Research Paper

Microbiological Quality of High-Demand Food from Three Major Cities in Ecuador

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ABSTRACT

Bacterial foodborne diseases are among the most important public health issues worldwide, but in Ecuador, reports on the microbiological quality of food are scarce. In this cross-sectional study, 450 samples of high-demand Ecuadorian food, including bolon, encebollado, sauces, ceviche, fruit, fruit juice, fruit salad, cheese, raw chicken, and ground beef, were collected from popular street markets in the cities of Guayaquil, Quito, and Cuenca. Populations of total aerobic mesophilic bacteria, total coliforms, fecal coliforms, *Escherichia coli, Salmonella enterica,* and *Listeria monocytogenes* were examined on composited samples by plate count following the local regulations (Norma Tecnica Ecuatoriana, Instituto Ecuatoriano de Normalización) for each kind of food. The individual and interaction effects of the city and food type on the levels of each bacterial group were assessed by two-way analysis of variance. Selected colonies from each culture were identified using Biolog OmniLog ID and sequencing of the V3 to V4 region on the 16S rRNA gene. Average total aerobic mesophilic bacteria, total coliform, fecal coliform, and *E. coli* levels were 5.10 ± 0.12, 2.50 ± 0.16, 1.09 ± 0.12, and 0.83 ± 0.12 log CFU/g or mL, respectively, with significant variations among the cities. The prevalence of *Salmonella* in chicken and sauces and *L. monocytogenes* in cheese and fruit salad was greater than 20%. Opportunistic pathogens including *Klebsiella pneumoniae, Staphylococcus sciuri,* and *Enterococcus* spp. were frequently identified in the samples from all three cities. High prevalence of spoilage microorganisms such as *Bacillus amyloliquefaciens* and biocontrol bacteria such as *Lactococcus lactis* was also observed. This is the first report on the microbiological quality of food from Ecuador.

HIGHLIGHTS

- Microbiological quality of 10 Ecuadorian food groups was examined.
- Prevalence of indicator microorganisms showed high levels of contamination in selected food groups.
- Foodborne pathogens were detected at high frequency in selected food groups.
- Significant intercity difference was observed for food microbiological quality.

It is estimated that 600 million people worldwide are affected by foodborne poisoning and 420,000 people die from this cause every year *(64),* with associated annual economic costs exceeding $14 billion *(8).* Foodborne bacterial pathogens such as *Salmonella enterica, Escherichia coli* (EC), *Listeria monocytogenes, Campylobacter jejuni, Clostridium* spp., *Shigella* spp., *Yersinia enterocolitica, Staphylococcus aureus, Vibrio* spp., and *Helicobacter pylori* are responsible for numerous outbreaks *(8).* In most cases, foodborne pathogens cause nausea, bloody diarrhea, vomiting, and fever, among other symptoms, but some bacteria such as *S. enterica* can invade the circulatory system and cause septicemia *(37, 40)* and even death *(8, 20).* In addition, brain damage and meningitis in infants can be caused by *L. monocytogenes (3),* whereas other pathogens, such as *Clostridium botulinum,* can produce toxins able to paralyze the nervous system and provoke the death of the affected individuals *(58).* In Ecuador, although outbreaks of salmonellosis are commonly reported *(15, 61)* other outbreaks may be underestimated as reported for other developing countries *(50).*

To control foodborne illnesses, most developed countries have established science-based food safety systems and

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implemented regulations supported by research. Research programs focusing on the prevalence of foodborne pathogens in the food chain, spread of foodborne illness within communities, and risk exposure within populations are considered the key to an effective national food safety system (63). However, research-based food safety programs are almost exclusively executed in industrialized countries (28, 63) and are missing from many developing countries such as Ecuador because of limitations in resources and lack of baseline data on the microbial contamination of food. In the United States, microbial baseline studies are carried out for commodities or regions for which pathogen prevalence data are not available (2). Similarly, baseline studies exploring the microbiological quality of ready-to-eat (RTE) food distributed in schools from China (66) and on popular street food from Pakistan (45) and Nigeria (52) can be found in the literature. However, reports on the microbiological quality of popular food in Ecuador are scarce.

Total counts of aerobic bacteria and the levels of coliforms have been used as food safety indicators for many years (13), and various regulations base the microbiological acceptability of food on the levels of indicator bacteria (49). Indicator organisms are usually quantified during baseline microbiological quality studies (2, 45, 52, 66). However, predominant isolates from both total aerobic and coliform tests have rarely been identified, precluding the detection of hazardous bacteria, because various foodborne pathogens can grow among the indicator microorganisms (52).

The objective of this study was to evaluate the microbiological quality of high-demand food from the three largest cities in Ecuador, identifying the prevalent isolates from indicator organism tests and assessing the presence of *Salmonella* spp. and *L. monocytogenes* in the samples.

