



## Introduction

- The extensive use of vancomycin and other antibiotics caused the emergence of vancomycin resistant bacteria (VRB) including *Enterococcus* which are major causes of hospital-acquired infections and which World Health Organization (WHO) placed on the list of bacterial priority pathogens list resistant to antimicrobials [1]. Resistance to vancomycin in VRB is primarily mediated by van genes (VRGs), among which the *vanA* and *vanB* phenotypes have the greatest clinical significance. They can be transferred between microbial cells via mobile genetic elements such as plasmids and transposons which determines the high epidemic potential of these strains [2] creating even more dangerous "superbugs" [3,4].
- Hospital wastewater (HW) discharged to wastewater treatment plants (WWTPs) is the main anthropogenic source of VRB, (VRGs and vancomycin in municipal wastewater, which are then finally released together with treated wastewater into surface water reservoirs and other elements of the environment [5,6].
- Therefore, the main goal of the study was to assess the concentration of VRGs in hospital wastewater collected from the whole territory of Poland, what was never done.

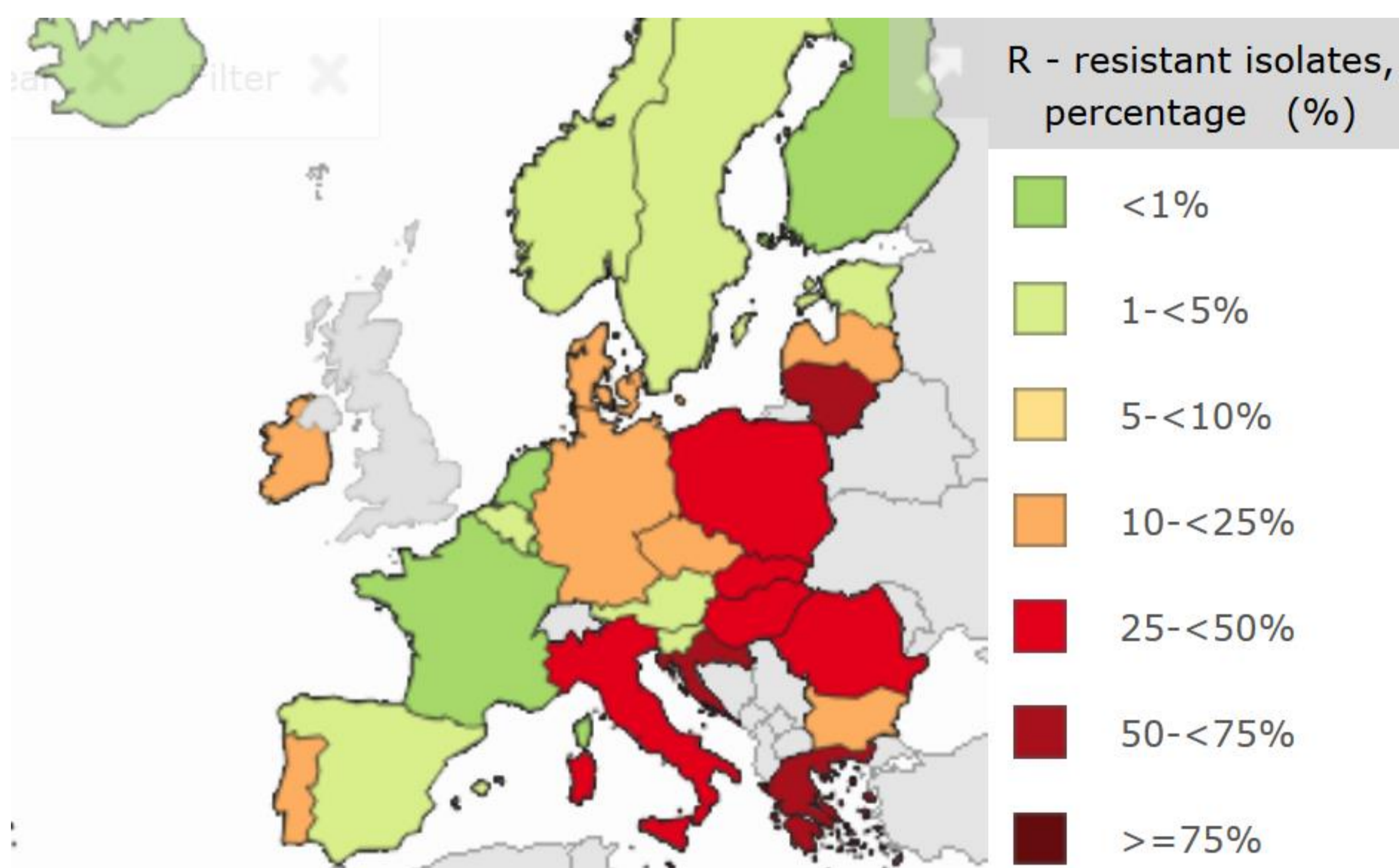


Fig. 1. Heatmap of vancomycin-resistant *Enterococcus faecium* in EU/EEA countries in 2024 based on the number of positive isolates among clinical isolates. Countries with the highest percentage of resistant strains are marked in dark red, countries with the lowest percentage of resistant strains are marked in green. No color indicates no data. Source: Surveillance Atlas of Infection Disease, ECDC, 2025 (<https://www.ecdc.europa.eu/en/publications-data/antimicrobial-resistance-eueea-ears-net-annual-epidemiological-report-2024>)

