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# Comparative analyses of bacterial contamination and microbiome of broiler carcasses in wet market and industrial processing environments

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ABSTRACT

The slaughtering environment is crucial for the food hygiene and safety of poultry products. Despite the global dominance of industrial processing, live bird slaughtering in wet markets persists due to cultural, religious, and economic reasons. This study aims to reveal the correlation between hygiene scales in wet markets and bacterial contamination levels on broiler carcasses, with a particular focus on pathobiont transmission risks and microbiome characteristics. Wet markets were categorized based on home-made ratings and the Hygiene and Biosecurity Assessment Tool (HABT). The study assessed total aerobic bacterial (TAB) levels, food spoilage and hygiene indicators (*Pseudomonas* and *E. coli*), foodborne pathogen *Salmonella*, and the microbiome of broiler carcasses, intestinal contents, and slaughtering facilities. Comparative analyses were conducted between market and industrial processing environments. TAB levels on broiler carcasses showed a significant negative correlation with hygiene scores, indicating that both HABT and home-made rating tools effectively assess and improve processing hygiene. Industrial processing consistently reduced bacterial contamination compared to wet markets. Although Salmonella spp. prevalence was lower in market-processed carcasses, the study identified significant cross-transmission of pathobionts and variations in bacterial composition with hygiene improvements. Notably, the microbiome analysis revealed overlaps in amplicon sequence variants (ASVs) between carcasses and contamination vectors, highlighting pathobiont transmission risks. The present study confirmed the scales of hygiene standards among wet markets reflect bacterial contamination on broiler carcasses. Enhancing slaughter practices to meet industrial hygiene standards is essential for reducing the transmission of foodborne pathogens and pathobionts, and improving food safety.

## 1. Introduction

The sale and slaughter of live broilers in markets represent a long-standing traditional practice prevalent in various regions, particularly within Asian and predominantly Islamic countries. This method of processing, often conducted in open and crowded environments, introduces numerous food safety risks (Cui et al., 2022). Despite pre-market veterinary inspections, the risk of zoonotic pathogen exposure and transmission remains elevated for broilers slaughtered on-site. Consequently, the slaughtering environments in these markets differ markedly from those in industrial slaughterhouses. This discrepancy in risk is highlighted by events such as the H5N1 avian influenza pandemic (Li et al., 2022), which underscores the potential hazards associated with market-based slaughtering practices. In response, more stringent measures have been introduced to mitigate the transmission of microorganisms from animals to humans in wet markets, particularly in the wake of the COVID-19 pandemic (Petrikova et al., 2020). For instance, China has enacted legislation prohibiting poultry slaughtering in markets within certain major cities to enhance animal welfare and reduce zoonotic risks (Cui et al., 2022). Nevertheless, the practice persists due to entrenched dietary customs and regional economic factors (Lin et al., 2017; Wong et al., 2017).

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Concerns about food safety in wet markets have intensified in light of outbreaks of various zoonotic pathogens. Previous research has established a correlation between bacterial contamination levels, pathogen detection rates, and factors such as hygiene standards, slaughtering facilities, and worker practices at these markets (Dhanalakshmi et al., 2018; Siddiky et al., 2022; Zakki et al., 2017). For example, studies in Bangladesh have reported total aerobic bacterial (TAB) levels on broilers slaughtered at markets exceeding 8.30 log10 CFU/g, with *Escherichia coli* levels at 5.53 log10 CFU/g (Julqarnain et al., 2022). Similarly, in Nepal, TAB levels have been recorded as high as 10.45 log10 CFU/g (Bohara, 2017). Such levels of contamination are indicative of advanced bacterial spoilage. Moreover, *Salmonella* detection rates have been reported to range from 48 % to 85 % in Indonesia (Wardhana et al., 2021; Yulistiani et al., 2019), 20 % in Bangladesh (Julqarnain et al., 2022), and 38 % in Nepal (Bohara, 2017). These figures exceed established food safety limits, which stipulate that foodborne pathogens, including *Salmonella*, *Campylobacter*, and *E. coli* O157:H7, should be absent in 25 g of raw meat (Zhong et al., 2023). Despite these concerns, comparative studies examining carcasses processed in market versus industrial settings remain scarce. Additionally, pathobionts, such as *Proteus mirabilis*, are commonly found on broiler carcasses (Yu et al., 2021), yet their risk has not been extensively assessed.

The slaughtering environment in wet markets, unlike industrial facilities, lacks consistent monitoring of food processing hygiene, relying instead on vendor practices. Siddiky et al. (2022) found that many chicken vendors in wet markets possess limited knowledge regarding foodborne pathogens, although they demonstrate a general understanding of food hygiene. This knowledge is significantly correlated with food processing practices and directly impacts food safety. Conversely, industrial processing procedures also contribute to bacterial contamination on poultry products, even within similar geographic regions (Yu et al., 2020). The slaughtering environment, including factors such as animal skin, intestines, and feces, contributes to bacterial contamination of poultry products, albeit with limited mitigation measures (Hauge et al., 2023). Unlike industrial slaughterhouses, the processing hygiene in wet market slaughter installations varies considerably depending on the vendor, and food safety satisfaction varies based on the Hygiene and Biosecurity Assessment Tool (HBAT, Soon and Abdul Wahab, 2021). However, there is a notable lack of research specifically addressing the relationship between bacterial contamination and hygiene practices in wet markets.

The present study aims to examine the impact of food hygiene practices in wet markets on bacterial contamination of broiler carcasses. Additionally, it seeks to characterize the predominant bacterial communities present in the processing environments of chicken vendors compared to those in industrial slaughterhouses, with the objective of assessing the transmission risk of pathobionts in wet markets.

## 2. Materials and methods

### 2.1. Market rating and carcasses collection

Markets distributed across Foshan, Guangdong, China, were visited and those with live broiler stalls were scored based on the rating scale shown in Table 1. Briefly, market rating was based on the differences in partition of function area (cages of live birds, slaughtering area, cutting table), the source of rinsing water, defeathering facility, knives in use, and general environmental hygiene. Due to the processing periods among these stalls were similar, around 12 min, the slaughtering period was not included in the rating scale. Markets with a total score lower than 8 were grouped to condition 1, with a score between 8 and 10 into condition 2, and markets with scores above10 were to condition 3. For each condition, at least three markets were selected as representative spots for sampling. The selected markets were rigorously assessed using HBAT, in accordance with the methodology outlined in the previous study (Soon and Abdul Wahab, 2021).

