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Contribution of poultry farm practice to the structure and composition of bacterial communities in the soil of poultry farms in Lagos, Nigeria

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Abstract

Background Poultry farming practices can impact soil health by altering its microbial and organic composition. On one hand, this could improve soil fertility, but on the other, it may pose health risks to humans and the environment. In this study, we assessed the microbial community structure and composition of poultry litter impacted soil in poultry farms in Lagos Nigeria.

Methods Composite soil samples were collected from two farms (PF01A and PF02B) in a poultry estate and from a pristine environment (CTR) without farming activity. Metagenomic analysis was performed using 16S rRNA gene amplicon sequencing and bioinformatics analysis.

Results The most abundant phyla in poultry-impacted soils were Firmicutes (54–69%), Bacteroidetes (18–19%), and Actinobacteria (3–17%), with a low relative abundance of Proteobacteria (7%). In contrast, CTR soil was dominated by the phylum Proteobacteria (30%) and had a low relative abundance of Bacteroidetes (2%). Bacteria strains, including *Jeotgalibaca arthritidis*, *Lysinibacillus jejuensis*, and the novel species *Enterococcus lemanii* and *Enterococcus eurekensis*, were among dominant taxa in PF01A and PF02B.Some dominant bacterial species such as *Corynbacterium xerosis* and *Ignatzschineria indica* have been implicated in human infections and are known to be multidrug resistant.

Conclusion In this study, a high abundance of bacterial operational taxonomic units (OTUs) were found in soil from poultry farms. The presence of poultry litter significantly changed the composition of the bacterial community in the soil. This alteration did not only affect the microbial structure and health of the soil, it also increased the presence of clinically important bacteria, which could pose potential risks to public health.

Keywords Bacterial pathogens, Environment, Microbiome, Poultry, Public health, Soil

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Introduction

The planetary crisis, driven by climate change, biodiversity loss, and the emergence of infectious diseases, stems from anthropogenic activities that show no signs of abating. Global temperatures have consistently risen, leading to increased precipitation, which, in turn, inadvertently impacts microbial communities in soils [1, 2]. The health of soil fundamentally influences human health in both positive and negative ways. This is evident in its role in food production, where it provides essential nutrition for humans, while also acting as a potential pathway for the transmission of pathogens and toxic elements to both humans and animals [3, 4]. Soil health is highly affected by the activities of humans such as cultivation, urbanization, and industrialization, which lead to large alterations in both the abiotic and biotic properties of the soil [5]. The activity and diversity of microorganisms, which constitute the biotic component of the soil, are closely linked to soil health [6]. Microorganisms are instrumental in humifying organic matter, mineralization, and the cycling of elements in the biogeochemical cycle [7]. The bacterial communities within the soil microbiota can be influenced, either positively or negatively, by agricultural practices [8]. One such practice is poultry farming, which can impact the environment through the introduction of poultry droppings.

Poultry litter is described as a complex material containing a diverse population of bacteria, viruses, and fungi, mixed with decomposing bedding of plant origin [9]. The use of poultry litter on soil is known to have a significant positive effect on the activity of nitrifying bacteria [10].

While poultry litter is considered an organic fertilizer due to its content of nitrogen (N), phosphorus (P_2O_5), and potassium (K_2O) in varying proportions [7, 11], it often increases phosphate levels in the soil. However, excessive phosphates can be transported into water bodies through runoff, promoting eutrophication [12]. Additionally, nitrate (NO_3-N) leaching into groundwater has been reported in croplands amended with poultry litter [13]. Untreated (non-composted) poultry litter is a source of saprophytic and disease causing bacteria, such as *Campylobacter* spp., *Listeria monocytogens*, *Staphylococcus aureus, Salmonella* spp., as well as other commensals and antibiotic residues [9, 14, 15]. Generally, poultry litter, due to its rich nutrient content, is often used as an organic fertilizer in the cultivation of vegetables and other food crops [16, 17]. However, Such farming practices have been linked to the transmission of foodborne pathogens within the food chain and environment [18]. Some foodborne pathogens present in poultry litters can survive for extended periods, increasing their potential for transmission [19]. In Southern Benin, a documented risk of food poisoning from *Campylobacter jejuni* and *Campylobacter coli* was associated with the consumption of leafy vegetables cultivated using poultry manure [20].