MATERIALS AND METHODS

Samples. Ten groups of commonly consumed Ecuadorian food were selected, and 15 samples for each group were taken from popular outdoor markets at Quito, Guayaquil, and Cuenca (Table 1), the three largest cities in Ecuador. The food groups sampled were raw chicken, ground beef, fresh cheese (unaged cheese made from pasteurized or boiled milk), bolon (mashed green plantains with cheese and pork, baked or fried), ceviche (cooked fish with raw onions, peppers, tomatoes, and cilantro marinated in lemon juice), encebollado (cooked fish and cassava soup with raw onions and cilantro), sauces (homemade mayonnaise and other tomato- and onion-based raw sauces), fruit (strawberries, apples, grapes, and pears), fruit juice (fresh homemade orange juice or lemonade with no thermal treatment), and fruit salad (mixed cut fruit with no thermal treatment). In total, 450 samples (3 cities × 10 food groups × 15 samples) were taken and immediately shipped in coolers with gel packs to el Centro de Investigaciones Biotecnológicas del Ecuador in Guayaquil for laboratory processing.

Upon receipt, solid food such as chicken, ground beef, fresh cheese, bolon, and fruit was aseptically cut into pieces of 2 to 5 g. The pieces from chicken samples were taken from the areas with skin, as suggested elsewhere (29). For samples containing a mix of liquid and solid food, such as ceviche, encebollado, fruit salad, and sauces, 10 g of each sample incorporating both liquid and solid parts of the food was aseptically weighted. Then, 10 g of three individual samples from the same food group and city was combined to make composite samples. Similarly, 10 mL of each juice sample was used for compositing three individual samples from the same city. A total of 150 composite samples were obtained.

Each composite sample was transferred to an unfiltered sterile sample bag containing 270 mL of peptone water (Neogen, Lansing, MI) and homogenized in a Stomacher 80 Biomaster (West Sussex, UK) at 265 rpm for 1 min to make a stock suspension. Chunks of food from the stock suspension were not allowed for serial dilution and plating.

Enumeration of indicator populations. Samples were processed following the protocols described in local regulations for each type of microbiological analysis (34). Table 1 describes the culture-based tests performed for each sample group. Stock suspensions from the composite samples were used for enumeration of total aerobic mesophilic bacteria (TAM), total coliforms (TC), fecal coliforms (FC), and EC. Indicator population enumeration was not performed for raw chicken samples.

For TAM analysis, decimal serial dilutions were prepared from each stock suspension, pour inoculated on plate count agar (PCA; Neogen) plates, and incubated at 37°C for 24 to 48 h. Colonies on PCA plates were enumerated in plates with counts up to 250 CFU. The limit of detection was 1 CFU/g. TAM enumeration was not performed for raw ground beef samples, because this is not a required indicator for this sample group (34).

The levels of TC were estimated by the most-probable-number (MPN) method (35). For this, 1-mL aliquots of each dilution prepared earlier for TAM were transferred to three individual tubes with 10 mL of sterile brilliant green lactose bile broth (BGLB; Neogen) with Durham tubes inside and incubated at 35 ± 2°C for 48 h. Solution in tubes showing gas production was streak inoculated onto eosin blue methylene (EMB; Neogen) agar and incubated at 35 ± 2°C for 24 h (35). Tubes with gas production yielding black or pink to orange colonies on EMB agar were considered positive for coliforms. The number of positive tubes observed at each dilution was recorded and loaded to the MPN calculator from the U.S. Environmental Protection Agency (62). Results were reported as MPN per gram (with a 3-MPN/g detection limit at 95% confidence intervals). Samples that tested positive for TC were subjected to FC analysis.

For MPN estimations of FC, 1-mL aliquots of the stock suspension and each of its serial dilutions were suspended into three tubes with 10 mL of BGLB and three tubes with 10 mL of tryptone water (TW) and incubated at 45.5 ± 0.2°C for 48 h. Three droplets of Kovac's reagent were added to the TW tubes after incubation. Tube pairs showing both gas production in BGLB and a red ring on TW after adding Kovac's reagent were considered positive for FC (33). The number of positive tube pairs at each dilution was used for MPN estimation of each sample as described for TC tests. Samples that tested positive for FC were subjected to EC analysis.

For MPN estimations of EC, BGLB tubes showing gas production were streak inoculated onto EMB agar and incubated at 35 ± 2°C for 24 h. Colonies showing a distinctive metallic green sheen were streak inoculated onto Simon's citrate agar (SCA; Neogen) slants and incubated at 35 ± 2°C for 24 h. Tubes were recorded as positive for EC if the SCA slants remained green (33). The number of positive tubes was then used for MPN estimation of EC in each sample as described for TC tests.