**Table 1**
Home-made rating for slaughter installs in wet markets.

| Category | | Score |
|---|---|---|
| Partition of function area(area for live birds, slaughtering, cutting) | 3 areas are well partitioned | 3 |
| | Only live birds are separated | 2 |
| | All in one | 1 |
| The source of rinsing water | Tap water | 3 |
| | Tap water and stored water | 2 |
| | Stored water | 1 |
| Defeather facility | Automation | 3 |
| | semi-automation | 2 |
| | Manual | 1 |
| Knife in use (bleeding, cutting) | Different uses and placed separated | 3 |
| | Different uses but placed together | 2 |
| | Mixed use | 1 |
| General environmental hygiene | no visible filth | 3 |
| | fresh filth | 2 |
| | old filth | 1 |

At each selected sampling stall, two broilers, originating from different farms, were purchased from the same stall and slaughtered by the butcher at the spot. The purpose of the study was explained to the butcher in the local language prior to purchasing the bird. After evisceration, the gastrointestinal tracts were collected in sterile bags, while the whole carcasses were placed into separate sterile bags after processing. Additionally, sterile swabs were used to sample the hands of workers and the knives used that were used during processing were sampled using sterile swabs by smearing. Sampling and data recording in this study were conducted by a designated staff member.

Additionally, industrial slaughtered broiler carcasses, processed within a day, were bought from local supermarkets, and based on the labels, information of the origin of the farm, and bird age were recorded. The study design is presented in Supplementary Fig. 1.

### 2.2. Sampling

All samples were cooled transported using a cooler box, and subsampled in the lab within maximum 1 h after arrival. Of each carcass, skins of neck, breast, and back were sterileally collected in three sterile stomacher bags separately. Each skin was around 10 g and homogenized with 1:10 (W/W) buffer peptone water (BPW, 023130, Huankai, China) for 2 min using a peristaltic homogenizer (MP-4, Dr. Ant, China). Swabs were rinsed with 1 ml BPW and the homogenates collected in sterile tubes. Additionally, sterile samples of jejunal and cecal contents were taken after carefully opening the gastrointestinal tract in sterile condition, with 0.1 g reserved for amplicon sequencing and 1 g added to 10 ml BPW for subsequent bacterial examination.

The age of birds was estimated using the length of leg bones based on a previous study (Breugelmans et al., 2007). In the present study, lengths of left femur, tibia and tarsometratarsus were measured to indicate the age of birds, after they were carefully exposed by dissecting the surrounding soft tissues.

### 2.3. Bacterial examination

Ten-fold serial dilutions of each carcass skin homogenate were prepared in BPW, followed by spread plating for quantitative bacterial isolation, in duplicate. Total aerobic bacterial counting (TAB) was performed on plate count agar (PCA, 022070, Huankai, China) and incubated at 30 °C for 48 h. Presumptive *Pseudomonas* spp. were isolated on cephalothin-sodium fusidate-cetrimide (CFC) agar with modified CFC selective supplement (CFC, 027382 with SR0320, Huankai), incubated at 30 °C for 48 h aerobically. *Escherichia coli* was selectively isolated on

*E. coli* Chromogenic Medium (HB7001, Haibo, China), incubated at 37 °C for 24 h aerobically according to the instructions of the manufacturer. Colonies on the agar plates were counted if bacterial growth ranged between 30 and 300 CFU/plate.

*Salmonella* was isolated according to ISO 6579-1. In brief, the homogenates of all samples were directly incubated at 37 °C for 18 ± 2 h, after which 100 μl was dropped on modified semi-solid rappaport vassiliadis medium with supplement (MSRV, 023025 and SR0530, Huankai), and incubated at 41.5 °C for 24 ± 3 h (ISO, 2017b). Subcultures were taken from each outside edge of the halo and transferred onto xylose lysine deoxycholate agar (XLD, 029999, Huankai), and plates were incubated at 37 °C for 24 ± 3 h. Typical black colonies with a white halo were picked, and confirmed with PCR assay. For this, DNA was extracted by alkaline lyses in which one colony was suspended into 20 μl of lysis buffer (2.5 ml 10%SDS, 5 ml 1 N NaOH and 92.5 ml Milli-Q water) and heated for 15 min at 95 °C. After a short spin, 180 μl Milli-Q water was added, and the supernatant was collected after centrifugation for 5 min at 10,000 ×*g* at 4 °C. A multiplex PCR was conducted according to a previous study (Han et al., 2020), using two sets of primers: invA (Forward: 5′-GTGAAATTATCGCCACGTTCGGGCAA-3′ and Reverse: 5′-TCATCGCACCGTCAAAGGAACC-3′) and hut (Forward: 5′-ACTGGCGTTATCCCTTTCTCTGCTG-3′ and Reverse: 5′-ATGTTGTCCTGCCCCTGGTAAGAGA-3′) with ratio 1:3 for the concentration. After amplification, for positive confirmation, two amplicons at 284 bp and 495 bp should be observed simultaneously under UV after size-separation after electrophoresis in pre-stained 1.5 % agarose for 30 min at 120 V.

### 2.4. 16S rRNA amplicon sequencing

Homogenates of skins, swabs, and intestinal contents were delivered to Shanghai Origin Gene Bio-pharm Technology Co. Ltd., China, for DNA extraction and 16S rRNA amplicon sequencing with the V3-V4 region (515F: 5′-GTGCCAGCMGCCGCGGTAAUT-3′ and 806R: 5′-GGACTACHVGGGTWTCTAATMUT-3′) on the Illumina HiSeq 2500 sequencing platform.

Clean raw reads were merged using FLASH software with default settings. The QIIME2 (version 2021.08) software pipeline was used for data analysis. Reads were demultiplexed with q2-demux2. Then, the DADA2 plugin was implemented for the quality control process, and all phiX reads and chimeric sequences were filtered (Bokulich et al., 2017; Lee et al., 2019). Based on the demux summary, merged sequences were truncated to a length of 500 bp. After denoising the data using the DADA2 denoise-paired method, representative sequences of each sample were retained and assigned to taxa using Naive Bayes classifiers pretrained on Sliva_99%_OTUs full-length.