## Materials and Methods

- To the study, 64 hospitals located in various provinces of Poland have been selected differing in terms of the number of hospital beds, the number of patients served and the type of hospital wards.
- Hospital wastewater samples have been collected in winter and summer seasons due to differentiate the seasonal level of antibiotic consumption.
- Environmental DNA (eDNA) has been extracted from HW samples using FastDNA™ Spin Kit for Feces (MP Biomedicals).
- The concentration of VRGs has been analysed by high-throughput long read sequencing based on nanopore technology.
- To assess the abundance and taxonomic affiliation of ARGs, two bioinformatic tools were employed: Abricate and ARGpore2. These enabled the quantification of gene copy numbers per gigabase pair (Gbp) of the obtained Oxford Nanopore sequencing reads.
- Contigs assembled using the FLYE method facilitated the identification of precise ARG locations along with their species-level annotations.

## Results

- In both sampling seasons (winter and summer, 2024), qualitative and quantitative analyses of VRGs revealed considerable differences in genes counts between seasons and regions of HW samples' collection.
- The abundance of VRGs ranged from 0 to  $6.55 \times 10^2$  gene copies/Gb and was highest in HW collected in winter from central-eastern Poland.
- Among ARGs responsible for resistance to vancomycin *vanA* and *vanB* genes were presented most frequently and the most common hosts of VRGs were *Enterococcus faecium* and *Staphylococcus aureus*.

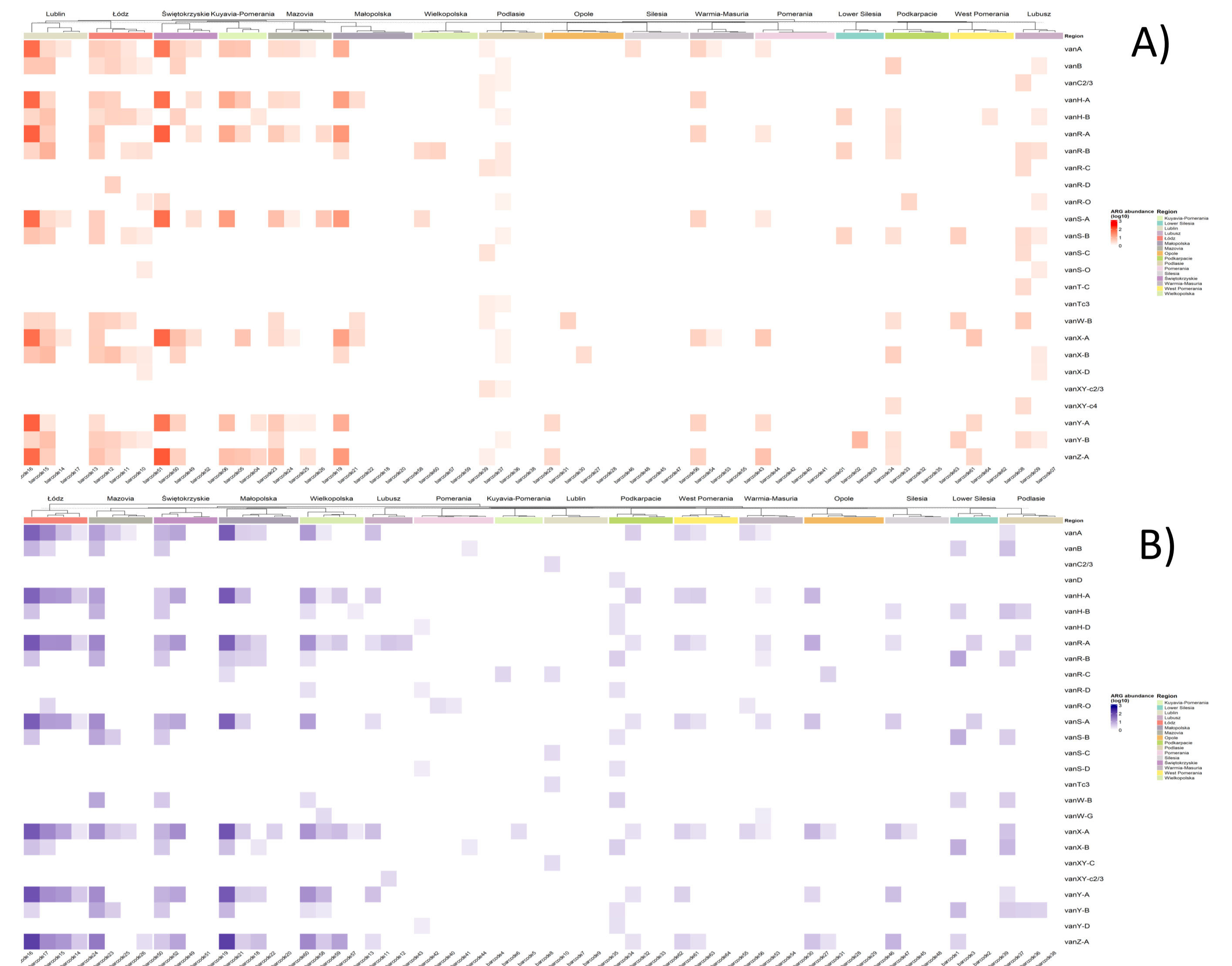


Fig. 2. Heat map of the mean regional abundance of antibiotic resistance genes (*van* group) in HWW samples collected from different regions in winter (A) and summer (B).