Not only are bacterial foodborne pathogens disseminated through poultry litter, but antibiotic resistant strains are also released into the environment [21]. In a Cameroonian urban area, 100% of *E. coli* and 36.4% of *Salmonella* spp. isolated from chicken litter manure were found to be multidrug resistant, posing a serious public health threat [22]. Some of these multi-drug resistant bacteria isolated from poultry litter harbor plasmids indicating the potential for horizontal gene transfer of genetic determinants, which further amplifies the dissemination of antimicrobial resistance [23].

While animal husbandry has significantly improved human well-being, it has also had a substantial impact on the natural environment and contributed to the spread of pathogens [24]. To sustain the progress made toward achieving the targets of the Sustainable Development Goals (SDGs) [25], it is crucial to understand the dynamics and impact of human activities, such as agriculture, on environmental health. With the advancement of molecular biology and bioinformatics tools, deeper insights into the perturbations of microbial communities caused by anthropogenic activities can be uncovered [26]. These insights enable the implementation of timely actions to mitigate adverse outcomes and promote environmental sustainability. Assessing the health of the soil microbiome in relation to the introduction of pathogens through agricultural practices is essential [27]. However, there is limited data on the contribution of poultry liter to the microbial composition of soils in Nigeria, despite the socioeconomic significance of agriculture, particularly livestock farming, in the lives of Nigerians [28].

Therefore, this study aimed to evaluate the composition and structure of the microbial community in soil receiving poultry litter at a poultry farm estate in Lagos, with the objective of elucidating the dynamic changes in the soil microbial community caused by poultry farming practices.

Materials and methods

Study design, sampling site and sample collection

This study was a cross sectional assessment of the contribution of poultry farming to the microbial community of the soil. Soil samples were obtained from two poultry farms located in poultry estates in Ikorodu (6°38′11.5008N, 3°32′23.7624E), Lagos State, Nigeria**.** Ikorodu is one of the 20 local government areas of Lagos State, the economic hub of Nigeria. It is a peri-urban settlement with an estimated population of 535,619, based on the 2006 census [29]. Soil samples contaminated with

poultry litter were collected using a soil auger (5.715 cm) from five different points in each farm, as well as from an unimpacted site, following the method described by Hegyi et al. [30]. Samples from each site were pooled by sieving (4 mm) to create a composite sample, which was packed then packed in labeled sterile zip lock bags. The samples were placed in a thermo-box and transported at 4 °C to the laboratory for further analysis.

Ethical approval

Ethical approval for this study was obtained from the Institutional Review Board of the Nigerian Institute of Medical Research with approval number IRBB/21/023. Social approval was granted by the Lagos state Ministry of Health with approval number LS/C.350/S.I/497.

Total DNA extraction

Total community DNA (tcDNA) was extracted as previously described by Oyetibo et al. [31]. Extraction was performed on 0.5 g of soil using Fast DNA® spin kit for soil (MP Biomedicals Solon, OH, USA.) according to the manufacturer's instructions. The FastPrep Cell Disruptor FP 120 (Qbiogene, Heidelberg, Germany) was used for the mechanical lysis of cells, with 20 mg of sterile skim milk (Sigma-Aldrich) added to the lysing matrix to prevent interference from humic substances during polymerase chain reaction (PCR). NanoDrop spectrophotometer (NanoDrop 2000; Thermo Fisher Scientific, Wilmington, DE, USA) was used to measure the purity and concentration of DNA isolated.

