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Microbial contamination, community diversity and cross-contamination risk of food-contact ice

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ABSTRACT

Ice is widely used in the food industry, as an ingredient (edible ice) directly added to food or as a coolant (food-contact ice) for fresh food preservation along the cold chain. However, it has been shown that food-contact ice are easily polluted by pathogens, potentially endangering the public's health. In the present study, the hygiene status of food-contact ice collected from various sources (local farmer markets, supermarkets, and restaurants) was evaluated through the quantitative estimation of total bacterial counts and coliform counts as well as the prevalence of foodborne pathogenic bacteria (*Staphylococcus aureus*, *Vibrio parahaemolyticus*, *Salmonella*, *Listeria monocytogenes*, *Shigella*). The average levels of total bacterial counts in the ice for preserving the aquatic products, poultry meat and livestock meat are 4.88, 4.18 and 6.11 $\log_{10}$ CFU/g, respectively. Over 90 % of the food-contact ice were positive for coliforms. The detection rate of *S. aureus* in all the food-contact ice samples was highest, followed by *Salmonella*, *V. parahaemolyticus* and *L. monocytogenes*, and *Shigella* was not detected. In addition, the bacterial community diversity of food-contact ice was analyzed with high-throughput sequencing. The dominant bacteria taxa in food-contact ice are heavily dependent on the environment of sampling sites. The predicted phenotypes of biofilm forming, oxidative stress tolerance, mobile element containing and pathogenesis were identified in the bacteria taxa of food-contact ice, which should be carefully evaluated in future work. Finally, the cross-contamination models of pathogen transfer during ice preservation were established. The results showed that the transfer rates of ice-isolated *S. aureus* between food and ice were significantly higher than that of *V. parahaemolyticus*. The binomial distribution B(n, p) exhibited a better fitness to describe the pathogen transfer during ice preservation when the transfer rate was low, in turn, the transfer rate-based probability model showed a better fit to the data when the transfer rate was high. Monte Carlo simulation with Latin-Hypercube sampling was carried out to predict the contamination levels of *S. aureus* and *V. parahaemolyticus* on food as the result of cross contamination during ice preservation ranging from −2.90 to 2.96 $\log_{10}$ CFU/g with a 90 % confidence interval. The findings of this work are conducive to a comprehensive understanding of the current hygiene status of food-contact ice, and lay a theoretical foundation for the risk assessment of cross-contamination during ice preservation.

1. Introduction

Ice has found wide use in large quantities as an ingredient (edible ice) and a coolant in the food industry (Hampikyan et al., 2017). During distribution, storage, and retail, fresh food is easily contaminated by microorganisms, which might lead to quality deterioration and food-borne diseases. Ice directly or indirectly contacts with fresh food (e.g., meats, fish, fruits, and vegetables) for preservation through effectively reducing temperature and inhibiting microbial growth (Katsaros, Koseki, Ding, & Valdramidis, 2021). Raw seafood, for instance, are commonly preserved in contact to a layer of ice before selling in the farm market or supermarket. Additionally, some perishable fruits (e.g., strawberries) and vegetables (e.g., lettuces) are also frequently stored with direct contact to ice before consumption to assure freshness. Under

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Table 1
The sequences of primers used in this study.

Pathogenic bacteria	Genes	Primer sequence (5′-3′)	Product size (bp)	References
Salmonella	*invA*	F: TCGCACCGTCAAAGGAACCGTAAAGC R: GCATTATCGATCAGTACCAGCCGTCT	284	Malorny et al., 2003
Staphylococcus aureus	*nuc*	F: GCGATTGATGGTGATACGGTT R: AGCCAAGCCTTGACGAACTAAAGC	447	Brakstad et al., 1992
Listeria monocytogenes	*hlyA*	F: ACTTCGGCGCAATCAGTGA R: TTGCAACTGCTCTTTAGTAACAGCTT	137	Zhang et al., 2015
Shigella	*ipaH*	F: GTTCCTTGACCGCCTTTCCGATACCGTC R: GCCGGTCAGCCACCCTCTGAGAGTAC	619	Shahin et al., 2019
Vibrio parahaemolyticus	*toxR*	F: GTCTTCTGACGCAATCGTTG R: ATACGAGTGGTTGCTGTCATG	368	Narayanan et al., 2020

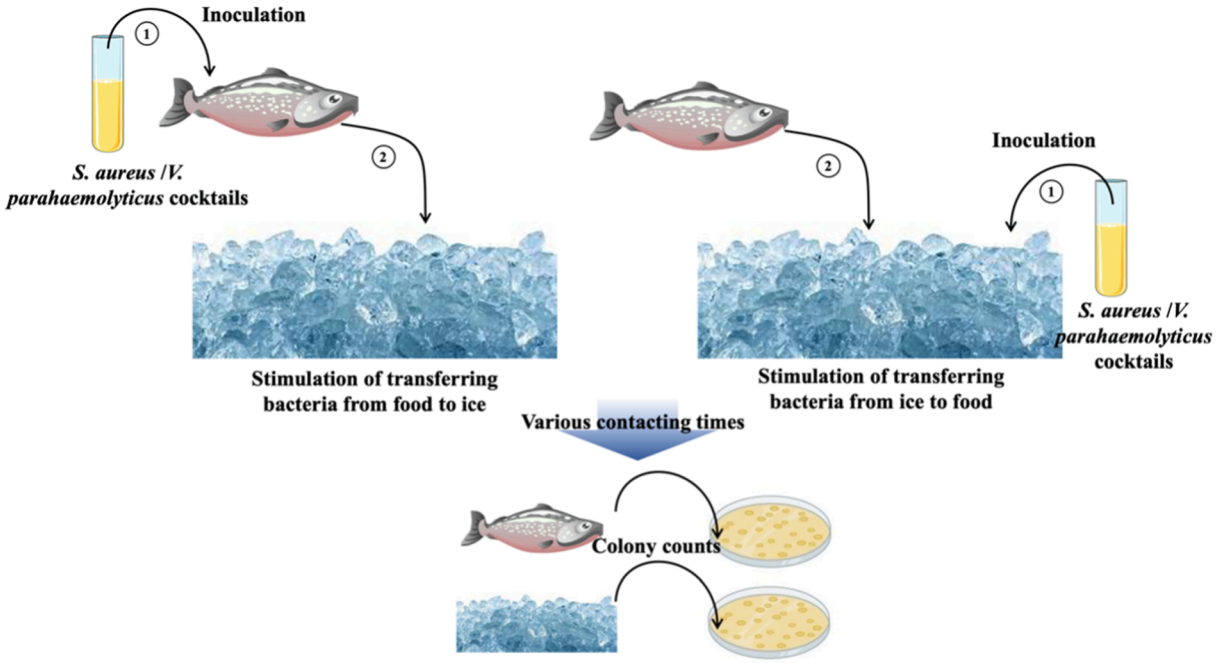

Fig. 1. Schematic diagram of modeling the pathogen transfer by cross-contamination during ice preservation.

Table 2
Sources and proportions of food-contact ice samples.

Sample sources	Sample counts (n)	Percentage (%)
Aquatic products	128	74.85
Poultry meat	27	15.79
Livestock meat	16	9.36
Sum	171	100

low temperatures, microorganisms experience complex physiological reactions, including compromises in membrane fluidity, lower stability of cellular macromolecules, and interference with ribosomes' normal transcription and translation functions (Phadtare et al., 2004; Gualerzi et al., 2003; Zangrossi et al., 2000). Some microbes, such as psychrophiles, may still maintain certain physiological activities and endure cold stress. The development of cold shock proteins, maintenance of membrane fluidity, structural modification of intracellular enzymes, and the presence of compatible solutes are all related to the survival of cold-adapted microorganisms (D'Amico, Gerday, & Feller, 2001; De Maayer, Anderson, Cary, & Cowan, 2014; Goyal, Swaroop, Prakash, & Pandey, 2022). Currently known common foodborne pathogenic bacteria, such as *Listeria monocytogenes* (Chan et al., 2008) and *Yersinia enterocolitica* (Li et al., 2020), have also been shown to be able to survive and grow under a low-temperature environment, thereby posing potential threats to the microbiological safety of ice.

The microbial contamination of edible ice has received attention widespread (Lateef et al., 2006; Mahale et al., 2008; Tuyet Hanh et al., 2020; Ukwo et al., 2011). Compared with edible ice, the hygiene condition of food-contact ice tends to be more serious, but the associated research is limited. Nichols et al. (2000) compared the microbial quality of edible ice used in drinks and ice contact with ready-to-eat food in the United Kingdom, and the results showed that only 9 % of the ice used in drinks contained coliforms, and 1 % of samples tested positive for *Escherichia coli*. While among the ice used for food preservation, 23 % of samples were found to contain coliforms and 5 % were positive for the contamination of *E. coli*. If the ice exhibit a poor hygiene status, it might cross contaminate the contacted food. The contaminated ice might be a vesicle for transmission of pathogenic bacteria to food and humans, resulting in the potential risk to food safety and public health. Food poisoning outbreaks have been reported to be associated with the direct or indirect consumption of contaminated ice (Fumian et al., 2021; Laussucq et al., 1988). Therefore, it is of importance to grasp a better understanding of the hygiene status of food-contact ice as well as the potential risks of pathogen transfer by cross-contamination during ice preservation.

In this study, the hygienic status of food-contact ice for preserving different food sources (aquatic products, poultry meat, livestock meat) collected from the farmer markets, local supermarkets and restaurants, was investigated and the prevalence of pathogenic bacteria was also determined in food-contact ice. Furthermore, a high throughput

