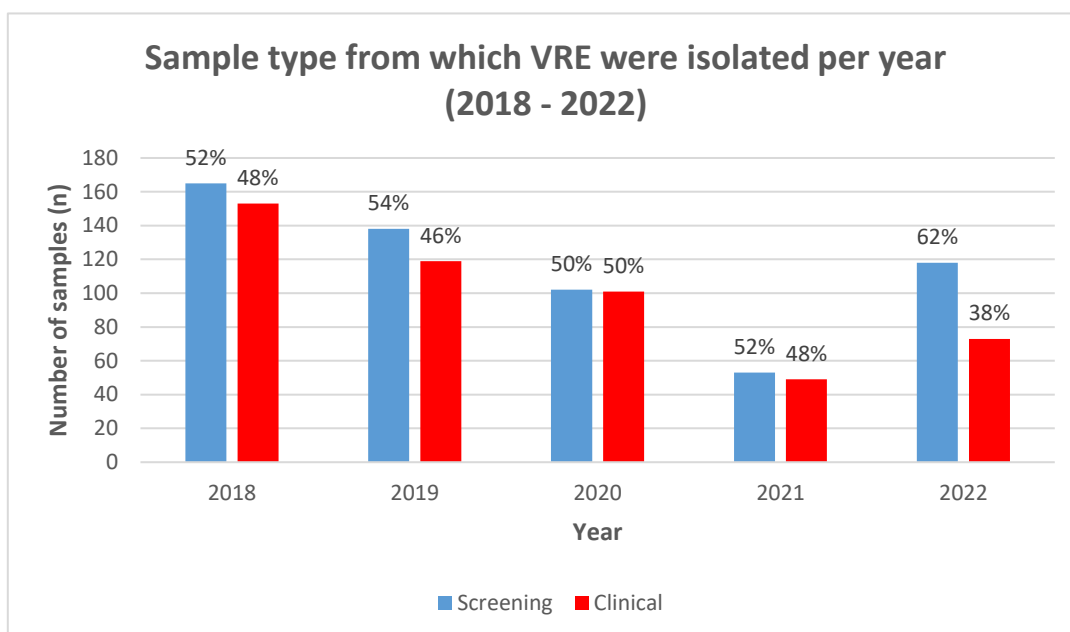


Figure 4: VRE isolated from screening and clinical samples per year (2018 -2022)

The clinical sample types where VRE were isolated from are shown in Table 1. The most common clinical sample type is urine, followed by blood, wounds, fluid and other sample types. The category “Fluid” consists of peritoneal, pleural and ascites fluid. “Other” consists mainly of respiratory samples, tissue and samples of unknown origin. Overall, the distribution of VRE by clinical sample types remains rather stable over the years. Noteworthy, in 2020 the proportion of blood isolates is higher than in other years.

Table 1: Most common clinical sample types from which VRE were isolated (2018 – 2022)

Year	Total N	Urine n (%)	Blood n (%)	Wounds n (%)	Fluid n (%)	Other n (%)
2018	153	46 (30)	19 (12)	17 (11)	5 (3)	66 (43)
2019	119	38 (32)	9 (8)	13 (11)	8 (7)	51 (43)
2020	101	48 (48)	22 (22)	13 (13)	3 (3)	15 (14)
2021	49	23 (47)	4 (8)	13 (27)	3 (6)	6 (12)
2022	73	27 (37)	8 (11)	14 (19)	7 (10)	17 (23)

Vancomycin resistance genes are determined by an in-house PCR. Figure 5 shows the prevalence of the vancomycin resistance genes between 2012 and 2022. The prevalence of *vanA* and *vanB* positive strains is similar in clinical and screening samples. Since 2017, the first year during which an increase in the proportion of *vanB* positive strains (25%) was reported, its proportion fluctuated over the recent years varying between 23 and 38%. In 2022 there seems to be a decrease in the proportion of *vanB* positive strains and a subsequent increase in the *vanA* gene proportion. *VanD* is rarely encountered. So far, the NRC has ten *vanD* positive isolates in its collection with vancomycin MIC values ranging from 16 to >256 µg/ml. Other *van* genes have not been detected so far, except for a rare detection of *vanP* in 2021 [5]. The presence of *vanA* and/or *vanB* in *E. gallinarum* and *E. casseliflavus* was confirmed in nine cases between 2012 and 2017, no other cases were found since 2018.

