

Article

Characterization of the Fecal Microbiota of Urban Pigeons (*Columba livia*) in Northern Mexico: Taxonomic Composition and Predicted Functional Profiles

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Abstract

Urban pigeons (*Columba livia*) are widely distributed synanthropic birds closely associated with environments of intense human activity, raising interest in their role in urban microbial dynamics. Here, we characterized the fecal bacterial microbiota of urban pigeons from northern Mexico using 16S rRNA gene amplicon sequencing (V3–V4). A total of 1479 amplicon sequence variants (ASVs) were identified across five pooled samples. Alpha diversity varied among pools, with observed richness ranging from 228 to 514 ASVs. The bacterial community was dominated by Proteobacteria and Firmicutes, particularly *Escherichia–Shigella* and *Enterococcus*. PICRUST2-based functional predictions suggested a predominance of predicted metabolic pathways related to carbohydrate degradation and energy acquisition. Conservative taxonomic screening identified 58 gut-associated taxa, including 15 bacteria previously reported in association with humans; however, only three (*Clostridium perfringens*, *Enterococcus faecalis*, and *Proteus mirabilis*) showed reported zoonotic associations, all at very low relative abundances (<0.07%). These findings indicate that the fecal bacterial communities characterized in this study were dominated by taxa commonly associated with the avian gastrointestinal tract, whereas taxa that could be conservatively linked to documented zoonotic reports represented only a minor fraction of the detected microbiota. Overall, the results contribute to a more ecologically informed understanding of urban pigeon-associated microbiota within a One Health framework.

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1. Introduction

From a One Health perspective, understanding the microbiota associated with urban wildlife is important for evaluating microbial circulation at the human–animal–environment interface. This is particularly relevant in regions where urbanization coexists with agricultural activities, generating complex ecological scenarios that may facilitate microbial exchange across environments [1,2]. More broadly, the avian gut microbiota remains comparatively understudied relative to mammalian systems, despite its ecological and evolutionary importance. Current evidence indicates that microbial community composition in birds is strongly influenced by host species, diet, environmental conditions, migration, physiology, and anthropogenic exposure, often resulting in substantial interspecific and geographic variability [3–6]. In addition, avian microbiota frequently exhibits phylum-level patterns similar to those observed in other vertebrates, particularly the predominance of major bacterial phyla such as Firmicutes and Proteobacteria [7,8]. Nevertheless, substantial gaps remain in our understanding of microbial diversity associated with synanthropic bird species, particularly in urban environments.

Urban pigeons (*Columba livia*) are among the most widely distributed synanthropic bird species in urban environments worldwide [9,10]. Their close association with human settlements, particularly in densely populated cities, has facilitated their establishment in public spaces such as parks, schools, and urban infrastructures, where they form large and persistent aggregations [11]. This ecological proximity increases opportunities for interactions between birds, humans, and urban environmental surfaces [12], potentially influencing patterns of microbial exposure associated with pigeon feces [13]. Consequently, this close coexistence has raised interest regarding the microorganisms associated with pigeon feces, as these birds are frequently discussed as potential carriers or dispersal agents of diverse microorganisms, including bacteria of possible clinical relevance [14,15]. The accumulation of fecal material on urban surfaces may facilitate microbial dispersion through processes such as wind resuspension, aerosolization, and direct contact, potentially contributing to environmental exposure pathways involving inhalation of bioaerosols and fecal–oral transmission [16,17]. In this context, synanthropic birds have been suggested to contribute to the persistence and circulation of microorganisms within urban ecosystems.

In urban ecosystems, pigeons are continuously exposed to anthropogenic food sources, waste accumulation, built infrastructure, and dense human activity, conditions that may strongly influence the composition and ecological dynamics of their associated microbiota [4,5]. Consequently, pigeon fecal microbiota may reflect complex interactions among host biology, environmental microbial reservoirs, and urban ecological conditions [3,5]. Understanding these microbial assemblages is therefore relevant not only from a microbiological perspective, but also for interpreting microbial circulation within highly human-modified environments [14,17].

Despite the ecological and public health relevance of synanthropic birds such as *C. livia*, important gaps remain regarding the structure and potential functional roles of their microbiota, particularly in Latin America and urban ecosystems of northern Mexico. Historically, studies of the pigeon microbiota relied primarily on culture-based approaches, which are known to underestimate microbial diversity because many bacterial taxa cannot be readily cultivated under standard laboratory conditions [18–20]. The advent of high-throughput sequencing technologies has substantially expanded our ability

to characterize avian microbial communities, enabling the detection of previously overlooked and low-abundance taxa [21]. Characterizing the fecal microbiota of urban pigeons may contribute to a better understanding of host–environment microbial interactions while providing ecological context for bacterial taxa detected in urban wildlife. We hypothesized that urban pigeon fecal communities would be dominated by typical avian gut-associated bacteria and that taxa previously reported in association with zoonotic transmission would represent only a minor fraction of the overall microbiota. Therefore, this study aimed to characterize the fecal bacterial microbiota of *C. livia* in the Comarca Lagunera region of northern Mexico using 16S rRNA V3–V4 amplicon sequencing. Specifically, we sought to describe bacterial community composition and alpha diversity, explore predicted functional profiles using PICRUST2, and screen for taxa previously reported in association with humans or zoonotic potential. This study provides baseline information for understanding the bacterial ecology of urban pigeon populations in northern Mexico and contributes to the growing body of knowledge on wildlife-associated microbiomes within a One Health framework. These data may also serve as a reference for future comparative studies using higher-resolution genomic approaches.

2. Materials and Methods

2.1. Study Area

The study was conducted between December 2023 and January 2024 in two public schools, one rural community (ejido, where pigeons are strongly associated with human settlements), and two urban parks located in the municipalities of Gómez Palacio (Durango), Torreón, and Matamoros (Coahuila), northern Mexico (25°32′38″ N, 103°25′08″ W; Figure 1). The region is characterized by a semi-arid climate, with mean annual temperatures ranging from 18 to 22 °C and annual precipitation between 100 and 400 mm, concentrated during the summer months [22]. Peripheral areas are dominated by xerophytic shrubland, primarily *Larrea tridentata* and *Prosopis glandulosa*, whereas urban sampling sites contain introduced ornamental vegetation, including tree species commonly used in parks and public spaces.

