Poultry Science

Comparative resistome, mobilome, and microbial composition of retail chicken originated from conventional, organic, and antibiotic-free production systems

Tatiana R. Vieira [*], Esther F. Cavinatto de Oliveira [*], Samuel P. Cibulski [†], Núbia M. V. Silva,[‡,§] Mauro R. Borba,[*] Celso J. B. Oliveira [§], and Marisa Cardoso [*,1]

[*]*Department of Preventive Veterinary Medicine, Federal University of Rio Grande do Sul (UFRGS), 91540-000, Porto Alegre, RS, Brazil;* [†]*Department of Biotechnology, Center for Biotechnology (CBiotec), Federal University of Paraiba (UFPB), 58397-000, Areia, PB, Brazil;* [‡]*Federal Institute of Education, Science and Technology of Sertão Pernambucano, Campus Salgueiro (IF-Sertão Pernambucano), 56000-000, Salgueiro, PE, Brazil; and* [§]*Department of Animal Sciences, College of Agricultural Sciences (CCA), Federal University of Paraiba (UFPB), 58397-000, Areia, PB, Brazil*

ABSTRACT The aim of this study was to investigate the microbial composition, and the profiles of antimicrobial resistance genes (**ARGs**, resistome) and mobile genetic elements (mobilome) of retail chicken carcasses originated from conventional intensive production systems (**CO**), certified antimicrobial-free intensive production systems (**AF**), and certified organic production systems with restricted antimicrobial use (**OR**). DNA samples were collected from 72 chicken carcasses according to a cross-sectional study design. Shot-gun metagenomics was performed by means of Illumina high throughput DNA sequencing followed by downstream bioinformatic analyses. Gammaproteobacteria was the most abundant bacterial class in all groups. Although CO, AF, and OR did not differ in terms of alpha- and beta-microbial diversity, the abundance of some taxa differed significantly across the groups, including spoilage-associated organisms such as *Pseudomonas* and *Acinetobacter*. The co-resistome comprised 29 ARGs shared by CO, AF and OR, including genes conferring resistance to beta-lactams ($bla_{\text{ACT-8, 10, 13, 29}}$; $bla_{\text{OXA-212;}}$ $bla_{\text{OXA-275}}$ and *omp*A), aminoglycosides (*aph*(3′)-IIIa, VI, VIa and *spd*), tetracyclines (*tet* KL (W/N/W and M), lincosamides (*inu* A,C) and fosfomycin (*fos*A). ARGs were significantly less abundant ($P < 0.05$) in chicken carcasses from AF and OR compared with CO. Regarding mobile genetic elements (**MGEs**), transposases accounted for 97.2% of the mapped genes. A higher abundance ($P = 0.037$) of MGEs was found in CO compared to OR. There were no significant differences in ARGs or MGEs diversity among groups according to the Simpsonś index. In summary, retail frozen chicken carcasses from AF and OR systems show similar ARGs, MGEs and microbiota profiles compared with CO, even though the abundance of ARGs and MGEs was higher in chicken carcasses from CO, probably due to a higher selective pressure.

Key words: antimicrobial resistance, food safety, metagenomics, one health, poultry industry

2023 Poultry Science 102:103002
https://doi.org/10.1016/j.psj.2023.103002

INTRODUCTION

Poultry meat figures among the most widely consumed animal-based foods, and its global trade is estimated to have reached 100.1 million tons in 2022 (USDA, 2016). As a low cost-high quality protein source, poultry meat plays an important role in human nutrition, both in developed and developing areas. Although the success of the poultry industry is mainly attributed to decades of continuous improvement in the areas of breeding, nutrition, precision production technology, health and management practices, including biosecurity, and the development of novel vaccines, the use of antimicrobials cannot be ignored as an important factor contributing to the high productivity levels in this industry (Saraiva et al., 2022).

In livestock, antimicrobials have been used for therapeutic, prophylactic (metaphylactic), and performance enhancing purposes. Despite its importance, the use of antimicrobials in food animals is a topic of increasing concern due to its potential contribution to the selection and dissemination of antimicrobial resistant bacteria, aggravating the burden caused by antimicrobial resistance in both human and veterinary medicine (O'Neill, 2016). A plethora of antimicrobial resistance determinants have

Received May 16, 2023.
Accepted July 31, 2023.

[1]Corresponding author: mcardoso@ufrgs.br

been identified in bacteria originated from the animal food chain, suggesting that foodstuff can play an important role as reservoirs of both antimicrobial resistant bacteria and antimicrobial resistance genes (**ARGs**, Duarte et al., 2021). Most importantly, many resistance genes have been identified in mobile genetic elements (**MGEs**, Partridge et al., 2018), reinforcing the potential impact of horizontal transmission mechanisms on the dissemination of these genes through the food chain (Silbergeld and Dailey, 2017). There is accumulated empirical evidence suggesting a positive association between the on-farm use of certain antimicrobials and the emergence of antimicrobial resistant zoonotic bacteria that are clinically relevant in human medicine (Vieira et al., 2011). Moreover, the use of certain antimicrobials on farm has been associated with a reduction in the susceptibility of bacteria to these drugs as well as to others with similar molecular structure, that is, drugs belonging to the same antimicrobial class (Catry et al., 2016).

There is a scientific consensus that the use of antimicrobials in the food industry must be reduced (FAO, 2016). Still, there is a lack of robust data originated from global harmonized integrated and continuous surveillance of antimicrobial usage and antimicrobial resistance to precisely determine the role of antimicrobials on the emergence and dissemination of antimicrobial resistance in the animal food chain (Aarestrup et al., 2000), particularly in animal-derived foodstuffs. Because decision-making regarding the use of antimicrobials in the poultry production systems has a profound economic impact on both industry and consumers, knowledge about the cost-effectiveness of the use of nonantimicrobial alternatives and rearing strategies, such as organic and antibiotic-free production systems, on the mitigation of antimicrobial resistance is critically needed. Although high-throughput genomics approaches could be used in food matrices for better understanding the dynamics of antimicrobial resistance in antibiotic-restricted poultry production systems, most of the studies focuses on the animals (Koorakula et al., 2022) or the farm environment (Luiken et al., 2020). Importantly, the high levels of ARGs in chicken carcasses compared with preharvest poultry samples (De Cesare et al., 2022) highlight the need to investigate chicken meat as a potential reservoir of ARGs.

This study aimed at assessing putative differences in microbial composition, and profiles of ARGs and MGEs in retail chicken carcasses originated from conventional intensive production systems, certified antimicrobial-free intensive production systems, and certified alternative (organic) production systems.

MATERIALS AND METHODS

Study Design

A cross-sectional study design was used to compare the microbiota, and the presence of ARGs and MGEs in retail frozen chicken carcasses originated from different production systems regarding the use of antimicrobials: conventional intensive production systems with no restriction on the use of antimicrobials for therapeutic purposes or as growth promoters (**CO**); QIMA/WQS (www.wqs.com.br) certified antimicrobial-free intensive production systems, in which the use of antimicrobials has been phased out (**AF**); SisOrg (Brasil, 2009) certified free-range organic production systems, in which birds have access to outdoor areas and antimicrobials are not used (**OR**). Importantly, the presence of this official certification seal in carcasses from OR systems is only allowed in flocks not treated with antimicrobials, as these may be used in OR production systems in sporadic circumstances, usually when the flock is severely affected by infectious diseases. Therefore, only certified frozen carcasses were included in groups AF and OR.

A total of 72 packaged frozen chicken carcasses originated from CO (n = 24), AF (n = 24), and OR (n = 24) were used in this study. Carcasses from different production dates were selected to assure that they did not originate from the same batch. The retail stores were selected by convenience considering the availability of products and access to points of sale.

