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Research Paper

Microbial Composition and Diversity of High-demand Street-vended Foods in Ecuador

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ABSTRACT

Developing countries such as Ecuador carry a heavy food safety burden but reports on the microbiological quality of their foods are scarce. In this investigation, the microbial diversity of 10 high-risk and mass-consumption street-vended foods including bolones, encebollado, food dressings, ceviche, chopped fruits, fruit juices, fruit salads, cheese, raw chicken, and ground beef in Quito, Guayaquil, and Cuenca, three major population centers in Ecuador, were evaluated using 16S rRNA gene High Throughput Sequencing. In total, 1,840 amplicon sequence variants (ASVs) were classified into 23 phyla, 253 families, 645 genera, and 829 species. In the tested food samples, Proteobacteria and Firmicutes were the most abundant phyla accounting for 97.41% of relative abundance (RA). At genus level, 10 dominant genera were identified: *Acinetobacter* (12.61% RA), *Lactococcus* (12.08% RA), *Vibrio* (8.23% RA), *Weissella* (7.43% RA), *Aeromonas* (6.18% RA), *Photobacterium* (6.32% RA), *Pseudomonas* (3.92% RA), *Leuconostoc* (3.51% RA), *Klebsiella* (3.49% RA), and *Cupriavidus* (2.86% RA). The highest microbial diversity indices were found in raw chicken, encebollados, fruit salads, and fruit juices from Guayaquil and Cuenca. From sampled foods, 29 species were classified as food spoilage bacteria and 24 as opportunistic pathogenic bacteria. Two groups associated with human diseases were identified, including 11 enteric species and 26 species of fecal bacteria. The occurrence of recognized and opportunistic pathogenic bacteria, as well as enteric and fecal microorganisms, in the street-vended foods indicated extensive risks for the consumers' health. This study demonstrated the application of culture-independent amplicon sequencing in providing a more comprehensive view of microbial safety for street-vended food, which could be a useful tool to facilitate the control of foodborne diseases.

Foodborne diseases (FBDs) encompass nearly 200 different illnesses ranging from diarrhea to cancer. The World Health Organization (WHO) estimates that 600 million people are annually affected, and half a million people die each year because of FBD (World Health Organization, 2020). Despite having highly functional national food safety programs, the burden of FBD is considered significant in high-income countries (HICs). In the US, the estimated annual cost of the diseases caused by 15 foodborne pathogens is $17.8 billion (Hoffmann, 2020; Hoffmann et al., 2017). Similarly, in the Netherlands, the total cost of diseases caused by 14 food-related pathogens and their sequelae is estimated at €468 million each year (Mangen et al., 2015). In low- and-middle-income countries (LMICs), the World Bank (2018) estimated that the total productivity loss associated with FBD was around $95.2 billion yearly. In Caribbean countries, foodborne illnesses are estimated to generate $700,000 to $19 million in annual health costs (PAHO & WHO, 2019). The data obtained by the WHO Foodborne Disease Burden Epidemiology Reference Group (FERG) regarding the global burden of FBD in LMICs are considered very conservative (Havelaar, 2019) because of the lack of accurate data-acquisition mechanisms, the absence of comprehensive baseline studies, and inadequate methodologies to establish nationwide food safety programs (Vipham et al., 2018). In this sense, the need to imple-

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ment precise, efficient, and low-cost technologies to generate data associated to the safety of foods in LMICs has been emphasized (WHO, 2018).

Street-vended foods are in high demand in Ecuador and many Latin American countries as a means of providing fast, inexpensive, and convenient access to food, and as an important income source for many families. Food sale on public streets increased recently in many cities in Ecuador, partially due to the economic downturns caused by the COVID-19 pandemic (Allison et al., 2021). Food safety is a major concern associated with these types of foods due to the lack of hygiene standards, required sanitizing supplies, and potable water, increasing the probability of contamination with foodborne pathogens and spoilage microorganisms during food preparation, storage, and sale (Birgen et al., 2020). However, the microbial safety and quality of street-vended foods from Ecuador are still poorly documented.

DNA sequencing technologies such as Next Generation Sequencing (NGS) have been rapidly improving and are becoming widely used to assess the microbiological quality of foods (Cardinali et al., 2017; FAO/WHO/AU, 2019; van Hoorde & Butler, 2018). NGS targeting the 16S rRNA gene is one of the commonly used methods to characterize bacterial communities (Brown et al., 2019; Lewis et al., 2020). Microbiome studies using NGS have been performed to evaluate the microbiological quality of foods in HIC such as Belgium, Italy, Republic of Korea, and USA (Ceuppens et al., 2017; Ferrario et al., 2017; Gu et al., 2018, 2020; Kim et al., 2019; Yi et al., 2020), but similar applications in LMICs such as Ecuador have not been reported. The present research aimed to evaluate the bacterial diversity in high-demand street-vended foods in Ecuador using NGS.

Materials and Methods

Study design and sample collection

Based on previously investigated food samples by Salazar-Llorente et al. (2020), samples of 10 common street-vended foods were purchased from popular farmers markets in the three largest cities in Ecuador, Cuenca (CUE), Guayaquil (GYE), and Quito (UIO) as shown in Table 1.

The food samples once purchased were immediately transported in insulated boxes with ice packs to the respective laboratories of the participating Universities of each sampled city within ~2 h, Escuela Politécnica Nacional (EPN) in Quito, University of Cuenca (UC) and the Escuela Superior Politécnica del Litoral (ESPOL) in Guayaquil. Food samples were purchased daily until 10 food commodities were completed. This was conducted based on the laboratory's capacity on sample processing and DNA extraction on the sampling day to avoid food deterioration.

Sample preparation, DNA extraction, and 16S rRNA gene high throughput (HT) sequencing

Bacterial cells from collected food samples were harvested according to a previous study (Gu et al., 2018) with a few modifications. Briefly, 20 g of each sample was transferred to sterile bags (Neogen, Lansing, MI) with 50 mL of 1x phosphate-buffered saline (PBS, pH 7.2, Thermo Fisher Scientific, Waltham, MA). The sample was then sonicated at 40 kHz for 1 min in an FS110D ultrasonic bath (Fisher Scientific, USA), followed by gentle hand rubbing. To remove food debris, 2 ml of the suspension was centrifuged for 2 min at 1,000 × *g* in a microcentrifuge 5424 with rotor FA-45-24-11 (Eppendorf®, USA). Then, 800 µl of the supernatant was centrifuged at 14,000 × *g* for 10 min to concentrate the bacterial cells. After removing the supernatant, the bacterial cell pellet was resuspended in 500 µl of 1 × PBS and centrifuged again at 14,000 × *g* for 10 min. DNA was extracted from cell pellets using the PureLink ™ Genomic DNA Mini Kit (Invitrogen, USA) following the manufacturer's instructions and stored at −80°C until further processing.

Sample DNAs were processed for 16S rRNA gene HT sequencing using the barcode primer sets 515F − 806R for MiSeq (Illumina, USA) following the Earth Microbiome Project protocol (Caporaso et al., 2011, 2012), and sequencing was conducted using MiSeq (Illumina, San Diego, CA) with MiSeq Reagent 600-cycles v3 kits as described in a previous study (Gu et al., 2018).

A total of 8,857,194 pairwise raw reads were generated for the 169 tested food samples. MiSeq sequence data were sorted based on unique barcodes and quality-controlled using Quantitative Insights into Microbial Ecology (QIIME2, version 2021.4) reads were demultiplexed using the demux plug-in (Bolyen et al., 2019). After quality check and merging of the forward and reverse reads, 6,711,008 reads in total were obtained with a range between 8,151 and 153,321 reads per sample. The 16S rRNA gene HT sequencing data were submitted to

Table 1
Ten types of foods tested from the three largest cities in Ecuador

Food	Description	Number of samples		
		CUE*	GYE	UIO
Bolones (B)	Fried green banana puree, round and filled with cheese	4	4	4
Ceviches (C)	Ecuadorian ceviche is made with seafood and shrimp already cooked and served in the same cooked juice with lemon, onion, and tomato	4	4	4
Chopped fruit (CF)	In Ecuador, watermelon, pineapple and mango are sold in plastic bags or cups, these fruits are prepared and chopped on trays, close to the selling point in the street.	4	5	4
Fresh cheeses (CHE)	Fresh cheese tested in this study was soft and moist, which was made using unpasteurized raw milk withoutaging.	2	15	5
Chicken (CHI)	The chicken is slaughtered near the place of sale, the meat is washed with tap water, and is not refrigerated. Usually, chicken carcasses are displayed on hooks or trays for sale.	8	15	6
Encebollados (E)	In Ecuador is an onion fish soup made with fresh tuna, yuca or cassava root, tomatoes, onions, cilantro, spices and is served with curtido or pickled onions and tomatoes on top or mixed in with the soup. The pickled or lime-marinated red onions are what give it the name “encebollado”.	4	4	4
Fruit salad (FS)	Also known as “*come y bebe*”, which translates as eat and drink. It is an Ecuadorian tropical fruit salad made with fruits including papaya, bananas, pineapple and orange juice, the fruit is cut into small pieces and usually has a good amount of orange juice. These salads are sold using buckets on busy street corners. Vendors add ice to the center of the bucket to preserve them.	4	4	4
Ground beef (GB)	Street vendors buy meat from wholesale slaughterers and sell it in carts that have refrigerated compartments with ice, the raw meat is displayed on hooks and ground when selling.	7	15	7
Fruit juices (J)	Soft drinks (watered-down fruit juices) made from pulp of seasonal fruit but mainly with orange, lemon and coconut.	3	5	4
Food dressing (S)	Ecuadorian lime pickled red onions, chili marinades and egg and oil based knows as homemade mayonnaise.	4	4	4

* Cuenca (CUE), Guayaquil (GYE), and Quito (UIO).

the National Center for Biotechnology Information (NCBI) under the accession number PRJNA629846.

Bioinformatic analysis

The paired-end fastq files were then further processed in the R environment v.4.1.3 (*R: The R Project for Statistical Computing*, 2021) through the DADA2 pipeline v.1.18.0 (Callahan et al., 2016, 2019). Taxonomic assignment of the amplicon sequence variants (ASVs) was performed using the DADA2-formatted reference SILVA database and optimized for classification of prokaryotic 16S rRNA gene sequencing data (v.138.1) at genus and species levels using "assignTaxonomy" and "addSpecies" packages, respectively. Species-level identification was based on 100% identity between the reference database and ASVs. When identification at certain taxonomic ranks was not available, the taxonomic name of its higher classification level with the word "Unspecified" was assigned (Edgar, 2018; McLaren & Callahan, 2021).

The tables with the number of reads per sequence of the 16S rRNA gene, the taxonomic lineage of these sequences, the phylogenetic tree that relates these sequences to each other, and the contextual samples metadata with the variables associated were combined in a single object using the R package "phyloseq" (v.1.34.0) (McMurdie & Holmes, 2013). Initial data exploration, filtering, and basic microbial community analysis were conducted using the R package phyloseq. To perform quality control for the dataset obtained in the phyloseq object, irrelevant taxa were filtered to exclude ambiguous annotations, non-bacterial, and non-archaeal ASVs. NA or unspecified taxa was formatted to show a higher taxonomic level.

Microbial community and statistical analysis

Through taxonomic and compositional analysis, a shared microbiome was identified for food samples across sampling cities, "trans_venn class" function was used for venn analysis from the "microeco" R package. Venn analysis was made at the ASV level using the sampling city as group factor where the integer is ASV number and percentage data resulted from division quotient between sequence number and total sequence number, average relative abundances of shared genera from all observed taxa were calculated among sampling cities. The most abundant phyla (RA > 1%) in food samples were presented by the taxa barplot. Identified species of potential foodborne bacterial pathogens were also presented by a heatmap using r-package "ampvis2", genera from foodborne grouped taxa were aggregated in y-axis and then relative abundance of each taxon in each food sample (x-axis) was calculated and indicated by gradient coloring of the tiles.

Alpha and beta diversity analyses were conducted based on the sequencing data after normalization. The differences among groups were analyzed by one-way ANOVA with multiple comparisons of Duncan's multiple-range test, and $P < 0.05$ was considered as statistically significant. The same objects used for the construction of the phyloseq object were used to create an object of microtable class (R6 class) using "microeco" R package (v.0.4.1) (Liu et al., 2021) to analyze differential abundance, redundancy, and functional profiles trough classes as functions included in the package.