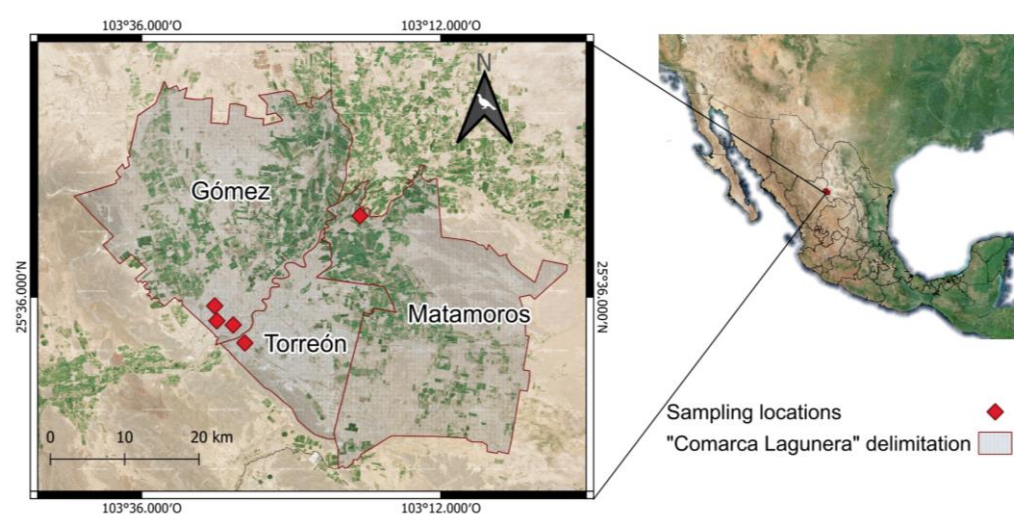


Figure 1. Study areas in the Comarca Lagunera region, northern Mexico, encompassing the municipalities of Torreón, Gómez Palacio, and Matamoros. The five red-marked points correspond to the sites where fecal samples from urban pigeons (*Columba livia*) were collected.

2.2. Fecal Sample Collection and Processing

Sampling locations were selected to represent common environments occupied by urban pigeon populations across the Comarca Lagunera region, including public parks, educational facilities, and a rural community (Figure 2a). These environments differ in human activity, food availability, infrastructure, and surrounding land use, providing a broad representation of the ecological conditions under which synanthropic pigeon populations persist in northern Mexico. Consequently, the selected sites were intended to capture community-level microbiota patterns associated with urban and peri-urban habitats rather than conditions specific to a single locality. Prior to sampling, individuals present at each site were visually confirmed to belong exclusively to this species in order to avoid interspecific contamination. Sterile paper bags were placed directly beneath perched individuals to collect fecal deposits immediately after defecation (Figure 2b). To minimize potential environmental contamination from surrounding surfaces or airborne particles, only freshly deposited fecal material with minimal visible environmental exposure was selected. To ensure independence among samples, one sterile bag was used per individual, and each fecal deposit was considered to represent a different bird (Figure 2c).



Figure 2. Sampling procedure for fecal microbiota collection in *Columba livia*. (a) Communal roosting sites in urban environments of the Comarca Lagunera, northern Mexico, where sterile paper bags were placed beneath perching structures (blue ovals). (b) Close-up of a freshly deposited fecal sample collected immediately after defecation. (c) Arrangement of sterile paper bags (blue ovals) positioned beneath perching sites for non-invasive sampling, with each fecal deposit considered to represent a different individual.

Ten individual fecal deposits were combined into a single composite sample to maximize representation of the microbial diversity associated with communal roosting sites while minimizing the influence of individual-level variation. Given the exploratory nature of the study, the objective was to obtain community-level microbiota profiles representative of each sampling location rather than to estimate variability among individual birds. A total of five pooled samples ($n = 5$) were obtained. Each pool was transferred into tubes containing 750 μL of BashingBead™ lysis/stabilization buffer (Zymo Research, Irvine, CA, USA) and silica beads. Samples were homogenized for 30 s using a TerraLyzer™ mechanical disruptor (Zymo Research, Irvine, CA, USA), transported under cold-chain conditions to the Laboratory of Conservation Medicine (Universidad Juárez del Estado de Durango), and stored at $-20\text{ }^{\circ}\text{C}$ until further processing.

2.3. DNA Extraction, PCR Amplification, and Sequencing

Bacterial DNA was extracted using the ZymoBIOMICS™ DNA MiniPrep kit (Zymo Research, Irvine, CA, USA), following the manufacturer's instructions. All procedures were performed within a UV-sterilized laminar flow cabinet to minimize potential contamination during sample processing. The V3–V4 region of the 16S rRNA gene was amplified using universal primers 341F (5'-CCTAYGGGRBGCASCAG-3') and 806R (5'-GGACTACNNGGTATCTAAT-3'). The V3–V4 region was selected because it provides a well-established compromise between phylogenetic resolution, sequencing accuracy, and compatibility with widely used Illumina-based microbiome workflows [23]. PCR reactions were carried out in a final volume of 15 μL using Phusion High-Fidelity PCR Master Mix (New England Biolabs, Ipswich, MA, USA), with 2 μM of each primer and approximately 10 ng of template DNA. Thermal cycling conditions consisted of an initial denaturation at $98\text{ }^{\circ}\text{C}$ for 1 min, followed by 30 cycles of denaturation at $98\text{ }^{\circ}\text{C}$ for 10 s, annealing at $50\text{ }^{\circ}\text{C}$ for 30 s, and extension at $72\text{ }^{\circ}\text{C}$ for 30 s. Amplicons were verified by electrophoresis on 2% agarose gels stained with SYBR™ Green (Thermo Scientific, Waltham, MA, USA). Amplicons generated from each pooled fecal sample were first purified using the QIAquick Gel Extraction Kit (Qiagen™, Hilden, Germany). Sequencing libraries were subsequently prepared individually using the TruSeq DNA PCR-Free Sample Preparation Kit (Illumina, San Diego, CA, USA), incorporating unique sample-specific indices (barcodes) to enable post-sequencing demultiplexing. Library quality and concentration were assessed using a Qubit 2.0 fluorometer (Thermo Scientific) and an Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA). Indexed libraries were then normalized and pooled at equimolar concentrations prior to sequencing on an Illumina NovaSeq 6000 platform (Illumina, San Diego, CA, USA), generating paired-end reads of 250 bp. This read configuration provides sufficient overlap for accurate reconstruction of the V3–V4 amplicon while maintaining high sequencing quality and compatibility with standard Illumina microbiome workflows. Because extraction blanks and PCR negative controls were not included in the experimental design, no formal bioinformatic decontamination methods (e.g., decontam) were applied. This limitation was explicitly considered during data interpretation.

