

Profiling and source tracking of the microbial populations and resistome present in fish products



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ARTICLE INFO

Keywords:

Catfish
Processing plant
Antimicrobial resistance
Listeria spp.
16S rRNA
Metagenomic sequencing

ABSTRACT

Microorganisms in processing environments significantly impact the quality and safety of food products and can serve as potential reservoirs for antibiotic-resistant genes, contributing to public health concerns about antimicrobial resistance (AMR). Fish processing plants represent an understudied environment for microbiome mapping. This study investigated the microbial composition, prevalence of *Listeria* spp., and resistome structures in three catfish processing facilities in the southeastern United States. The 16S rRNA gene sequencing revealed that the observed richness and Shannon diversity index increased significantly from fish to fillet. Beta diversity analysis showed distinct clustering of microbial communities between fish, environment, and fillet samples. Fast expectation-maximization microbial source tracking (FEAST) algorithm demonstrated that the microbiota presents in the processing environment contributed 48.2 %, 62.4 %, and 53.7 % to the microbiota present on fillet in Facility 1 (F1), F2, and F3, respectively. Food contact surfaces made larger contributions compared to the non-food contact surfaces. The linear discriminant analysis of effect size (LEfSe) identified specific microbial genera (e.g., *Plesiomonas*, *Brochothrix*, *Chryseobacterium* and *Cetobacterium*) that significantly varied between *Listeria* spp. positive and negative samples in all three processing plants. The metagenomic sequencing results identified 212 antimicrobial resistance genes (ARGs) belonging to 72 groups from the raw fish and fish fillet samples collected from three processing plants. Although there was a significant decrease in the overall diversity of ARGs from fish to fillet samples, the total abundance of ARGs did not change significantly ($P > 0.05$). ARGs associated with resistance to macrolide-lincosamide-streptogramin (MLS), cationic antimicrobial peptides, aminoglycosides, and beta-lactams were found to be enriched in the fillet samples when compared to fish samples. Results of this study highlight the profound impact of processing environment on shaping the microbial populations present on the final fish product and the need for additional strategies to mitigate AMR in fish products.

1. Introduction

Consumption of fish and shellfish in the United States (U.S.) has increased over the past decade, from 16.8 pounds per capita in 2012 to 19.2 pounds per capita in 2019 (Hanson, 2022). Catfish is one of the leading finfish production species in the U.S., constituting more than half of domestic freshwater production (NOAA Fisheries, 2020). Each American annually consumes a half-pound of domestic farm-raised

catfish on average (Hanson, 2022). Currently, 95 % of all catfish sales for food purposes were made directly to processing plants, where whole live fish are converted into a variety of food products such as fillets, nuggets, and steaks (USDA, 2021). During catfish processing, the microorganisms on the skin, gills, and gut can be transferred to processing equipment, workers, and the fillet (Ramos and Lyon, 2000). At the same time, microbes that are already colonizing the processing plant can contaminate the sterile tissue through cross-contamination (Bagge-Ravn

Abbreviations: AMR, Antimicrobial resistance; APC, Aerobic plate counts; ARB, Antibiotic resistant bacteria; ARGs, Antimicrobial resistance genes; ASV, Amplicon Sequence Variant; BWA, Burrows-Wheeler aligner; CAPs, Cationic-antimicrobial-peptides; ECC, Enterobacteriaceae counts; FB, Fraser broth; FCS, Food-contact surfaces; FEAST, Fast expectation-maximization microbial source tracking; LEfSe, Linear discriminant analysis of effect size; LM, *Listeria monocytogenes*; MLS, Macrolide-Lincosamide-Streptogramin; MOX, Modified oxford agar; NFCS, Nonfood-contact surfaces; TCC, Total coliform counts; TSA, Tryptic soy agar; TSB, Tryptic soy broth; UVM, Modified University of Vermont broth.

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<https://doi.org/10.1016/j.ijfoodmicro.2024.110591>

Received 24 October 2023; Received in revised form 8 January 2024; Accepted 18 January 2024

Available online 25 January 2024

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et al., 2003). While microorganisms continuously accumulate and change in the food processing environment through the entry of new raw materials and utensils and the flow of workers, some can persist in processing plants on surfaces that are difficult to clean or disinfect, creating protected microenvironments to support microbial growth (Carpentier and Cerf, 2011). Therefore, the control of microbial contamination during processing has become one key factor for ensuring the microbial safety and shelf life of fish products.

Among all the common foodborne pathogens, *Listeria monocytogenes* (LM), despite a low incidence in fish outbreaks, is the most common bacterial cause for recalls of fish and fish products (Sheng and Wang, 2021). Listeriosis caused by LM is a life-threatening disease for newborns, elderly, and immunocompromised people (Bintsis, 2017). The prevalence of LM is of specific concern for seafood because the growth rate of this pathogen is higher on catfish and shrimp than on beef and chicken due to the inherent pH difference of the fresh tissues (Augustin et al., 2005; B. Y. Chen et al., 2010b; Chou et al., 2006; Shineman and Harrison, 1994). LM has previously been isolated from several locations within catfish processing facilities as well as from chilled and frozen catfish products (B. Y. Chen et al., 2010b; Chou et al., 2006; Chou and Wang, 2006; Erdenlig et al., 2000; Ramos and Lyon, 2000). Genotypic characterization of LM isolates from food contact surfaces, chiller water, and (chilled) fresh fillets within catfish processing facilities revealed nearly identical fingerprint profiles, indicating that the processing environment rather than whole raw catfish is the primary source of *Listeria* spp. contamination in the catfish fillets (B. Y. Chen et al., 2010a; B. Y. Chen et al., 2010b). It has long been recognized that interspecies interactions between LM and other microbes comprising the microbiome of the food processing environment affect LM's ability to survive and colonize facilities (Giaouris et al., 2013; Zwirzitz et al., 2021). For example, the presence of *Listeria* spp. on meat samples was found to be positively associated with known biofilm formers *Pseudomonas*, *Acinetobacter*, and *Janthinobacterium* species (Zwirzitz et al., 2021). On the contrary, some lactic acid bacterium isolates were shown to inhibit the growth of LM in floor drains of the poultry processing plant (Zhao et al., 2006). Therefore, another function of microbial profiling of processing plants is to facilitate the identification of factors that correlated with LM persistence in processing environments.

The injudicious use of antibiotics in aquaculture has led to the selection of antibiotic resistant bacteria (ARB) in the normal intestinal and other microbiomes of fish (Cabello et al., 2016; Chuah et al., 2016; Watts et al., 2017). Studies have shown that post-harvest interventions in the processing plant may lower the risk of AMR in the final meat product at the retail level (Geser et al., 2012; Noyes et al., 2016; Schmidt et al., 2015). For example, in the study conducted by Noyes et al. (2016), while antimicrobial resistance genes (ARGs) were identified during beef production, the final beef products were ARG-free owing to the effective sanitary protocols and processing treatments used. However, there is very limited information about how fish processing impacts the ARGs' abundance in processed fish products compared to raw fish. The transfer of ARGs or ARB from fish or the environment to humans may pose significant health hazards.

Over the last decade, next generation sequencing has become an increasingly popular strategy for environmental microbiome mapping, with the aim of improving quality and safety in the food industry (Hultman et al., 2015; Mørætø et al., 2016; Parker et al., 2018; Stellato et al., 2015). While this method has been employed in dairy and raw meat processing environments, very few studies have explored its potential in fish processing plants. Furthermore, previous research has indicated that the bacterial loads and compositions of the skin and intestinal microbiota of farm-raised catfish have seasonal fluctuations (Fernandes et al., 1997). With this in mind, the objectives of this research were threefold: 1. to apply an amplicon sequencing approach to characterize factors (including season, facility, and collection sections) that may impact the microbial compositions on intact fish skin, fish intestines, fish fillets, and environment samples from three catfish

processing facilities; 2. to investigate the associations and co-occurrences between LM and other members of the in-plant microbiome; and 3. to use metagenomic sequencing to assess systematic changes in resistome compositions of the processed fish fillets compared to raw fish.

2. Materials and methods

2.1. Study design and sample collection

A total of 288 samples were collected from three catfish processing facilities located in the Southern United States during two seasons (Spring and Fall) in 2021. Four visits (two visits per season) were conducted for each facility. A survey acquiring basic information about the cleaning and sanitizing frequency of each facility was conducted at each visit. At each facility, food-contact surfaces (FCS), including the blade of the de-heading/evisceration machine, the surface of the conveyor belt, and the gloves of line workers, were sampled by swabbing areas of 10 cm × 10 cm with FLOQSwabs® (COPAN Diagnostics Inc., CA, USA) (Gong and Jiang, 2017). Nonfood-contact surfaces (NFCS), including the exterior of fillet transferring bins (for transferring fillets from the processing area to the packaging area) and floors of the sorting, processing, and packaging areas, were sampled using the same method. Samples collected from FCS and NFCS were denoted as environmental samples in subsequent analyses. Whole unprocessed fish and fish fillet (fresh and frozen) were taken directly from the processing lines. All samples (FCS, NFCS, whole unprocessed fish, fresh and frozen fish fillets) were kept on ice right after collection and brought to the microbiology laboratory located at the Alabama Fish Farming Center (AFFC) immediately. The intestine and skin of the whole unprocessed fish were sampled at the AFFC lab by squeezing out the luminal contents (intestinal samples) or swabbing areas of 5 cm × 5 cm (skin samples) with FLOQSwabs®. Fish intestine and skin samples were denoted as fish samples in subsequent analyses. Similarly, fish fillets were also sampled by swabbing a 5 cm × 5 cm area on each fillet; these samples were denoted as fillet samples in the following analysis. Every type of sample was sampled in duplicate, with one sample used for culture-based analysis and the other used for culture-independent analysis. Swabs collected for microbial plating were placed in culture tubes filled with 1 ml of 1 x phosphate buffered saline (PBS; pH = 7.00) and stored at 4 °C for a short period of time (<2 h) while preparing for shipping. Swabs for DNA extraction were placed directly in the Qiagen PowerBead Tubes (QIAGEN, Valencia, CA) and stored at -20 °C. Upon completion of the preparation of shipping, all samples were shipped on ice to the microbiology laboratory located at University of California Davis overnight. These samples were processed immediately after being received by the UCD laboratory.

2.2. Culture-based microbiological analyses

Collected fish, environmental, and fillet samples were analyzed for total aerobic plate counts (APCs) and Enterobacteriaceae counts (ECCs) by plating serial dilutions onto plate count agar (PCA; Difco, Sparks, MD, USA) and CHROMagar™ ECC (CHROMagar, Paris, France), respectively. The detection of *Listeria* spp. was conducted following the Food and Drug Administration bacteriological analytical manual with modifications (FDA, 2015). Briefly, primary enrichment was performed by using Modified University of Vermont broth (UVM; Difco) followed by a secondary enrichment with Fraser broth (FB; Difco). After enrichment, FB was streaked onto Modified Oxford (MOX; Difco) agar to examine the potential presence of *Listeria* spp. Presumptive positive colonies grown on MOX were then further streaked on to CHROMagar™ *Listeria* (CHROMagar) for confirmation. From CHROMagar™, one single colony confirmed as potential *Listeria* was streaked on to tryptic soy agar (TSA) for isolation of pure cultures. The isolated suspect *Listeria* spp. was further confirmed by streaking back onto CHROMagar™ *Listeria* before being preserved in tryptic soy broth (TSB) supplemented with 20 %

glycerol at -80°C . Presumptive *Listeria* spp. isolates were then checked with a SYBR® Green real-time PCR assay following the protocol described by Barbau-Piednoir et al. (2013) (Barbau-Piednoir et al., 2013). All real-time PCR reactions were performed on an Applied Bio-systems QuaratStudio3 Real-Time PCR System (Applied Biosystems, MA, USA).

2.3. Culture-independent 16S rRNA gene sequencing and data processing

DNA was extracted by using the Qiagen DNeasy PowerSoil Pro Kit (QIAGEN). The purity and concentration of DNA were evaluated with a plate reader spectrophotometer (NanoDrop Technologies, DE, USA) at 260 and 280 nm. Further quantification of DNA was performed using the Qubit fluorometer (Thermo Fisher, MA). A total of 162 samples with high DNA qualities were then subjected to 16S rRNA gene sequencing. The 16S amplicon library prep was performed using the QIAseq 16S/ITS Screening Panel kit targeting the V3-V4 region (QIAGEN) following the manufacturer's instructions. The sizes and concentrations of the library were checked by a bioanalyzer and sequenced with the Illumina MiSeq platform at the UC Davis Genome Center to obtain 300-bp paired-end reads.