Detection of *S. enterica* and *L. monocytogenes*. *S. enterica* isolation was carried out for all food group samples except fruit,

TABLE 1. *Food groups sampled at each city with corresponding analyses performed*

Food[a]			Analysis performed[b]					
Group	Type	Specialty	TAM	TC	FC	EC	SE	LM
Chicken	Raw	Common	—	—	—	—	X	—
Ground beef	Raw	Common	—	X	X	X	X	—
Fresh cheese	RTE	Ecuador	X	X	X	X	X	X
Bolon	RTE	Ecuador	X	X	X	X	X	X
Ceviche	RTE	Ecuador	X	X	X	X	X	—
Encebollado	RTE	Ecuador	X	X	X	X	X	—
Sauces	RTE	Ecuador	X	X	X	X	X	—
Fruit	Fresh	Common	X	X	X	X	—	—
Fruit juice	RTE	Common	X	X	X	X	—	X
Fruit salad	RTE	Common	X	X	X	X	—	X

[a] Food group description provided in "Materials and Methods." RTE, ready-to-eat.
[b] TAM, total aerobic mesophile bacteria; TC, total coliforms; FC, fecal coliforms; EC, *E. coli*; SE, *S. enterica* isolation; LM, *L. monocytogenes* isolation; —, analysis not performed; X, analysis performed.

fruit juice, and fruit salad. For isolation of *S. enterica,* 25 g (solid) or 25 mL (liquid sauces) from composited food samples was suspended in 225 mL of 3% saline peptone water and incubated at 37°C for 24 h. Then, 1 mL of the suspension was inoculated into 9 mL of Rapapport broth (Neogen) and incubated at 37°C for 24 h. The enrichment culture was streak onto triple sugar iron (TSI; Neogen) slants and onto brilliant green phenol red lactose sucrose (BPLS; Neogen) agar plates, followed by incubation at 37°C for 24 h. Samples were considered positive for *Salmonella* if BPLS plates turned dark red and TSI slants turned dark red on the surface and yellow at the bottom with or without darkening of the media because of hydrogen sulfide production *(34)*.

L. monocytogenes isolation was carried out for fresh cheese, bolon, fruit juice, and fruit salad. For this, 25 g (solid) or 25 mL (liquid) of the composited samples was suspended in 225 mL of buffered *Listeria* enrichment broth (Neogen) and incubated at 37°C for 24 h. Then, 1 mL of the suspension was transferred to 9 mL of Fraser broth with Fraser supplements and incubated at 37°C for 24 h. Presumptively positive tubes with *Listeria* spp. (darkening of Fraser broth) were streaked onto Oxford agar and incubated at 37°C for 24 h. Black colonies with a translucent or white halo were selected for identification of *L. monocytogenes* using biochemical and molecular methods described in the next section.

Biochemical and molecular identification of bacteria. Three colonies from the PCA plates selected for colony counting and all EMB, BPLS, TSI, and Oxford plates of each composite sample—regardless of the TC, FC, EC, *Salmonella,* or *L. monocytogenes* outcome—were randomly selected and streaked onto tryptic soy agar (TSA) for purification. All colonies that tested positive for *Salmonella* spp. and *L. monocytogenes* were also purified on TSA. After incubation at 37°C for 24 h, one isolated colony from each TSA plate was used for each biochemical and molecular identification method.

Biochemical identification was carried out using the Omni-Log ID kit (Biolog, Hayward, CA) following the manufacturer's instructions. TSA colonies were transferred to blood agar (Apracom, Guayaquil, Ecuador) and incubated at 33°C for 24 h. The resulting colonies were suspended in the Biolog's inoculating fluids A and B to a final 95% transmittance as measured in the 3587 turbidimeter (Biolog). A volume of 100 μL of the suspensions was placed in each well of a GEN III MicroPlate (Biolog) and incubated at 33°C for 24 h. Wells that turned purple

were considered positive, whereas colorless wells were reported as negative. The results were compared with those in the OmniLog Biolog database for identification of the isolates.

For molecular identification, a partial colony from each TSA plate was suspended in 50 μL of 20 mM NaOH and microwaved at high intensity to boiling *(24)*. The microwave-boiled cell suspension was directly used as template for PCR reaction. The DNA sequence corresponding to a partial 16S rRNA gene (V3 to V4 region) was amplified using 1.5 μL of the DNA template prepared as earlier, 12.5 μL of GoTaq Colorless master mix (Promega, Madison, WI), 10 μL of double distilled water, 0.5 μL of each primer (341F: 5′-CCTACGGGNGGCWGCAG, 806R: 5′-GACTACHVGGGTATCTAATCC) in a 25-μL reaction *(41)*. A negative control (master mix without DNA) was included in each amplification run. The PCR amplification conditions were as follows: 95°C for 5 min, followed by 30 cycles of 95°C for 1 min, 55°C for 2 min, and 72°C for 2 min, with a final extension at 72°C for 2 min. The PCR products, after checking for purity by electrophoresis on 2% agarose gel, were sent to the Macrogen-Korea service laboratory for sequencing, and sequencing data were then submitted to BLAST (NCBI, Bethesda, MD) to obtain the bacterial identifier *(11)*.