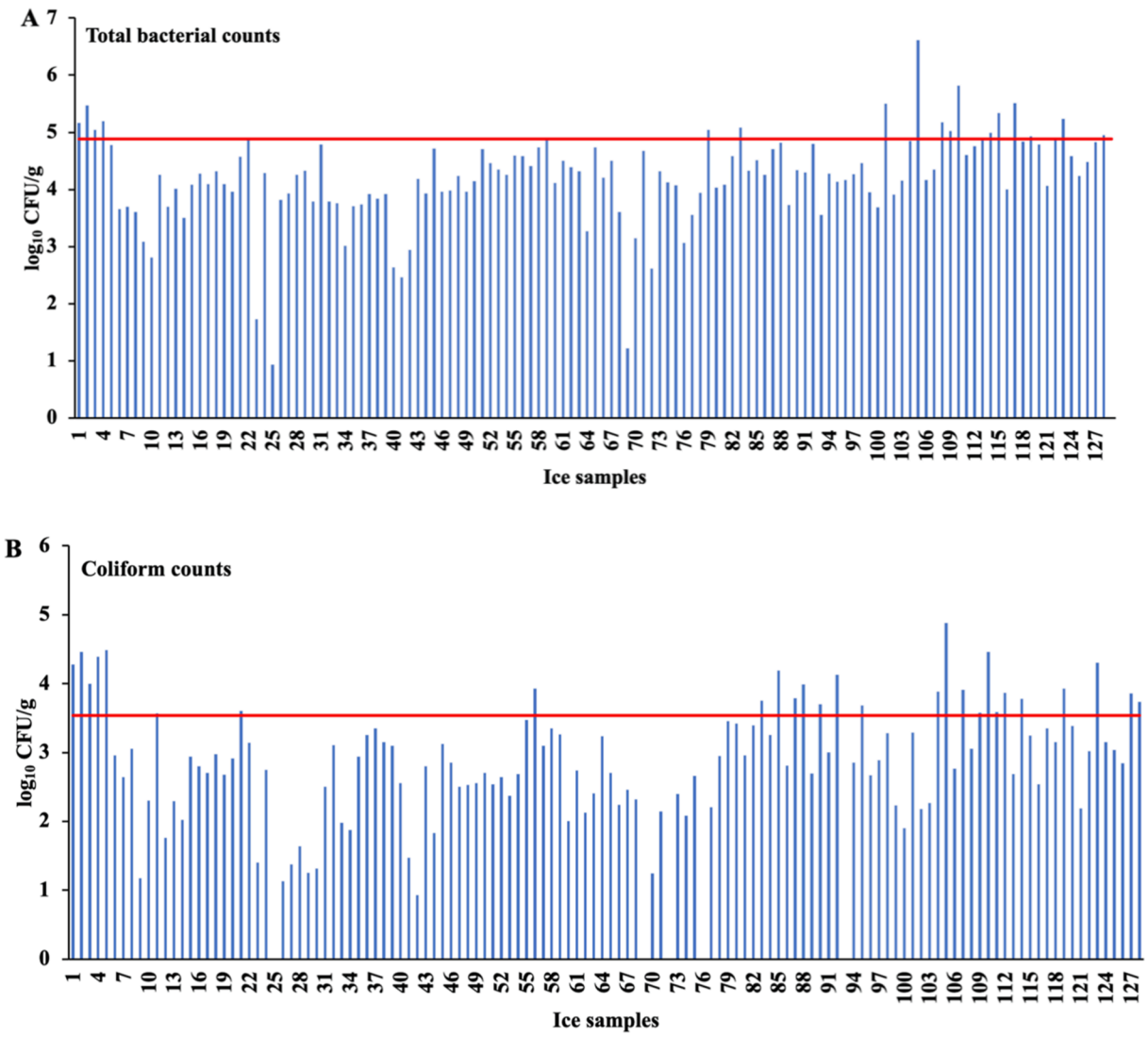

Fig. 2. Colony counts (log_{10}CFU/g) of ice samples contacting aquatic products: (A) Total bacterial counts; (B) Coliform counts. Red lines exhibit the average value of colony counts. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

sequencing technique was applied for the exploration of bacterial community diversity as well as the dominant bacterial taxa in food-contact ice. Finally, the cross-contamination models were established to predict the transfer rates of foodborne pathogens to food products/ice during preservation, and a Monte Carlo simulation was conducted to estimate the bacteria levels in food products due to cross-contamination.

2. Materials and methods

2.1. Sampling food-contact ice

Ice samples were gathered in sterile polyethylene containers from farmer markets, local supermarkets, and restaurants. The samples were transported to the laboratory and melted at room temperature for analysis within 2 h.

2.2. Total bacterial count and coliform count

Total bacterial and coliform counts in ice samples were determined according to the procedure described in the Chinese National Food Safety Standards GB 4789.1–2016 and GB 4789.3–2016 (China, 2016a; b), respectively. A 25 g of ice sample was added into 225 mL sterilized 0.85 % saline solution and homogenized thoroughly. Tenfold serial dilutions were conducted with sterilized 0.85 % saline solution. A 1 mL of the appropriate diluents was added into ~ 20 mL plate count agar (PCA) and violet red bile agar (VRBA) followed by incubation at 36 ± 1 °C for 48 ± 2 h and 18–24 h for the enumeration of total bacterial and coliform counts, respectively. The red colonies surrounded by reddish precipitation zones on VRBA were selected and transferred into brilliant green lactose bile broth with the following incubation at 36 ± 1 °C for 24–48 h. The formation of gas indicates the positive for coliforms.

2.3. The determination of pathogenic bacteria in food-contact ice samples

(1) *Salmonella*: According to the Chinese National Food Safety Standards GB 4789.4–2016 (China, 2016c), 25 mL sample was added into 225 mL peptone water and cultured at 36 ± 1 °C for 8–18 h. One milliliter of the above culture suspension into 10 mL tetrathionate broth base (TTB) and selenite cystine broth base (SC) broth followed by incubation at 42 ± 1 °C for 18–24 h (TTB broth) and 36 ± 1 °C for 18–24 h (SC broth), respectively. The above culture suspensions were then streaked on bismuth sulfite (BS) and xylose lysine desoxycholate (XLD) mediums, and cultured at 36 ± 1 °C for 40–48 h (BS medium) or 18–24 h (XLD medium), respectively. The suspected colonies were selected

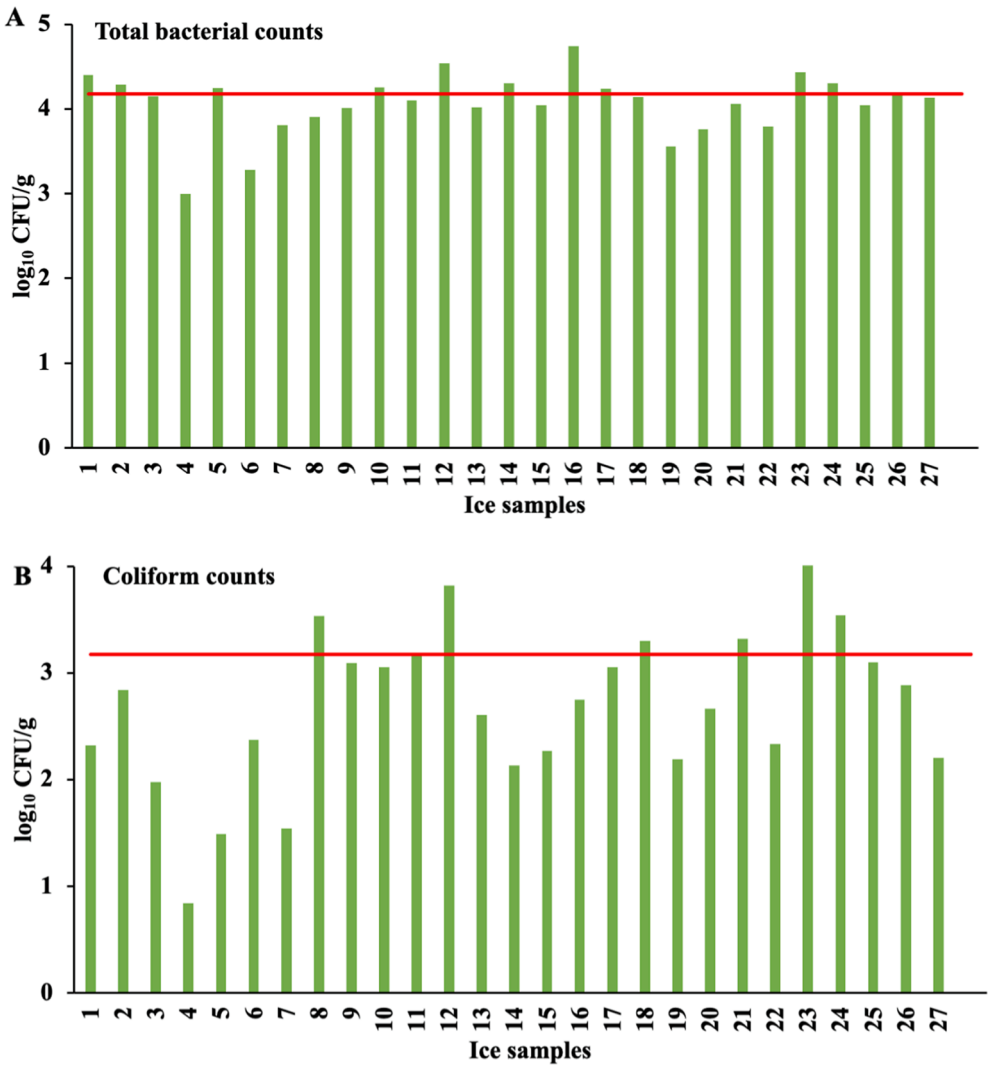

Fig. 3. Colony (log_{10} CFU/g) of ice samples contacting poultry products: (A) Total bacterial counts; (B) Coliform counts. Red lines exhibit the average value of colony counts. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

from the plate for PCR identification, and the isolated strains were stored in 25 % glycerol at −80 °C.

(2) *Staphylococcus aureus*: Refer to the Chinese National Food Safety Standard GB 4789.10–2016 (China, 2016d) for the detection of *S. aureus*. The ice samples were melted at room temperature, and then 25 mL of the melted ice samples were transferred into 225 mL broth containing 7.5 % sodium chloride, and cultured at 36 ± 1 °C for 18–24 h. The culture suspension was streaked on Baird-Parker medium and incubated at 37 °C for 48 ± 2 h. The suspicious colonies were selected for PCR identification, and the isolated strain was stored in 25 % glycerol at −80 °C.

(3) *Shigella*: In accordance with the Chinese National Food Safety Standard GB 4789.5–2012 (China, 2012) for *Shigella* detection, the melted ice samples (25 mL) were transferred into 225 mL broth, followed by an anaerobic incubation at 41.5 ± 1 °C for 16–20 h. The culture suspension was streaked on XLD and MacConkey (MAC) mediums, followed by incubation at 36 ± 1 °C for 20–24 h. Typical colonies were selected for PCR identification and the collected strain was stored in 25 % glycerol at −80 °C.

(4) *Listeria monocytogenes*: Refer to the Chinese National Food Safety Standard GB 4789.30–2016 (China, 2016e) for *L. monocytogenes* detection, the melted ice samples (25 mL) were transferred into 225 mL LB_1 enrichment solution, and cultured at 30 ± 1 °C for 24 ± 2 h. The culture solution (0.1 mL) was further transferred in 10 mL LB_2 and cultured at 30 ± 1 °C for 24 ± 2 h. The culture suspension was inoculated on PALCAM medium and cultured at 36 ± 1 °C for 24–48 h. Suspicious colonies were selected and identified by PCR. The isolated strain was stored in 25 % glycerol at −80 °C.

(5) *Vibrio parahaemolyticus*: Refer to the Chinese National Food Safety Standard GB 4789.7–2013 (China, 2013) for *Vibrio parahaemolyticus* detection, the melted ice samples (25 mL) were transferred into 225 mL 3 % sodium chloride alkaline peptone water, and cultured at 36 ± 1 °C for 8–18 h. The culture suspension was inoculated on thiosulfate-citrate-bile salts-sucrose (TCBS) agar and cultured at 36 ± 1 °C for 18–24 h. Suspicious colonies were selected and transferred into tryptose soya agar supplemented with 3 % NaCl followed by incubation at 36 ± 1 °C for 18–24 h. Suspicious colonies were further selected and identified by PCR. The isolated strain was stored in 25 % glycerol at −80 °C.