Raw FASTQ files were denoised by using DADA2 in the QIIME2 pipeline (Callahan et al., 2016) with default parameters. Taxonomy was assigned by using the QIIME2 q2-feature-classifier plugin and a Naïve Bayes classifier that was trained on the SILVA database (Bolyen et al., 2019; Quast et al., 2013). Sequences classified as Archaea, Eukaryota, chloroplasts, or mitochondria were culled. Additional filters were applied in R to remove amplicon sequence variants (ASVs) with fewer than ten copies across all samples, as well as ASVs present in less than five samples.

Statistical analysis was performed in R (version 4.2.2) (R Core Team, 2022). The alpha-diversity was calculated by using both observed species and the Shannon index as implemented in the phyloseq package (McMurdie and Holmes, 2013). Here, the observed richness counted the number of different taxa present in a sample at a given taxonomic level, while the Shannon index was a measure that incorporates both the variety and the evenness of given species in a sample. Beta-diversity was estimated by using the Bray-Curtis distance and visualized in Principal Coordinates Analysis (PCoA). Variations in microbial composition and microbial structure between sample type, sampling site, facility, the presence of *Listeria* spp. and season were assessed by using the Kruskal-Wallis test (Kruskal and Wallis, 1952) and the permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2017), as implemented in the adonis function of the vegan R package (version 2.6.4) (Dixon, 2003). Pairwise comparisons were conducted by using the Wilcoxon test (stats package; version 4.2.2; Team RC 2013) and pairwise adonis (pairwiseAdonis package) (Arbizu, 2020) with the Benjamini-Hochberg correction. The linear discriminant analysis (LDA) effect

$$\text{Abundance} = \sum_1^n \left(\frac{N_{\text{ARG-like sequence}} \times L_{\text{reads}} / L_{\text{ARG reference sequence}}}{N_{16S \text{sequence}} \times L_{\text{reads}} / L_{16S \text{sequence}}} \right) \quad (1)$$

size (LEfSe) (Segata et al., 2011) was applied to search for biomarkers between samples with or without presumptive *Listeria* spp. In addition, Fast expectation-maximization microbial source tracking (FEAST; version 1.6.0) (Shenav et al., 2019) was used to quantify the contribution of different sources (i.e., fish and environmental samples) for the fillet samples using the ASV tables as the input.

2.4. Shotgun metagenomic sequencing and taxonomy analysis

To understand how the ARG abundance changes from the point of harvest (fish samples) to processed products (fillet samples), samples collected from fish (intestine and skin, $n = 16$) and fillet ($n = 29$) with high DNA quality were submitted for shotgun metagenomic sequencing. The library was prepared and sequenced by the UC Davis Genome Center. Briefly, barcode-indexed sequencing libraries were generated from genomic DNA samples sheared on an E220 Focused Ultrasonicator (Covaris, Woburn, MA). Sheared DNAs were converted to sequencing libraries using the DNA KAPA Hyper Prep Kit (Kapa Biosystems-Roche, Basel, Switzerland). The libraries were amplified with 5 PCR cycles and analyzed with a Bioanalyzer 2100 instrument (Agilent, Santa Clara, CA), quantified by fluorometry on a Qubit instrument (LifeTechnologies, Carlsbad, CA), and combined into pools at equimolar ratios. The pools were quantified by qPCR with a Kapa Library Quant kit (Kapa Biosystems-Roche), and each pool was sequenced on the Illumina NovaSeq (Illumina, San Diego, CA) with paired-end 150 bp reads.

Raw sequencing reads were quality-trimmed using Trimmomatic (version 0.39) (Bolger et al., 2014) with default parameters. Next, host removal was performed by Bowtie 2 (version 2.4.2) (Langmead and Salzberg, 2012) by removing the reads aligning to the catfish host genome from all samples. Quality filtered and host removed reads were then classified using Kraken2 (version 2.0.8-beta) against the RefSeq database (O'Leary et al., 2016). The relative abundance of the bacteria at genus level was estimated using Bracken (version 2.0) (Lu et al., 2017).

2.5. Antibiotic resistance gene analysis based on the metagenomic sequencing data

Filtered reads without assembly were aligned to the MEGAREs (version 3.0) to identify antibiotic resistance genes following the AMR ++ pipeline (Bonin et al., 2022). Briefly, sequencing reads were mapped to the resistance gene database using Burrows-Wheeler aligner with maximal exact matches (BWA-MEM; version 2.0_x64-linux) (H. Li and Durbin, 2010). The resulting SAM file was processed with ResistomeAnalyzer with an 80 % gene fraction threshold for ARG characterization. The filtered data are summarized as the number of aligned reads for each gene accession in each sample, and the counts are aggregated hierarchically from gene ID to group, mechanism, class, and resistant type levels. The gene level data was used to calculate ARG abundance. To avoid the bias caused by varying ARG reference sequence lengths, the ARG abundance was normalized by the ARG sequence length and depth, and expressed as "copy of ARG per copy of 16S rRNA gene" (B. Li et al., 2015; J. Liu et al., 2019) using the following equation:

n denotes for each individual ARG. $N_{\text{ARG-like sequence}}$ is the number of reads mapped to the MEGAREs database. L_{reads} is the length of the sequence reads. $L_{\text{ARG reference sequence}}$ is the length of the corresponding ARG reference sequence in the database. $N_{16S \text{sequence}}$ represents the number of 16S rRNA genes identified per sample using METAXA2 (version 2.1.3) (Bengtsson-Palme et al., 2015). $L_{16S \text{sequence}}$ denotes the average length of a 16S rRNA gene (1432 bp) in the Greengenes database.

Bacterial origins of ARGs were predicated by assigning taxonomy to

Megahit assembled contigs harboring ARGs. Specifically, the ResistomAnalyzer-obtained ARG-aligned sequencing reads were mapped back to the Megahit-assembled contig using minimap2 (version 2.24). ARGs containing contigs were then retrieved by using seqtk (version 1.3r106) for taxonomy assignment. Sourmash (version v4.1.1), a k-mers-based taxonomy classification software (Brown and Irber, 2016), was performed against the GTDB R07-RS207 database (317,542 genomes) with threshold-bp = 1 and k = 31 to assign taxonomy to ARG-containing contigs at the family level.

3. Results

3.1. Impact of fish and fish-processing facility resident microbiota on the microbial composition of fillet

During the spring and fall seasons, various samples were collected from three catfish processing facilities at different points in the processing line. Fish samples were obtained from both the intestines and skin of the raw whole fish, and environmental samples were taken from both food contact as well as non-food contact surfaces. Additionally, fillet samples were collected from both fresh and frozen fillets, and the total aerobic plate counts (APC) of all collected samples were determined (Fig. 1). In the spring, the APC for fish samples ranged from 2.90 ± 0.29 to 5.06 ± 0.24 Log CFU/swab, while the APC for environmental samples ranged from 1.54 ± 1.78 to 3.76 ± 0.36 Log CFU/swab, and for fillet samples ranged from 2.60 ± 0.37 to 3.70 ± 0.84 Log CFU/swab. During the fall season, the ranges for APC were 2.69 ± 0.86 to 5.01 ± 2.18 Log CFU/swab for fish samples, 2.97 ± 0.50 to 5.74 ± 1.37 Log CFU/swab for environmental samples, and 3.43 ± 0.80 to 4.52 ± 0.80 Log CFU/swab for fillet samples. Coliforms were only detected in certain samples, with F3 exhibiting the highest total coliform counts (TCC) for both fish and environmental samples, quantified at 3.42 ± 1.54 and 4.35 ± 1.14 Log CFU/swab, respectively. Highest TCCs for fillet samples were found in F2, with a value of 4.50 ± 2.21 Log CFU/swab (Supplementary material: Table S1–3).

The 16S rRNA gene sequencing of 162 samples yielded an average of $16,432 \pm 12,630$ quality-filtered reads per sample, for a total of ~ 3 million quality-filtered reads. In general, the average values of observed richness and Shannon diversity index increased significantly ($P < 0.05$) from fish to fillet regardless of season, except for the Shannon diversity index in fall (Fig. 2; Supplementary material: Table S4). Nine genera

including *Acinetobacter*, *Aeromonas*, *Cetobacterium*, *Chryseobacterium*, *Clostridium*, *Flavobacterium*, *Pseudomonas*, *Psychrobacter*, and *Weeksellaceae*_unclassified were determined to be the most abundant taxa in all sample types. Among them, *Cetobacterium* and *Acinetobacter* were most dominant in the fish samples, with mean relative abundances of 13.67 % and 9.40 %, respectively. In contrast, *Acinetobacter* (10.52 %) was more prevalent than *Cetobacterium* (9.18 %) in the environmental samples. *Acinetobacter* and *Aeromonas* were most abundant in fillet samples, accounting for 18.04 % and 9.68 % of the microbiota, respectively (Fig. 3A; Appendix A). The relative abundance of *Acinetobacter*, *Chryseobacterium*, *Pseudomonas*, *Aeromonas* and *Weeksellaceae*_unclassified increased significantly in fillet compared to fish ($P < 0.05$; Fig. 3A). Ordination analysis revealed that 13.31 % and 7.67 % variances in bacterial composition were represented by the PC1 and PC2, respectively, with the microbial communities clustering distinctly between fish, environment, and fillet samples (PERMANOVA; $P = 9.99E-05$; Fig. 3B; Supplementary material: Table S5).

The beta diversity of bacterial communities in fish, the environment, and fillet samples across seasons and facilities was then analyzed separately, and the results were presented in the PCoA plots (Fig. 4). Differences in the beta diversity of fish samples were only significant between seasons (PERMANOVA; $P = 9.99E-05$; Fig. 4A; See Supplementary material: Table S6), but not between facilities (PERMANOVA; $P = 8.85E-02$; Fig. 4B; Supplementary material: Table S7). Environment samples clustered differently between seasons (PERMANOVA; $P = 1.70E-03$; Fig. 4C; Supplementary material: Table S6) and facilities (PERMANOVA; $P = 9.99E-05$; Fig. 4D; See Supplementary material: Table S7). In particular, the microbiota from F3 samples formed a distinct cluster, while the microbiota of samples collected from F1 and F2 appeared to be more closely related to each other than to the microbiota from F3 (Pairwise Adonis; Supplementary material: Table S8). Consistent with the environment samples, the microbial structures of the fillet samples were significantly different both between seasons (PERMANOVA; $P = 0.01$; Fig. 4E; Supplementary material: Table S6) and between facilities (PERMANOVA; $P = 1.00E-04$; Fig. 4F; Supplementary material: Table S7); however, the difference was less evident between seasons than facilities.

3.2. FEAST analyses for microbial source tracking

The FEAST program was run on ASV-level taxonomy tables to

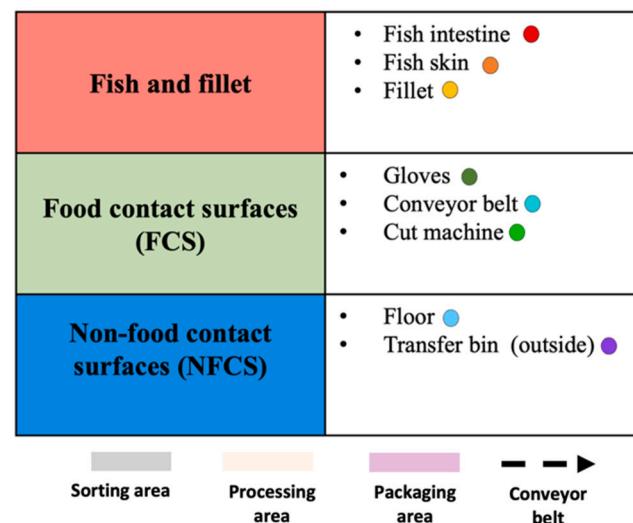
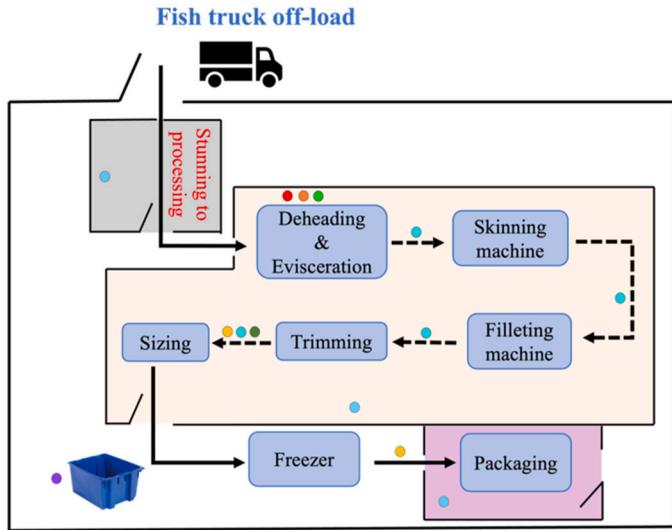


Fig. 1. Schematic map of catfish processing facilities with sampling sites labeled. Four visits to each facility were performed over the two seasons (spring and fall) in one year. Sample collected at each area are indicated in the map, together with their classification as fish ($n = 48$) and fillet ($n = 72$), food contact surfaces (FCS; $n = 72$) or non-food contact surfaces (NFCS; $n = 96$).

