Journal of Global Antimicrobial Resistance 37 (2024) 129–134

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

When it rains it pours: An increased prevalence of intestinal carriage of vancomycin-resistant *Enterococcus faecium* related to higher use of oral vancomycin in a tertiary care Hungarian clinical centre during the years of the COVID-19 pandemic

László Orosz*

Department of Medical Microbiology, University of Szeged, Szeged, Hungary

ARTICLE INFO

Article history:
Received 14 February 2024
Accepted 5 March 2024
Available online 27 March 2024

Editor: Professor E. Giamarellos-Bourboulis

Keywords:
Vancomycin-resistant *Enterococcus*
Intestinal carriage
Oral vancomycin therapy
Fourier transform infrared spectroscopy

ABSTRACT

Objectives: This study aims to investigate the association between oral vancomycin consumption and intestinal vancomycin-resistant *Enterococcus* carriage in the pre- and COVID era in the clinical centre of the University of Szeged, Hungary.

Methods: This retrospective microbiological examination was carried out using electronically collected data, corresponding to the period between 1 January 2018 and 31 December 2022, at the Department of Medical Microbiology. Data included isolated species and the according antimicrobial susceptibility patterns. Annual consumption data for oral vancomycin consumption were exported from the database of the central pharmacy of the clinical centre. As a strain typing procedure, Fourier transform infrared spectroscopy analysis was used.

Results: There was a significant increase in the number of faecal vancomycin-resistant *Enterococcus* isolates throughout the study. The prevalence increased significantly during the years of the pandemic. The use of orally administered vancomycin in the clinical centre increased significantly. A strong positive correlation existed between the two phenomena. Several strains with different resistance patterns spread in the clinical centre. Two of these occurred in greater numbers, differing in their high-level aminoglycoside resistance. However, the overall resistance of these strains was stagnating. FTIR analysis revealed that 59 of the 62 strains were also divided into 2 large clusters differing partially in their high-level aminoglycoside resistance.

Conclusions: During the pandemic, intestinal VRE carriage among clinical centre patients increased significantly, linked to increased oral vancomycin use. Different strains spread, with aminoglycoside resistance being the primary distinction. This highlights the negative impact of the pandemic on VRE carriage.

1. Introduction

In recent years, antibiotic resistance has become a global health concern, with increasing attention directed toward multidrug-resistant bacteria [1]. Among these pathogens, vancomycin-resistant *Enterococcus* spp. (VRE) has gained prominence because of its ability to cause a wide range of infections and its high level of resistance to many commonly used antibiotics [2]. *Enterococcus* species are commensal bacteria that inhabit the gastrointestinal tract of humans and animals. They can also colonize other body sites, including the urinary tract, skin, and wounds [3]. Although *Enterococcus faecalis* and *E. faecium* are the most common species associated with human infections, VRE is predominantly *E. faecium* [3]. Transmission of VRE occurs mainly through person-to-person contact, healthcare workers, contaminated surfaces, and medical devices [4]. The incidence of VRE infections has increased throughout the world, posing a substantial burden on healthcare systems [5].

Abbreviations: ARI, antibiotic resistance index; CDI, *Clostridioides difficile* infection; EUCAST, European Committee on Antimicrobial Susceptibility Testing; FTIR, Fourier transform infrared; VRE, vancomycin-resistant *Enterococcus*.

* Corresponding author: Mailing address: László Orosz, Department of Medical Microbiology, University of Szeged, H-6720 Szeged Semmelweis str. 6/b., Szeged, Hungary.
E-mail address: orosz.laszlo@med.u-szeged.hu

https://doi.org/10.1016/j.jgar.2024.03.004

The COVID-19 pandemic has disrupted healthcare systems around the world, particularly in the management of VRE [6]. Prioritizing infection control measures to prevent SARS-CoV-2 transmission has compromised routine practices, leading to increased VRE transmission and increased incidence [7]. The increase in cases has strained resources, causing difficulties in the management of patients with VRE infections, leading to delayed diagnosis, inadequate treatment, and higher mortality rates [7]. The increased use of antibiotics has accelerated the evolution and dissemination of VRE strains within healthcare settings [7].