Data analysis. The study followed an observational cross-sectional research plan as suggested in previous reports *(18, 48, 51)*. For this, data from 5 composite samples (replicates) were obtained for each of the 10 food groups at each city, yielding 150 composite samples (5 composites $\times$ 10 food groups $\times$ 3 cities) in total. For comparison purposes, MPN counts from TC, FC, and EC were reported as CFU and all bacterial counts were log transformed. For data analysis, the sampling city and the food group were considered independent variables, whereas the TAM, TC, FC, and EC counts were the individual dependent variables. The normality and homoscedasticity of each dependent variable were verified using Kolmogorov-Smirnov and Levene's tests, respectively. The levels of TAM, TC, FC, and EC were compared between cities and food types, as was their interaction, using two-way analysis of variance (ANOVA) followed by post hoc Tukey tests. All tests were run in Matlab R2020a (MathWorks, Natick, MA), and significance was reported for $P < 0.05$.

The prevalence of each bacterial species was estimated as the percentage of samples in which the species was detected. Thus, prevalence values were reported as 20, 40, 60, 80, or 100% if the species was detected in one, two, three, four, or five composite
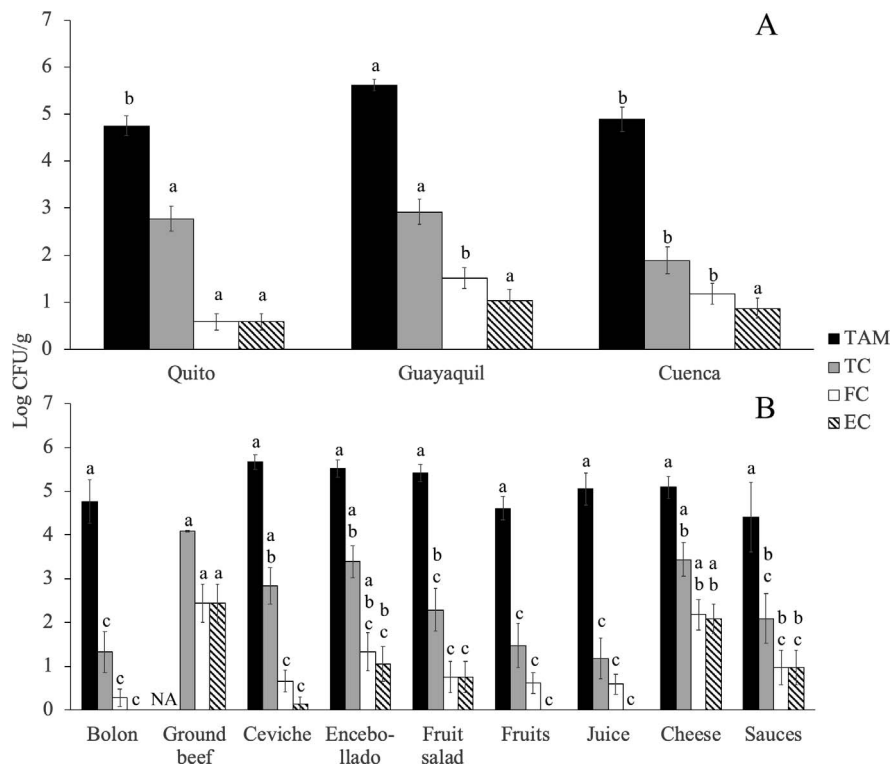
FIGURE 1. *Individual effect of city (A) and food type (B) on total aerobic mesophilic bacteria (TAM), total coliforms (TC), fecal coliforms (FC), and* E. coli *(EC). Different letters above the bars represent significant differences between cities (A) or food groups (B). NA, not analyzed. Bars denote the averages and standard errors of 45 replicates (A) or 15 replicates (B).*

samples, respectively, that were tested for each food group and city. A heatmap was built in Heatmapper.ca (7) using complete linkage clustering and Pearson's correlations applied to the prevalence data.

RESULTS

Microbiological quality of Ecuadorian food. The levels of TAM ranged from 0.3 to 7.0 log CFU/g (solid) or log CFU/mL (liquid) across all samples tested, whereas the overall counts of TC, FC, and EC were from nondetectable (under the detection limit of 0.48 log CFU/g) to 4.11 log CFU/g for all three tests. Two-way ANOVA showed significant individual and interaction effects (Supplemental Table S1). A significant independent variable effect of the city was observed for TAM, TC, and FC counts (Fig. 1A). Samples from Guayaquil showed significantly higher TAM levels (5.16 ± 0.12 log CFU/g) when compared with those from Quito (4.75 ± 0.20 log CFU/g) and Cuenca (4.89 ± 0.25 log CFU/g). Similarly, the TC counts from Quito (2.77 ± 0.27 log CFU/g) and Guayaquil (2.92 ± 0.27 log CFU/g) were significantly higher than those from Cuenca (1.89 ± 0.29 log CFU/g), whereas the FC levels from Guayaquil (1.51 ± 0.23 log CFU/g) and Cuenca (1.18 ± 0.22 log CFU/g) significantly exceeded those from Quito (0.58 ± 0.17 log CFU/g).

