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RESEARCH ARTICLE

Abundance and Diversity of The Faecal Resistome and Microbiome in Broilers

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Abstract

Antimicrobial resistance (AMR) is a growing problem, posing a threat to human and animal health. The use of antimicrobials in livestock selects for AMR that can subsequently be transferred to humans. This flow of AMR between reservoirs demands continuous surveillance in livestock and in humans. Although there are numerous studies to determine the fecal carriage of antibiotic-resistant bacteria in broiler flocks, there is a lack of comprehensive metagenomic research targeting the resistome in broilers in Türkiye. The aim of this study was to investigate the microbial composition and the profiles of antimicrobial resistance genes (ARGs, resistome) in three selected broiler farms in Hatay, using a shotgun metagenomics approach. The microbiota of broilers from Flock A and C was dominated by Bacillota (formerly Firmicutes) (46.83% and 43.87%, respectively), whereas Flock B exhibited a high relative abundance of Pseudomonadota (formerly *Proteobacteria*) (82.5%). At the genus level, *Brachybacterium, Escherichia*, and *Ligilactobacillus* were significantly more abundant in Flocks A, B, and C, respectively. It was also noticed that the most abundant KEGG pathways belonged to metabolism and genetic information processing. Furthermore, xenobiotics biodegradation and metabolism pathways were more abundant in Flock B than in Flocks A and C. Similarly, a higher ARG diversity was observed in Flock B. A total of 137 ARGs were identified, comprising different resistance mechanisms. The *MLS23S* gene, conferring macrolide resistance through a 23S rRNA gene mutation, was the predominant ARG in all flocks. These findings provide a baseline characterization of the broiler gut resistome, highlighting the importance of metagenomic surveillance in poultry production despite the unavailability of farm-level antimicrobial usage records.

Keywords: Broilers, Metagenomics, Microbiome, Bacillota, Pseudomonadota, *Ligilactobacillus*, Resistome

INTRODUCTION

According to World Health Organization (WHO), antimicrobial resistance (AMR) is a growing health concern worldwide, promoting international cooperation due to depth, breadth and complexity [1]. The emergence of resistance to antibiotics in bacteria is mainly associated with target mutations and horizontal transfer of resistance genes [2]. The poultry sector employs a variety of antimicrobials to maintain flock health and enhance production efficiency. These antimicrobials serve different functions and are categorized into therapeutic, prophylactic, and growth promotion uses [3]. Therefore, the widespread and intensive use of antibiotics in poultry farming has significantly contributed to the selection and emergence of antibiotic-resistant bacteria (ARB) and antibiotic-resistant genes (ARGs) [4].

Several national surveillance programs have been implemented to monitor the occurrence of AMR in different reservoirs using indicator bacteria (e.g. *Escherichia coli, Enterococcus* spp.) and to follow its trends over time [5-7]. There is currently no national program in the field of veterinary medicine in Türkiye for the determination of AMR. However, previous studies have reported have showed high levels resistance against critically important antimicrobials, especially in broiler flocks [8-11] and chicken meats [12,13]. Although these studies are important in demonstrating the emergence and spread of resistance, they target a limited number of species present in the gut microbiota and their associated resistance genes, thus representing only a limited portion of the resistome. Shotgun metagenomics has the potential to overcome the drawbacks of these approaches by directly identifying and characterizing the microbiome and resistome [14]. This technique provides a substantial overview of the abundance, diversity, and structure of the acquired broiler resistomes. Understanding circulating resistomes is thus important to provide critical insights for developing robust, tailored strategies to mitigate AMR risks and safeguard both public and environmental health [15]. For instance, the presence of mobile genetic elements (MGEs) such as the *mcr-1* gene in broiler gut microbiota can lead to the transmission of colistin resistance to human pathogens through the food chain or environmental runoff, posing a significant threat to public health.

In this study, it was aimed to perform comprehensive analysis of broiler's gut microbiomes and resistomes using shotgun sequencing.

MATERIAL AND METHODS

Ethical Approval

This study was approved by Hatay Mustafa Kemal University Animal Experiments Local Ethics Committee (Decision number: 2025/07-11)

Animals, Sample Collection and Processing

Three commercial broiler flocks, labeled flock A, flock B and flock C and located about 50 km from each other in Hatay, Türkiye, were selected for this study, between 10 and 20 September 2025 to minimize seasonal influences. On each farm, 25 undisturbed, fecal droppings were collected in different areas covering the whole house (a minimum of 3 g faeces per sample). All samples were collected aseptically in plastic containers and stored at 4°C and transported to the laboratory within 1-2 h after sampling. Upon laboratory arrival, individual fecal samples were homogenized by stirring thoroughly with a sterile wooden spatula for a few minutes. To obtain a representative profile of the entire house, a composite sample was created for each flock by pooling equal weights (approximately 2 g) of the 25 homogenized individual samples. These composite pools (one per flock) were then used for downstream DNA extraction.

DNA Extraction and Sequencing

DNA was extracted from 200 mg of each homogenized sample using the QIAamp Fast DNA Stool Mini Kit (Qiagen) based on the manufacturer's instructions. A bead beating step was applied at the beginning of the DNA extraction process. The concentration and quality of DNA were checked using NanoDrop spectrophotometer. To evaluate the flock-level microbial and resistance profiles, three metagenomic libraries (one per flock) were constructed from the homogenized composite DNA pools using the Nextera XT DNA library kit (Illumina). This pooling approach allowed for a comprehensive surveillance of the diverse genetic material present across the entire broiler house within a single sequencing run per farm. Fragment size was evaluated by an automated capillary electrophoresis system (Fragment Analyzer, Agilent Technologies, Santa Clara,CA, USA). The constructed libraries were subjected to high-throughput sequencing on the NovaSeq 6000 platform (Illumina), using 2 × 150-bp paired-end sequencing.

