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Common Wood Pigeon (*Columba palumbus*): An Avian Bioindicator of Antimicrobial Resistance at the Human–Wildlife Interface

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Abstract

Antimicrobial resistance (AMR) is a silently escalating global crisis, presenting a specific challenge for the One Health approach. Landscapes can serve as reservoirs of AMR bacteria and genes, while synurban wildlife may act as vectors of bidirectional exchange. However, these species can also be utilised as sentinels of landscape AMR load. Herbivorous avian bioindicators, such as the Common Wood Pigeon (*Columba palumbus*), continuously sample the landscape during foraging and drinking, providing a homogenous spatial overview on the state of AMR. This study aimed to investigate the potential of this species for assessing the impact of landscape diversity on bacterial communities and their AMR patterns. Toward this objective, two spatial units of 4 km in diameter located at an upstream and a downstream section of a river, relative to a provincial town, were compared using 16 cloacal samples per site. Heterotrophic plate count techniques resulted in 60 isolates, of which 48 were identified, and 35 were tested for AMR using the VITEK 2 Compact system. The association between bacteriological findings and landscape diversity was analysed using Rényi diversity profiles. The Gram-positive/Gram-negative ratio was the only parameter that proved to be significantly different between the two study sites. The investigation detected six MDR isolates, with two methicillin-resistant phenotypes (*Staphylococcus gallinarum*, *Mammaliicoccus lentus*) and two carbapenem-resistant *Pseudomonas fluorescens* isolates. We established that the small sample size limits drawing definitive conclusions. However, exploring the link between landscape diversity and the cultivable faecal microbiota of herbivorous birds is a promising approach for more extensive research.

Keywords: Common Wood Pigeon; Rényi entropy; antimicrobial resistance; gram-positive/gram-negative ratio; landscape; sentinel

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1. Introduction

The One Health approach is predicated on the recognition that pathogens circulate dynamically across human, animal, and environmental domains [1–3]. While this phenomenon is conspicuous in the case of emerging infectious diseases, the dissemination of antimicrobial resistance (AMR) is far more insidious. Due to its stealthy progression across these interfaces, AMR is widely characterised as a 'silent pandemic' [2].

Evidence suggests that wildlife [2,4], surface water (including groundwater), and soil [3] can act as reservoirs for AMR bacteria and antimicrobial resistance genes (ARGs). The circulation of water throughout the ecosystem facilitates the movement of these bacteria and genes between the domains of health. Furthermore, AMR transmission via rivers and stormwater runoff is a well-documented phenomenon, highlighting critical environmental exposure pathways [3,5]. As the AMR crisis subtly escalates, adopting a holistic surveillance strategy, integrating all ecosystem components, is essential for estimating the situation's severity [3]. Avian species are frequently utilised as bioindicators of environmental AMR load because their active movement allows them to sample extended areas within their home ranges, providing a spatially comprehensive representation of the landscape's microbial state [1,6]. In contrast to small mammal populations, which provide point-source data [4], birds integrate landscape variations, offering a broader cross-sectional snapshot [6]. During feeding and water consumption, wildlife can contact resistant bacteria and their genes. The trophic transmission and accumulation of AMR in the ecosystem are well-documented [7,8]. Migratory species can transmit resistant bacteria between continents, causing long-distance influence of AMR sources [9]. On the other hand, ringing stations along flyways serve as potential surveillance points to collect faecal or oral samples from captured animals [10]. Birds with limited home ranges are extensively utilised as bioindicators of small-scale areas. For Feral Pigeons (*Columba livia*), the typical foraging range has a radius of approximately 2 km [11], whereas for Common Wood Pigeons (*Columba palumbus*), this distance varies with food availability (1–5.7 km). In mosaic landscapes with abundant forage sources, their flight range can be as small as <1 km [12,13]. However, it is worth noting that during migration, the animals encounter various landscapes with distinct nutrient sources and environmental stressors, hence, both the intestinal microbiome [14] and AMR status [7,8] change. The intestinal microbiome responds to dietary changes within weeks [15,16]. In the Hungarian populations of Common Wood Pigeons, spring migration ends by the beginning of April [17], while Feral Pigeons are non-migratory [11]. Although Wood Pigeons are opportunistic feeders, their diet is strictly based on plant materials [18]. In contrast, Feral Pigeons primarily exploit anthropogenic waste [11,19].

Trophic interactions facilitate the transmission of pathogens and pollutants from lower to higher trophic levels, and thus biomagnification in secondary consumer species [8,20]. Species with scavenger or predatory diets are considered bioaccumulators of pollutants in a specific habitat [21]; therefore, they are frequently employed as sentinels to detect even rare bacteria or resistant genes in the ecosystem. However, the use of accumulating organisms introduces a selection bias, which means that biological accumulation disproportionately enhances the effect of a hot spot within the sampled landscape [2,22]. Conversely, herbivorous animals can serve as proxies for background environmental health, providing a baseline reflection of the current ambient AMR load [2]. Unlike feral pigeons [19], gulls (Laridae family) and storks (*Ciconia* spp.) [23], granivorous and frugivorous birds typically avoid feeding at landfills. Consequently, their exposure to AMR sources is directly proportional to the frequency of these sources within the investigated landscape [2,24].

The Common Wood Pigeon has undergone a significant population expansion across Europe, with the breeding population nearly doubling since the 1990s, now estimated at 25.5–36.5 million pairs [25]. This demographic explosion is a complex process driven by shifting agricultural practices and the species' remarkable adaptive capacity to diverse environments [18,26]. From an epidemiological perspective, climate change is a critical driver; earlier spring arrivals and extended breeding seasons [27] have been accompanied by significant shifts in migratory patterns. The species' range has expanded northward, and an increasing number of individuals now overwinter in regions like the Carpathian

Basin, where this was previously uncommon [28]. In parallel, its local population nearly quadrupled [17].

The synurbanisation of the species has been ongoing in Western Europe since the 19th century and has intensified in Central Europe since the 1980s [29]. Today, population densities in urban and suburban areas often significantly exceed those of rural populations [30]. This ecological shift carries profound environmental health implications: the presence of Wood Pigeons in human-dominated landscapes leads to the accumulation of organic matter (faecal deposition), which directly alters the microbial composition of local ecosystems [31]. Although Wood Pigeons do not consume anthropogenic waste [24], they feed on intensive arable lands [18] and rely on potentially polluted surface waters, which can serve as reservoirs of AMR [8]. As Wood Pigeons can serve as reservoirs for antimicrobial-resistant (AMR) bacteria [32], their role as biological vectors has gained prominence due to increased human–wildlife contact. Expanding populations within synanthropic environments [17] might contribute to the rising health risk caused by faecal contamination of urban spaces [11,19].

In accordance with the Birds Directive, the Common Wood Pigeon is a huntable species across Europe. Due to its significant impact on crop damage, hunting is widely employed as a population control measure in most Member States [33,34]. Hunter-harvested individuals are suitable for sample collection for bacteriological investigation. Furthermore, the species' large populations facilitate large-scale, minimally invasive swab sampling of live birds at ringing stations throughout Europe [6]. Such surveillance can provide critical data on the AMR carriage of these birds, thereby reflecting the AMR load along their migratory flyways [35].

Heterotrophic plate count (HPC) bacteria are extensively used in drinking water quality control, as well as in environmental monitoring programs. These bacteria are non-fastidious, aerobic or facultatively anaerobic, and mesophilic, allowing for rapid growth on general culture media. These characteristics make them highly suitable for surveillance efforts regarding environmental AMR dynamics [36–38]. Although HPC techniques possess limited sensitivity and specificity, these are generally applied to detect cultivable pathogenic bacteria and AMR in wildlife [39–41].

This pilot study aimed to develop an exploratory method to search for potential AMR reservoirs within limited spatial units of a landscape. We hypothesised that Common Wood Pigeons, as herbivore birds, interact with vegetation, soil and surface water throughout their home range, while they avoid anthropogenic food sources. Therefore, faecal samples of these birds reflect the AMR load of their habitat, including cryptic sources. Our research objectives were

(1) To investigate the cultivable bacterial community composition of Wood Pigeon faecal samples using the HPC technique;
(2) To determine the phenotypic resistance pattern of the isolated HPC bacteria;
(3) To analyse the potential association between bacteriological findings and landscape features, like landscape diversity and longitudinal river sections (upstream vs. downstream).

The HPC technique has limited accuracy, and phenotypic resistance cannot provide comprehensive insight into AMR load of the landscape, while the landscape diversity profile only approximately corresponds to biodiversity. For this reason, this study aimed to test this high-throughput screening method to demonstrate its suitability as a preliminary survey to highlight potential investigation directions for a more accurate AMR surveillance within a specific landscape.

