Seasonal Dynamics of the Airborne Resistome and Mobilome across Diverse Environments in the Belgrade Metropolitan Area

Katarina Novović[1*], Milka Malešević[1], Danka Matijašević[1], Nemanja Kljajević[1], Lazar Gardijan[1] & Stefan Stanovčić[1]

[1]Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Serbia
*Corresponding author: katarina.novovic@imgge.bg.ac.rs

Antibiotics 2026 Conference

Introduction

The role of the environment in the dissemination of antimicrobial resistance is increasingly recognized within the One Health framework. The airborne transmission route of antibiotic resistance genes (ARGs) is particularly significant since ARG-carrying bioaerosols could travel long distances and remain in the atmosphere for a long period of time. In addition, mobile genetic elements (MGEs) can promote the spread of ARGs in the environment.

Objective

This study aimed to characterize the seasonal dynamics of the airborne resistome and mobilome across diverse environments in the Belgrade metropolitan area, Serbia.

Methods

Outdoor air samples were collected at several urban, suburban, and rural locations across four seasons. Airborne DNA was subjected to shotgun metagenomic sequencing (Illumina NovaSeq X Plus), followed by bioinformatic analysis. Resistance genes were annotated using AMR++ v3.0 pipeline in conjunction with the MegaRes database v3.0, while integrons, insertion sequences (ISs), and plasmids were identified using Integrall v1.2 , ISFinder v2.0, and PLSDB v2024_05_31_v2, respectively. Seasonal differences were assessed using the Skillings–Mack test with Wilcoxon post hoc comparisons and Benjamini–Hochberg FDR correction, while associations were evaluated using Spearman's rank correlations.

Results

Resistome

Shotgun metagenomic analysis revealed pronounced seasonal variation in the composition and abundance of the airborne resistome across all sampled environments (Fig. 1). ARGs dominated the resistome across all seasons, followed by metal resistance genes (MRGs) and multi-compound resistance genes (MCRGs) (Fig. 1A). Resistome abundance and gene richness increased progressively from spring to winter, accompanied by a higher number of season-specific resistance genes during autumn and winter (Fig. 1B-E).