To further confirm the pathogenic bacteria, PCR combined with Sanger sequencing analysis was performed on the suspected isolates based on the specific genes of *invA*, *nuc*, *hlyA*, *ipaH*, and *toxR* for *Salmonella*, *S. aureus*, *L. monocytogenes*, *Shigella* and *V. parahaemolyticus*, respectively. Asingle bacterial colony was transferred to 10 mL Nutrient Broth (NB) for overnight incubation. One milliliter of the culture was

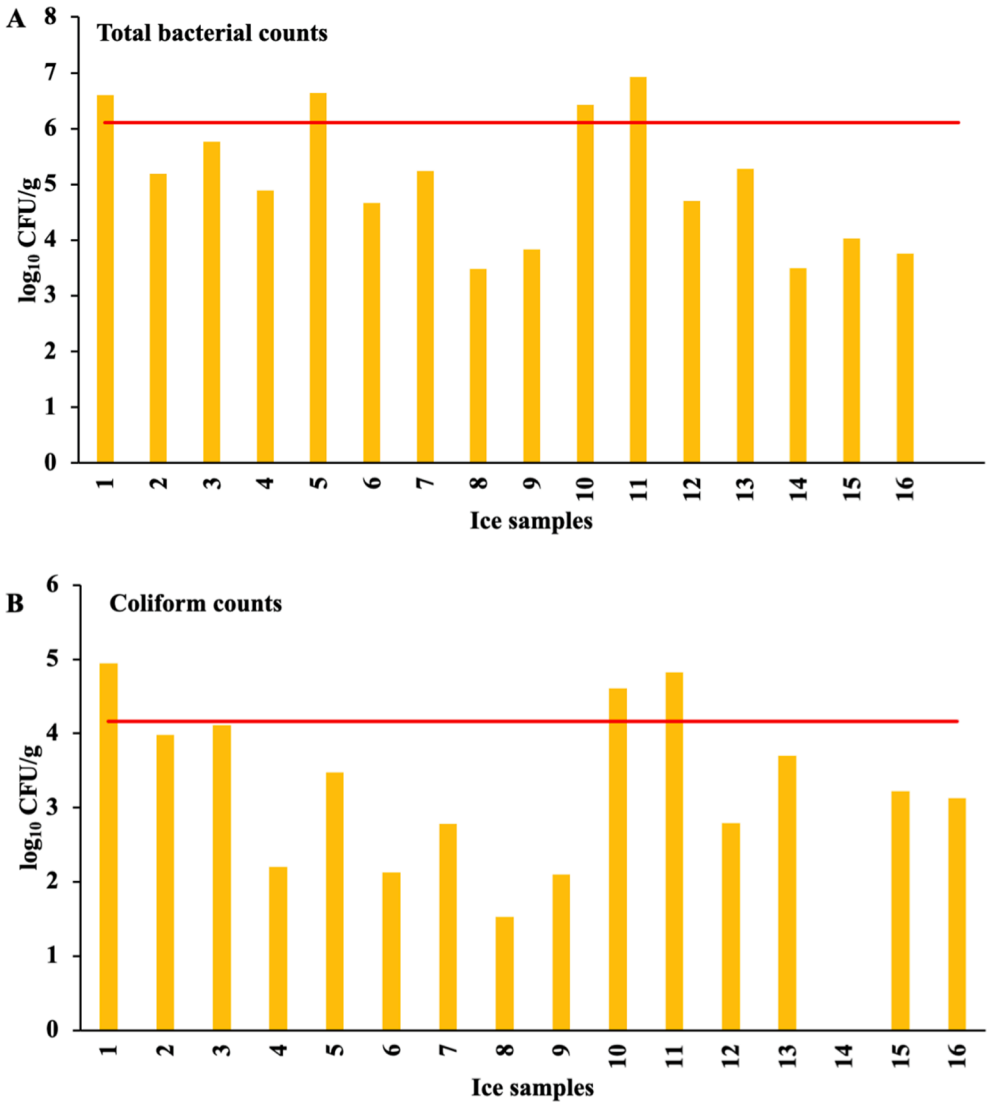

Fig. 4. Colony counts of ice samples for preservation of livestock meat products: (A) Total bacterial counts; (B) Coliform counts. Red lines exhibit the average value of colony counts. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
The prevalence of pathogenic bacteria in food-contact ice samples.

Food sources	Sample counts	Prevalence (%) *Staphylococcus aureus*	*Salmonella*	*Vibrio parahaemolyticus*	*Listeria monocytogenes*	*Shigella*
Aquatic products	128	48.44 (62)*	5.47 (7)	5.47 (7)	1.56 (2)	0.00 (0)
Poultry products	27	48.15 (13)	7.41 (2)	11.11 (3)	0.00 (0)	0.00 (0)
Livestock meat	16	6.25 (1)	6.25 (1)	0.00 (0)	0.00 (0)	0.00 (0)

* The number within bracket represents the counts of positive samples.

Table 4
The acceptable rates of various indicators in food-contact ice samples.

Food sources	Sample counts	Acceptance rate (%) Total bacterial counts	Coliform counts	*Staphylococcus aureus*	*Salmonella*	*Vibrio parahaemolyticus*	*Listeria monocytogenes*	*Shigella*
Aquatic products	128	4.69	3.91	51.56	94.53	94.53	98.44	100.00
Poultry products	27	0.00	0.00	51.85	92.59	88.89	100.00	100.00
Livestock meat	16	0.00	6.25	93.75	93.75	100.00	100.00	100.00

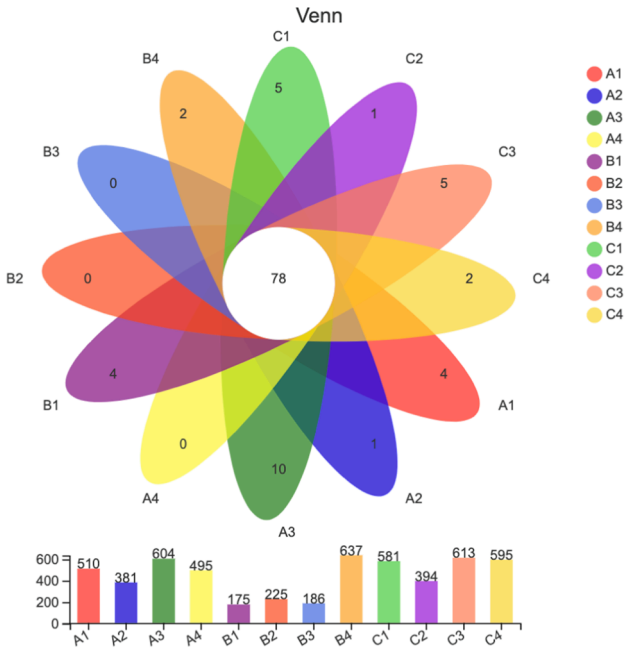

Fig. 5. Venn image of OTUs of different food-contact ice samples.

Table 5
Bacterial diversity indexes of food-contact ice samples.

Group	Samples	Shannon	Simpson	Ace	Chao	Coverage
A	A1	3.46	0.10	651.63	639.73	0.996
	A2	3.28	0.09	473.28	449.30	0.998
	A3	4.05	0.06	688.65	684.93	0.997
	A4	3.54	0.08	631.94	634.04	0.996
B	B1	1.90	0.30	314.38	277.00	0.998
	B2	2.22	0.18	377.08	322.23	0.997
	B3	2.28	0.20	318.66	269.20	0.998
	B4	3.98	0.05	750.29	730.56	0.997
C	C1	3.82	0.05	745.90	736.42	0.995
	C2	3.51	0.05	731.04	613.12	0.995
	C3	4.06	0.04	776.02	769.45	0.994
	C4	4.04	0.04	722.10	708.67	0.995

taken and centrifuged at 6,000 × g for 5 min and resuspended twice in PBS for washing. Add 95 μL DEPC treated water and 4 μL lysozyme (50 mg/ml) for cell lysis with incubation at 37 °C for 15 min. Add 1 μL protease K (20 mg/ mL) to the mixture, and incubate at 58 °C for 50 min, subsequent by incubation at 95 °C for 8 min. Centrifugation was performed at 6,000 × g for 5 min, and the supernatant was collected for PCR analysis. Each PCR system includes 25 μL PCR master mix (Sangon Biotech (Shanghai) Co., ltd., Shanghai, China), 20 μL DEPC treated water, 2 μL forward and reverse primers (10 μM) (Table 1) and 1 μL the extracted DNA. PCR procedure was conducted as follows: 95 °C, 5 min; 95 °C, 10 s, 60 °C, 30 s and 72 °C, 30 s for 40 cycles; 72 °C, 10 min. The quality and size of PCR products were analyzed with Nanodrop (Thermo Fisher, MA, USA) and gel electrophoresis (1 %). PCR products were then purified using the AxyPrep DNA gel recovery kit and were sequenced by ABI3730-XL (Applied Biosystems, USA). The sequencing information was aligned to the NCBI Genebank database with Basic Local Alignment Search Tool (BLAST) analysis. The sequencing alignment results were exhibited in Table S1.

2.4. High throughput sequencing for bacterial diversity

Bacterial cells were collected through the filtration of melted ice with 0.22 μm sterile filters. DNA was then extracted and DNA quality was checked by 1 % agarose gel electrophoresis. Libraries were constructed by amplifying V1-V3 regions of the *16S rRNA* bacterial gene in a PCR thermocycle instrument (GeneAmp® 9700, ABI, USA). Amplicons were detected by 2 % gel electrophoresis and AxyPrepDNA Gel Recovery Kit (Axygen, Darmstadt, Germany) was used for the purification of the target products. The recovered PCR amplicons were quantified by the QuantiFluor™-ST blue fluorescence quantification system (Promega, Inc., WI, USA). According to the quantitative results, the samples were mixed properly to meet the sequencing requirements. The removal of the prominent base at the 5′ end of DNA sequence, and the addition of a phosphate group to complement the missing base at the 3′ end of DNA sequence was achieved through End Repair Mix2. Adenine was added to the 3′ end of DNA sequence to prevent the self-linking of DNA fragments as well as to ensure the connection between the target sequence and sequencing connector. A library specific tag (i.e., Index sequence) was added to the 5′end of DNA sequence to fix DNA molecule on the Flow Cell. BECKMAN AMPure XP beads were then used to remove the joint self-connecting sections and purify the library system. PCR amplification was conducted to enrich the sequencing library templates, and BECKMAN AMPure XP beads were used to purify the products. Agilent High Sensitivity DNA Kit was used for quality inspection of the library in Agilent Bioanalyzer. Subsequently, Quant-iT PicoGreen dsDNA Assay Kit was employed for the library quantification. The qualified libraries (without repeated Index sequences) were diluted appropriately, and mixed according to the required amount for sequencing. The MiSeq sequencer was used for 2 × 300 bp double-ended sequencing with MiSeq Reagent Kit V3. PE reads obtained by Miseq sequencing are spliced according to the overlap relationship, and the sequence quality is determined and filtered. The data were analyzed on the online platform of Majorbio Cloud Platform (https://www.majorbio.com). OTU was used to merge and divide the obtained sequences, and the representative sequences of each OTU were used for classification and phylogenetic analysis. Based on OTU cluster analysis results, the species abundance and diversity of the bacterial populations in different ice samples were analyzed.

2.5. Cross-contamination scenario settings

The transfer between ice and fresh food was simulated, and the contact times of 5, 10, 20, and 30 min were used in this work. Chilled salmon and chicken breast meat were purchased from a local supermarket and cut into 1.8 × 1.8 cm pieces in the laboratory. The meat pieces were then sterilized for 30 min in 75 % alcohol followed by 30-min UVC irradiation. Colony count analysis on the plate count agar (PCA) medium was conducted to confirm the completed decontamination of meat after incubating at 36 ± 1 °C for 48 ± 2 h.