### 2.5. Statistical analysis

Quantitative data were first assessed for normality and homogeneity of variance using the Shapiro–Wilk and Levene's tests, respectively. One-way ANOVA tests and *t*-test were used to assess significant differences, with statistical analyses and plots generated using GraphPad software. Standardized coefficients between bacterial level and factors (bone length and market rates) were calculated using linear regression analysis in SPSS 26.0. Reported significant differences were corrected for multiple testing using the Benjamini–Hochberg correction with a 5 % false discovery rate.

Default plugins in QIIME2 were utilized for alpha diversity analysis, while ImageGP software was used for similarity analysis (ANOSIM), principal coordinate analysis (PCoA), and plotting. Differences in taxon abundance between groups were analyzed using Lefse, with only biomarkers possessing LDA scores exceeding 4 included.

The percentage (pe) of the same ASVs or taxa between skin homogenates and contamination vectors was calculated based on the following formula:

$$pe = n2/n1$$

n1: the counts of ASVs or taxa with reads over 0 in skin homogenate; n2: the counts of ASVs or taxa with reads over 0 both in homogenate of skin and contamination vectors.

## 3. Results

### 3.1. Market rating and selection

A total of 18 markets in Foshan, China, were visited, with 13 markets housing stalls for live poultry processing. The scaling of these wet markets, based on the home-made rating system and HBAT, is presented in Table 2 (with details provided in Supplementary Tables 1 and 2). This assessment highlights the distinct differences in processing environments and procedures. From each of the final condition representative of the home-made rating scale, a total of 26 carcasses from 13 markets, along with related samples for contamination vectors, were examined. With the total rating scores lower than 8, there were three markets grouped as condition 1, where the slaughter procedure were more primitive and processing environment was less hygienic. Another five markets, rating scores between 8 and 10, were grouped as condition 2 with a few automation facilities and tap water, as well as better hygiene environments. And five markets, rating scores over 10, were grouped as condition 3 with well-equipped facilities and hygienic environment. Rating these 13 wet markets using HBAT, three markets, along with those assessed by the home-made rating system, were classified as poor, requiring major improvements. Two markets under condition 3 were rated as good, while the remaining markets were rated as moderate, with some improvements needed. The home-made rating system generally aligned with the HBAT results.

Additionally, 12 carcasses processed at three different industrial facilities from the same region of the market ones were included in the study. According to the butcher and label information, all broilers were aged between 100 and 180 days and originated from various farms in Guangdong province.

The range length of bone from these carcasses was shown in Table 3

**Table 2**
The hygiene scales of wet markets rated by two scoring methods.

| Location | Home-made rating | | HBAT | |
|---|---|---|---|---|
| | Scores | Category | Scores | Category |
| C | 5 | Condition 1 (<8) | 3.7 | Poor and major improvement necessary |
| B | 6 | Condition 1 (<8) | 2.9 | Poor and major improvement necessary |
| D | 6 | Condition 1 (<8) | 3.1 | Poor and major improvement necessary |
| K | 8 | Condition 2 (8–10) | 4.7 | Moderate and some improvement required |
| G | 9 | Condition 2 (8–10) | 4.7 | Moderate and some improvement required |
| J | 9 | Condition 2 (8–10) | 4.0 | Moderate and some improvement required |
| F | 10 | Condition 2 (8–10) | 4.2 | Moderate and some improvement required |
| L | 10 | Condition 2 (8–10) | 4.9 | Moderate and some improvement required |
| A | 11 | Condition 3 (>10) | 5.7 | Moderate and some improvement required |
| H | 12 | Condition 3 (>10) | 6.3 | Moderate and some improvement required |
| I | 12 | Condition 3 (>10) | 5.0 | Moderate and some improvement required |
| E | 14 | Condition 3 (>10) | 7.2 | Good |
| M | 14 | Condition 3 (>10) | 7.1 | Good |
| Y | Industrial | | Pre-packed chicken from supermarket | |

**Table 3**
Coefficients of bone length to the TAB level in the linear regression.

| | Neck | Breast | Back | Market vs Industry (cm) |
|---|---|---|---|---|
| Femural length | 0.356 (0.065) | 0.179 (0.468) | 0.236 (0.259) | 8.48 ± 0.56 vs 7.93 ± 0.50 (0.007) |
| Tibial length | 0.093 (0.635) | −0.195 (0.441) | −0.265 (0.224) | 11.77 ± 0.56 vs 10.83 ± 0.69 (0.0001) |
| Tarsometatarsal length | −0.372 (0.043) | −0.058 (0.797) | 0.027 (0.892) | 8.78 ± 0.53 vs 8.11 ± 0.46 (0.0008) |

*p* value was indicated in the brackets.

to indicate the presumptive age of birds. The broilers slaughtered at industry was significantly younger than those from wet markets, while no significantly difference was observed within broilers slaughtered at different wet markets.

### 3.2. Bacterial examination

The level of total aerobic bacteria showed to be correlated to the processing hygiene conditions, irrespective of the sampling site (Fig. 1 & Supplementary Table 3). Carcasses slaughtered at markets consistently displayed significantly higher TAB levels on the neck skin (7.40 ± 0.44 $\log_{10}$ CFU/g) compared to those processed in industrial settings (6.57 ± 0.56 $\log_{10}$ CFU/g, $p < 0.0001$). Similarly, the TAB level was also significantly higher on the breast skin of carcasses slaughtered at markets (7.25 ± 0.61 $\log_{10}$ CFU/g) than those processed at industry (6.74 ± 0.57 $\log_{10}$ CFU/g, $p = 0.0272$), as well as for the back skin (market: 7.37 ± 0.48 $\log_{10}$ CFU/g; industry: 6.76 ± 0.49 $\log_{10}$ CFU/g, $p = 0.0013$). In the linear regression model, slaughter environment always contributed