Sample Processing and Conventional Microbiological Procedures

Frozen chickens were transported in isothermal containers to the laboratory and stored under refrigeration until complete thawing. After draining excess liquid from defrost and removing the giblets, if any, the samples were transferred to sterile plastic containers, weighed and rinsed as recommended by ISO 17604. Rinsing was performed by pouring 400 mL of 1% buffered peptone water (**BPW**) followed by homogenization for 1 min allowing BPW to rinse the entire internal and external surface of the carcass. The resulting liquid was transferred to a sterile container for further analyses.

Metagenomics for Microbial Composition, Resistome, and Mobilome Analyses

Total DNA from BPW originated from rinsed carcasses was extracted as previously described (Shi et al., 2022) with the following modifications: duplicate 40 mL aliquots of the initial broth (400 mL) were transferred to 50 mL sterile conical tubes and submitted to a 2-step filtering process: the first step consisted of using a 70 μm membrane (Falcon, Corning, Glendale, AZ) followed by a vacuum filtration using a 8 μm membrane (Merck Millipore, Burlington, MA). The filtrate was centrifuged (1,735 × *g*) for 30 min at 4°C (Routine 380R, Hettich, Germany) in 2 steps. After the first centrifugation round, the supernatant was discarded and the contents of the second aliquot (40 mL) were added and centrifuged under the same condition. After discarding the supernatant, the pellet was resuspended in 1mL sterile phosphate buffer solution (**PBS**) and transferred to sterile microtubes.

We used a protocol for the reduction of contaminating (host) DNA (Shi et al., 2022). Briefly, samples were

centrifuged at 8,000 × *g* for 10 min and the supernatant discarded; 450 μL of sterile ultrapure water, 45 μL of 10 × DNase buffer (Tris-HCl/$MgCl_2$) and 5μL of DNase (28 U/μL Sigma) were then added, incubated at 37°C for 1 h followed by incubation at 65°C for 10 min for DNase inactivation. Afterwards, a centrifugation step at 8000 × g for 10 min was performed and the supernatant was discarded. The pellet was resuspended in 350 μL of lysis buffer (1M Tris-HCl, 0,5M EDTA, Triton x100) plus lysozyme (20 mg/mL) and 30μL lysostaphin (1 mg/mL, Sigma). After incubation at 36 °C for 12 to 18 h in water bath, a further centrifugation was carried out at 8,000 × g for 10 min. The supernatant was discarded and the pellet was suspended in 400 μL PBS. Aliquots (200 μL) of this suspension were used for total DNA extraction by means of a commercial extraction kit (Magmax, Applied Biosystems, Foster City, CA) following the manufacturer's recommendation.

The concentration and quality of the DNA were checked using the Quantus Fluorometer (Promega, Madison, WI) and NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA), respectively. DNA integrity was assessed by agarose gel electrophoresis (0.5%). Purified DNA samples were stored at -20°C.

High Throughput Sequencing

All 24 DNA samples from each CO, AF and OR groups were pooled into 4 composite samples of 6 samples each. The libraries were prepared using the Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA) according to the manufacturer's guidelines. After DNA fragmentation, samples were indexed using the Nextera XT Index Kit v2 Set A (Illumina). Fragment size was evaluated by an automated capillary electrophoresis system (Fragment Analyzer, Agilent Technologies, Santa Clara, CA). Total metagenome paired-end sequencing was performed in Illumina MiSeq using a 500 (2 × 250 cyles) v2 reagent kit. A total of 3 distinct sequencing rounds were performed. Four randomly selected pools were sequenced in each round, that is, each sequencing round included samples from all experimental groups.

Downstream Bioinformatic Analyses

The quality of the reads was checked by means of FastQC (Wingett and Andrews, 2018). After removal of low-quality reads and Illumina adapters, the metagenome was assembled by means of MetaSPAdes (Nurk et al., 2017) using default parameters. Given the high quality of the generated sequences, no trimming was performed. The metagenome assemblies were performed on the Galaxy 20.05 platform (https://usegalaxy.org/).

The paired-end sequences were aligned against the host reference genome (*Gallus* galGal4) using the Bowtie2 tool (Langmead and Salzberg, 2012). After alignment, the mapped sequences, that is, reads derived from the host, were subtracted, and discarded. The characterization of the meta-resistome was performed by mapping the sequences against the Comprehensive Antibiotic Resistance Database - CARD 3.1.0. After downloading the database (https://card.mcmaster.ca/download), the resistance genes were mapped using the Bowtie2 program, as described by Pärnänen et al. (2018).

The mobilome characterization was performed as described by Pärnänem et al. (2018). Reads were mapped against a comprehensive nonredundant database composed of data from MGEs with 278 annotations of different gene names and more than 2,000 unique sequences.

The mapped reads were adjusted according to the total number of generated sequences, after subtraction of sequences related to the host genome, and the size of each gene using the fragments per kilobase per million (**FPKM**) approach.

The alpha diversity of resistance genes and MGEs was calculated using the Simpson (1-D) and Shannon indices, with each production group being considered as a distinct community. Each sequenced pool was considered as a sample, with each group representing a distinct habitat. The profile of resistance genes was analyzed descriptively within each group and between groups.

The microbial composition analysis was performed using Kraken2 (Wood et al., 2019). This tool uses exact k-mers alignments for the classification of metagenomic sequences. Data generated after the alignment of sequences for taxonomic classification by Kraken2 were further analyzed using the Pavian Metagenomics Data Explorer tool (Breitwieser and Salzberg, 2020) available at https://fbreitwieser,shinyapps,io/pavian/.

For principal coordinate analysis (**PCoA**), Bray-Curtis dissimilarity between samples was obtained from the normalized gene array by means of the Community Ecology Package Vegan in R (Oksanen et al., 2007). The scatter plot was obtained using the ggplot2 tool (Wickham, 2016).

The shotgun metagenomic sequences used in this study are publicly available (NCBI BioProject: PRJNA867482; SRAs accession: SRX16990708 to SRX16990719)

RESULTS

High Throughput Sequencing

The number of paired-end reads generated from each pooled sample, in each sequencing round, is shown in Table S1 (Supplementary material). There was no difference ($P = 0.779$) in the number of mapped reads across the groups (CO, AF, and OR). Likewise, there was no difference ($P = 0.276$) in the number of reads that mapped to the reference genome (Table S2; Supplementary Material).

Microbial Composition Analysis

From the 19 million mapped reads, 8.8 million belonged to Bacteria domain. Bacterial reads represented 94% of all classified microbial reads, including a total of 38 bacterial phyla and 72 different classes. Proteobacteria (90.9%), Firmicutes (5.6%),

Bacteroidetes (1.9%) and Actinobacteria (1.2%) represented 99.7% of all identified phyla. The relative abundance of these phyla was similar across the 3 groups. There was no difference ($P = 0.966$) in the absolute abundance across the groups. Gammaproteobacteria (90.03%), Bacilli (5.48%), Flavobacteria (1.84%), Actinobacteria (1.20%), and Betaproteobacteria (0.58%) were the most abundant bacterial classes in all 3 groups and there was no difference ($P = 0.397$) in their abundances across the groups. Gammaproteobacteria was the most abundant class in the 3 groups, representing 91.4% of all identified classes in CO, 88.4% in AF and 90.2% in OR. Bacilli was the second most abundant class accounting for 3.5%. 4.6% and 6.3% in CO, AF, and OR, respectively. A higher abundance of Flavobacteria was observed in groups CO (2.6%) and AF (2.5%) compared with OR, in which they represented only 0.9%.

A total of 351 bacterial families were identified. Pseudomonadaceae (51.7%), Moraxellaceae (24.8%), Enterobacteriaceae (7.9%), Yersiniaceae (2.6%), and Staphylococcaceae (2.0%) were the 5 most abundant families in all 3 groups. While Pseudomonadaceae was the most abundant family identified in both AF and OR, higher relative abundances of Moraxellaceae and Enterobacteriaceae were identified in CO. In this group, Moraxellaceae (39.1%). Pseudomonadaceae (33.8%) and Enterobacteriaceae (17.4%) represented over 90.0% of the total bacterial families. However, no significant differences ($P > 0.05$) among groups were observed neither by Simpson nor Shannon diversity indexes (Table 1).