2. Materials and Methods

2.1. Study Area

The study area is part of the Zagyva River catchment system, located in the northern part of the Great Hungarian Plain. The river originates within Hungary, approximately 100 river kilometres (rkm) upstream from the sampling sites. Four small industrial towns are situated along the Zagyva River: Salgótarján, Bátonyterenye, Pásztó, and Hatvan. Among these, Hatvan is located closest to the study area. Since more than half of the river's water supply is effluent from municipal and industrial wastewater treatment plants [42], riverside towns represent a significant urban-industrial influence. This lowland region lies at an altitude of approximately 86 m within the continental temperate zone. Both sampling sites (with a 4 km diameter) were situated in the suburban zone of Jászberény, a small provincial town characteristic of the region. This size of the investigated area was set based on the average home range calculated according to the literature data [12,13]. Our analyses considered the two spatial units as proxies of the pigeons' home ranges where they sought food and water, and thus quasi-sampled the environment evenly.

By the upper streams of the Zagyva River, Salgótarján and Bátonyterenye were heavy industrial centres with significant and long-term (170 years) coal mining and metal industrial activity, and glassworks [43,44]. Consequently, the urban soil of these towns contains more than average heavy metals and metalloids, which originate from the ruins of former smelters, coal mines, coal-fired power plants, 'slag hill', 'ash cone', etc. These local point sources pollute their environment and surface waters [45]. The heavy metal and metalloid pollution of the river is detectable, especially in samples collected at low water discharges [46].

The landscape around Jászberény town was formed by the Zagyva River, which was not regulated until the middle of the 20th century [47]. The dominant upper soil is meadow soil that originated from sediments deposited by the river [48]. The upper stream has a stream gradient of 16.7 m/km, which decreases to 0.64 m/km when the Zagyva reaches the plain above Jászberény town [47]. Consequently, a thick layer of fluvial sediment characterises the topsoil of both study sites [48]. However, the soil water regime at the JFGY site is more advantageous than at JB [49], probably due to the meandering river section here, which facilitates the water retention capacity of the landscape [47].

The river flows through the town, originating from the direction of the Jászfelsőszentgyörgy sampling site (JFGY; 47.498276/19.848227) toward the northern outskirts of Jászberény (JB; 47.515572/19.935496). The upstream section relative to the town is characterised by a meandering riverbed with semi-natural floodplain forests along the banks. These forests provide essential foraging and nesting habitats for the Common Wood Pigeon.

Within the town, the river is primarily confined between artificial embankments. Few sections retain canopy cover or riparian vegetation; consequently, the flow velocity is higher within this urban reach. The JB sampling site is situated on the downstream section relative to the town centre. The studied river reaches at JFGY and JB are 5.1 km and 4.8 km in length, respectively (Figure 1).

Although wastewater management has improved remarkably since Hungary acceded to the European Union, the Zagyva River was historically a well-known example of heavy water pollution. The legacy of this industrial and communal contamination, spanning decades of the 19th and 20th centuries, is well-documented across the river and its tributaries [50–52].

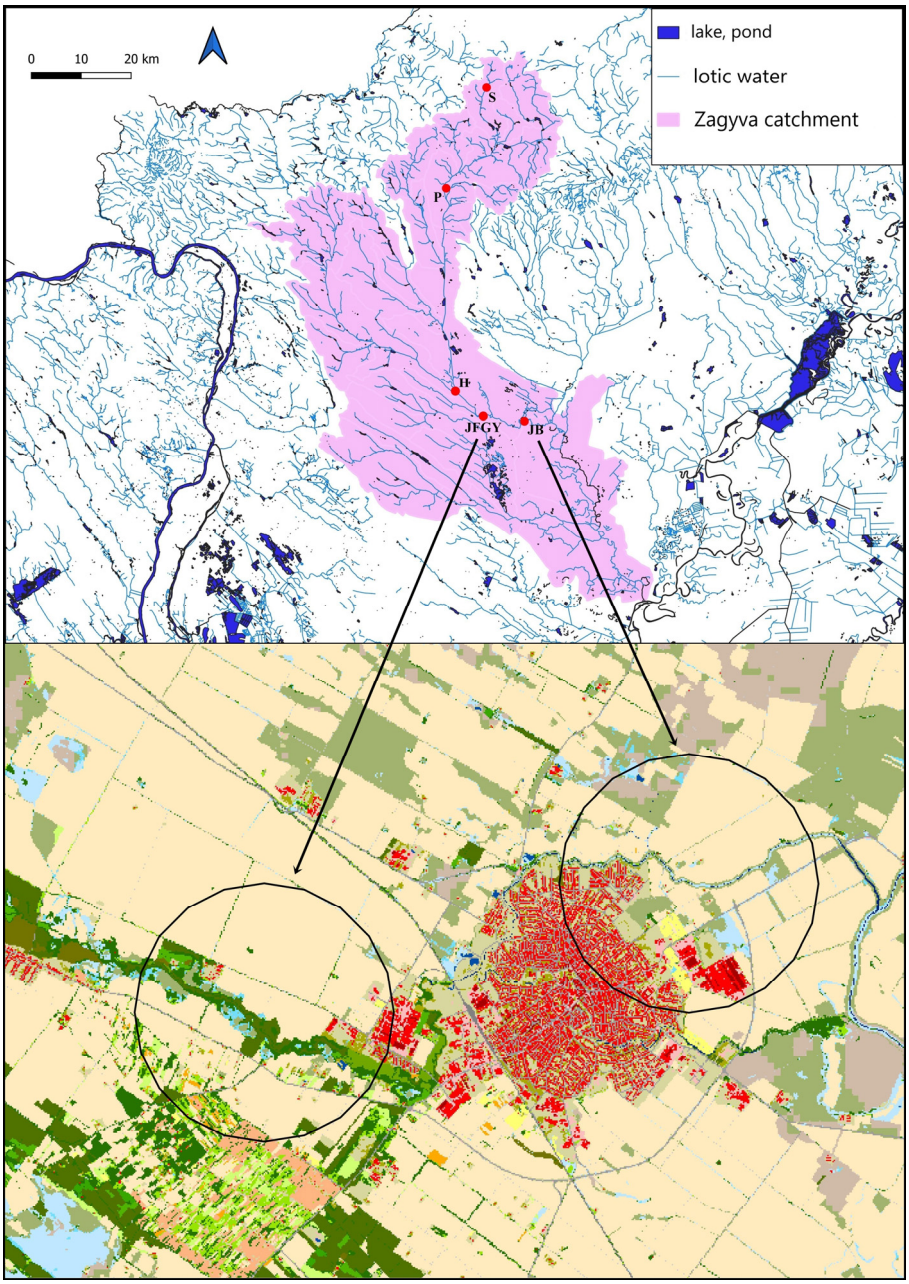


Figure 1. Location of the two study sites, Jászfelsőszentgyörgy (JFGY) and Jászberény (JB) within the Zagyva River's catchment (upper map): Salgótarján (S), Pásztó (P), and Hatvan (H) are industrial hubs along the river's upstream sections. Land cover characteristics of the two study sites (lower map): Red blocks: buildings (shades indicate height), grey blocks: roads (shades by hierarchy, lightest: unpaved), brownish blocks: agricultural land (shades by vegetation height, sand: arable land), green blocks: seminatural vegetation (shades by height, lightest: grassland), khaki blocks: urban greenery (shades by height, lightest: grass), blue blocks: surface water (shades by depth, lightest blue: temporary coverage). Diameters of the sampling sites (black framed circles): 4 km.

2.2. Sample Collection

The carcasses (N = 32) involved in the study were obtained from regular pest control activities regulated by Hungarian law, specifically Act No. 1996/LV on Wildlife and Act No. 79/2004. (V. 4.) FVM Regulation on its implementation. In the Great Plain of Hungary, the urbanisation of Common Wood Pigeon is a general phenomenon [17]. Compared to natural habitats, in urban and suburban environments, the nesting success significantly increased due to reduced nest predation [53]. Consequently, in specific hot spots, the popu-

lation size of Common Wood Pigeon expanded [17], and the species became a significant agricultural pest [18,24]. In densely populated areas, the regional Hunting Authority issues permits for population control through out-of-season hunting. The activity must comply with Article 7 of Directive 2009/147/EC and remains limited to crop protection, ensuring it does not jeopardise conservation efforts within the species' distribution area [34].

At the JFGY and JB sites, sampling events took place on 27 April and 31 May 2024, respectively. Only carcasses showing no visible signs of health issues were included in the study. To avoid sex-based bias, the sample size was limited by the smallest group, female harvested at the JFGY site. This group consisted of eight suitable carcasses; therefore, eight specimens were collected from each of the other groups as well. Consequently, 32 carcasses (8 of each sex per site) were included in the study.