Results

Overview of 16S rRNA gene HT sequencing

The taxonomic assignment using the Silva 138.1 database identified a total of 2,500 ASVs, including 10 archaea, 2,486 bacteria, three eukaryotes, and one unassigned ASV. After filtering eukaryotic and false-positive taxa, 1,840 bacterial ASVs were classified into 23 phyla, 253 families, 645 genera, and 829 species.

Dominant bacteria among three cities

From the 23 phyla annotated in samples from the three cities, five dominant phyla together accounted for 99.95% of total RA (Fig. 1), including Proteobacteria (63.95%), Firmicutes (33.46%), Bacteroidota (1.03%), Actinobacterota (0.85%), and Campylobacterota (0.65%).

From the 645 genera annotated, 13 belonging to Proteobacteria showed RA > 1% including *Acinetobacter* (12.61%), *Vibrio* (8.23%), *Photobacterium* (6.32%), *Aeromonas* (6.18%), *Tatumella* (5.03%), *Pseudomonas* (3.92%), *Klebsiella* (3.49%), *Cupriavidus* (2.86%), *Shewanella* (1.96%), *Marinomonas* (1.64%), Unspecified-Enterobacterales (1.39%), *Pseudoalteromonas* (1.34%), and *Pantoea* (1.09%), as well as

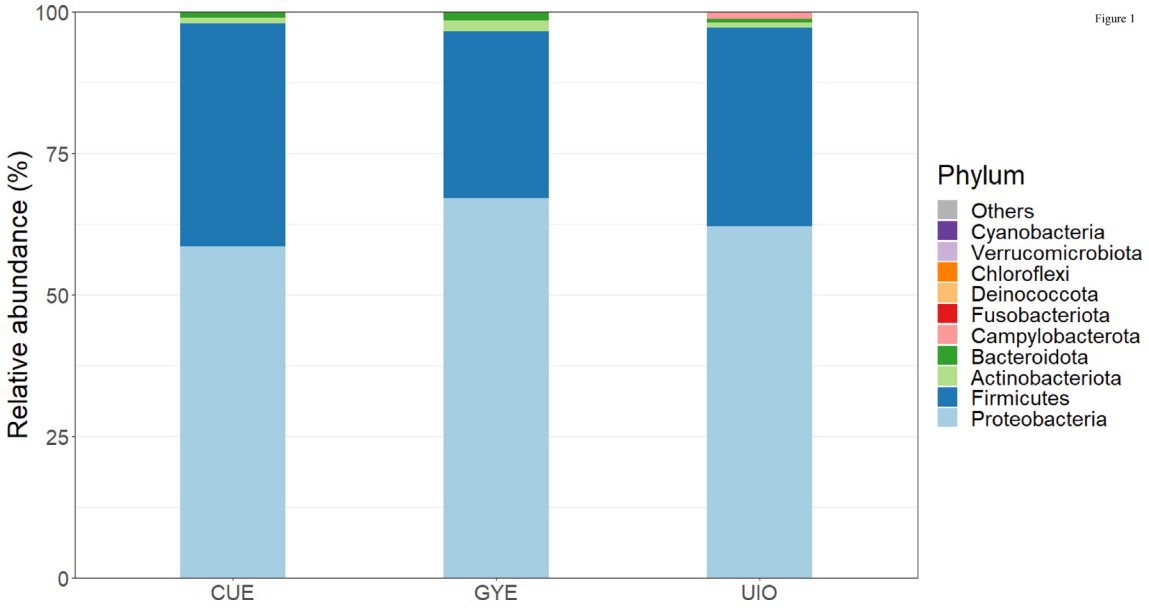

Figure 1. Top 10 most abundant phyla annotated from each sampling city. Less abundant Phyla were grouped as "Others". CUE: Cuenca, GYE: Guayaquil, and UIO: Quito.

six Firmicutes genera, including *Lactococcus* (12.08%), *Weissella* (7.43%), *Leuconostoc* (3.51%), *Streptococcus* (1.60%), *Lactiplantibacillus* (1.46%), and *Kurthia* (1.31%) (Fig. 2). Although the RA of these genera varied, a very comparable trend was observed for these genera among the most dominant foods from all the three cities.

Bacterial composition of the 10 food types

General composition of major bacteria in street-vended foods in Ecuador were Proteobacteria and Firmicutes showing the highest relative abundance overall (Fig. 3). From the 10 types of food samples, raw meat from chicken and ground beef (22.76% and 17.40%, respectively), cheese (16.91%), encebollados (8.35%), and food dressings (6.52%) together accounted for almost 72% of bacterial taxa classification.

Since the foods are not categorized into specific groups because of the nature of each commodity (i.e. variations in a food preparation among cities), top genera are presented in Table 2 according to RA showing only taxa above 1% of the threshold.

Venn diagram based on ASVs was further applied to compare the composition of shared taxa of each food sample among sampling cities (Fig. 4). For each food type, the dominant taxa tended to be shared by all three cities. Hence, although a moderate number of ASVs were shared among the cities, they accounted for over 90% of bacterial reads. On the other hand, Unique ASVs were identified for each food type and city. While they represented a significant portion of the ASVs identified, these ASVs accounted for only a minor percentage of the

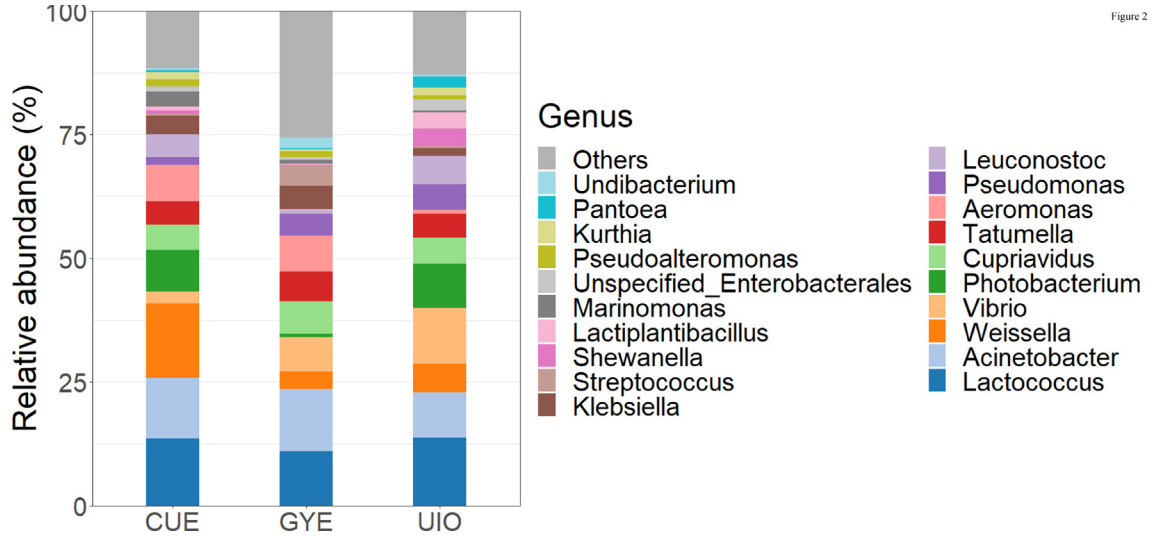

Figure 2. Top 20 most abundant genera annotated from each sampling city. Less abundant genera were grouped as "Others". CUE: Cuenca, GYE: Guayaquil, and UIO: Quito.

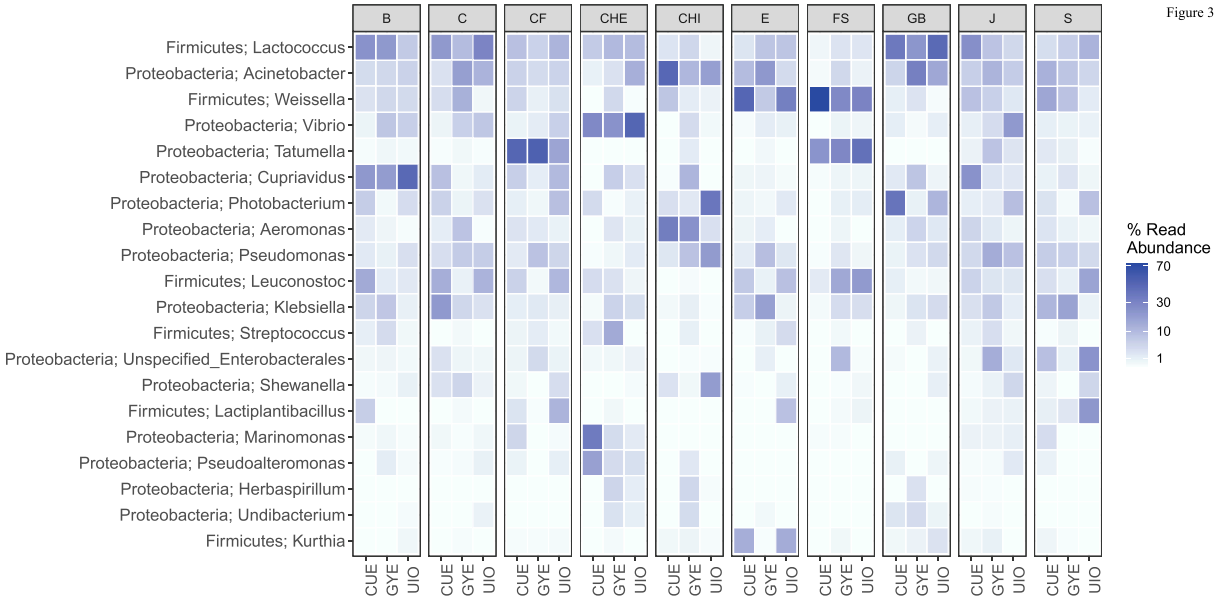

Figure 3. Heatmap of the top 20 genera detected in food samples and their relative abundances (RA%). Values in each row denote the relative abundance for a given genus (Annotated as Phylum, Genus) in different food samples, and those in each column denote the relative abundance (RA%) of different genera on a given food type (Top code) from a given city (Bottom code). Codes for food types are: Bolones (B), Ceviches (C), Chopped fruit (CF), Fresh cheeses (CHE), Chicken (CHI), Encebollados (E), Fruit salad (FS), Ground beef (GB), Fruit juices (J), and Food dressing (S). Cities codes are: Cuenca (CUE), Guayaquil (GYE), and Quito (UIO).

Table 2
Top genera with a relative abundance (RA %) above 1% in each type of food