The COVID-19 pandemic has significantly affected *Clostridioides difficile* infection (CDI), a major cause of healthcare-associated diarrhoea [8]. The increased use of antibiotics has disrupted the intestinal microbiota, which is crucial for the development of CDI [8,9]. The interaction between antibiotic use, CDI, and oral vancomycin therapy deserves attention during the pandemic [8]. For this reason and due to the limited data available on this topic in Hungary, we investigated the association between oral vancomycin consumption and faecal VRE carriage in the pre- and COVID era in the clinical centre of the University of Szeged.

2. Materials and methods

2.1. Study setting

The present retrospective microbiological study was carried out using collected data, corresponding to the period between 1 January 2018 and 31 December 2022, at the Department of Medical Microbiology, University of Szeged, Hungary. This clinical microbiology laboratory serves the Albert Szent-Györgyi Clinical Centre, which is an 1800-bed primary and tertiary care teaching hospital in the southern Great Plain of Hungary. Data collection was carried out electronically, in the laboratory information system records, corresponding to faecal samples positive for VRE.

2.2. Microbiological data set

This study was carried out using local data that were exported from the laboratory clinical microbiology information system (MedBakter, Asseco Central Europe Ltd., Hungary) and was reported in a customized database. Data included isolated species and the according antimicrobial susceptibility patterns. The results of the antimicrobial susceptibility tests were determined and interpreted according to EUCAST breakpoints.

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of the University of Szeged (protocol code 8/2021-SZTE RKEB; 25 January 2021).

2.3. Data analysis

The data were exported from the laboratory information system into the MS Excel 2016 (Microsoft Corp., Redmond, WA, USA) and GraphPad Prism version 8 (GraphPad Software, San Diego, CA, USA) software. MS Excel 2016 was used to store the data and to determine the different antibiotic resistance index (ARI) curves. GraphPad Prism 8 was used for statistical analysis and plotting. All values are expressed as means and ranges, where appropriate. An unpaired, two-tailed *t*-test was used to compare the data from pre-COVID era and COVID era years and between clusters. The *P* values <0.05 were considered statistically significant. The Spearman correlation function of the GraphPad Prism 8 was used to determine the correlation between the amounts of oral vancomycin consumption (mg) and the number of faecal VRE-positive patients. Annual consumption data for oral vancomycin consumption were exported from the database of the central pharmacy of the clinical centre.

2.4. Calculating antibiotic resistance index

To calculate the ARI, the model for measuring antibiotic resistance used by De Socio et al. was followed [10]. Briefly, for each antibiotic tested, a score of 0 for susceptibility, 0.5 for intermediate resistance, or 1 for resistance was assigned, and the ARI was calculated by dividing the sum of these scores by the number of antibiotics tested, giving a maximum score of 1.

2.5. Strain typing by Fourier transform infrared spectroscopy

To confirm the differences found in the antibiotic resistance pattern with other typing methods, an advanced methodology, InfraRed Biotyper® (Bruker Daltonics GmbH & Co. KG, Bremen, Germany), was used. The IR Biotyper® analyses characteristic molecular vibrations induced by infrared light absorption. The absorption bands are assigned to specific chemical structures, whereas the wavelength range of the carbohydrates plays the most important role or contributes the most to strain differentiation. The analysis is based on Fourier transform infrared (FTIR) spectroscopy. The FTIR technique is used to obtain the absorption or emission spectrum of a solid, liquid, or gas. During these tests, we had the opportunity to analyse 62 VRE strains from the period under study. All typed isolates were cultured at 37 °C for 24 h on Mueller-Hinton Agar (MHA; Bio-Rad, Hercules, CA, USA). The first step was to collect a loopful of bacterial culture (~1 µL) and suspend it in 50 µL of 70% ethanol in a 1.5 mL microcentrifuge tube with sterile metal rods provided by the kit manufacturer. Using a vortexer, a uniform suspension was achieved. 50 µL of sterile water was added after 1 min of vortexing, and the remaining 100 µL of the solution was vortexed again for 1 min. Then, 12 µL of the two infrared (IR) test standard 1 (IRTS1) and IR test standard 2 (IRTS2) suspensions were spotted onto the IRBT silicon plate with 15 µL of the bacterial suspension and dried at 37 °C for 30 min, until a film was formed from the drops. The dried silicon plate was then put into the IRBT spectrometer (Bruker Daltonics GmbH & Co. KG, Bremen, Germany) with the analytical parameters at their defaults. OPUS 7.5 software was used to collect the isolates' spectra (Bruker Daltonics GmbH & Co. KG, Bremen, Germany). The spectra that met the default quality criteria of absorption [0.4 arbitrary unit (AU) < D value < 2 AU], signal/noise ($<150 \times 10^{-6}$ AU), signal/water ($<300 \times 10^{-6}$ AU), and fringes ($<100 \times 10^{-6}$ AU) were determined as 'quality pass' in the IRBT analysis. To create the 2D scatter plots and dendrograms, the spectra acquired with 'quality pass' were used. The software includes a function that automatically suggests a cut-off value that establishes the minimum distance at which two spectra are regarded as belonging to the same cluster.