After harvesting and a vision-only health check by a trained person, all carcasses were subjected to biometric measurements (body weight, body, wing, tail, and bill length) according to the method described by [54]. A bacteriological sample was taken with a sterile swab from the cloacal lumen and placed into transport medium (A0590 Amies transport medium with charcoal, Sigma-Aldrich, Merck KGaA, Darmstadt, Germany). Inoculated transport media were stored and transported at 4 °C until laboratory processing. Samples were transported to the laboratory on the day after collection. Bacteriological procedures were initiated within 36 h of collection.

2.3. Bacteriological Investigation

Transport swabs were incubated in 5 mL of buffered peptone water (BPW) liquid medium (produced in-house from buffered peptone water, GranuCult® prime; Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) at 37 °C temperature for 24 h to ensure the revival of bacteria. From each tube of BPW, 100 µL inoculum was spread on the surface of plate count agar (PCA; prepared in-home with plate count agar, GranuCult® prime; Sigma-Aldrich, Merck KGaA, Darmstadt, Germany in accordance with the manufacturer's instructions). Inoculated PCA plates were incubated for 48 h at 37 °C and were checked every 24 h.

Pure cultures were obtained by transferring separate colonies onto PCA plates using a four-quadrant streak plate method. Gram staining was performed on pure cultures using the Gram-Nicolle Kit (VWR International, LLC, Radnor, PA, USA) in accordance with the manufacturer's instructions to differentiate between Gram-positive (GP) and Gram-negative (GN) bacteria.

For identification, the VITEK 2 Compact system (bioMérieux, Marcy-l'Étoile, France) was employed. For GP and GN bacteria, VITEK 2 GP (Ref. No. 21342) and VITEK 2 GN (Ref. No. 21341) cards were used, respectively. Antimicrobial susceptibility testing (AST) was carried out on bacterial isolates identified with at least 80% probability. Bacterial species lacking an AST record in the VITEK database (e.g., *Kocuria* spp.) were excluded from this phase. For GPs, the investigated antibiotics included: cefoxitin (FOX), benzylpenicillin (B-PEN), oxacillin (OXA), gentamicin (GEN), ciprofloxacin (CIP), moxifloxacin (MOX), erythromycin (ERY), clindamycin (CLIN), linezolid (LIN), teicoplanin (TEI), vancomycin (VAN), tetracycline (TET), tigecycline (TIGE), fosfomycin (FOM), fusidic acid (FUS), rifampicin (RIF), and trimethoprim (TRIM). For GNs, ampicillin/sulbactam (AMP/SAM), ticarcillin/clavulanic acid (TIC/CLA), piperacillin (PIP), piperacillin/tazobactam (TZP), ceftazidime (CZD), cefepime (CFP), aztreonam (AZT), imipenem (IMI), meropenem (MER), amikacin (AMI), gentamicin (GEN), tobramycin (TOB), ciprofloxacin (CIP), levofloxacin (LEV), colistin (COL), and trimethoprim/sulfamethoxazole (SXT) were tested. These analyses were performed using VITEK 2 AST-P592 (Ref. No. 222887) and VITEK 2 AST-N331

(Ref. No. 418675) cards, respectively. Findings were exported to Excel sheets (Microsoft® Excel® for Microsoft 365 MSO, Version 2511).

Regarding quality control, while external reference strains were not processed in parallel with test specimens, strict internal validation criteria were applied. Only isolates achieving a 'Good' or higher confidence level in identification were included. Isolates with low discrimination levels were excluded unless the CLSI interpretive breakpoints for both potential species were identical, ensuring the validity of the AMR data. Furthermore, all inocula were standardised to a turbidity of 0.5–0.63 McFarland via VITEK DensiCHEK device (bioMérieux, Marcy-l'Étoile, France), and only 'Consistent' antibiograms validated by the VITEK 2 Advanced Expert System (AES) were reported.

It is worth noting that this automated system was developed for clinical use; therefore, it is optimised for pathogenic bacteria. For this reason, its database contains the biochemical features and antimicrobial phenotypes of a few environmental bacteria. Furthermore, its diagnostic accuracy is lower than the gold standard methods [41,55].

2.4. Data Analysis

For spatial analysis, land cover data were obtained from http://alapterkep.termeszetem.hu/ (accessed on 6 December 2025). Spatial representation and analysis were performed using QGIS version 3.16 (Hannover). To calculate the proportion of land cover categories, we used the software's "Raster layer unique values report" algorithm. The specific patch types were classified into subcategories based on their functional relevance, such as grouping orchards and vineyards as intensively cultivated areas and potential foraging sites for pigeons. These subcategories were aggregated into main categories: urban areas (URBs), agricultural lands (AGROs), semi-natural habitats (SEMINATs), and surface waters (WATERs). Sunburst diagrams were generated to visualise the hierarchical distribution of patch types and their respective categories across the two study sites. As the river provides the most significant water source for the regional wildlife, the same land cover classification was conducted within a 50 m-wide buffer zone along the river.

Several methods have been introduced in ecology to estimate diversity. Since species richness alone is insufficient for comparing communities—for instance, a community dominated by a few species is less diverse than an even one with the same number of species—various indices have been developed [56,57]. However, standard indices often fail to address extreme scenarios, such as the presence of numerous rare species or the absolute dominance of a few. These metrics remain biased toward specific aspects of diversity: the widely used Shannon index is sensitive to rare species, the Simpson index emphasises dominant species, while the Berger–Parker index reflects only the single most abundant taxon [56,58].

Rényi entropy (H_α) offers a more robust approach by providing a comprehensive diversity profile instead of a single value. It is based on a family of functions that simultaneously accounts for both rare and dominant elements, characterised by the scale parameter alpha (α). At $\alpha = 0$, the value corresponds to species richness; thus, this section of the profile is highly sensitive to rare species. As α approaches 1, the value converges to the Shannon index. At $\alpha = 2$, the curve represents quadratic diversity (Simpson index), reflecting the influence of dominant species. As alpha tends toward infinity, the curve flattens and yields the Berger–Parker index. Consequently, Rényi entropy is uniquely suited for analysing complex communities where both rare and dominant species play critical roles [56,57].

To evaluate differences in landscape heterogeneity between the two sampling sites, landscape diversity was quantified using Rényi's generalised entropy, calculated as follows [56,58]:

$$H_\alpha = \frac{1}{1-\alpha} \ln\left(\sum_{i=1}^{s} p_i^\alpha\right) \quad (1)$$

where H_α is the Rényi entropy, α is the scale parameter, and p_i is the relative frequency of the given land cover type in the sample.

The diversity profiles were visualised to compare landscape evenness and richness across a range of scale parameters (α). The calculation and visualisation of Rényi's entropy were conducted within the R (v4.4.2) statistical environment [59] using vegan [60], plotly [61], and ggplot2 [62] packages.

In contrast to single-value indices, Rényi's entropy provides a more comprehensive insight into the structural complexity of the investigated landscape. Instead of providing a static parameter, it characterises the balance between dominant habitat types and the preservation of rare habitats. Since this study aimed to evaluate the anthropogenic impact on the background AMR load, assessing landscape naturalness through Rényi's diversity profiles proved essential. These curves enabled us to investigate the specific association between the degree of naturalness and the spatial distribution of AMR load within distinct landscape units [56,63].

Since a preliminary health check was conducted by a game meat inspector following the steps of a visual-only meat inspection, the morphometric analyses aimed to compare the populations of the two sampling sites and to identify outlying individuals that might indicate specific health issues. Mean ($\bar{x}$) and standard deviation (SD) were calculated for all morphometric parameters at both sites (JFGY and JB). After confirming normality using the Shapiro–Wilk test, population datasets were compared using independent-samples *t*-tests in the PAST (version 4.17) statistical software [64]. It was hypothesised that consistent body measurements in full-grown individuals correlate with a healthy population with a faecal microbiome free from pathological alterations.

With respect to the bacteriological data, the GP/GN ratios, the diversity of bacterial communities, and resistance phenotypes, the Multiple Antibiotic Resistance (MAR) indices and the prevalence of multidrug-resistant (MDR) bacteria were determined for both sampling sites. The GP/GN ratio was employed as a proxy for environmental integrity, as anthropogenic disturbances, such as the removal of vegetation coverage [65], intensive agricultural activity [66], pesticide contamination [67], or industrial wastewater discharge [68], typically shift the community structure toward a predominance of GN taxa. Previous studies conducted in heavy industrial centres by the Zagyva River revealed a dominance of Proteobacteria and a high occurrence of MDR genotypes in polluted urban soil [43,44,69]. The Chi-square test was used to compare these parameters between the two sites (R software version 4.4.2).