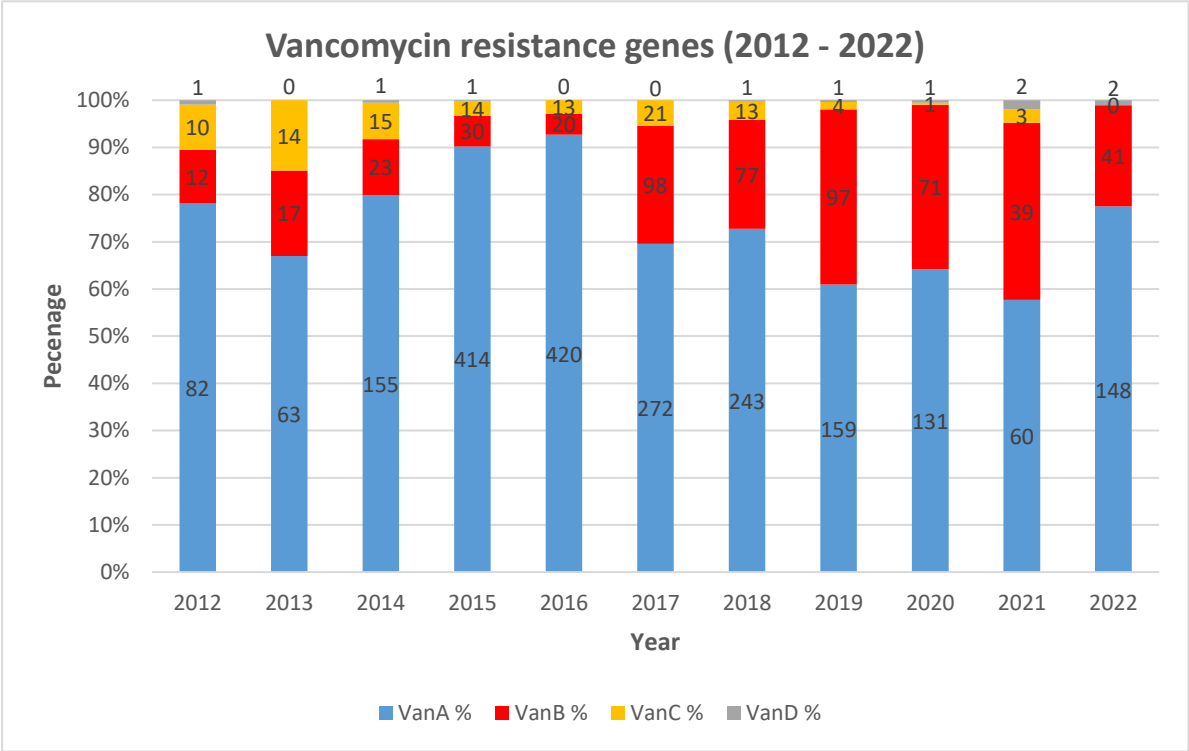


Figure 5: Prevalence of vancomycin resistance genes of enterococci isolates (2012 – 2022)

On an annual basis, the NRC receives an average of five to ten requests for detection of vancomycin variable enterococci (VVE). Three VVE isolates were detected so far at the NRC. These are vancomycin susceptible enterococci with the ability to switch to a vancomycin resistant phenotype upon exposure to glycopeptides as they harbour the *vanA* gene. These VVE's are identified at the NRC by the absence of parts of the *vanA* operon (orf1, orf2, vanS, vanR, vanH, vanX, vanYZ and IS 1251 are targeted in the PCR reaction).

3. LINEZOLID RESISTANCE

The absolute number of linezolid-resistant strains sent to the NRC from 2013 – 2022 is shown in figure 6 and increases over the years. In 2012, there was no screening for linezolid resistance at the NRC. Between January 2013 and December 2022, 124 linezolid-resistant enterococci (LRE) isolates, of which 103 (83%) *E. faecalis* and 21 (17%) *E. faecium* strains, were submitted to the NRC. Due to the observed increase in LRE strains, the NRC created awareness around this topic by launching a communication to all Belgian laboratories in October 2022 asking to send all LREs to the NRC for further typing.