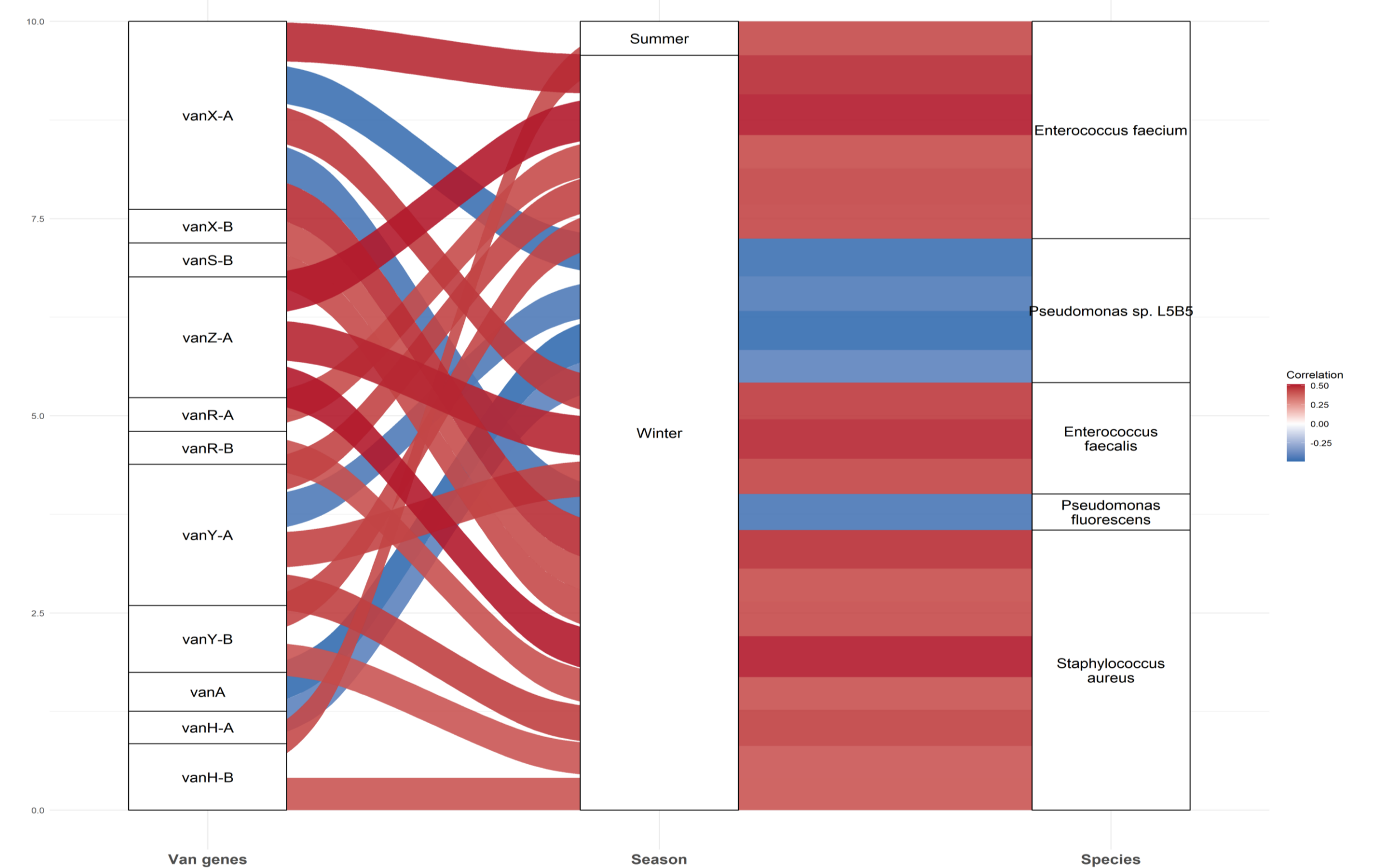


Fig. 3. Mean relative abundance of *van* genes and their relationship with priority pathogens classified by WHO across both seasons.

## Conclusions

- The results of the study carried out on the vancomycin resistance genes obtained from HW samples collected from hospitals located in various Polish districts were proved that untreated hospital wastewater could be the hotspots of VRGs and VRB enter into the environment.
- As a final result, the research can be used to clarify the requirements for hospitals and other health care facilities regarding the use of disinfection of wastewater discharged from individual facilities, as well as the requirements for microbiological quality of wastewater entering the WWTPs.

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