3. Results

3.1. A significant increase in the number of faecal VRE isolates

As a first step in the cautery, we looked at how the number of VRE strains isolated from faeces evolved each year. Fig. 1A clearly shows that there was a marked increase from year to year over the period studied. The rate of increase is particularly remarkable since 2020. To analyse the impact of the pandemic, the years in question were divided into two periods: 2018 and 2019 were the pre-COVID era, while 2020–2022 were the COVID era. In this comparison, there is a significant difference between the two periods in favour of the COVID era (Fig. 1B). These data suggest that the pandemic has had a significant impact on the spread of VRE in the clinical centre.

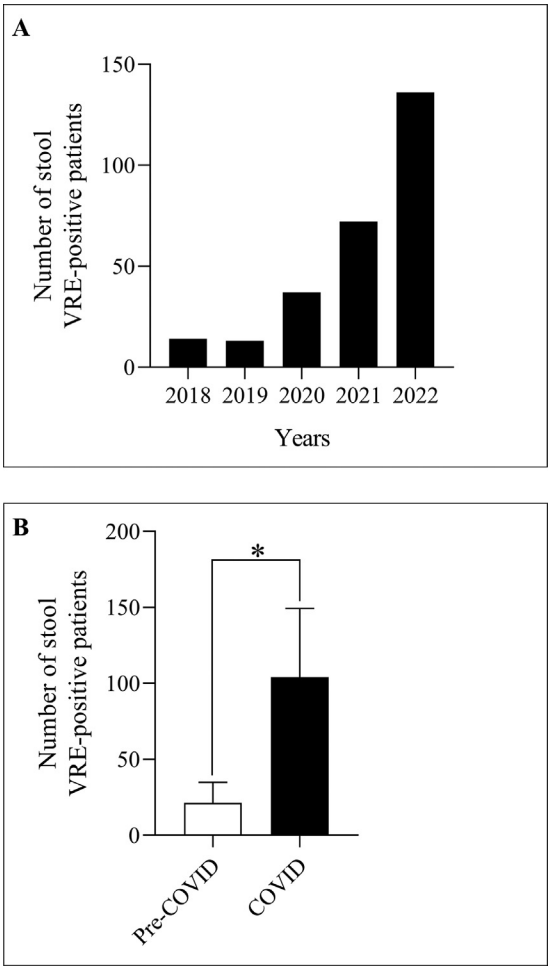

Fig. 1. Trends in the number of patients carrying intestinal VRE between 2018 and 2022. (A) The number of VRE carrier patients per year between 2018 and 2022. (B) VRE-positive patients prior to and during the COVID era.

3.2. Strong positive correlation between VRE incidence and oral vancomycin consumption

Then the association between the consumption of oral vancomycin in the treatment of CDIs and the number of patients with VRE in their faeces was investigated. Oral vancomycin use increased significantly in 2020, followed by an increase in the number of VRE-positive patients (Fig. 2). The correlation between the subsamples of the two parameters was determined using Spearman's method. The correlation coefficient® is 0.8, which indicates a strong positive correlation (Fig. 2). All these data suggest that increased oral vancomycin use is closely related to the prevalence of VRE in the clinical centre.