BOLONES		CEVICHE		CHOPPED FRUITS		CHEESE		CHICKEN	
GENERA	RA %	GENERA	RA %	GENERA	RA %	GENERA	RA %	GENERA	RA %
Lactococcus	20.61	*Lactococcus*	22.93	*Tatumella*	44.86	*Vibrio*	37.70	*Acinetobacter*	25.18
Cupriavidus	16.46	*Klebsiella*	16.64	*Lactococcus*	7.37	*Marinomonas*	8.58	*Aeromonas*	22.11
Leuconostoc	8.23	*Acinetobacter*	9.06	*Leuconostoc*	4.29	*Lactococcus*	7.58	*Photobacterium*	12.39
Klebsiella	4.77	*Leuconostoc*	7.92	*Cupriavidus*	4.20	*Streptococcus*	6.47	*Pseudomonas*	8.33
Vibrio	4.61	*Unspecified_Carnobacteriaceae*	5.64	*Pseudomonas*	3.91	*Pseudoalteromonas*	5.43	*Shewanella*	6.66
Acinetobacter	3.83	*Pseudomonas*	4.42	*Acinetobacter*	3.85	*Acinetobacter*	5.25	*Weissella*	3.14
Weissella	3.75	*Vibrio*	4.00	*Lactiplantibacillus*	3.73	*Lactobacillus*	3.07	*Unspecified_Proteobacteria*	3.10
Celerinatantimonas	3.32	*Aeromonas*	3.53	*Photobacterium*	2.59	*Unspecified_Arcobacteraceae*	2.76	*Lactococcus*	2.82
Companilactobacillus	3.10	*Citrobacter*	2.73	*Weissella*	2.27	*Klebsiella*	2.73	*Cupriavidus*	1.98
Staphylococcus	2.78	*Cupriavidus*	2.69	*Vibrio*	1.87	*Celerinatantimonas*	2.36	*Vibrio*	1.73
Photobacterium	2.70	*Weissella*	2.51	*Klebsiella*	1.47	*Leuconostoc*	1.97		
Streptococcus	2.41	*Photobacterium*	2.15	*Erwinia*	1.36	*Cupriavidus*	1.71		
Renibacterium	2.33	*Unspecified_Enterobacterales*	1.47	*Unspecified_Enterobacterales*	1.35	*Weissella*	1.29		
Lactiplantibacillus	2.05			*Aeromonas*	1.35	*Raoultella*	1.20		
Ralstonia	1.84			*Pantoea*	1.29	*Herbaspirillum*	1.11		
Pseudomonas	1.33			*Marinomonas*	1.27	*Aeromonas*	1.04		
Citrobacter	1.26								
Aeromonas	1.06								
Brochothrix	1.02								
ENCEBOLLADO		**FRUIT SALADS**		**GROUND BEEF**		**JUICES**		**SAUCES**	
GENERA	RA %	GENERA	RA %	GENERA	RA %	GENERA	RA %	GENERA	RA %
Weissella	33.97	*Weissella*	41.49	*Lactococcus*	32.37	*Pantoea*	16.45	*Unspecified_Enterobacterales*	14.12
Kurthia	11.51	*Tatumella*	39.56	*Acinetobacter*	20.52	*Vibrio*	12.26	*Lactiplantibacillus*	12.75
Acinetobacter	9.28	*Leuconostoc*	10.94	*Photobacterium*	13.51	*Lactococcus*	12.13	*Leuconostoc*	10.21
Leuconostoc	5.71	*Klebsiella*	1.95	*Aeromonas*	2.63	*Pseudomonas*	9.19	*Klebsiella*	8.68
Klebsiella	5.50	*Lactococcus*	1.14	*Myroides*	2.49	*Acinetobacter*	6.46	*Lactococcus*	8.42
Paeniclostridium	5.18			*Cupriavidus*	2.46	*Cupriavidus*	5.20	*Lentilactobacillus*	7.16
Lactococcus	4.28			*Citrobacter*	2.40	*Photobacterium*	5.13	*Weissella*	5.80
Pseudomonas	2.92			*Pseudomonas*	2.36	*Weissella*	4.06	*Acinetobacter*	4.86
Lactiplantibacillus	2.85			*Klebsiella*	1.81	*Klebsiella*	2.97	*Pseudomonas*	3.63
Streptococcus	1.61			*Hafnia-Obesumbacterium*	1.47	*Leuconostoc*	2.74	*Companilactobacillus*	3.31
Exiguobacterium	1.38			*Undibacterium*	1.44	*Shewanella*	2.20	*Photobacterium*	3.05
Clostridium sensu stricto 1	1.32			*Clostridium sensu stricto 1*	1.30	*Unspecified_Enterobacterales*	1.59	*Levilactobacillus*	2.33
				Weissella	1.27	*Aeromonas*	1.50	*Shewanella*	1.38
				Kurthia	1.21	*Tatumella*	1.39	*Yersinia*	1.35
						Unspecified_Arcobacteraceae	1.28	*Pectobacterium*	1.30
						Streptococcus	1.14		
						Unspecified_Planococcaceae	1.01		

sequence reads, indicating their low relative abundance in the food samples.

Occurrence and identification of pathogenic and spoilage bacteria

From the 829 ASVs detected in the taxonomic classification of DADA2 pipeline, bacteria taxa were grouped according to the literature. A total of 573 taxa were grouped as "Unspecified" as there was no hit in species classification, this group accounted for 93.52% of RA. Among those with species classification, 164 (4.4% of total reads) were classified as environmental bacteria (EVB), 29 (1.31%) as food spoilage bacteria (SPB), and 24 (0.48%) as opportunistic pathogenic bacteria (OPB). Two groups that could be associated with human disease were identified, including enteric (ENB) and fecal bacteria (FCB) which are commonly involved in large numbers of foodborne outbreaks. ENB included 11 species (0.17% of total reads), whereas FCB involved 26 species (0.08%). Collectively, these bacteria constituted 2.04% of the total Amplicon Sequence Variants (ASVs) classified in our analysis. Finally, two species of plant pathogenic bacteria (0.01%) were also identified in 305 ASVs, including *Pseudomonas fluorescens* (0.005%) and *Gluconacetobacter liquefaciens* (0.001%).

Raw chicken samples showed the highest SPB reads accounting for 2.6% of RA from detected taxa for this food and distributed across sampling cities in Cuenca (1.8%), Guayaquil (0.5%), and Quito (0.3%). Raw ground beef showed 1.7% of the total SPB abundance and were present in samples from Guayaquil (1%), Quito (0.4%), and Cuenca (0.2%). SPB reads were also detected in food dressings (1.5%), bolones and fruit juices (1.3%), chopped fruits (0.8%), ceviches and encebollados (0.6%), cheese (0.3%), and fruit salads (0.1%) from the three cities.

Top SPB species across the three sampling cities included *Weissella ceti* (50.36% of FSB reads), *Brochothrix thermosphacta* (32.16%), *Chryseobacterium antarcticum* (5.40%), *Weissella koreensis* (2.37%), *Weissella viridescens* (2.32%), and *Leuconostoc carnosum* (1.22%). The distribution of SPB in food samples from each city is illustrated in Figure 5.

Reads classified as OPB were identified mainly in ground beef (1.11% of total reads), raw chicken (0.59%), and bolones (0.37%) in Guayaquil, which is the city with the highest abundance of this bacteria group (0.32%; 18,829 reads). In Quito, OPB was detected in cheese (0.35%), ceviches (0.17%), and fruit salads (0.02%); while in Guayaquil fruit juices (0.31%), encebollados and chopped fruits (0.26%), and food dressings (0.14%) were sources of OPB.

The main identified OPB reads were *Acinetobacter lwoffii* (23.06% RA from OPB reads), *Myroides phaeus* (20.61% RA), *Arcobacter nitrofigilis* (8.94% RA), and *Corynebacterium stationis* (5.72%). Figure 6 shows the OPB in food samples from each city.

As depicted in Figure 7, Enteric Bacteria (ENB) were primarily detected in raw ground beef (2.41%) and raw chicken (0.08%) from Guayaquil city. Similarly, bolones from Cuenca (0.08%), as well as juice (0.05%), cheese and chopped fruits (0.03%), food dressings

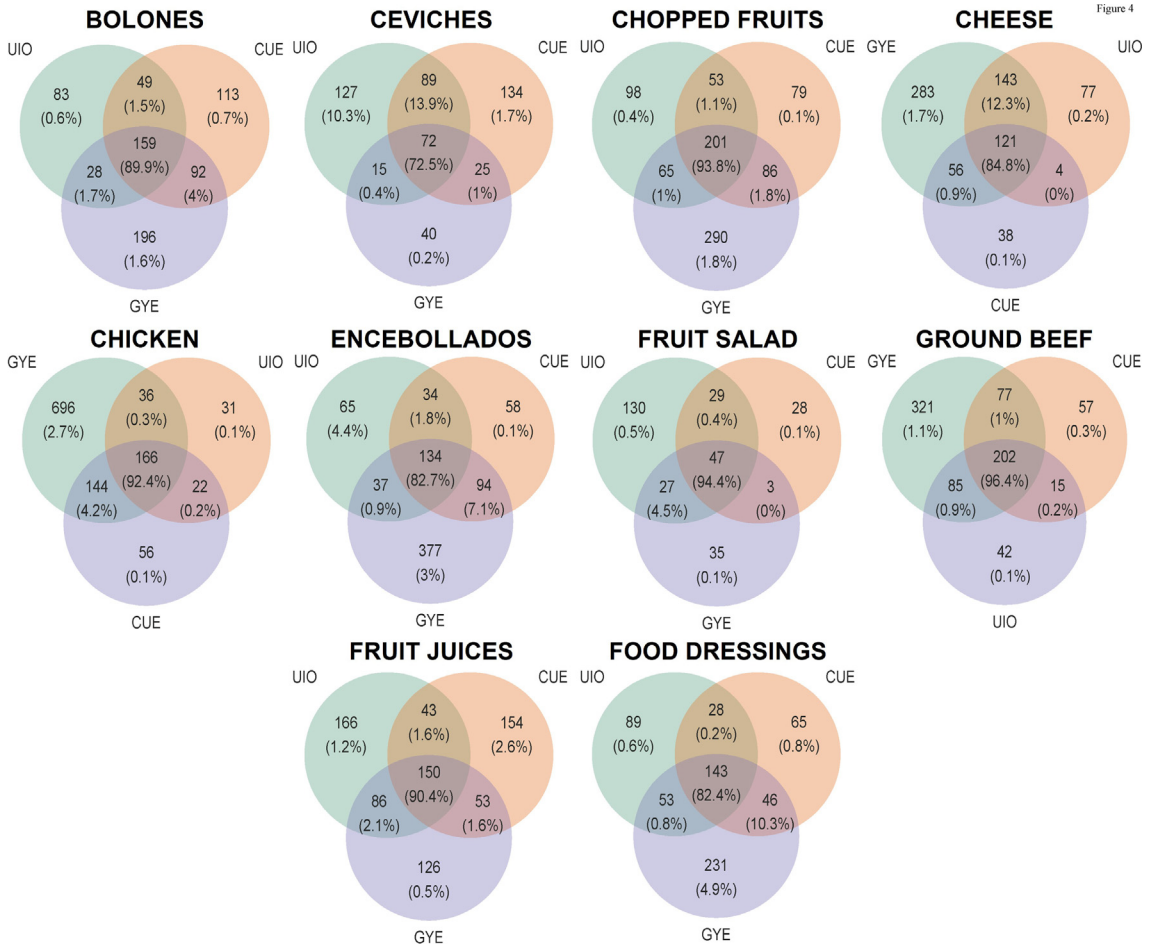

Figure 4. Proportion of shared taxa among food samples from the different sampling cities. The integers denote the ASV numbers for the food sample and the percentage data denote the relevant sequence number over the total sequence number. Cities codes are: Cuenca (CUE), Guayaquil (GYE), and Quito (UIO).

(0.02%), encebollados and ceviches (0.01%), and fruits salads (0.002%) from Quito were sources of ENB. Top taxa within this group were *Myroides phaeus* (54.91% RA), *Kurthia zopfii* (4.92% RA), and *Psychrobacter phenylpyruvicus* (2.23% RA), which were predominant in raw meat from beef and chicken, with their combined abundance accounting for over 62% of the identified ASVs.

Fecal Bacteria (FCB) group was mostly dominated by *Corynebacterium stationis* (15.24% RA), *Streptococcus dysgalactiae* (4.52% RA), *Photobacterium damselae* (2.04% RA), and *Brevibacterium luteolum* (1.07% RA). Guayaquil was the city where these foodborne bacteria were mostly found. The proportions of FCB detected in total reads of each food were chopped fruits (0.29%), raw chicken and fruit juices (0.14%), ceviches (0.10%), bolones (0.09%), raw ground beef (0.06%), cheese (0.04%), encebollados, fruits salads, and encebollados (0.01%). *Vibrio cholerae* (0.60% RA), a known pathogen, was also detected in low abundance (RA < 1%) from raw chicken, food dressings, raw ground beef, bolones, cheese, and encebollados in Guayaquil samples. Figure 8 provides a visual representation of the FCB in food samples from each city.

Diversity of bacterial communities in food samples

The microbial biodiversity of food samples across sampling cities were estimated based on alpha-diversity indices. The alpha diversity measured as Shannon index showed a highly significant difference in sampling cities ($P = 0.000339$), with higher diversity in food from Guayaquil (Fig. 9A). Significant difference in food sample diversity ($P = 0.00001$) was contributed by lower diversity in fruit salad samples than the other nine foods. In addition, Shannon diversity in chicken samples was significantly higher than others (Fig. 9B). Diversity in encebollados and fruit juices in Guayaquil, and fruit salads from Cuenca showed significant difference among all combinations of food type and city ($P = 0.005$) (Fig. 9C).

Bacterial communities differed significantly among food samples and sampling cities (Bray-Curti's dissimilarity: $\text{PERMANOVA}_{10,000\ \text{permutations}}$: $F = 2.629$, R2 = 0.183, $P = 0.0009$; Weighted UniFrac distance: $\text{PERMANOVA}_{10,000\ \text{permutations}}$: $F = 3.079$, R2 = 0.2018, $P = 0.0009$). Figure 10 shows Principal coordinate analysis (PcoA) using Bray and Unifrac distances measures of beta-diversity to determine the relativeness of bacterial communities' food samples among the three sampled cities Cuenca, Guayaquil, and Quito. Each dot in the PcoA plot represents an individual sample, plots showed no changes to the phylogenetic diversity of food samples among cities as no specific cluster was shown. It was notable that ASVs indicated in gray arrows (Fig. 10) composed by *Photobacterium* (ASV-0002), *Aeromonas* (ASV-0003), *Tatumella* (ASV-0004), *Lactococcus* (ASV-0006), *Acinetobacter* (ASV-0009), *Cupriavidus* (ASV-0011), and *Weissella* (ASV-0013) belonging to raw chicken, raw ground beef, chopped fruits, and fruits salads, respectively, were apart from centroid suggesting these samples contribute to phylogenetic variation in foods among cities.