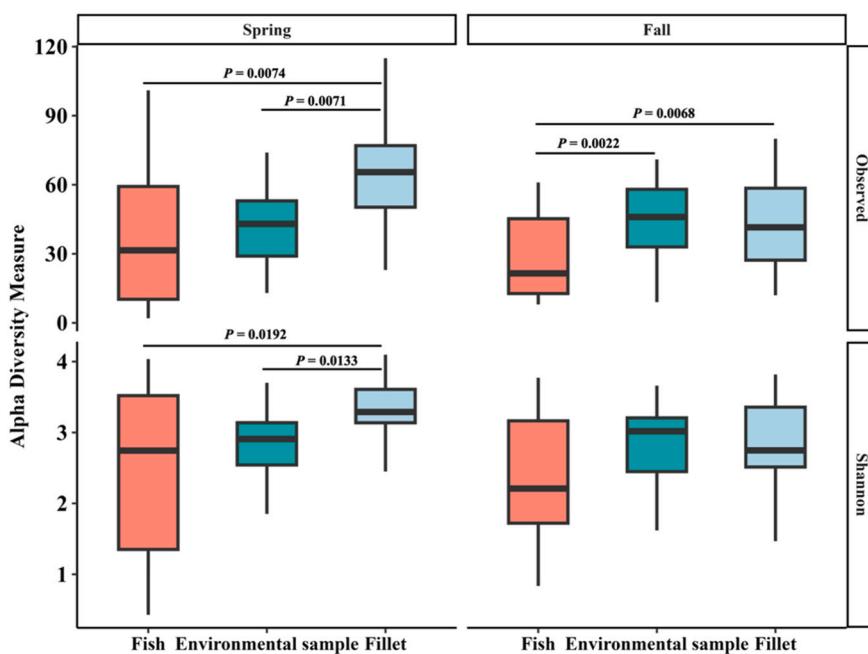


Fig. 2. Observed richness and Shannon indexes. A total of 162 samples were collected from fish ($n = 46$), environment ($n = 34$), and fillet ($n = 46$) in two seasons (spring and fall). P -values of <0.05 obtained from the Kruskal-Wallis and Dunn tests are indicated in the Figure.

estimate the contributions of the indigenous microbiota of fish and the processing environment to the fillet microbiota at each facility. Results showed that fish accounted for 42.7 %, 27.6 %, and 38.4 % of the fillet microbiota in F1, F2, and F3, respectively (Fig. 5). A total of 48.2 %, 62.4 %, and 53.7 % of the microbiota in fillet were potentially sourced from the processing environment in F1, F2, and F3, respectively, where FCS had larger contributions of 25 %, 40.5 %, and 30.2 % compared to NFCS (F1: 23.2 %; F2: 21.9 %; F3: 23.5 %) (Fig. 5; Appendix B).

3.3. *Listeria* spp. detection and biomarkers identification

Given the significant impact that processing-plant microbiota may have on the quality and safety of the processed fillet, the presence of *Listeria* spp. was surveyed, and the biomarkers associated with *Listeria*-positive samples were profiled. As shown in Fig. 6, F3 had the highest *Listeria* spp. positive rate (28/56; 50 %) as determined by PCR, while 25 % (14/56) and 20 % (11/56) of samples were positive in F1 and F2 (Supplementary material: Table S9). Most of the *Listeria*-positive samples were received in the fall, when higher total APC and coliform counts were observed (Supplementary material: Table S1–3). Linear discriminant analysis of effect size (LEfSe) analysis was then performed to identify microbial genera that varied substantially between *Listeria* spp. positive and negative samples, with an LDA score of >2 indicating the presence of significant biomarkers for that sample type. In F1 samples, *Sphingobacterium*, *Undibacterium*, and SD04E11 were identified to be biomarkers for *Listeria* spp., with relative abundances of 0.1794 %, 0.2086 %, and 0.0443 %, respectively. In F2 samples, *Plesiomonas*, *Turicibacter*, and *Oligoflexus*, with relative abundances of 3.9437 %, 0.2422 %, and 0.0724 % respectively, were identified to be *Listeria* spp. biomarkers. In F3 samples, biomarkers *Brochothrix*, *Acinetobacter*, and *Rhodococcus* were present at relative abundances of 4.2702 %, 17.4667 %, and 1.6778 %, respectively (Fig. 7). It is worth noting that *Listeria* spp. was only detected via enrichment and culture-dependent analysis in this study; the level of *Listeria* spp. was low and could not be detected with the amplicon sequencing approach. Therefore, the identification and detection of these biomarkers is likely more feasible than the detection of low-level *Listeria* spp.

3.4. The ARG profile of fish and fillet samples

To elucidate the resistome structure change of fish in the processing environment, shotgun metagenomic sequences were used to compare the ARGs found in fillet (processed) to the ARGs found in fish (unprocessed). A total of 141 gigabytes of Illumina sequencing data were received from 45 samples submitted (fish: 16 and fillet: 29), and around 40 million reads with an average length of 147 bp were left after quality trimming and host removal. ARGs were detected in 15 samples, including 6 fish and 9 fillet samples, as shown by the output of AMR ++ analysis. In total, 212 ARGs belonging to 72 groups were identified, representing 41 antibiotic resistance mechanisms (Appendix C). The detected ARGs were predicted to confer resistance to 17 classes of antibiotics in fish and 10 classes of antibiotics in fillet samples, with an abundance range of 1.85E-04 to 6.80 and 0.05 to 6.62 copies of ARG per 16S rRNA gene per ARG class, respectively (Appendix C). Although the total richness of antimicrobial resistance genes (ARGs) decreased significantly from fish to fillet ($P = 0.028$; Fig. 8A), the total abundance of ARGs did not exhibit a significant change ($P = 0.15$; Fig. 8B). Moreover, particular types of ARGs were found to be enriched in fillet samples as compared to fish samples (Fig. 8C). For example, the normalized copy number of ARGs imparting resistance to Macrolide-Lincosamide-Streptogramin (MLS), cationic antimicrobial peptides (CAPs), aminoglycosides, and beta-lactams increased from 0.075 to 0.368, from 0.053 to 0.146, from 0.072 to 0.274, and from 2.687E-04 to 0.050, respectively ($P < 0.05$; Fig. 8C; Appendix C).

The bacterial origin of the observed ARGs was predicted by assigning the taxonomy to metagenomic contigs containing ARGs. ARGs detected in fish belong to 708 bacterial families, with Mycobacteriaceae, Peptostreptococcaceae, Microtrichaceae, Fusobacteriaceae and Clostridiaceae being the dominant taxa harboring the ARGs (Fig. 9A). On the contrary, only 87 bacterial families were responsible for the detected ARGs in the fillet samples, with Xanthomonadaceae, Aeromonadaceae, Enterobacteriaceae, Moraxellaceae and Carnobacteriaceae found to be responsible for the majority of the ARGs (Fig. 9B).

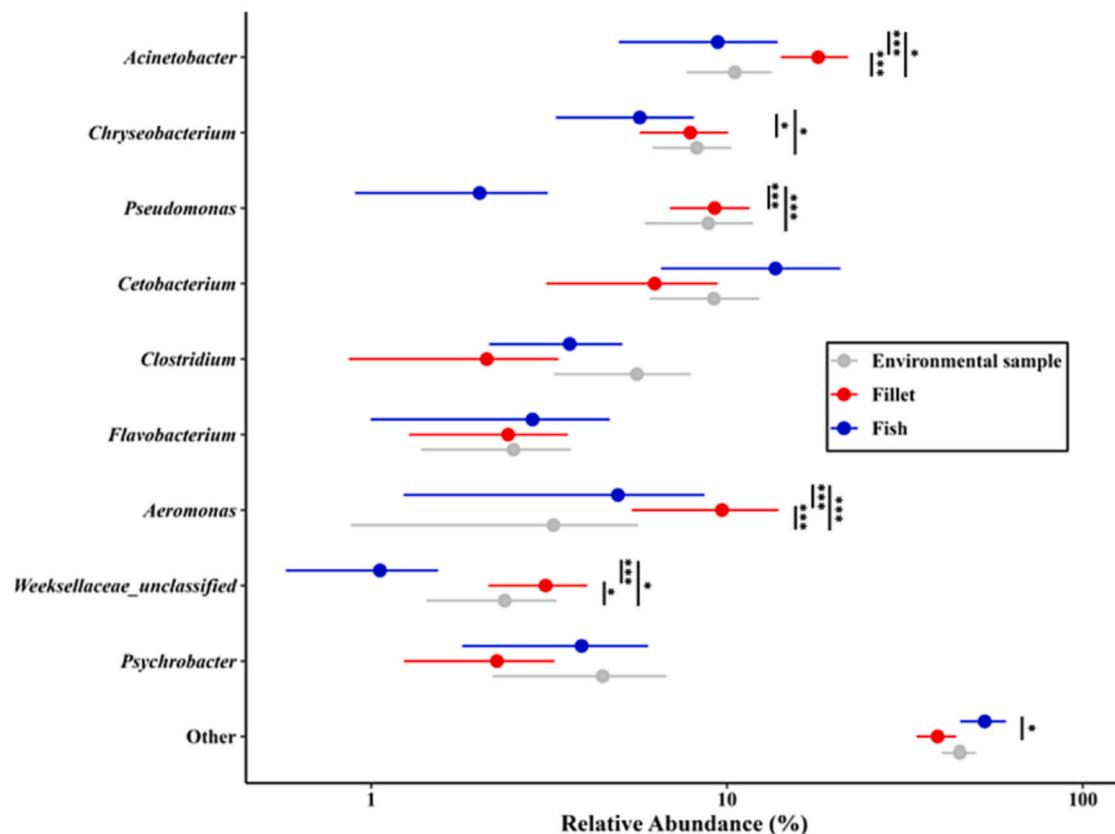
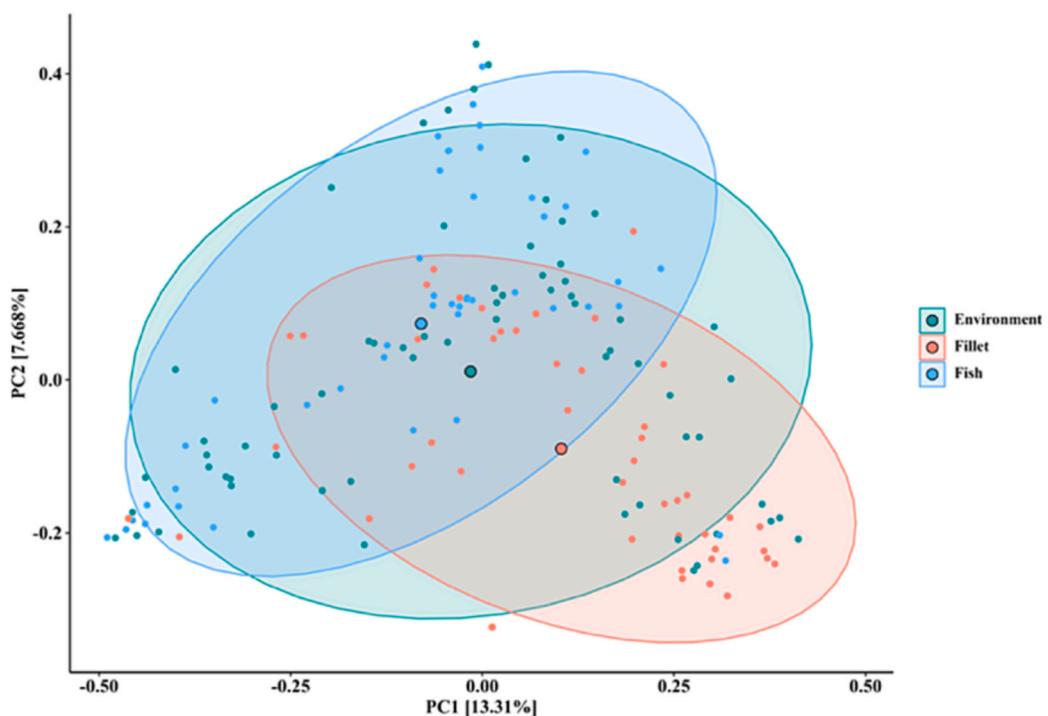
A.**B.**

Fig. 3. Microbial composition of fish, environmental and fillet samples. A. Relative abundance of taxa found in different sample types at the genus level. For each genus, the dot denotes mean relative abundance; the outer line extends from the 25th to the 75th percentile of each group's distribution of values. Genus with median relative abundance $\leq 0.5\%$ are grouped into "Other". Asterisks represent statistical significance.