Food type significantly affected the levels of TC, FC, and EC (Fig. 1B). Ground beef (4.09 ± 0.02 log CFU/g), ceviche (2.84 ± 0.42 log CFU/g), encebollado (3.39 ± 0.38 log CFU/g), and cheese (3.44 ± 0.48 log CFU/g) were the food groups with the highest significant TC levels, whereas bolon (1.32 ± 0.47 log CFU/g), fruit (1.48 ± 0.51 log CFU/g), and juice (1.18 ± 0.46 log CFU/g) samples showed the lowest TC counts. Similarly, FC levels were

significantly higher on ground beef (2.43 ± 0.44 log CFU/g), encebollado (1.33 ± 0.44 log CFU/g), and cheese (2.17 ± 0.35 log CFU/g) than on samples of bolon (0.28 ± 0.19 log CFU/g), ceviche (0.66 ± 0.25 log CFU/g), fruit salad (0.75 ± 0.35 log CFU/g), fruit (0.60 ± 0.24 log CFU/g), juice (0.59 ± 0.23 log CFU/g), and sauces (0.97 ± 0.40 log CFU/g). Ground beef (2.44 ± 0.44 log CFU/g) and cheese (2.09 ± 0.34 log CFU/g) were the food types with the highest EC counts, whereas this species was not detected in bolon, fruit, and juice samples.

The interaction between city and food type significantly affected TAM, TC, FC, and EC counts. Sauce (6.65 ± 0.04 log CFU/g) and cheese (5.98 ± 0.47 log CFU/g) samples from Guayaquil, fruit salad from Quito (5.81 ± 0.40 log CFU/g), and encebollado (6.05 ± 0.08 log CFU/g), ceviche (5.99 ± 0.11 log CFU/g), and bolon (6.25 ± 0.43 log CFU/g) from Cuenca showed the highest TAM levels, which were significantly higher than those observed in sauces (2.70 ± 1.11 log CFU/g) from Cuenca and fruit (3.31 ± 0.30 log CFU/g) and bolon (2.91 ± 0.30 log CFU/g) from Quito (Fig. 2A). Encebollado from Quito and ground beef from the three cities showed the highest overall TC levels, with average counts exceeding 4.00 log CFU/g, whereas this bacterial group was absent from fruit salad, fruit, and juice samples from Cuenca and juice from Quito (Fig. 2B). Ground beef from Guayaquil showed the highest FC levels at 3.29 ± 0.82 log CFU/g, whereas no FC was detected in bolon from Quito and Guayaquil, encebollado from Quito, or fruit salad, fruit, and juice samples from Quito and Cuenca (Fig. 2C). Similarly, ground beef from Guayaquil showed the highest EC counts at 3.29 ± 0.82 log CFU/g, whereas this species was not observed in ceviche from Guayaquil and Cuenca, encebollado from Quito, fruit salad