(1) Transfer from food to ice.

S. aureus/ V. parahaemolyticus cocktails (four strains isolated from ice in 2.3) were inoculated evenly on the salmon/chicken breast surface (Fig. 1). The inoculated meat samples were stored on 40 ± 2 g ice for different times (5, 10, 20, 30 min). Then, the salmon/chicken breast piece was transferred to 10 mL sterile normal saline for homogenizing. One milliliter of homogenate and melted ice was mixed with PCA medium, respectively, followed by incubation at 37 °C for 18–24 h, to determine the bacterial counts in the salmon/chicken breast and ice before and after contact. The experiment was independently performed by 5 times.

(2) Transfer from ice to food.

One milliliter of *S. aureus/V. parahaemolyticus* cocktails was inoculated on 40 ± 2 g ice. Sterilized salmon/chicken breast pieces were placed on ice inoculated with *S. aureus/V. parahaemolyticus* for 5, 10, 20, and 30 min (Fig. 1). The bacterial counts of ice and salmon/chicken breast were determined by pour plate method.

2.6. Cross-contamination modeling

The transfer rates of bacteria in different scenarios were fitted with

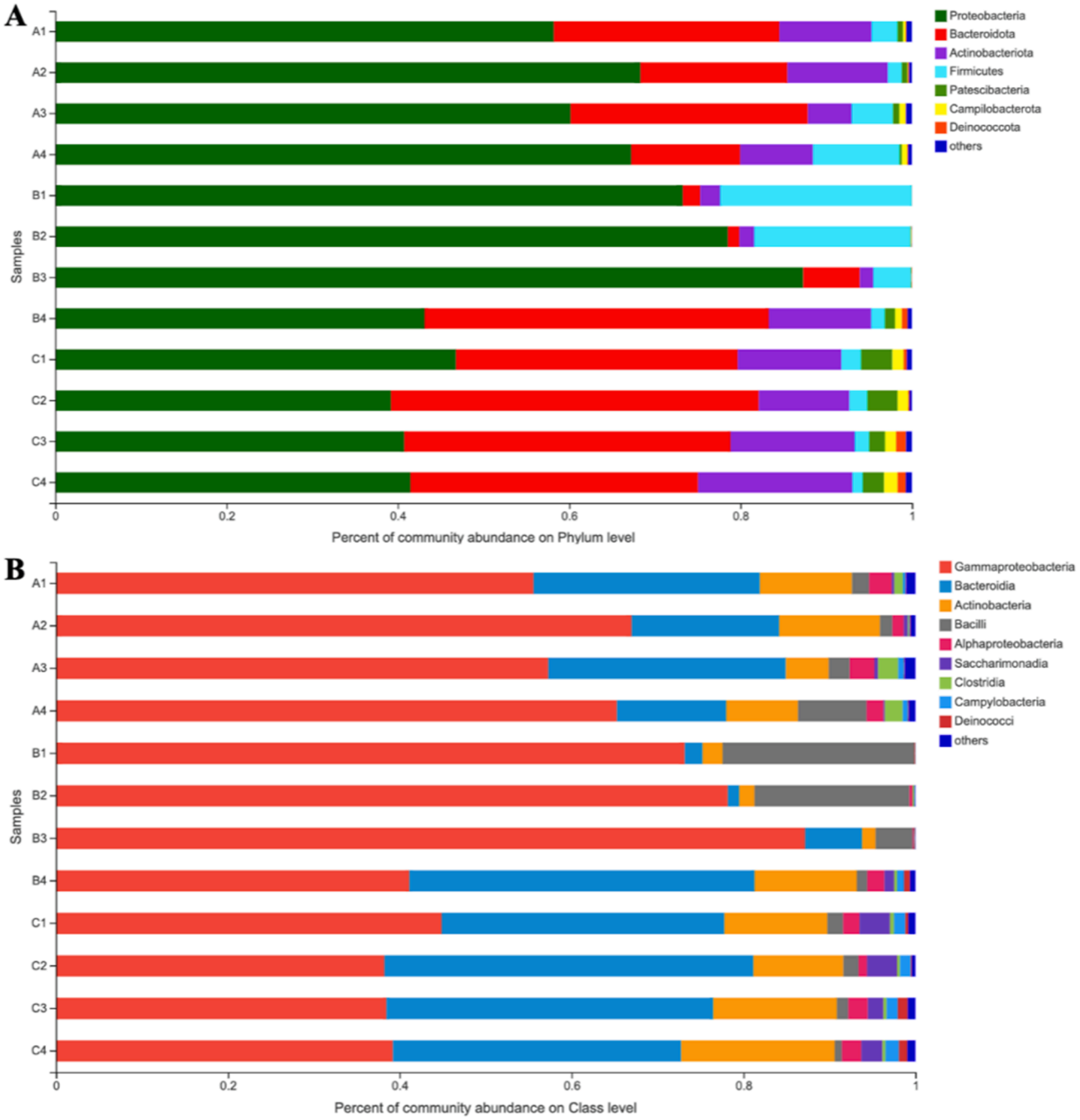

Fig. 6. The bacterial community abundance in (A) phylum and (B) class level.

@risk software, and the optimal distribution model was selected according to the KS and Chi-square test results. The transfer rates of *S. aureus* and *V. parahaemolyticus* between food and ice at different exposure times were calculated according to Equation (1).

$$TR(\%) = N_{recipient}/N_{donor} \times 100 \quad (1).$$

Where TR is the transfer rate, and $N_{recipient}$ and N_{donor} are the bacteria amount (CFU/g) on the ice after cross-contamination and the initial bacterial amount on the foods, respectively.

The cross-contamination model based on the probability distribution of transfer rates was established and is shown as Equation (2) (den Aantrekker et al., 2003).

$$N = N_0 \times TR_1 \times TR_2$$

Where TR_1 and TR_2 are the transfer rate of food-to-ice and ice-to-food, respectively.

The above model is more suitable to describe the cross-contamination process when the transfer level is high. Referring to Pérez-Rodríguez et al. (2006), the binomial distribution B (n, P) was used for modeling the low transfer rate cross-contamination process as exhibited in Eq. (3). P is the transfer probability and n is the number of bacteria. This model assumes that microorganisms are evenly distributed in food.

$$N_{meat1} \sim binomial\left(N_{meat0}, 1 - TR_{meat,ice}\right) + binomial\left(N_{ice}, TR_{ice,meat}\right)$$

Where N_{meat1} is the pollution level (CFU/g) of salmon/chicken breast after cross-contamination; N_{meat0} is the initial contamination level (CFU/g) of salmon/chicken breast. $TR_{meat,ice}$ represents the transfer rate from fresh meat to ice; $TR_{ice,meat}$ is the transfer rate of ice to raw meat.

Goodness-of-Fit of the model is usually assessed by the indexes of the determination coefficient (R^2), root mean square error (RMSE), accuracy

Table 6
Abundance of major bacterial genera in food-contact ice samples.

Samples	Bacterial genera	Abundance (%)
A1	*Polaromonas*	26.20
	Flavobacterium	18.02
	Acinetobacter	16.71
	Staphylococcus	0.01
	Vibrio	<0.01
A2	*Acinetobacter*	24.93
	Polaromonas	15.32
	Pseudomonas	12.85
	Flavobacterium	11.21
	Arthrobacter	10.26
	Vibrio	<0.01
	Staphylococcus	<0.01
A3	*Polaromonas*	19.19
	Flavobacterium	17.31
	Psychrobacter	12.23
	Acinetobacter	10.01
	Staphylococcus	0.01
	Vibrio	<0.01
A4	*Polaromonas*	18.84
	Psychrobacter	18.17
	Acinetobacter	16.70
	Staphylococcus	0.02
B1	*Acinetobacter*	52.70
	Psychrobacter	17.63
	Brochothrix	17.42
	Vibrio	<0.01
	Staphylococcus	<0.01
B2	*Psychrobacter*	59.68
	Acinetobacter	16.42
	Vibrio	<0.01
	Staphylococcus	<0.01
B3	*Pseudomonas*	38.34
	Acinetobacter	26.41
	Psychrobacter	11.68
	Vibrio	<0.01
	Staphylococcus	<0.01
B4	*Flavobacterium*	29.48
	Psychrobacter	18.35
	Vibrio	0.04
	Staphylococcus	0.02
C1	*Psychrobacter*	25.98
	Flavobacterium	20.87
	Staphylococcus	0.01
	Vibrio	<0.01
C2	*Flavobacterium*	27.31
	Psychrobacter	20.84
	Chryseobacterium	12.31
	Staphylococcus	0.01
	Vibrio	<0.01
C3	*Flavobacterium*	25.53
	Psychrobacter	17.59
	Vibrio	0.17
	Staphylococcus	0.01
C4	*Flavobacterium*	21.19
	Psychrobacter	18.84
	Arthrobacter	10.43
	Vibrio	0.08
	Staphylococcus	0.01

factor (A_f) and deviation factor (B_f) (Vorst et al., 2006) as follows:

$$R^2 = 1 - \frac{\sum_1^n (Y_i - \widehat{Y}_i)^2}{\sum_1^n (Y_i - \overline{Y})^2}$$

$$RMSE = \sqrt{\frac{\sum_1^n (Y_i - \widehat{Y}_i)^2}{n}}$$

$$A_f = 10^{\left(\frac{\sum \left|\log\left(\mu_p/\mu_0\right)\right|}{n}\right)}$$

$$B_f = 10^{\left(\frac{\sum \log\left(\mu_p/\mu_0\right)}{n}\right)}$$

Where Y_i is the observed value, $\widehat{Y}_i$ is the predicted value and $\overline{Y}$ is the mean value. μ_p is the predicted value of fresh food contamination level, μ_0 is the observed value of fresh food contamination level, and n is the number of observed values.

2.7. Statistical analysis

All the experiment was conducted at least three times. SPSS statistical analysis software was used for chi-square test analysis of the data, and $p < 0.05$ indicated a statistically significant difference.

3. Results and discussions

3.1. Sources of ice samples

Table 2 shows the sources and percentages of food-contact ice samples investigated in this study. A total of 171 ice samples used for food preservation were collected from farmer markets, supermarkets and restaurants in China. The ice samples used for preserving aquatic products accounted for 74.85 %, and those for cooling poultry and livestock meat had shares of 15.79 % and 9.36 %, respectively.

3.2. The sanitary status of food-contact ice samples

To investigate the sanitary status of food-contact ice, the total bacterial counts and coliform counts as well as the prevalence of pathogenic bacteria in food-contact ice samples were determined. As shown in Fig. 2A, total bacterial counts of the ice samples used to preserve aquatic products were in the range of 0.93 to 6.61 $\log_{10}$ CFU/g (an average level of 4.88 $\log_{10}$ CFU/g), and the coliforms were detected in 123 out of 128 (96.09 %) ice samples with an average contamination value of 3.54 $\log_{10}$ CFU/g (Fig. 2B). As shown in Fig. 3A, total bacterial counts of 27 ice samples for cooling poultry meat ranged from 3.00 to 4.74 $\log_{10}$ CFU/g, with an average level of 4.18 $\log_{10}$ CFU/g. And all the ice samples for preserving poultry were positive for coliform contamination at an average level of 3.17 $\log_{10}$ CFU/g (Fig. 3B). Regarding the ice exposed to livestock meat, the total bacterial counts of 16 ice samples were up to 6.92 $\log_{10}$ CFU/g, and the average pollution level was 6.11 $\log_{10}$ CFU/g. Coliform was negative in only one of 16 ice samples (Fig. 4B).