Sequencing and sequence reads analysis

Sequencing and analysis of reads were performed as previously described by Oyetibo et al. [31]. Using primer sets 341F and 805R, the bacterial 16S rRNA gene was amplified. The V3 – V4 hypervariable regions of the bacterial 16S rRNA gene was targeted for amplification to effectively discriminate between diverse bacterial taxa and generate high accuracy data. Libraries were constructed and checked for quality with Agilent 2100 Bioanalyzer System (Agilent Technologies, Palo Alto, CA, USA) through a DNA 7500 chip. Quantification was then done using the Quanti-iT™ PicoGreen™ dsDNA Assay kit (Invitrogen) following the manufacturer's instructions. Short DNA fragments were removed using CleanPCR™ (CleanNA, Netherlands) and sequencing was performed with the Illumina MiSeq Reagent Kit v2 (500-cycles) to obtain 20–30 M reads. Sequencing of libraries was performed on the Illumina MiSeq platform (Illumina, San Diego, CA, USA) at ChunLab Inc. Seoul Korea. The quality of the generated data was assessed, and low quality reads (< Q25) were sorted using Trimmomatic 0.32 software [32]. PANDAseq software was used to merge pair-end sequences of similar strand of the PCR amplicon that overlapped [33]. The 16S rRNA PCR primer sequences were removed using ChunLab's pipeline in-house algorithms. Identification and removal of non-specific amplicons was done through an HMMER program-based search to exclude Singleton sequences [34]. Additionally, using DUDE-Seq software, sequences were denoised [35], and extraction of de-replicated and non-redundant reads was done by UCLUST-clustering [34]. Chimera were detected and removed using UCHIME against BIOiPLUG's chimera-free reference database. Non-chimeric sequences were then clustered into operational taxonomic units (OTUs) as previously described by Lee et al. [35]. Taxonomic assignment was conducted by comparing the sequence reads against the EzBioCloud 16S database (https://www.ezbiocloud.net/), using a combination of the BLAST-based searches and pairwise similarity comparisons with a 97% similarity threshold. Reads with less than the 0.97 cut-off value were assigned to an unclassified group [36].

The sequence data generated in this study have been deposited in the Sequence Read Archive (SRA) database of NCBI under BioProject accession number **PRJNA939722.**

Statistical analysis

GraphPad Prism version 5 (GraphPad Software San Deigo, CA, United States of America) statistical software was used to generate bar charts. The coverage of constructed 16S rRNA libraries were estimated using the formula $C = 1 - \left(\frac{n}{N}\right) \times 100$,where N represents the total number of sequences in the initial dataset and n is the number of Singletons after assembly [37]. Diversity and richness indices, including Shannon–Weaver index, abundance-base coverage estimator (S_{ACE}) and the bias corrected Choa1 were computed using pre-calculated program of CLcommunity™ software package [31].

Results

Microbial community structure of soil samples from poultry farms

Quality filtering of sequence data yielded 17,714, 16,975 and 24,218 valid bacterial reads of for PF02B, PF01A, and CTR, respectively. Reads from PF02B were clustered into 1,036 operational taxonomic units (OTUs), those from PF01A into 1,189 OTUs, and CTR had its reads clustered into 3,037 OTUs as shown in Table 1. Based on Good's library coverage estimate, more than 98.37%, 98.16% and 96.09% of the microbial community were represented by bacterial OTUs in PF02B, PF01A and CTR soil samples, from the poultry estate and pristine site, respectively. Furthermore, microbial richness and diversity were proportional to the values of the abundance-based coverage

Table 1 Alpha diversity of the microbial community, indicating evenness, richness, and species variety in soil samples

Sample name	Target reads	OTUs	ACE	CHAO	Jackknife	NPShannon	Shannon	Simpson	Phylogenetic Diversity	Good's coverage of library (%)
PF02B	17,714	1036	1286.37	1223.00	1325.88	4.61	4.52	0.08	1400	98.37
PF01A	16,975	1189	1457.27	1350.18	1501.00	5.11	5.01	0.03	1615	98.16
CTR	24,218	3037	3922.19	3613.22	3989.25	6.59	6.43	0.01	4004	96.09

estimator (ACE), Chao1, and Jacknife (Table 1). Bacterial diversity in PF01A was greater than that in PF02B, as indicated by alpha diversity indices, including NPShannon, Shannon, and inverse Simpson and phylogenetic diversity.

Bacterial community composition of soil samples from poultry farms

The relative abundance of bacterial communities at the phylum and family levels in the soil samples is shown in Figs. 1 and 2, respectively. In the PF02B sample, four dominant phyla were Firmicutes (69.1%), Bacteroidetes (19.1%), Proteobacteria (7.4%), and Actinobacteria (3.4%). In contrast, sample PF01A was dominated by Firmicutes (55%), Bacteroidetes (18.5%), Actinobacteria (2.3%), Proteobacteria (7.4%), and Tenericutes (1%). The CTR soil sample, which had no history of contamination with poultry droppings, exhibited nine dominant phyla in the following order: Proteobacteria (30%), Firmicutes (28.1%), Actinobacteria (22.3%), Acidobacteria (7.5%), Verrucomicrobia (3.4%), Chloroflexi (2.9%), Bacteroidetes (2%), Gemmatimonadetes (1.4%) and Nitrospirae (1.1%) (Fig. 1). The relative abundance of Firmicutes was observed in all samples, including CTR. However, the relative abundance of Bacteroidetes in the CTR sample was barely 2%, despite being a dominant phylum in the impacted soils, as shown in Fig. 2.