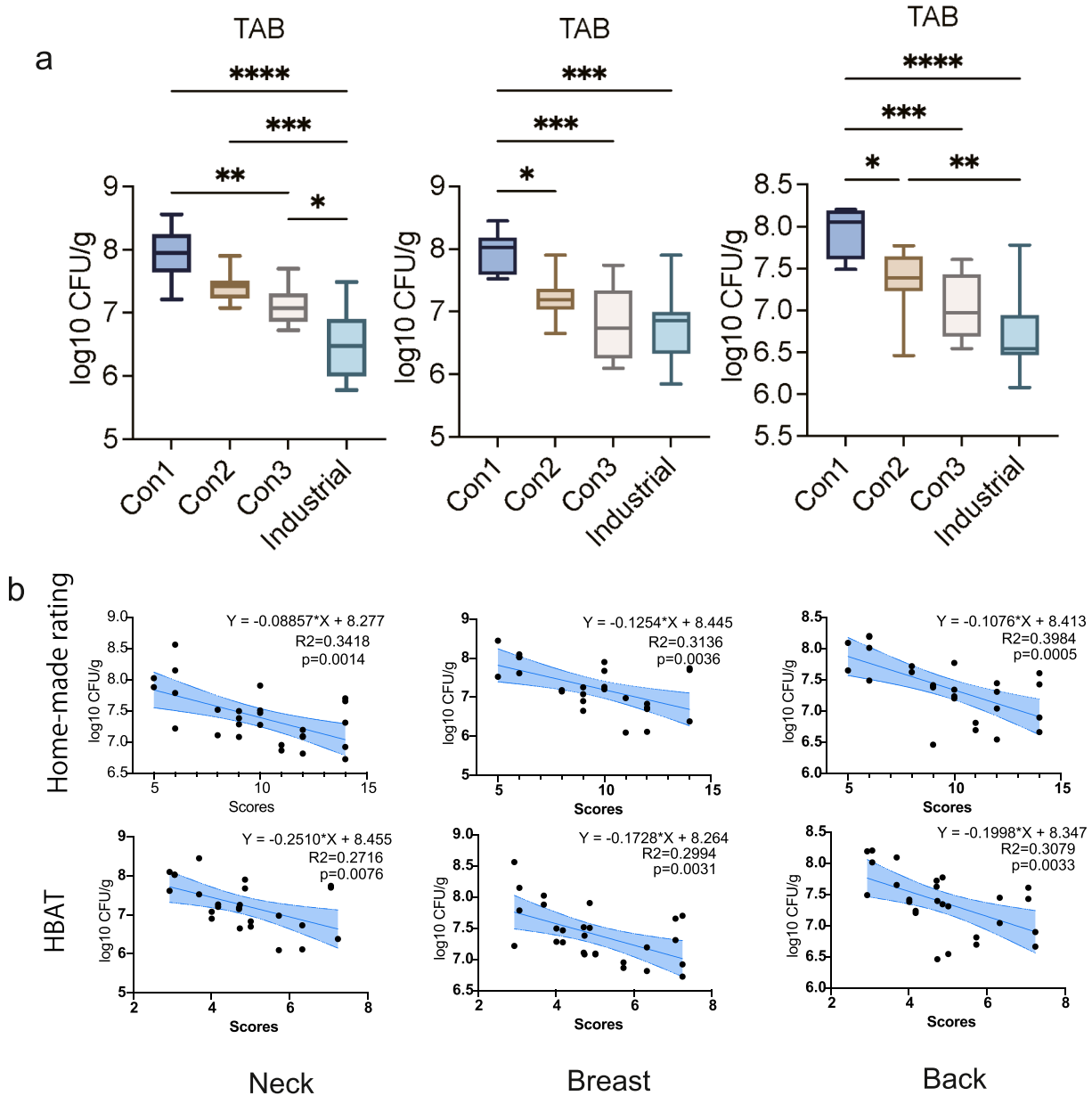


**Fig. 1.** a. Total aerobic bacteria level on carcasses processed in markets with different hygiene and at industry; b. fitted curve from linear regression analysis for TAB level and hygiene scales (ANOVA - *: $p < 0.05$, **: $p < 0.01$; ***: $p < 0.001$, ****: $p < 0.0001$).

significantly to the TAB level on carcasses, while the length of bones, indicating the bird age, had limited impact on the TAB level regardless of sampling site, as standardized coefficients of slaughter environment were larger than those for bone length (Table 3).

At market level, carcasses processed under condition 1, corresponding with the lowest hygienic level, exhibited significantly higher TAB levels (neck: 7.93 ± 0.44 $\log_{10}$ CFU/g; breast: 7.96 ± 0.34 $\log_{10}$ CFU/g; back:7.94 ± 0.30 $\log_{10}$ CFU/g) than those under condition 2 (neck: 7.40 ± 0.24 $\log_{10}$ CFU/g, $p = 0.0742$; breast: 7.23 ± 0.34 $\log_{10}$ CFU/g, $p = 0.0355$; back:7.35 ± 0.37 $\log_{10}$ CFU/g, $p = 0.0383$), and condition 3 (neck: 7.12 ± 0.32 $\log_{10}$ CFU/g, $p = 0.0022$; breast: 7.22 ± 0.35 $\log_{10}$ CFU/g, $p = 0.0006$; back:7.04 ± 0.38 $\log_{10}$ CFU/g, $p = 0.0007$). No significant difference in TAB levels was observed between carcasses processed under conditions 2 and 3. However, linear regression analysis revealed a significant negative correlation between TAB levels and the wet market scores, as assessed by both the home-made rating system and HBAT, regardless of the sampling sites (Fig. 1b).

Regarding presumptive *Pseudomonas* spp., only minimal differences were observed between slaughtering environments, except for significantly higher contamination levels on the skin in condition 2 (breast: 6.15 ± 0.71 $\log_{10}$ CFU/g; back:6.25 ± 0.54 $\log_{10}$ CFU/g) compared to condition 3 (breast:5.26 ± 0.46 $\log_{10}$ CFU/g, $p = 0.008$; back:5.51 ±