The prevalence of pathogenic bacteria in food-contact ice samples is exhibited in Table 3. Regarding the ice used to preserve aquatic products, the detection rate of *S. aureus* contamination was the highest with 62 positive samples. The number of samples positive for *Salmonella* or *V. parahaemolyticus* was 7, and two samples were found to be contaminated by *L. monocytogenes*, but *Shigella* was not detected in all samples. As for the ice preserving poultry meat, the prevalence rate of foodborne pathogens arranged from high to low: *S. aureus* (48.15 %), *V. parahaemolyticus* (11.11 %), *Salmonella* (7.41 %), *L. monocytogenes* (0.00 %) and *Shigella* (0.00 %). In this result, it was found that the detection rate of *V. parahaemolyticus* in the aquatic product-contact ice

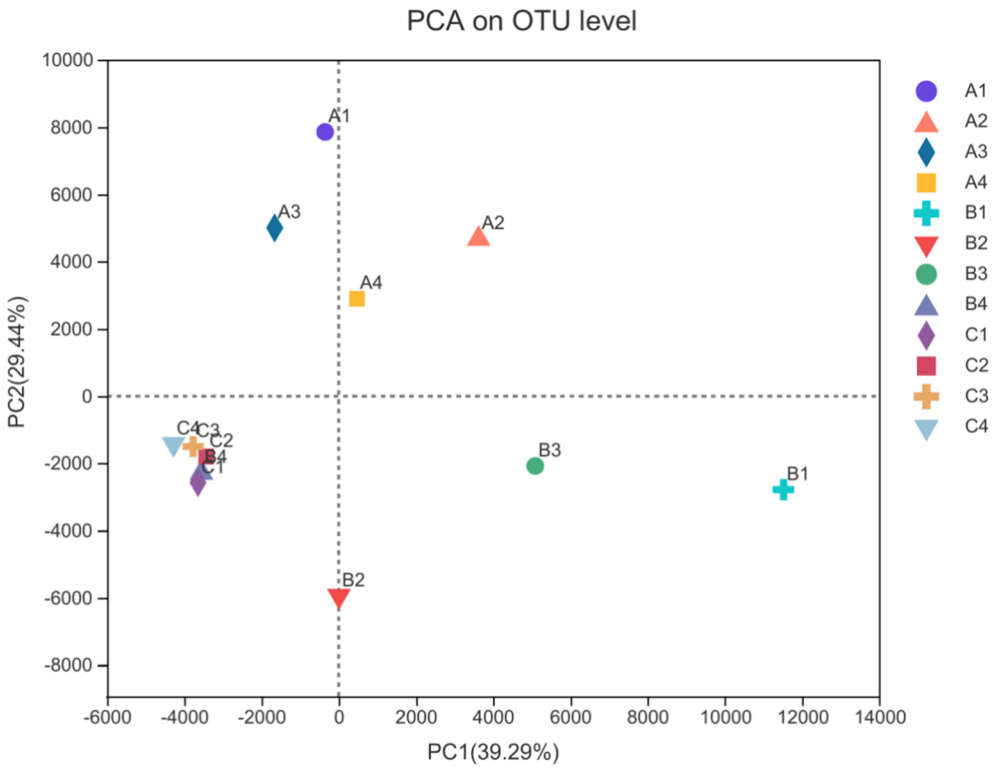

Fig. 7. Principal component analysis of food-contact ice samples.

was lower than that in the ice contacting to poultry meat. It may be due to the unhygienic placement of samples in the market, that is, the ice cubes of fresh-keeping aquatic products are used for the preservation of other fresh foods, as a potential medium for cross-contamination. In general, the main sources of *Salmonella* contamination include infected livestock and raw poultry. *Salmonella* can contaminate food through drinking water, feces, etc., and cause infection through the digestive tract (Levantesi, Bonadonna, Briancesco, Grohmann, Toze, & Tandoi, 2012). The results of this work confirmed that food contact ice also has *Salmonella* contamination, probably being a potential risk of infection. The contamination rates of pathogenic bacteria in the ice for preserving the livestock meat were relatively low, with only one sample positive for *S. aureus* or *Salmonella.* Most of the livestock meat has been stored at low temperatures for a long time, which might cause the death of pathogenic bacteria and result in low contamination levels.

According to the hygiene requirements of International Packaged Ice Association (IPIA) on packaged ice (IPIA, 2005), the acceptance of food-contact ice is defined as: total bacterial counts $\leq$ 500 CFU/g, and coliform and other foodborne pathogens ought to not be detected. As shown in Table 4, the rates of ice samples with acceptable total bacterial and coliform counts were low, especially for those exposed to poultry meat with zero acceptance rate. The contamination of coliform in food associated ice has been reported in previous studies (Mako, Harrison, Sharma, & Kong, 2014; Nichols, Gillespie, & De Louvois, 2000; Noor Izani, Zulaikha, Mohamad Noor, Amri, & Mahat, 2012; Schmidt & Rodrick, 1999). Regarding the pathogenic bacteria, it was found that the rate of ice samples with acceptable counts of *S. aureus* was the lowest, and it was 51.56 % and 51.85 % for those cooling the aquatic products and poultry meat, respectively (Table 4). Similarly, *S. aureus* was detected in fish contact ice with a median value of 271 CFU/100 mL (Teixeira et al., 2019). The survey conducted by Caggiano et al. (2020) also reported the presence of *S. aureus* was detected in 11.1 % of ice samples collected from catering establishments. The contamination of ice might be attributed to the contaminated ice-making machines, unhygienic handling operations and poor water sources (Northcutt and Smith, 2010). Of note, the contamination of pathogenic bacteria in food-contact ice might pose a potential risk to food safety and human through cross contamination.

3.3. Bacterial diversity and dominant taxa in food-contact ice

In arrange to determine the bacterial diversity of food-contact ice, a total of 12 ice samples obtained from three different sites (denoted as group A, B, C) were utilized for high throughput sequencing analysis. The average values of total bacterial counts in food-contact ice collected from A, B, and C were 4.89, 4.27, and 4.61 $\log_{10}$ CFU/g, respectively. Shannon-wiener curves indicated that the sequencing depth of ice samples is suitable for subsequent analysis (Figure S1) and the sequencing information was exhibited in Table S2. Cluster analysis was first conducted on OTUs data to summarize the common or unique OTUs among each sample group. OTU statistics were performed on different ice samples from different sites. A total of 961 OTUs were obtained and the comparisons of OTUs among the samples were shown in Fig. 5. The results indicated that there were 78 OTUs in common among all the ice samples, and the specific OTUs of A and C ice samples were higher that of B ice sample.

Shannon and Simpson indexes are commonly used to estimate bacterial community diversity. The larger the Shannon index and the smaller the Simpson index, the higher the community diversity is implicated (Simpson, 1949). As shown in Table 5, the Shannon indexes of group A and C ice samples were larger than that of group B ice sample (except B4), while Simpson index exhibited the opposite result, indicating that the community diversity of ice samples in A and C was higher than those in group B. Ace and Chao indexes are usually used to reflect the actual number of species (community richness) in the bacterial community. The Chao indexes of A and C ice samples were much higher than that of B ice sample (except B4), indicating a higher abundance of bacteria in groups A and C.

Species annotations were performed for OTUs in food-contact ice samples to compare the species abundance of bacteria in different ice

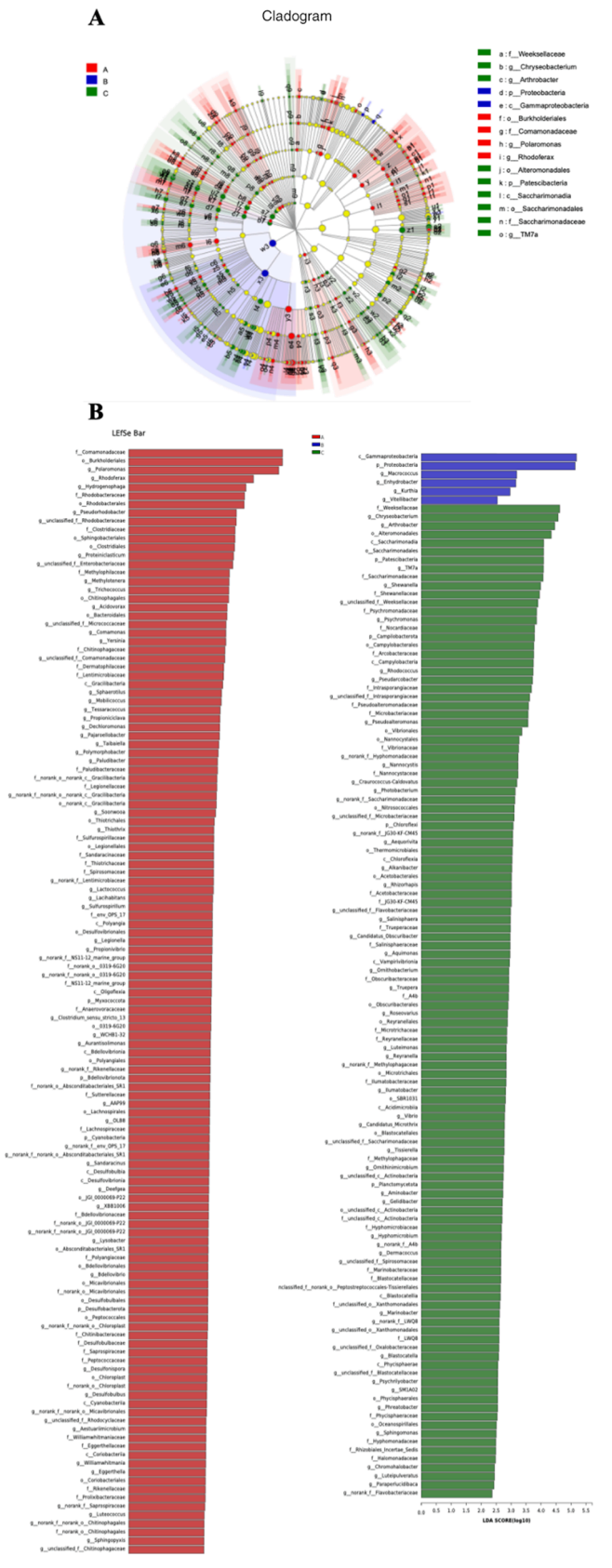

Fig. 8. LEfSe identified the most differentially abundant taxa among various groups of ice samples: (A) Taxonomic cladogram obtained from the LEfSe analysis with a cutoff value of over 2.0. (B) The bacteria taxa with significant abundances among groups A, B, and C.

samples at phylum and class levels (Fig. 6). The phylum of Proteobacteria was widely present in the food-contact samples from A, B, and C groups with the abundance in the range of 58.2 %-68.3 %, 43.1 %-87.3 %, and 39.2 %-46.8 %, respectively. Among the phylum of Proteobacteria, the highest prevalent bacterial class in the food-contact ice was Gammaproteobacteria, which contains approximately 250 genera, such as *Vibrio*, *Escherichia*, and some species of *Salmonella*, corresponding to the results in Section 3.2.