Discussion

Street-vended foods are an important component of the food supply in Ecuador but might pose a risk to public health due to preparation

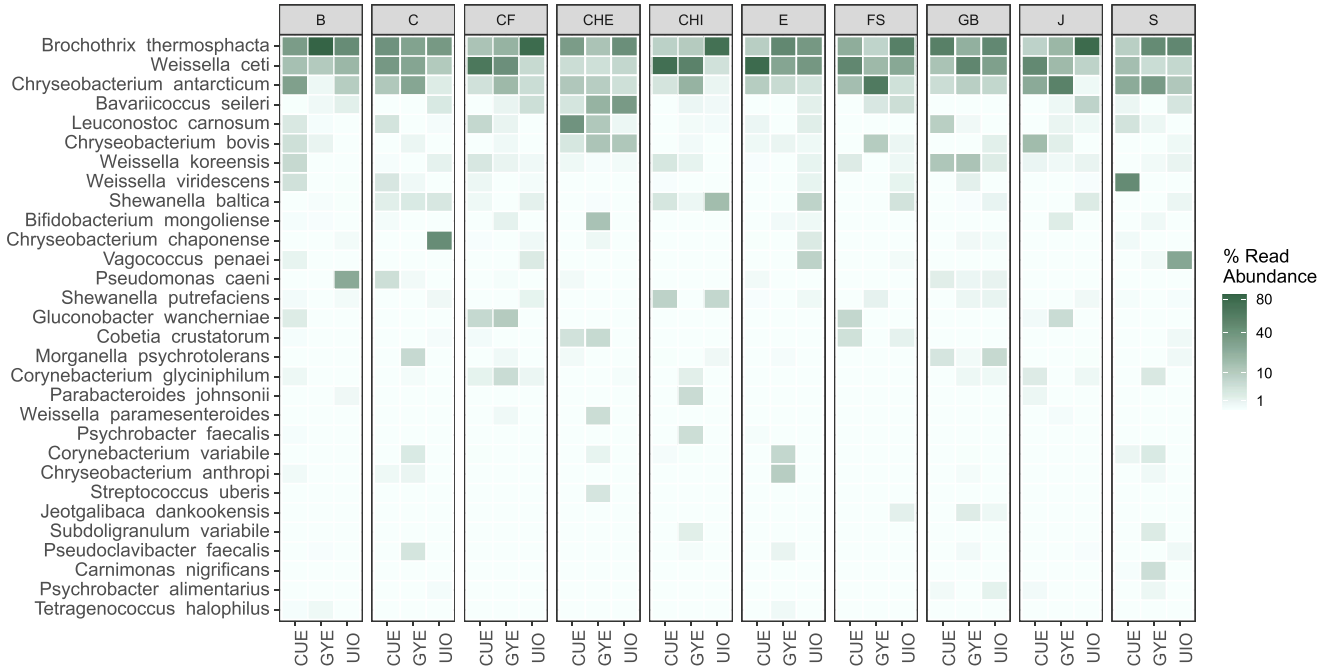

Figure 5. Heatmap showing identified food spoilage bacteria species in food samples. Values denote the relative abundance (%) of ASV reads of a given species (left) among all identified SPB ASVs in a given sample type (Top) and city (Bottom). Codes for food types are: Bolones (B), Ceviches (C), Chopped fruit (CF), Fresh cheeses (CHE), Chicken (CHI), Encebollados (E), Fruit salad (FS), Ground beef (GB), Fruit juices (J) and Food dressing (S). Cities codes are: Cuenca (CUE), Guayaquil (GYE), and Quito (UIO).

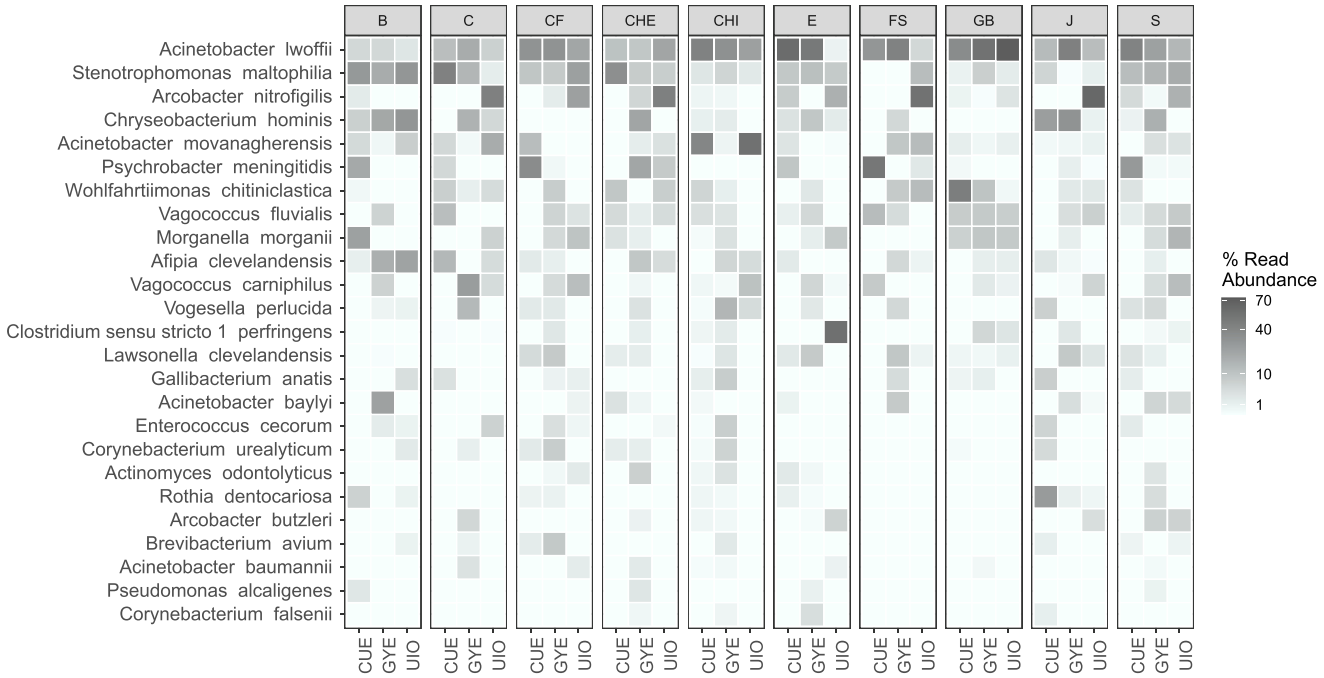

Figure 6. Heatmap showing identified opportunistic pathogenic bacteria species detected in food samples. Numbers denote the relative abundance (%) of ASV reads of a given species (left) among all identified OPB ASVs in the given sample type (Top) and city (Bottom). Codes for food types are: Bolones (B), Ceviches (C), Chopped fruit (CF), Fresh cheeses (CHE), Chicken (CHI), Encebollados (E), Fruit salad (FS), Ground beef (GB), Fruit juices (J), and Food dressing (S). Cities codes are: Cuenca (CUE), Guayaquil (GYE), and Quito (UIO).

and handling issues, including the lack of a cold chain for food, tap water for washing hands and ingredients, and other food handling practices. Traditionally, the taxonomy classification of bacteria is based on the isolation and cultivation of microorganisms. However, most of the microorganisms present in natural environments are not yet culturable by traditional methods (Hugerth & Andersson, 2017; L. Li et al., 2014; Wu et al., 2020). Innovative molecular techniques such as microarrays, sequencing, and others make it possible to reveal the unculturable microbes. The culture-independent methods based on NGS improve the elucidation of the biodiversity of various samples,

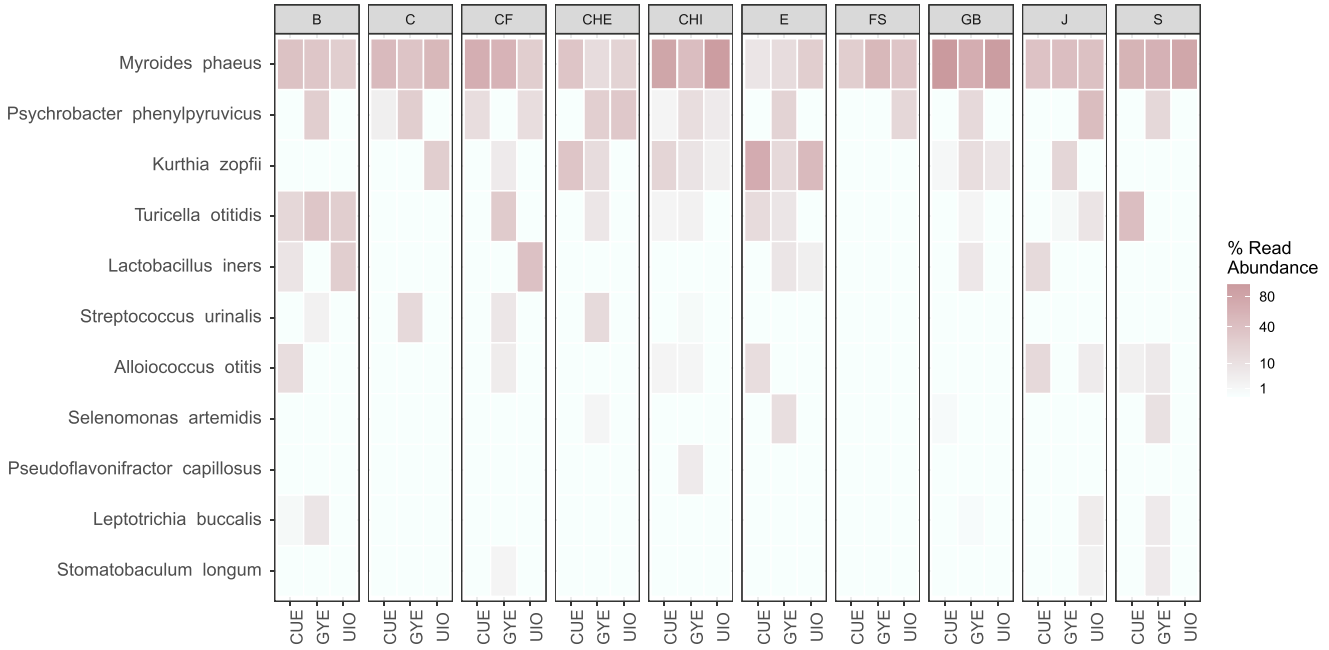

Figure 7. Heatmap showing identified enteric bacteria species detected in food samples. Numbers denote the relative abundance (%) of ASV reads of a given species (left) among all identified ENB ASVs in the given sample type (Top) and city (Bottom). Codes for food types are: Bolones (B), Ceviches (C), Chopped fruit (CF), Fresh cheeses (CHE), Chicken (CHI), Encebollados (E), Fruit salad (FS), Ground beef (GB), Fruit juices (J), and Food dressing (S). Cities codes are: Cuenca (CUE), Guayaquil (GYE), and Quito (UIO).