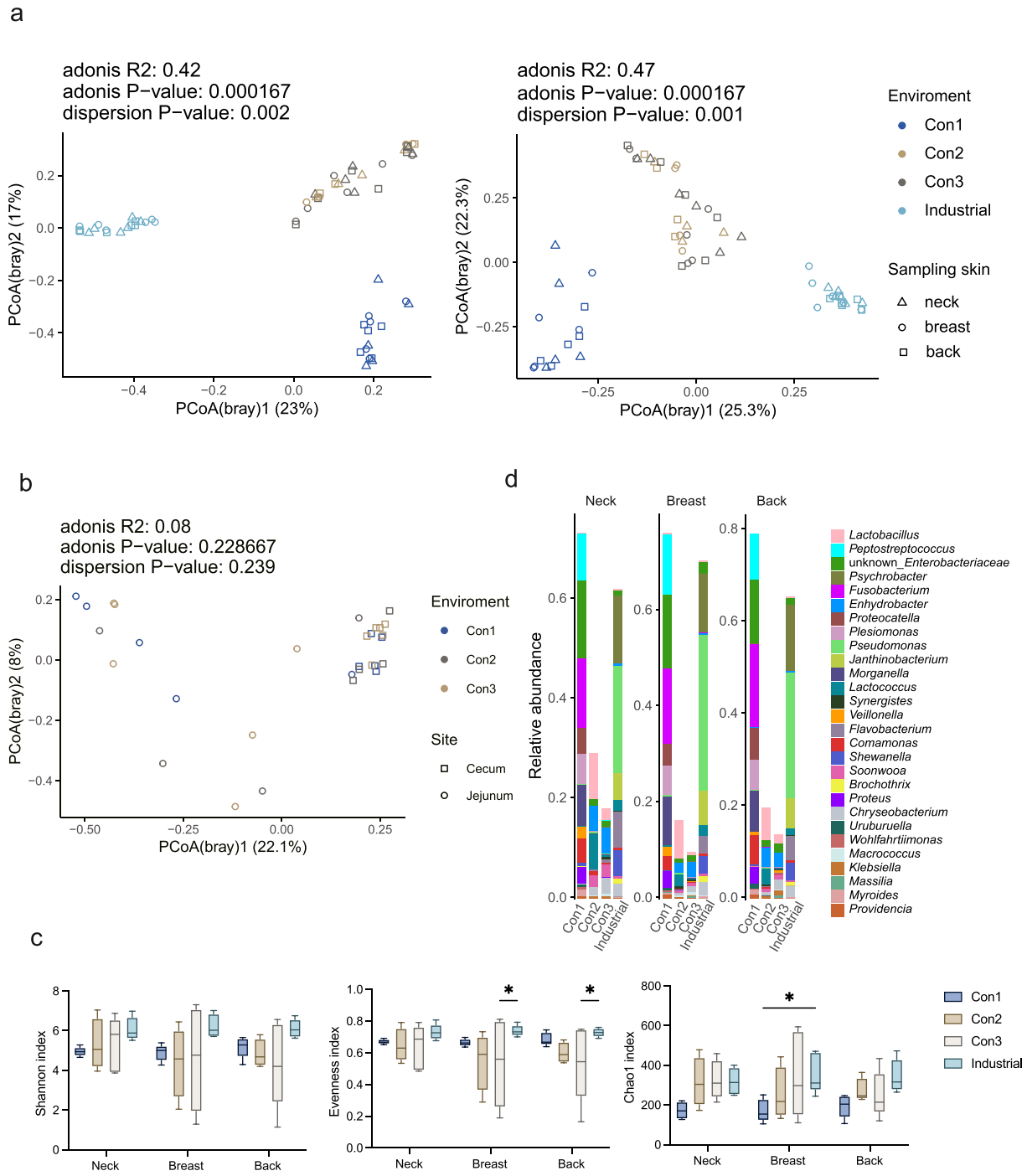


**Fig. 2.** Bacterial communities on carcasses processed at market and industry. a. PCoA analysis of bacterial communities based on ASV level (left) and genera level (right); b. PCoA analysis of gut microbiota in broilers slaughtered at market; d. top 10 abundant genera that are significantly different between groups based on LEFse analysis; c. alpha diversity of bacterial communities on carcasses (ANOVA - *: $p < 0.05$).

0.59 $\log_{10}$ CFU/g, $p = 0.0138$), and industrial setting (back:5.41 ± 0.36 $\log_{10}$ CFU/g, p = 0.002), as shown in Fig. 1b & Supplementary Table 1. Conversely, *E. coli* levels were significantly lower on carcasses processed in industrial settings (neck: 2.92 ± 0.71 $\log_{10}$ CFU/g; breast: 3.21 ± 0.83 $\log_{10}$ CFU/g; back:3.14 ± 0.79 $\log_{10}$ CFU/g) than those processed at markets across all sampling sites (neck: 4.40 ± 0.64 $\log_{10}$ CFU/g, $p <$ 0.0001; breast: 4.68 ± 0.72 $\log_{10}$ CFU/g p < 0.0001; back:4.59 ± 0.53 $\log_{10}$ CFU/g, p < 0.0001). With the exception of the bacterial loads of *Pseudomonas* spp. and *E. coli* on the breast skin, which showed a significant negative correlation with the scores scaled by HBAT, no correlation was observed between the sampling sites and the wet market ratings (Supplementary Fig. 2).

A total of 13 carcasses were contaminated with *Salmonella* spp., among which seven from markets (26.92 %) and seven from industry (58.33 %). Detailed information regarding the isolation of *Salmonella* from various samples is provided in Supplementary Table 4. Notably, *Salmonella* spp. were isolated from different carcass parts, including the neck, breast, and back skin, as well as from a cutting knife.

### 3.3. Bacterial composition between carcasses processed at market and industry

A total of 9,254,799 reads were generated from 111 samples, resulting in 1,873,722.989 ASVs after filtering out non-bacterial reads. Following normalization and retention of reads above the sample size threshold ($n = 111$), a total of 1,684,068.589 ASVs were included for further analysis. ASVs were assigned at genus-level, resulting in the identification of 1348 taxonomic groups.

Bacterial compositions on carcasses were significantly different between carcasses processed at markets and those at industry, regardless of ASVs and taxonomy level (Fig. 2a & Supplementary Table 5). Additionally, carcasses processed at markets under different conditions exhibited significantly distinct bacterial compositions, whereas the gut microbiota had no significant differences between broilers processed in different markets (Fig. 2b). Notably, the dissimilarity of bacterial communities on carcass skin of those processed under condition 2 and those of 3 was smaller compared to that between condition 1 and the two other conditions.

Alpha diversity analyses revealed minor differences between carcasses processed under different conditions (Fig. 2c), although larger variances were observed within groups from conditions 2 and 3. Specifically, the evenness of bacterial compositions on breast and back skin was significantly lower in condition 3 than in industrial settings. Furthermore, Chao 1 was significantly lower on breast skin from carcasses under condition 1 compared to those from industrial settings.

