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Pathogenic Microorganisms in the Environment of Sheep Slaughterhouse

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ABSTRACT

***Background*:** As critical nodes for zoonotic disease transmission, the microbial community composition in sheep slaughterhouse environments directly impacts meat safety and public health. While existing studies have focused on single-pathogen detection, there remains a lack of systematic understanding of the diversity, distribution patterns, and transmission risks of pathogenic microorganisms in complex environments. This study aims to elucidate the microbial community characteristics of mutton slaughterhouse environments using metagenomics approaches, with a focus on analyzing the ecological distribution of zoonotic pathogens and their potential threats.

***Materials, Methods & Results*:** A total of 12 environmental samples (n=3 per zone) were collected from a sheep slaughterhouse in Suzhou, Jiangsu Province, including slaughter workshop, slaughter tools, pre-slaughter holding area, and production wastewater. Samples were preserved in liquid nitrogen, and total DNA was extracted using the HiPure Stool DNA Kit. Paired-end sequencing (PE150) was performed on the Illumina HiSeq platform. Raw reads were quality-filtered (Cutadapt), and host-contaminant sequences removed (BWA). Clean reads were assembled with MEGAHIT, and unigenes predicted via Prodigal. Clustering by MMseq2 generated a non-redundant gene set, which was annotated against the NCBI NT database using Diamond to obtain taxonomic assignments. Species abundance was calculated at phylum, genus, and species levels, and principal component analysis (PCA) assessed variation between zones. At the phylum level, microbial communities were dominated by Proteobacteria (31.39% - 75%), Firmicutes (11.89% - 43.66%), Bacteroidota (9.15% -41.61%), and Actinobacteria (3% - 14.69%), with significant clustering by functional zones. Genus-level analysis revealed *Chryseobacterium*, *Comamonas*, *Acinetobacter*, *Psychrobacter*, and *Prevotella* as top taxa across samples. Species-level profiling detected 6 categories of zoonotic pathogens, notably *Staphylococcus aureus* (7.07% relative abundance in slaughter tools), *Mycobacteroides abscessus* (3.56% in slaughter workshop), *Vibrio cholerae*, and *Klebsiella pneumoniae*. Slaughter tools harbored the highest pathogen load; *Lactococcus garvieae* accounted for 10.39%. Production wastewater contained elevated levels of aquatic pathogens, suggesting environmental dissemination risks. An upset plot showed 116 species shared across all samples, with unique species counts ranging from 88 to 505 per sample.

***Discussion*:** This study elucidates the ecological niches of drug-resistant and opportunistic pathogens in a sheep slaughterhouse, identifying production wastewater and slaughter tools as high-risk contamination sources. High abundances of *Staphylococcus aureus* and *Mycobacteroides abscessus* underscore occupational exposure risks for abattoir workers, while detection of *Vibrio cholerae* highlights vulnerabilities in wastewater treatment and potential for zoonotic outbreaks. The prevalence of biofilm-forming Firmicutes on tools indicates that conventional disinfectants may be insufficient. Horizontal transfer of antibiotic resistance genes among dominant phyla could exacerbate public health threats. We recommend implementing targeted disinfection protocols, routine metagenomic monitoring for early pathogen detection, stricter PPE use and worker training, and optimization of wastewater pH control. Future studies should expand geographic sampling and integrate functional metagenomics to unravel resistance mechanisms and cross-species gene flow. These strategies will strengthen slaughterhouse biosafety and mitigate zoonotic disease transmission.

Keywords: sheep slaughterhouse, public health, environment microbiology, zoonoses, pathogens, metagenomics.

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INTRODUCTION

In recent years, the incidence of zoonotic diseases has significantly increased with the continuous expansion of sheep farming, posing substantial challenges to public health and the livestock industry [4]. Zoonotic diseases are estimated to account for approximately 60% of known human infectious diseases and 75% of emerging and re-emerging infectious diseases in the past three decades [5,8,20,24]. These diseases, caused by a wide range of pathogens and transmitted through diverse routes, have severe implications for human and animal health as well as the economy.