Out of the 1,246 identified bacterial genera, 1,227 were present in CO, 1,238 in AF, and 1,241 in OR. There was a significant difference ($P < 0.001$) in the relative abundance of certain genera across the groups. *Pseudomonas, Acinetobacter* and *Enterobacter* represented 54.0%, 22.9%, and 3.7% of all detected genera, respectively. While *Pseudomonas* was more abundant than *Acinetobacter* in AF (55.1% and 29.9%) and OR (56.2% and 17.5%), respectively (Figure S1; Supplementary Material), similar abundances of these genera were observed in CO, that is, *Acinetobacter* (39.6%) and *Pseudomonas* (34.4%). Considering the 2 major foodborne agents associated with poultry products, *Salmonella enterica* represented 0.50%, 0.10%, and 0.28% in CO, AF and OR groups, respectively, while *Campylobacter* spp. was identified in lower relative abundances in the carcasses, representing 0.02% in CO and AF and 0.01% in OR.

Antimicrobial Resistance Determinants

A total of 896,986 hits associated with resistance genes were detected in CO, while 389,002 and 414,564 hits were detected in AF and OR, respectively. The relative abundance of ARGs after normalization (FPKM) is shown in Figure S2 (Supplementary Material). There was no difference ($P = 1.00$) in the abundance of ARGs between OR (1.57; 0.20−80.03) and AF (2.00; 0.31−107.27) groups. There was no difference ($P = 1.00$) in the abundance of ARGs between OR (1.57; 0.20−80.03) and AF (2.00; 0.31−107.27) groups. On the other hand, a higher abundance of ARGs was observed in carcasses originated from CO (3.07; 0.42−169.71) compared with either AF and OR ($P < 0.001$) (Table 1).

The PCoA showed that CO group was more dissimilar to the other 2 groups (AF and OR), which were closely related to each other. The principal coordinate PCoA1 explained 24% of the total variation across the resistome of the groups (Figure 1).

Reads were mapped to 152 different antimicrobial resistance encoding genes. Most of them were associated with resistance to beta-lactams (569,459), and aminoglycosides (336,170). A high abundance of genes associated with efflux pump mechanisms were detected (335,746). This pattern was also observed within each individual group: ARGs associated with resistance to beta-lactams accounted for 312,404 hits in CO, 124,823 in AF and 132, 232 in OR; while ARGs related to aminoglycoside resistance accounted for 167,784 hits in CO, 91,067 in AF and 77,318 in OR. However, we observed a higher number of ARGs associated with efflux pump mechanisms in CO (207,169) compared with AF (44,607) and OR (83,969) (Figure 2).

Table 1. Diversity of microbiota at the family level, antimicrobial resistance genes (ARGs) and mobile genetic elements (MGEs) in pooled chicken carcass samples from broilers raised under conventional production systems (CO), broilers in conventional production systems but fed antibiotic-free diets (AF), or raised in organic production systems (OR), as determined by Shannon and complimentary Simpsonś (1-D) diversity indexes.

Diversity index	CO				AF				OR				*P*-value[1]
	1	2	3	4	1	2	3	4	1	2	3	4	
Microbiota[2]													
Shannon	1.37	1.56	1.44	1.55	1.55	1.76	1.38	0.99	1.60	1.59	1.46	1.46	*0.590*
Simpson[3]	0.62	0.61	0.62	0.70	0.65	0.69	0.55	0.40	0.63	0.64	0.62	0.65	*0.826*
ARGs													
Shannon	3.19	3.04	2.86	3.05	2.51	3.11	2.25	3.15	3.28	3.45	3.00	2.63	*0.551*
Simpson[3]	0.92	0.94	0.90	0.92	0.82	0.92	0.83	0.94	0.92	0.96	0.89	0.84	*0.634*
MGEs													
Shannon	1.40	1.24	1.19	1.29	1.41	1.53	1.67	1.76	1.58	1.71	1.53	1.36	*0.036*
Simpson[3]	0.65	0.63	0.62	0.63	0.62	0.67	0.70	0.71	0.67	0.69	0.67	0.64	0.141

[1]Nonparametric Kruskal-Wallis test at 5% significance level.
[2]Based on the families of bacteria identified in the pools.
[3]Complementary Simpsonś diversity index (1-D).

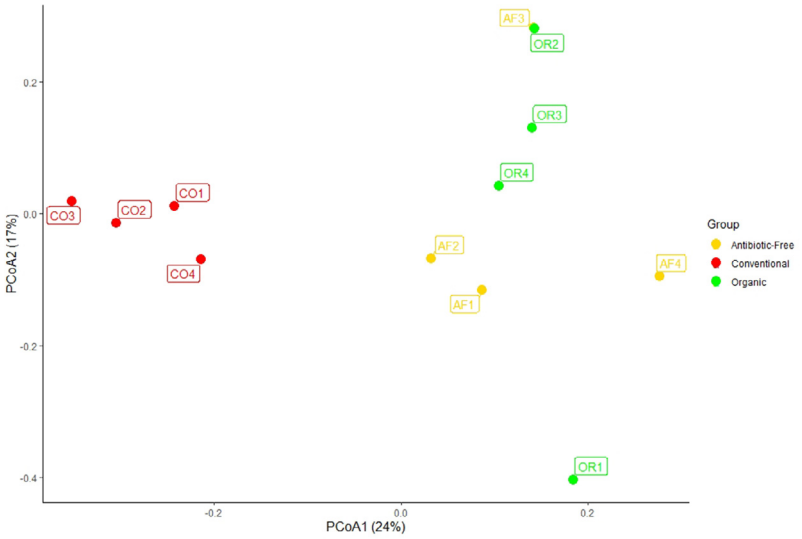

Figure 1. Principal coordinate analysis (PCoA) of normalized gene array. Bray-Curtis dissimilarity was calculated between samples using the R package Vegan. The scatter plot was performed using the ggplot2 tool.

Despite the plethora of different ARGs identified in this study, there was no significant difference in the diversity of ARGs across the 3 groups (Table 1). On the other hand, we observed differences among groups regarding the presence of certain ARGs. While genes encoding resistance to macrolide-lincosamide-streptogramin B (**MLSB**) were not found in OR, resistance genes to fluoroquinolones were not identified in AF (Table S3; Supplementary Material).

The co-resistome was determined by the sum of all ARGs shared by CO, AF, and OR) and comprised by 29 genes commonly found in these groups, including genes conferring resistance to beta-lactams ($bla_{\text{ACT-8, 10, 13, 29}}$; $bla_{\text{OXA-212}}$, $bla_{\text{OXA-275}}$ and *omp*A), aminoglycosides (*aph* (3′)-IIIa, VI, VIa and *spd*), tetracyclines (*tet* KL (W/N/W and M), lincosamides (*inu* A,C) and fosfomycin (*fos*A) (Figure 3). In addition, genes associated with 8 different efflux pump mechanisms conferring resistance to more than one class of antimicrobials were also found in all groups. However, 17 ARGs were more abundant in CO compared with AF and OR. Marked differences across the groups were observed for the most abundant genes, such as $bla_{\text{OXA-275}}$, *aph*(3')-VI and *acr*A.