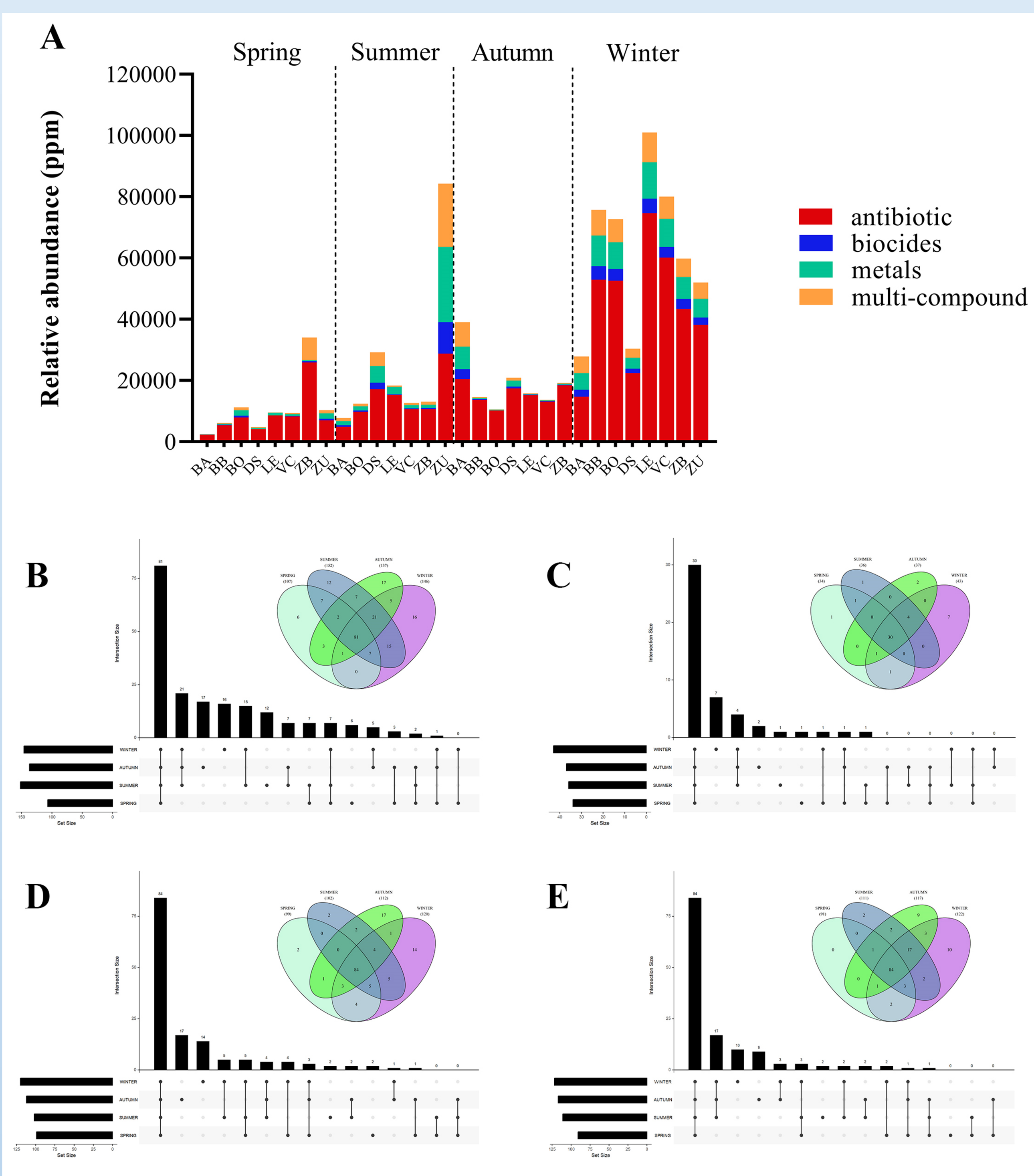

Figure 1. Seasonal abundance and diversity patterns of the airborne resistome. (A) Seasonal variation in abundance (ppm) of resistome components (ARGs, BRGs, MRGs and MCRGs). (B-E) UpSet plots with corresponding Venn diagrams illustrating the overlap and season-specific occurrence of genes across the four seasons for each resistome category: (B) ARGs, (C) BRGs, (D) MRGs and (E) MCRGs.

Mobilome

The airborne mobilome was dominated by integron- and plasmid-associated sequences, while ISs accounted for a smaller proportion of the total mobilome (Fig. 2A). Total MGE abundance exhibited a modest seasonal trend, with winter tending to show higher levels than other seasons. MGE diversity and the number of season-specific elements increased toward winter, driven mainly by plasmid-derived sequences (Fig. 2B-D).