Nevertheless, Lactobacillaceae (PF02B, 34.1%; PF01A, 20.4%), and Bacteroidaceae (PF02B, 11.8%; PF01A, 9.2%) were the dominant family taxa in the impacted soils. Additionally, Lachnospiraceae (8.5%) and Enterococcaceae (5.4%) constituted the remaining dominant family taxa in PF02B. In contrast, PF01A was predominantly characterized by Lactobacillaceae (20.4%), Corynebacteriaceae (15.4%), Bacteroidaceae (9.2%), Peptoniphilaceae(6.5%), Planococcaceae (5.8%) and Ruminococcaceae (5.5%). In comparison, CTR which was not impacted by poultry droppings, was dominated by nine families, including Bacillaceae (22.5%), Bradyrhizobiaceae (7.0%), Gaiellaceae (6.0%) and Sphingomonadaceae(5.3%) (Fig. 3).

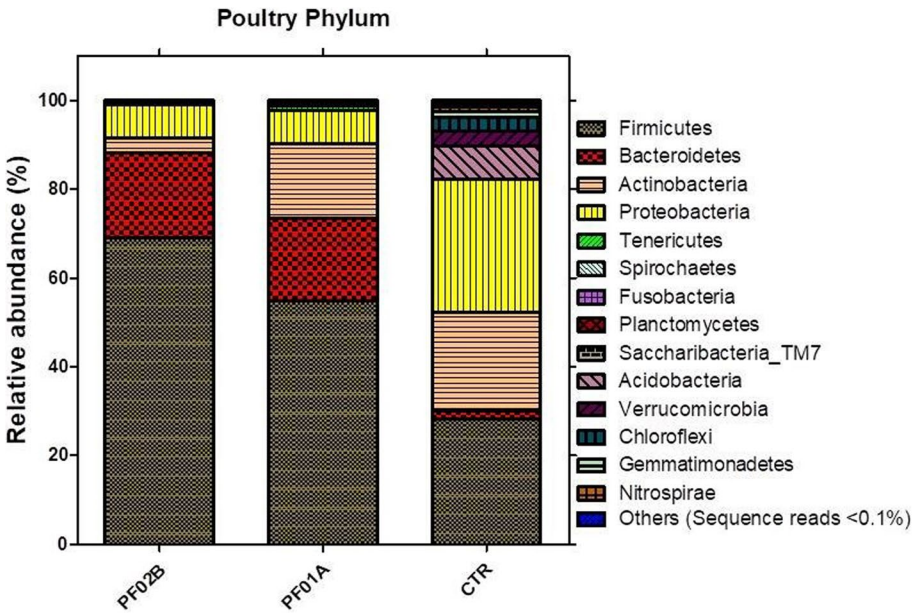

Fig. 1 Taxonomic composition at the phylum level of soil samples from a poultry estate and a pristine environment. PF02B and PF01A represent poultry-impacted soil, while CTR represent pristine soil unaffected by poultry litter. Others includes phyla with sequence reads less than 0.1%