2.4. Bioinformatic Analysis

Sequence data were processed using QIIME2 version 2023.5 [24] within a Linux–Ubuntu environment. The analytical workflow followed a previously established pipeline for avian fecal microbiota studies conducted in northern Mexico [25]. Paired-end reads were subjected to quality filtering, denoising, and merging using the DADA2 algorithm [26], which also enabled the removal of chimeric sequences and the inference of high-resolution amplicon sequence variants (ASVs). Rarefaction curves were generated for all samples using PAST version 5.3 to assess sequencing depth and sampling completeness. Sequencing depth sufficiency was further evaluated using Good’s coverage [27], with values approaching 1.0 interpreted as indicative of adequate sampling completeness. A first taxonomic classification was performed using a Naïve Bayes classifier trained on the Greengenes2 (2022.10) reference database [28]. Because the most abundant ASV remained poorly resolved taxonomically using this database, a complementary classification was performed using the SILVA 138.1 reference database [29]. This secondary classification was used exclusively to improve the taxonomic interpretation of unresolved dominant taxa and was not intended as a complete replacement of the original Greengenes2-based classification. Taxonomic assignments obtained from both databases were qualitatively compared to assess consistency at higher taxonomic ranks and to improve genus-level interpretation where necessary. Relative abundance patterns at the phylum, family, and genus levels were visualized through heatmaps generated using BioRender (<https://www.biorender.com>) .

Because each sample represented a composite pool of fecal deposits, results were interpreted as community-level profiles associated with communal roosting sites. Consequently, analyses were conducted under a descriptive framework without formal inferential statistical testing. Putative species-level affiliations were explored using BLAST+ (version 2.17.0) under stringent criteria (E-value = 0.0, $\geq 98\%$ sequence identity, and 100% query coverage). To minimize ambiguity in species-level assignments, only BLAST matches meeting these criteria and yielding a single unambiguous best hit were retained. In cases where multiple species shared equivalent sequence identity and query coverage values, species-level identification was not attempted and taxonomic assignment was conservatively retained at the genus level. Consequently, subsequent ecological and zoonotic interpretations were restricted to taxa supported by a single unambiguous BLAST assignment. However, given the limited taxonomic resolution of partial 16S rRNA sequences and the known ambiguity of some bacterial groups, these affiliations were considered provisional and interpreted conservatively [30]. Alpha diversity was estimated using observed ASVs, Shannon diversity index, and Pielou’s evenness, calculated with the QIIME2 diversity plugin, to characterize richness, diversity, and evenness, respectively [31,32].

2.5. Functional Prediction Analysis Using PICRUSt2

The functional potential of the bacterial microbiome associated with *C. livia* was inferred using PICRUSt2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) version 2.4.1, which enables the prediction of functional profiles from 16S rRNA gene sequencing data [33]. For this analysis, amplicon sequence variants (ASVs) derived from the V3–V4 region of the 16S rRNA gene, previously processed through the metabarcoding pipeline, were used as input.

The PICRUSt2 workflow included phylogenetic placement of representative ASVs into a reference tree using the EPA-NG and GAPP algorithms. Subsequently, the potential gene content of each ASV was predicted using the Hidden State Prediction (HSP) approach implemented in the Castor (version 1.6.5) software. Based on these predictions, gene family abundance profiles were generated and categorized into

functional orthologs (KEGG Orthologs, KOs), which were then used for the reconstruction of MetaCyc metabolic pathways. In addition, the Nearest Sequenced Taxon Index (NSTI) was calculated for each sample as part of the PICRUSt2 pipeline. NSTI values quantify the average phylogenetic distance between the ASVs in each sample and organisms with available reference genomes, providing an estimate of prediction accuracy [33]. Lower NSTI values indicate higher reliability of functional inference. In this study, NSTI scores were used as an approximate indicator of prediction reliability of predicted functional profiles and were considered when interpreting downstream analyses. Relative abundance patterns of the 20 most abundant predicted metabolic pathways were visualized as a heatmap in R (version 2025.09) using the ggplot2 package (version 3.5.2). Functional predictions were interpreted cautiously, as they are based on inferred gene content rather than direct metagenomic or metatranscriptomic evidence.

2.6. Conservative Screening of Tentatively Assigned Taxa

Taxa tentatively assigned at the species level based on BLAST results were evaluated using a context-oriented screening framework designed to support ecological interpretation rather than formal contaminant removal. This approach was adapted from previous work on avian microbiomes in the region [25] and applied to the present dataset.

First, each taxon was examined for potential associations with known sources of background contamination, including DNA extraction kits, laboratory reagents, and human skin microbiota, based on published reports. To further contextualize these associations, taxa were annotated using the EFILTER tool [34]; <https://efilter.shinyapps.io/EFilter-app/> (accessed on 10 March 2026), which classifies microorganisms according to their typical environmental habitats. Taxa primarily linked to non-gut environments (e.g., skin, laboratory settings, or built environments) were flagged as “potential contaminants” for interpretative caution. Importantly, this classification did not result in their exclusion but was used to guide ecological interpretation. In a second step, taxa not flagged as potential contaminants were considered plausible components of the fecal microbiota and were evaluated for reported clinical relevance. This assessment was conducted using the Bacteria–Human Pathogens Database (BaHPD) [35], complemented by targeted literature searches. Information on associated clinical contexts and potential exposure routes was compiled. Finally, literature reports describing zoonotic associations were reviewed. Throughout this process, detections were interpreted strictly as evidence of environmental presence, without inferring microbial viability, pathogenicity, or transmission risk. This conservative framework was applied to minimize overestimation of the public health relevance of detected taxa.

3. Results

3.1. Sequencing Output and Data Processing

Sequencing generated an average of 193,424 raw reads per pool. After quality filtering and denoising using the DADA2 algorithm, the mean number of non-chimeric reads was 155,496, representing 80.86% of the initial reads. The analysis of pooled fecal samples from *C. livia* collected in the Comarca Lagunera region yielded a total of 1479 amplicon sequence variants (ASVs) across five composite samples. Rarefaction curves approached asymptotic trends in all pooled samples (Figure S1), indicating that sequencing depth was sufficient to recover most of the bacterial diversity present in each community. Although ASV richness varied among pools, the progressive plateauing of

all curves and the high Good's coverage values (0.999; Table S1) support adequate sampling completeness.