To determine the GP/GN ratio, all pure bacterial isolates were included based on Gram staining results, regardless of identification success. To avoid bias, if multiple isolates of the same species were recovered from a single animal, only one was included in the analysis. The MAR index was calculated as the proportion of the total number of phenotypic resistance occurrences in the total number of AST tests performed. These indices were determined globally for both sampling sites, as well as separately for isolates within each location. The prevalence of MDR was calculated both within the sampled bird populations and among all AST-tested isolates. Bacterial strains exhibiting resistance to at least three different antimicrobial classes were classified as MDR.

For better insight into the ecological interdependence of landscape composition, bacterial community structure, and AMR pattern, the diversity of these parameters was assessed and compared between sites using Rényi's entropy analysis in the R environment (version 4.4.2) [59] employing vegan [60], plotly [61], and ggplot2 [62] packages.

Our research data are available in Zenodo repository at https://zenodo.org/records/19286829 (accessed on 3 April 2026) with a README file adapted from Conzett and Dijkstra Haugstvedt [70].

2.5. Use of Generative AI

During the preparation of this manuscript, the authors used Google Gemini 1.5 Pro version 3.1 for the purposes of correcting grammatical errors and improving fluency. The authors have reviewed and edited the output and take full responsibility for the content of this publication.

3. Results

Visual-only game meat inspection, conducted by a trained person, revealed no visible signs of disease in the carcasses. Morphometric analysis revealed that the average body mass of the individuals was 496 ± 32 g at the JB site and 476 ± 28 g at the JGYF site. The mean body length was 422 ± 12 mm and 425 ± 12 mm, while the mean wing length was 242 ± 5 mm and 244 ± 7 mm for the JB and JGYF sites, respectively. Statistical comparison showed no significant differences between the two locations regarding body mass ($t(30) = 1.839$; $p = 0.076$), body length ($t(30) = 0.674$; $p = 0.505$), or wing length ($t(30) = 1.014$; $p = 0.318$).

Although both sites are characterised by a dominant habitat class (AGRO) consisting primarily of cropland, alongside numerous smaller habitat patches, the sunburst diagrams revealed remarkable structural differences between the two units (Figure 2).

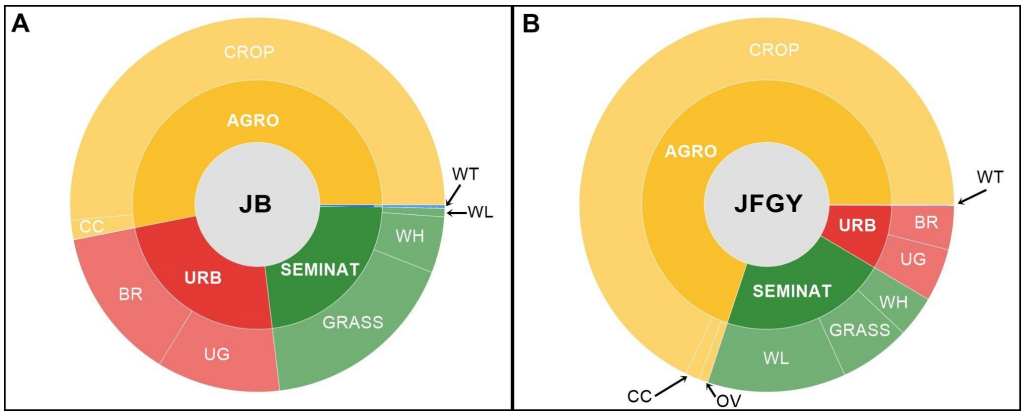


Figure 2. Comparison of sunburst diagrams regarding landscape compositions at the study sites Jászberény (**A**) and Jászfelsőszentgyörgy (**B**). Abbreviations: JB = Jászberény, JFGY = Jászfelsőszentgyörgy, AGRO = agricultural land, SEMINAT = seminatural habitat, URB = urban area, CROP = cropland, CC = complex cultivation, OV = orchard and vineyard, GRASS = grassland, WH = wet habitat, WL = woodland, WT = surface water, BR = buildings and roads, UG = urban greenery.

Comparing the land cover characteristics of the two study sites, it is conspicuous that both sites are dominated by agricultural-type habitats. The main distinctions are that at sites JB and JFGY, a slightly higher proportion of urban and seminatural habitats was observed, respectively (Table 1).

The Rényi entropy profiles of the two sampling sites demonstrated that overall landscape diversity had higher evenness at site JB; however, the riverine buffer zone was significantly more diverse at site JFGY (Figure 3).

Table 1. Land cover subcategories in percentage (%) rounded to one decimal place at the study sites, Jászberény (JB) and Jászfelsőszentgyörgy (JFGY).

Land Cover Subcategories	JB	JFGY
urban greenery	10.7%	4.5%
buildings and roads	13.2%	3.8%
cropland	51.4%	67.9%
orchard and vineyard	0.0%	0.8%
complex cultivation	1.7%	1.2%
grassland	17.2%	6.1%
wet habitat	4.8%	3.6%
woodland	0.7%	11.9%
surface water	0.4%	0.1%

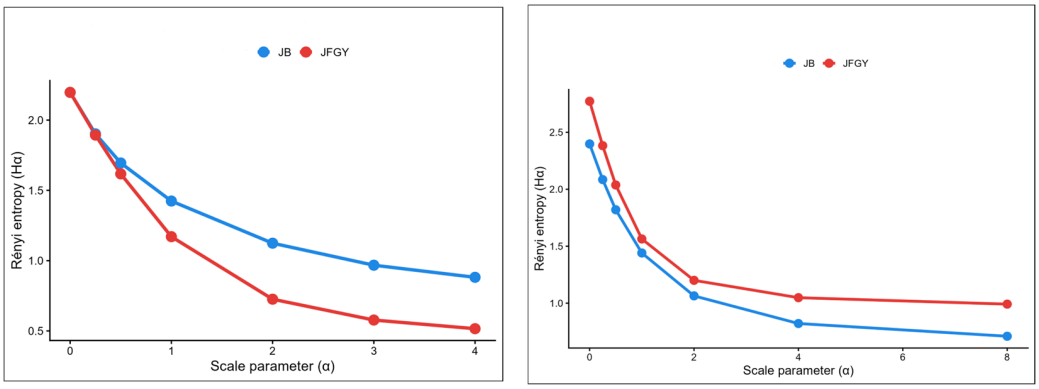


Figure 3. Rényi landscape diversity profiles of the study sites (**a**) and their 50 m-wide riverine buffer zones (**b**). H_α represents the Rényi entropy, and α is the scale parameter. Specific values shown: $\alpha = 0$ (landscape type richness), $\alpha \rightarrow 1$ (Shannon diversity index), $\alpha = 2$ (Simpson dominance index). Abbreviations: JB—Jászberény; JFGY—Jászfelsőszentgyörgy.

Of the 32 cloacal swabs examined, 31 tested positive for HPC bacteria, resulting in 60 bacterial isolates. Of these, 48 were successfully identified, and 35 were AST-tested using the VITEK 2 Compact system (Table 2). The number of isolates per site was equal ($N = 30$), while the number of isolates per carcass ranged from 0 to 4. Interestingly, the GP/GN ratios were reversed between the two sites, being 20:10 at JB and 10:20 at JFGY.

Table 2. Heterotrophic plate count bacteria isolated from faecal samples of Common Wood Pigeon at the study sites, Jászberény (JB) and Jászfelsőszentgyörgy (JFGY), their antimicrobial resistance (AMR) pattern with minimal inhibitory concentration (MIC) in µg/mL in parenthesis following the drug name, and multiple antibiotic resistance (MAR) index with 95% confidence intervals (CI 95%).