Bioinformatic Analysis

The quality of the reads was checked by FastQC tool [16]. After removal of low-quality reads and Illumina adapters by using Trimmomatic [17], the paired end sequences were aligned against the host reference genome (*Gallus gallus*) using the Bowtie2 tool [18]. After alignment, the mapped sequences, that is, reads derived from the host were removed. The integrated pipeline SqueezeMeta v1.4.0 was applied for further processing and annotation of the trimmed and host-removed reads from each sample [19]. Clean reads were assembled by MEGAHIT (v1.2.9) using default parameters and the Open Reading Frames (ORFs) were detected by Prodigal v2.6.3 (Li et al. 2016). Cleaned sequences were analyzed for antimicrobial resistance genes (ARGs) using the AMR++ pipeline v3 as well as for taxonomic assignment using Kraken2 [20]. For resistome profiling, clean reads were aligned against the MEGARes v3.0 database. A gene was identified as present only if it met a gene fraction coverage cutoff of 0.80 (80%), ensuring that the majority of the reference gene sequence was represented in our data. Furthermore, an identity threshold of 90% and a minimum alignment length of 75 bp were maintained to ensure the specificity of the ARG detection. Transcripts per million (TPM) normalization adjusts raw read counts so that the total expression across all genes is constant among samples, allowing each TPM value to represent the relative expression level of a gene within a sample. Statistical analyses and data visualizations were conducted using RStudio (R version 4.5.1) and the STAMP software [21]. For each sample, the Chao1, Shannon, and Simpson indices were calculated using the R package vegan, based on taxonomic units at the genus level. To find the correlation between the relative abundances of ARGs and bacterial genera, Spearman's rank correlation coefficients were calculated using the rcorr() function in the Hmisc package [22].

A correlation network was constructed based on strong and statistically significant associations, defined by a Spearman's rho (ρ) >0.8 and a P-value <0.01. Network graphs were created using the igraph and ggraph packages in R [23,24], applying the Fruchterman-Reingold force-directed layout for optimal visualization of the nodes and edges. Venn diagrams were plotted using ggvenn package [25] for the number of shared and unique anti-microbial resistance genes in the flocks.

RESULTS

Microbial Composition and Diversity

A total of 223,331,799 paired-end reads were generated after host removal and quality trimming. The sequencing depth per flock ranged from 74 million to 80 million reads, with an average of 77 million reads per sample. In addition, a total of 1,239,781 contigs were assembled during the metagenomic analysis, with a total assembly length of 1.15 Gbp and an N50 value of 1366 bp. At the taxonomic level, 84 phyla, 96 classes, 161 orders, 268 families, 879 genera and 1663 species were identified. The taxonomic distribution of the broiler gut microbiota at the phylum level was dominated by Bacillota (formerly Firmicutes) and Pseudomonadota (formerly Proteobacteria). Specifically, Bacillota accounted for 46.83% and 43.87% of the total sequences in Flocks A and C, respectively, while Pseudomonadota was the most prevalent phylum in Flock B (82.5%) *(Fig. 1)*. There were significant differences in the absolute abundances among the flocks *(Table 1)*. Notably, relative abundance of Pseudomonadota (82.5%) in flock B was significantly greater than that of other flocks *(Fig. 2)*. Whereas Bacillota (46.83%) and Actinomycetota (39.02%) in flock A and, Bacillota (43.87%) and Bacteroidota (28.51%) in flock C were noted as the dominant phyla. At the genus level, *Ligilactobacillus*, *Escherichia*, and *Brachybacterium* were identified as the most abundant taxa. The relative abundance of *Ligilactobacillus* was significantly higher in Flock C compared to the other groups. At the species level, *Escherichia coli* was significantly more abundant in Flock B, whereas *Ligilactobacillus salivarius* was more prevalent in Flock A *(Fig. 2-a)*

It was calculated several alpha-diversity indexes for each flock. The range of microbial diversity was much higher in Flock A and B than Flock C *(Table 2)*.

Table 1. *Relative abundance (%) of the dominant bacterial phyla and their associated major genera across the three sampled broiler flocks (Flock A, B, and C)*

Phylum	**Genus**	**Flock A (%)**	**Flock B (%)**	**Flock C (%)**
Bacillota	*Ligilactobacillus*	12	0.2	23
	Streptococcus	7	0.1	0.1
	Lactobacillus	12	0.4	6
	Faecalibacterium	2	0.5	1.7
	Staphylococcus	0.9	2.7	0.6
Bacteroidota	*Bacteroides*	0.2	0.1	6
	Alistipes	0.6	0.7	3
	Parabacteroides	0.2	0.3	1.9
Actinomycetota	*Corynebacterium*	16	1.8	0.06
	Brachybacterium	17	4	0.5
	Brevibacterium	3.9	1.4	0.05
	Dietzia	4	0.01	0.005
Fusobacteriota	*Fusobacterium*	0.009	0.05	9
Lentisphaerota	*Candidatus Spyradenecus*	0.0002	8	6
Pseudomonadota	*Escherichia*	10	83	9
Others (<1%)		12	5	31

The ARG Diversity of Flocks

The relative abundance of AMR to the corresponding class of antimicrobial's level for each flock was presented in *Fig. 3*. Shannon, Richness, and Simpson indexes were also calculated to assess ARG diversity. It was found that ARG diversity was relatively higher in Flock B *(Table 2)*. Both within and among poultry farms, the relative abundances of AMR per drug were more varied *(Fig 4)*. As seen in *Fig. 3*, macrolide AMR was more abundant in all flocks, but the relative abundance of tetracycline, aminoglycoside and beta-lactam drug classes differed in flocks. For example, relative abundance of beta-lactam resistance genes was higher in flock B, was much lower in other flocks. Aminoglycoside and tetracycline AMR genes were the most abundant in flock C, but flock A and