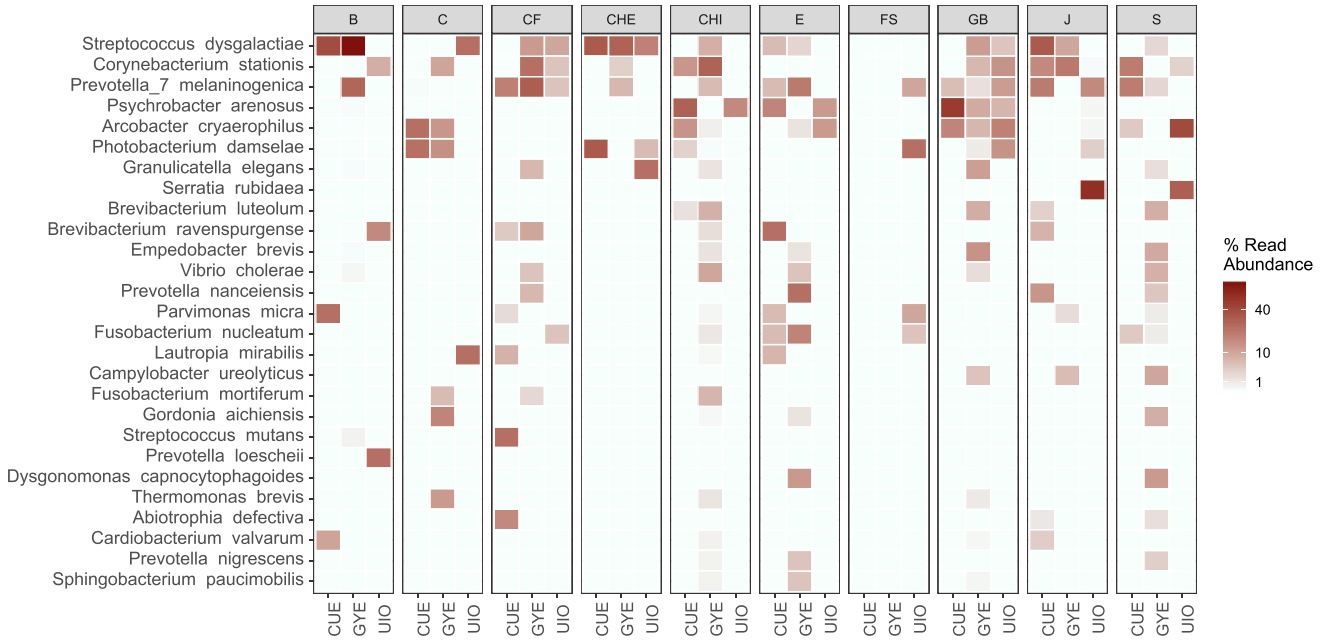

Figure 8. Heatmap showing identified fecal bacteria species detected in food samples. Numbers denote the relative abundance (%) of ASV reads of a given species (left) among all identified FCB ASVs in the given sample type (Top) and city (Bottom). Codes for food types are: Bolones (B), Ceviches (C), Chopped fruit (CF), Fresh cheeses (CHE), Chicken (CHI), Encebollados (E), Fruit salad (FS), Ground beef (GB), Fruit juices (J) and Food dressing (S). Cities codes are: Cuenca (CUE), Guayaquil (GYE), and Quito (UIO).

which can lead to a better understanding of the composition and diversity of bacterial communities and benefit the cultivation and isolation of target bacteria from samples with complex microbiomes (Dash & Das, 2018).

Following a previous research (Salazar-Llorente et al., 2020), we investigated the foodborne bacteria from 16S rRNA sequencing data of 10 high-demand street-vended foods collected from the three largest cities in Ecuador. These foods are the most popular in the 3 cities because they are easy to make by street vendors and are highly demanded by consumers who need ready-to-eat low-cost foods. Similar street-vended foods are very common in most Latin America cities as ingredients are chosen by financial availability and consumer demand (Simopoulos & Bhat, 2000).

To provide a baseline information about foodborne pathogens using 16S rRNA sequencing data, we used the DADA2 method to make species-level assignments based on exact matching between ASVs and

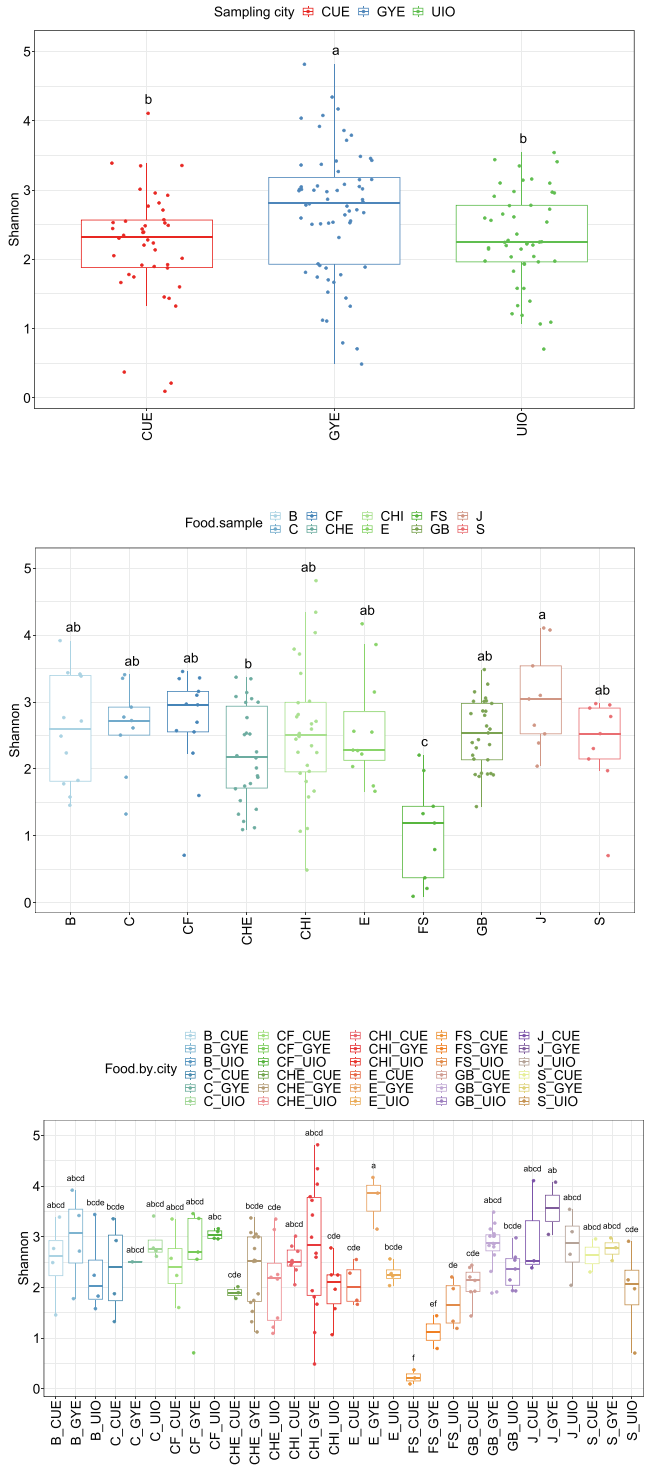

Figure 9. Shannon diversity among three sampling cities (A), ten food types (B) and food by cities (C). Letters over each data set denote Duncan's groups' significant difference. Boxes denote the interquartile range of the data. Whiskers extend to the most extreme value within 1.5 times interquartile range and black dots represent outliers beyond that range. Codes for food types are: Bolones (B), Ceviches (C), Chopped fruit (CF), Fresh cheeses (CHE), Chicken (CHI), Encebollados (E), Fruit salad (FS), Ground beef (GB), Fruit juices (J) and Food dressing (S). Cities codes are: Cuenca (CUE), Guayaquil (GYE), and Quito (UIO).

sequenced reference strains in SILVA 138 (100% identity) indicated as a valid appropriate procedure to assign species to 16S rRNA gene fragments (Callahan et al., 2016; Edgar, 2018; McLaren & Callahan, 2021); and as no previous studies using molecular data to investigate microbial communities of foodborne bacteria in street-vende food in Ecuador have been developed, similar to previous studies using NGS and 16S rRNA molecular data (Lewis et al., 2020; Mira Miralles et al., 2019; Syromyatnikov et al., 2020). We identified important bacterial profiles that accounted for 2.04% of the total classified ASVs and have implications for both food quality and human health, including food spoilage, opportunistic, enteric, and fecal bacteria.

The occurrence of spoilage bacteria in food samples may help estimate the safety and time of exposure to the environment of the food before serving. Freshly prepared street-vended foods are not common in Latin America, as street vendors prefer to store, recook, or freeze unsold foods to try to sell them again the next day (Simopoulos & Bhat, 2000).

When analyzing microbial composition, each food showed Proteobacteria and Firmicutes as the most dominant phyla, with almost 98% of RA. The remaining abundance (>1%) was completed by Bacteroidota, which is in agreement with previous food investigations of street-vended foods (Jarvis et al., 2018; Y. M. Li et al., 2020; Rodríguez-López et al., 2020). Intra-group abundance assessment showed notable differences in phyla distribution across sampling cities, with Guayaquil showing the highest phyla diversity, followed by Quito and Cuenca.

We found that ground beef, chicken, cheese, encebollados, and food dressings together contributed to almost 72% of the bacteria taxa classified, while ceviches, fruit salads, bolones, chopped fruits, and fruit juices shared approximately 5% of the detected ASVs, accounting for 28.05% of the bacteria abundance. These foods are commonly sold to consumers in Ecuador cities with no consideration of the cold chain management even in hot weather like that in Guayaquil. They were also found carrying much higher counts of various bacteria, especially in samples from Guayaquil (Salazar-Llorente et al., 2020). The high bacterial counts could be related to the hot weather of this city that allows bacterial proliferation during display and selling on the streets. Similar findings of higher counts in raw food were presented by Raza et al. (2021) that food quality was worse in summer months and that most of the RTE food tested presented high levels of microbial contamination. The authors noticed that food carts were often placed above drainage or very close to roads, and that food items for sale might be contaminated with dust and dirt. All these factors could serve as a source of microbial contamination for street-vended foods in the cities among these developing countries.

As shown in Figures 3 and 4, *Acinetobacter, Lactococcus, Vibrio*, *Weissella*, *Photobacterium*, *Aeromonas,* and *Tatumella* accounted for almost 58% of the RA in all food samples. These genera are showed based on its relative abundance (>1%). Different foods, including raw meat and cheese, cooked fish, fruits, vegetables, and food dressings present diverse surface morphology, tissue composition, and metabolic activities that make each product a unique ecological niche for specific microbial groups (Beuchat, 2002). These factors might explain the significantly different biodiversity that we observed in food samples. Most of these genera detected belong to groups of coliforms and aerobic mesophiles that previously has been found in the investigations of Salazar-Llorente et al. (2020) and in other countries related to foodborne bacteria in street-vended food (Moloi et al., 2021b; Salamandane et al., 2021).

For each food type, we identified commonly shared taxa among sampling cities. Unique ASVs of *Lactococcus* were present as shared taxa in bolones, ceviches, raw ground beef, and food dressings. Species of *Lactococcus* are commonly found in fermented foods, and their detection at high level in street-vended foods would indicate spoilage, as most of these foods are stored for several days despite its quality degradation by lactic acid bacteria (LAB) influenced by temperature and low hygienic handling that subserves LAB proliferation (Hwanhlem et al., 2017; Kharel et al., 2016; Song et al., 2017).

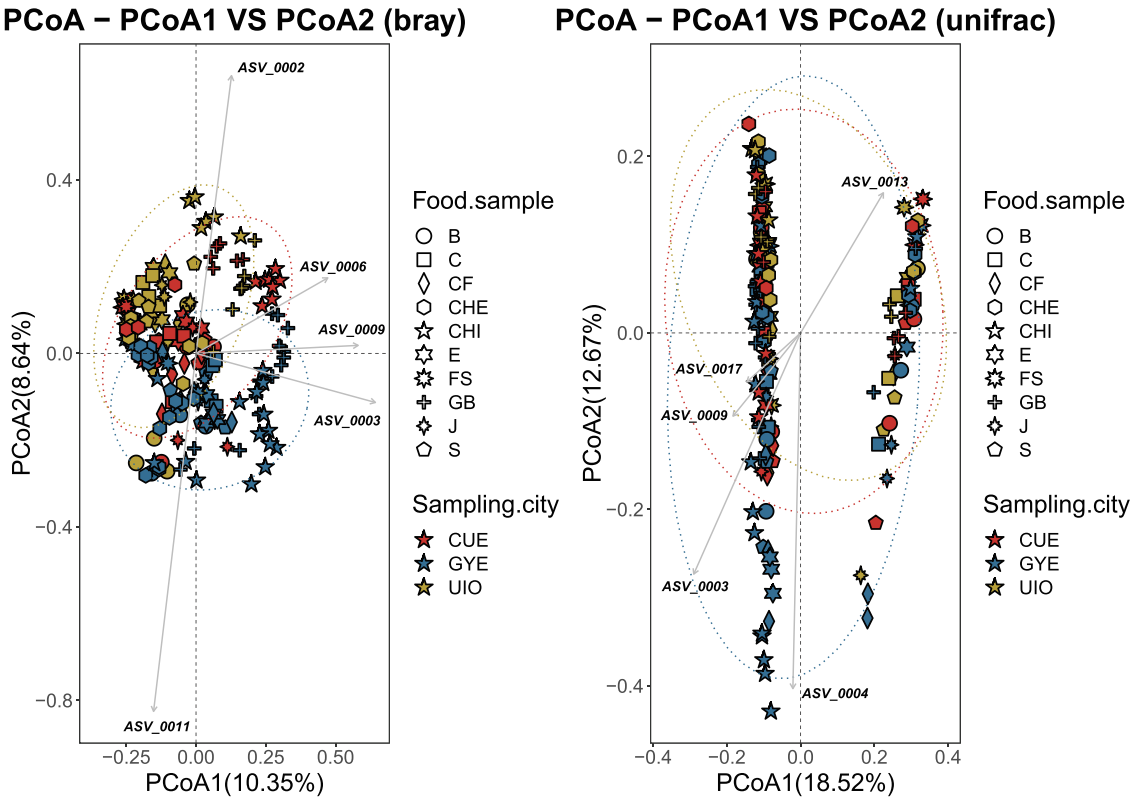

Figure 10. Principal coordinates analysis (PcoA) reveals no specific clustering using Bray Curtis distance (left) of food samples by sampling city. PCoA plot computed from unweighted UniFrac distances (right) reveals a clustering from a group of samples with similar composition that also drives differences in the microbiome. The 10 food types are represented by different symbols and three sampled cities by different colors. Normal confidence ellipses indicate 95% confidence intervals of multivariate t-distribution around centroids of the groupings. Codes for food types are: Bolones (B), Ceviches (C), Chopped fruit (CF), Fresh cheeses (CHE), Chicken (CHI), Encebollados (E), Fruit salad (FS), Ground beef (GB), Fruit juices (J) and Food dressing (S). Cities codes are: Cuenca (CUE), Guayaquil (GYE), and Quito (UIO).