3.2. Taxonomic Composition and Alpha Diversity

A total of 23 phyla, 40 classes, 116 orders, 197 families, 256 genera, and 412 bacterial species were identified in the dataset. Among these, 66 taxa with Latinized nomenclature were further examined using NCBI BLAST to support their tentative assignment at the species level (Table S2). At the phylum level, the bacterial community was dominated by Proteobacteria sequences (47.5%), followed by Firmicutes_D (44.2%) (Figure 3). At the family level, Enterobacteriaceae (44.9%) showed the highest relative abundance, followed by Enterococcaceae (33.4%) (Figure 4). At the genus level, the most abundant taxa were *Escherichia-Shigella* (44.4%), *Enterococcus* (33.2%), and *Turicibacter* (7.7%) (Figure 5).

Alpha diversity metrics revealed variability among pooled samples, indicating differences in community composition and structure across pigeon flocks (Table S3). Observed ASVs ranged from 228 to 514 (mean = 365.2), while Shannon diversity values varied between 2.09 and 3.25 (mean = 2.82). Pielou's evenness ranged from 0.24 to 0.38 (mean = 0.33), reflecting differences in the distribution of relative abundances among taxa within each community. Consistent with the observed alpha diversity variation, considerable differences in relative abundance were observed among pooled samples, particularly for dominant genera such as *Escherichia-Shigella* and *Enterococcus*. For example, the relative abundance of *Escherichia-Shigella* ranged from 19.3% to 72.9% across pools, whereas *Enterococcus* ranged from 2.7% to 64.5% (Figure 5).

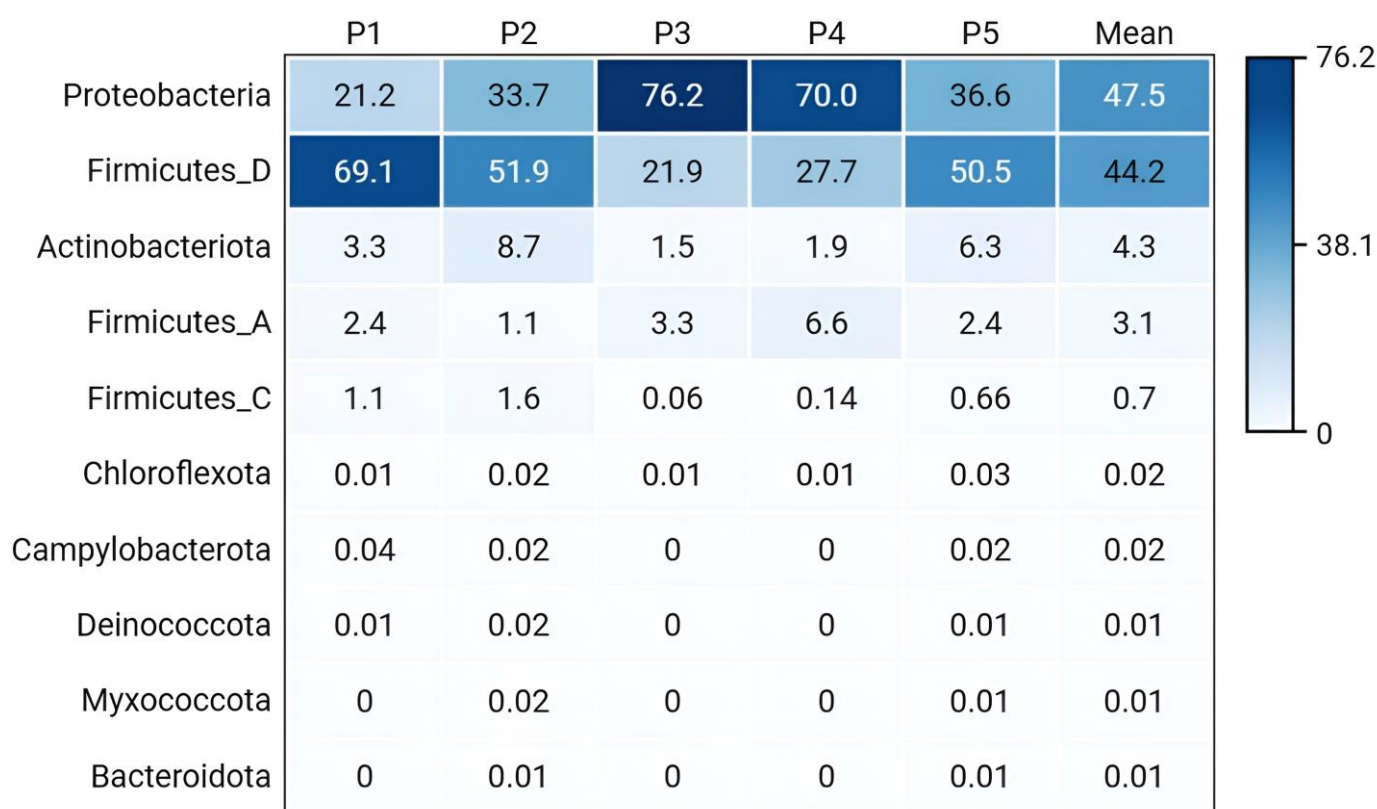


Figure 3. Heatmap showing the relative abundance (%) of the 10 most abundant bacterial phyla across five pooled fecal samples of *Columba livia* from the Comarca Lagunera region, northern Mexico.