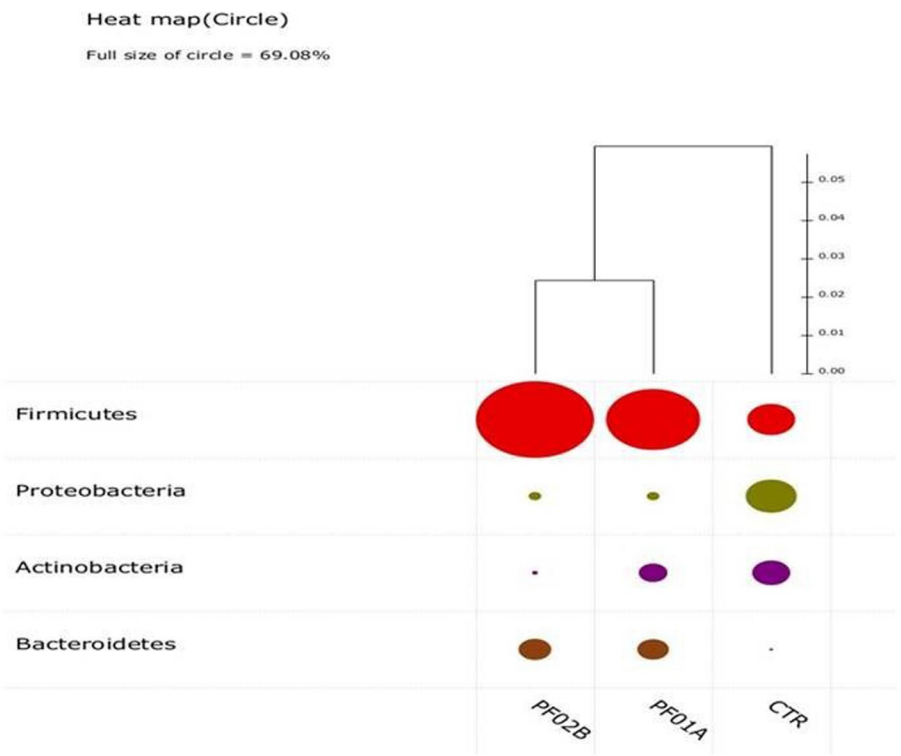

Fig. 2 Heat map representing abundance of bacterial phyla above the cut off threshold. Each taxon and its proportion are depicted by a circle, with the full size of circle representing 69.08%. PF02B and PF01A represent poultry-impacted soil, while CTR represents pristine soil unaffected by poultry litter

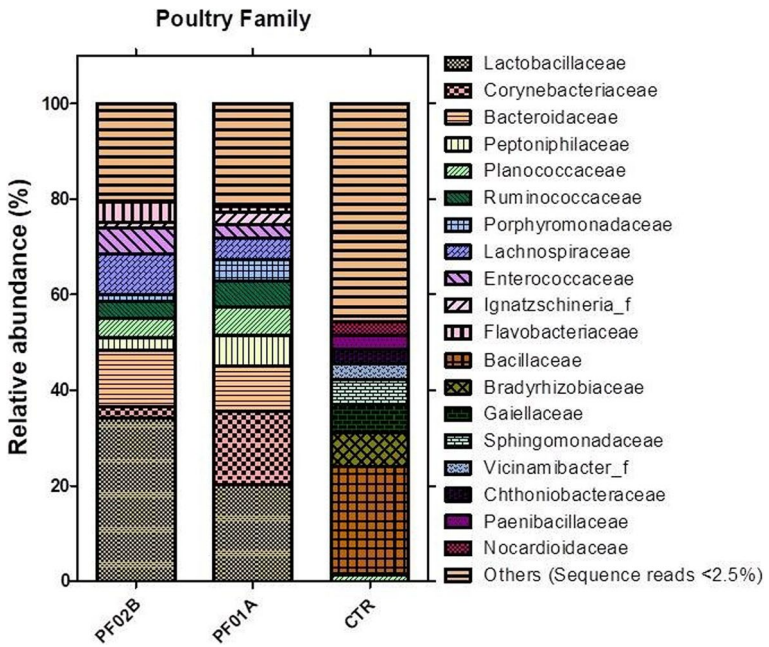

Fig. 3 Taxonomic composition at the family level of soil samples from poultry estate and a pristine environment. PF02B and PF01A represent poultry impacted soil, while CTR represents pristine soil unaffected by poultry litter. Others represent families with sequence read less than 2.5%

It is imperative to note that none of the dominant family taxa in the poultry-droppings-impacted soils had relative sequence reads exceeding 1% in the unimpacted (CTR), except for Planococcaceae, which comprised a mere 1.3% relative abundance. Interrelationships between soil samples revealed that the impacted soils (PF01A and PF02B) were closely related but distinctly separated from CTR, which is free from poultry droppings, as depicted by the heat map analysis (Fig. 4).

However, Bacillaceae, which was absent in the impacted soils (PF01A and PF02B), was the only dominant family in CTR, that was not impacted by poultry droppings. Conversely, Corynebacteriaceae and Bacteroidaceae were present in PF01A and PF02B but absent in CTR, which had no history of contamination with poultry droppings. The phylogeny and evolutionary relationships of the dominant bacterial strains from the impacted samples is shown in Fig. 5. The dominant bacterial strains were grouped into four clades based on monophyletic and polyphyletic relationships. Notably, the closely related *Enterococcus eurekensis*, *Enterococcus lemanii*, *Jeotgalibaca arthritidis,* and *Aerococcaceae* AJ279038_g clustered together in one clade. Similarly, *Lactobacillus* and *Corynebacterium* strains formed a distinct clade as Gram-negative bacilli *Ignatzschineria indica* and *Acinetobacter towneri* clustered together, highlighting their evolutionary relatedness.