A total of 28 genes were uniquely identified in CO, while 15 genes were only detected in AF, and 31 genes were exclusively found in OR (Table S2; Supplementary Material). Considering only the genes encoding resistance to high priority antimicrobials in human medicine uniquely identified in each group, those related to efflux pump mechanisms conferring resistance to fluoroquinolones predominated in CO, while ARGs conferring resistance by enzyme inactivation were found in CO

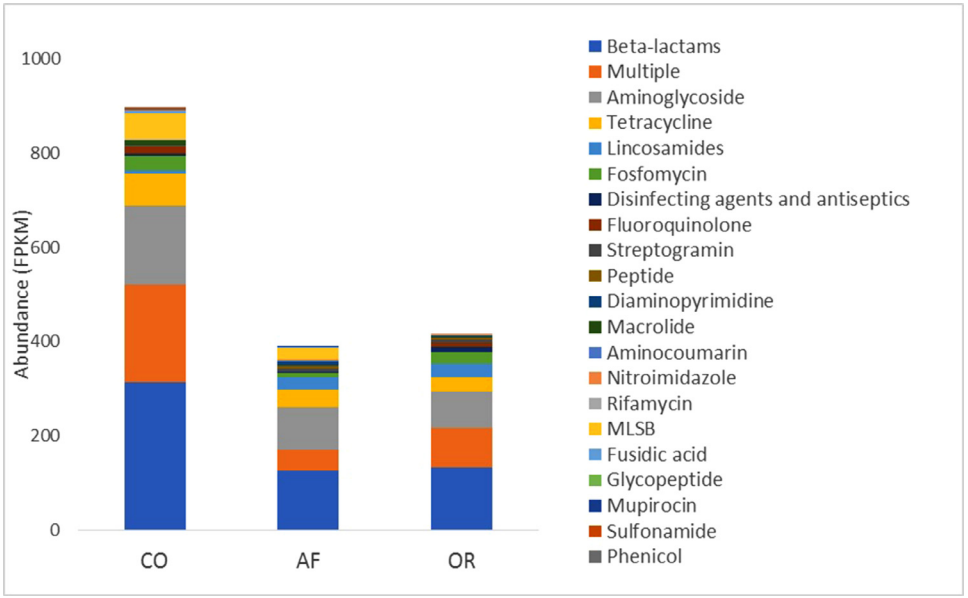

Figure 2. Abundance of resistance genes to antimicrobial classes in groups CO, AF and OR. CO: conventional intensive production systems. Abbreviations: AF, certified antimicrobial-free intensive production systems; MLSB, macrolide-lincosamide-streptogramin B; OR, certified organic production systems.

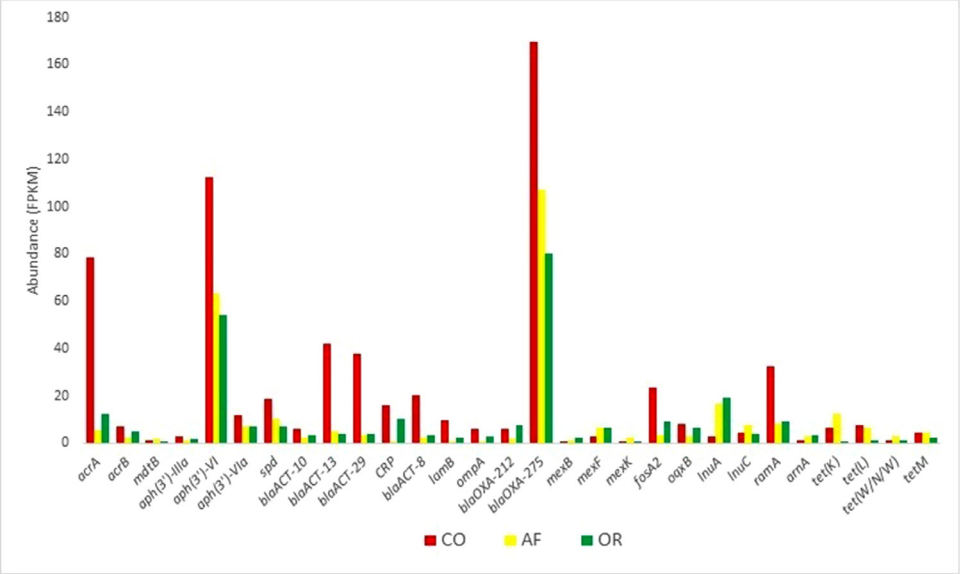

Figure 3. Abundance of antimicrobial resistance genes commonly found across all treatment groups. These genes composed the co-resistome. Bars represent the abundance of each gene per experimental group (FPKM). CO: conventional intensive production systems. Abbreviations: AF, certified antimicrobial-free intensive production systems; FPKM, fragments per kilobase of transcript per million fragments mapped; OR, certified organic production systems.

($bla_{ACT-38;}$ $bla_{OXA-309}$), AF (bla_{ACC-1}) and OR (CARB-5, $bla_{OXA-280}$, $bla_{OXA-281}$).

Mobile Genetic Elements

A total of 152 genes associated with MGEs such as plasmids, integrases, transposases, and transposons were identified. Transposases were the most frequently identified MGE, representing 97.2% of the mapped genes (97.9 in CO, 96.1 in AF, and 97.2% in GO), followed by plasmids and Transposon NTP-binding protein (IstB), both representing 1.2% of the identified MGEs (Table S1 Table S4; Supplementary Material). There was no difference in the abundance of FPKM normalized MGEs genes between AF and OR and between CO and AF groups. However, a higher frequency ($P = 0.036$) of MGEs was found in chicken carcasses from conventional production (CO) compared with organic systems (OR).

From the 152 detected MGEs, 54 were mapped in all 3 groups, while 14 were detected only in CO, 17 in AF and 20 in OR. There was no difference in MGE diversity among groups ($P = 0.141$) according to Simpson's index. On the other hand, we observed a lower MGE diversity ($P = 0.048$) in carcasses from CO as indicated by the Shannon diversity index (Table 1).

DISCUSSION

Although this study was designed to test the hypothesis that chicken meat carcasses originated from antibiotic-restricted rearing systems harbor a lower abundance and diversity of ARGs compared with those originated from conventional systems, a comprehensive analysis of the microbial composition is needed, considering that changes in the microbial composition could directly affect the diversity of ARGs across the investigated groups.

According to our results, there were no marked differences in the abundance and diversity of bacteria at phylum, class or family levels in chicken meat from the 3 groups. These results are supported by a previous culture-based investigation showing no differences in the overall microbial composition of chicken meat originated from conventional and free-range broilers (Marmion et al., 2023), except for pathogenic bacteria such as *Salmonella* and *Campylobacter*. Since preharvest broiler chickens represent the main sources of chicken carcass contamination (Rouger et al., 2017), the similar microbial composition observed in CO, AF, and OR groups could be explained by the fact that antimicrobial-mediated shifts in the microbial structural composition of birds are limited or restricted to less abundant members of the microbiota, as previously demonstrated (Costa et al., 2017; Turcotte et al., 2020).

Proteobacteria was the dominant phylum in our study among the 38 different phyla, corroborating previous studies reporting Proteobacteria as the most abundant bacterial phylum in chicken carcasses, regardless the sampling point in the slaughter plant (Handley et al., 2018) or storage temperature of chicken carcasses (Meng et al., 2019). Within Gammaproteobacteria, the most abundant bacterial class detected in our study, we observed dominance of Pseudomonadaceae and Moraxellaceae families. These families comprise most spoilage microorganism populations found in foodstuff, such as *Pseudomonas* and *Acinetobacter*, which are frequently associated with economic losses in the food industry (Quintieri et al., 2019; Li et al., 2020).