3.3. Several VRE strains with different resistance patterns spread in the clinical centre

To better understand the spread of different VRE strains, the diverse resistance patterns that occurred during the study period were analysed. To this end, a new approach was used whereby the sensitivity results for each antibiotic were replaced by ARI values and then merged into a single cell using the CONCAT function in MS Excel (resistance profile analysis – a precise description of the method is submitted for publication in another journal). This series represents the resistance pattern for each strain. The prevalence of the five most common VRE strains with resistance patterns was then plotted by year (Fig. 3A). The two most common strains have a significantly higher prevalence than the others. The only difference between the two resistance patterns was high-level gentamicin susceptibility (Fig. 3A). Plotting the percentage prevalence of the different resistance profiles by year, the occurrence of high-level gentamicin-resistant strains decreased in the years of the COVID era, while the sensitive strain increased (Fig. 3B). All these data show that in aminoglycoside resistance two different VRE strains dominated, with the sensitive one prevailing.

3.4. Resistance to different VRE strains is stagnating

In the next part of our investigation, the ARI values of VRE strains with different resistance profiles were examined. By plotting the mean ARI values of the five most common susceptibility patterns, a trend line can be created for the scores using linear regression (Fig. 4). From the equation of the trendline, the slope was determined by using the linear regression calculator of GraphPad (https://www.graphpad.com/quickcalcs/linear1/). Its value is 0.009, which indicates that the trend line is slightly emasculated towards the less frequent VRE strains (Fig. 4). It can be concluded that the resistance decreases very slightly, almost negligibly, with the occurrence of the strains (e.g., aminoglycoside resistance disappears for the first two strains). In other words, the resistance of VRE strains is roughly constant, stagnating.

3.5. The results of the resistance profile analysis were partially confirmed by FTIR

In the next part of the work, 62 VRE strains were typed using IR Biotyper to reproduce the results of the resistance profile analysis with other typing methods. As a result of the FTIR analysis, 59 of the 62 strains were divided into two large clusters (Fig. 5). Cluster #1 contains 34 strains, whereas cluster #2 comprises 25. In terms of antibiotic susceptibility, the only remarkable difference between the two groups is high-level gentamicin resistance. This rate is 30% for cluster 1, whereas it is 0% for cluster 2 (Fig. 5).

Comparing the ARI values of the two clusters reveals that cluster 1 is substantially more resistant (Fig. 6). The disparity is primarily due to high-level aminoglycoside susceptibility. The results of the resistance profile analysis were partially supported by the FTIR method based on all these data.

4. Discussion

This study, which aimed to analyse VRE strains isolated from faeces at the clinical centre of the University of Szeged, revealed that the prevalence of these strains increased between 2018 and 2022 (Fig. 1A). There was a substantial distinction between the pre-COVID era and the COVID era (Fig. 1B). Following the findings of other researchers, the prevalence of VRE is increasing globally, as demonstrated by the current findings [11,12]. Even though many publications address the issue of intestinal VRE carriage, only a small number of studies examine the effect of pandemic measures on this parameter.

Since the number of CDIs increased markedly during the pandemic [8], the effect of oral vancomycin therapy on intestinal VRE carriage was investigated. During the years of the pandemic, the use of orally administered vancomycin in the clinical centre increased dramatically, as did the prevalence of intestinal VRE carriage (Fig. 2). The coefficient of Spearman correlation between the two data series is 0.80, indicating an intensely positive relationship (Fig. 2). Even though the relationship between the two factors appears logical, the literature provides contradictory evalua-