Site	Sample ID	Sex [1]	Isolate ID	Gram Type [2]	Organism	Profile of Decreased Susceptibility (MIC in µg/mL)	Tested AM [3]	MAR Index [CI 95%]
JB	JB-01	M	JB_01.1	GP	*Enterococcus faecium*	Trimethoprim/Sulfamethoxazole (≤10) [4]	9	0.111 [0.003–0.482]
			JB_01.2	GN	*Enterobacter cloacae* complex	Colistin (≤0.5) [5]	14	0.071 [0.002–0.339]
JB	JB-02	M	JB_02.1	GP	*Enterococcus faecium*	Trimethoprim/Sulfamethoxazole (≤10) [4]	9	0.111 [0.003–0.482]
			JB_02.2	GN	*Klebsiella oxytoca*	Piperacillin (16.0)	16	0.062 [0.002–0.302]

Table 2. *Cont.*

Site	Sample ID	Sex [1]	Isolate ID	Gram Type [2]	Organism	Profile of Decreased Susceptibility (MIC in µg/mL)	Tested AM [3]	MAR Index [CI 95%]
JB	JB-03	F	JB_03.1	GP	*Enterobacter cloacae* complex	n.i.	n.a.	n.a.
			JB_03.2	GN	Unidentified Organism	n.i.	n.a.	n.a.
JB	JB-04	F	JB_04.2	GP	*Enterococcus faecium*	Trimethoprim/Sulfamethoxazole (≤10) [4]	9	0.111 [0.003–0.482]
JB	JB-05	F	JB_05.1	GN	*Klebsiella* spp. (low discr.)	Ampicillin/Sulbactam (16.0), Ticarcillin/Clavulinic acid (≤8), Piperacillin (8.0)	15	0.200 [0.043–0.481]
JB	JB-06	M	JB_06.1	GN	*Enterobacter cloacae* complex	Colistin (≤0.5) [5]	15	0.067 [0.002–0.319]
			JB_06.2	GP	*Enterococcus raffinosus*	none	1	0.000 [0.000–0.975]
JB	JB-07	F	JB_07.3	GP	*Enterococcus faecium*	Trimethoprim/Sulfamethoxazole (≤10) [4]	9	0.111 [0.003–0.482]
			JB_07.1	GP	*Kocuria rhizophila*	n.i.	n.a.	n.a.
			JB_07.2	GN	Unidentified Organism	n.i.	n.a.	n.a.
JB	JB-08	F	JB_08.1	GP	Dermacoccaceae (low discr.)	n.i.	n.a.	n.a.
			JB_08.3	GP	*Enterococcus faecium*	n.i.	n.a.	n.a.
JB	JB-09	M	JB_09.3	GP	*Staphylococcus gallinarum*	Cefoxitin Screen (POS), Benzylpenicillin (≥0.5), Erythromycin (≥8), Tetracycline (2.0), Fosfomycin (≥128), Fusidic Acid (4.0), Rifampicin (1.0) [4]	14	0.500 [0.230–0.770]
			JB_09.1	GP	Unidentified Organism	n.i.	n.a.	n.a.
			JB_09.2	GP	Unidentified Organism	n.i.	n.a.	n.a.
JB	JB-10	F	JB_10.0	GP	Unidentified Organism	n.i.	n.a.	n.a.
JB	JB-11	M	JB_11.1	GP	*Enterococcus faecium*	Trimethoprim/Sulfamethoxazole (≤10) [4]	9	0.111 [0.003–0.482]
			JB_11.2	GN	Unidentified Organism	n.i.	n.a.	n.a.
JB	JB-12	F	JB_12.2	GP	*Rothia kristinae*	n.i.	n.a.	n.a.
			JB_12.0	GN	*Enterobacter cloacae* complex	Colistin (≤0.5) [5]	14	0.071 [0.002–0.339]
JB	JB-13	F	JB_13.2	GN	*Enterobacter cloacae* complex	n.i.	n.a.	n.a.
JB	JB-14	M	JB_14.1	GP	*Staphylococcus* (*syn. Mammaliicoccus*) *lentus*	Cefoxitin Screen (POS), Benzylpenicillin (≥0.5), Oxacillin (≥4), Fosfomycin (≥128), Rifampicin (≥32)	16	0.312 [0.110–0.587]
			JB_14.2	GP	Unidentified Organism	n.i.	n.a.	n.a.
JB	JB-15	M	JB_15.1	GN	Escherichia coli	none	15	0.000 [0.000–0.218]
			JB_15.3	GP	Enterococcus faecium	Trimethoprim/Sulfamethoxazole (≤10) [4]	9	0.111 [0.003–0.482]

Table 2. *Cont.*

Site	Sample ID	Sex [1]	Isolate ID	Gram Type [2]	Organism	Profile of Decreased Susceptibility (MIC in µg/mL)	Tested AM [3]	MAR Index [CI 95%]
JB	JB-16	M	JB_16.1	GP	*Enterococcus faecalis*	Trimethoprim/Sulfamethoxazole (≤10) [4]	9	0.111 [0.003–0.482]
			JB_16.2	GP	Unidentified Organism	n.i.	n.a.	n.a.
JFGY	JFGY-01	M	JFGY_01.1	GP	*Staphylococcus* (*syn. Mammaliicoccus*) *sciuri*	Benzylpenicillin (0.5), Clindamycin (≤0.25), Fusidic Acid (8.0)	16	0.188 [0.040–0.456]
JFGY	JFGY-02	M	JFGY_02.3	GP	*Kocuria rosea*	n.i.	n.a.	n.a.
			JFGY_02.2	GN	*Sphingomonas paucimobilis*	n.i.	n.a.	n.a.
			JFGY_02.1	GN	*Stenotrophomonas maltophilia*	n.i.	n.a.	n.a.
JFGY	JFGY-03	F	JFGY_03.1	GN	*Aeromonas hydrophila*	Ciprofloxacin (1.0)	3	0.333 [0.008–0.906]
			JFGY_03.2	GN	*Ewingella americana*	none	15	0.000 [0.000–0.218]
			JFGY_03.3	GN	*Ac. lwoffii*/*Ps.* spp. (low discr.)	n.i.	n.a.	n.a.
			JFGY_03.4	GN	*Sphingomonas paucimobilis*	Amikacin (32.0) [4], Gentamicin (8.0) [4], Tobramycin (≥16), Ciprofloxacin (≥4)	4	1.000 [0.398–1.000]
JFGY	JFGY-04	F	JFGY_04.1	GN	*Serratia liquefaciens* group	Colistin (≥16)	14	0.071 [0.002–0.339]
			JFGY_04.2	GN	*Serratia fonticola*	Colistin (≤0.5) [5]	14	0.071 [0.002–0.339]
JFGY	JFGY-06	F	JFGY_06.1	GP	*Enterococcus durans*	n.i.	n.a.	n.a.
			JFGY_06.2	GN	*Sphingomonas paucimobilis*	Tobramycin (8.0) [4]	4	0.250 [0.006–0.806]
			JFGY_06.3	GP	*Enterococcus faecalis*	Trimethoprim/Sulfamethoxazole (≤10) [4]	9	0.111 [0.003–0.482]
JFGY	JFGY-07	M	JFGY_07.1	GN	*Stenotrophomonas maltophilia*	n.i.	n.a.	n.a.
			JFGY_07.2	GN	*Aeromonas salmonicida*	n.i.	n.a.	n.a.
			JFGY_07.3	GN	*Serratia fonticola*	n.i.	n.a.	n.a.
JFGY	JFGY-08	M	JFGY_08.1	GN	*Sphingomonas paucimobilis*	none	4	0.000 [0.000–0.602]
			JFGY_08.4	GP	*Staphylococcus equorum*	Erythromycin (≥8), Clindamycin (≤0.25), Fosfomycin (≥128)	15	0.200 [0.043–0.481]
JFGY	JFGY-09	M	JFGY_09.1	GP	Unidentified Organism	n.i.	n.a.	n.a.
			JFGY_09.2	GN	*Citrobacter braakii*	Ampicillin/Sulbactam (1.0)	15	0.067 [0.002–0.339]
JFGY	JFGY-10	F	JFGY_10.1	GN	*Acinetobacter lwoffii*	Ticarcillin/Clavulinic acid (32.0) [4]	10	0.100 [0.003–0.445]
			JFGY_10.2	GN	*Serratia fonticola*	Colistin (≥16)	14	0.071 [0.002–0.339]
JFGY	JFGY-11	F	JFGY_11.1	GN	*Pseudomonas fluorescens*	Ticarcillin/Clavulinic acid (≥128), Ceftazidime (≥64), Aztreonam (≥64), Meropenem (4.0) [4], Amikacin (≥64), Gentamicin (8.0), Tobramycin (≥16), Colistin (≥16)	8	1.000 [0.631–1.000]

Table 2. *Cont.*

Site	Sample ID	Sex [1]	Isolate ID	Gram Type [2]	Organism	Profile of Decreased Susceptibility (MIC in µg/mL)	Tested AM [3]	MAR Index [CI 95%]
JFGY	JFGY-12	M	JFGY_12.0	GP	*Enterococcus durans*	none	8	0.000 [0.000–0.369]
JFGY	JFGY-13	F	JFGY_13.1	GP	*Enterococcus faecalis*	Trimethoprim/Sulfamethoxazole (≤10) [4]	9	0.111 [0.003–0.482]
			JFGY_13.2	GN	*Pseudomonas fluorescens*	Ticarcillin/Clavulinic acid (≥128), Aztreonam (≥64), Imipenem (8.0), Meropenem (4.0) [4], Gentamicin (≤1) [4]	8	0.625 [0.245–0.915]
JFGY	JFGY-14	M	JFGY_14.1	GN	*Acinetobacter baumannii* complex	Piperacillin (32.0)	11	0.091 [0.002–0.413]
JFGY	JFGY-15	M	JFGY_15.1	GN	*Enterobacter cloacae* complex	Colistin (≤0.5) [5]	14	0.071 [0.002–0.339]
JFGY	JFGY-16	F	JFGY_16.1	GP	*Granulicatella adiacens*	n.i.	n.a.	n.a.
			JFGY_16.2	GP	*Kocuria rosea/rhizophila* (low)	n.i.	n.a.	n.a.