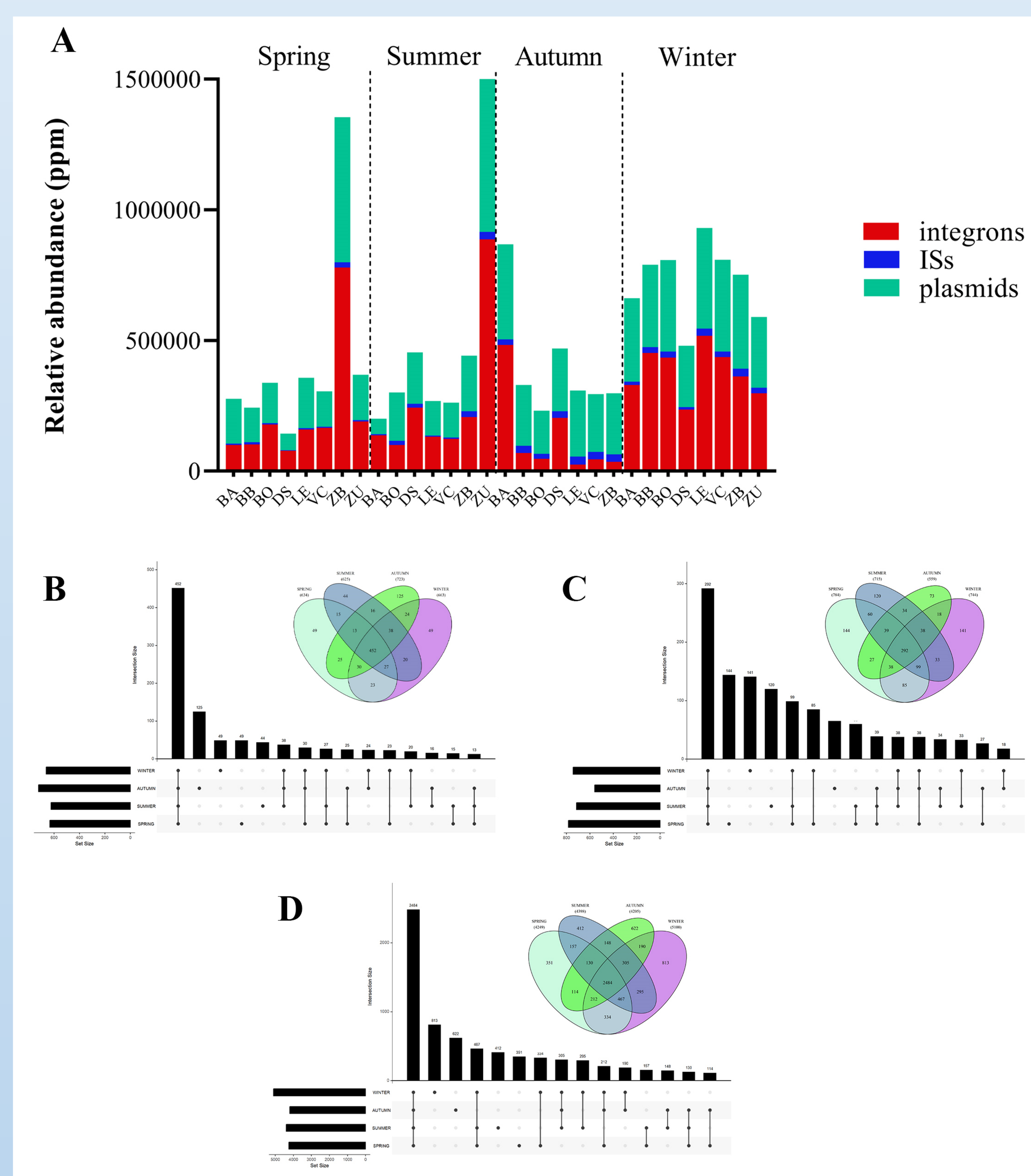

Figure 2. Seasonal abundance and diversity patterns of the airborne mobilome. (A) Seasonal variation in abundance (ppm) of mobilome components (integrons, ISs, and plasmid-derived sequences). (B–D) UpSet plots with corresponding Venn diagrams illustrating the overlap and season-specific occurrence of mobilome elements across the four seasons: (B) integrons, (C) ISs and (D) plasmid-associated sequences.

Correlation analysis

Significant positive correlations were observed among all resistome categories, while plasmid-associated sequences showed the strongest associations with resistome abundance. Within the mobilome, plasmids showed strong associations with both integrons and ISs, whereas integrons and ISs were not significantly correlated (Fig. 3).

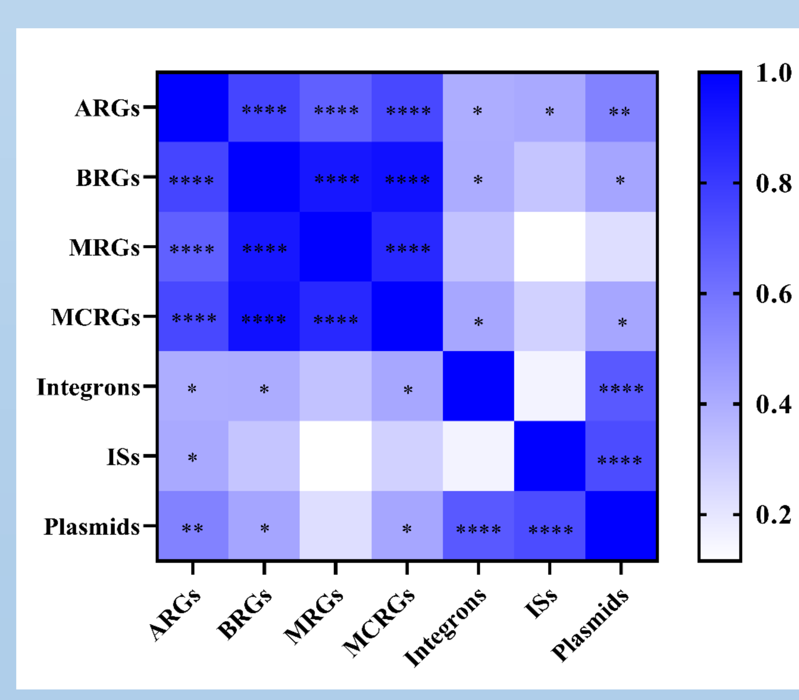

*Figure 3. Heatmap of Spearman correlation coefficients (ρ) among resistome categories (ARGs, BRGs, MRGs, MCRGs) and mobilome components (integrons, ISs, and plasmids). Color intensity reflects the strength of the correlation. Asterisks indicate statistically significant correlations (*p < 0.05; **p < 0.01; ****p < 0.0001).*

Conclusion

Air in Belgrade represents a reservoir and potential dissemination pathway for antimicrobial resistance, highlighting the need to include airborne environments in One Health surveillance.

This research was supported by the Science Fund of the Republic of Serbia (Promis, GRANT No. 10933, Project title – AirPollRes), Alfa Genetics d.o.o. and Novos d.o.o.