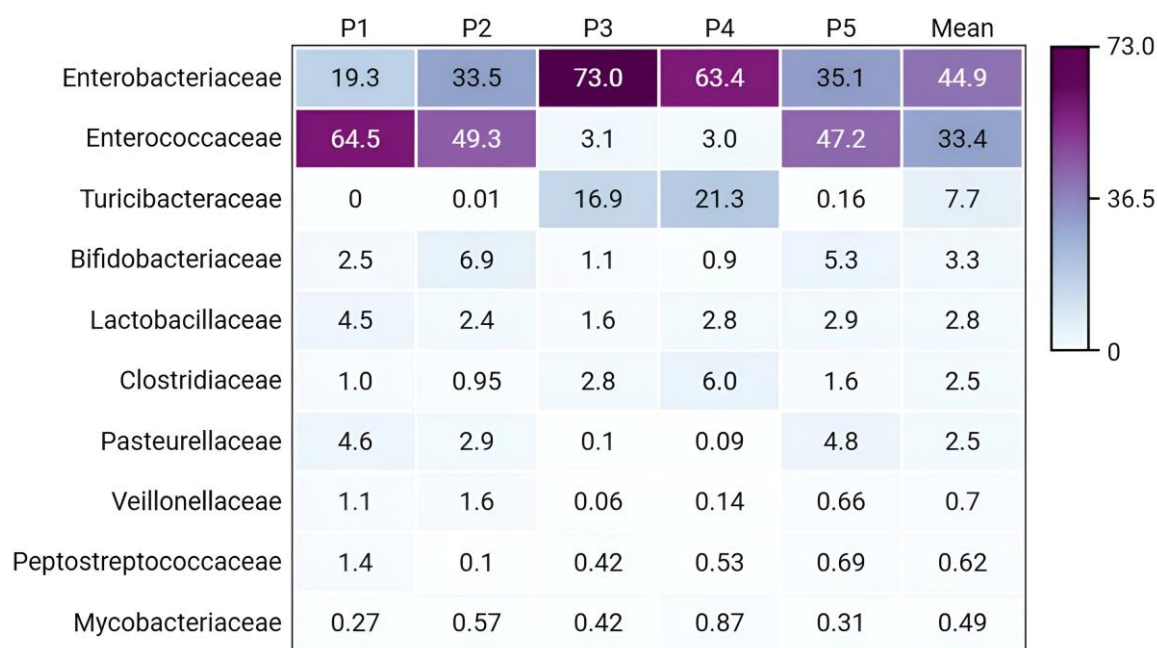


Figure 4. Heatmap showing the relative abundance (%) of the 10 most abundant bacterial families across five pooled fecal samples of *Columba livia*.

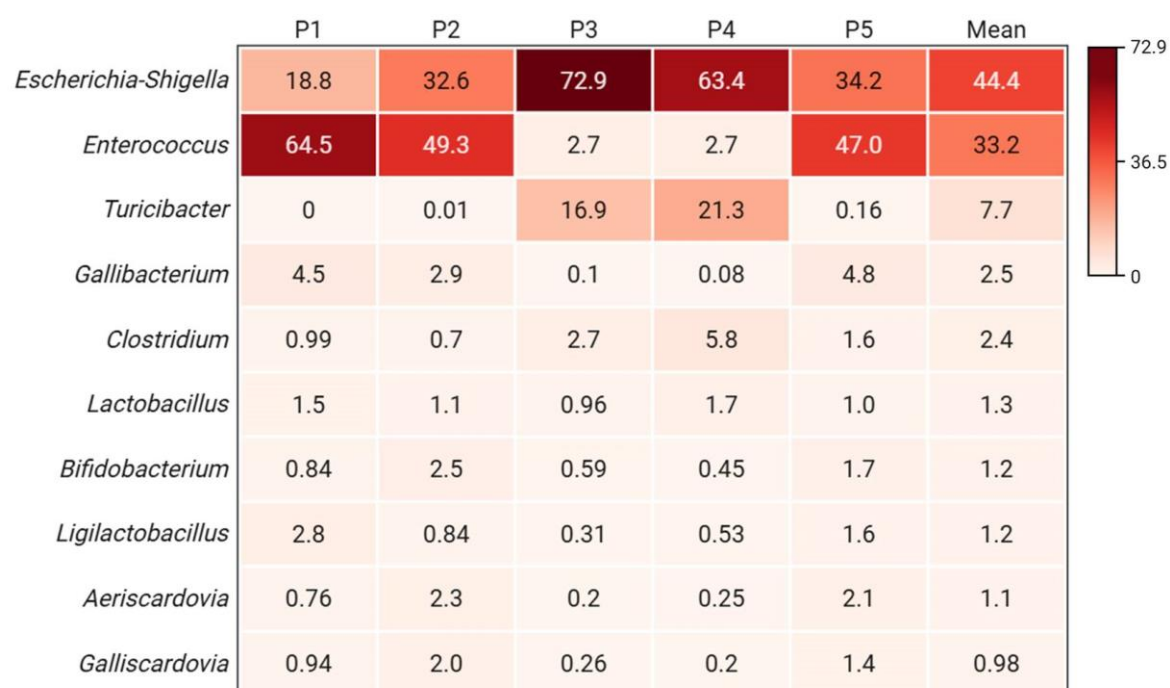


Figure 5. Heatmap showing the relative abundance (%) of the 10 most abundant bacterial genera across five pooled fecal samples of *Columba livia*.

3.3. Functional Prediction of the Fecal Microbiota (PICRUST2)

Functional prediction using PICRUST2 identified a total of 402 predicted metabolic pathways within the fecal microbiome of *C. livia*. The analysis showed high predictive reliability, with NSTI (Nearest Sequenced Taxon Index) values ranging from 0.023 to 0.053 (mean = 0.039) (Table S1). These values are well below the thresholds commonly reported for wildlife samples (typically < 0.15), supporting the robustness of the inferred metabolic profiles and indicating adequate representation of the detected taxa in reference databases. The predicted functional profile of the 20 most abundant pathways re-

vealed a predominance of processes associated with carbohydrate metabolism and energy acquisition. The most abundant pathway across samples was sucrose degradation III (sucrose invertase; PWY-621), with a mean relative abundance of 1.28%. This was followed by the pentose phosphate pathway (non-oxidative branch; NONOX-IPENT-PWY) at 0.81%, and acetylene degradation (P161-PWY) at 0.74% (Figure 6, Table S4).

Predicted metabolic profiles were relatively consistent across pooled samples, with carbohydrate metabolism and energy acquisition pathways dominating all communities (Figure 6). Although minor differences in relative abundance were observed, no major shifts in overall pathway composition were evident across samples. A consistent distribution of the most abundant pathways was observed across all pooled samples (P1–P5), suggesting relatively similar predicted functional profiles despite the taxonomic variation detected among communities. This pattern may reflect functional redundancy within the pigeon-associated microbiota and the persistence of central metabolic functions associated with dietary substrate utilization and energy production in urban environments. However, these interpretations should be considered cautiously, as functional predictions are based on inferred gene content rather than direct metagenomic evidence.