The relative abundance of the top 10 taxa differed between groups (Fig. 2d). Notably, unidentified *Enterobacteriaceae*, *Peptostreptococcus*, *Fusobacterium*, *Proteocatella*, *Plesiomona*, and *Morganella* were

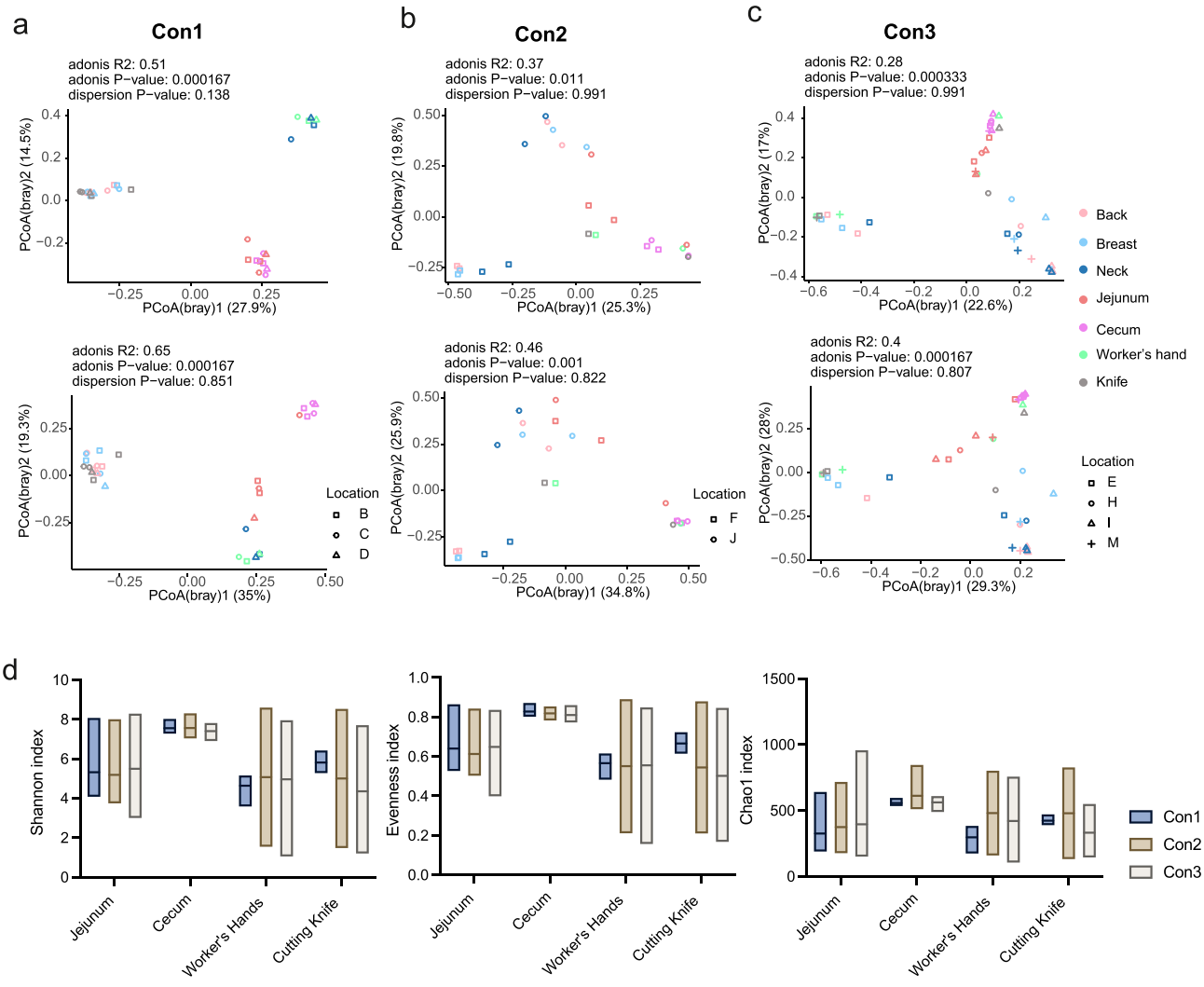


**Fig. 3.** Bacterial compositions on carcasses and associated contamination vectors in different conditions. a. Condition 1; b. condition 2; c. condition 3; d. alpha diversity index.

significantly abundant on carcasses processed under condition 1 at markets. In contrast, *Lactobacillus*, *Enhydrobacter*, *Soonwooa*, and *Chryseobacterium* were significantly more prevalent on carcasses processed under conditions 2 and 3 at markets. *Psychrobacter*, *Pseudomonas*, *Janthinobacterium*, *Flavobacterium*, *Shewanella*, and unidentified *Enterobacteriaceae* were significantly higher on carcasses processed at industrial facilities.

### 3.4. Bacterial similarity between carcasses and contamination vectors

Co-analyses between reads from skin homogenates and associated contamination vectors in condition 1 revealed significant dissimilarity