Historically, cholera, brucellosis, and rabies have triggered widespread outbreaks and public health crises. More recently, COVID-19, H7N9 avian influenza, and Ebola have underscored the persistent global threat of zoonoses [18,23]. Slaughterhouses represent critical interfaces for zoonotic transmission, where pathogens such as *Vibrio cholerae*, *Klebsiella pneumoniae,* and *Streptococcus agalactiae* disseminate via water, soil, and feed [6,12-14,21,22,25]. Traditional detection methods (culture, molecular assays, and immunological tests) are limited in comprehensiveness and throughput. In contrast, metagenomic sequencing provides an unbiased, high-resolution view of microbial communities, capturing both taxonomic diversity and functional potential of pathogens and resistance determinants.

This study aims to address current knowledge gaps, this study employed metagenomic sequencing to characterize the overall microbial community structure in 4 functional zones of a mutton sheep slaughterhouse, identify and quantify key zoonotic pathogens, and assess their distribution patterns and potential transmission risks.

MATERIALS AND METHODS

Sample collection

Samples were collected from a sheep slaughterhouse in Suzhou, Jiangsu Province, China. The sample groups included: Slaughter Workshop Group (TZCJ), Slaughter Tools Group (TZGJ), Pre-slaughter Workshop Group (DZCJ), and Production Wastewater Group (SCFS). Three samples per group, a total of 12 samples. All samples were preserved in liquid nitrogen immediately after collection for subsequent experiments.

Slaughter Workshop Group (TZCJ): 5 areas were evenly selected within the workshop. Sterile cotton swabs moistened with PBS were used to swab the surfaces of each area. Each sample was a mixture of swabs from five sampling points.

Slaughter Tools Group (TZGJ): Knives and other tools were placed in sterile containers with PBS. The containers were shaken to dislodge surface microorganisms, and the PBS suspension was collected. After centrifugation at low temperature and high speed, the sediment was stored at -80°C.

Production Wastewater Group (SCFS): Using sterile syringes, 3 mL of wastewater was collected and stored in sterile tubes.

Pre-slaughter Workshop Group (DZCJ): Sampling methods were consistent with those used in the slaughter workshop.

Metagenomic sequencing and data processing

DNA was extracted using the HiPure Stool DNA Kit[1] following the manufacturer's protocol. Purity and concentration of the extracted DNA were assessed using a NanoDrop 2000 spectrophotometer, with quality determined by OD260/280 and OD260/230 ratios.

For each sample, 200 ng of genomic DNA was fragmented into 300-350 bp segments using an ultrasonic DNA fragmenter. The fragments underwent end repair and adapter ligation with P5 (5'-AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT-3') and P7 (5'-AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC-3') primers. Paired-end sequencing (PE150) was performed using the Illumina HiSeq platform.

Raw image data were processed using bcl-2fastq (v2.17.1.14) to generate FASTQ files. Adapter sequences and low-quality reads were removed using Cutadapt (v1.9.1), producing clean data. Host contamination was filtered using BWA (v0.7.12).

Clean data were assembled using MEGAHIT (v1.1.3) with a de Bruijn graph strategy, employing K-mer values of 59, 79, 99, 119, and 141. Optimal assembly results for each sample were integrated.

Gene prediction was performed using Prodigal (v3.02). Gene sequences from all samples were consolidated and clustered using MMseq2, with a sequence identity threshold of 95% and coverage of 95%, to generate a non-redundant unigene set. Unigene sequences were aligned to the clean reads to calculate their abundance in each sample.

The unigene sequences were aligned against the NCBI NT database via Diamond software to obtain

species annotation information. On the basis of the annotation results and gene abundance, the species abundance was calculated at different taxonomic levels (phylum, genus, and species).