The dominant bacterial genera in food-contact ice samples are exhibited in Table 6. It is found that *Polaromonas* occupied the highest abundance with the range of 15.32–26.20 % in the ice samples of group A. The dominant bacterial genus detected in the food-contact ice from the B group included *Acinetobacte* and *Psychrobacter* with an abundance of over 10 %. *Polaromonas* and *Psychrobacter* are well-known psychrophilic genera with a strong ability to thrive in a cold environment (Kanekar & Kanekar, 2022; Paul, Kazy, Gupta, Pal, & Sar, 2015; Ayala-del-Río, 2010), which could explain the presence of those genera in food-contact ice. At low temperatures, psychrophilic bacteria counteract the kinetic temperature effect by increasing the concentrations of ATP and ADP (key substrates in metabolic and energy conservation reactions), thereby keeping up the reaction rate at low temperature and being able to withstand cold stressors (Amato & Christner, 2009). *Acinetobacter* widely exists in soil, water as well as food (e.g., raw poultry meat), and this bacterial genus is one of the common spoilage organisms, which is able to outlive under a low-temperature habitat (Carvalheira et al., 2021). It has been reported that *Acinetobacter* may colonize the digestive tract by eating contaminated food, coming about in symptoms such as gastroenteritis (Carvalheira et al., 2021), and *Acinetobacter* has also been associated with the dissemination of antibiotic resistance, which may pose an additional concern (Gurung et al., 2013; Marí-Almirall et al., 2019). Regarding C group ice sample, the most abundant genus (>20 %) was *Flavobacterium*, which is characterized by the production of yellow pigment, including over 100 species. Several species of *Flavobacterium* (e.g., *F. psychrophilum*, *F. columnare*) have been associated with the infection of fish (Bernardet et al., 1996). Among the species, *F. psychrophilum* is capable of growing under low temperatures extending 4 to 20 °C (Hesami et al., 2011), which might result in their presence in food-contact ice. All the ice samples from three groups were positive for the genera of *Staphylococcus* and *Vibrio*, which were in accordance with the results of 3.1. The differences in the abundance of these two bacterial genera may be ascribed to the distinct sampling sites. With principal component analysis (PCA) at OUT levels, it is also found that the confidence intervals of bacterial communities among ice samples from groups A, B, and C (from different sampling sites) exhibited high deviations (Fig. 7). Within the same group, the similarity of ice samples from A and C groups were small, but a large heterogeneity of bacterial community structure was observed within group B. Therefore, the bacterial community diversity of food-contact ice was profoundly dependent on the sampling sites.

Linear discriminant analysis (LDA) analysis was used to identify a total of 251 significantly different taxa from phylum to genus in abundance among three groups of ice samples. As shown in Fig. 8, the amounts of significantly different taxa in groups A, B, and C were 129, 6, and 116, respectively. The significantly different taxa in group A ice sample included the order of Burkholeriales, the family of Comamonadaceae, the genera of *Polaromonas*, *Rhodoferax*, and *Hydrogenophaga,* etc. The phylum of Proteobacteria and the class of Gammaproteobacteria were the most specific taxa in group B ice sample. Among group C, the phylum of Patescibacteria, the orders of Alteromonadales, and Saccharimonadales, the families of *Weeksellaceae* and *Saccharimonadaceae*, the genera of *Chryseobacterium*, *Arthrobacter*, and *TM7a*, were the dominant bacteria taxa. Wilcoxonrank-sum test, a non-parametric test for two groups of independent samples, was utilized to identify the differences in bacterial diversity of food-contact ice samples from various sites (Rosner and Grove, 1999). In Wilcoxonrank-sum test, the original assumption is that there is no significant difference in the

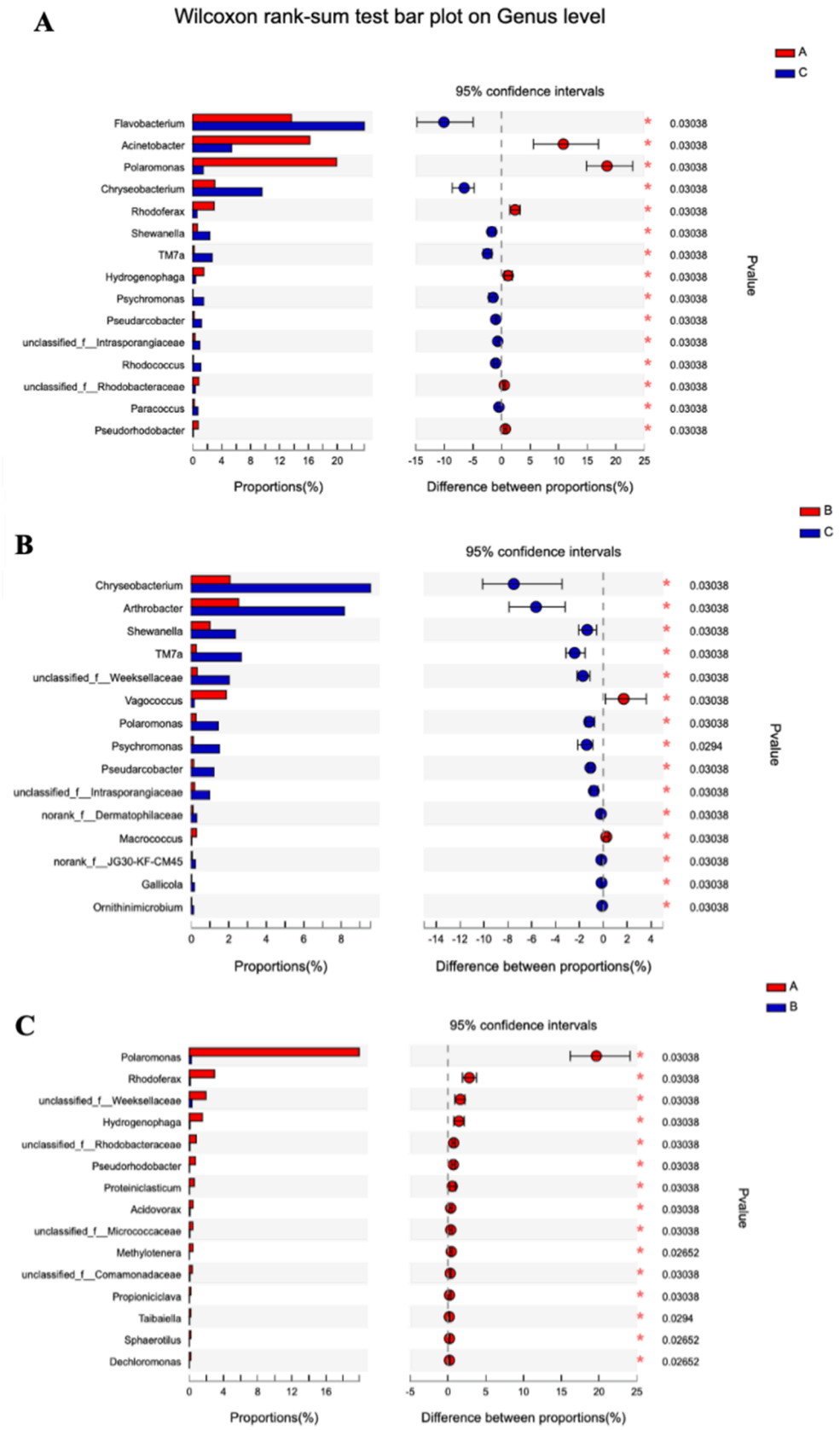

Fig. 9. Wilcoxon rank sum test for analysis of the significantly different bacterial taxa between (A) A and C groups, (B) B and C groups, (C) A and B groups.

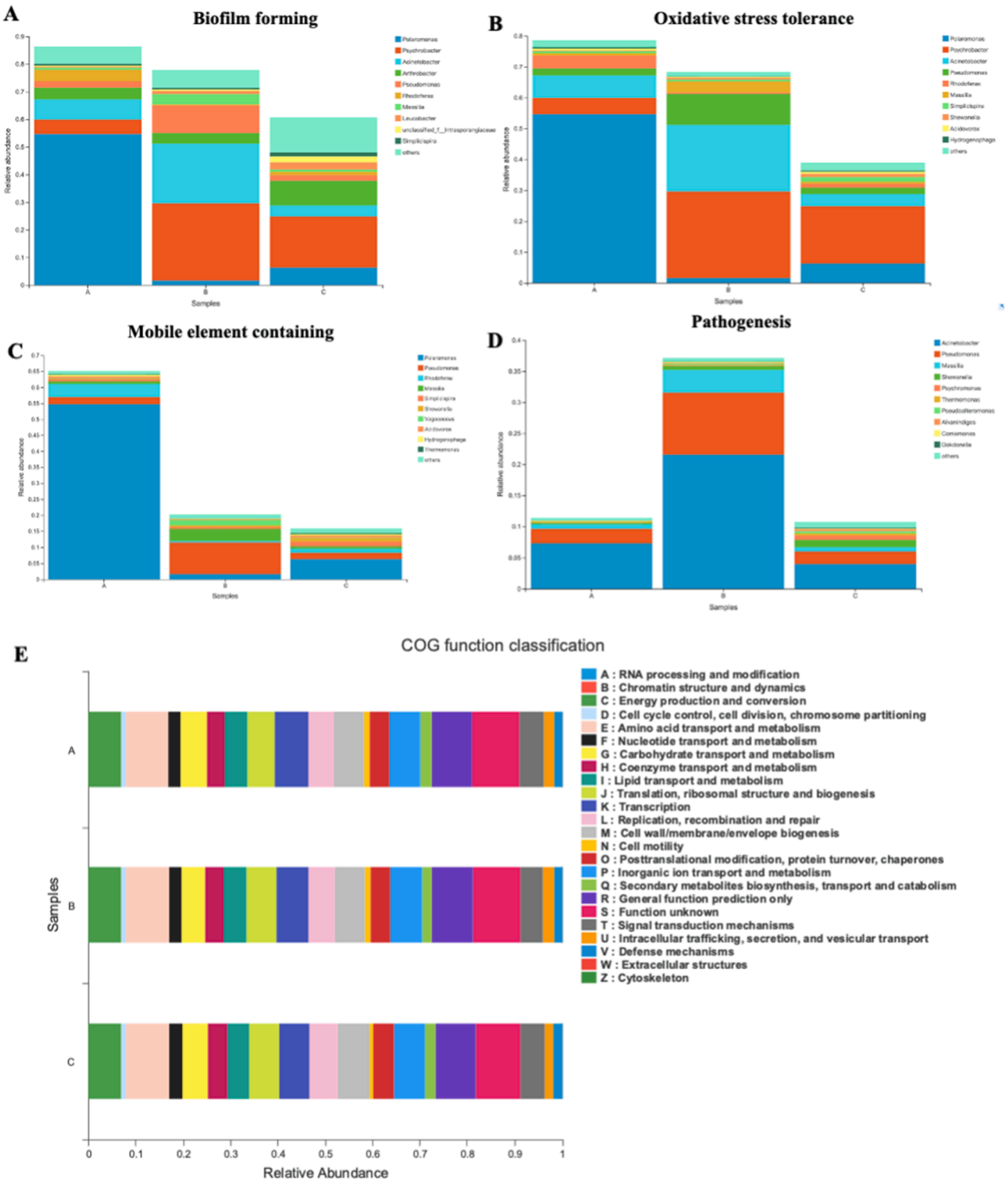

Fig. 10. BugBase analysis of the abundance of bacteria taxa with the phenotypes of (A) biofilm forming, (B) stress tolerance, (C) mobile element containing, and (D) pathogenesis; (E) PICRUSt1 analysis of the bacterial Cluster of Orthologous Groups (COG) profile.

population distribution of two groups of independent samples. The average rank of the two groups of samples is used to determine whether there is a difference in the population distribution of the two groups of samples. The false detection rate (FDR) Q value was calculated for p value. As appeared in Fig. 9, within the genus level, there were noteworthy differences ($p < 0.05$) in the abundance of *Flavobacterium*, *Acinetobacter,* and *Polaromonas* between groups A and C ice samples. The significantly different bacteria taxa between groups A and C ice samples included *Chryseobacterium*, *Arthrobacter*, *Shewanella*, TM7a, etc. Regarding the ice from A and B groups, a total of 15 bacterial taxa with a significant difference in abundance were determined, such as *Polaromonas*, *Rhodoferax*, etc.