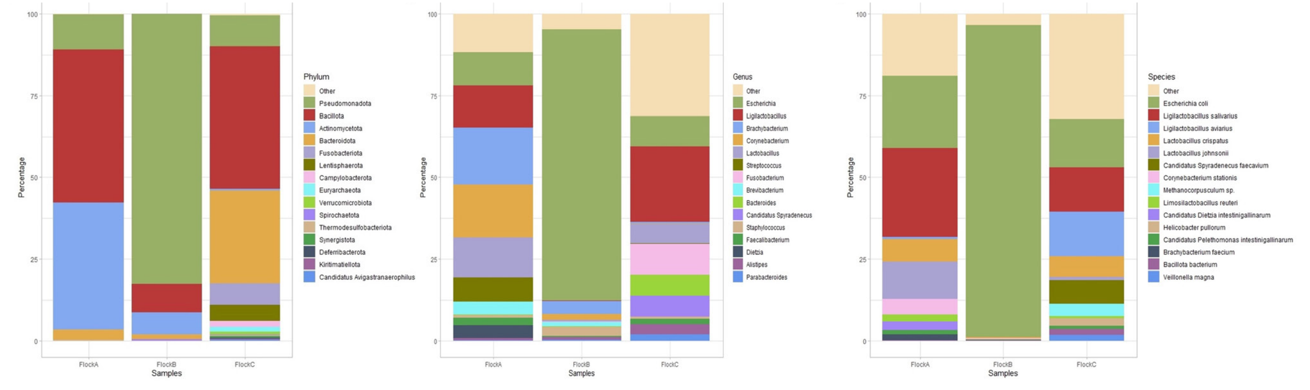

Fig 1. Taxonomic distribution of the microbial communities at the phylum, genus, and species level across three broiler flocks (**a, b,** and **c**). The 'Others' category includes phyla with a relative abundance of less than 1%. Data represent pooled samples from each flock

***Table 2.** Summary of alpha-diversity indices to determine the Shannon, Simpson, and Chao1 of microbial genera and ARGs*

Parameter	Flocks	Shannon	Chao1	Simpson
Microbiome	Flock A	3.18	1021	0.92
	Flock B	1.44	756	0.5
	Flock C	3.71	969	0.95
ARG	Flock A	0.97	1055	5.06
	Flock B	0.99	1078	5.51
	Flock C	0.96	673	4.59

ARG: antimicrobial resistance genes

B had low relative abundance *(Fig. 4)*. Similarly, flock A had a notably larger proportion of phenicol AMR than flock B and C. In addition, while oxazolidinone resistance was only observed in flock A, mupirocin resistance was observed in flock B *(Fig. 4)*.

The overall structure and composition of the resistome and AMR genes are presented in *Fig. 4*. A total of 137 different antimicrobial resistance genes were detected across all flocks. Most of them were associated with resistance to macrolides in all flocks. *MLS23S* were more abundant and ubiquitous in all flocks *(Fig. 4)*.

In flock C, A16S and *tet*16S were more common AMR genes following *MLS23S* gene. Among beta-lactam AMR genes, *bla*$_{\text{CTX}}$ and *bla*$_{\text{TEM}}$ were relatively higher in flock B, which is beta-lactam resistance higher than other flocks. Among several phenicol AMR genes, *cmx* and *fexB* were much more abundant in flock A than in the other flocks. Of the resistant genes related to the last resort antibiotics (colistin, carbapenem, linezolid), *optrA* and *poxtA* were only detected in one flock (flock A). *mcr* gene variants and carbapenemase genes (eg, KPC, IMP, VIM, NDM, OXA-48-like) were not detected in flocks. The relative abundances of other AMR genes varied across flocks *(Fig. 4)*. Among beta-lactam resistance genes, *bla*$_{\text{CTX}}$ and *bla*$_{\text{TEM}}$ were relatively more abundant in Flock B, which exhibited higher overall beta-lactam resistance than the other flocks. Regarding phenicol resistance genes, *cmx* and *fexB* were significantly more abundant in Flock A. Notably, resistance genes associated with last-resort antibiotics (e.g., colistin, carbapenems, linezolid), specifically *optrA* and *poxtA*, were only detected in Flock A. Conversely, *mcr* gene variants and carbapenemase genes (e.g., *bla*$_{\text{KPC}}$, *bla*$_{\text{IMP}}$, *bla*$_{\text{VIM}}$, *bla*$_{\text{NDM}}$, *bla*$_{\text{OXA-48-like}}$) were not detected in any of the flocks.

The relative abundances of other AMR genes varied across flocks *(Fig. 4)*. A Venn diagram illustrated the number of common and unique genes among the groups *(Fig. 5-a)*. Furthermore, network diagrams based on the co-occurrence of bacterial genera and ARGs were presented to visualize statistical associations and identify potential resistance carriers *(Fig. 5-b)*. The majority of ARGs