Fruit salads and chopped fruits harbored *Weissella* spp. and *Tatumella* spp. as shared taxa, similar to encebollados that also had *Weisella* species as dominant taxa. *Tatumella* spp. could indicate food exposure to fruit flies (*Drosophila suzukii*), as these bacteria were typically related to fruit ripening (Chandler et al., 2014; Gurung et al., 2022). The presence of *Weissella* in fruit samples could indicate the overripening of the fruits, which could lead to ethylene production that in turn attracts insects such as flies. As a subgroup of LAB, *Weisella spp.* are often associated with spoilage of fish or seafood (Lorenzo et al., 2018; Petruzzi et al., 2017).

Several species of *Vibrio* were also frequently detected in some food like bolones, ceviches, cheese, and fruit juices with a RA < 1%. While some *Vibrio* species are pathogenic, others are commonly used for curing in cheese preparation (Woods et al., 2020). Seafood usually carries *Vibrio* species because of their ubiquitous presence in water bodies. Sometimes these bacteria could be rendered unculturable by food preparation such as sauce marination, which would allow them to pass culture-based food quality controls and to cause severe illness once they reach the human digestion system (Herrera et al., 2010; Mathur & Schaffner, 2013; Ramirez Martinez et al., 2022; Woods et al., 2020).

LABs were dominantly present in raw food like ground beef and chicken, and in fruit juices and food dressing samples. Taxa in this group were associated with spoilage of meat, consistent with findings by Tsafrakidou et al. (2021), who also detected several subgroups of LABs including *Acinetobacter*, *Lactococcus,* and *Lactiplantibacillus* when evaluating storage conditions for raw chicken. Hwang et al. (2020) using NGS reported several subgroups of LABs that influenced shifts in microflora in raw beef, with refrigeration temperatures and environmental factors like weather influencing microflora alteration during processing, storage, and display of the meat for selling. Street-vended juices and food dressings in Ecuador are often exposed to sun light and ambient temperature. Food handlers generally lack knowledge of food safety guidelines to store and preserve these foods, although sometimes food handlers add citric fruits like lemon or orange to preserve taste (Lobo et al., 2019; Niu et al., 2020). In addition, containers of these foods are often kept open, further increasing the risk of contamination from humans and environments, which could be one of the reasons *Klebsiella* was detected in food dressings, similar to previous reports by Hartantyo et al. (2020). *Lactobacillus plantarum* is naturally present on fruits (Li et al., 2021). Their detection in NGS reads could indicate a bad sanitization process of the fruits or prolonged time in exposition to ambience causing spoilage.

In concur with our previous report (Salazar-Llorente et al., (2020), sequencing data of this research confirmed that alpha diversities of bacterial communities were significantly higher in Guayaquil food samples than in the other two cities ($P < 0.05$). The significant difference observed in microbial diversity among food types could be attributed to lower diversity in fruit salads and higher diversity in chicken samples (Fig. 9B). Alpha diversity in encebollados, fruit juices, and fruit salads was statistically significant ($P = 0.005$) showing differences among samples (Fig. 9C). Encebollado preparation requires a high level of postcooking ingredient manipulation same as fruit preparations as RTE foods are also hand manipulated. Moreover, vendors often manipulate raw and cooked food at the same time facilitating

cross-contamination; this improper hygiene could contribute to the high bacterial load observed in these foods (Muriuki et al., 2021; Salazar-Llorente et al., 2020).

Bacterial communities showed no differences among food samples (Fig. 10). Principal coordinate analysis (PcoA) using Bray and UniFrac distances was used to measure beta-diversity to determine the relatedness of bacterial communities' food samples among the three cities. We observed no specific clustering in relation to any given city, indicating the lack of difference in phylogenetic diversity of food samples among these cities.

Food spoilage bacteria (SPB) are bacteria that cause undesirable changes in the appearance, smell, taste, or texture of food, making it unfit for consumption. These bacteria can grow on various types of food, such as meat, dairy, fruits, vegetables, grains, etc. And they can affect the nutritional value and safety of food (Azad et al., 2019; Sanguyo et al., 2021). As shown in Figure 5, there were 29 species grouped as FSB, of which *Weissella ceti* (50.36%) and *Brochothrix thermosphacta* (32.16%) were present in most of the food types sampled, including raw chicken and beef. Microbiological food spoilage is caused by the growth of microorganisms which produce enzymes and objectionable metabolites in the food (Petruzzi et al., 2017). *B. thermosphacta* has been associated with the spoilage and the production of off-odors in meat and seafood products (Illikoud et al., 2018). These bacteria are part of the most common bacteria isolated from raw meat (Eshamah et al., 2020) and directly influence the quality and integrity of food with potential implications to human health (Teixeira, 2021).

SPB are microorganisms that cause foods to develop unpleasant odors, tastes, and textures. These bacteria can create an environment that is conducive for the growth of OPB (Gram et al., 2002; Noor et al., 2019). In this study, OPB were also abundant in raw foods like meat and cheese, and on cooked food like encebollados from Guayaquil and Quito (Fig. 6). *Acinetobacter lwoffii* (23.06% RA), *Myroides phaeus* (20.61% RA), *Arcobacter nitrofigilis* (8.94% RA), and *Corynebacterium stationis* (5.72%) were the most abundant OPB in these food types. Nowadays, *Acinetobacter* and *Arcobacter* spp. are considered as potential pathogens of major public health concern due to their resistance to multiple antibiotics and their association with a wide range of nosocomial and community-acquired infections like bacteremia, endocarditis, peritonitis, gastroenteritis, and diarrhea in humans. Isolation of these Gram-negative bacteria from food sources is difficult but molecular techniques help elucidate their abundance (de Amorim & Nascimento, 2017). Similarly, *M. phaeus* has been associated with bacteremia (Pérez-Lazo et al., 2020). Although there have not been reported human infections, *C. stationis* is considered pathogenic (Reimer et al., 2022) as some strains from *Corynebacterium* genus are associated with upper respiratory infections (Cavalieri & Knoop, 2007). Despite the use of several *Weissella* strains in the biotechnological field, certain species of this genus have been found to act as opportunistic pathogens, while some strains of *W. ceti* were recognized to be pathogenic (Abriouel et al., 2015).

Enteric bacteria (ENB) usually inhabit the gastrointestinal tract of both humans and animals. These bacteria can exhibit a range of pathogenicity, with some strains being benign and others potentially harmful. Fecal bacteria (FCB), on the other hand, serve as indicators of fecal contamination in various environmental samples, including water and food. While FCBs themselves may not be inherently pathogenic, their presence often implies the potential existence of other pathogenic organisms originating from fecal matter. Therefore, the detection of FCB is crucial in assessing the sanitary quality of environmental samples. (Getie et al., 2019). Both bacteria groups can cause foodborne diseases, such as diarrhea, dysentery, and typhoid fever, if they contaminate food during production, processing, or handling (Randazzo et al., 2021; Vicentini et al., 2021).

M. phaeus (54.91% RA from ENB group), *Kurthia zopfii* (4.92% RA), and *Psychrobacter phenylpyruvicus* (2.23% RA) were the most abundant human microbiota bacteria, whereas *C. stationis* (5.53% RA), *Streptococcus dysgalactiae* (1.62% RA), *Photobacterium damselae* (2.04% RA), and *Brevibacterium luteolum* (1.07% RA) were the most abundant ENB. These bacteria were found in food samples of chicken and beef, chopped fruit, and fruit juices. For *Myroides*, there is a lack of information on this genus for public health management, although there has been at least one case of *M. phaeus* causing bacteremia, suggesting the need of more extensive surveillance programs. Zhao et al. (2022) also found *K. zopfii* presenting in almost 80% of raw pork samples. Moloi et al. (2021) reported the detection of pathogenic bacteria including *Staphylococcus aureus*, *Escherichia coli,* and other bacteria from human sources such as *Prevotella bivia* or *Corynebacterium jeikeium* in street-vended raw meats, suggesting poor sanitary practices from food handlers.

Corynebacterium spp. are part of the human microbiota and also have been found ubiquitously in the environment (Hahne et al., 2018). Scarce information has been established on the genus Corynebacterium in foods but may it be involved in the spoilage or ripening of cheese and meats (Alibi et al., 2016). Infections caused by *S. dysgalactiae* subspecies *equisimilis* have been previously reported (Brandt & Spellerberg, 2009). However, there is a lack of information regarding its genetic diversity, profiles of virulence factors (VFs), and antimicrobial resistance (AMR), especially in regard to food-related isolates (Xu et al., 2021).

Vibrio was also detected in different food commodities like raw meat and food dressings, including pathogenic species causing vibriosis, which is mainly associated with the consumption of raw or undercooked seafood (Baker-Austin et al., 2018; Dutta et al., 2021). Recently, *P. damselae* has been associated with skin infections and histamine fish poisoning in humans (Matanza & Osorio, 2020; Yoon et al., 2020). Using NGS, Khayyira et al. (2020) detected *Brevibacterium luteolum* as an opportunistic pathogen in various sites of the human body, such as the urinary tract, lacrimal apparatus, and peritoneum.

Campylobacter infections have been associated most often with poultry, raw (unpasteurized) dairy products, untreated water, and produce. However, *Campylobacter* was identified in sauces, ground beef, and juices but not on the tested chicken samples in this study. The prevalence of the foodborne pathogens on street-vended foods might be different from typical commercial products provided by large food producers in developed countries. Contamination of *Campylobacter* in sauces, ground beef, and juices with an RA $< 1\%$ indicated possible cross-contamination from food handlers as they usually work close to other street-vended foods, i.e. markets, food fairs, etc. (bioMérieux Industry, 2020; Donnison & Ross, 2014).

It is important to mention that from 879 ASVs detected only 192 were identified and classified taxonomically to species level and several important species could have been present but no reported in this study. As highlighted by Hoffman et al. (2021), the proper choice of taxonomic database and variable region of the 16S rRNA gene sequence makes species-level identification possible. However, one important limitation is the short region used for the analysis (V3-V4) making further research such as shotgun metagenomics and third-generation sequencing analysis necessary tools to help elucidate ambiguities and additional species.

The presence of these bacterial profiles in sampled ecuadorian street-vended food indicates several factors that need to be included in a hazard analysis such, as the unhealthy conditions provided by food manipulators as a major cause of food contamination (Amare et al., 2019; Moloi et al., 2021a; Raza et al., 2021). Often, the unavailability of safe tap water prevents proper cleaning and disinfection of hands and utensils, including dishes and cutlery that are reused by consumers (Salamandane et al., 2021). These conditions could have

serious consequences to consumer health, especially among the more vulnerable population, where the opportunistic pathogens, among normal human microflora, could result in disease outbreaks upon consumption of these contaminated foods (Noor et al., 2019). Further sensory evaluation and longitudinal microbiome studies on different food commodities in developing countries can benefit the improvement of food quality and safety.