Discussion

The use of organic fertilizers, particularly poultry litter, in improving soil health has been widely reported [8, 17]. Agricultural practices results in substantial alterations to the microbial community structure of the soil [8, 38]. This could explain the large numbers of bacteria OTUs observed in this study and the proportional relationship between diversity and microbial richness to diversity index values. Similarly, Parente et al. [21] reported relatively high richness in a related study, with confirmed bacterial OTUs ranging from 1,243 to 1,279 in soil samples fertilized with poultry litter over time. In this study, the dominance of Firmicutes and Bacteroidetes in soils impacted by poultry litter contradicted the expected dominance of Proteobacteria

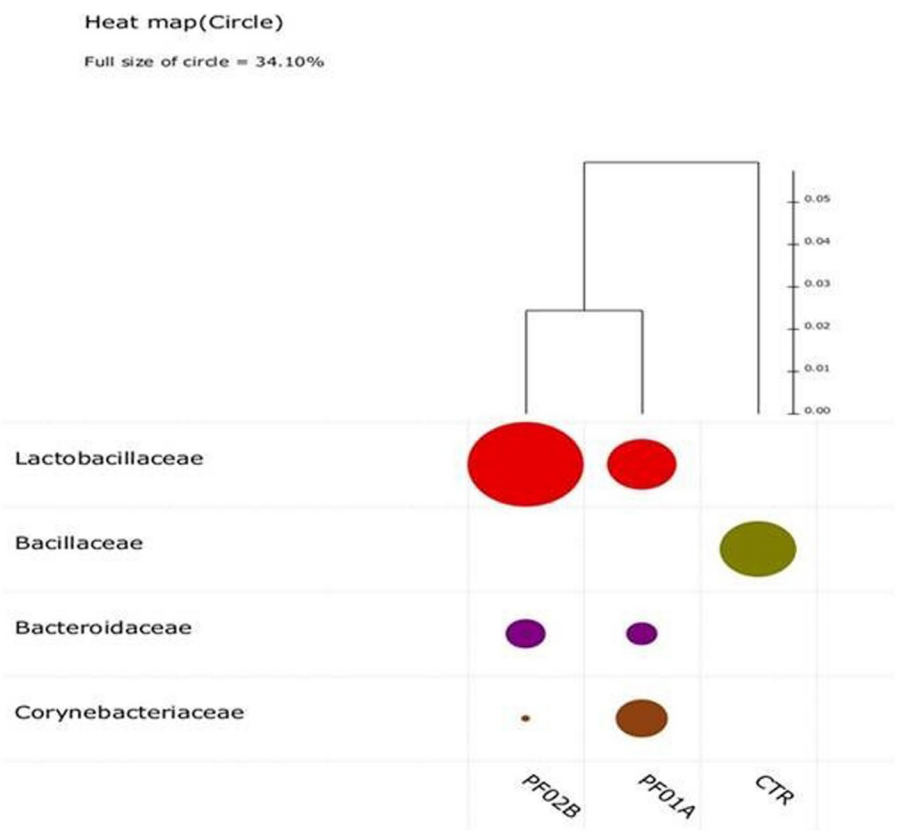

Fig. 4 Heat map representing the abundance of bacterial families above the cut off threshold. Each taxon and its proportion are depicted by a circle, with the full size of the circle representing 34.10%. PF02B and PF01A represent poultry-impacted soil, while CTR represent pristine soil unaffected by poultry litter