FIGURE 2. *Total aerobic mesophilic bacteria (A), total coliforms (B), fecal coliforms (C), and* E. coli *(D) from different food sampled from Quito, Guayaquil, and Cuenca. Different letters above the bars represent significant differences between cities and food group combinations. NA, not analyzed. Bars denote the averages and standard errors of five replicates.*
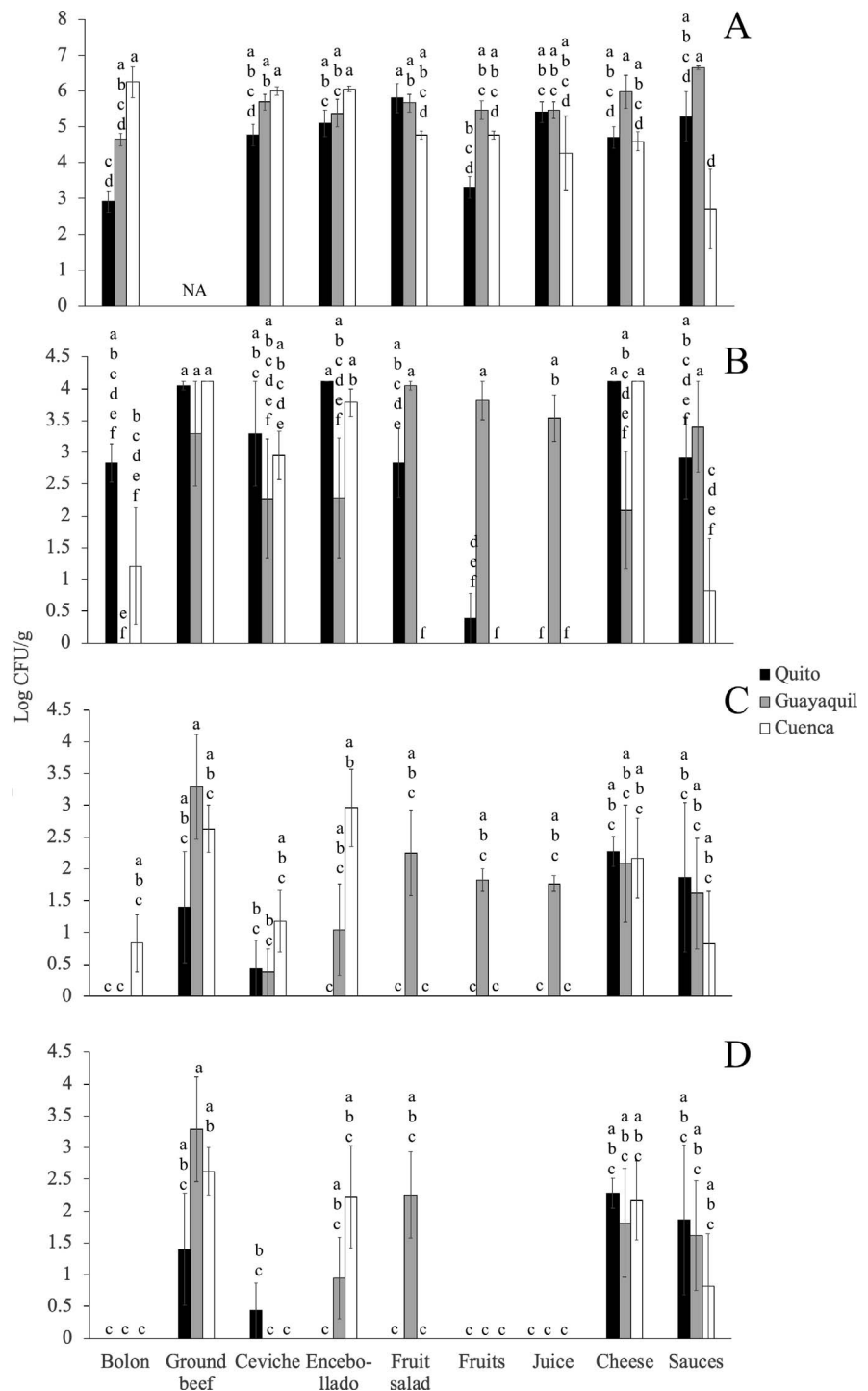
from Quito and Cuenca, and bolon, fruit, and juice samples from the three cities (Fig. 2D).

Prevalence of EC, *S. enterica,* and *L. monocytogenes*. Presumptive *Salmonella, L. monocytogenes,* and EC isolates obtained from the culture-based methods were confirmed using both Biolog OmniLog ID and 16S rRNA gene sequencing. The prevalence of EC, *Salmonella,* and *L. monocytogenes* in selected food groups is shown in Figure 3.

EC, although generally commensal, encompasses deadly foodborne pathogens such as enterohemorrhagic serotypes and is treated as a potential pathogen in this study.

It was highly prevalent in ground beef (40 to 100%) and in fresh cheese (60 to 100%) from all three cities, with national averages of 73 and 80%, respectively. It was also frequently detected among samples of sauces (20 to 40%) and encebollado (0 to 80%), with national averages of 33 and 40%, respectively. EC was not detected in samples of bolon, fruit, and fruit juice but was detected in fruit salad from Guayaquil (80%) and ceviche from Quito (20%).