[1] Sex: female (F), male (M). [2] Gram-type: Gram-positive (GP), Gram-negative (GN). [3] Tested AM: number of tested antimicrobials. [4] Indicates intermediate susceptibility. [5] Interpretation by the VITEK 2 Advanced Expert System (AES). Abbreviations: n.i. = not investigated, n.a. = not applicable, POS = positive.

Besides the low isolate recovery rate, a high taxonomic diversity, primarily at site JFGY, was observed. The GP/GN ratio was the sole bacteriological parameter exhibiting a significant difference ($p = 0.0105$) between the two sampling sites. No other comparisons yielded statistically significant differences regarding the bacterial communities of the locations (Figure 4).

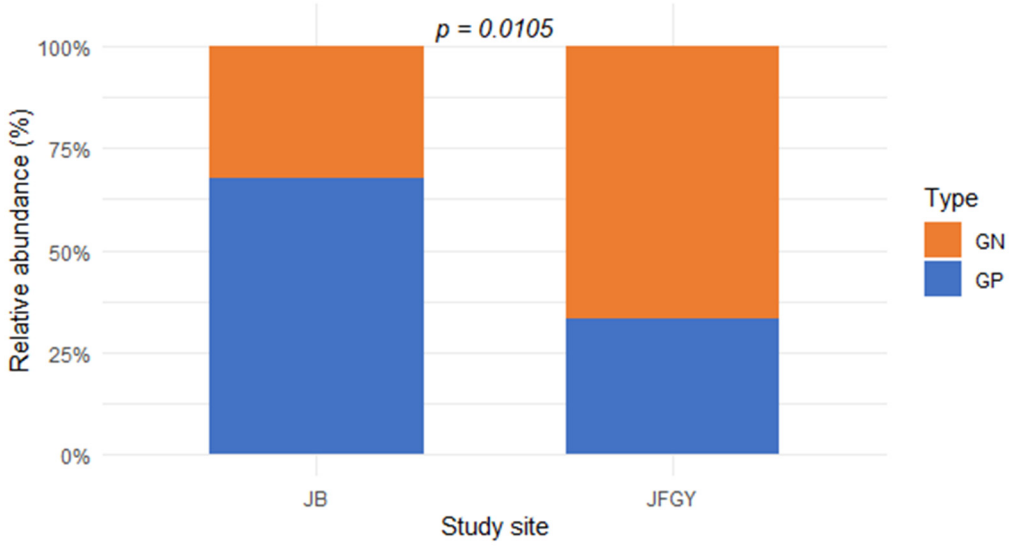


Figure 4. Ratio of Gram-positive (GP) and Gram-negative (GN) heterotrophic plate count (HPC) isolates at the study sites Jászberény (JB, $N = 30$) and Jászfelsőszentgyörgy (JFGY, $N = 30$).

At site JB, *Enterococcus faecium* (23% of the isolates) and *Enterobacter cloacae* complex (17%) were the dominant GP and GN species, respectively. At site JFGY, all GP species were represented with only one isolate, while the most frequently detected GN species was *Sphingomonas paucimobilis* (13%). This investigation found two and four MDR isolates

at sites JB and JFGY, respectively. Consequently, the overall frequency of detected MDR isolates was 10%, with 6.7% and 13.3% at sites JB and JFGY, respectively. At site JB, MDR phenotypes were detected in only GPs. At site JFGY, two GPs and two GNs proved to be MDR.

All four isolates belonging to the *Staphylococcus* and *Mammaliicoccus* genera proved to be MDR; notably, two isolates (*S. gallinarum* and *S./Mammaliicoccus lentus*, both from the JB site) exhibited a methicillin-resistant phenotype. Conversely, the most abundant GP genus, *Enterococcus*, showed only intermediate susceptibility to trimethoprim/sulfamethoxazole, while remaining susceptible to all other tested antimicrobials. Among GNs, two *Pseudomonas fluorescens* and one *S. paucimobilis* isolate from the JGYF site were classified as MDR. Remarkably, aztreonam, carbapenem and colistin resistance were detected in the *P. fluorescens* species. In addition to these, colistin resistance was observed in the *Enterobacter cloacae* complex (at both sites), as well as in the *Serratia fonticola* and *Serratia liquefaciens* group (at the JGYF site). The only *Escherichia coli* isolate at site JB was susceptible to all tested antimicrobials. Other species of the GN class showed resistance to none or only one antimicrobial (Figure 5).

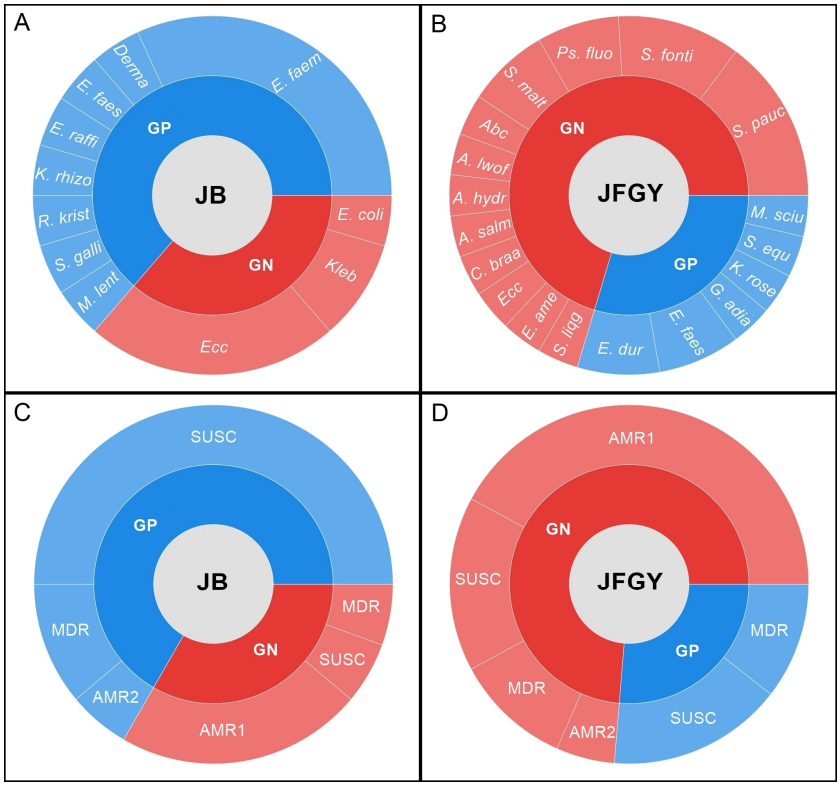


Figure 5. Comparison of sunburst diagrams of bacterial communities (**A**,**B**), and antimicrobial patterns (**C**,**D**) of the study sites Jászberény (JB) and Jászfelsőszentgyörgy (JFGY). Abbreviations: GP = Gram-positive, *Derma.* = *Dermacoccaceae* sp., *E. faem.* = *Enterococcus faecium*, *E. faes* = *E. faecalis*, *E. raffi.* = *E. raffinosus*, *E. dur.* = *E. durans*, *G. adia.* = *Granulicatella adiacens*, *K. rhizo.* = *Kocuria* rhizophila, *M. lent.* = *Mammaliicoccus lentus*, *M. sciu.* = *M. sciuri*, *R. krist.* = *Rosea* kristiae, *S. galli.* = *Staphylococcus gallinarum*, *S. equ.* = *S. equorum*, GN = Gram-negative, *Abc* = *Acinetobacter* baumanii complex, *A. lwof.* = *A. lwoffii*, *A. hydr.* = *Aeromonas hydrofila*, *A. salm.* = *A. salmonicida*, *C. braa.* = *Citrobacter* braakii, *Ecc* = *Enterobacter cloacae* complex, *E. ame.* = *Ewingella* americana, *E. coli* = *Escherichia* coli, *Kleb* = *Klebsiella* sp., *Ps. fluo.* = *Pseudomonas fluorescens*, *S. liqg* = *Serratia liquefaciens* group, *S. fonti* = *S. fonticola*, *S. malt.* = *Strenotrophomonas maltophilia*, *S. pauc.* = *Sphingomonas paucimobilis*, SUSC = susceptible isolate, AMR1 = isolate resistant to one antimicrobial class, AMR2 = isolate resistant to two antimicrobial classes, MDR = isolate resistant to at least three antimicrobial classes.

Comparing the diversity profiles of bacterial communities showed higher overall diversity at site JFGY (Figure 6).