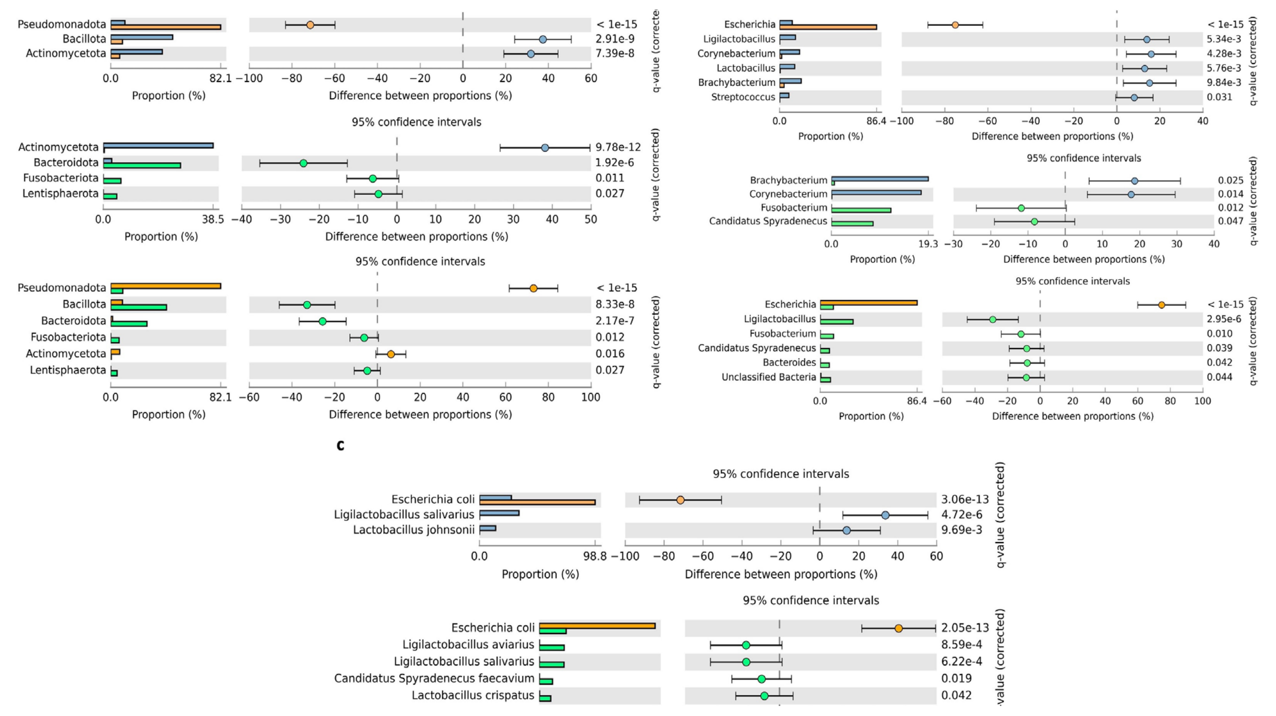

Fig 2. Extended error bar plots comparing microbial composition across the three flocks at different taxonomic levels: **a)** phylum, **b)** genus, and **c)** species. Each bar represents the proportion of taxa within each flock, with error bars indicating variability (e.g., standard error or confidence interval, if applicable). Colors denote flocks as follows: Flock A *(blue)*, Flock B *(orange)*, and Flock C *(green)*. Statistical differences between flocks were assessed using two-tailed Fisher's exact test with false discovery rate (FDR) correction (q-values <0.05)

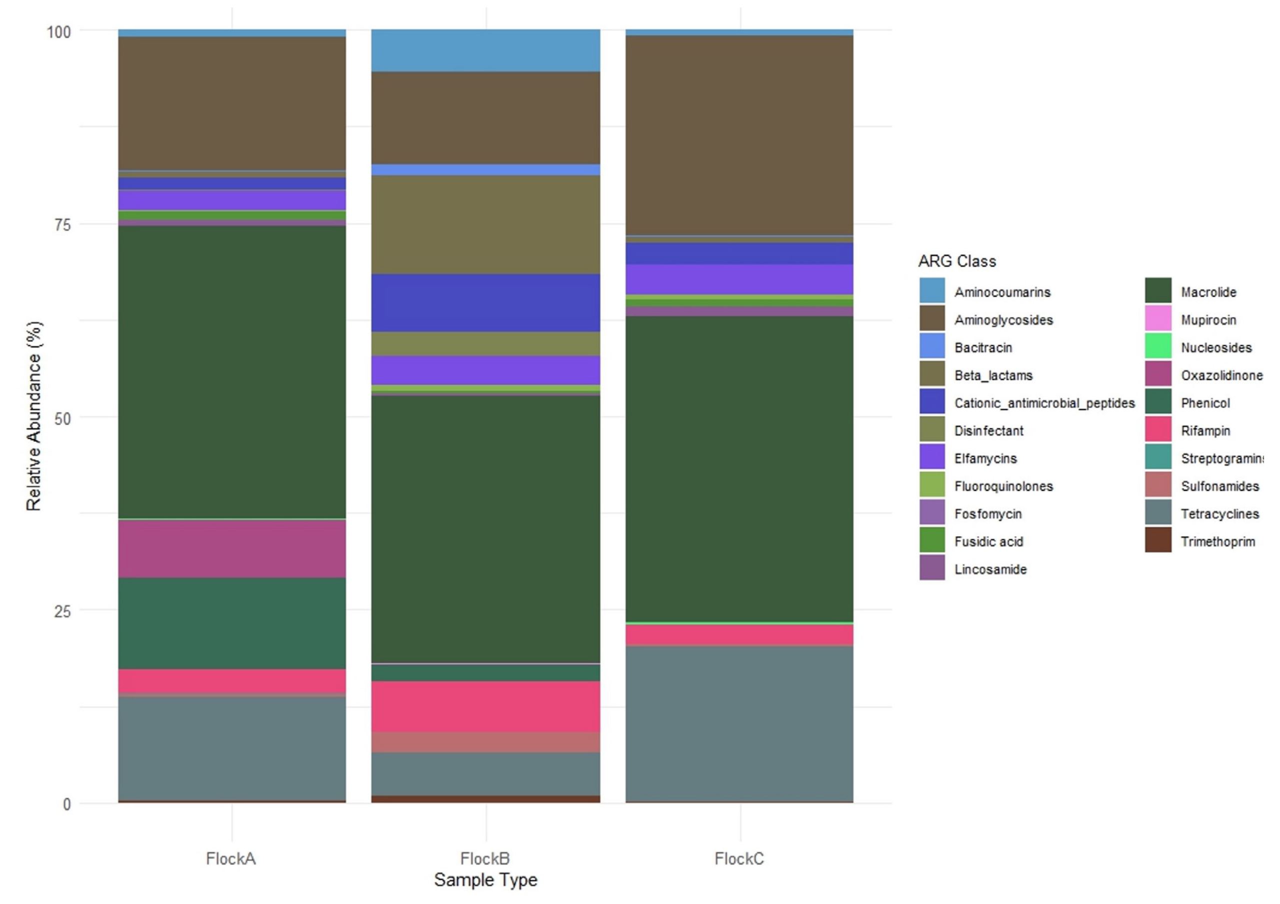

Fig 3. Stacked bar plots illustrating the relative abundance of antimicrobial resistance associated with different drug classes across the three flocks. Each bar represents an individual flock, and each segment within the bar corresponds to the proportion of resistance attributed to a specific drug class. Colors indicate distinct drug classes, as defined in the legend. The total height of each bar reflects the overall normalized abundance, enabling comparison of resistance profiles between flocks