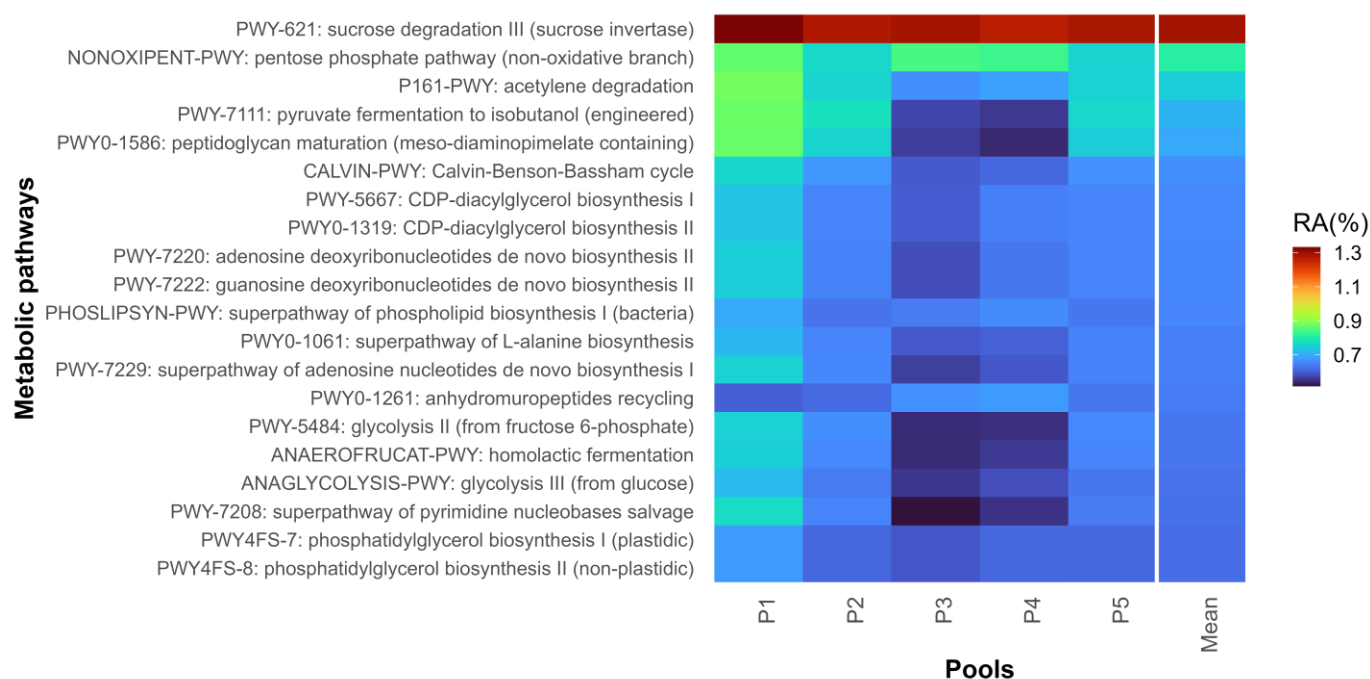


Figure 6. Heatmap showing the relative abundance (RA%) of the 20 most abundant predicted metabolic pathways inferred using PICRUST2 across the fecal microbiome of *Columba livia*. Columns represent pooled samples and rows represent metabolic pathways. Color intensity reflects relative pathway abundance.

3.4. Conservative Screening of Tentatively Assigned Taxa and Ecological Interpretation

Out of the 66 bacterial taxa tentatively assigned at the species level and supported through manual BLAST validation, eight were identified as taxa requiring cautious ecological interpretation due to their recurrent association with DNA extraction kits, laboratory reagents, or human skin microbiota in low-biomass microbiome studies (Table S5). The remaining 58 taxa were subsequently cross-referenced against the Bacteria–Human Pathogens Database (BaHPD), including available information on their reported sources and clinical associations [35]. Of these, 15 taxa have been previously reported in association with human clinical contexts under a variety of conditions [17,36–49]. Among the

taxa retained after conservative BLAST screening, only three tentatively assigned species, *Clostridium perfringens*, *Enterococcus faecalis*, and *Proteus mirabilis*, were associated with documented zoonotic reports in the literature [17,48,49]. These taxa were detected at very low relative abundances (<0.07%) across the analyzed samples (Table S6). Importantly, species-level assignments were considered tentative and were based on partial 16S rRNA gene sequences. Consequently, the absence of additional zoonotic associations should not be interpreted as evidence that other taxa lacked zoonotic relevance, but rather that no further taxa could be conservatively linked to documented zoonotic reports under the criteria applied in this study. Within these limitations, taxa tentatively associated with documented zoonotic reports were detected at low relative abundances and represented only a minor fraction of the detected microbial community.

4. Discussion

4.1. Ecological Structure and Taxonomic Patterns

The present study provides a comprehensive characterization of the fecal bacterial microbiota of *C. livia* inhabiting urban environments in northern Mexico, offering ecological insights relevant to urban microbial ecology and One Health research. At higher taxonomic levels, the bacterial community was dominated by Proteobacteria and Firmicutes. This pattern aligns with previous reports describing Firmicutes as a major component of avian gut microbiota, frequently associated with fermentative metabolism and energy acquisition [7,8]. Additionally, the high relative abundance of Proteobacteria observed in the present study is consistent with recent reports in urban pigeons and other avian systems, where members of this phylum may become highly abundant under specific ecological or host-associated conditions, including environmental exposure and anthropogenic influences [50,51].