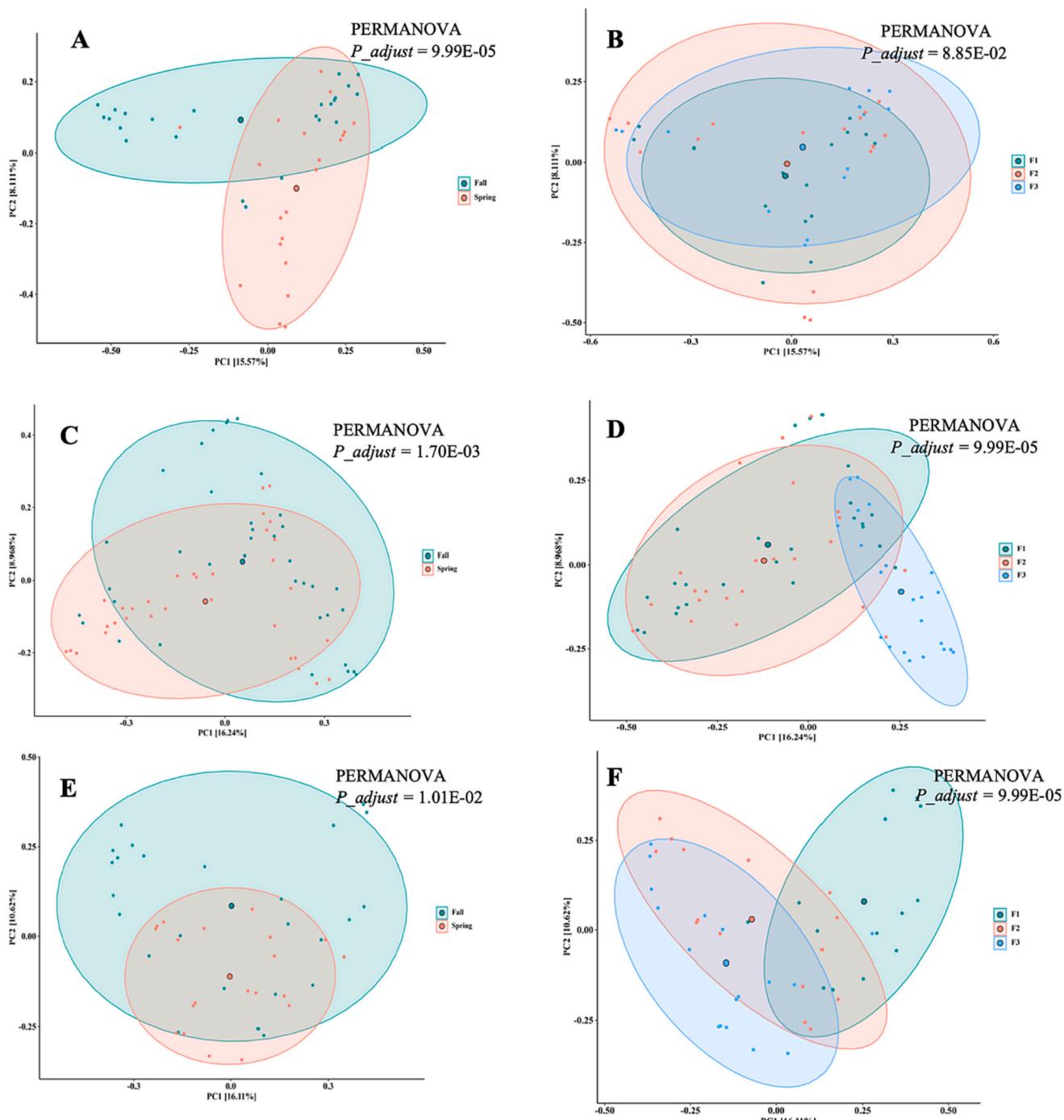


Fig. 4. Principal Coordinates Analysis (PCoA) of fish (A, B), environment (C, D), and fillet (E, F) samples using the Bray–Curtis distances. Samples were separated between seasons (left column) or between facilities (right column). The centroid of each ellipse represents the group mean, and the shaded polygons, indicating the 95 % confidence region of each cluster, are applied to differentiate sample types. Adonis test values are shown in each Figure.

4. Discussion

Recent progress in sequencing technologies has enabled high-resolution mapping of the environmental microbiome in food industries, offering unprecedented insights to support overall quality and safety management plans (De Filippis et al., 2021). In this study, we employed an integrated approach combining culture-based, amplicon-based, and metagenomic sequencing techniques to evaluate the microbial and resistome structure and dynamics of microbiomes in three catfish processing facilities situated in the southeastern U.S.

APC is useful for identifying spoilage and suboptimal handling

practices in fish processing, and the presence of coliform bacteria can indicate unsanitary working surfaces, fecal matter contamination, and potentially harmful pathogens (Tortorello, 2003; Varga and Anderson, 1968). Although no clear pattern was observed for APC or TCC across different sampling locations in this study, certain samples, such as those collected from the sorting area floors and fillets, consistently exhibited significantly higher APC during the fall (September), compared to the spring (March), regardless of facility. This finding is consistent with similar studies conducted by Fernandes et al. (1997) and McCoy et al. (2011), where higher counts of aerobic, psychrotropic, total coliform, *Escherichia coli*, and *Salmonella* were detected on farm-raised catfish

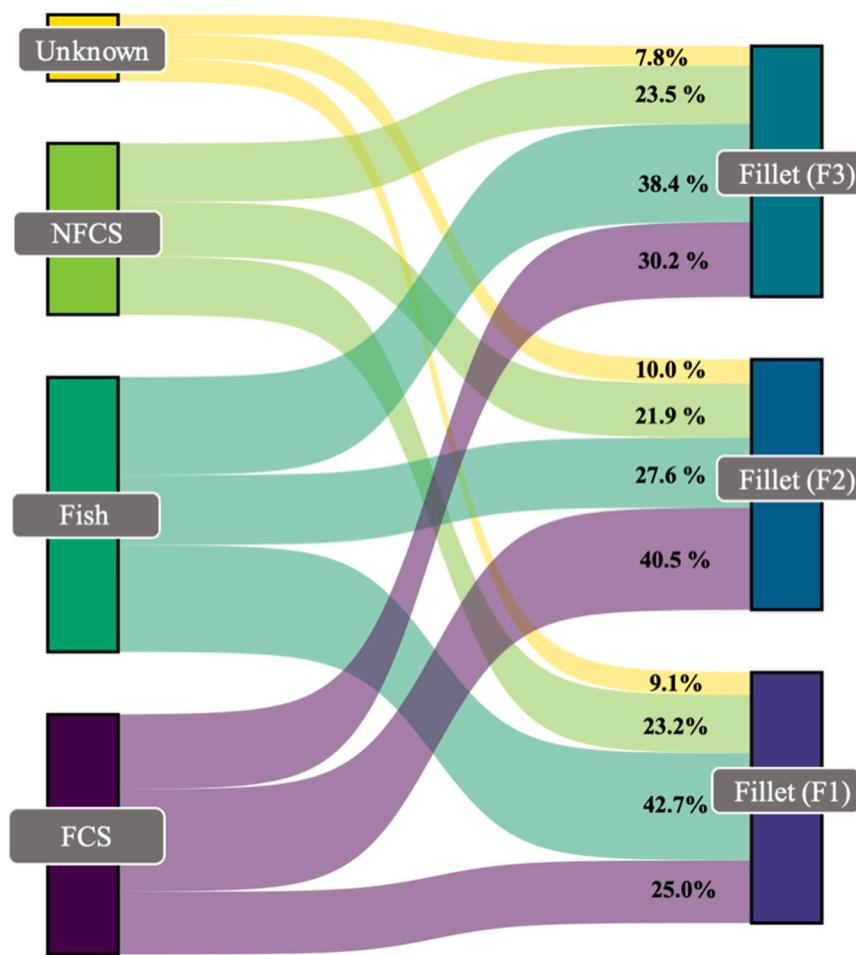


Fig. 5. Source environment proportions for fillet samples estimated by using the fast expectation-maximization (FEAST) estimation. Environmental (food contact surfaces (FCS) and non-FCS (NFCS)) and fish source samples are represented on the left and fillet samples, as sinks, are shown on the right. The average contribution of microorganisms from source samples to the microbial population of corresponding sink (fillet) samples is shown by the line width of individual flows between them. The contribution proportions from each source sum up to 100 % on the fillet samples.

fillets from July to September, as opposed to January to March (Fernandes et al., 1997; McCoy et al., 2011). The authors attributed the observed seasonal trend to the increased microbial loads in the fish intestinal tract during warmer weather, and the intestinal tract of catfish was considered an important source of contamination for fish fillets (Macmillan and Santucci, 1990; McCoy et al., 2011).

The genera *Acinetobacter*, *Chryseobacterium*, *Pseudomonas*, *Cetobacterium*, *Flavobacterium*, *Clostridium*, and *Aeromonas* were identified as the predominant bacterial taxa shared between fish, environmental surfaces, and fillet samples. Notably, *Acinetobacter*, *Aeromonas*, and *Pseudomonas* were the most frequently detected genera on the fillet. *Acinetobacter* spp. are commonly found in food processing environments with high humidity, such as fish processing plants, as they are vulnerable to desiccation (Kramer et al., 2006; Møretrø and Langsrød, 2017). Indeed, Langsrød et al. (2016) found that *Acinetobacter* spp. were more prevalent than *Pseudomonas* spp. in salmon processing plants (Langsrød et al., 2016). Recently, there has been a growing recognition of the potential association between *Acinetobacter* spp. and foodborne illnesses. This is especially true for *A. baumannii*, which is one of the most commonly observed species responsible for human infections and is frequently resistant to antimicrobial treatments (Amorim and Nascimento, 2017; Campos et al., 2019; Malta et al., 2020). In the past few decades, *Aeromonas* spp. have emerged as important foodborne pathogens as well as spoilage microorganisms (Hoel et al., 2019). Strains associated with foodborne illness can grow at refrigeration temperatures and produce heat-stable enterotoxins (Tomás, 2012). The ubiquity of

Aeromonas in aquatic environments raises the likelihood of contamination of aquatic animal food products during processing, preparation, and storage steps. For instance, a study conducted in the U.S. showed that *A. hydrophila* comprised 37.5 % of gram-negative bacterial isolates recovered from 39 swab samples collected from catfish processing equipment in two plants (Praveen et al., 2016). *Pseudomonas* is another frequently reported genus that acts as a significant spoilage agent of fish and fish products, especially in cold storage conditions (Bagge-Ravn et al., 2003; Fagerlund et al., 2017; Sterniša et al., 2020). *Pseudomonas*, *Aeromonas* and *Acinetobacter* spp. can coexist in the same niche and can form biofilms, which may contribute to their resistance to sanitizers and persistence in food processing environments, hence increasing their chance of transfer to food products (Gedefie et al., 2021; N. T. Liu et al., 2013; Talagrand-Reboul et al., 2017).