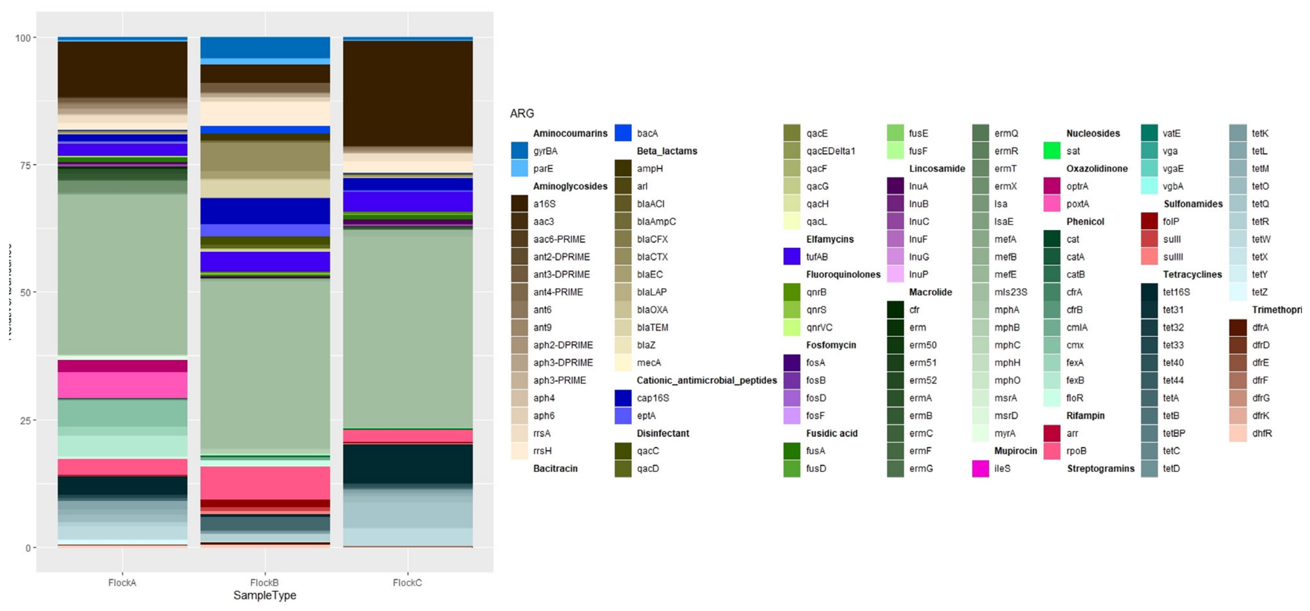

Fig 4. Stacked bar plots illustrating the relative abundance of antimicrobial resistance genes (ARGs) within each flock. Each bar represents a single flock, and each segment within the bar corresponds to the proportion of a specific ARG or ARG class. Colors indicate different ARGs (or ARG classes) as defined in the legend. The total height of each bar reflects the normalized relative abundance, allowing comparison of resistome composition across flocks

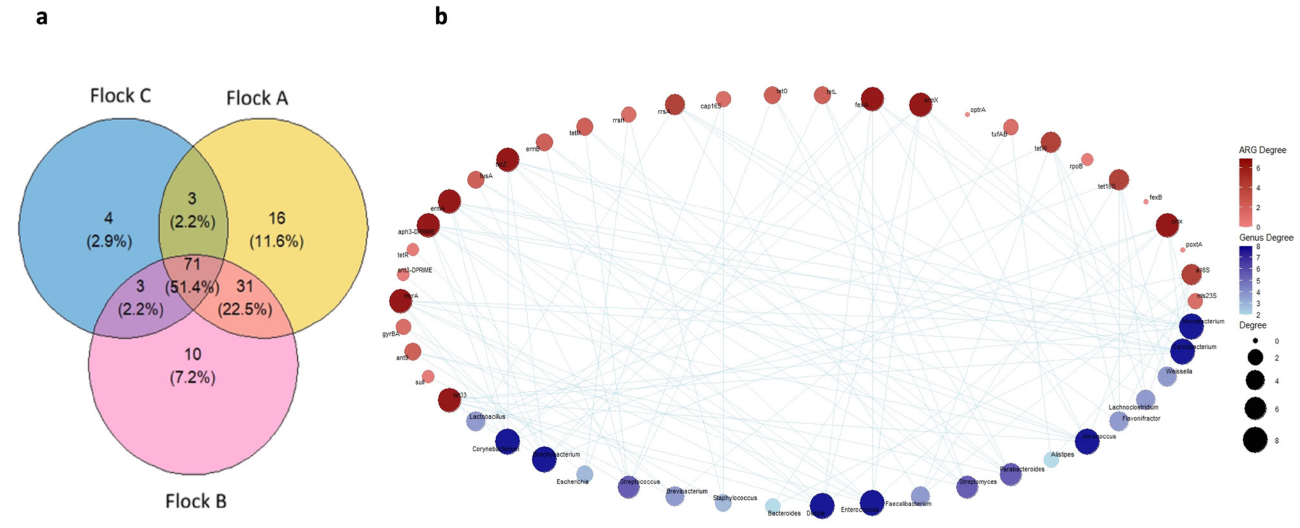

Fig 5. **(a)** Venn diagram showing the number of shared and unique ARGs among the flocks, **(b)** Co-occurrence network analysis identifying putative associations between bacterial genera *(red)* and ARGs *(red)*. Edges represent strong and statistically significant Spearman's correlations (rho >0.8, P<0.01). Node size is proportional to the relative abundance of each feature