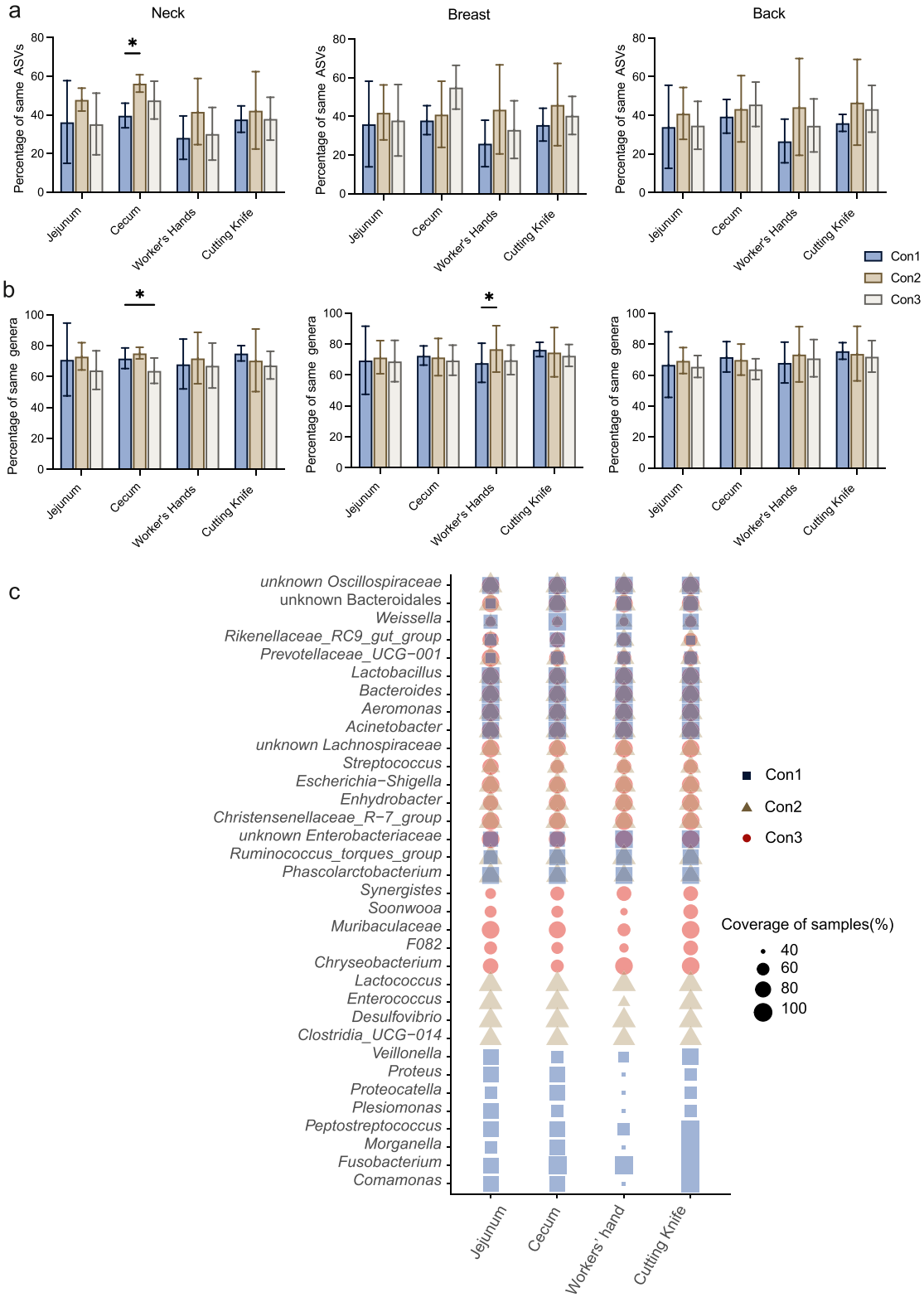


**Fig. 4.** Similarity of bacteria between skin of carcasses and associated contamination vectors. a. Percentage of same ASVs between carcass skin and certain factors; b. percentage of same genera between skin and certain factors; c. top 20 genera that are same in carcass skin and associated factors.

between carcasses and these factors, regardless of ASVs and taxonomy, as shown in Fig. 3a. Under this condition 1, bacterial communities showed greater similarity within skin homogenates, intestinal content, or environmental samples (such as workers' hands and cutting knife). In condition 2, bacterial communities in skin homogenates were more akin to the associated jejunal content at the taxonomy level, but less so at the ASVs level (Fig. 3b). Additionally, similar bacterial communities between cecal content and environmental samples (workers' hands and cutting knife) were observed, independent of ASVs and taxonomy level. Condition 3 exhibited more similar communities between carcass skin homogenates and contamination vectors (Fig. 3c). Bacterial communities on the skin partly resembled worker's hands and cutting implements, though still maintaining similarities with jejunal and cecal bacterial compositions. While there was no significant difference in alpha diversity of bacterial communities in these contamination vectors between different conditions, the variance of alpha diversity in samples from condition 2 and 3 was larger than in condition 1, especially on workers' hands and cutting knives (Fig. 3d). Bacterial communities were more diverse in the content of the cecum than the jejunum and environmental facilities.

The cross-transmission between carcasses and contamination vectors was evaluated using the percentage of the same ASVs or taxa between skin homogenates and contamination vectors, as shown in Fig. 4a–b. Generally, taxonomic similarity was higher than at the ASVs level. On the neck skin, more similar ASVs were shared between skin homogenates and cecal content, with condition 2 having a significantly higher percentage than condition 1, while significantly higher similarity in genera was observed in condition 1 than in condition 3 concerning the taxonomy level (Fig. 4b). On the breast, carcass skin from condition 2 also showed higher similarity in ASVs with jejunal content and environmental samples than other conditions, while skin from condition 3 had a higher percentage of the same ASVs as cecal content than conditions 1 and 2. Percentages of the same genera between breast skin and other samples were similar, while significantly higher similarity in genera between skin and workers' hands was found in condition 2 than in condition 1. Back skin exhibited similar observations as breast skin, with condition 2 showing higher similarity of ASVs between skin and jejunal content, while environmental samples, except for condition 3, had a slightly higher percentage between skin and cecum. The trend of the same genera between back skin and other samples was similar to that shown in breast skin, though without any significance.

The relative abundance of the same genera between skin homogenates and contamination vectors was calculated, and the top 20 genera from each condition were shown in Fig. 4c. *Comamonas*, *Fusobacterium*, *Morganella*, *Peptostreptococcus*, *Plesiomonas*, *Proteocatella*, *Proteus*, and *Veillonella* were among the top 20 taxa present in both carcasses and environmental samples only in condition 1, although some were found on a few workers' hands. *Clostridia* UCG-014, *Desulfoibrio*, *Enterococcus*, and *Lactococcus* were found as top 20 taxa in both carcasses and environmental samples only in condition 2, while *Chryseobacterium*, F082, *Muribaculaceae*, *Snoonwooa*, and *Synergistes* were present in condition 3. Besides these taxa identified in only one condition, there were other taxa shared among conditions. Unidentified *Oscillospiraceae*, unidentified *Bacteroidales*, *Weissella*, *Rikenellaceace* RC9, *Prevotellaceace* UCG001, *Lactobacillus*, *Bacteroides*, *Aeromonas*, and *Acinetobacter* were common taxa present in both carcasses and contamination vectors identified in all conditions. *Lachnospiraceae*, *Streptococcus*, *Escherichia-Shigella*, *Enhydrobacter*, and *Christensenellaceae* R-7 were identified in both condition 2 and 3, while unidentified *Enterobacteriaceae* was identified in conditions 1 and 3. *Ruminococcus torques* and *Phascolarctobacterium* were identified in conditions 2 and 3.