In this study, the alpha-diversity of fillet samples was significantly greater than that of the fish samples, indicating an increased species richness in the processed fillet compared to the raw fish. Notably, certain genera such as *Weeksellaceae*_unclassified and *Pseudomonas* exhibited low abundance in fish samples but were found to be much more prevalent in both environmental and fillet samples. Furthermore, distinct clustering of fillet microbiota was observed among different processing plants, despite the insignificant differences in fish microbiota across the facilities. These findings suggest that the resident microbes in processing plants may play an important role in shaping the microbiota of the processed fillet. Previous research has demonstrated that environmental microbes may serve as the main source of contamination for final food

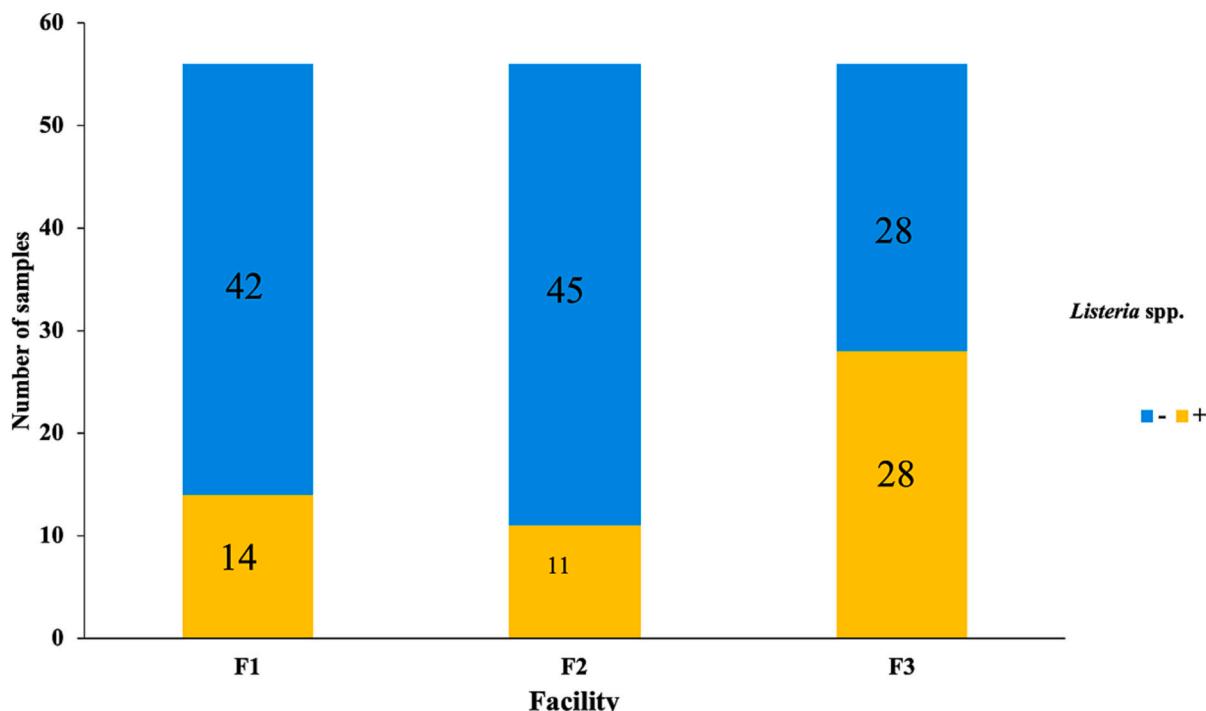


Fig. 6. Summary of the presence (+) and absence (−) of *Listeria* spp. in environmental samples collected from three fish processing facilities F1, F2, and F3 throughout spring and fall seasons.

products. For example, Syropoulou et al. (2021) found *Enterobacter ludwigii*, *Staphylococcus warneri*, *Serratia grimesii* and *Rouxiella* spp. were only observed on gutted sea bass or sea bass fillets but not on the whole fish from the same batch (Syropoulou et al., 2021). Møretrø et al. (2016) discovered that some *Pseudomonas* and *Shewanella* isolates from equipment/machine surfaces and salmon fillets shared similar genomic signatures (Møretrø et al., 2016). Similarly, other authors found that the processing plant is the primary site of *Listeria* contamination of fish products, rather than live fish from farm ponds (Autio et al., 1999; B. Y. Chen et al., 2010b; Hansen et al., 2006). However, most of these studies only focused on specific spoilage or pathogenic microorganisms. Our study provides more insight regarding the impact of the processing environment on fillet products by considering the entire resident microbiota as a cohesive unit.

The FEAST program was then used to further confirm the bacterial origins of the fillet microbiota. Results suggested nearly or more than half of the fillet microbiota was sourced back to the processing environment, with FCS having a higher contribution than NFCS in all three facilities. FEAST is an efficient statistical model that effectively utilizes an expectation-maximization-based algorithm to assess the contribution of each source environment to the sink community (Shenhan et al., 2019). The FEAST algorithm has been widely used in a wide range of studies as a source tracker for both microbial communities and antibiotic resistant genes (H. Chen et al., 2020; J. Chen et al., 2023; Jiang et al., 2022; Peng et al., 2021). Here we present the feasibility of using FEAST as a microbial tracking tool in food processing settings to quantify the contribution of different source environments to a target microbial community. This information can be used to identify sites where more attention should be paid to improve hygiene design and sanitation plans.

Considering the significant impact of processing environment on the final food product, the implementation of pathogen testing in food processing environments is instrumental in identifying and eliminating environmental pathogen sources. This measure is crucial in mitigating the risk of food contamination, recalls, and foodborne illnesses. Globally, *L. monocytogenes* caused detention of fish products in 4 % of recorded cases (Ababouch et al., 2005) and has resulted in many

products recalls due to contamination (Wan Norhana et al., 2010; Zhu et al., 2005). Data from the European Food Safety Authority in 2016 showed that fish and fishery products were the primary source of *L. monocytogenes* infection (European Food Safety, 2017). In the present study, the prevalence of *Listeria* spp. was tested, which has been used by many testing programs to indicate potential *L. monocytogenes* contamination. Greater proportions of *Listeria* spp. positive samples were observed in environment samples than in fish and fish samples, particularly in F3, where 50 % of environment samples were *Listeria* spp. positive. According to the survey conducted, the cleaning schedules were identical across all three facilities, occurring at the beginning and end of the production shift. Despite this uniformity, there were minor differences in temperature and relative humidity across different operational areas of the facilities (Table S10). However, these differences in temperature and humidity did not significantly contribute to clarifying the results. Moreover, the facilities did not disclose their specific cleaning and sanitation protocols, or the disinfectants used.

The abundances of *Leuconostoca*, *Sphingomonas Romboutsia* and *Chryseobacterium* were negatively correlated with the presence of *Listeria* spp. in the environment samples. Certain *Leuconostoca* spp. such as *mesenteroides* and *gelidum* can produce bacteriocins with an anti-*Listeria* effect (Hastings et al., 1991; Héchard et al., 1992; Morisset et al., 2004). Similarly, Mageswari et al. (2015) discovered that *Sphingomonas* inhibited the growth of *L. monocytogenes* by production of astaxanthin (Mageswari et al., 2015). While no study has examined the direct interactions between *Romboutsia* with *Listeria* spp., Magruder et al. (2020) found an inverse relationship between the levels of *Romboutsia* and *Enterobacteriaceae*, a causative agent of urinary tract infections (Magruder et al., 2020). This study suggested that *Romboutsia* could inhibit *Enterobacteriaceae* growth through the production of short-chain fatty acids, which have also been shown to inhibit colonization and virulence factor production in *L. monocytogenes* at the transcriptional level (Magruder et al., 2020; Sun and O'Riordan, 2013; Sun et al., 2012). In our study, the negative correlation between *Chryseobacterium* and *Listeria* spp. was observed in F2. In support of our finding, Zwirzitz et al. (2021) also reported *Chryseobacterium* together with a few other

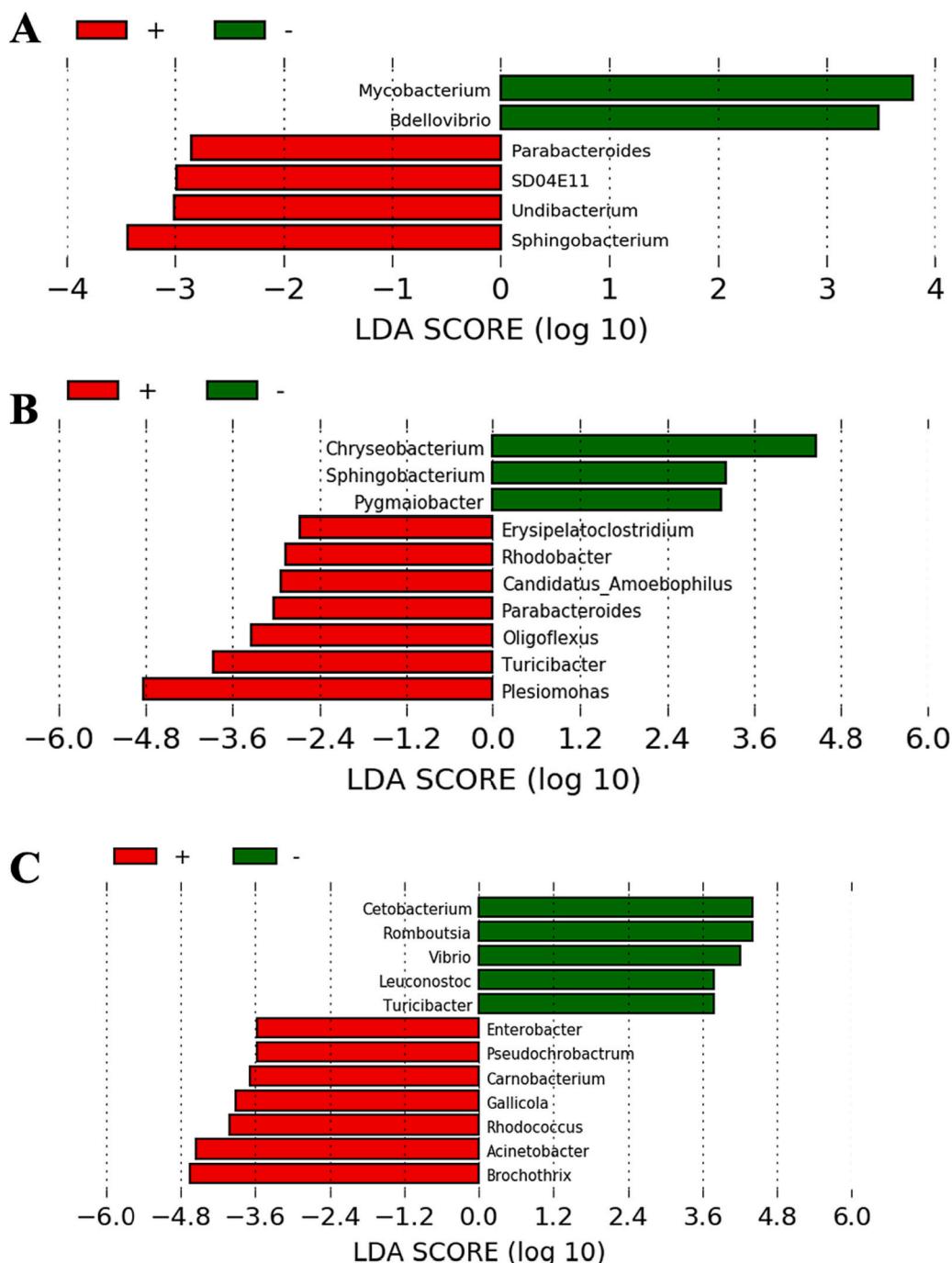


Fig. 7. Taxonomic differences of processing plant microbiota in *Listeria* spp. + and – samples. Linear discriminant analysis (LDA) effect size (LEfSe) analysis revealed significant bacterial differences in *Listeria* spp. + and – environmental samples at three facilities. (A) F1, (B) F2, and (C) F3. LDA scores (log10) > 2 and $P < 0.05$ are shown.

members of the meat processing plant core microbiome correlated negatively with *Listeria* spp. on meat samples; however, the exact mechanism was still unclear (Zwirzitz et al., 2021).

On the other hand, positive correlations were found between *Listeria* spp. and other genera such as *Acinetobacter*, *Brochothrix* and *Carnobacterium*. The co-occurrence of spoilage microorganisms including *Acinetobacter*, *Brochothrix*, and *Listeria* spp. in the processing environment has been established by many previous studies, most likely inside the biofilm (Fagerlund et al., 2017; Zwirzitz et al., 2021). Contrary to our findings, dos Reis et al. (2011) and Duffes et al. (1999) found that certain bacteriocin-producing *Carnobacterium* strains could inhibit *L. monocytogenes* effectively in fish model systems (dos Reis et al., 2011;

Duffes et al., 1999). This contrasting trend may be due to the varying behaviors of different *Carnobacterium* subpopulations, and the interactions could be species- or even strain-specific (Zwirzitz et al., 2021).