The phenotypic function of the bacterial community in different food-contact ice samples were predicted with BugBase . As shown in Fig. 10, the taxa phenotypes associated with biofilm forming, pathogenic, mobile element containing and oxidative stress tolerant was

Table 7
Transfer rates of *Staphylococcus aureus* and *Vibrio parahaemolyticus* during ice preservation.

Transfer rate (%) *	Time (min) 5	10	20	30
$TR_{S,\ salmon\text{-}ice}$	11.28 ± 1.43^{a}	10.81 ± 2.02^{a}	13.39 ± 0.79^{a}	12.50 ± 1.15^{a}
$TR_{V,\ salmon\text{-}ice}$	5.23 ± 1.09^{c}	6.55 ± 0.38^{c}	6.71 ± 1.22^{c}	5.78 ± 0.36^{c}
$TR_{S,ice\text{-}salmon}$	3.40 ± 0.29^{cd}	5.28 ± 1.01^{c}	5.44 ± 1.13^{c}	5,79 ± 2.10^{c}
$TR_{V,\ ice\text{-}salmon}$	1.33 ± 0.90^{de}	2.01 ± 0.39^{de}	2.81 ± 0.59^{d}	2.93 ± 0.34^{de}
$TR_{S,chicken\text{-}ice}$	8.91 ± 1.60^{b}	7.62 ± 0.33^{b}	7.91 ± 1.04^{b}	8.31 ± 0.58^{b}
$TR_{V,\ chicken\text{-}ice}$	3.91 ± 1.01^{c}	2.82 ± 0.48^{d}	2.79 ± 0.92^{d}	3.20 ± 0.49^{de}
$TR_{S,ice\text{-}chicken}$	2.31 ± 1.48^{d}	3.42 ± 0.34^{d}	3.74 ± 0.49^{d}	4.09 ± 1.31^{d}
$TR_{V,\ ice\text{-}chicken}$	1.01 ± 0.33^{e}	1.32 ± 0.71^{e}	2.09 ± 0.43^{e}	2.31 ± 1.20^{e}

* The subscripts of *S* and *V* in TR (transfer rate) represent *Staphylococcus aureus* and *Vibrio parahaemolyticus*, respectively. Salmon-ice, ice-salmon, chicken-ice and ice-chicken represent the pathogen transfer rates from salmon to ice, ice to salmon, chicken to ice, and ice to chicken, respectively. Different lowercase letters in the same column indicated significant difference in the level of metastasis rate at the same time ($p < 0.05$).

determined at the genus level among various groups. The food-contact ice in A group contained the most abundant taxa with biofilm forming, stress tolerance, and mobile element containing characteristics compared with those in the B and C groups. The creation of biofilms can improve pathogenicity and bacterial community stability, enhancing bacterial stress resistance, and the mobile elements is closely related to the transfer of bacterial antibiotic resistance genes, which may lead to the increase and spread of antibiotic resistance (Davey and Otoole, 2000). The predominant bacterial genus *Acinetobacter* found in food-contact ice, for example, has the ability to form biofilms and contains mobile genetic elements such as insertion sequence (IS) elements, which are crucial to the development of drug resistance (Noel, Petrey, & Palmer, 2022). Ice-to-food cross-contamination might lead to the colonization of bacteria with high stress adaptability and antibiotic resistance, posing a risk to the safety of the food. To better understand the physiological function of the bacterial community in food-contact ice samples, PICRUSt1 was employed for the functional prediction of bacterial 16S amplicon sequencing data. As a result, a total of 4309 functional proteins or enzymatic COGs belonging to 24 COG functional classes were identified. Fig. 10E demonstrates that the bacterial community in food-contact ice samples from groups A, B, and C were highly enriched in the activities of amino acid transport and metabolism, transcription, energy generation, and conversion, and inorganic ion transport and metabolism. It demonstrates that the bacteria colonized on food-contact ice are active in protein metabolism and utilization, and they produce amino acids by hydrolyzing protein. These amino acids can then be used to synthesize the substances necessary to ensure survival in the low-temperature environment on ice. The alpha-keto acids generated from the catabolism of amino acids are oxidized into carbon dioxide and water through the tricarboxylic acid cycle, providing energy for self-maintenance.

3.4. Transfer of foodborne pathogens during ice preservation

As shown in Table 7, the transfer rate of *S. aureus* from fresh meat to ice was significantly higher than that of *V. parahaemolyticus* ($p < 0.05$). The transfer rate of pathogenic bacteria from salmon to ice was essentially higher than that from the chicken breast (except for the transfer rate at 5 min, no significant difference was exhibited). The transfer rate of pathogenic bacteria from fresh meat to ice was significantly higher than that from ice to fresh meat ($p < 0.05$). In fact, in the activity test of the two pathogenic bacteria isolated from ice, it was found that under the same culture conditions (37 °C, 18–20 h, 180 r/min), the level of *S. aureus* isolates was about 2×10^9 CFU/ mL, while the average level of *V. parahaemolyticus* isolates was only 10^8 CFU/ mL. The activity of isolated *S. aureus* was higher than that of *V. parahaemolyticus* (data not given). Previous studies have reported that the higher bacterial activity rendered higher transfer rates (Luber et al., 2006; Montville et al., 2003), which could explain the result of higher transfer rates of *S. aureus* than that of *V. parahaemolyticus* in this work. Additionally, the fat content of the food matrix was also found to pose a critical impact on the transfer rate of bacteria, and the higher fat content of the food makes bacteria more difficult to transfer (Lindqvist & Lindblad, 2008; Montville, Chen, & Schaffner, 2001). Salmon contains a lower fat content than chicken breast, which seems to contribute to the higher transfer rate of salmon-ice than the chicken breast-ice observed in this work.

Cross-contamination models during ice preservation were constructed by the transfer rate-based probability model and the binomial distribution B (n, P). The indexes of R^2, A_f and B_f are commonly used for implicating the goodness-of-fit of models, and the values of R^2, A_f and B_f closer to 1 indicate a good fitting of models to data (Perez-Rodriguez & Valero, 2013; Ross, 1996). As shown in Table 8, for the group 1 to 12, the R^2 values of 0.90–0.93, A_f values of 1.08–1.12, and B_f values of 0.94–1.15 were determined for the established transfer rate-based probability models, which indicated better fitness in comparison to

Table 8
Experimental results of 16 groups.

Group*	Time (min)	V_{p1} (CFU/g)	V_{p2} (CFU/g)	V_{ob} (CFU/g)	A_{f1}	A_{f2}	B_{f1}	B_{f2}	R_1^2	R_2^2
1	5	5.30×10^5	5.10×10^5	5.90×10^5	1.12	1.20	0.96	0.97	0.91	0.85
2	10	4.30×10^5	5.00×10^5	3.70×10^5						
3	20	5.30×10^5	5.00×10^5	5.90×10^5						
4	30	5.40×10^5	5.40×10^5	6.00×10^5						
5	5	8.20×10^5	9.20×10^5	8.10×10^5	1.15	1.25	1.15	1.25	0.90	0.77
6	10	8.80×10^5	9.80×10^5	7.10×10^5						
7	20	9.50×10^5	9.50×10^5	7.40×10^5						
8	30	8.50×10^5	9.50×10^5	7.80×10^5						
9	5	5.40×10^5	5.00×10^5	6.00×10^5	1.08	1.19	0.94	0.85	0.93	0.81
10	10	6.10×10^5	5.10×10^5	6.80×10^5						
11	20	5.40×10^5	5.40×10^5	5.30×10^5						
12	30	5.70×10^5	5.00×10^5	6.10×10^5						
13	5	8.90×10^5	9.90×10^5	8.60×10^5	1.18	1.10	1.18	1.10	0.89	0.92
14	10	1.00×10^5	1.00×10^5	8.50×10^5						
15	20	9.90×10^5	9.90×10^5	9.70×10^5						
16	30	9.90×10^5	9.90×10^5	9.20×10^5						

* Groups 1–4 and 5–8 were the transfer of *Staphylococcus aureus* and *Vibrio parahaemolyticus* between salmon and ice, respectively, and groups 9–12 and 13–16 were the transfer of *Staphylococcus aureus* and *Vibrio parahaemolyticus* between chicken breast and ice, respectively. The subscripts 1 and 2 represent the cross-contamination models constructed by using Equations (2) and (3), respectively. The subscripts p and ob represent the predicted and observed values, respectively.