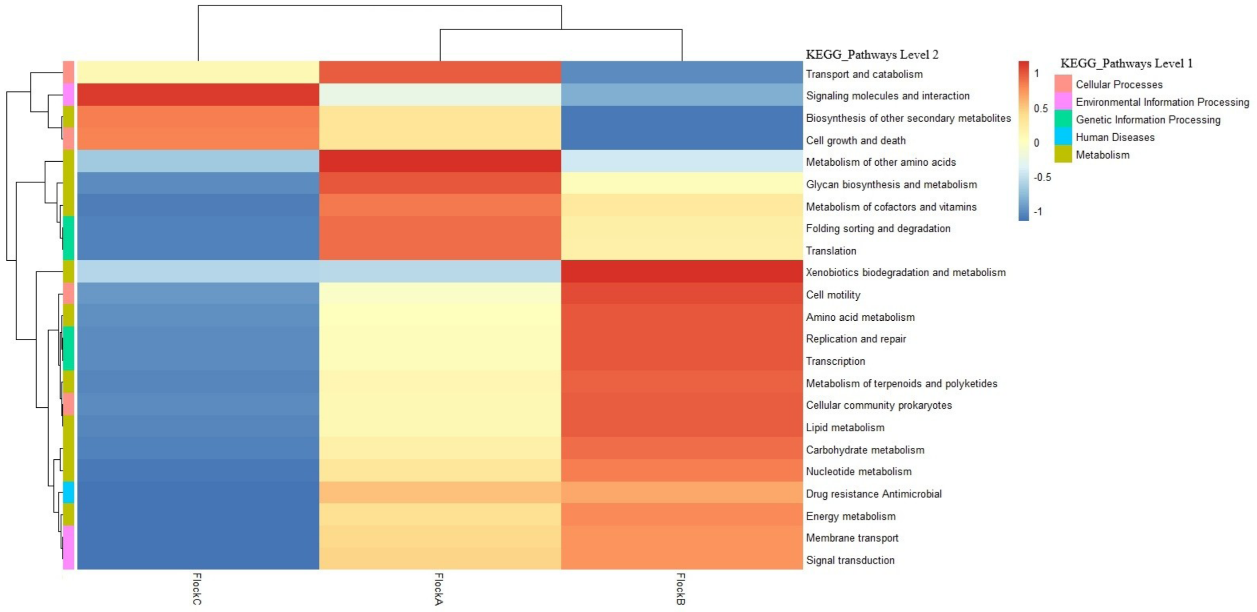

Fig 6. Heatmap illustrating the distribution of KEGG pathway abundances across the three flocks, organized according to hierarchical Levels 1 and 2. Rows represent individual KEGG pathways, while columns correspond to the different flocks. Color intensity indicates the normalized relative abundance of each pathway, with gradients reflecting lower to higher abundance levels. Pathways are grouped based on their functional hierarchy, enabling comparison of broad (Level 1) and more specific (Level 2) functional categories across flocks

were predicted to be associated with *Corynebacterium, Microbacterium, Dietzia, Enterococcus, Aerococcus, Carnobacterium,* and *Streptococcus*, suggesting their putative roles as resistance gene hosts. Specifically, the *tetZ* gene showed significant correlations with *Corynebacterium, Brachybacterium, Enterococcus, Dietzia,* and *Microbacterium*. Additionally, one of the most abundant genes, *MLS23S*, exhibited co-occurrence with *Alistipes* and *Bacteroides*. Moreover, *fexA* and *ermX* were associated with *Enterococcus, Dietzia, Carnobacterium,* and *Microbacterium* (Fig. 5-b).

Significant variations in ARG abundance and diversity were observed across the three flocks, particularly in Flock B, which showed a distinct profile characterized by a higher abundance of beta-lactam resistance genes. While these differences highlight the variability of the broiler gut resistome under different commercial conditions, explicit records regarding on-farm management and antimicrobial usage (AMU) could not be retrieved for the sampled facilities.

The KEGG pathway analysis revealed that the most abundant pathways belonged to metabolism including carbohydrate, energy metabolism, biosynthesis of other secondary metabolites, amino acid, nucleotide, glycan biosynthesis and metabolism of cofactors and vitamins, replication and repair and genetic information processing which are folding, sorting and degradation, transcription, and translation *(Fig. 4)*. Drug resistance and antimicrobial pathway were more abundant in Flock A and B than Flock C. Xenobiotics biodegradation and metabolism pathway was the most abundant in Flock B than Flock A and C *(Fig. 6)*.

DISCUSSION

This study aimed to comprehensively characterize the gut microbiome and resistome of broiler flocks using a shotgun metagenomics approach. The gut microbiota plays a central role in broiler health and productivity by maintaining intestinal homeostasis, inhibiting pathogen colonization, supporting intestinal development, and modulating host immune responses [26].

Previous studies have reported variable dominance patterns of major bacterial phyla, particularly Bacillota, Pseudomonadota, and Actinomycetota, in broiler gut microbiota [27,28]. In line with these findings, we observed marked compositional differences among flocks, with Bacillota predominating in Flocks A and C, while Pseudomonadota dominated Flock B. Such variation is likely driven by a complex interplay of host-related factors (e.g., age, genetics, immune status) and environmental conditions, including management practices, diet, and housing systems [29].

At the genus level, *Escherichia, Ligilactobacillus, Brachybacterium,* and *Corynebacterium* were among the most abundant taxa, consistent with previous reports [28,30]. *Ligilactobacillus salivarius,* in particular, has been associated with improved gut health and growth performance in broilers [31]. However, shifts in microbial composition may also arise under antimicrobial pressure, as antibiotic exposure has been shown to alter gut communities and increase the relative abundance of certain taxa, including *Ligilactobacillus* spp. and *Enterobacteriaceae* [32].

The increased abundance of xenobiotics biodegradation and metabolism pathways observed in Flock B may reflect greater exposure to exogenous compounds, such as pharmaceuticals or agrochemicals. These compounds can impose selective pressures on microbial communities, potentially contributing to shifts in community structure and the persistence of antimicrobial resistance [33,34]. In agreement with this observation, Flock B also exhibited higher ARG diversity. Nevertheless, due to the absence of detailed antimicrobial usage (AMU) and farm-level management data, these findings should be interpreted cautiously as indicative patterns rather than evidence of direct causal relationships.

The broiler gut microbiota constitutes an important reservoir of antimicrobial resistance genes. The widespread use of antimicrobials in poultry production has been associated with increased ARG abundance and diversity [35]. In the present study, ARGs conferring resistance to macrolides, aminoglycosides, and tetracyclines were predominant, which is consistent with previous studies [35,36]. In contrast, β-lactam resistance genes were generally less abundant, although a relatively higher proportion was observed in Flock B. These differences may reflect variations in selective pressures across farms; however, in the absence of AMU data, they should be regarded as baseline resistome characteristics rather than direct consequences of antimicrobial exposure.

Notably, resistance genes associated with critically important antimicrobials, such as *mcr,* bla_{NDM}, and bla_{KPC}, were not detected. However, the detection of linezolid resistance-associated genes *(optrA* and *poxtA)* in one flock highlights the potential emergence of resistance to last-resort antibiotics [36]. Similarly, plasmid-mediated quinolone resistance *(qnr)* genes were present at low abundance, which may not fully capture the extent of quinolone resistance, as high-level resistance is often mediated by chromosomal mutations or alternative mechanisms.

Network analysis revealed co-occurrence patterns between bacterial genera and ARGs, suggesting potential ecological associations between taxa and resistance determinants. However, these relationships should be interpreted with caution. Correlation-based analyses do not provide evidence of direct genetic linkage or host attribution. Rather, they identify statistical associations that may reflect shared ecological niches or selective pressures. High-resolution approaches, such as proximity ligation (Hi-C) sequencing or functional metagenomics, would be required to confirm host-gene relationships and horizontal gene transfer Dynamics.