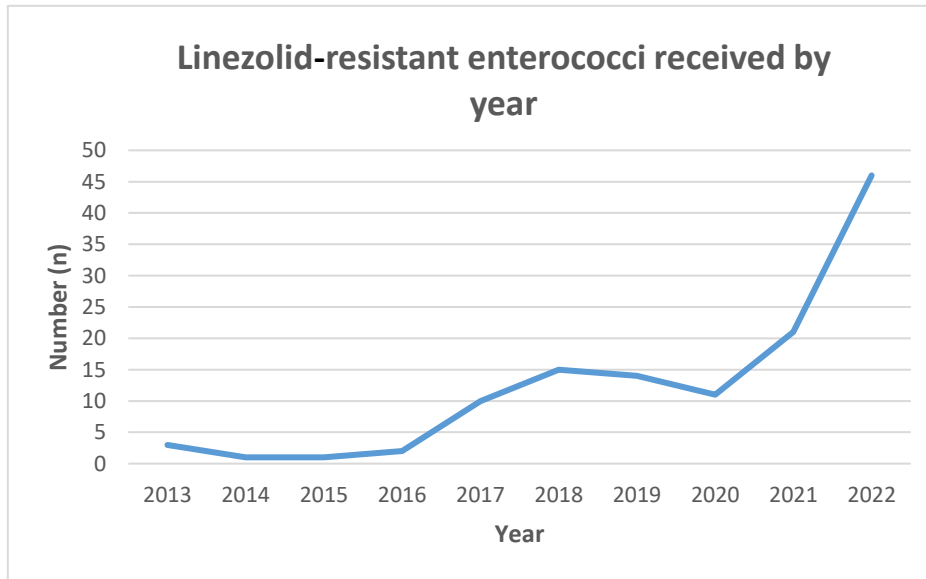


Figure 6: Prevalence linezolid resistant enterococci 2013 – 2022 received by NRC

An in-house real-time (RT) multiplex PCR is performed on the phenotypical linezolid-resistant strains for detection of the transferable linezolid resistance genes *cfr*, *cfr(B)*, *optrA* and *poxtA*, on request of the sending laboratories. Whole genome sequencing (WGS) is performed on all *cfr/cfr(B)/optrA/poxtA* negative-LRE strains in search for 23S rRNA point mutations.

Of the *E. faecalis* strains, *optrA* (98%) is the most prevalent resistance gene (Figure 7). For *E. faecium* the resistance mechanism is more diverse with G2576T rRNA point mutation (57%) most frequently detected (Figure 8). Of the 21 LRE *E. faecium* LRE strains, 14 also harbour a glycopeptide resistance-gene. Infection with these types of strains poses a serious health threat as very few therapeutic options remain.

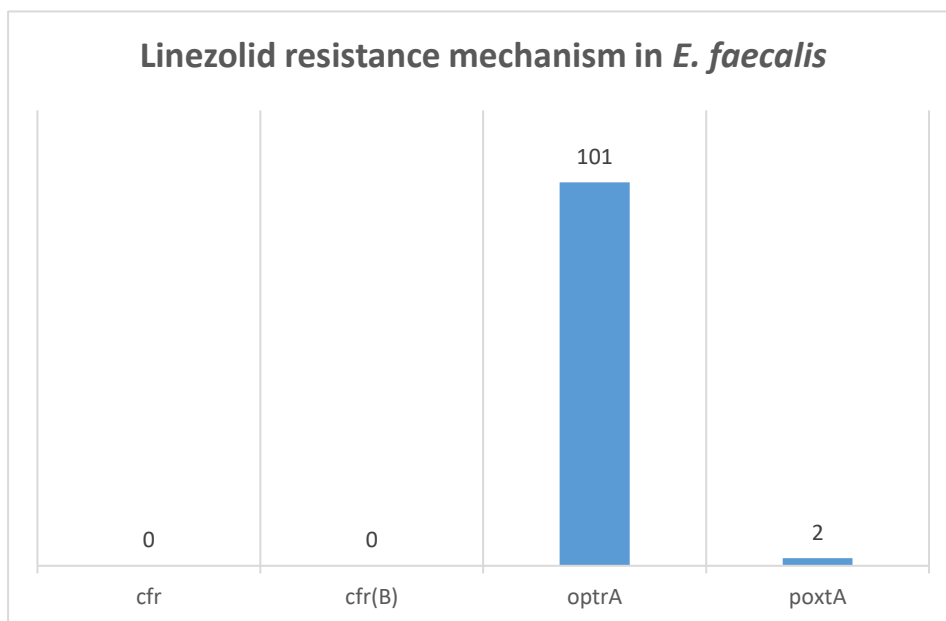


Figure 7: Detected linezolid resistance mechanisms in the isolated *E. faecalis* strains from 2013–2022 (n=103)