Along with the contamination of fish processing plants with human pathogens, other contamination agents such as ARGs and ARBs also pose food safety concerns. The prolonged use of antibiotics in aquaculture may result in harvested fish serving as a reservoir of ARGs and ARBs. However, scarce data is currently available on the effectiveness of fish processing in reducing the risk of AMR on fish products before entering the market. In this study, we compared the resistome structures of fillet and fish and found that fish processing did not significantly decrease the

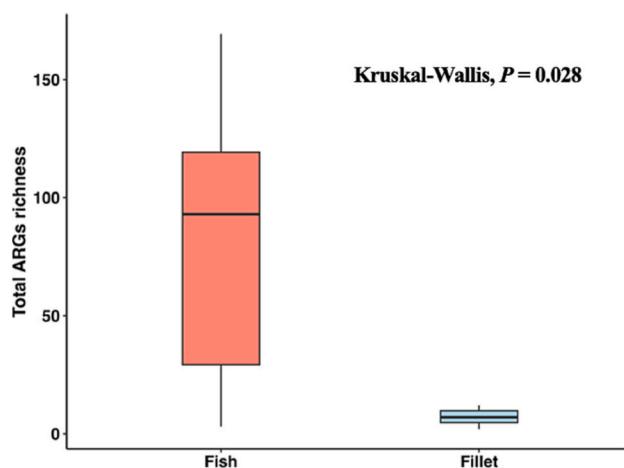
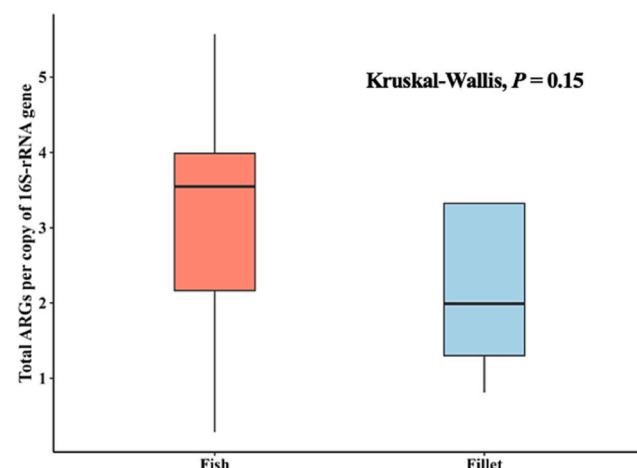
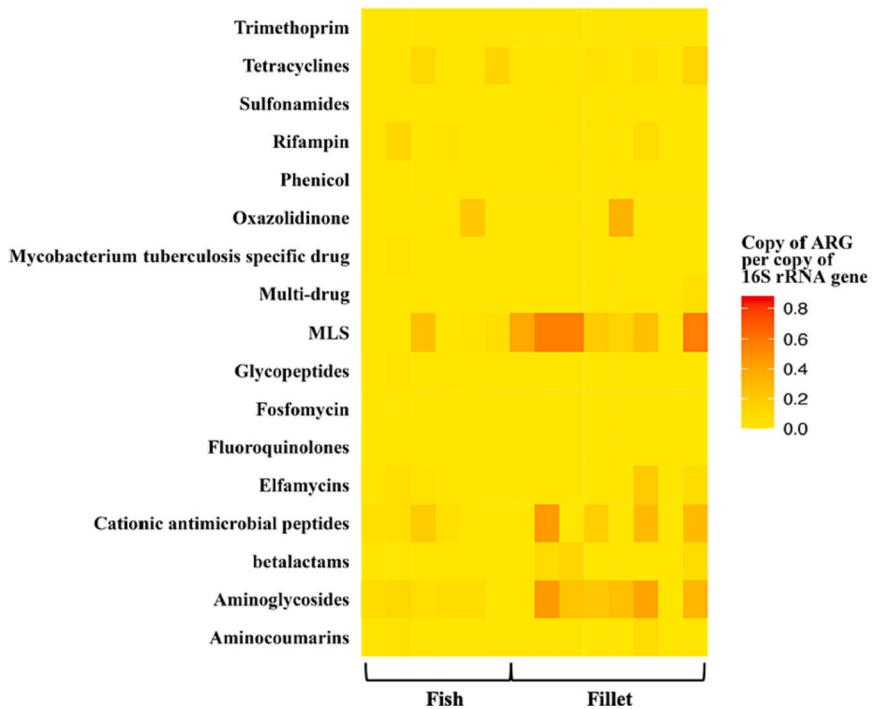
A.**B.****C.**

Fig. 8. Comparison of resistome changes in fish and fillet Samples. A. Boxplot representing the distribution of the count of unique antimicrobial resistance genes (ARGs) detected in fish and fillet samples. B. Boxplot of the distribution of total ARG abundance observed in fish and fillet samples. C. Heatmap showing the antibiotic resistance profile of fish and fillet samples. Each column represents a sample. The counts data were normalized by 16S rRNA gene and the ARG abundance was expressed as "copy of ARG per copy of 16S rRNA gene". MLS: Macrolide-Lincosamide-Streptogramin.

total ARG abundance. In contrast to our findings, previous research conducted with the beef product system suggested that interventions implemented in the post-harvest processing stage can effectively lower the risk of AMR in the final products sold in retail markets. For example, Noyes et al. (2016) observed that despite the presence of ARGs in cattle production, the final beef products were ARG-free (Noyes et al., 2016). Comparable results were obtained by Geser et al. (2012) and Schmidt et al. (2015), where ARGs were detected in the pre-evisceration stage of

cattle production but had low prevalence or were even absent in the final beef products, including minced beef and sirloin (Geser et al., 2012; Schmidt et al., 2015). Current sanitary dressing procedures and processing interventions such as steam vacuuming, carcass washing, organic acid rinses and thermal pasteurization were therefore thought to be effective in reducing the risk of ARG transmission to consumers (Bacon et al., 2000; Noyes et al., 2016). In contrast to beef processing, fish processing is comparatively simple, involving a sequence of

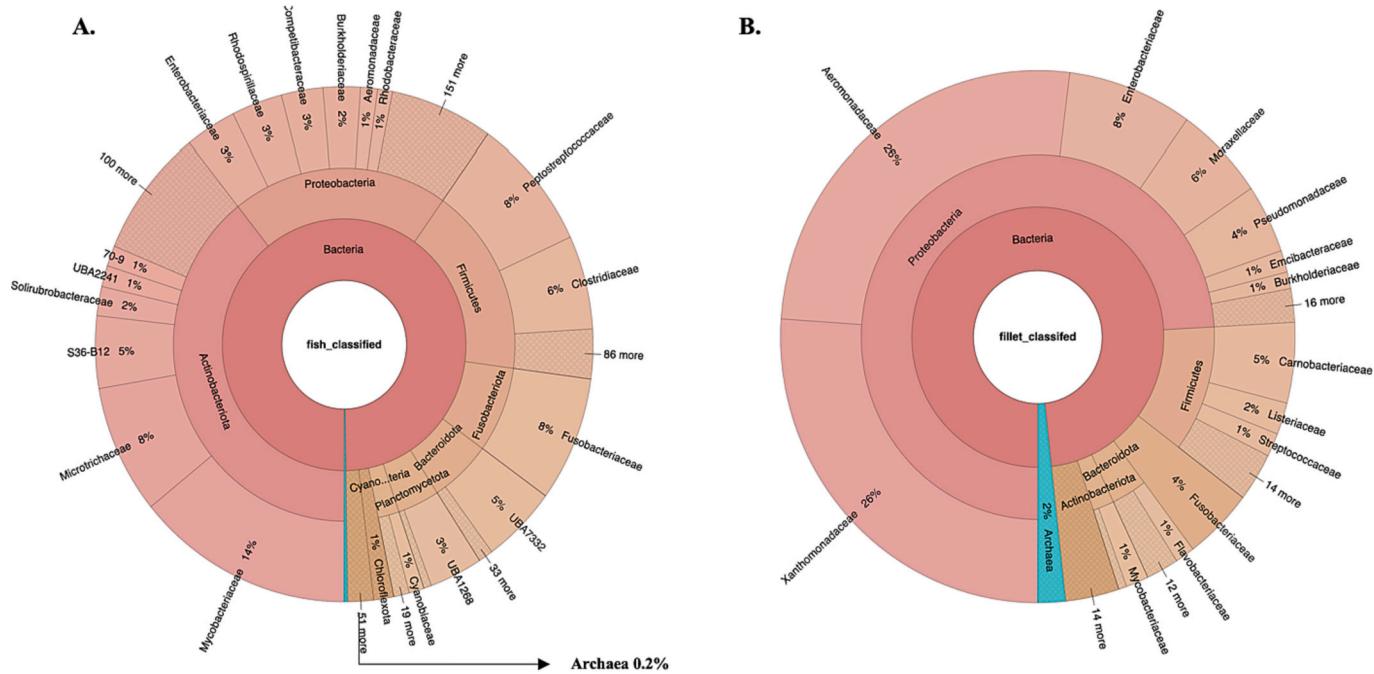


Fig. 9. Taxonomy classification of antimicrobial resistance genes (ARG)-aligned contigs in fish (A) and fillet (B). The three circles represent taxonomic ranks from the family outwards to phylum and kingdom levels. Percentages correspond to the relative abundance of each classified taxonomic family.

activities such as washing, de-heading, filleting, skinning, chilling, trimming, packaging, metal detection, and freezing/shipping. Within these procedures, the sole decontamination measure involved is the chilling stage, during which some processors introduce up to 20 ppm of chlorine to the chiller water or rinse water (Silva et al., 2001). Notably, in the case of chlorine, free chlorine is the primary compound responsible for its antimicrobial effects. However, the presence of organic matter can readily diminish the effectiveness of free chlorine, resulting in a significant decline in its bactericidal activity (Paul et al., 2017). Both Abdallah-Ruiz et al. (2022) and Chen et al. (2010b) reported processing chiller water was a significant source of cross-contamination during catfish processing, as the water is frequently recycled and used throughout the day (Abdallah-Ruiz et al., 2022; B. Y. Chen et al., 2010b). The absence of dedicated antibacterial procedures in fish processing may explain the lack of a significant reduction in total ARG abundance observed in the current study.

Interestingly, although the overall abundance of ARGs did not change, there was a discernible alteration in the resistome structure of fillet in comparison to fish. The richness of ARGs, i.e., the number of unique ARG types, showed a significant reduction. Furthermore, it was observed that certain types of ARGs, specifically those that confer resistance to MLS, CAPs, beta-lactams, and aminoglycosides, appeared to be more abundant in fillet samples compared to fish samples. The majority of ARGs detected in fillet samples were associated with Xanthomonadaceae, Aeromonadaceae, and Enterobacteriaceae, which have previously been linked to resistance against multiple classes of antibiotics, including CAPs, MLS, beta-lactams, and aminoglycosides (Andersson et al., 2016; Roberts, 2011; Zdanowicz et al., 2020; Zhou et al., 2019). Except for Enterobacteriaceae, which exhibited a similar relative abundance in fish and fillet samples, the relative abundances of the other two bacterial families were greater in fillet samples than in fish samples. Therefore, the differences in resistome structures observed between fish and fillet samples can be explained by the varying microbial communities present on these sample types as a result of processing. This hypothesis is supported by previous research conducted in other food processing settings (Forsberg et al., 2014; Noyes et al., 2016).

5. Conclusions

Considering the significant economic importance of fish and fish products, it is imperative to direct greater public attention and research efforts towards ensuring their safety and quality. To the best of our knowledge, this is the first study dedicated to characterizing the microbiome of the built environment of catfish processing plants in the U.S. The results obtained highlight the substantial impact of the resident microbiota in the processing environment on the microbial communities present in fish fillets, thereby influencing the safety and quality of the final fillet products. The further identification of discriminatory genera from *Listeria*-positive and *Listeria*-negative environmental samples could potentially contribute to the development of effective pathogen detection protocols in processing facilities.

On top of that, distinct ARG profiles were observed in processed fish products compared to raw fish. Specifically, a higher abundance of ARGs associated with resistance to MLS, CAPs, beta-lactams, and aminoglycosides was identified in processed fillet samples compared to raw fish samples. This suggests that existing fish processing practices may not be sufficient for reducing AMR in fish products before they enter the market. Therefore, novel mitigation strategies should be incorporated into fish handling practices to better address the spread of AMR in the seafood supply chain.

(*P < 0.05, **P < 0.01, *** P < 0.001) obtained from pairwise Wilcoxon tests. P values were adjusted using Benjamini-Hochberg method. B. Principal Coordinates Analysis (PCoA) of 162 samples, including fish (n = 46), environment (n = 70), and fillet (n = 46), collected from three processing plant based on the Bray–Curtis distances. The 70 environmental samples included 36 food contact surface (FCS) samples and 34 non-FCS (NFCs) samples. The centroid of each ellipse represents the group mean, and the shaded polygons, indicating the 95 % confidence region of each cluster, are applied to differentiate sample types. Adonis test values are indicated in the Figure.

Funding

This research is funded by the U.S. Department of Agriculture National Institute of Food and Agriculture, under grant number 2019-06093.

Ethics declarations

Ethics approval and consent to participate: Not applicable.

Consent for publication

Not applicable.

CRediT authorship contribution statement

Xiran Li: Data curation, Formal analysis, Writing – original draft.
Hongye Wang: Data curation, Formal analysis.
Hisham Abdelrahman: Data curation.
Anita Kelly: Data curation, Writing – review & editing.
Luke Roy: Writing – review & editing.
Luxin Wang: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no competing interests.

Data availability

The data support the findings of this study are available under NCBI Bioproject ID PRJNA97559.

Acknowledgements

The authors wish to acknowledge the personnel of the processing plants for their availability and support throughout the study. The team appreciates the financial support from the U.S. Department of Agriculture National Institute of Food and Agriculture (Grant number: 2020-68015-30855). The sequencing was carried out at the DNA Technologies and Expression Analysis Cores at the UC Davis Genome Center, supported by NIH Shared Instrumentation Grant 1S10OD010786-01. We are grateful for their work and support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijfoodmicro.2024.110591>.