S. enterica was most prevalent in raw chicken samples, ranging from 20 to 60% in the three cities for a national average of 40%. The pathogen was also isolated in sauces (20% prevalence) from Quito and Cuenca, as well as
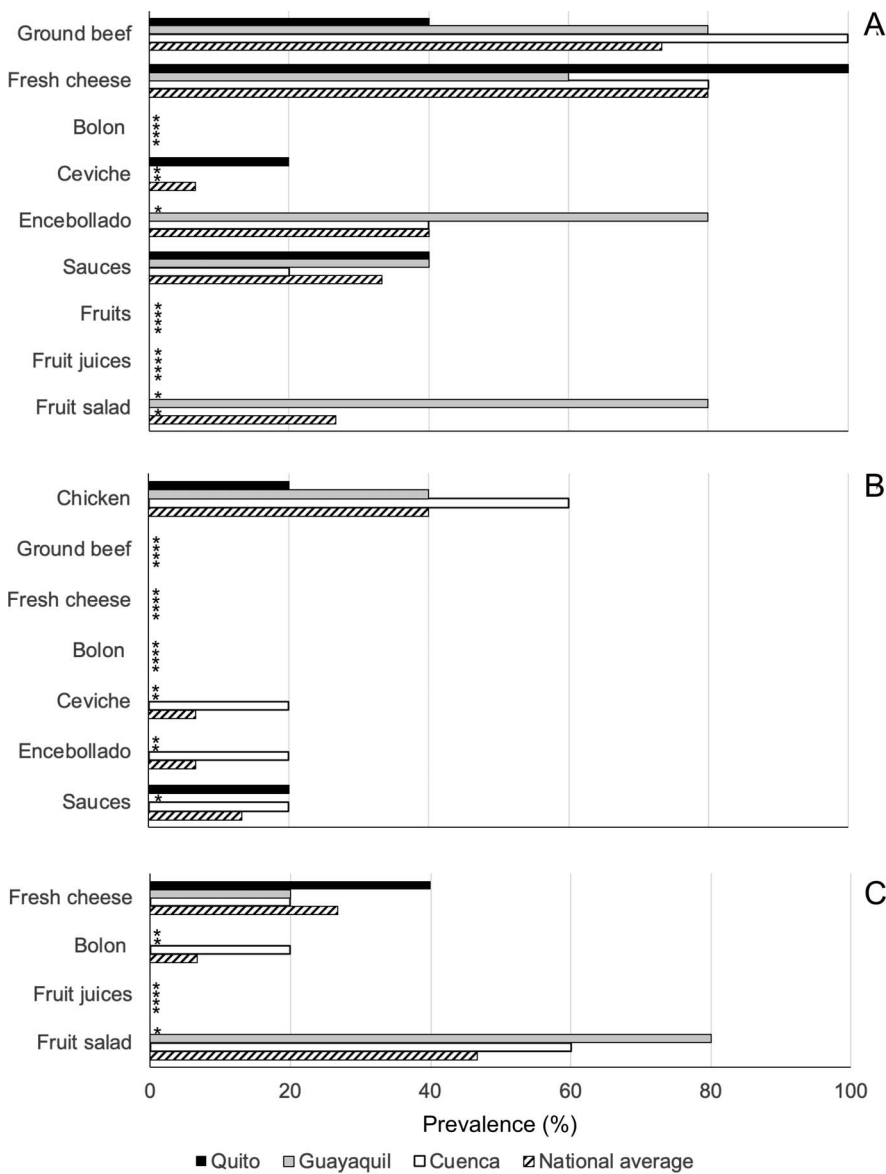
FIGURE 3. *Prevalence (%) of* E. coli *(A),* S. enterica *(B), and* L. monocytogenes *(C) in the five composite samples of the selected food groups from each of the three Ecuadorian cities. National averages are the mean prevalence values of all cities. *, not detected.*

ceviche (20% prevalence) and encebollado from Cuenca (20% prevalence). *S. enterica* was not isolated from ground beef, fresh cheese, or bolon.

L. monocytogenes was detected in cheese (20 to 40% prevalence) from all three cities, with a national average of 27%, and in bolon (20% prevalence) from Cuenca. The pathogen showed high prevalence in fruit salad from Guayaquil (80% prevalence) and Cuenca (60% prevalence) but was not detected in fruit salad from Quito.

Prevalence of other bacteria. In addition to EC, *S. enterica,* and *L. monocytogenes,* which were isolated in targeted analyses, 97 bacteria species were identified by Biolog OmniLog ID and 16S rRNA gene sequencing from the randomly picked colonies that were isolated by enumerating indicator microorganisms (Table S2). All isolates showed identity scores of at least 80% using OmniLog ID and at least 99% by 16S sequencing with Blast E values of zero. The 50 most prevalent species excluding EC, *S. enterica,* and *L. monocytogenes* were shown as a heatmap for each food group-and-city combination (Fig. 4).

Each identified species was classified based on literature descriptions as a potential foodborne pathogen, opportunistic pathogen, food spoilage bacterium, biocontrol agent, or other bacterium. The pathogen *Shigella flexneri* was detected in encebollado and fruit samples from Cuenca and sauces from Guayaquil. In addition, EC was detected in ground beef, cheese, and sauce samples from the three cities, encebollado from Cuenca and Guayaquil, ceviche from Quito, and fruit salad from Guayaquil.