In addition, the compositional nature of metagenomic data introduces inherent limitations. Because sequencing data are relative, an apparent increase in one taxon may lead to a proportional decrease in others, potentially generating spurious correlations. Therefore, the associations identified in this study should be considered hypothesis-generating rather than definitive.

A pooling strategy was employed to characterize the resistome at the flock level. Although individual sampling can provide insights into within-flock variation, pooled samples offer a cost-effective and representative overview of dominant microbial and resistance profiles across the production unit. This approach is widely used in large-scale surveillance programs, such as DANMAP [36].

Several limitations should be acknowledged. First, the study was limited to three flocks, which may not fully capture the diversity of broiler production systems. Second, the absence of detailed antimicrobial usage and management data restricts the ability to establish causal relationships. Finally, the pooling strategy does not account for individual bird-level variation within flocks.

In conclusion, this study provides a high-resolution snapshot of the broiler gut microbiome and resistome in Türkiye. The observed differences among flocks highlight the variability of microbial and resistance profiles under commercial production conditions. However, these findings should be interpreted as baseline observations rather than evidence of causality. Future longitudinal and multi-center studies incorporating detailed antimicrobial usage and management data will be essential to better understand the drivers of antimicrobial resistance in poultry production systems.

DECLARATIONS

Availability of Data and Materials: Data are available via NCBI BioProject PRJNA1401269.

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Conflict of Interest: The authors have no conflicts of interest to disclose.

Ethical Statement: This study was approved by Hatay Mustafa Kemal University Animal Experiments Local Ethics Committee (Decision number: 2025/07-11)

Generative Artificial Intelligence (AI): The authors declare that the article and/or tables and figures were not written/created by AI and AI-assisted technologies.

Further Considerations: We declare that the project and the information in the article are compatible and no other publications have been produced from the project, order of the researchers in the project proposal are compatible with those in the article.

Author Contributions: ÖA conceptualization, supervision, writing-review & editing, SŞD bioinformatic analysis, writing-review & editing

REFERENCES

1. **WHO:** Antimicrobial resistance. https://www.who.int/health-topics/antimicrobial-resistance; *Accessed:* 24.10.2025.
2. **Singh S, Kriti M, Anamika KS, Sharma P, Pal N, Sarma DK, Tiwari R, Kumar M:** A one health approach addressing poultry-associated antimicrobial resistance: Human, animal and environmental perspectives. *The Microbe*, 7:100309, 2025. DOI: 10.1016/j.microb.2025.100309
3. **Hedman HD, Vasco KA, Zhang L:** A review of antimicrobial resistance in poultry farming within low-resource settings. Animals (Basel), 10 (8):1264, 2020. DOI: 10.3390/ani10081264
4. **Collignon P, Voss A:** China, what antibiotics and what volumes are used in food production animals? *Antimicrob Resist Infect Control,* 4, 1-4, 2015. DOI: 10.1186/s13756-015-0056-5
5. **Aarestrup FM, Bager F, Jensen NE, Madsen M, Meyling A, Wegener HC:** Resistance to antimicrobial agents used for animal therapy in pathogenic-, zoonotic- and indicator bacteria isolated from different food animals in Denmark: A baseline study for the Danish Integrated Antimicrobial Resistance Monitoring Programme (DANMAP). *APMIS,* 106, 745-770, 1998. DOI: 10.1111/j.1699-0463.1998.tb00222.x
6. **Gilbert JM, White DG, McDermott PF:** The US national antimicrobial resistance monitoring system. *Future Microbiol,* 2 (5): 493-500, 2007. DOI: 10.2217/17460913.2.5.493
7. **Schwarz S, Alesík E, Grobbel M, Lübke-Becker A, Wallmann J, Werckenthin C, Wieler LH:** The BfT-GermVet monitoring program - Aims and basics. *Berl Münch Tierärztl Wochenschr,* 120 (9-10): 357-362, 2007. DOI: 10.2376/0005-9366-120-357
8. **Aslantaş Ö:** High occurrence of CMY-2-type beta-lactamase-producing *Escherichia coli* among broiler flocks in Turkey. *Trop Anim Health Prod,* 52 (4): 1681-1689, 2020. DOI: 10.1007/s11250-019-02167-8
9. **Aslantaş Ö, Küçükaltay K:** Investigation and molecular characterization of colistin resistance in commensal *Escherichia coli* strains isolated from broiler flocks. *Isr J Vet Med,* 78 (3): 25-35, 2023.
10. **Aslantaş Ö:** Molecular and phenotypic characterization of enterococci isolated from broiler flocks in Turkey. *Trop Anim Health Prod,* 51 (5):1073-1082, 2019. DOI: 10.1007/s11250-018-01784-z
11. **Aslantaş Ö:** Antimicrobial resistance among commensal *Escherichia coli* from broilers in Turkey. *Isr J Vet Med,* 73 (3): 19-25, 2018.
12. **Yılmaz EŞ, Aslantaş Ö, Pehlivanlar Önen S, Türkyılmaz S, Kürekci C:** Prevalence, antimicrobial resistance and virulence traits in enterococci from food of animal origin in Turkey. *LWT - Food Sci Technol,* 66, 20-26, 2016. DOI: 10.1016/j.lwt.2015.10.009
13. **Pehlivanlar Önen S, Aslantaş Ö, Şebnem Yılmaz E, Kürekci C:** Prevalence of β-lactamase producing *Escherichia coli* from retail meat in Turkey. *J Food Sci,* 80 (9): M2023-M2029, 2015. DOI: 10.1111/1750-3841.12984
14. **Cason C, D'Accolti M, Soffritti I, Mazzacane S, Comar M, Caselli E:** Next-generation sequencing and PCR technologies in monitoring the hospital microbiome and its drug resistance. *Front Microbiol,* 13:969863, 2022. DOI: 10.3389/fmicb.2022.969863
15. **Szoke Z, Fauszt P, Mikolas M, David P, Szilagyi-Tolnai E, Pesti-Asboth G, Homoki JR, Kovacs-Forgacs I, Gal F, Stundl L, Czegledi L, Stagel A, Biro S, Remenyik J, Paholcsek M:** Comprehensive analysis of antimicrobial resistance dynamics among broiler and duck intensive production systems. *Sci Rep,* 15 (1):4673, 2025. DOI: 10.1038/s41598-025-89432-z
16. **Wingett SW, Andrews S:** FastQ screen: A tool for multigenome mapping and quality control. *F1000Res,* 7:1338, 2018. DOI: 10.12688/f1000research.15931.2
17. **Bolger AM, Lohse M, Usadel B:** Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics,* 30, 2114-2120, 2014. DOI: 10.1093/bioinformatics/btu170