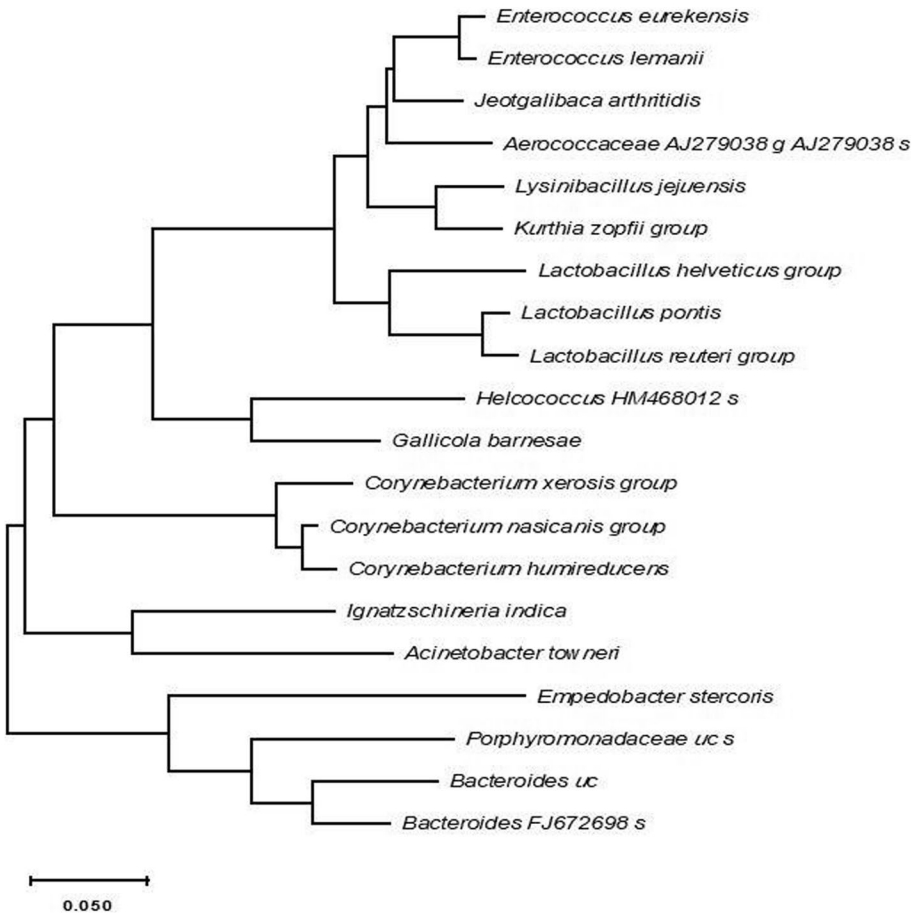

Fig. 5 Evolutionary relationships of the most abundant taxa in PF01 and PF02 sequences. The evolutionary trend was derived using the Neighbor-Joining method. Genetic divergence was computed using the Maximum Composite Likelihood method, with the scale bar (0.050) representing the number of substitutions per site. The final dataset included a total of 431 positions

observed in CTR and other copiotrophic systems [31]. This indicates that the enrichment of soils with poultry droppings is likely responsible for the observed skewness, as previously reported [21]. Peng et al. [23] observed a significant alternation in the bacterial community structure of soli that received poultry manure for three years, with the presence of Firmicutes, Proteobacteria, Actinobacteria and Bacteroidetes. Thus, the shift in the bacterial community from Proteobacteria dominace to Firmicutes and Bacteroidetes dominace reveals the clear impact of poultry farming practice on the soil. Additionally, it can be inferred that the OTUs and diversity in the CTR soil were less resilient to the changes imposed by the introduction of poultry litter, as bacteria phyla such as Acidobacteria, Chloroflexi, Verrucomicrobia, and Nitrospirae were depleted or eliminated in PF02A and PF02B. Our findings align with those of Parente et al. [21], who reported similar dominant phyla, including Firmicutes, Bacteroidetes, Proteobacteria and Actinobacteria, in poultry litter-impacted soils, with a reduced presence of Firmicutes in the control soil sample. Firmicutes and Bacteroidetes have been reported as dominant bacterial phyla in the duodenum, ileum, and cecum of chickens [39], which explain the observed change in dominant phyla. However, it should be noted that the bacterial composition of the chicken gut is not stable, as age is an important factor influencing changes in its taxonomic composition [40]. This suggests that the microbial community profile observed in this study may reflect the age of the birds from which the poultry litter was obtained. In terms of bacterial families, Lactobacillaceae dominated among other bacterial families in samples PF01A and PF02B. Additionally, Corynebacteriaceae was a dominant family in PF01A, while it was present in lower abundance in PF02B. Conversely, the control sample (CTR) had Bacillaceae as the dominant bacterial family. Neher et al. [41] have reported similar bacterial community dynamics in a study demonstrating the effect of poultry compost on the alteration of

microbial communities within field soils planted with spinach.