RESULTS

Microbial composition in the sheep slaughterhouse environment

Metagenomic sequencing analysis revealed that the microbial community in the slaughterhouse environment consisted of 98.93% bacteria, 0.6% archaea, 0.4% eukaryotes, and 0.07% viruses. Across 12 samples, a total of 46 phyla, 75 classes, 174 orders, 355 families, 1062 genera, and 2968 species were identified.

Principal Component Analysis (PCA) at the species level showed that the 1st and 2nd principal components (PC1 and PC2) accounted for 32.45% and 23.51% of the variation, respectively. Each point on the PCA plot represents the information of 1 sample, with proximity of points indicating greater similarity in microbial composition between samples. Conversely, distant points reflect larger differences in composition. PCA demonstrated clear clustering patterns among the samples, indicating variation in microbial composition (Figure 1).

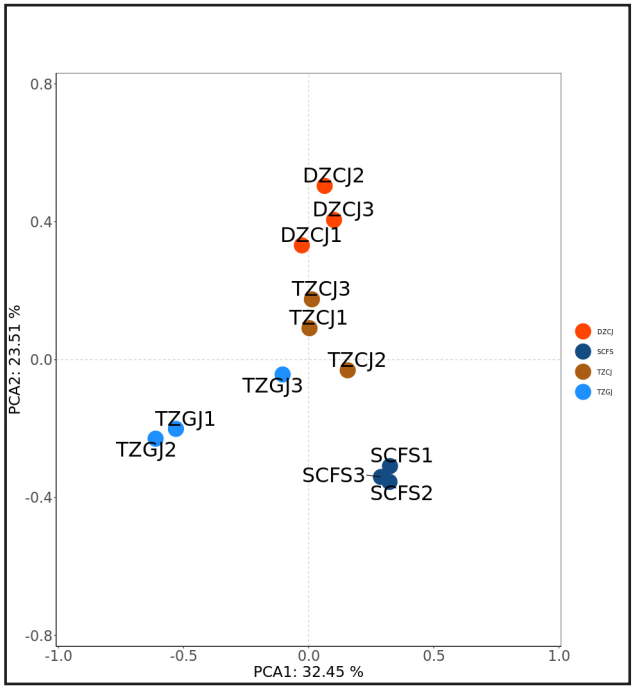

Figure 1. PCA diagram of microbial species composition at sheep slaughterhouse.

Relative abundance analysis of sheep slaughterhouse microbiota

At the phylum level, excluding Chordata, the dominant taxa were Proteobacteria, Actinobacteria, Bacteroidota, and Firmicutes, which together accounted for over 90% of the relative abundance across all samples (Figure 2). In slaughter workshop group, the top 2 dominant phyla were Proteobacteria and Bacteroidota, together comprising over 50% of the relative abundance. The 3rd and 4th dominant phyla varied slightly among the 3 samples but were consistently Actinobacteria and Firmicutes. In pre-slaughter workshop group, the dominant phyla were Bacteroidota, Firmicutes, and Proteobacteria. Actinobacteria accounted for less than 3% in this group. In production wastewater group, Proteobacteria and Firmicutes were the top 2 dominant phyla, with their combined relative abundance exceeding 75%, while Bacteroidota ranked 3rd with an average relative abundance of 13.7%. In slaughter tools group, sample 1 and sample 2 exhibited similar dominant phyla, with the average relative abundances of Firmicutes (43.66%), Proteobacteria (31.39%), Bacteroidota (9.15%), and Chordata (9.4%); In Sample 3, the dominant phyla were Bacteroidota (41.61%), Proteobacteria (24.49%), Actinobacteria (14.69%), and Firmicutes (11.89%).