Pseudomonas was the main bacterial genus detected in our study, corroborating a previous report (Oakely et al., 2013). Besides their role as spoilage bacteria in foodstuff, these 2 genera also include some important

antimicrobial-resistant opportunistic pathogen species (Cheng et al., 2020). Considering family taxa potentially associated with foodborne pathogens, Enterobacteriaceae was the most abundant family in our study. Moreover, *Salmonella enterica* was detected in all groups, highlighting its importance for the poultry production system in Brazil. On the contrary, *Campylobacter*, which is a leading foodborne agent associated with the consumption of chicken globally (Myintzaw et al., 2021) was less abundant in all groups. Although a previous report (De Cesare et. al, 2022) detected bacterial genera that were found only in chicken carcasses from either conventional rearing (*Anoxybacillus, Bacillus, Flavobacterium, Pedobacter, Geobacillus,* and *Sphingobacterium*) or antibiotical-free production systems (*Aeromonas, Burkholderia, Endoriftia, Prevotella, Ruminococcus,* and *Shewanella*), these taxa were detected at similar abundances across all groups in our study. These results suggest that further investigations are needed to address the putative association between on-farm antimicrobial use and chicken meat microbial composition.

The presence of ARGs is deemed to be directly associated with the bacterial composition in each habitat (Hu et al., 2010). Therefore, our results indicating that the diversity of ARGs did not significantly differ across CO, AF and OR were expected, since no major differences in the microbial composition among the groups were detected. Another possible explanation related to the similar ARGs profiles observed in CO, AF and CO could rely on the evolutionary aspects of antimicrobial resistance acquisition by bacteria. It has been demonstrated that despite the fitness cost of carrying resistance determinants (Andersson and Hughes, 2010), disappearance of ARGs may need long periods do take place after interruption of antimicrobial exposure (Diarra et al., 2021). Therefore, the impact of the use of nonantimicrobial alternative systems on the reduction of ARGs should be observed in much longer time frames. To test this hypothesis, we suggest the use of longitudinal study designs covering various producing cycles.

The ARGs associated with beta-lactam resistance, which accounted for the most frequently detected genes in all groups, included the types bla_{ACC}, bla_{ACT} and bla_{OXA}. While bla_{ACC} and bla_{ACT} are AmpC beta-lactamases conferring resistance to a broad spectrum of beta-lactams including penicillins, several cephalosporins and aztreonam (Jacoby, 2009), bla_{OXA} encodes OXA-type betalactamases against amino- and carboxipenicillins (Evans and Amyes, 2014). Some bla_{OXA} determinants are capable of hydrolyzing carbapenems, which are considered a last resort drug for treatment of multiresistant bacteria in human medicine. Interestingly, bla_{TEM}, $bla_{\text{CTX-M}}$ and bla_{CMY}, which have been frequently reported in poultry in Brazil (Botelho et al., 2015; Rodrigues et al., 2017; Koga et al., 2019), were not found among the identified ARGs in our study.

Although we observed no significant differences in ARGs diversity among the groups, some variation in the antimicrobial resistance profiles can be observed by the presence of genes detected in each group only. For instance, although *tet*(A) and *sul*2 genes have been frequently reported in poultry farm environments (Mazhar et al., 2021), they were only detected in the CO group in our study. Considering high priority antimicrobials to humans, the genes *qnr*D1 and *qnr*B19 conferring transferable quinolone resistance were detected only in OR. These genes have been detected in plasmids harbored by multiple *Salmonella enterica* serovars (Cavaco et al., 2009). ARGs associated with beta lactam resistance were also detected only in some groups. For instance, genes codifying plasmid-mediated AmpC beta-lactamases, which confer resistance to extended-spectrum cephalosporins and monobactams, were found in CO ($bla_{\text{ACT-38}}$) and AF ($bla_{\text{ACC-1}}$). On the other hand, $bla_{\text{OXA-280,281}}$ and CARB-5, conferring resistance to carbapenems and carbenicillin, respectively, and commonly harbored by *Acinetobacter* spp., were detected in OR only. Importantly, neither *mcr*-1 nor its homologues have been identified in our study, corroborating a previous report (Salerno et al., 2022). In terms of resistance to polymyxins, only *ept*A (*pmr*C) was detected in groups CO and OR. Mutations in *pmr*CAB operon are associated with polymyxin resistance in Enterobacteriaceae due to LPS modifications (Olaitan et al., 2014).

Although significant differences in the diversity of microbiota and ARGs were not observed, a difference ($P < 0.05$) was observed in the abundance of ARGs among groups. ARGs were more abundant in chicken carcasses from CO compared with either AF or. Considering that CO and AF intensive production systems and diets are very similar and differ only regarding the restriction of antibiotics in AF, our results suggest that the selective pressure associated with the on-farm use of antimicrobials in CO group could play a role on the increase of ARG abundance in postharvest chicken. ARGs conferring resistance to beta-lactams and aminoglycosides represented the most abundant resistance determinants in the 3 groups, corroborating previous findings (Chng et al. 2020). These results are also in accordance with the most frequent phenotypic resistance profiles reported for foodborne pathogens associated with chicken carcasses in Brazil, such as *Salmonella enterica* and *E. coli* (Cerutti et al., 2020; Rau et al., 2021). Moreover, these antimicrobial classes are among the most commonly drugs used in the poultry industry over the years for prophylactic and therapeutic purposes (Saraiva et al., 2022).

Considering the most abundant genes in the co-resistome, $bla_{\text{oxa-275}}$, *aph*(3′)-VI, and acrA were more abundant in CO compared with AF and OR. The number of identified OXA-type betalactamases has significantly increased in the last years, and the group of OXA-51 has played an important role on the carbapenem resistance in *Acinetobacter baumannii* in Brazil (Martins et al., 2012). The $bla_{\text{OXA-275}}$ gene belongs to the OXA228 group, which presents a 56-57% identity with the OXA51 group. This gene is carried in the chromosome of *Acinetobacter guillouiae,* a species commonly found in sewage and human ear (Nemec et al., 2010). Although highly associated with *A. guillouiae* species, OXA228

group may pose a risk if transferred to pathogenic species of this genus (Périchon et al., 2014). Gene *aph*(3′)-VI, the second most abundant ARG in the co-resistome, codifies a modifying enzyme that is active against most aminoglycosides, including amikacin. Previously described in *A. baumannii* (Martin et al., 1988), it has been recently emerged as a key mechanism associated with the accumulation of aminoglycoside modifying enzymes in KPC-2-carrying *Enterobacter aerogenes* causing infections in Brazil (Firmo et al., 2019).

ARGs associated with efflux pump mechanisms were highly abundant in all 3 groups. These results were expected, since efflux is a common mechanism of antimicrobial resistance through readily acquired exogenous genes for drug-specific resistance and reduced biocide susceptibility, or chromosomal genes that contribute to intrinsic or acquired multidrug resistance mechanisms, which are widespread in bacteria (Poole, 2005). Among the ARGs related to efflux pumps detected in the co-resistome, *acr*A was the most abundant gene, particularly in the CO group. Overexpression of *ramA,* also detected in the co-resistome, is associated with the *acrA* upregulation facilitating resistance against tigecycline, which presents remarked activity against gram-negative bacteria (Livermore, 2005). Moreover, the expression of the periplasmic adaptor protein AcrA in the resistance-nodulation-division (**RND**) family of efflux pumps, such as AcrAB-TolC efflux pump, is associated with resistance to multiple antibiotics contributing to the virulence of foodborne pathogens such as *Salmonella* (Blair et al., 2009).

The horizontal transfer of resistance genes is a major mechanism of antimicrobial resistance dissemination between and within bacterial species; therefore, we also investigated MGEs sequences in the 3 groups. The results were similar to those observed for ARGs, as a higher abundance of MGEs was found in CO group compared with OR. Although there were no significant differences in MGE diversity among groups according to the Simpsonś index, a lower MGE abundance ($P = 0.037$) was found in CO compared to OR by Shannon diversity index. This difference was expected since transposases were highly abundant (above 95%) in all groups. Therefore, while Simpsonś index considers not only the number of MGEs but also their abundance, Shannonś index, as a better predictor for richness, reflected the diversity of low abundant MGEs that probably differed among the treatment groups.