References

- Ababouch, L., Gandini, G., Ryder, J., 2005. Causes of Detentions and Rejections in International Fish Trade. Food & Agriculture Org.
- Abdullah-Ruiz, A., Wood, L.S., Kim, T., Schilling, W., White, S.B., Chen, B.Y., Durango-Villadiego, A., Silva, J.L., 2022. Microbial indicators and possible focal points of contamination during production and processing of catfish. Foods 11.
- Amorim, A.M., Nascimento, J.D., 2017. *Acinetobacter*: an underrated foodborne pathogen? J. Infect. Dev. Ctries. 11, 111–114.
- Anderson, M.J., 2017. Permutational Multivariate Analysis of Variance (PERMANOVA). Statistics Reference Online, Wiley StatsRef, pp. 1–15.
- Andersson, D.I., Hughes, D., Kubicek-Sutherland, J.Z., 2016. Mechanisms and consequences of bacterial resistance to antimicrobial peptides. Drug Resist. Updat. 26, 43–57.
- Arbizu, M., 2020. pairwiseAdonis: pairwise multilevel comparison using adonis, version 0.4 ed, p. R package.
- Augustin, J.C., Zuliani, V., Cornu, M., Guillier, L., 2005. Growth rate and growth probability of *Listeria monocytogenes* in dairy, meat and seafood products in suboptimal conditions. J. Appl. Microbiol. 99, 1019–1042.
- Autio, T., Hielm, S., Miettinen, M., Sjöberg, A.-M., Aarnisalo, K., Björkroth, J., Mattila-Sandholm, T., Korkeala, H., 1999. Sources of *Listeria monocytogenes* contamination in a cold-smoked rainbow trout processing plant detected by pulsed-field gel electrophoresis typing. Appl. Environ. Microbiol. 65, 150–155.
- Bacon, R., Belk, K., Sofos, J., Clayton, R., Reagan, J., Smith, G., 2000. Microbial populations on animal hides and beef carcasses at different stages of slaughter in plants employing multiple-sequential interventions for decontamination. J. Food Prot. 63, 1080–1086.
- Bagge-Ravn, D., Ng, Y., Hjelm, M., Christiansen, J.N., Johansen, C., Gram, L., 2003. The microbial ecology of processing equipment in different fish industries—analysis of the microflora during processing and following cleaning and disinfection. Int. J. Food Microbiol. 87, 239–250.
- Barbau-Piednoir, E., Botteldoorn, N., Yde, M., Mahillon, J., Roosens, N.H., 2013. Development and validation of qualitative SYBR®/green real-time PCR for detection and discrimination of *Listeria* spp. and *Listeria monocytogenes*. Appl. Microbiol. Biotechnol. 97, 4021–4037.
- Bengtsson-Palme, J., Hartmann, M., Eriksson, K.M., Pal, C., Thorell, K., Larsson, D.G., Nilsson, R.H., 2015. METAXA2: improved identification and taxonomic classification of small and large subunit rRNA in metagenomic data. Mol. Ecol. Resour. 15, 1403–1414.
- Bintsis, T., 2017. Foodborne pathogens. AIMS Microbiol 3, 529–563.
- Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30, 2114–2120.
- Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J.E., Bittinger, K., Brejnrod, A., Brislawn, C.J., Brown, C.T., Callahan, B.J., Caraballo-Rodríguez, A.M., Chase, J., Cope, E.K., Da Silva, R., Diener, C., Dorrestein, P.C., Douglas, G.M., Durall, D.M., Duvallet, C., Edwardson, C.F., Ernst, M., Estaki, M., Fouquier, J., Gauglitz, J.M., Gibbons, S.M., Gibson, D.L., Gonzalez, A., Gorlick, K., Guo, J., Hillmann, B., Holmes, S., Holste, H., Huttenhower, C., Huntley, G.A., Janssen, S., Jarmusch, A.K., Jiang, L., Kaehler, B.D., Kang, K.B., Keeffe, C.R., Keim, P., Kelley, S.T., Knights, D., Koester, I., Koscielak, T., Kreps, J., Langille, M.G.I., Lee, J., Ley, R., Liu, Y.-X., Loftfield, E., Lozupone, C., Maher, M., Marotz, C., Martin, B.D., McDonald, D., Mciver, L.J., Melnik, A.V., Metcalf, J.L., Morgan, S.C., Morton, J.T., Naimay, A.T., Navas-Molina, J.A., Nothias, L.F., Orchanian, S.B., Pearson, T., Peoples, S.L., Petras, D., Preuss, M.L., Pruesse, E., Rasmussen, L.B., Rivers, A., Robeson, M.S., Rosenthal, P., Segata, N., Shaffer, M., Shiffer, A., Sinha, R., Song, S.J., Spear, J.R., Swafford, A.D., Thompson, L.R., Torres, P.J., Trinh, P., Tripathi, A., Turnbaugh, P.J., Ul-Hasan, S., van der Hooft, J.J.J., Vargas, F., Vázquez-Baeza, Y., Vogtmann, E., von Hippel, M., Walters, W., Wan, Y., Wang, M., Warren, J., Weber, K. C., Williamson, C.H.D., Willis, A.D., Xu, Z.Z., Zaneveld, J.R., Zhang, Y., Zhu, Q., Knight, R., Caporaso, J.G., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37, 852–857.
- Bonin, N., Doster, E., Worley, H., Pinnell, L.J., Bravo, J.E., Ferm, P., Marini, S., Prosperi, M., Noyes, N., Morley, P.S., Boucher, C., 2022. MEGARes and AMR++, v3.0: an updated comprehensive database of antimicrobial resistance determinants and an improved software pipeline for classification using high-throughput sequencing. Nucleic Acids Res. 51, D744–D752.
- Brown, C.T., Irber, L., 2016. Sourmash: a library for MinHash sketching of DNA. JOSS 1, 27.
- Cabello, F.C., Godfrey, H.P., Buschmann, A.H., Döhl, H.J., 2016. Aquaculture as yet another environmental gateway to the development and globalisation of antimicrobial resistance. Lancet Infect. Dis. 16, e127–e133.
- Campos, A., Lopes, M.S., Carvalheira, A., Barbosa, J., Teixeira, P., 2019. Survival of clinical and food *Acinetobacter* spp. isolates exposed to different stress conditions. Food Microbiol. 77, 202–207.
- Carpentier, B., Cerf, O., 2011. Review — persistence of *Listeria monocytogenes* in food industry equipment and premises. Int. J. Food Microbiol. 145, 1–8.
- Chen, B.Y., Pyla, R., Kim, T.J., Silva, J.L., Jung, Y.S., 2010a. Incidence and persistence of *Listeria monocytogenes* in the catfish processing environment and fresh fillets. J. Food Prot. 73, 1641–1650.
- Chen, B.Y., Pyla, R., Kim, T.J., Silva, J.L., Jung, Y.S., 2010b. Prevalence and contamination patterns of *Listeria monocytogenes* in catfish processing environment and fresh fillets. Food Microbiol. 27, 645–652.
- Chen, H., Li, Y., Sun, W., Song, L., Zuo, R., Teng, Y., 2020. Characterization and source identification of antibiotic resistance genes in the sediments of an interconnected river-lake system. Environ. Int. 137, 105538.
- Chen, J., Chen, H., Liu, C., Huan, H., Teng, Y., 2023. Evaluation of FEAST for metagenomics-based source tracking of antibiotic resistance genes. J. Hazard. Mater. 442, 130116.
- Chou, C.-H., Wang, C., 2006. Genetic relatedness between *Listeria monocytogenes* isolates from seafood and humans using PFGE and REP-PCR. Int. J. Food Microbiol. 110, 135–148.
- Chou, C.-H., Silva, J.L., Wang, C., 2006. Prevalence and typing of *Listeria monocytogenes* in raw catfish fillets. J. Food Prot. 69, 815–819.
- Chuah, L.-O., Effarizah, M.E., Goni, A.M., Rusul, G., 2016. Antibiotic application and emergence of multiple antibiotic resistance (MAR) in global catfish aquaculture. Curr. Environ. Health Rep. 3, 118–127.
- De Filippis, F., Valentino, V., Alvarez-Ordóñez, A., Cotter, P.D., Ercolini, D., 2021. Environmental microbiome mapping as a strategy to improve quality and safety in the food industry. Curr. Opin. Food Sci. 38, 168–176.
- Dixon, P., 2003. VEGAN, a package of R functions for community ecology. J. Veg. Sci. 14, 927–930.
- Duffes, F., Leroi, F., Boyaval, P., Dousset, X., 1999. Inhibition of *Listeria monocytogenes* by *Carnobacterium* spp. strains in a simulated cold smoked fish system stored at 4°C. Int. J. Food Microbiol. 47, 33–42.
- Erdenlig, S., Ainsworth, A.J., Austin, F.W., 2000. Pathogenicity and production of virulence factors by *Listeria monocytogenes* isolates from channel catfish. J. Food Prot. 63, 613–619.
- European Food Safety, 2017. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016. EFSA J. 15.
- Fagerlund, A., Møretø, T., Heir, E., Briandet, R., Langsrød, S., 2017. Cleaning and Disinfection of Biofilms Composed of *Listeria Monocytogenes* and Background Microbiota from Meat Processing Surfaces. Appl. Environ. Microbiol. p. 83.
- FDA, 2015. Testing Methodology for *Listeria* species or *L. monocytogenes* in environmental samples, US Food and Drug Administration, Bacteriological analytical manual.