However, notable differences emerge when comparing these results with those reported in urban pigeon populations from other regions. For instance, a recent study conducted in Seoul, South Korea [52], identified *Lactobacillus*, *Escherichia-Shigella*, and *Clostridium* as the most abundant genera. Although these taxa were also detected in pigeons from the Comarca Lagunera, their relative abundances differed, with *Escherichia-Shigella* exhibiting comparatively high relative abundances in the present study. This observation is consistent with previous findings indicating that members of this group can reach high relative abundances in *C. livia*, particularly in response to diet, environmental exposure, or host physiological conditions [50]. Importantly, the dominance of *Escherichia-Shigella* should be interpreted cautiously, as 16S rRNA gene-based approaches do not allow discrimination between commensal and pathogenic lineages within this complex. In avian systems, *Escherichia* has been reported as a common and sometimes dominant genus during microbiota establishment, reflecting its ecological role in early colonization and microbial community dynamics rather than evidence of active pathogenic processes [50]. These differences highlight the strong context dependency of pigeon-associated microbiota and emphasize the role of local environmental conditions, diet, and urban ecological dynamics in shaping microbial community composition. The substantial variation observed among pooled samples in the present study further supports this interpretation. Dominant genera such as *Escherichia-Shigella* and *Enterococcus* exhibited marked differences in relative abundance among sampling locations, suggesting that pigeon-associated microbiota is strongly influenced by local ecological conditions. Differences in food availability, habitat characteristics, anthropogenic inputs, and host-associated factors may contribute to this variability. In particular, variation in food sources, including anthropogenic inputs such as processed foods and organic waste, as well as differences in environmental exposure, may drive the contrasting microbial profiles observed across studies. These findings support the notion that the microbiota of *C.*

livia cannot be generalized across urban systems and must be interpreted within its specific ecological context. This pattern is also consistent with the ecological plasticity of this species and previous reports of microbiome variability in urban wildlife [8,53].

4.2. Functional Potential and Ecological Adaptation (PICRUSt2)

From a functional perspective, PICRUSt2-based predictions suggested a predominance of metabolic pathways associated with carbohydrate degradation and energy acquisition, including sucrose degradation and pentose phosphate metabolism. In contrast to previous avian microbiome studies based solely on taxonomic profiles, the integration of predictive functional profiling in the present work provides exploratory insights into the predicted functional profiles of pigeon-associated microbiota. Predicted pathways were primarily associated with central carbohydrate metabolism and energy acquisition processes commonly observed in bacterial communities. Although these pathways were consistently detected across pooled samples, their ecological significance should be interpreted cautiously because PICRUSt2 predictions represent inferred functional potential rather than direct measurements of gene content or metabolic activity [52]. The recurrence of these pathways across pooled samples suggests relatively similar predicted functional profiles despite taxonomic variability, a pattern that has also been reported in other avian microbiome studies [8]. However, these functional inferences should be interpreted cautiously, as they are based on predicted gene content rather than direct metagenomic evidence. Thus, these predictions should be interpreted as ecological potential rather than realized metabolic activity. Similar limitations have been highlighted in previous studies using amplicon-based functional prediction approaches [33].

4.3. Zoonotic Potential and Public Health Implications

Despite the detection of several bacterial taxa with reported clinical relevance, only a limited number of tentative species (*C. perfringens*, *E. faecalis*, and *P. mirabilis*) have been previously reported with documented zoonotic associations, and all were detected at very low relative abundances (<0.07%). These findings suggest that taxa previously associated with zoonotic transmission represent only a minor component of the overall microbiota identified in this study. Importantly, the detection of these taxa through DNA-based approaches should be interpreted with caution. The presence of bacterial DNA does not necessarily indicate microbial viability, infectivity, or transmission potential, particularly when detected at low relative abundances.

Comparative studies in urban pigeon populations have reported a broader diversity of bacteria with potential clinical relevance, including genera such as *Campylobacter*, *Chlamydia*, *Enterococcus*, *Escherichia*, *Klebsiella*, *Listeria*, *Salmonella*, *Shigella*, *Staphylococcus*, *Streptococcus*, *Peptostreptococcus*, and *Pseudomonas*, frequently discussed in the context of potential zoonotic risk [51,52,54]. However, these interpretations frequently rely on presence/absence data or relative abundances without integrating ecological context or quantitative thresholds that distinguish environmental occurrence from epidemiological relevance. In the present study, although *Escherichia-Shigella* was the most abundant taxon, this designation corresponds to a broad taxonomic complex that includes both commensal and pathogenic lineages and cannot be resolved at the species level using V3-V4 16S rRNA sequencing. Therefore, its high relative abundance should not be interpreted as direct evidence of pathogenic lineages, but rather as an indicator of enteric-associated bacterial communities commonly found in avian hosts. Consequently, the absence of documented zoonotic associations for other taxa should not be interpreted as evidence that zoonotic organisms were absent, but rather that no additional taxa could be conservatively linked to zoonotic reports under the criteria applied in this study. In this context, taxa conservatively linked to documented zoonotic reports were detected exclu-

sively at low relative abundances within this dataset. Collectively, these observations highlight the importance of distinguishing between taxonomic detection and actual public health risk, particularly in studies based on amplicon sequencing, where ecological interpretation is essential to avoid overestimation of zoonotic relevance.

4.4. One Health Perspective

From a public health perspective, these findings highlight both the relevance and the existing knowledge gaps regarding the role of *C. livia* in urban microbial dynamics. Although pigeons are often discussed as potential carriers of microorganisms of public health interest, the results obtained here indicate that their fecal microbiota is predominantly composed of bacteria commonly associated with the gastrointestinal tract, with only a minor fraction corresponding to taxa previously reported with zoonotic associations. This pattern is consistent with previous studies in wild and synanthropic birds, where most detected microorganisms are interpreted as components of typical enteric microbial communities rather than pathogen-dominated assemblages [3,4].

In this context, urban pigeons may reflect microbial patterns associated with anthropogenic environmental settings, integrating signals from diverse ecological sources and reflecting the microbial landscape of the habitats they occupy. Rather than providing direct evidence of sanitary risk, these birds may function as biological interfaces associated with environmental microbial exposure. Thus, the microbiota associated with urban pigeons can be interpreted as part of everyday microbial exposure in cities, influenced by factors such as food availability, contact with waste, and constant interaction with human infrastructure [55,56]. Within a One Health framework, this interpretation gains particular relevance because it acknowledges the interconnectedness of human, animal, and environmental health. From this perspective, the presence of microorganisms—including those with reported zoonotic associations—should not be evaluated in isolation, but rather as part of a complex ecological system in which multiple factors determine the potential risk of transmission. Elements such as population density, sanitary conditions, waste management, and the frequency of contact between humans and synanthropic fauna are key determinants that may modulate the probability of exposure and eventual infection [57,58].

Collectively, these observations highlight the importance of avoiding oversimplified interpretations based solely on the detection of potentially pathogenic microorganisms. Instead, integrative approaches that consider urban microbial ecology as a whole are needed to distinguish between presence, abundance, and actual epidemiological relevance. Ultimately, understanding the role of synanthropic species such as *C. livia* within this framework may contribute to more ecologically informed surveillance and management approaches aligned with One Health principles and aimed at a more realistic interpretation of microbial exposure within urban environments [59].