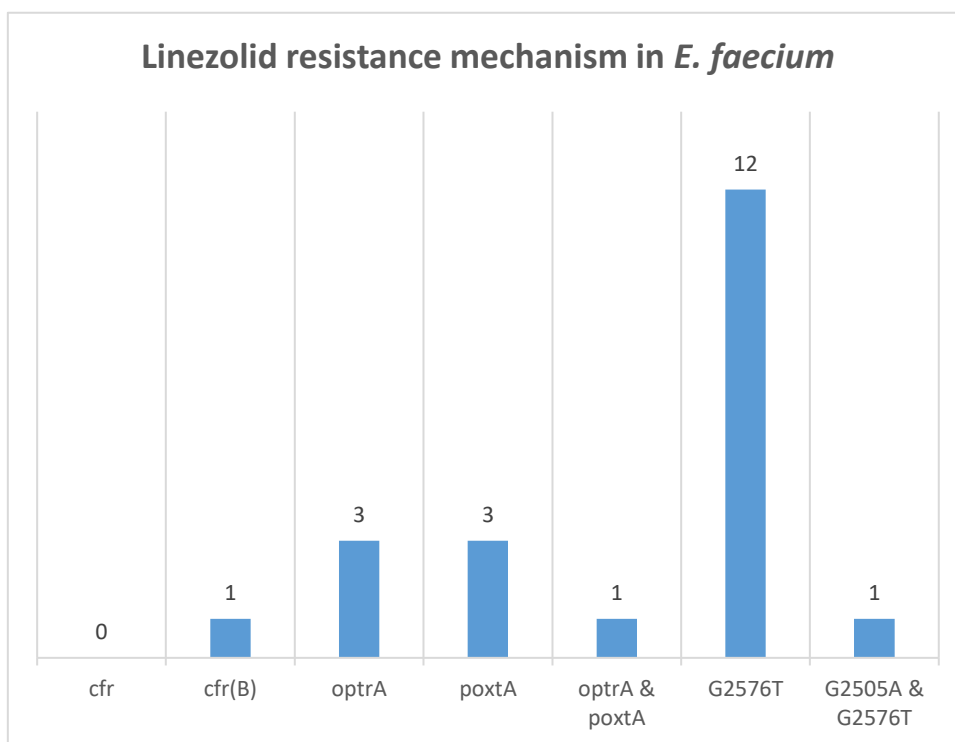


Figure 8: Detected linezolid resistance mechanisms in the isolated *E. faecium* strains from 2013–2022 (n=21)

4. STRAIN TYPING AND OUTBREAK INVESTIGATION

The NRC supports Belgian hospitals with VRE outbreak investigation using WGS since 2017. The number of hospitals where an outbreak was detected and the NRC was notified is shown in table 2. The request for outbreak detection was more frequent before the COVID-19 pandemic, but has increased again in 2022. In total 343 outbreak strains originating from 41 hospitals with outbreaks were sequenced in the period 2017-2022. Only outbreaks involving *E. faecium* strains were reported to the NRC. The most prevalent ST-types of all *E. faecium* outbreak strains are ST117 (41%) and ST80 (38%). The ST117 strains were often associated with a *vanB* resistance gene and the ST80 strains with the *vanA* resistance gene.

Table 2: Outbreak with detected ST types (2017-2022)

Year	Hospitals with outbreak(s)	Detected ST types
2017	19	ST6, ST17, ST18, ST80, ST117, ST203
2018	18	ST17, ST80, ST117, ST191, ST280, ST612, ST1465
2019	14	ST18, ST80, ST117, ST203, ST563, ST612
2020	1	ST117
2021	1	ST80
2022	8	ST17, ST18, ST80, ST117, ST192, ST1336

Figure 9 presents a cgMLST analysis of an outbreak in 2022 of *E. faecium* ST80 strains. Clonality was inferred from the genetic distance between the strains, expressed as alleles difference shown in the upper line in the graph.