- Fernandes, C.F., Flick Jr., G.J., Silva, J.L., McCaskey, T.A., 1997. Influence of processing schemes on indicative bacteria and quality of fresh aquacultured catfish fillets. *J. Food Prot.* 60, 54–58.
- Forsberg, K.J., Patel, S., Gibson, M.K., Lauber, C.L., Knight, R., Fierer, N., Dantas, G., 2014. Bacterial phylogeny structures soil resistomes across habitats. *Nature* 509, 612–616.
- Gedefie, A., Demsis, W., Ashagrie, M., Kassa, Y., Tesfaye, M., Tilahun, M., Bisetegn, H., Sahle, Z., 2021. *Acinetobacter baumannii* biofilm formation and its role in disease pathogenesis: a review. *Infect Drug Resist* 14, 3711–3719.
- Geser, N., Stephan, R., Hächler, H., 2012. Occurrence and characteristics of extended-spectrum β-lactamase (ESBL) producing Enterobacteriaceae in food producing animals, minced meat and raw milk. *BMC Vet. Res.* 8, 21.
- Giaouris, E., Chorianopoulos, N., Doulgeraki, A., Nychas, G.-J., 2013. Co-culture with *Listeria monocytogenes* within a dual-species biofilm community strongly increases resistance of *Pseudomonas putida* to benzalkonium chloride. *PLoS One* 8, e77276.
- Gong, C., Jiang, X., 2017. Characterizing *Salmonella* contamination in two rendering processing plants. *J. Food Prot.* 80, 265–270.
- Hansen, C.H., Vogel, B.F., Gram, L., 2006. Prevalence and survival of *Listeria monocytogenes* in Danish aquatic and fish-processing environments. *J. Food Prot.* 69, 2113–2122.
- Hanson, T., 2022. US Farm-Raised Catfish Industry – 2021 Review and 2022 Outlook.
- Hastings, J., Sailer, M., Johnson, K., Roy, K., Vederas, J., Stiles, M., 1991. Characterization of leucocin A-UAL 187 and cloning of the bacteriocin gene from *Leuconostoc gelidum*. *J. Bacteriol.* 173, 7491–7500.
- Héchard, Y., Dérijard, B., Letellier, F., Cenatiempo, Y., 1992. Characterization and purification of mesentericin Y105, an anti-*Listeria* bacteriocin from *Leuconostoc mesenteroides*. *Microbiology* 138, 2725–2731.
- Hoel, S., Vadstein, O., Jakobsen, A.N., 2019. The significance of mesophilic *Aeromonas* spp. in minimally processed ready-to-eat seafood. *Microorganisms* 7, 91.
- Hultman, J., Rahkila, R., Ali, J., Rousu, J., Björkroth, K.J., 2015. Meat processing plant microbiome and contamination patterns of cold-tolerant bacteria causing food safety and spoilage risks in the manufacture of vacuum-packaged cooked sausages. *Appl. Environ. Microbiol.* 81, 7088–7097.
- Jiang, G., Zhang, Y., Gan, G., Li, W., Wan, W., Jiang, Y., Yang, T., Zhang, Y., Xu, Y., Wang, Y., Shen, Q., Wei, Z., Dini-Andreote, F., 2022. Exploring rhizo-microbiome transplants as a tool for protective plant-microbiome manipulation. *ISME Commun.* 2, 10.
- Kramer, A., Schwebke, I., Kampf, G., 2006. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. *BMC Infect Dis* 6, 130.
- Kruskal, W.H., Wallis, W.A., 1952. Use of ranks in one-criterion variance analysis. *J. Am. Stat. Assoc.* 47, 583–621.
- Langmead, B., Salzberg, S.L., 2012. Fast gapped-read alignment with bowtie 2. *Nat. Methods* 9, 357–359.
- Langsrød, S., Moen, B., Mørætro, T., Løype, M., Heir, E., 2016. Microbial dynamics in mixed culture biofilms of bacteria surviving sanitation of conveyor belts in salmon-processing plants. *J. Appl. Microbiol.* 120, 366–378.
- Li, B., Yang, Y., Ma, L., Ju, F., Guo, F., Tiedje, J.M., Zhang, T., 2015. Metagenomic and network analysis reveal wide distribution and co-occurrence of environmental antibiotic resistance genes. *ISME J.* 9, 2490–2502.
- Li, H., Durbin, R., 2010. Fast and accurate long-read alignment with burrows-Wheeler transform. *Bioinformatics* 26, 589–595.
- Liu, J., Taft, D.H., Maldonado-Gomez, M.X., Johnson, D., Treiber, M.L., Lemay, D.G., DePeters, E.J., Mills, D.A., 2019. The fecal resistome of dairy cattle is associated with diet during nursing. *Nat. Commun.* 10, 4406.
- Liu, N.T., Lefcourt, A.M., Nou, X., Shelton, D.R., Zhang, G., Lo, Y.M., 2013. Native microflora in fresh-cut produce processing plants and their potentials for biofilm formation. *J. Food Prot.* 76, 827–832.
- Lu, J., Breitwieser, F.P., Thielen, P., Salzberg, S.L., 2017. Bracken: estimating species abundance in metagenomics data. *PeerJ Comput. Sci.* 3, e104.
- Macmillan, J.R., Santucci, T., 1990. Seasonal trends in intestinal bacterial flora of farm-raised channel catfish. *J. Aquat. Anim. Health* 2, 217–222.
- Mageswari, A., Subramanian, P., Srinivasan, R., Karthikeyan, S., Gothandam, K.M., 2015. Astaxanthin from psychrotrophic *Sphingomonas faeni* exhibits antagonism against food-spoilage bacteria at low temperatures. *Microbiol. Res.* 179, 38–44.
- Magruder, M., Edusei, E., Zhang, L., Albakry, S., Satlin, M.J., Westblade, L.F., Malha, L., Sze, C., Lubetzky, M., Dadhania, D.M., Lee, J.R., 2020. Gut commensal microbiota and decreased risk for Enterobacteriaceae bacteriuria and urinary tract infection. *Gut Microbes* 12, 1805281.
- Malta, R.C.R., Ramos, G., Nascimento, J.D.S., 2020. From food to hospital: we need to talk about *Acinetobacter* spp. *Germs* 10, 210–217.
- McCoy, E., Morrison, J., Cook, V., Johnston, J., Ebles, D., Guo, C., 2011. Foodborne agents associated with the consumption of aquaculture catfish. *J. Food Prot.* 74, 500–516.
- McMurdie, P.J., Holmes, S., 2013. Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* 8, e61217.
- Mørætro, T., Langsrød, S., 2017. Residential bacteria on surfaces in the food industry and their implications for food safety and quality. *Compr. Rev. Food Sci. Food Saf.* 16, 1022–1041.
- Mørætro, T., Moen, B., Heir, E., Hansen, A.Å., Langsrød, S., 2016. Contamination of salmon fillets and processing plants with spoilage bacteria. *Int. J. Food Microbiol.* 237, 98–108.
- Morisset, D., Berjeaud, J.-M., Marion, D., Lacombe, C., Frère, J., 2004. Mutational analysis of mesentericin Y105, an anti-*Listeria* bacteriocin, for determination of impact on bactericidal activity, *in vitro* secondary structure, and membrane interaction. *Appl. Environ. Microbiol.* 70, 4672–4680.
- NOAA Fisheries, 2020. Fisheries of the United States, 2020.
- Noyes, N.R., Yang, X., Linke, L.M., Magnuson, R.J., Dettenwanger, A., Cook, S., Georaras, I., Woerner, D.E., Gow, S.P., McAllister, T.A., 2016. Resistome diversity in cattle and the environment decreases during beef production. *elife* 5, e13195.
- O'Leary, N.A., Wright, M.W., Brister, J.R., Ciuffo, S., Haddad, D., McVeigh, R., Rajput, B., Robertse, B., Smith-White, B., Ako-Adjei, D., Astashyn, A., Badretdin, A., Bao, Y., Blinkova, O., Brover, V., Chetverin, V., Choi, J., Cox, E., Ermolaeva, O., Farrell, C., Goldfarb, T., Gupta, T., Haft, D., Hatcher, E., Hlavina, W., Joardar, V.S., Kodali, V.K., Li, W., Maglott, D., Masterson, P., McGarvey, K.M., Murphy, M.R., O'Neill, K., Pujar, S., Rangwala, S.H., Rausch, D., Riddick, L.D., Schoch, C., Shkedla, A., Storz, S.S., Sun, H., Thibaud-Nissen, F., Tolstoy, I., Tully, R.E., Vatsan, A.R., Wallin, C., Webb, D., Wu, W., Landrum, M.J., Kimchi, A., Tatusova, T., DiCuccio, M., Kitts, P., Murphy, T.D., Pruitt, K.D., 2016. Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. *Nucleic Acids Res.* 44, D733–D745.
- Parker, M., Zobrist, S., Donahue, C., Edick, C., Mansen, K., Hassan Zade Nadjari, M., Heerkhuisen, M., Sybesma, W., Molenaar, D., Diallo, A.M., 2018. Naturally fermented milk from northern Senegal: bacterial community composition and probiotic enrichment with *Lactobacillus rhamnosus*. *Front. Microbiol.* 9, 2218.
- Paul, N.C., Sullivan, T.S., Shah, D.H., 2017. Differences in antimicrobial activity of chlorine against twelve most prevalent poultry-associated *Salmonella* serotypes. *Food Microbiol.* 64, 202–209.
- Peng, S., Wang, Y., Chen, R., Lin, X., 2021. Chicken manure and mushroom residues affect soil bacterial community structure but not the bacterial resistome when applied at the same rate of nitrogen for 3 years. *Front. Microbiol.* 12.
- Praveen, P.K., Debnath, C., Shekhar, S., Dalai, N., Ganguly, S., 2016. Incidence of *Aeromonas* spp. infection in fish and chicken meat and its related public health hazards: a review. *Vet World* 9, 6–11.
- Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* 41, D590–D596.
- R Core Team, 2022. R: A Language and Environment for Statistical Computing, 4.2.2 ed. R Foundation for Statistical Computing, Vienna, Austria.
- Ramos, M., Lyon, W.J., 2000. Reduction of endogenous bacteria associated with catfish fillets using the grovac process. *J. Food Prot.* 63, 1231–1239.
- dos Reis, F.B., de Souza, V.M., Thomaz, M.R.S., Fernandes, L.P., de Oliveira, W.P., de Martinis, E.C.P., 2011. Use of *Carnobacterium maltaromaticum* cultures and hydroalcoholic extract of *Lippia sidoides* Cham. Against *Listeria monocytogenes* in fish model systems. *Int. J. Food Microbiol.* 146, 228–234.
- Roberts, M., 2011. Environmental macrolide-Lincosamide-Streptogramin and tetracycline resistant bacteria. *Front. Microbiol.* 2.
- Schmidt, J.W., Agga, G.E., Bosilevac, J.M., Brichta-Harhay, D.M., Shackelford, S.D., Wang, R., Wheeler, T.L., Arthur, T.M., 2015. Occurrence of antimicrobial-resistant *Escherichia coli* and *Salmonella enterica* in the beef cattle production and processing continuum. *Appl. Environ. Microbiol.* 81, 713–725.
- Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W.S., Huttenhower, C., 2011. Metagenomic biomarker discovery and explanation. *Genome Biol.* 12, R60.
- Sheng, L., Wang, L., 2021. The microbial safety of fish and fish products: recent advances in understanding its significance, contamination sources, and control strategies. *Compr. Rev. Food Sci. Food Saf.* 20, 738–786.
- Shenhai, L., Thompson, M., Joseph, T.A., Briscoe, L., Furman, O., Bogumil, D., Mizrahi, I., Pe'er, I., Halperin, E., 2019. FEAST: fast expectation-maximization for microbial source tracking. *Nat. Methods* 16, 627–632.
- Shineman, T.L., Harrison, M.A., 1994. Growth of *Listeria monocytogenes* on different muscle tissues. *J. Food Prot.* 57, 1057–1062.
- Silva, J.L., Ammerman, G.R., Dean, S., 2001. Processing Channel Catfish. Mississippi State University, South Regional Aquaculture center.
- Stellato, G., De Filippis, F., La Storia, A., Ercolini, D., 2015. Coexistence of lactic acid bacteria and potential spoilage microbiota in a dairy processing environment. *Appl. Environ. Microbiol.* 81, 7893–7904.
- Sterniša, M., Bucar, F., Kunert, O., Smole Možina, S., 2020. Targeting fish spoilers *Pseudomonas* and *Shewanella* with oregano and nettle extracts. *Int. J. Food Microbiol.* 328, 108664.
- Sun, Y., O'Riordan, M.X.D., 2013. Chapter three - regulation of bacterial pathogenesis by intestinal short-chain fatty acids. In: Sariaslani, S., Gadd, G.M. (Eds.), *Adv. Academic Press*, *Appl. Microbiol.*, pp. 93–118.
- Sun, Y., Wilkinson, B.J., Standiford, T.J., Akinbi, H.T., O'Riordan, M.X., 2012. Fatty acids regulate stress resistance and virulence factor production for *Listeria monocytogenes*. *J. Bacteriol.* 194, 5274–5284.
- Syropoulou, F., Parlapani, F.F., Kakasis, S., Nychas, G.E., Boziaris, I.S., 2021. Primary processing and storage affect the dominant microbiota of fresh and chill-stored sea bass products. *Foods* 10.
- Talagrand-Reboul, E., Jumas-Bilak, E., Lamy, B., 2017. The social life of *Aeromonas* through biofilm and quorum sensing systems. *Front. Microbiol.* 8, 37.
- Tomas, J.M., 2012. The Main *Aeromonas* pathogenic factors. *ISRN Microbiology* 2012, 256261.
- Tortorella, M.L., 2003. Indicator organisms for safety and quality—uses and methods for detection: minireview. *J. AOAC Int.* 86, 1208–1217.
- USDA, 2021. Catfish Production.
- Varga, S., Anderson, G.W., 1968. Significance of coliforms and enterococci in fish products. *Appl. Microbiol.* 16, 193–196.
- Wan Norhana, M.N., Poole, S.E., Deeth, H.C., Dykes, G.A., 2010. Prevalence, persistence and control of *Salmonella* and *Listeria* in shrimp and shrimp products: a review. *Food Control* 21, 343–361.
- Watts, J.E.M., Schreier, H.J., Lanska, L., Hale, M.S., 2017. The rising tide of antimicrobial resistance in aquaculture: sources, sinks and solutions. *Mar. Drugs* 15.

- Zdanowicz, M., Mudryk, Z.J., Perliński, P., 2020. Abundance and antibiotic resistance of *Aeromonas* isolated from the water of three carp ponds. *Vet. Res. Commun.* 44, 9–18.
- Zhao, T., Podtburg, T.C., Zhao, P., Schmidt, B.E., Baker, D.A., Cords, B., Doyle, M.P., 2006. Control of *Listeria* spp. by competitive-exclusion bacteria in floor drains of a poultry processing plant. *Appl. Environ. Microbiol.* 72, 3314–3320.
- Zhou, Y., Yu, L., Nan, Z., Zhang, P., Kan, B., Yan, D., Su, J., 2019. Taxonomy, virulence genes and antimicrobial resistance of *Aeromonas* isolated from extra-intestinal and intestinal infections. *BMC Infect. Dis.* 19, 158.
- Zhu, M., Du, M., Cordray, J., Ahn, D.U., 2005. Control of *Listeria monocytogenes* contamination in ready-to-eat meat products. *Compr. Rev. Food Sci. Food Saf.* 4, 34–42.
- Zwirzitz, B., Wetzels, S.U., Dixon, E.D., Fleischmann, S., Selberherr, E., Thalguter, S., Quijada, N.M., Dzieciol, M., Wagner, M., Stessl, B., 2021. Co-occurrence of *Listeria* spp. and spoilage associated microbiota during meat processing due to cross-contamination events. *Front. Microbiol.* 12.