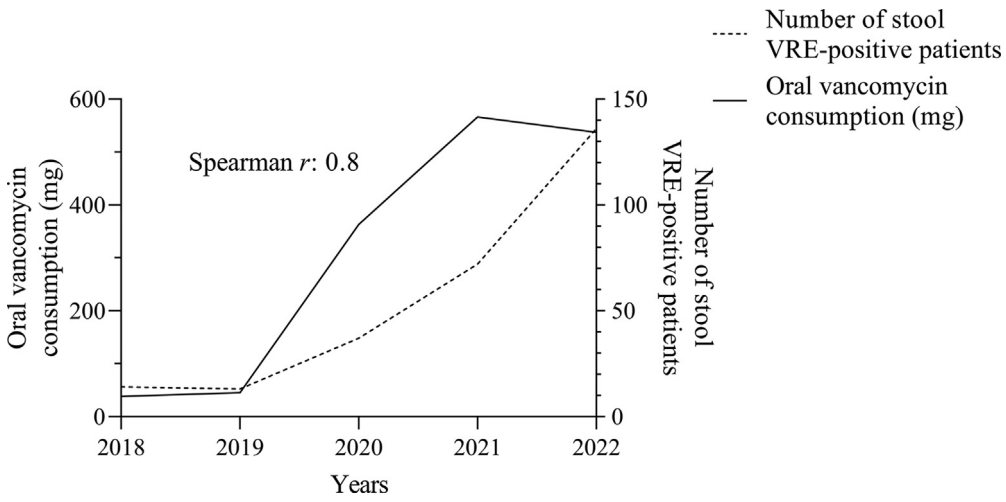

Fig. 2. Relationship between oral administration of vancomycin and intestinal VRE carriage.