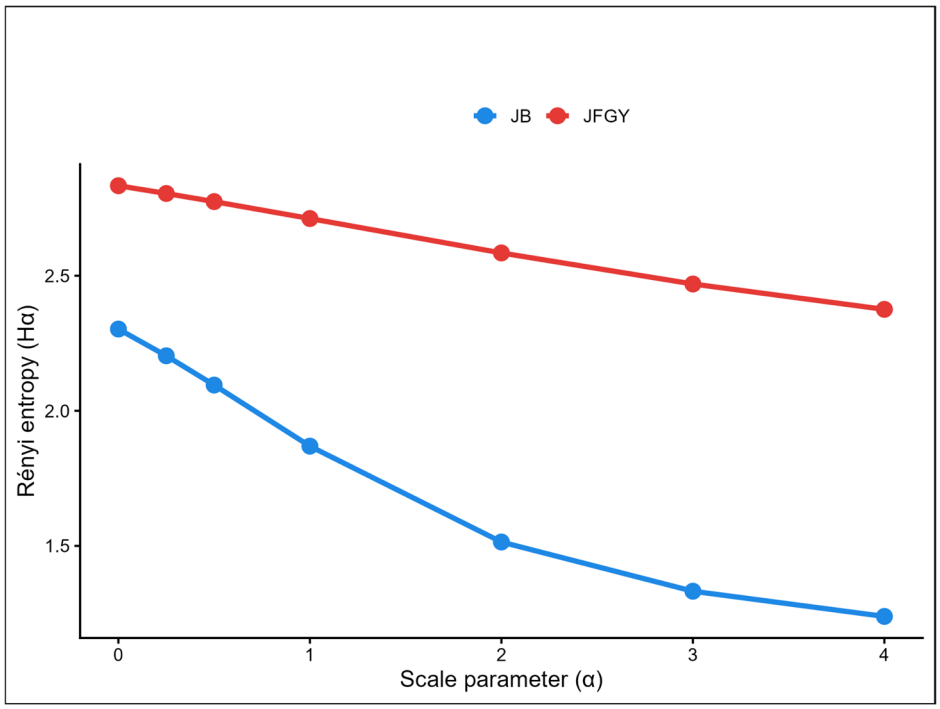


Figure 6. Rényi diversity profiles of bacterial communities of the two study sites. Hα represents the Rényi entropy, and α is the scale parameter. Specific values shown: $\alpha = 0$ (species richness), $\alpha \rightarrow 1$ (Shannon diversity index), $\alpha = 2$ (Simpson dominance index). Abbreviations: JB—Jászberény; JFGY—Jászfelsőszentgyörgy.

4. Discussion

This pilot study was based on a limited number of faecal samples; therefore, the results did not provide a qualitative assessment on AMR status of the investigated landscape. Only 60 distinct isolates, 0–4 per sample, were obtained with our method. This highlights the limitations of our laboratory techniques. Although the pre-enrichment step was applied to enhance the recovery rate of cultivable bacteria, a 24 h incubation in a non-selective medium possibly created a highly competitive environment. Among these conditions, microorganisms with the most colony-forming units overgrew their counterparts. In a future study, a shorter period of pre-enrichment or complete elimination of this step might improve the efficiency of the laboratory technique.

However, it is noteworthy that detection bias was very similar at the two sites, resulting in equal numbers of isolates with conspicuous distinctions between the taxonomic composition of HPC bacteria at the two sites.

Although the morphometric assessment of the sampled bird populations demonstrated remarkable uniformity, indicating generally good health status, several HPC bacteria with pathogenic potential were isolated from both sites. Among the taxa isolated in this study, *Staphylococcus* spp., *Enterococcus* spp., *E. cloacae* complex, *A. baumannii* complex, *Klebsiella* spp., *Pseudomonas* spp. [71], and *S. paucimobilis* [72] are frequently reported as hospital-acquired pathogens with AMR characteristics [71]. Apart from *Enterococcus* spp., our findings correspond with studies conducted in public health and veterinary care systems. Although our small sample size needs caution during interpretation, the detection of seven MDR isolates in 32 faecal samples of a herbivorous host indicates an environmental risk of AMR.

The most abundant GP genus was *Enterococcus*, although with a low recovery rate, especially at site JFGY. Both the low detection success and the lack of antimicrobial resistance appear to be peculiar characteristics of these *Enterococcus* communities, since faecal contamination is one of the richest sources of enterococci [73,74]. The low detection rate may be attributed to the non-selective culture method, which likely favoured competitors with higher initial cell counts or faster growth rates, potentially outcompeting enterococci during the incubation period. Regarding the observed susceptibility levels, the lack of phenotypic resistance in these eight isolates does not necessarily imply that the investigated *E. faecium* population is entirely free from antimicrobial resistance. This finding suggests that, while resistance was not detected, its presence in the environment cannot be ruled out. Consequently, our experiences regarding the detection of enterococci and their AMR pattern highlight the limitations of non-selective cultivation of this genus. Although the HPC technique aimed to obtain minimally biased data on AMR phenotypes, our approach resulted in the loss of relevant information.

Interestingly, all *Staphylococcus* isolates were multidrug-resistant (MDR), with those from site JB also exhibiting methicillin resistance. This aligns with previous environmental studies, which frequently report both MDR and methicillin resistance in staphylococci [75]. Notably, higher MAR indices were observed in this taxon at site JB, despite this site appearing less contaminated based on other AMR indicators. Two isolates of the *Mammaliicoccus* genus (formerly *Staphylococcus*), namely *M. lentus* and *M. sciuri*, exhibited AMR patterns similar to their staphylococcal counterparts at the same site. This is an important observation, as mammaliicocci are often considered reservoirs and potential sources of resistance genes for other *Staphylococcus* species [76].

Due to the low number of isolates (N = 4), the apparently higher AMR load in the staphylococcal community at site JB remains indicative rather than conclusive. However, it is noteworthy that water sources for Wood Pigeons at site JB are subject to more direct anthropogenic impacts than those at site JFGY. The latter is located further upstream; for instance, Hatvan, the nearest major urban centre, is approximately 35–40 river kilometres (rkm) from site JFGY. Although staphylococci are ubiquitous and resilient, the distance from potential anthropogenic sources, combined with intense competition from more pollution-tolerant GN bacteria at site JFGY, likely hindered their detection. Given that all recovered isolates were MDR, these findings underscore the necessity of further studies using selective, high-salt culture media to better characterise the halotolerant staphylococcal community.

Among GN bacteria, two *P. fluorescens* isolates, one MDR, and one with co-resistance to carbapenems, aztreonam, and colistin were detected at site JFGY. While intrinsic carbapenem resistance in *P. fluorescens* has been documented [77], the MDR phenotype suggests extreme selection pressure on the bacterial population [78,79]. Such phenomena are frequently associated with heavy-metal pollution from industrial effluents [68,80,81]. Due to the lack of comprehensive environmental sampling of the river sediment, historical industrial pollution of the Zagyva River [50–52] is only hypothesised as a potential cause of this phenomenon. However, a study on urban soil by the upper stream of the river is consistent with the pollution hypothesis, as a high frequency of heavy-metal resistant Pseudomonas spp. was detected in samples originating from former industrial areas [44]. Regarding colistin resistance in *Pseudomonas* spp., it often manifests as heteroresistance, where a generally susceptible population harbours a resistant subpopulation. This phenomenon complicates AST testing, as isolates may appear susceptible in vitro despite clinical treatment failure [82]. In this study, one of the two MDR *P. fluorescens* isolates exhibited resistance to colistin with a high ($\geq$16) MIC. Since automated systems, like VITEK, often produce a high frequency of very major errors (false negative evaluation of resistant

phenotypes) and a lower frequency of major errors (false positive evaluation of susceptible isolates) in the case of colistin [55,83], these findings do not support broader generalisation.

Furthermore, colistin resistance was observed in all six *Enterobacter cloacae* complex isolates from both sites, although low MIC values were recorded according to the AES evaluation. Although colistin resistance is common in the *E. cloacae* complex, it is also frequently masked by heteroresistance [84]. Therefore, the high prevalence of colistin resistance detected here is noteworthy and might be attributed to the pre-enrichment of samples in buffered peptone water. Studies on heteroresistance have confirmed that specific culture conditions, such as osmolarity, pH and oxygen availability, can alter the growth of colistin-resistant subpopulations; similarly, broth-based cultivation may promote the proliferation of resistant cells [84].

Although the *E. cloacae* complex is ubiquitous, only six isolates were recovered. Interestingly, at site JB, where GPs dominated, this species was detected five times more frequently than at site JFGY. This irregular detection pattern was likely due to the non-selective culture technique, as previously noted for *Enterococcus* spp.