At the genus level, excluding unclassified genera, the top 10 dominant genera in the slaughterhouse environmental samples were as follows: *Chryseobacterium* (9.60%), *Comamonas* (7.11%), *Acinetobacter* (6.45%), *Psychrobacter* (6.19%), *Prevotella* (5.53%), *Flavobacterium* (2.70%), *Corynebacterium* (2.41%), *Pseudomonas* (2.34%), *Lactococcus* (2.22%), and *Staphylococcus* (1.69%) [Figure 3]. Across all samples, 19 genera exhibited relative abundances exceeding 1%. In slaughter workshop group, the top dominant genus was *Psychrobacter* (15.49%), followed by *Chryseobacterium* (12.03%) and *Acinetobacter* (10.32%). In production wastewater group, *Comamonas* ranked 1st (21.04%), followed by *Acinetobacter* (8.67%) and *Pseudomonas* (6.82%). In pre-slaughter workshop group, *Prevotella* was the most abundant genus (18.20%), followed by *Chryseobacterium* (8.49%) and *Flavobacterium* (5.06%). In slaughter tools group, *Chryseobacterium* was the most abundant genus (15.76%), followed by *Lactococcus* (10.04%), *Staphylococcus* (7.57%), and *Shigella* (7.08%). In pre-slaughter workshop group, the unclassified genera accounted for 16.42% of relative abundance (Figure 3).

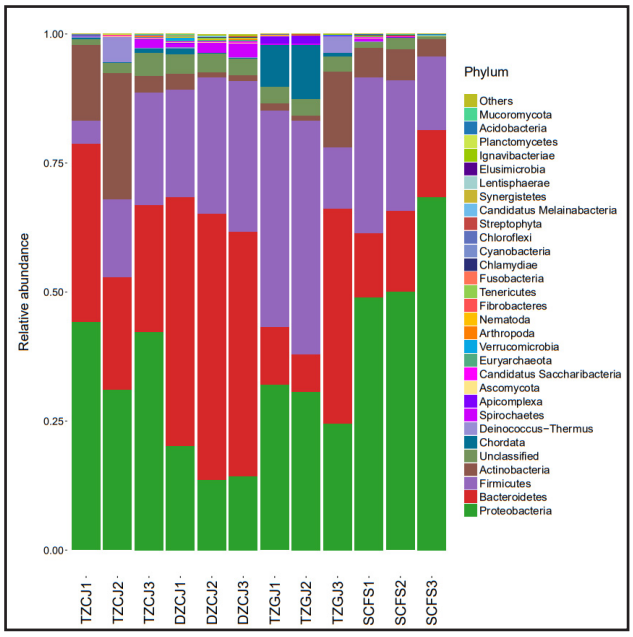

Figure 2. The relative abundance at phylum level.

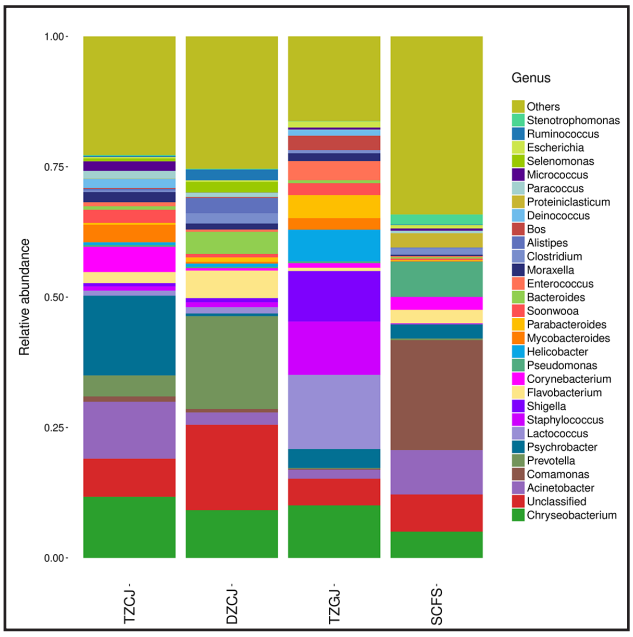

Figure 3. The relative abundance at genus level.