Although integrases and transposases can serve as vehicles for ARGs dissemination, plasmids play a key role on the horizontal transfer of ARGs (Partridge et al., 2018). Both IncFIB and IncFII plasmids were commonly detected in chicken carcass across the 3 groups. Considering that these plasmids are associated with increased virulence in *E. coli, Salmonella* and *Klebsiella* (Khajanchi et al., 2017), their frequent presence in chicken carcasses, particularly at greater abundances in CO group, may play a role in public health. Considering that MGEs and ARGs were significantly more abundant in CO, we encourage further studies to investigate the role of postharvest chicken, particularly from intensive rearing systems in which antimicrobials are used, as vehicles for the transference of ARGs to the human microbiota.

Comparative studies investigating antimicrobial resistance levels in bacteria from animal production systems with and without the use of antimicrobials have been previously performed (Millman et al., 2013; Much et al., 2019; Pesciaroli et al., 2020). Such studies usually focus on indicator microorganisms for the assessment of antimicrobial resistance levels. However, these microorganisms may represent only a small fraction of the whole microbiota, even in complex samples such as feces. Moreover, ARGs may also be harbored in nonculturable bacteria. Therefore, shot-gun metagenomics approaches used in the present study can provide more accurate data regarding the bacterial population, as well as resistance and virulence genes present in the samples. Despite the high potential of metagenomics approaches to better understanding the aspects associated with antimicrobial resistance in the food industry (Costa et al., 2017; Munk et al., 2018; Van Gompel et al., 2020), there is still a scarcity of studies addressing the dynamics of antimicrobial resistance in foodstuff. Moreover, the significant differences in the abundance of ARGs between cecal contents of broilers on-farm and their respective carcasses after slaughter (De Cesare et al., 2022) support our conviction that further studies are needed to investigate the potential role of poultry meat as ARGs to consumers.

The present study has some limitations. Considering the proposed shotgun sequencing approach, the low concentration of microbial cells in chicken carcasses compared with somatic host cells had been considered a critical aspect during the conceptualization of our study. Therefore, the protocol described in the present study, which included ultrafiltration and DNAse treatment steps, resulted from a series of pilot studies (data not shown) aiming at providing maximum depletion of host DNA in chicken samples. In this sense, the total yield attributed to microbial DNA (62.65% per sample on average) provided enough DNA sequencing depth (8,836,477) to support downstream analysis. Most importantly, there was no significant difference in the yield of sequenced DNA across the 3 treatment groups (CO, AF, and OR). Regarding both quantity and quality of DNA, the protocol used herein might be useful as a reference method in studies involving high throughput shotgun sequencing of microbial DNA in meat samples.

Moreover, as an observational study, its limitation relies on the putative effects of uncontrolled factors, including both on-farm as well as postharvest variables. Therefore, causal associations between on farm antimicrobial use and ARGs profile in packaged chicken carcasses should be interpreted with caution. On the other hand, a positive aspect of the transversal observational approach performed herein is its capability to provide real-world evidence of the abundance of ARGs and microbial composition in retail chicken meat commercially available for consumers. Therefore, the microbial composition, resistome, and mobilome features observed in our study reflects the real impact of the alternative

production systems towards the restriction of antimicrobial use (AF and OR) considering all comprehensive variables from a farm to table perspective. These considerations are important in view of the high complexity of antimicrobial resistance issue and the need to use the "One Health" approach involving not only animal and human components but also the environment.

In summary, retail frozen chicken carcasses from certified organic production systems and certified antibiotic-free production had similar microbial composition, and profiles of ARGs and MGEs compared with those carcasses from broilers raised under conventional production systems. Nonetheless, the abundance of ARGs and MGEs was lower in chicken carcasses from certified organic systems and antibiotic-free production compared with conventional production systems, which probably resulted from a lower selective pressure for antimicrobial resistance in antibiotic restricted production settings. Further investigations are needed to understand the role of animal-derived products as vehicle of ARGs and their real implications for the consumer's health. This information is of crucial importance for the food animal industry and policy makers to support decisions involving the use of antimicrobials in livestock.

ACKNOWLEDGMENTS

The authors wish to express their thanks for the financial support of Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, financial code 001; PDSE, 88881.131934/2016-01; Projeto CAPES-PrInt UFPB "Omic sciences applied to the prevention of antimicrobial resistance at the human-animal-environment interface-a one health approach" (88881.311776/2018-01/Bioma Caatinga, biodiversidade e sustentabilidade), Conselho Nacional de Desenvolvimento Científico (CNPq − Brasil) − Universal (407039/2016-3) and Financiadora de Estudos e Projetos (FINEP/CT-Infra).

Author contributions: TRV, SPC and MRIC: Study conception and design. TRV, EFC, SPC, NMVS, MRB, CJBO and MRIC: Acquisition of data, analysis and interpretation of data. TRV, CJBO, MRIC: Drafting of the manuscript. CJBO, SPC, and MRIC: Supervision. All authors read and approved the final manuscript.

Data availability statement: Data available on request from the authors.

DISCLOSURES

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2023.103002.

REFERENCES

Aarestrup, F. M., Y. Agerso, P. Gerner−Smidt, M. Madsen, and L. B Jensen. 2000. Comparison of antimicrobial resistance phenotypes and resistance genes in *Enterococcus faecalis* and *Enterococcus faecium* from humans in the community, broilers, and pigs in Denmark. Diagn. Microbiol. Infect. Dis. 37:127–137.

Andersson, D. I., and D. Hughes. 2010. Antibiotic resistance and its cost: is it possible to reverse resistance? Nat. Rev. Microbiol. 8:260–271.

Blair, J. M. A., R. M. la Ragione, M. J. Woodward, and L. J. V. Piddock. 2009. Periplasmic adaptor protein AcrA has a distinct role in the antibiotic resistance and virulence of *Salmonella* enterica serovar Typhimurium. J. Antimicrob. Chemother. 64:965–972.

Brasil. Ministério da Agricultura, Pecuária e. Abastecimento. 2009. Institui o selo único oficial do Sistema Brasileiro de Avaliação da Conformidade Orgânica, na forma dos Anexos à presente Instrução Normativa, e estabelecer os requisitos para a sua utilização nos produtos orgânicos.

Breitwieser, F. P., and S. L. Salzberg. 2020. Pavian: interactive analysis of metagenomics data for microbiome studies and pathogen identification. Bioinformatics 36:1303–1304.

Botelho, L. A. B., G. B. Kraychete, J. Costa e Silva, D. V. V. Regis, R. C. Picão, B. M. Moreira, and R. R. Bonelli. 2015. Widespread distribution of CTX-M and plasmid-mediated AmpC β-lactamases in *Escherichia coli* from Brazilian chicken meat. Mem. Inst. Oswaldo. Cruz. 110:249–254.

Catry, B., J. Dewulf, D. Maes, B. Pardon, B. Callens, M. Vanrobaeys, G. Opsomer, A. Kruif, and F. Haesebrouck. 2016. Effect of antimicrobial consumption and production type on antibacterial resistance in the bovine respiratory and digestive tract. PLoS One 11: e0146488.

Cavaco, L. M., H. Hasman, S. Xia, and F. M. Aarestrup. 2009. qnrD, a novel gene conferring transferable quinolone resistance in *Salmonella* enterica serovar Kentucky and Bovismorbificans strains of human origin. Antimicrob. Agents Chemother. 53:603–608.

Cerutti, M. F., T. R. Vieira, K. S. Zenato, G. O. Werlang, C. Pissetti, and M. Cardoso. 2020. *Escherichia coli* in chicken carcasses in Southern Brazil: absence of shigatoxigenic (STEC) and isolation of atypical Enteropathogenic (aEPEC). Braz. J. Poult. Sci. 22:1–7.