Conclusion

The purpose of this study was to define the baseline microbiomes of the 10 most popular street foods consumed in the three biggest cities in Ecuador by 16S rRNA gene HT sequencing. In addition to conventional microbial enumeration, examining food microbiomes based on HT sequencing data provides a more comprehensive characterization of the diversity and dynamics of microbial communities in different foods with low safety standards, which is essential for the improvement of microbial food safety in developing countries such as Ecuador. Microbiome profiling can facilitate the detection of adulteration or contamination, and the identification of potential antagonistic bacteria as biocontrol tools for the food industry. Results derived from this study expand the knowledge on the bacterial profiling in popular foods and food sources, which can benefit the development of preventive strategies to reduce the spoilage and pathogenic bacteria in street-vended foods. The information can be utilized in many ways in food-related pursuits and to develop food safety and security programs for the regulation and management of street vendors.

CRediT authorship contribution statement

B. Diaz Cardenas: Data curation, Writing – original draft, Visualization, Investigation, Validation, Formal analysis. **E. Salazar Llorente:** Data curation, Investigation, Formal analysis. **G. Gu:** Conceptualization, Writing – review & editing, Validation, Supervision, Resources. **X. Nou:** Conceptualization, Writing – review & editing, Validation, Resources. **J. Ortiz:** Conceptualization, Writing – review & editing, Methodology, Resources. **P. Maldonado:** Conceptualization, Writing – review & editing, Methodology, Resources. **J. Cevallos-Cevallos:** Conceptualization, Funding acquisition, Writing – review & editing, Supervision, Resources, Project administration.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Juan Manuel Cevallos-Cevallos reports financial support was provided by Corporación Ecuatoriana para el Desarrollo de la Investigación y la Academia (CEDIA). Juan Manuel Cevallos-Cevallos reports financial support was provided by Flemish Interuniversities Council and University Development Co-operation (VLIR-UOS).

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References

Abriouel, H., Lerma, L. L., Casado Muñoz, M. del C., Montoro, B. P., Kabisch, J., Pichner, R., Cho, G.-S., Neve, H., Fusco, V., Franz, C. M. A. P., Gálvez, A., & Benomar, N. (2015). The controversial nature of the Weissella genus: Technological and functional aspects versus whole genome analysis-based pathogenic potential for their application in food and health. *Frontiers in Microbiology, 6*(OCT), 1–14. https://doi.org/10.3389/fmicb.2015.01197.

Alibi, S., Ferjani, A., & Boukadida, J. (2016). Implication of Corynebacterium species in food's contamination. *Journal of Coastal Life Medicine, 4*(5), 416–419. https://doi.org/10.12980/JCLM.4.2016J6-26.

Allison, N., Ray, K., & Rohel, J. (2021). Mobilizing the streets: The role of food vendors in urban life. *Food, Culture and Society, 24*(1), 2–15. https://doi.org/10.1080/15528014.2020.1860454.

Amare, A., Worku, T., Ashagirie, B., Adugna, M., Getaneh, A., & Dagnew, M. (2019). Bacteriological profile, antimicrobial susceptibility patterns of the isolates among street vended foods and hygienic practice of vendors in Gondar town, Northwest Ethiopia: A cross sectional study. *BMC Microbiology, 19*(1), 1–9. https://doi.org/10.1186/s12866-019-1509-4.

de Amorim, A. M. B., & Nascimento, J. D. S. (2017). Acinetobacter: An underrated foodborne pathogen? *The Journal of Infection in Developing Countries, 11*(02), 111–114. https://doi.org/10.3855/jidc.8418.

Azad, Z. R. A. A., Ahmad, M. F., & Siddiqui, W. A. (2019). Food spoilage and food contamination. In *Health and safety aspects of food processing technologies* (pp. 9–28). Springer International Publishing https://doi.org/10.1007/978-3-030-24903-8_2.

Baker-Austin, C., Oliver, J. D., Alam, M., Ali, A., Waldor, M. K., Qadri, F., & Martinez-Urtaza, J. (2018). Vibrio spp. infections. *Nature Reviews Disease Primers, 4*(1), 1–19. https://doi.org/10.1038/s41572-018-0005-8.

Beuchat, L. R. (2002). Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. *Microbes and Infection, 4*(4), 413–423. https://doi.org/10.1016/S1286-4579(02)01555-1.

bioMérieux Industry (2020). *Campylobacter: a leading foodborne pathogen*. *Newsletter* https://www.biomerieux-industry.com/es/calidad-seguridad-alimentaria/recursos/biblioteca-de-microorganismos-alimentarios/2020-05-12#:~:text=Campylobacterinfectionshavebeenassociated,mostcommonsourceofinfection.

Birgen, B. J., Njue, L. G., Kaindi, D. M., Ogutu, F. O., & Owade, J. O. (2020). Determinants of microbial contamination of street-vended chicken products sold in Nairobi county, Kenya. *International Journal of Food Science, 2020*. https://doi.org/10.1155/2020/2746492.

Brandt, C. M., & Spellerberg, B. (2009). Human infections due to Streptococcus dysgalactiae subspecies equisimilis. *Clinical Infectious Diseases, 49*(5), 766–772. https://doi.org/10.1086/605085.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods, 13*(7), 581–583. https://doi.org/10.1038/nmeth.3869.

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S. M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J. A., Smith, G., & Knight, R. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *The ISME Journal, 6*(8), 1621–1624. https://doi.org/10.1038/ismej.2012.8.

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh, P. J., Fierer, N., & Knight, R. (2011). Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences, 108*(supplement_1), 4516–4522. https://doi.org/10.1073/pnas.1000080107.

Cavalieri, S. J., & Knoop, F. C. (2007). Corynebacterium Infections. *XPharm: The Comprehensive Pharmacology Reference*, 1–5. https://doi.org/10.1016/B978-008055232-3.60858-X.

Chandler, J. A., James, P. M., Jospin, G., & Lang, J. M. (2014). The bacterial communities of Drosophila suzukii collected from undamaged cherries. *PeerJ, 2*. https://doi.org/10.7717/peerj.474 e474.

Dash, H. R., & Das, S. (2018). Molecular methods for studying microorganisms from atypical environments. *Methods in Microbiology, 45*, 89–122. https://doi.org/10.1016/BS.MIM.2018.07.005.

Donnison, A. M., & Ross, C. M. (2014). Microbiological Safety of Meat | Thermotolerant Campylobacter. *Encyclopedia of Meat Sciences*. Elsevier https://doi.org/10.1016/B978-0-12-384731-7.00038-6.

Dutta, D., Kaushik, A., Kumar, D., & Bag, S. (2021). Foodborne pathogenic Vibrios: Antimicrobial resistance. *Frontiers in Microbiology, 12*(June), 1–10. https://doi.org/10.3389/fmicb.2021.638331.

Edgar, R. C. (2018). Updating the 97% identity threshold for 16S ribosomal RNA OTUs. *Bioinformatics, 34*(14). https://doi.org/10.1093/bioinformatics/bty113.

Eshamah, H. L., Naas, H. T., Garbaj, A. M., Azwai, S. M., Gammoudi, F. T., Barbieri, I., & Eldaghayes, I. M. (2020). Extent of pathogenic and spoilage microorganisms in whole muscle meat, meat products and seafood sold in Libyan market. *Open Veterinary Journal, 10*(3), 276. https://doi.org/10.4314/OVJ.V10I3.6.

Getie, M., Abebe, W., & Tessema, B. (2019). Prevalence of enteric bacteria and their antimicrobial susceptibility patterns among food handlers in Gondar town, Northwest Ethiopia. *Antimicrobial Resistance & Infection Control, 8*(1), 111. https://doi.org/10.1186/s13756-019-0566-7.

Gram, L., Ravn, L., Rasch, M., Bruhn, J. B., Christensen, A. B., & Givskov, M. (2002). Food spoilage—Interactions between food spoilage bacteria. *International Journal of Food Microbiology, 78*(1–2), 79–97. https://doi.org/10.1016/S0168-1605(02)00233-7.

Gu, G., Ottesen, A., Bolten, S., Ramachandran, P., Reed, E., Rideout, S., Luo, Y., Patel, J., Brown, E., & Nou, X. (2018). Shifts in spinach microbial communities after chlorine washing and storage at compliant and abusive temperatures. *Food Microbiology, 73*, 73–84. https://doi.org/10.1016/j.fm.2018.01.002.

Gurung, K., Vink, S. N., Salles, J. F., & Wertheim, B. (2022). More persistent bacterial than fungal associations in the microbiota of a pest insect. *Journal of Pest Science*. https://doi.org/10.1007/s10340-022-01524-1.

Hahne, J., Kloster, T., Rathmann, S., Weber, M., & Lipski, A. (2018). Isolation and characterization of Corynebacterium spp. from bulk tank raw cow's milk of different dairy farms in Germany. *PLOS ONE, 13*(4), e0194365. https://doi.org/10.1371/JOURNAL.PONE.0194365.

Hartantyo, S. H. P., Chau, M. L., Koh, T. H., Yap, M., Yi, T., Cao, D. Y. H., Gutiérrez, R. A., & Ng, L. C. (2020). Foodborne Klebsiella pneumoniae: Virulence potential, antibiotic resistance, and risks to food safety. *Journal of Food Protection, 83*(7), 1096–1103. https://doi.org/10.4315/JFP-19-520.

Herrera, A., Espinosa, B. J., Nuñez, G., Espinoza, N., Maves, R. C., & Martin, G. J. (2010). The effect of preparation of cebiche on the survival of enterotoxigenic Escherichia coli, Aeromonas hydrophila, and Vibrio parahaemolyticus. *Journal of Travel Medicine, 17*(6), 395–399. https://doi.org/10.1111/j.1708-8305.2010.00465.x.

Hoffman, C., Siddiqui, N. Y., Fields, I., Gregory, W. T., Simon, H. M., Mooney, M. A., Wolfe, A. J., & Karstens, L. (2021). Species-level resolution of female bladder microbiota from 16s rRna amplicon sequencing. *MSystems, 6*(5). https://doi.org/10.1128/mSystems.00518-21.

Hoffmann, S. (2020). Cost Estimates of Foodborne Illnesses. *In Economic Research Service* https://www.ers.usda.gov/data-products/cost-estimates-of-foodborne-illnesses/.

Hoffmann, S., Devleesschauwer, B., Aspinall, W., Cooke, R., Corrigan, T., Havelaar, A., Angulo, F., Gibb, H., Kirk, M., Lake, R., Speybroeck, N., Torgerson, P., & Hald, T. (2017). Attribution of global foodborne disease to specific foods: Findings from a World Health Organization structured expert elicitation. *PLoS ONE, 12*(9), 1–26. https://doi.org/10.1371/journal.pone.0183641.

Hugerth, L. W., & Andersson, A. F. (2017). Analysing microbial community composition through amplicon sequencing: From sampling to hypothesis testing. *Frontiers in Microbiology, 8*(SEP), 1–22. https://doi.org/10.3389/fmicb.2017.01561.

Hwang, B. K., Choi, H. L., Choi, S. H., & Kim, B. S. (2020). Analysis of microbiota structure and potential functions influencing spoilage of fresh beef meat. *Frontiers in Microbiology, 11*(July), 1–11. https://doi.org/10.3389/fmicb.2020.01657.

Hwanhlem, N., Ivanova, T., Haertlé, T., Jaffrès, E., & Dousset, X. (2017). Inhibition of food-spoilage and foodborne pathogenic bacteria by a nisin Z-producing Lactococcus lactis subsp. lactis KT2W2L. *LWT - Food Science and Technology, 82*, 170–175. https://doi.org/10.1016/j.lwt.2017.04.052.

Illikoud, N., Jaffrès, E., & Zagorec, M. (2018). Brochothrix thermosphacta. *Reference Module in Life Sciences*. Elsevier https://doi.org/10.1016/B978-0-12-809633-8.12106-5.

Jarvis, K. G., Daquigan, N., White, J. R., Morin, P. M., Howard, L. M., Manetas, J. E., Ottesen, A., Ramachandran, P., & Grim, C. J. (2018). Microbiomes associated with foods from plant and animal sources. *Frontiers in Microbiology, 9*(OCT), 1–13. https://doi.org/10.3389/fmicb.2018.02540.

Kharel, N., Palni, U., & Tamang, J. P. (2016). Microbiological assessment of ethnic street foods of the Himalayas. *Journal of Ethnic Foods, 3*(3), 235–241. https://doi.org/10.1016/j.jef.2016.01.001.