An upset plot was generated to show the microbial species composition in each environmental sample with a relative abundance exceeding 0.01%. As illustrated, the microbial species count in samples TZGJ1 and TZGJ2 was below 1,000, while the rest exceeded this number. A total of 116 species were shared across all samples. The lowest number of unique species was found in sample TZGJ2 (88 species), whereas SCFS2 contained the highest number of unique species (505 species). Other samples also had unique species, with counts falling between these 2 extremes (Figure 4). In slaughter tools group, the top 10 pathogenic or opportunistic pathogens by relative abundance included *Lactococcus garvieae* (10.39%), Staphylococcus *aureus* (7.07%), and *Mycobacteroides abscessus* (3.5%). In production wastewater group, the top pathogenic genus was *Acinetobacter indicus* (0.49%). In slaughter workshop group, pathogenic species in the top 10 included *Mycobacteroides abscessus* (3.56%), *Moraxella osloensis* (1.37%), and *Corynebacterium dentalis* (3.02%). In pre-slaughter workshop group, *Lactococcus garvieae* ranked among the top 10 pathogens with a relative abundance of 0.98% (Figure 5).

DISCUSSION

This study systematically analyzed the microbial community characteristics in the environment of a sheep slaughterhouse using metagenomic sequencing technology, revealing for the 1st time the distribution patterns of zoonotic pathogens in this setting and their potential public health risks. The results demonstrate the presence of a complex microbial ecosystem in the slaughterhouse environment, dominated by Proteobacteria, Firmicutes, Bacteroidota, and Actinobacteria. Notably, the high abundance of Firmicutes (43.66%) in the slaughter tools group may be related to their biofilm-forming capabilities on surfaces, raising concerns about their resistance to conventional disinfectants [2,15]. Meanwhile, the significant dominance of *Proteobacteria* (over 75%) in production wastewater group suggests potential inefficiencies in the wastewater treatment system. The presence of various antibiotic resistance genes within these bacterial groups may exacerbate public health risks through horizontal gene transfer [16,19].

At the species level, the study detected multiple zoonotic pathogens of significant public health importance. The high detection rate of *Staphylococcus aureus* (7.07%) in slaughter tools group is closely associated with biofilm colonization on knife surfaces due to micro-damage, corroborating the European Food Safety Authority (EFSA) warning report on cross-contamination via slaughter tools [3]. Of particular concern is the simultaneous detection of *Mycobacteroides abscessus* in both slaughter workshop group and slaughter tools group. As a representative of non-tuberculous mycobacteria (NTM), this pathogen