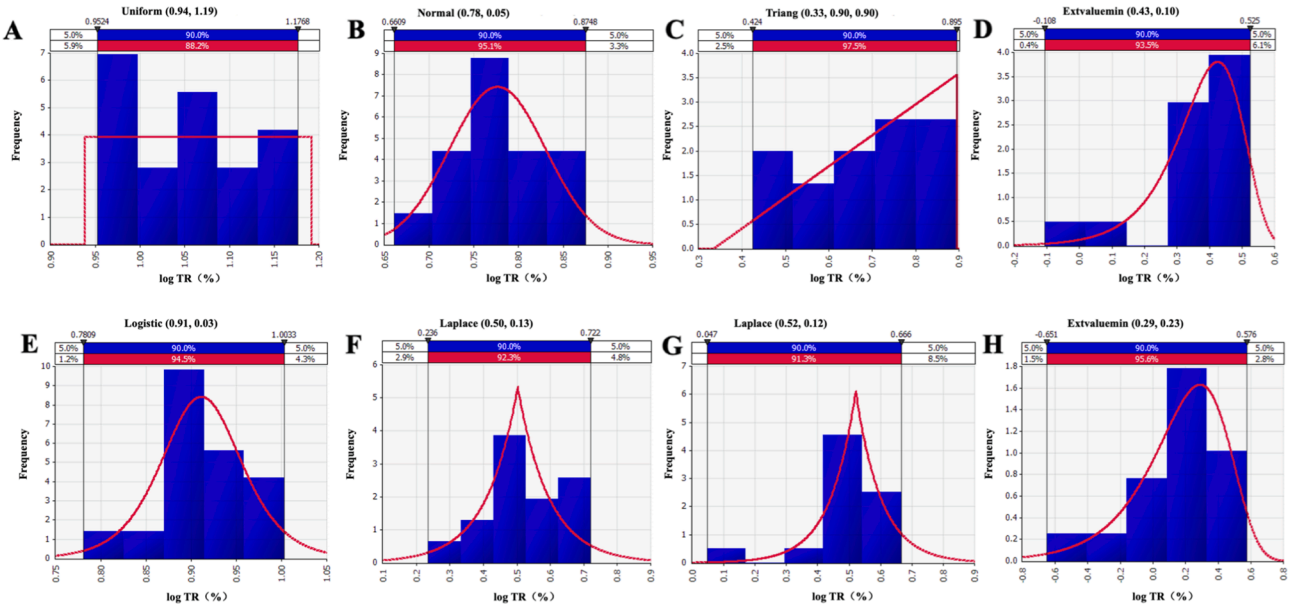

Fig. 11. Distribution models of transfer rate: (A) $TR_{S,\ salmon\text{-}ice}$, (B) $TR_{V,\ salmon\text{-}ice}$, (C) $TR_{S,\ ice\text{-}salmon}$, (D) $TR_{V,\ ice\text{-}salmon}$, (E) $TR_{S,\ chicken\text{-}ice}$, (F) $TR_{V,\ chicken\text{-}ice}$, (G) $TR_{S,\ ice\text{-}chicken}$, (H) $TR_{V,\ ice\text{-}chicken}$.

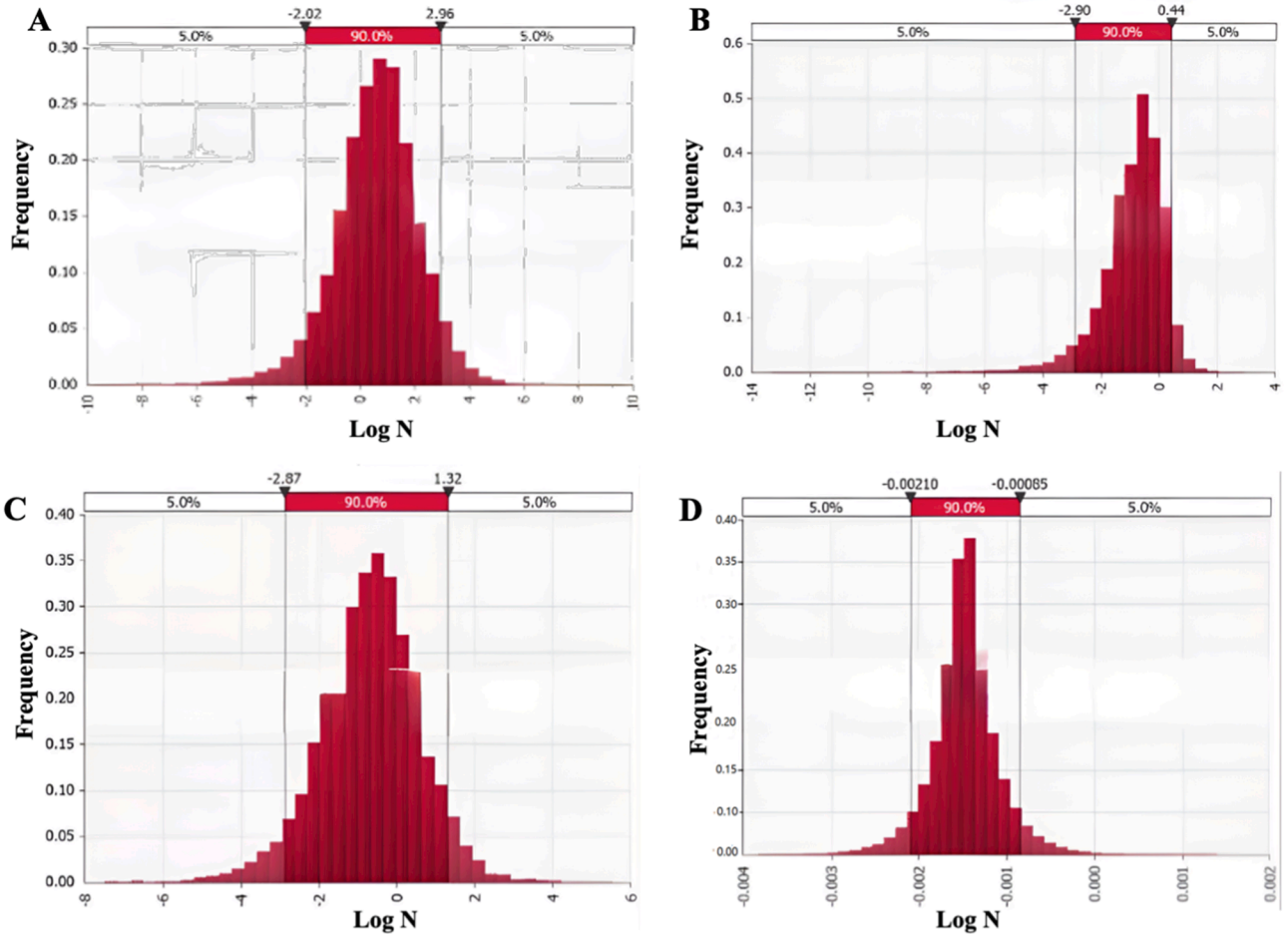

Fig. 12. Probability distribution of contamination levels of (A) *Staphylococcus aureus* and (B) *Vibrio parahaemolyticus* during the ice preservation of salmon, (C) *S. aureus* and (D) *V. parahaemolyticus* during the ice preservation of fresh chicken breast.

binomial distribution B (n, P) model. But in groups of 13–16, the fitness of the binomial distribution B (n, P) model exhibited a better fit to the data. As shown in Table 7, the transfer rates of groups 1 to 12 were higher than those of groups 13–16, indicating that the transfer rate-based probability model was more suitable to describe the cross-contamination during ice preservation in the case of high transfer rates, while regarding the low transfer rates, the binomial distribution B (n, p) was better to describe the cross-contamination process during ice preservation.

The @risk software was used to fit the logarithmic transfer rate data of bacteria in different scenarios. According to the KS and Chi-square test, the best fitted distributions are shown in Fig. 11. $TR_{S,salmon\text{-}ice}$, $TR_{V,\ salmon\text{-}ice}$, $TR_{S,ice\text{-}salmon}$, $TR_{V,\ ice\text{-}salmon}$, $TR_{S,chicken\text{-}ice}$, $TR_{V,\ chicken\text{-}ice}$, $TR_{S,ice\text{-}chicken}$ and $TR_{V,\ ice\text{-}chicken}$ were best fitted by Uniform (0.94, 1.19), Normal (0.78, 0.05), Triang (0.33, Extvaluemin (0.43, 0.10), Logistic (0.91, 0.03), Laplace (0.50, 0.13), Laplace (0.52, 0.12), and Extvaluemin (0.29, 0.23), respectively. Uniform distribution, Normal distribution, and Logistics distribution are widely used to describe bacterial growth, transfer and other processes (Lindqvist and Lindblad, 2008). A normal distribution is ued to describe the uncertainty and variability of bacterial transfer (Hoelzer et al., 2012; Jensen et al., 2015; Jiang et al., 2018). Logistics distribution is widely used to fit the logarithmic transfer rates of contact (Montville et al., 2001), cleaning (Jensen et al., 2015), cutting (Hoelzer et al., 2012), and other cross-contamination processes. As shown in Fig. 10, it indicated that the Extvaluemin distribution was a good fit for the logarithmic transfer rate of *V. parahemolyticus* from ice to salmon or chicken breast. Extremum distribution refers to the probability distribution of maximum (or minimum) values in probability theory (Lin, 2014). In this work, it is found that compared with Normal distribution, Laplace distribution was better to describe the logarithmic transfer rate of *V. parahaemolyticus* from chicken breast to ice and the logarithmic transfer rate of *S. aureus* from ice to chicken breast according to the Chi-square test.

All parameters (transfer rate distribution, initial contamination level, initial contamination rate) were substituted into the constructed cross-contamination models as random variables. The initial contamination levels of food-contact ice were generated from 3.1, and the initial contamination levels of salmon were obtained from the results of Liang et al. (2016) and Tang (2013). The initial contamination levels of chicken breast meat were obtained based on the results of Hurley et al. (1983). The transfer rate distribution models were derived from the above fitting results. Monte Carlo simulation was used to randomly assign values from the distribution interval of each parameter to express the uncertainty and variability of parameters. Latin-Hypercube sampling was used in the simulation, with 10,000 iterations each time, to simulate the final pollution level of fresh food in the process of ice preservation. As shown in Fig. 12, during ice preservation, the ice-mediated cross contamination of fresh food contributed to the contamination levels of *S. aureus* and *V. parahaemolyticus* in salmon ranging from −2.02 to 2.96 ($\log_{10}$ CFU/g) and −2.90 to 0.44 ($\log_{10}$ CFU/g) (90 % confidence interval), respectively. The ice-mediated cross-contamination induced the transfer amounts of *S. aureus* to the fresh chicken breast was −2.87 to 1.32 ($\log_{10}$ CFU/g), much higher than *V. parahaemolyticus* with a range of −0.00210–0.00065 ($\log_{10}$ CFU/g).

4. Conclusions

In this work, the hygiene status of food-contact ice from farmer markets, local supermarkets, and restaurants was determined through the estimation of total bacterial counts, coliform counts, and pathogenic bacteria contamination levels. It is found that the ice for preserving poultry meat and aquatic products had a high prevalence of *S. aureus*, *Salmonella*, *V. parahaemolyticus*, and *L. monocytogenes*. High-throughput sequencing investigation demonstrated that the dominant bacteria taxa in food-contact ice were distinctive and intensely influenced by the environment of inspecting destinations. The anticipated phenotypes of biofilm forming, oxidative stress tolerance, mobile element containing and pathogenesis were identified within the bacteria taxa of food-contact ice, which should be carefully assessed in future work. Moreover, the cross-contamination models during ice preservation were established upon the conditions of high or low transfer rates between ice and foods. Monte Carlo simulation with Latin-Hypercube sampling was further carried out and it is anticipated the defilement levels of *S. aureus* and *V. parahaemolyticus* on raw salmon or chicken breast as the result of cross contamination via ice contacting ranging from −2.90 to 2.96 $\log_{10}$ CFU/g with a 90 % confidence interval. The results of this study indicated that unhygienic food-contact ice could be the vesicles of microbial cells to food and human, exhibiting potentially tremendous health implications. The hygienic management of food-contact ice is of significance and urgency in future research.

CRediT authorship contribution statement

Xinyu Liao: Investigation, Data curation, Writing – original draft, Visualization. **Wangwang Shen:** Investigation, Methodology, Visualization. **Yeru Wang:** Writing – review & editing. **Li Bai:** Writing – review & editing. **Tian Ding:** Supervision, Conceptualization, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2022.112335.

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