Khayyira, A. S., Rosdina, A. E., Irianti, M. I., & Malik, A. (2020). Simultaneous profiling and cultivation of the skin microbiome of healthy young adult skin for the development of therapeutic agents. *Heliyon, 6*(4), e03700. https://doi.org/10.1016/j.heliyon.2020.e03700.

Lewis, E., Hudson, J. A., Cook, N., Barnes, J. D., & Haynes, E. (2020). Next-generation sequencing as a screening tool for foodborne pathogens in fresh produce. *Journal of Microbiological Methods, 171*. https://doi.org/10.1016/j.mimet.2020.105840 105840.

Li, L., Mendis, N., Trigui, H., Oliver, J. D., & Faucher, S. P. (2014). The importance of the viable but non-culturable state in human bacterial pathogens. *Frontiers in Microbiology, 5*(JUN), 1. https://doi.org/10.3389/FMICB.2014.00258/BIBTEX.

Li, S., Tao, Y., Li, D., Wen, G., Zhou, J., Manickam, S., Han, Y., & Chai, W. S. (2021). Fermentation of blueberry juices using autochthonous lactic acid bacteria isolated from fruit environment: Fermentation characteristics and evolution of phenolic profiles. *Chemosphere, 276*. https://doi.org/10.1016/j.chemosphere.2021.130090 130090.

Li, Y. M., Cao, W. W., Liang, S. L., Yamasaki, S., Chen, X., Shi, L., & Ye, L. (2020). Metagenomic characterization of bacterial community and antibiotic resistance genes in representative ready-to-eat food in southern China. *Scientific Reports, 10*(1), 1–14. https://doi.org/10.1038/s41598-020-72620-4.

Liu, C., Cui, Y., Li, X., & Yao, M. (2021). Microeco: An R package for data mining in microbial community ecology. *FEMS Microbiology Ecology, 97*(2). https://doi.org/10.1093/femsec/fiaa255.

Lobo, A., Zúñiga, C., Worobo, R. W., Padilla-Zakour, O. I., & Usaga, J. (2019). Fate of spoilage and pathogenic microorganisms in acidified cold-filled hot pepper sauces. *Journal of Food Protection, 82*(10), 1736–1743. https://doi.org/10.4315/0362-028X.JFP-19-071.

Lorenzo, J. M., Munekata, P. E., Dominguez, R., Pateiro, M., Saraiva, J. A., & Franco, D. (2018). Main groups of microorganisms of relevance for food safety and stability: General aspects and overall description. *Innovative Technologies for Food Preservation: Inactivation of Spoilage and Pathogenic Microorganisms, 53–107*. https://doi.org/10.1016/B978-0-12-811031-7.00003-0.

Matanza, X. M., & Osorio, C. R. (2020). Exposure of the opportunistic marine pathogen Photobacterium damselae subsp. Damselae to human body temperature is a stressful condition that shapes the transcriptome, viability, cell morphology, and virulence. *Frontiers in Microbiology, 11*. https://doi.org/10.3389/fmicb.2020.01771.

Mathur, P., & Schaffner, D. W. (2013). Effect of lime juice on vibrio parahaemolyticus and salmonella enterica inactivation during the preparation of the raw fish dish ceviche. *Journal of Food Protection, 76*(6), 1027–1030. https://doi.org/10.4315/0362-028X.JFP-12-526.

McLaren, M. R., & Callahan, B. J. (2021). *Silva 138.1 prokaryotic SSU taxonomic training data formatted for DADA2*. https://doi.org/10.5281/ZENODO.4587955

Mira Miralles, M., Maestre-Carballa, L., Lluesma-Gomez, M., & Martinez-Garcia, M. (2019). High-throughput 16s rRna sequencing to assess potentially active bacteria and foodborne pathogens: A case example in ready-to-eat food. *Foods, 8*(10), 480. https://doi.org/10.3390/foods8100480.

Moloi, M., Lenetha, G. G., & Malebo, N. J. (2021a). Microbial levels on street foods and food preparation surfaces in Mangaung Metropolitan Municipality. *Health SA Gesondheid, 26*, 1–7. https://doi.org/10.4102/HSAG.V26I0.1407.

Moloi, M., Lenetha, G. G., & Malebo, N. J. (2021b). Microbial levels on street foods and food preparation surfaces in Mangaung Metropolitan Municipality. *Health SA Gesondheid, 26*. https://doi.org/10.4102/hsag.v26i0.1407.

Muriuki, S. W., Rengan, M. S., & Budambula, N. L. M. (2021). Prokaryotic diversity and potentially pathogenic bacteria in vended foods and environmental samples. *Annals of Microbiology, 71*(1), 27. https://doi.org/10.1186/s13213-021-01640-w.

Niu, C., Xue, Y., Liu, C., Zheng, F., Wang, J., & Li, Q. (2020). Identification of gas-forming spoilage bacteria in chili sauce and its control using nisin and salt. *LWT, 118*. https://doi.org/10.1016/j.lwt.2019.108658 108658.

Noor, R., Maniha, S. M., & Review, M. (2019). Opportunistic food borne infections: A brief review. *Acta Scientific Microbiology, 2*(7), 2581–3226. https://doi.org/10.31080/ASMI.2019.02.0273.

Pérez-Lazo, G., Morales-Moreno, A., Soto-Febres, F., Jove-Químper, H., Morales-Castillo, L., Palomares-Reyes, C., Del Valle-Mendoza, J., Aguilar-Luis, M., & Silva-Caso, W. (2020). First report of Myroides phaeus bacteraemia identified by Polymerase chain reaction and genetic sequencing. *IDCases, 19*. https://doi.org/10.1016/J.IDCR.2020.E00695.

Petruzzi, L., Corbo, M. R., Sinigaglia, M., & Bevilacqua, A. (2017). Microbial spoilage of foods: Fundamentals. *The Microbiological Quality of Food: Foodborne Spoilers, 1–21*. https://doi.org/10.1016/B978-0-08-100502-6.00002-9.

Ramirez Martinez, M. E., Rodriguez Castillejos, G. C., Hernandez Jimenez, M. C., Ramirez Quintanilla, L. Y., Siller Lopez, F., Acosta Cruz, E., & Martinez Montoya, H. (2022). Analysis of bacterial diversity in raw fish ceviche. *Journal of Microbiology, Biotechnology and Food Sciences, 11*(5), e3321. https://doi.org/10.55251/jmbfs.3321.

Randazzo, W., Zuber, S., D'Souza, D. H., & Sánchez, G. (2021). Editorial: Enteric viruses in food: Survival and inactivation methods. *Frontiers in Microbiology, 12*. https://doi.org/10.3389/fmicb.2021.722368.

Raza, J., Asmat, T. M., Mustafa, M. Z., Ishtiaq, H., Mumtaz, K., Jalees, M. M., Samad, A., Shah, A. A., Khalid, S., & Rehman, H. ur. (2021). Contamination of ready-to-eat street food in Pakistan with Salmonella spp.: Implications for consumers and food safety. *International Journal of Infectious Diseases, 106*, 123–127. https://doi.org/10.1016/J.IJID.2021.03.062.

Reimer, L. C., Sardà Carbasse, J., Koblitz, J., Ebeling, C., Podstawka, A., & Overmann, J. (2022). BacDive in 2022: The knowledge base for standardized bacterial and archaeal data. *Nucleic Acids Research, 50*(D1), D741–D746. https://doi.org/10.1093/NAR/GKAB961.

Rodríguez-López, P., Rodríguez-Herrera, J. J., & Cabo, M. L. (2020). Tracking bacteriome variation over time in Listeria monocytogenes-positive foci in food industry. *International Journal of Food Microbiology, 315*. https://doi.org/10.1016/J.IJFOODMICRO.2019.108439 108439.

Salamandane, A., Silva, A. C., Brito, L., & Malfeito-Ferreira, M. (2021). Microbiological assessment of street foods at the point of sale in Maputo (Mozambique). *Food Quality and Safety, 5*. https://doi.org/10.1093/fqsafe/fyaa030.

Salazar-Llorente, E., Morales, M., Sornoza, I., Mariduena-Zavala, M., Gu, G., Nou, X., Ortiz, J., Maldonado-Alvarado, P., & Cevallos-Cevallos, J. (2020). Microbiological quality of high-demand foods from three major cities in Ecuador. *Journal of Food Protection*. https://doi.org/10.4315/jfp-20-271.

Sanguyo, F. H. C., Angeles, F. L. A., Deborde, S. M. V., Jumarang, K. C., Mahait, J. A., Onayan, R. S. M., Pacada, M. J. V., Pitong, C. R., & Hagosojos, B. M. (2021). Bacteriocin and its current application as a food packaging film component against spoilage: A narrative review. *Asian Journal of Biological and Life Sciences, 10*(2), 325–339. https://doi.org/10.5530/ajbls.2021.10.45.

Simopoulos, A. P., & Bhat, R. V. (2000). *Street foods*. S. Karger AG https://doi.org/10.1159/isbn.978-3-318-00473-1.

Song, A.-A.-L., In, L. L. A., Lim, S. H. E., & Rahim, R. A. (2017). A review on Lactococcus lactis: From food to factory. *Microbial Cell Factories, 16*(1), 55. https://doi.org/10.1186/s12934-017-0669-x.

Syromyatnikov, M. Y., Kokina, A. V., Solodskikh, S. A., Panevina, A. V., Popov, E. S., & Popov, V. N. (2020). High-throughput 16s rRna gene sequencing of butter microbiota reveals a variety of opportunistic pathogens. *Foods, 9*(5), 608. https://doi.org/10.3390/foods9050608.

Teixeira, C. G. (2021). The Weissella genus in the food industry: A review. *Research Society and Development, 2021*, 1–15.

Tsafrakidou, P., Sameli, N., Bosnea, L., Chorianopoulos, N., & Samelis, J. (2021). Assessment of the spoilage microbiota in minced free-range chicken meat during storage at 4 C in retail modified atmosphere packages. *Food Microbiology, 99*. https://doi.org/10.1016/j.fm.2021.103822 103822.

Vicentini, F. A., Keenan, C. M., Wallace, L. E., Woods, C., Cavin, J.-B., Flockton, A. R., Macklin, W. B., Belkind-Gerson, J., Hirota, S. A., & Sharkey, K. A. (2021). Intestinal microbiota shapes gut physiology and regulates enteric neurons and glia. *Microbiome, 9*(1), 210. https://doi.org/10.1186/s40168-021-01165-z.

Woods, D. F., Kozak, I. M., & O'Gara, F. (2020). Microbiome and functional analysis of a traditional food process: Isolation of a novel species (Vibrio hibernica) with industrial potential. *Frontiers in Microbiology, 11*(April), 1–15. https://doi.org/10.3389/fmicb.2020.00647.

World Health Organization (2020). Foodborne diseases. *Overview* https://www.who.int/health-topics/foodborne-diseases#tab=tab_1.

Wu, X., Spencer, S., Gushgari-Doyle, S., Yee, M. O., Voriskova, J., Li, Y., Alm, E. J., & Chakraborty, R. (2020). Culturing of "unculturable" subsurface microbes: Natural organic carbon source fuels the growth of diverse and distinct bacteria from groundwater. *Frontiers in Microbiology, 11*(December). https://doi.org/10.3389/fmicb.2020.610001.

Xu, S., Liu, Y., Gao, J., Zhou, M., Yang, J., He, F., Kastelic, J. P., Deng, Z., & Han, B. (2021). Comparative genomic analysis of Streptococcus dysgalactiae subspecies dysgalactiae isolated from bovine mastitis in China. *Frontiers in Microbiology, 12*. https://doi.org/10.3389/fmicb.2021.751863.

Yoon, S., Cho, Y., Lee, K., Lim, S. R., Park, S. Y., Kwon, H., Lee, Y. S., Han, J. E., & Kim, J. H. (2020). Complete genome sequence of Photobacterium damselae subsp. damselae KC-Na-NB1, a potential histamine producer isolated from finless porpoise (Neophocaena asiaeorientalis). *Korean Journal of Microbiology, 56*(4), 397–400. https://doi.org/10.7845/kjm.2020.0091.

Zhao, F., Wei, Z., Zhou, G., Kristiansen, K., & Wang, C. (2022). Effects of different storage temperatures on bacterial communities and functional potential in pork meat. *Foods, 11*(15), 2307. https://doi.org/10.3390/FOODS11152307/S1.