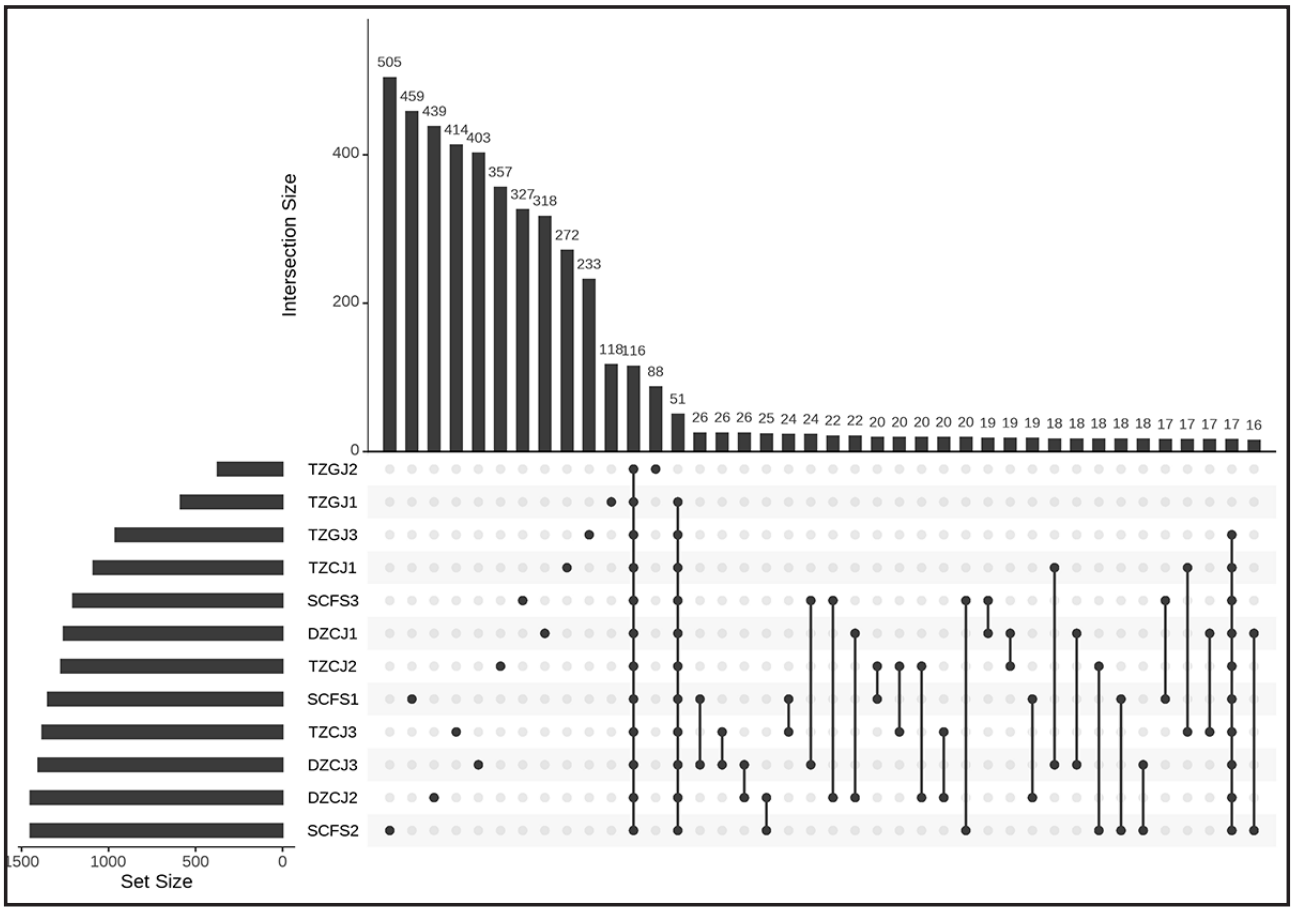

Figure 4. Species composition number upset diagram.