18. **Langmead B, Salzberg SL:** Fast gapped-read alignment with Bowtie2. *Nat Methods,* 9, 357-359, 2012. DOI: 10.1038/nmeth.1923

19. **Tamames J Puente-Sánchez F:** SqueezeMeta, a highly portable, fully automatic metagenomic analysis pipeline. *Front Microbiol,* 9:3349. DOI: 10.3389/fmicb.2018.03349

20. **Bonin N, Doster E, Worley H, Pinnell LJ, Bravo JE, Ferm P, Marini S, Prosperi M, Noyes N, Morley PS, Boucher C:** MEGARes and AMR++, v3.0: An updated comprehensive database of antimicrobial resistance determinants and an improved software pipeline for classification using high-throughput sequencing. *Nucleic Acids Res,* 51, D744-D752, 2023. DOI: 10.1093/nar/gkac1047

21. **Parks DH, Tyson GW, Hugenholtz P, Beiko RG:** STAMP: Statistical analysis of taxonomic and functional profiles. *Bioinformatics,* 30, 3123-3124, 2014. DOI: 10.1093/bioinformatics/btu494

22. **Harrell FE Jr, Dupont C:** Hmisc: Harrell Miscellaneous. R Package Version 4.2-0, 2019. https://CRAN.R-project.org/package=Hmisc23; *Accessed:* October 12, 2025.

23. **Csardi G, Nepusz T:** The Igraph Software Package for Complex Network Research. InterJournal, Complex Systems, 1695, 1-9, 2006. http://igraph.org; *Accessed:* October 12, 2025.

24. **Wickham H, Chang W, Henry L, Pedersen TL, Takahashi K, Wilke C, Woo K, Yutani H, Dunnington D:** *ggplot2:* Create elegant data visualisations using the grammar of graphics (R package version 3.4.0) [Computer software]. Comprehensive R Archive Network (CRAN), 2007. https://CRAN.R-project.org/package=ggplot2; *Accessed:* October 12, 2025.

25. **Yan L:** *ggvenn:* Draw Venn diagram by *"ggplot2"* (Version 0.1.9) [R package]. Comprehensive R Archive Network (CRAN), 2021. https://CRAN.R-project.org/package=ggvenn; *Accessed:* October 12, 2025.

26. **Jia M, Lei J, Dong Y, Liu X, Zhang H, Wang Q:** The interactive effects of nutrient density and breed on growth performance and gut microbiota in broilers. *Animals,* 14 (23):3528, 2024. DOI: 10.3390/ani14233528

27. **Zhou Q, Lan F, Li X, Chen Y, Zhang T, Wang H:** The spatial and temporal characterization of gut microbiota in broilers. *Front Vet Sci,* 8:712226, 2021. DOI: 10.3389/fvets.2021.712226

28. **Khan MM, Mushtaq MA, Suleman M, Ahmad A, Iqbal Z:** Fecal microbiota landscape of commercial poultry farms in Faisalabad, Pakistan: A 16S rRNA gene-based metagenomics study. *Poult Sci,* 104:105089, 2025. DOI: 10.1016/j.psj.2025.105089

29. **Diaz Carrasco JM, Casanova NA, Fernández Miyakawa ME:** Microbiota, gut health and chicken productivity: What is the connection? *Microorganisms*, 7 (10):374, 2019. DOI: 10.3390/microorganisms7100374

30. **Alvarenga BO, Paiva JB, Souza AIS, Rodrigues DR, Tizioto PC, Ferreira AJP:** Metagenomics analysis of the morphological aspects and bacterial composition of broiler feces. *Poult Sci,* 102 (2):102401, 2023. DOI: 10.1016/j.psj.2022.102401

31. **Yang J, Wang J, Liu Z, Chen J, Jiang J, Zhao M, Gong D:** *Ligilactobacillus salivarius* improve body growth and anti-oxidation capacity of broiler chickens via regulation of the microbiota-gut-brain axis. *BMC Microbiol,* 23 (1):395, 2023. DOI: 10.1186/s12866-023-03135-x

32. **Mancabelli L, Ferrario C, Milani C, Mangifesta M, Turroni F, Duranti S, Lugli GA, Viappiani A, Ossiprandi MC, van Sinderen D, Ventura M:** Insights into the biodiversity of the gut microbiota of broiler chickens. *Environ Microbiol,* 18, 4727-4738, 2016. DOI: 10.1111/1462-2920.13363

33. **Das A, Srinivasan M, Ghosh TS, Mande SS:** Xenobiotic metabolism and gut microbiomes. *PLoS One,* 11:e0163099, 2016. DOI: 10.1371/journal.pone.0163099

34. **Gonçalves M, Vale N, Martins da Costa P, Silva P:** Xenobiotics and broiler microbiota: Molecular insights into bacterial antimicrobial resistance and food-safety implications for human health. *J Xenobiotics,* 15:129, 2025. DOI: 10.3390/jox15040129

35. **Liang C, Wei Y, Wang X, Gao J, Cui H, Zhang C, Liu J:** Analysis of resistance gene diversity in the intestinal microbiome of broilers from two types of broiler farms in Hebei Province, China. *Antibiotics,* 12 (12):1664, 2023. DOI: 10.3390/antibiotics12121664

36. **Munk P, Knudsen BE, Lukjancenko O, Duarte ASR, Van Gompel L, Luiken REC, Smit LAM, Schmitt H, Garcia AD, Hansen RB, Petersen TN, Bossers A, Ruppé E; EFFORT Group; Lund O, Hald T, Pamp SJ, Vigre H, Heederik D, Wagenaar JA, Mevius D, Aarestrup FM:** Abundance and diversity of the faecal resistome in slaughter pigs and broilers in nine European countries. *Nat Microbiol,* 3 (8): 898-908, 2018. DOI: 10.1038/s41564-018-0192-9