Notably, only one *E. coli* isolate was detected during this study. This finding contradicts general observations, as *E. coli* is a common member of faecal microbiota in wild birds [9]. Furthermore, AMR is also a frequent phenomenon in this bacterial species [10,40,85]. The low prevalence of *E. coli* is assumed to be a consequence of sample processing. Transport time of more than 24 h and the pre-enrichment step in our laboratory protocol might have facilitated competitive bacteria.

Furthermore, an isolate of the typical environmental bacterium *S. paucimobilis* was detected at site JFGY, exhibiting resistance to ciprofloxacin and tobramycin, and intermediate susceptibility to amikacin and gentamicin. This species is known for its adaptability to extreme conditions, such as heavy-metal pollution, and is therefore utilised in bioremediation [72]. In highly polluted environments, co-selection for resistance to both heavy metals and antimicrobials is well-documented in this species [86]. Additionally, *S. paucimobilis* is an oligotrophic bacterium, allowing it to thrive in nutrient-poor environments. This trait facilitates its role as a healthcare-associated pathogen, as it can survive and proliferate on the surfaces of tracheal tubes and catheters [72]. However, this study did not collect environmental samples, like river sediment or arable land soil, which could support the pollution hypothesis. The only rationale behind the theory is that environmental studies conducted recently in the Zagyva valley detected diverse communities of Gram-negative bacteria with a high frequency of MDR genotypes [43,44]. Furthermore, water samples collected from the river are also evidence that the legacy of historic water pollution continues to this day [42,46].

In a broader context, Rényi's diversity profiles highlighted potential landscape-level mediators of AMR load. A comparison of landscape diversity revealed higher evenness at site JB. While JB encompasses a larger urban area, the significant proportion of urban green space contributes to a more heterogeneous urban ecosystem. Traditionally, anthropogenic areas have been viewed primarily as sources of pollution, often dominated by non-ecological elements [87]. However, urban-to-rural gradients are not simple geographical transects; they represent complex patterns of diverse functional land use that can, in some cases, support increased ecosystem function and biodiversity [88]. Consequently, the higher landscape-level evenness at JB may foster a more resilient environmental microbiome than the intensively cultivated, more homogenous croplands dominating site JFGY [67,68]. At the latter site, high landscape diversity is confined to a narrow riparian buffer zone, while the surrounding area is dominated by agricultural land use. Paradoxically, the higher landscape diversity at JB was accompanied by lower bacterial alpha-diversity compared to JFGY. It is important to note that surveillance focusing on heterotrophic plate count

(HPC) bacteria provides only a partial view of the total bacterial profile. Nevertheless, the seemingly higher bacterial diversity at JFGY is characterised by a profound shift toward GN dominance, with twice as many GN isolates detected as at JB. This ratio was reversed at site JB. Consequently, the difference in the GP/GN ratio between the two sites was statistically significant ($p = 0.0105$). If the GP/GN ratio is considered a proxy for naturalness in bacterial communities [67,68], the apparent contradiction between higher landscape diversity and lower bacterial diversity at JB becomes explainable [89]. Furthermore, the AMR profiles of the two sites appear to support the hypothesis that landscape-level diversity may mitigate AMR load, even within human-impacted environments. However, the lack of statistical significance precludes a definitive conclusion.

Nevertheless, it is worth noting that a pilot study with two sampling sites is not suitable to determine a causal correlation between the diversity of a landscape and a bacterial community. For this reason, the application of Rényi entropy in this study aimed to demonstrate the utility of this approach in very similar habitats. The two sites were located close to each other on the opposite side of a lowland town. Both sites were dominated by cropland, which is an important feeding area for Wood Pigeons. The difference between their diversities was determined by habitats occupying a minority of the areas. Using a single indicator cannot explore fine-scale dissimilarities, although they might contribute to landscape functioning.

This study suggests that higher landscape diversity is associated with a lower rate of MDR bacteria among isolates obtained with the HPC technique. Interestingly, a lower frequency of AMR phenotypes was detected in a less diverse bacterial community. This phenomenon seemingly contradicts previous observations suggesting that environmental microbiome diversity provides a protective buffer against AMR accumulation [90]. On the other hand, the shifted GP/GN ratio within the diverse collection of HPC bacteria at site JFGY reveals that this apparent diversity is largely restricted to the GN population [89]. Coupled with increased AMR richness, this suggests that intense selective pressure allowed GN species to outcompete GP bacteria at JFGY, leading to a taxonomically skewed community. This theory is also supported by a study carried out on industrial soil in the upper Zagyva valley, finding a high bacterial diversity, a dominance of GN species, and a high frequency of MDR genotypes in heavy metal-polluted soil samples [44]. Additionally, the most diverse microhabitats at site JFGY are located within a narrow, 50 m-wide riparian buffer zone. Notably, Klümper et al. [90] observed that the protective role of biodiversity often fails in riparian ecosystems, as riverbeds are highly dynamic environments where various anthropogenic and natural stressors converge, potentially facilitating the persistence of resistant taxa.

In the current study, both sites encompassed a river section of similar length, serving as the primary water source for the investigated pigeon populations. However, these sections exhibited remarkable hydrological differences. Site JB features a downstream section with increased flow velocity due to channelisation, whereas the upstream section at JFGY is characterised by a meandering, slow-flowing (lentic-like) character. Despite the high biological diversity of the riparian vegetation along this meandering reach, the reduced water velocity promotes sedimentation; this process may act as a filter, trapping pollutants and associated bacteria transported from the upper catchment. It is probable that this natural ecological filter above the city maintains a relatively high water quality within the urban river section, resulting in a more natural and less AMR-burdened bacterial community in pigeons utilising urban water sources.

Interestingly, the proximity of anthropogenic effluents appeared to have a less detrimental impact on the downstream bacterial community than anticipated. Both the GP/GN ratio and the AMR profiles exhibited a more ‘natural’ state here than at the upstream

section. However, the presence of methicillin resistance in *Staphylococcus* species at site JB and its absence at the upstream site suggest that local urban sources may emit resistant bacteria or antibiotic resistance genes (ARGs) into the ecosystem [3,5].

Despite these compelling findings, the study has certain limitations. As a pilot investigation, the sample size (N = 16 per site) was not intended to determine the absolute prevalence of specific AMR phenotypes. While the heterotrophic plate count (HPC) method provides a valuable snapshot of bacterial community diversity and resistance patterns, it is less suited for precise quantification of these parameters. The relatively low mean isolate number per sample represents a potential constraint, suggesting a possible loss of certain bacterial taxa during laboratory processing. Nevertheless, even this limited sample size revealed a reduced naturalness and elevated AMR load at site JFGY, consistent with a parallel occurrence of lower landscape diversity [56] and skewed GP/GN [67,68] ratio.

5. Conclusions

This study aimed to assess the utility of a screening approach for detecting AMR in a landscape. For this, faecal samples from an herbivorous avian bioindicator were processed by the HPC technique and VITEK-based AMR testing. The potential association between laboratory findings and landscape diversity was analysed using Rényi's diversity profiles of the two sites.

Although the findings of this research revealed a taxonomic difference between the investigated spatial units and the presence of MDR phenotypes, and bacteriological findings showed a moderate association with landscape diversity, the authors recommend this approach as a preliminary, high-throughput screening. To identify exact sources of AMR in a landscape, a more comprehensive environmental sampling and a more accurate diagnostic technique, such as DNA-based methods, are required.

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Abbreviations

The following abbreviations are used in this manuscript:

AMI	amikacin
AMR	Antimicrobial Resistance
AMP/SAM	ampicillin/sulbactam

ARG	Antimicrobial Resistance Gene
AST	Antimicrobial Susceptibility Testing
AZT	aztreonam
B-PEN	benzylpenicillin
CFP	cefepime
CIP	ciprofloxacin
CLIN	clindamycin
COL	colistin
CZD	ceftazidime
ERY	erythromycin
FOM	fosfomycin
FOX	cefoxitin
FUS	fusidic acid
GEN	gentamicin
GN	Gram-negative
GP	Gram-positive
HPC	Heterotrophic Plate Count
IMI	imipenem
JB	Jászberény (study site)
JFGY	Jászfelsőszentgyörgy (study site)
LEV	levofloxacin
LIN	linezolid
MAR	Multiple Antibiotic Resistance
MDR	Multidrug Resistance
MER	meropenem
MOX	moxifloxacin
OXA	oxacillin
PCA	Plate Count Agar
PIP	piperacillin
RIF	rifampicin
SD	Standard Deviation
SXT	trimethoprim/sulfamethoxazole
TEI	teicoplanin
TET	tetracycline
TIC/CLA	ticarcillin/clavulanic acid
TIGE	tigecycline
TOM	tobramycin
TRIM	trimethoprim
TZP	piperacillin/tazobactam
VAN	vancomycin

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