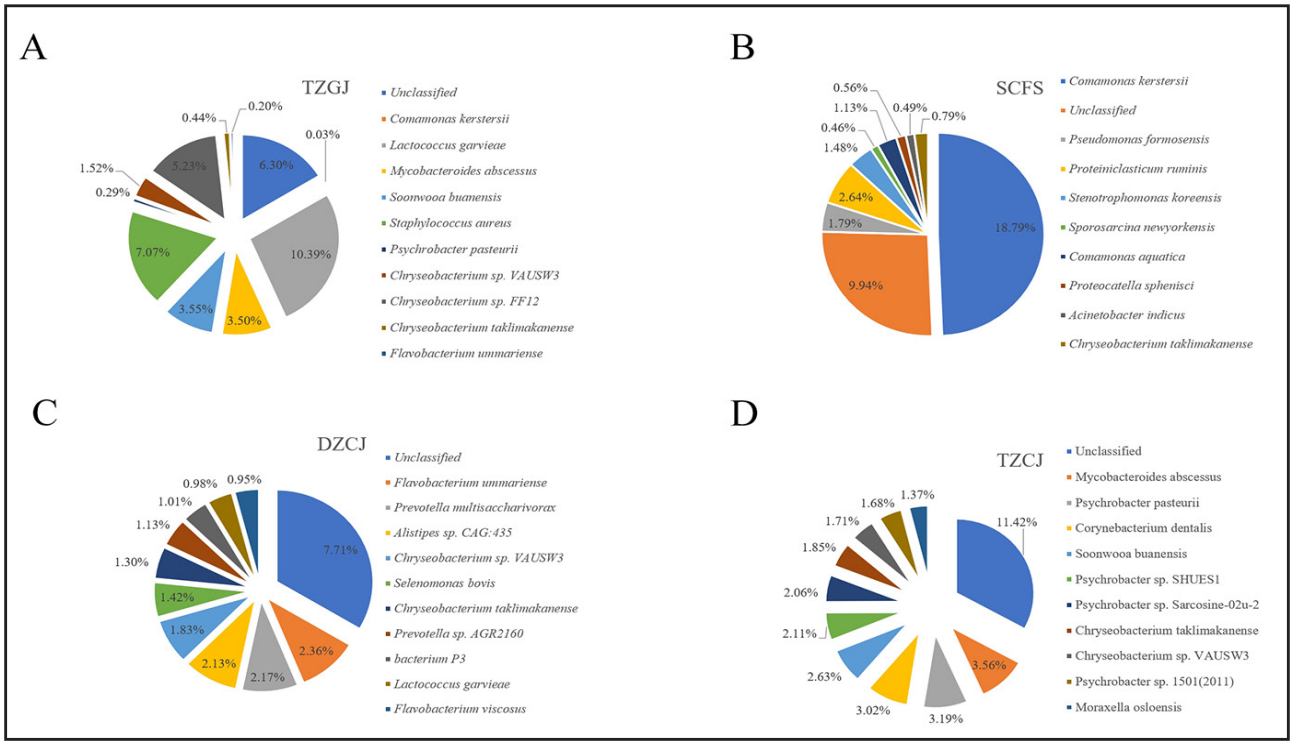

Figure 5. Pie chart of relative abundance at species level. A- TZGJ. B- SCFC. C- DZCJ. D- TZJC.

can cause chronic lung infections in workers through aerosol transmission, with its environmental tolerance likely enhanced by the temperature and humidity conditions in the slaughter workshop [7,17]. Additionally, the abnormal proliferation of *Prevotella* (18.20%) in pre-slaughter workshop group may be linked to stress-induced intestinal dysbacteriosis in sheep awaiting slaughter, highlighting the need for targeted biosecurity measures during livestock transportation [9-11].

Although *Vibrio cholerae* was detected at a relatively low abundance (0.02%), its presence in production wastewater reveals significant vulnerabilities in the wastewater treatment system. Given *V. cholerae*'s strong survival capability in alkaline wastewater [1], dynamic monitoring of wastewater pH is recommended. The multi-point detection of *Klebsiella pneumoniae* underscores the potential role of this environment as a reservoir for antibiotic resistance genes, necessitating further investigation into the presence of resistance genes. Notably, the high abundance of *Lactococcus garvieae* (10.39%) in slaughter tools group suggesting potential water source contamination during tool cleaning procedures.

In this study, the geographic scope was confined to a single region, potentially limiting the generalizability of the results. Future research should focus on expanding the geographic scope of sampling. This will be instrumental in developing prevention strategies to mitigate microbial risks in slaughterhouse environments and safeguard public health.

CONCLUSIONS

This study employed metagenomic sequencing to elucidate the distribution characteristics of dominant microbial phyla, including Proteobacteria and Firmicutes, in the environment of a sheep slaughterhouse. Multiple zoonotic pathogens were detected, such as *Staphylococcus aureus* (7.07%) and *Mycobacteroides abscessus* (3.56%), with slaughter tools and production wastewater identified as high-risk areas. These findings provide a scientific basis for optimizing cleaning protocols and blocking pathogen transmission. We recommend the development of targeted disinfection strategies and the establishment of a real-time monitoring system to enhance biosafety levels.

MANUFACTURER

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***Declaration of interest*.** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The authors alone are responsible for the content and writing of the paper.

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