Chng, K. R., C. Li, D. Bertrand, A. H. Q. Ng, J. S. Kwah, H. M. Low, C. Tong, M. Natrajan, M. H. Zhang, L. Xu, K. K. Ki Ko, E. X. P. Ho, T. V. Av-Shalom, J. W. P. Teo, C. C. Khor, MetaSUB. Consortium, S. L. Chen, C. E. Mason, O. T. Ng, K. Marimuthu, B. Ang, and N. Nagarajan. 2020. Cartography of opportunistic pathogens and antibiotic resistance genes in a tertiary hospital environment. Nat. Med. 26:941–951.

Costa, M. C., J. A. Bessegatto, A. A. Alfieri, J. S. Weese, J. A. Filho, and A. Oba. 2017. Different antibiotic growth promoters induce specific changes in the cecal microbiota membership of broiler chicken. PLoS One 12:e0171642.

De Cesare, A., C. Oliveri, A. Lucchi, F. Savini, G. Manfreda, and C. Sala. 2022. Pilot study on poultry meat from antibiotic free and conventional farms: can metagenomics detect any difference? Foods 11:249.

Diarra, M. S., X. Zhao, and P. Butaye. 2021. Antimicrobial use, antimicrobial resistance, and the microbiome in food animals. Front. Vet. Sci. 7:638781.

Duarte, A. S. R., T. Röder, L. Van Gompel, T. N. Petersen, R. B. Hansen, I. M. Hansen, A. Bossers, F. M. Aarestrup, J. A. Wagenaar, and T. Hald. 2021. Metagenomics-based approach to source-attribution of antimicrobial resistance determinants—identification of reservoir resistome signatures. Front. Microbiol. 11:601407.

Evans, B. A., and S. G. B. Amyes. 2014. OXA β-lactamases. Clin. Microbiol. Rev. 27:241–263.

FAO - Food and Agriculture Organization of the United Nations. 2016. The FAO action plan on antimicrobial resistance 2016 −2020. Accessed Oct. 2022. www.fao.org/publications.

Firmo, E. F., A. B. Cabral, É. M. Oliveira, and A. C. S. Lopes. 2019. Emergence of aph(3')-VI and accumulation of aminoglycoside modifying enzyme genes in KPC-2-possessing *Enterobacter aerogenes* isolates from infections and colonization in patients from Recife-PE, Brazil. Rev. Soc. Bras. Med. Trop. 52:e20180460.

Handley, J. A., S. H. Park, S. A. Kim, and S. C. Ricke. 2018. Microbiome profiles of commercial broilers through evisceration and immersion chilling during poultry slaughter and the identification of potential indicator microorganisms. Front. Microbiol. 9:345.

Hu, Y., X. Yang, J. Qin, N. Lu, G. Cheng, N. Wu, Y. Pan, J. Li, L. Zhu, X. Wang, Z. Meng, F. Zhao, D. Liu, J. Ma, N. Qin, C. Xiang, Y. Xiao, L. Li, H. Yang, G. F. Gao, L. Jakobsen, D. J. Spangholm, K. Pedersen, L. B. Jensen, H. D. Emborg, Y. Agersø, F. M. Aarestrup, A. M. Hammerum, and N. Frimodt-Møller. 2010. Broiler chickens, broiler chicken meat, pigs and pork as sources of ExPEC related virulence genes and resistance in *Escherichia coli* isolates from community-dwelling humans and UTI patients. Int. J. Food Microbiol. 142:264–272.

Jacoby, G. A. 2009. AmpC β-lactamases. Clin. Microbiol. Rev. 22:161–182.

Khajanchi, B. K., N. A. Hasan, S. Y. Choi, J. Han, S. Zhao, R. R. Colwell, C. E. Cerniglia, and S. L. Foley. 2017. Comparative genomic analysis and characterization of incompatibility group FIB plasmid encoded virulence factors of Salmonella enterica isolated from food sources. BMC Genomics. 18:570.

Koga, V. L., R. P. Maluta, W. D. da Silveira, R. A. Ribeiro, M. Hungria, E. C. Vespero, G. Nakazato, and R. K. T. Kobayashi. 2019. Characterization of CMY-2-type beta-lactamase-producing *Escherichia coli* isolated from chicken carcasses and human infection in a city of South Brazil. BMC Microbiol. 19:1–9.

Koorakula, R., M. Schiavinato, M. Ghanbari, G. Wegl, N. Grabner, A. Koestelbauer, V. Klose, J. C. Dohm, and K. J. Domig. 2022. Metatranscriptomic analysis of the chicken gut resistome response to in-feed antibiotics and natural feed additives. Front. Microbiol. 13:833790.

Langmead, B., and S. L. Salzberg. 2012. Fast gapped-read alignment with Bowtie 2. Nat. Methods 9:357–359.

Li, H., X. Sun, X. Liao, and M. Gänzle. 2020. Control of pathogenic and spoilage bacteria in meat and meat products by high pressure: challenges and future perspectives. Compr. Rev. Food Sci. Food Saf. 19:3476–3500.

Livermore, D. M. 2005. Tigecycline: what is it, and where should it be used? J. Antimicrob. Chemother. 56:611–614.

Luiken, R. E. C., L. Van Gompel, A. Bossers, P. Munk, P. Joosten, R. B. Hansen, B. E. Knudsen, S. García-Cobos, J. Dewulf, F. M. Aarestrup, J. A. Wagenaar, L. A. M. Smit, D. J. Mevius, D. J. J. Heederik, and H. Schmitt. 2020. EFFORT-group. 2020. Farm dust resistomes and bacterial microbiomes in European poultry and pig farms. Environ. Int. 143:105971.

Marmion, M., A. B. Soro, P. Whyte, and A. G. M. A. Scanell. 2023. A culture-based assessment of the microbiota of conventional and free-range chicken meat from Irish processing facilities. Food Microbiol. 114:104306.

Martin, P., E. Jullien, and P. Courvalin. 1988. Nucleotide sequence of *Acinetobacter baumannii* aphA-6 gene: evolutionary and functional implications of sequence homologies with nucleotide-binding proteins, kinases and other aminoglycoside-modifying enzymes. Mol. Microbiol. 2:615–625.

Martins, A. F., R. S. Kuchenbecker, K. O. Pilger, M. Pagano, and A. L. Barth. 2012. High endemic levels of multidrug-resistant *Acinetobacter baumannii* among hospitals in southern Brazil. Am. J. Infect. Control 40:108–112.

Mazhar, S. H., X. Li, A. Rashid, J. Su, J. Xu, A. D. Brejnrod, J. Q. Su, Y. Wu, Y. G. Zhu, S. G. Zhou, R. Feng, and C. Rensing. 2021. Co-selection of antibiotic resistance genes, and mobile genetic elements in the presence of heavy metals in poultry farm environments. Sci. Total Environ. 755:142702.

Meng, J., X. Huang, L. Song, B. Hou, M. Qiao, P. Zhang, Q. Zhao, B. Zhang, and F. Liu. 2019. Effect of storage temperature on bacterial diversity in chicken skin. J. Appl. Microbiol. 126:854–863.

Millman, J. M., K. Waits, H. Grande, A. R. Marks, J. C. Marks, L. B. Price, and B. A. Hungate. 2013. Prevalence of antibiotic-resistant *E. coli* in retail chicken: comparing conventional, organic, kosher, and raised without antibiotics. F1000Res 2:155.

Much, P., H. Sun, H. Lassnig, S. Koeberl-Jelovcan, H. Schliessnig, and H. P. Stueger. 2019. Differences in antimicrobial resistance of commensal *Escherichia coli* isolated from caecal contents of organically and conventionally raised broilers in Austria, 2010-2014 and 2016. Prev. Vet. Med. 171:104755.

Munk, P., B. E. Knudsen, O. Lukjancenko, A. S. R. Duarte, L. Van Gompel, R. E. C. Luiken, L. A. M. Smit, H. Schmitt, A. D. Garcia, R. B. Hansen, T. N. Petersen, A. Bossers, E. Ruppé, EFFORT. Group. 2018O. Lund, T. Hald, S. J. Pamp, H. Vigre, D. Heederik, J. A. Wagenaar, D. Mevius, and F. M Aarestrup. 2018. Abundance and diversity of the faecal resistome in slaughter pigs and broilers in nine European countries. Nat. Microbiol. 3:898–908.