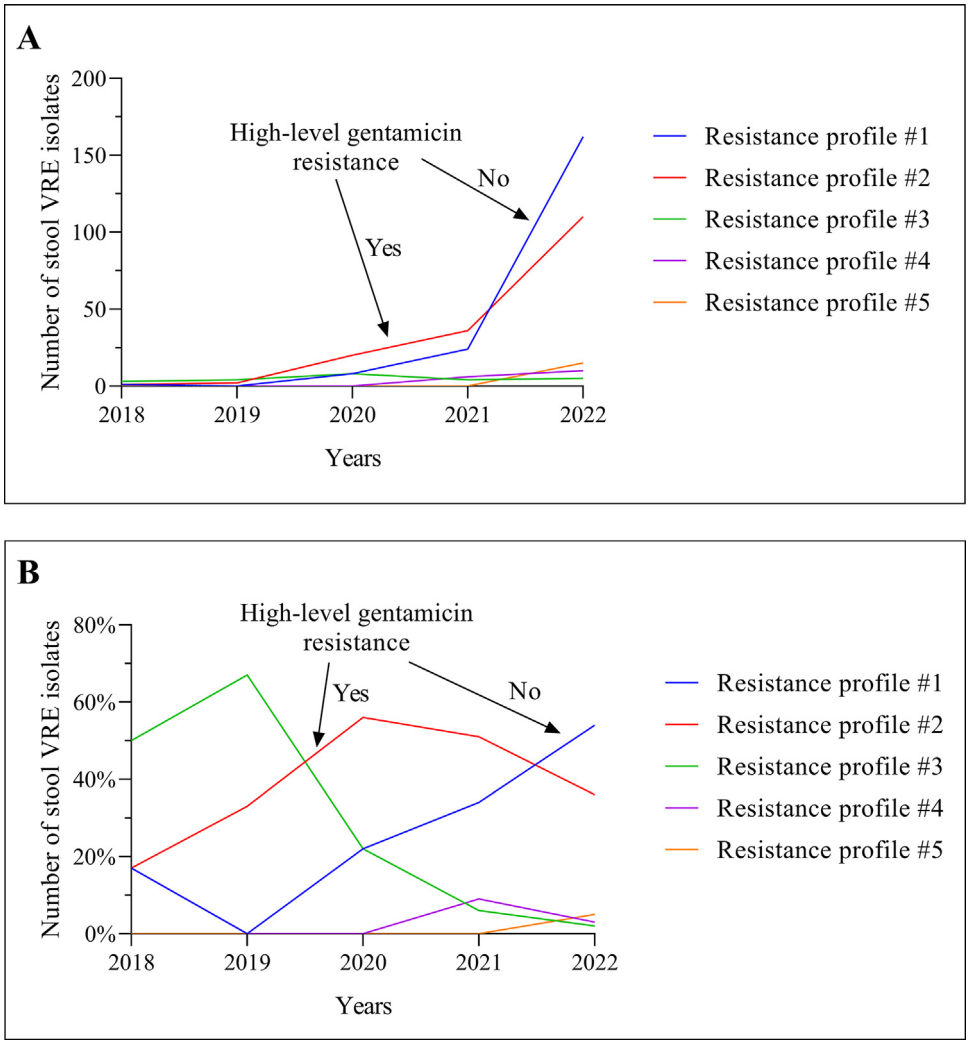

Fig. 3. Analysis of the resistance profiles of intestinally carried VRE strains. (A) The annual evolution of the number of variants with distinct resistance profiles. (B) Percentage of strains with different patterns of resistance per year.

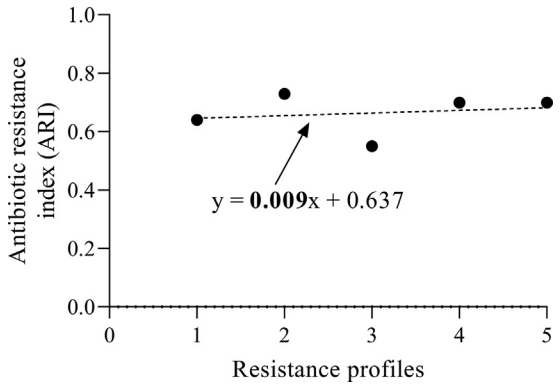

Fig. 4. Plots of the antibiotic resistance indices of the five most prevalent VRE strains with varying patterns of resistance. The dashed line represents the trendline that can be fitted to the data using linear regression; its equation is also displayed. The number in bold represents the slope value.

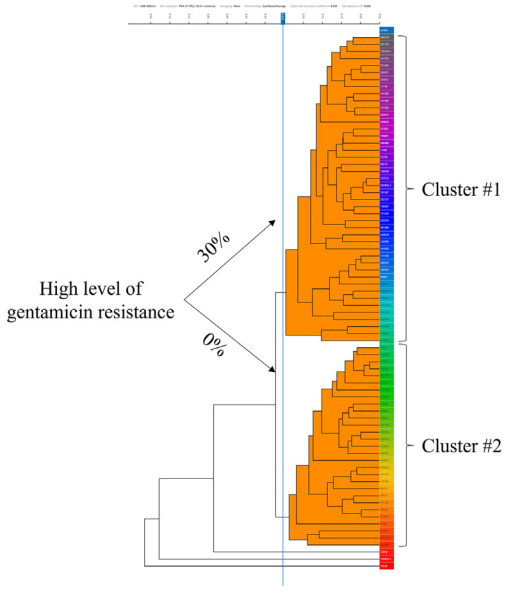

Fig. 5. FTIR analysis results of the VRE strains. The prevalence of high-level gentamicin resistance in the two large clusters is also reported.

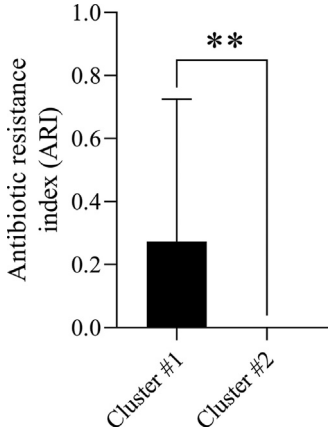

Fig. 6. Comparison and statistical analysis of the antibiotic resistance indices of two clusters determined by the IR Biotyper.

tions. According to some authors, oral vancomycin therapy or other antibiotic use, including anti-anaerobic therapy, may not be a substantial independent risk factor for patients who have never been exposed to VRE to develop a culture-positive infection [13]. That study suggests that oral vancomycin is unlikely to induce VRE culture positivity in patients who have not yet been exposed to VRE; rather, it may merely exacerbate previously undetectable colonization [13]. One of the limitations of the present study is that it did not examine this topic from this perspective, but it can be presumed that the VRE strains detected as intestinal invaders have spread throughout the clinical centre and that the inpatients have become colonized with them.