Gram positive bacterial strains such as *Lysinibacillus jejuensis*, *Jeotgalibaca arthritidis*, *Enterococcus lemanii* and *Enterococcus eurekensis* were among dominant bacterial strains identified in sample PF01A and PF02B in this study. Another dominant Gram positive bacterial species identified in this study was *Lactobacillus*. *Lactobacillus* species are known to play a crucial role in cycling nitrogen-containing compounds such as NH_3, in poultry manure, thereby creating a safe environment for both humans and birds [42].On the other hand, safety concerns regarding human and environmental health have been raised over the use or introduction of poultry litter into soil. Contamination of soil with pathogenic bacteria (both antibiotic and non-antibiotic resistant strains) and antibiotic resistance genes are among key safety issues highlighted by Smoglica et al. [43]. Although the focus of this study was not on antimicrobial resistance, the phyla and families to which known pathogenic, antibiotic resistant bacteria belong were prominent. Enterococci species, which were found to be dominant in this study, thrive in diverse environments, including food, feed, soil, and water. However, enterococcal resistance to important antibiotics raises public health concerns [44]. Fatoba et al. [45] advocated for a reassessment of the use of chicken litter as manure following a study that revealed 56% of *Enterococcus* species isolated from agricultural soils in South Africa were resistant to at least one antibiotic.

Similarly, *Acinetobacter towneri*, a non-*baumannii Acinetobacter* species [46], was a dominant bacterial strain in both PF01A and PF02B. Although *Acinetobacter towneri* is not typically implicated in infections, it is considered a public health threat due to its ability to accumulates clinically relevant antimicrobial resistance genes, with the potential for transmission to other strains such as *Acinetobacter baumannii*, a known cause of several nosocomial infections [47].

Corynebacterium xerosis, one of the *Corynebacterium* species frequently reported as a human pathogen, is implicated in ear infections, osteomyelitis, maternal ventriculoperitoneal shunt infections, and brain abscesses [48]. It was also among the dominant strains identified in both samples in this study. Another emerging human pathogen [49] that dominated among bacterial strains in both samples in this study was the Gram negative asaccharolytic bacillus *Ignatzschineria indica*. This bacterium has been reported as a causative agent of bacteremia in patients with maggot-infected wounds [49, 50]. The prevalence of *Ignatzschineria indica* in soil receiving poultry litter is not surprising, given that poultry litter has an odor that attracts numerous flies. Notably, *Ignatzschineria indica* is associated with the parasitic flesh fly *Wohlfahrtia magnifica* [50].

The findings from this study have practical implications that extend beyond nutrient enrichment and soil health to potential risks of disease outbreaks, which may arise from the use of poultry litter in soil augmentation or its indiscriminate disposal into pristine environments. Khodadadi et al. [51] propose drying as a practical technology for reducing poultry litter pollution.

Conclusion

The analysis of soil from poultry farms in this study revealed a high abundance of bacterial OTUs, with Firmicutes, Bacteroidetes, and Actinobacteria being the most dominant phyla. The bacterial community composition in the soil showed noticeable shifts, likely influenced by the application of poultry litter. Among the identified strains, pathogenic bacteria such as *Corynebacterium xerosis* and *Ignatzschineria indica* were prominent, underscoring potential public health risks associated with the improper disposal of untreated poultry litter. While poultry litter can enhance soil microbial diversity and health, it is essential to implement measures to mitigate its adverse effects. Considering the critical role of poultry farming in providing income and nutrition for many households, adopting improved practices such as treating poultry litter prior to disposal or its use as manure will promote both environmental sustainability and public health.

Limitation

This study is constrained by the limited number of farms and samples included. The Data generated may not fully represent the dynamics on a larger scale. However, the data provided here can serve as baseline information for further studies.

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Authors' contributions

AA conceptualized the study. AA, BA, KO, EC, JY, RA, PE, JO, and SS designed the study. SS and BS obtained the grant. AA, BA, KO, EC, EUU, JY, RA, PE, JO, YAT, STA, RM, AO, RY and SS participated in advocacy visits and sample collection. AA, EC, EUU, JY, and JO carried out laboratory analysis. GOO and AA analyzed data and did initial draft of manuscript. All the authors participated in the discussion and reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data availability
Sequences generated in this study are available in the sequence read archive (SRA) database of NCBI under BioProject with the accession number PRJNA939722 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA939722).

Declarations

Ethics approval and consent to participate
Approval for this study was granted by the Institutional Review Board, Nigerian Institute of Medical Research with code number IRBB/21/023. Social approval was obtained from the Lagos state Ministry of Health with approval number LS/C.350/S.I/497.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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