Myintzaw, P., A. K. Jaiswal, and S. Jaiswal. 2021. A review on Campylobacteriosis associated with poultry meat consumption. Food Rev. Int. 39:2107–2121.

Nemec, A., M. Musílek, O. Šedo, T. De Baere, M. Maixnerová, T. J. K. van der Reijden, Z. Zdráhal, M. Vaneechoutte, and L. Dijkshoorn. 2010. *Acinetobacter bereziniae* sp. nov. and *Acinetobacter guillouiae* sp. nov., to accommodate *Acinetobacter* genomic species 10 and 11, respectively. Int. J. Syst. Evol. Microbiol. 60:896–903.

Nurk, S., D. Meleshko, A. Korobeynikov, and P. A. Pevzner. 2017. metaSPAdes: a new versatile metagenomic assembler. Genome Res. 27:824–834.

Oakley, B. B., C. A. Morales, J. Line, M. E. Berrang, R. J. Meinersmann, G. E. Tillman, M. G. Wise, G. R. Siragusa, K. L. Hiett, and B. S. Seal. 2013. The poultry-associated microbiome: network analysis and farm-to-fork characterizations. PLoS One 8:e57190.

Oksanen, J. A., F. Blanchet, R. Kindt, P. Legendre, R. O'Hara, G. Simpson, P. E. Minchin, and R. O'Hara. 2007. Vegan : Community Ecology Package. R package version 1.85.

Olaitan, A. O., S. Morand, and J.-M. Rolain. 2014. Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria. Front. Microbiol. 5:643.

O'Neill, J. 2016. Review on antimicrobial resistance: tackling drug-resistant infections globally: final report and recommendations.

Pärnänen, K., A. Karkman, J. Hultman, C. Lyra, J. Bengtsson-Palme, D. G. J. Larsson, S. Rautava, E. Isolauri, S. Salminen, H. Kumar, R. Satokari, and M. Virta. 2018. Maternal gut and breast milk microbiota affect infant gut antibiotic resistome and mobile genetic elements. Nat. Commun. 9:3891.

Partridge, S. R., S. M. Kwong, N. Firth, and S. O. Jensen. 2018. Mobile Genetic elements associated with antimicrobial resistance. Clin. Microbiol. Rev. 31 e00088-17.

Périchon, B., S. Goussard, V. Walewski, L. Krizova, G. Cerqueira, C. Murphy, M. Feldgarden, J. Wortman, D. Clermont, A. Nemec, and P. Courvalin. 2014. Identification of 50 class D β-lactamases and 65 *Acinetobacter*-derived cephalosporinases in *Acinetobacter* spp. Antimicrob. Agents Chemother. 58:936–949.

Pesciaroli, M., C. F. Magistrali, G. Filippini, E. M. Epifanio, C. Lovito, L. Marchi, C. Maresca, F. R. Massacci, S. Orsini, E. Scoccia, S. Tofani, and G. Pezzotti. 2020. Antibiotic-resistant commensal *Escherichia coli* are less frequently isolated from poultry raised using non-conventional management systems than from conventional broiler. Int. J. Food Microbiol. 314:108391.

Poole, K. 2005. Efflux-mediated antimicrobial resistance. J. Antimicrob. Chemother. 56:20–51.

Quintieri, L., F. Fanelli, and L. Caputo. 2019. Antibiotic resistant *Pseudomonas* Spp. spoilers in fresh dairy products: an underestimated risk and the control strategies. Foods 8:372.

Rau, R. B., A. R. Ribeiro, A. dos Santos, and A. L. Barth. 2021. Antimicrobial resistance of *Salmonella* from poultry meat in Brazil: results of a nationwide survey. Epidemiol. Infect. 149:e228.

Rodrigues, I. B. B. E., K. F. S. Ferreira, R. L. Silva, S. A. Machado, E. R. Nascimento, D. P. Rodrigues, M. H. C. Aquino, and V. L. A. Pereira. 2017. Amoxicillin/clavulanic acid and cefotaxime resistance in *Salmonella* Minnesota and *Salmonella* Heidelberg from broiler chickens. Poult. Sci. 5:123–129.

Rouger, A., O. Tresse, and M. Zagorec. 2017. Bacterial contaminants of poultry meat: sources, species, and dynamics. Microorganisms 5:50.

Salerno, B., M. Furlan, R. Sabatino, A. Di Cesare, M. Leati, M. Volanti, L. Barco, M. Orsini, C. Losasso, and V. Cibin. 2022. Antibiotic resistance genes load in an antibiotic free organic broiler farm. Poult. Sci. 101:101675.

Saraiva, M. M. S., K. Lim, D. F. M. do Monte, P. E. N. Givisiez, L. B. R. Alves, O. C. de Freitas Neto, S. Kariuki, A. B. Júnior, C. J. B. de Oliveira, and W. A. Gebreyes. 2022. Antimicrobial

resistance in the globalized food chain: a one health perspective applied to the poultry industry. Braz. J. Microbiol. 53:465–486.

Shi, Y., G. Wang, H. C. H. Lau, and J. Yu. 2022. Metagenomic sequencing for microbial DNA in human samples: emerging technological advances. Int. J. Mol. Sci. 23:2181.

Silbergeld, E. K., and J. L. Dailey. 2017. Biological plausibility of associations between antimicrobial use in food-producing animals and increased risks of human exposures to, and infections by, antimicrobial resistant zoonotic pathogens. WHO Guidelines on Use of Medically Important Antimicrobials in Food-Producing Animals. World Health Organization, Geneva, Switzerland.

Turcotte, C., A. Thibodeau, S. Quessy, E. Topp, G. Beauchamp, P. Fravalo, M. Archambault, and M. Gaucher. 2020. Impacts of short-term antibiotic withdrawal and long-term judicious antibiotic use on resistance gene abundance and cecal microbiota composition on commercial broiler chicken farms in Québec. Front. Vet. Sci. 7:547181.

USDA. 2016 Livestock and poultry: world markets and trade. United States Department of Agriculture and Foreign Agricultural Service. Accessed Oct. 2022. https://www.fas.usda.gov/data/livestock-and-poultry-world-markets-and-trade.

Van Gompel, L., R. E. C. Luiken, R. B. Hansen, P. Munk, M. Bouwknegt, L. Heres, G. D. Greve, P. Scherpenisse, B. G. M. Jongerius-Gortemaker, M. H. G. Tersteeg-Zijderveld, S. García-Cobos, W. Dohmen, A. Dorado-García, J. A. Wagenaar, B. A. P. Urlings, F. M. Aarestrup, D. J. Mevius, D. J. J. Heederik, H. Schmitt, A. Bossers, and L. A. M. Smit. 2020. Description and determinants of the faecal resistome and microbiome of farmers and slaughterhouse workers: a metagenome-wide cross-sectional study. Environ. Int. 143:105939.

Vieira, A. R., P. Collignon, F. M. Aarestrup, S. A. McEwen, R. S. Hendriksen, T. Hald, and H. C. Wegener. 2011. Association between antimicrobial resistance in *Escherichia coli* isolates from food animals and blood stream isolates from humans in Europe: an ecological study. Foodborne. Pathog. Dis. 8:1295–1301.

Wickham, H. 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York. ISBN:978-3-319-24277-4 https://ggplot2.tidyverse.org

Wingett, S. W., and S. Andrews. 2018. FastQ screen: a tool for multi-genome mapping and quality control. F1000Res 7:1338.

Wood, D. E., J. Lu, and B. Langmead. 2019. Improved metagenomic analysis with Kraken 2. Genome Biol. 20:257.