To characterize the faecal VRE isolates, a method that is pending publication, resistance profile analysis was used. Consequently, it was determined that the isolates can primarily be categorized into two larger groups with distinct resistance patterns (Fig. 3A). The high-level gentamicin resistance was the only difference between these strains. The dynamics of the two groups can be observed more clearly if their annual occurrence percentages are plotted (Fig. 3B). During the outbreak years, the prevalence of gentamicin-sensitive strains with resistance profile #1 increased, while the prevalence of gentamicin-resistant strains decreased. To the best of the author's knowledge, this method has not previously been used to characterize VRE strains.

To further characterize the strains having different resistance profiles, the distinct ARI values were determined. This analysis revealed that the ARI values of the various strains are quite comparable (Fig. 4). To analyse the resistance trend, a trend line was fitted to the values using linear regression. The line had a slope that, despite being insignificant, increased in the direction of the rarer isolates (Fig. 4). All of this may suggest that the resistance of the strains decreases slightly as they become more widespread. In the case of the two most prevalent isolates, this is exemplified by the loss of high-level aminoglycoside resistance. Although this argument is somewhat speculative, there are known instances of resistance decreasing as strain frequency increases [14].

To support this claim, 62 VRE isolates were analysed using a contemporary typing method. The FTIR analysis confirmed that the VRE population is divided into two clusters (Fig. 5). Examining the difference between the two groups regarding antibiotic resistance, it can be concluded that they differ only in terms of the high-level aminoglycoside resistance (Fig. 5). It is important to note that this difference is only partial, as the aforementioned resistance trait is only detectable in 30% of cluster #1. Since high-level aminoglycoside resistance in enterococci is primarily based on the production of intracellular enzymes and FTIR examines cell surface structures, a plausible explanation for this issue requires additional research. Nevertheless, a significant advancement in recent years is the growing body of literature that validates the occurrence of membrane vesicle production in Gram-positive bacteria [15]. Enterococci have also been shown to have aminoglycoside resistance enzymes inside these membrane vesicles [16,17]. One of the proteins that provide resistance to aminoglycosides, such as gentamicin, is Aac6′-Ie-Aph2′′-Ia, which is a bifunctional protein with broad-spectrum activity [17,18]. This indicates that the distinction between the two groups may originate from the manifestation of membrane vesicles that are observable on their external surface and in some instances provide aminoglycoside resistance.

The ARI values of the two clusters were also compared statistically to corroborate that the difference was not coincidental. This analysis demonstrated that the difference between the two groups is statistically significant and cannot be explained by chance alone (Fig. 6). Since the only difference between the two groups was high-level aminoglycoside resistance, this demonstrates the validity of the FTIR results.

5. Conclusion

In conclusion, during the pandemic years, the prevalence of intestinal VRE carriage among the patients of the clinical centre increased substantially. This phenomenon has a close relationship with the increased use of oral vancomycin. Diverse VRE strains with varying resistance profiles have spread, with aminoglycoside resistance being the primary distinction. Additionally, this was partially confirmed using a modern typing method. All of this draws attention to the negative impact of the pandemic in terms of the transmission and intestinal carriage of VRE, which can lead to life-threatening infections later on.

Declarations

Funding: None.

Ethical approval: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of the University of Szeged (protocol code 8/2021-SZTE RKEB; 25 January 2021).

Competing interests: None declared.