## 4. Discussion

The slaughtering environment is associated with food hygiene and safety of poultry products. While industrial processing dominates in many regions globally, live bird slaughtering in markets persists due to gastronomic customs, religious culture, and economic constraints. The present study revealed the correlation between the processing environment and bacterial contamination levels on broiler carcasses and indicated the transmission risk of pathobionts in wet markets where hygiene is poor and needs major improvement.

The markets included in the present study were grouped into three scales, dependent on the home-made rating and HABT. Different from HABT, which assesses general food hygiene and biosecurity (Soon et al., 2022), the home-made rating, addressed in the present study, focuses on the details of poultry slaughter procedures and environmental hygiene. The present study was the first to assess the practicability of HABT for slaughter installations, as well as the home-made rating, correlating scores with the loads of food hygiene and safety indicators. The TAB level, indicating the general hygiene of broiler carcasses, had a significantly negative correlation with the scores of wet markets, indicating that these two rating tools are effective in assessing processing hygiene and aiding in improving food safety. Although spoilage and food safety indicators, namely the loads of *Pseudomonas* and *E. coli*, only showed a correlation with HABT for the breast skin, it is plausible to optimize these tools for market evaluation to improve food safety (Astbury et al., 2022). Furthermore, studies aimed at revealing the more precise factors affecting processing hygiene should be designed, considering the detailed scales in HABT and the home-made rating.

Previous studies have shown that broiler carcasses processed in wet markets suffer from higher contamination with general bacteria and foodborne pathogens compared to industrial settings (Asuming-Bediako et al., 2022; Julqarnain et al., 2022; Pavon et al., 2022). However, there have been few studies comparing the bacterial contamination levels on carcasses processed at markets versus those processed industrially in the same location. The present study revealed disparities in bacterial contamination levels on carcasses processed in these two settings, with broilers raised under similar conditions. Generally, the TAB and *E. coli* levels were significantly reduced when broilers were processed industrially, consistent with previous studies. Notably, differences in bird restraint procedures between markets and industrial facilities may contribute to varying contamination levels (Khairunnesa et al., 2020), with birds in market settings having more exposure to potential contamination sources such as feathers and feces. However, the present study subgrouped samples from wet markets according to hygiene scales and revealed that a higher hygiene scale in wet markets decreased the general bacterial level, suggesting that improvements in processing hygiene can mitigate bacterial contamination in wet markets (Soon and Abdul Wahab, 2021).

Unlike general food hygiene indicators, the foodborne pathogen *Salmonella* spp. was less frequently detected on carcasses processed in markets compared to industrial settings. This finding contrasts with recent reports indicating a higher prevalence of *Salmonella* spp. on chicken from wet markets compared to supermarkets in Malaysia and Vietnam (Nhung et al., 2018; Thung et al., 2016), and a study from China that found no significant difference (Huang et al., 2016). The present study only included broiler carcasses, particularly those slaughtered on-site at wet markets. Given that birds in industrial settings are generally younger than those in wet markets, as indicated by bone length, this aligns with previous research suggesting that younger birds carry more pathogens (Beal et al., 2004). To improve public health outcomes, targeting high-virulence *Salmonella* serotypes in risk management strategies and food safety regulations for poultry products is crucial. Therefore, isolates from the present study will undergo further identification and serotyping in the subsequent research.

Moreover, although research on products from wet markets is increasing (Fearnley and Zheng, 2023; Oluwatoyin et al., 2024; Williams et al., 2023), few studies have addressed the potential risk of pathobionts, as chickens harbor several pathobionts (Oladele et al., 2024; Yu et al., 2021). The present study assessed the microbiome of broiler carcasses, intestinal contents, and slaughtering facilities, revealing

overlaps in amplicon sequence variants (ASVs) and taxa between the carcass and contamination vectors, suggesting cross-transmission among birds and processing environments. Thus, the transmission of pathobionts resident in the gut of broilers is notable in wet markets. Interestingly, bacterial composition also changed with the hygiene scale of the wet market, regardless of the carcasses and contamination vectors, suggesting the slaughter procedure contributes to the microbiome of both product and environment as it to those at industry (Kunert et al., 2022).

Industrial carcasses exhibit distinct bacterial signatures compared to those processed in markets, with *Psychrobacter*, *Pseudomonas*, and *Janthinobacterium* predominating, likely originating from the chill-chain processing environment (Park et al., 2023). Improvements in processing hygiene resulted in bacterial compositions more aligned with industrial standards, featuring dominant bacteria such as *Lactobacillus*, *Enhydrobacter*, and *Lactococcus*. Carcasses processed under poor hygiene conditions showed abundant families and genera that harbor several pathobionts, such as *Proteus*, *Morganella*, *Enterobacteriaceae* and *Peptostreptococcus* (Cordell, 2023; Park et al., 2023), suggesting that poor hygiene in wet markets increases the transmission risks of pathobionts.

In conclusion, the hygiene scale of wet markets varies and is significantly correlated with the bacterial contamination levels on broiler carcasses, affecting food quality and safety. Enhancements in slaughter practices at wet markets should be considered to achieve hygiene standards comparable to industrial settings to reduce the cross-transmission of foodborne pathogens and pathobionts.

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## CRediT authorship contribution statement

**Yuanyuan Zhou:** Writing – review & editing, Writing – original draft, Conceptualization. **Nan-lin Wang:** Resources, Methodology, Investigation, Data curation. **Jin-qi Cen:** Resources, Methodology, Investigation. **Jia-tong Han:** Resources, Methodology. **Yu-xuan Tang:** Methodology. **Zi-qi Xu:** Methodology. **Hang Zeng:** Resources, Methodology. **Kurt Houf:** Writing – review & editing, Validation, Conceptualization. **Zhongjia Yu:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Funding acquisition, Data curation, Conceptualization.

## Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT 3.0 in order to improve readability and language. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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## Declaration of competing interest

None.

## Data availability

The raw amplicon sequencing data has been deposited in NCBI as a BioProject PRJNA1103958.

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