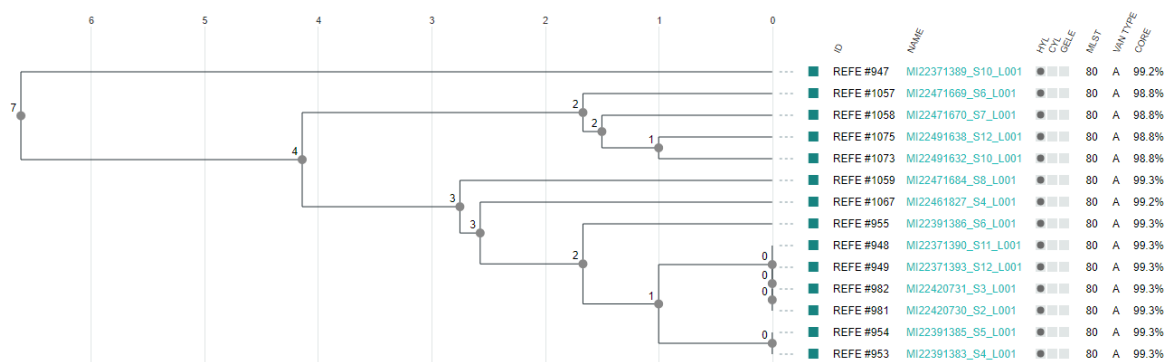


Figure 9: cgMLST analysis of an outbreak in 2022 of *E. faecium* ST80 strains

Some strains of outbreaks in different hospitals are closely related. In 2022, the NRC detected clonal spread of *vanB* ST117 *E. faecium* covering 2 hospitals in close vicinity. Figure 10 presents *vanB* ST117 *E. faecium* strains of two hospitals which differ in merely 3 alleles and therefore considered clonally related.

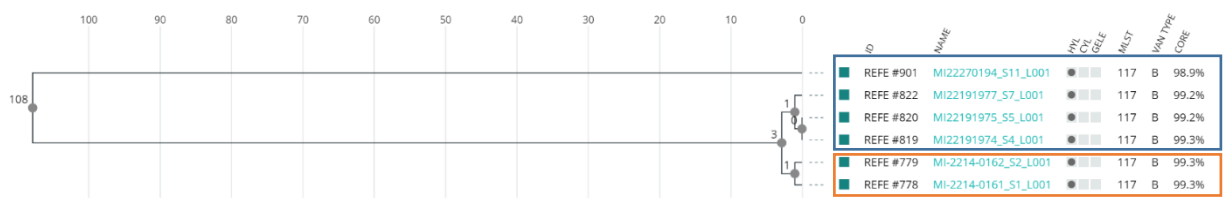


Figure 10: cgMLST analysis of ST117 *E. faecium* outbreak strains in 2022 of two different hospitals

5. SUMMARY

The results presented in this report are based on the data of the National Reference Centre (NRC) for Enterococci during the period 2012 – 2022. The strains received at the NRC are sent for various reasons, but mainly for confirmation of glycopeptide resistance. Less common requests are for confirmation of linezolid resistance (linezolid resistant enterococci, LRE) and for vancomycin resistant enterococcus (VRE) outbreak investigation.

Since the start of the NRC the number of strains received increased every year with a peak in 2015 (n=595). Thereafter a slow decline was observed up until 2021 in which a remarkably lower number of strains was received. In 2022 the number increase again but not up to the pre-pandemic level, with 239 *E. faecium*, 77 *E. faecalis* and 6 other enterococcal species. Strains are from all over Belgium, with the highest prevalences in urban areas correlating with the population densities.

The VRE **incidence** increases with age with the 80+ (n=39) elderly representing one fifth of all strains. The most common **clinical sample** type for VRE strains is urine (n=27, 37%), followed by wounds (n=14, 19%), blood (n=8, 11%), fluid (n=7, 10%) and other sample types (n=17, 23%). The VRE strains isolated from **screening** samples slightly dominated in the years before COVID-19. However, 2022 is the first year where the proportion of screening samples over clinical samples increased to over 60%. The prevalence of **vanA** and **vanB** positive strains in VRE is similar in clinical and screening samples and **vanA** dominates (> 58%).

The absolute number of **linezolid-resistant strains** sent to the NRC from 2013 – 2022 increases over the years, with 46 received isolates in 2022. Of the *E. faecalis* strains, *optrA* (98%) is the most prevalent resistance gene. For *E. faecium* the resistance mechanism is more diverse with G2576T rRNA point mutation (57%) most frequently detected.

In total 343 **outbreak strains** originating from 41 hospitals with outbreaks were sequenced in the period 2017-2022. The most prevalent ST-types of all *E. faecium* outbreak strains are ST117 (41%) and ST80 (38%).

6. REFERENCES

- [1] J. Cole and E. Barnard, "The impact of the COVID-19 pandemic on healthcare acquired infections," *American Journal of Infection Control*, vol. 49, pp. 653-654, 2021.
- [2] F. Van Laer, E. Van Cauwenberg and H. Jansens, "Welk impact van de COVID-19-pandemie op de incidentie van zorginfecties in het UZA?," NOSOinfo, Edegem, 2021.
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7. RECENT NRC PUBLICATIONS

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