References

[1] Dadgostar P. Antimicrobial resistance: implications and costs. Infect Drug Resist 2019;12:3903–10. doi:10.2147/IDR.S234610.
[2] Sood S, Malhotra M, Das B, Kapil A. Enterococcal infections & antimicrobial resistance. Indian J Med Res 2008;128:111–21.
[3] Franyó D, Kocsi B, Lesinszki V, Pászti J, Kozák A, Bukta EE, et al. Characterization of clinical vancomycin-resistant *Enterococcus faecium* isolated in Eastern Hungary. Microb Drug Resist 2018. doi:10.1089/mdr.2018.0074.
[4] Verma D, Sinha S, Prakash V. Prevalence and comparison of high-level aminoglycoside resistance in vancomycin-sensitive and vancomycin resistant enterococcus at a tertiary care hospital in Rohillkhand region. Int J Med Res Rev 2015;3:1162–6. doi:10.17511/ijmrr.2015.i10.210.
[5] Sparo M, Delpech G, García Allende N. Impact on public health of the spread of high-level resistance to gentamicin and vancomycin in enterococci. Front Microbiol 2018;9:3073. doi:10.3389/fmicb.2018.03073.
[6] Mustapha A, Nikau J, Isa T. COVID-19 and antibiotic resistance: parallel pandemics and different intercessions. Microbes Infect Dis 2021;2:15–24. doi:10.21608/mid.2020.49732.1087.
[7] Toc DA, Butiuc-Keul AL, Iordache D, Botan A, Mihaila RM, Costache CA, et al. Descriptive analysis of circulating antimicrobial resistance genes in vancomycin-resistant *Enterococcus* (VRE) during the COVID-19 pandemic. Biomedicines 2022;10:1122. doi:10.3390/biomedicines10051122.
[8] Spigaglia P. *Clostridioides difficile* infection (CDI) during the COVID-19 pandemic. Anaerobe 2022;74:102518. doi:10.1016/j.anaerobe.2022.102518.
[9] Langford BJ, So M, Raybardhan S, Leung V, Soucy J-PR, Westwood D, et al. Antibiotic prescribing in patients with COVID-19: rapid review and meta-analysis. Clin Microbiol Infect 2021;27:520–31. doi:10.1016/j.cmi.2020.12.018.
[10] De Socio GV, Rubbioni P, Botta D, Cenci E, Belati A, Paggi R, et al. Measurement and prediction of antimicrobial resistance in bloodstream infections by ESKAPE pathogens and Escherichia coli. J Glob Antimicrob Resist 2019;19:154–60. doi:10.1016/j.jgar.2019.05.013.
[11] Abubakar U, Al-Anazi M, Alanazi Z, Rodríguez-Baño J. Impact of COVID-19 pandemic on multidrug resistant gram positive and gram negative pathogens: a systematic review. J Infect Public Health 2023;16:320–31. doi:10.1016/j.jiph.2022.12.022.
[12] Fukushige M, Syue L-S, Morikawa K, Lin W-L, Lee N-Y, Chen P-L, et al. Trend in healthcare-associated infections due to vancomycin-resistant *Enterococcus* at a hospital in the era of COVID-19: more than hand hygiene is needed. J Microbiol Immunol Infect 2022;55:1211–18. doi:10.1016/j.jmii.2022.08.003.
[13] Salgado CD, Giannetta ET, Farr BM. Failure to develop vancomycin-resistant *Enterococcus* with oral vancomycin treatment of *Clostridium difficile*. Infect Control Hosp Epidemiol 2004;25:413–17. doi:10.1086/502415.
[14] Hughes D, Andersson DI. Evolutionary trajectories to antibiotic resistance. Annu Rev Microbiol 2017;71:579–96. doi:10.1146/annurev-micro-090816-093813.
[15] Briaud P, Carroll RK. Extracellular vesicle biogenesis and functions in gram-positive bacteria. Infect Immun 2020;88 e00433–e00420. doi:10.1128/IAI.00433-20.
[16] Lehmkuhl J, Schneider JS, vom Werth KL, Scherff N, Mellmann A, Kampmeier S. Role of membrane vesicles in the transmission of vancomycin resistance in *Enterococcus faecium*. Sci Rep 2024;14:1895. doi:10.1038/s41598-024-52310-1.
[17] Wagner T, Joshi B, Janice J, Askarian F, Škalko-Basnet N, Hagestad OC, et al. *Enterococcus faecium* produces membrane vesicles containing virulence factors and antimicrobial resistance related proteins. J Proteomics 2018;187:28–38. doi:10.1016/j.jprot.2018.05.017.
[18] Azrad M, Matok LA, Leshem T, Peretz A. Comparison of FT-IR with whole-genome sequencing for identification of maternal-to–neonate transmission of antibiotic-resistant bacteria. J Microbiol Methods 2022;202:106603. doi:10.1016/j.mimet.2022.106603.