4.5. Methodological Considerations, Limitations, and Future Directions

A key methodological consideration in this study is the absence of extraction and PCR negative controls, which precluded the application of formal bioinformatic decontamination approaches. In response, a conservative interpretative framework, similar to those previously applied in avian microbiome studies [25], was adopted to contextualize potential sources of bias. This approach combined ecological interpretation, strict BLAST validation, and cross-referencing against known contamination sources to minimize overinterpretation of taxa potentially influenced by laboratory or environmental contamination [7,25,60]. Nevertheless, the lack of negative controls limits the ability to directly assess the contribution of contaminant DNA to the dataset. Consequently, low-abundance taxa should be interpreted cautiously, as the possibility that some rare

detections originated from laboratory reagents or environmental contamination cannot be completely excluded.

Although the V3–V4 region provides a well-established compromise between phylogenetic resolution, sequencing performance, and compatibility with widely used Illumina-based microbiome workflows, it cannot reliably discriminate among several closely related bacterial taxa. Consequently, the use of partial 16S rRNA gene sequencing constrains taxonomic resolution for certain bacterial groups. For instance, the assignment of sequences to the *Escherichia–Shigella* complex reflects the inability of the V3–V4 region to distinguish between these genera, encompassing both commensal and pathogenic lineages. Therefore, the high relative abundance of this taxon should be interpreted as indicative of enteric bacterial communities rather than as direct evidence of transmission risk. Furthermore, reliance on relative abundance data limits the ability to infer absolute microbial loads or assess quantitative thresholds potentially associated with infection risk.

The use of pooled samples was intended to obtain community-level microbiota profiles representative of communal roosting sites while minimizing the influence of individual-level variation. However, although this strategy facilitated characterization of site-level bacterial communities, it reduced the number of analytical units available for downstream analyses and limited the assessment of individual-level variability. Consequently, the findings should be interpreted as descriptive community-level observations rather than population-level estimates of microbiota variation among individual pigeons. Collectively, these factors highlight the importance of integrating ecological interpretation with methodological awareness when assessing microbiome data.

Another limitation relates to the use of PICRUSt2, which infers functional potential from phylogenetic information rather than directly measuring gene content or expression. Consequently, predicted pathways should be interpreted as exploratory hypotheses that require confirmation through shotgun metagenomics, metatranscriptomics, or functional assays.

Future studies employing high-resolution approaches and the inclusion of appropriate negative controls will be essential to refine taxonomic resolution and improve confidence in functional predictions. In particular, these approaches could facilitate the identification of virulence-associated genes, antimicrobial resistance determinants, and strain-level variation, supporting a more comprehensive understanding of the ecological and epidemiological significance of pigeon-associated microbiota.

5. Conclusions

This study provides a comprehensive characterization of the fecal bacterial microbiota of urban *C. livia* in northern Mexico, revealing microbial communities largely dominated by typical gut-associated bacteria rather than profiles dominated by bacteria previously associated with pathogenic lineages. Although several bacterial taxa with reported clinical relevance were detected, only a small subset of tentatively assigned species could be conservatively linked to documented zoonotic associations in the literature. From a functional perspective, PICRUSt2-based predictions suggested a predominance of metabolic pathways associated with carbohydrate degradation and energy acquisition, indicating relatively consistent predicted metabolic profiles across pooled samples. These findings suggest that similar predicted functional trends may occur despite taxonomic variability, potentially reflecting shared ecological pressures associated with urban environments and diet.

Overall, the results of this study suggest that urban pigeons may reflect patterns of environmental microbial exposure rather than serve as clear indicators of direct infection risk. Future studies employing high-resolution approaches, such as shotgun meta-

genomics, quantitative PCR, and improved experimental controls, will be important for refining taxonomic resolution and improving confidence in functional predictions. Such integrative efforts may contribute to a more comprehensive understanding of the ecological and epidemiological significance of pigeon-associated microbiota within urban environments and the human–animal–environment interface.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/microbiolres17070127/s1>, Figure S1: Rarefaction curves showing ASV richness as a function of sequencing depth in pooled fecal microbiota samples from *Columba livia* collected in northern Mexico. Each curve corresponds to one pooled sample (P1–P5), and shaded regions indicate 95% confidence intervals. The asymptotic trend observed across samples suggests that sequencing depth was sufficient to capture most of the bacterial diversity present in each community. Table S1: Summary of read-processing statistics generated using DADA2 for V3–V4 amplicon sequences, including Good’s coverage estimates and NSTI (Nearest Sequenced Taxon Index) values obtained from fecal samples of the urban pigeon (*Columba livia*). Table S2: Mean relative abundances of bacterial taxa detected in fecal samples of the Urban Pigeon (*Columba livia*); tentative species-level assignments were obtained using conservative NCBI BLAST validation; BLAST query coverage, E-value, percent identity, and accession numbers are reported only for taxa meeting these criteria. Table S3: Alpha diversity metrics across pooled fecal samples of the Urban Pigeon (*Columba livia*). Table S4: Relative abundance (%) of MetaCyc metabolic pathways predicted using PICRUSt2 from 16S rRNA gene sequences across five pooled fecal samples (P1–P5) of *Columba livia*. Pathways are shown with their corresponding MetaCyc identifiers and descriptions. Mean values indicate average relative abundance across samples. Table S5: Conservative screening of tentatively identified bacterial taxa for potential kit-, reagent-, or skin-associated contamination based on published low-biomass microbiome studies and environmental metadata (screening was used for contextual interpretation only and did not constitute contaminant removal) [61]. Table S6: Tentatively identified fecal bacterial species with reported clinical relevance and documented zoonotic potential detected in the Urban Pigeon (*Columba livia*).

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Data Availability Statement: The raw datasets underlying the findings of this study have been deposited in the National Center for Biotechnology Information under accession number PRJNA1455177.

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Abbreviations

The following abbreviations are used in this manuscript:

DNA	Deoxyribonucleic acid
rRNA	Ribosomal ribonucleic acid
QIIME2	Quantitative Insights into Microbial Ecology
ASVs	Amplicon sequence variants
BLAST	Basic Local Alignment Search Tool
DADA2	Divisive Amplicon Denoising Algorithm
NCBI	National Center for Biotechnology Information
PICRUSt	Phylogenetic Investigation of Communities by Reconstruction of Unobserved States
NSTI	Nearest Sequenced Taxon Index

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