Among the opportunistic pathogens, *Enterococcus faecalis, Psychrobacter sanguinis, Staphylococcus sciuri, Enterococcus hirae, Klebsiella pneumoniae, Bacillus pumilus, Proteus mirabilis,* and *Hafnia alvei* showed the highest prevalence across all samples, whereas *Bacillus amyloliquefaciens, Serratia* spp., *Acinetobacter* spp., and *Obesumbacterium* spp. were the only observed food spoilage bacteria. Potential biocontrol bacteria—including *E. faecalis, E. hirae, E. durans, E. faecium,* and other *Enterococcus* spp.; *B. amyloliquefaciens, B. subtilis, B. velezensis,* and other *Bacillus* spp.; and *Lactococcus lactis*—were detected in most food groups (Fig. 4).
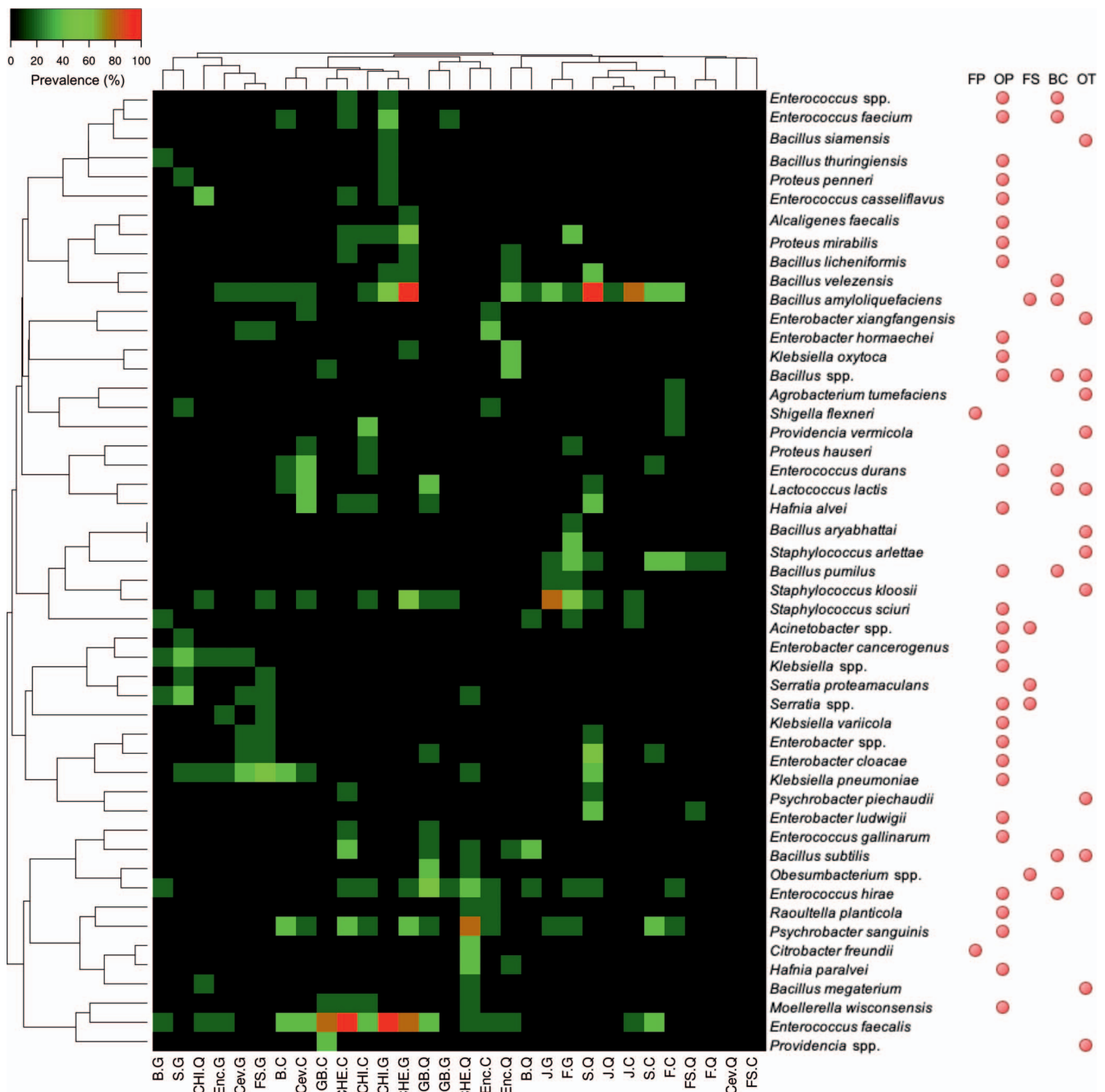
FIGURE 4. *Heatmap showing Pearson's correlation and complete linkage clustering of the 50 most prevalent bacterial species. Food inputs are labeled (food type)-(city). Food types were fruit salad (FS), cheese (CHE), bolon (B), encebollado (E), chicken (CHI), sauces (S), ground beef (GB), juice (J), ceviche (CEV), and fruit (F). Samples were from the cities of Quito (Q), Guayaquil (G), and Cuenca (C). Species detected were classified as foodborne pathogens (FP), opportunistic pathogens (OP), food spoilage bacteria (FS), biocontrol (BC), or other bacteria (OT).*

DISCUSSION

Overall microbiological quality of Ecuadorian food.
Ecuador is a developing agricultural country in South America. Food safety awareness, practices, and regulations have traditionally been lagging in this country in comparison to most industrialized and some other developing countries. Amid a new national awareness of food safety as a critical component of public health, development and implementation of science-based food safety practices and

regulations require baseline data for the microbiological quality of national food supplies. In this study, we assessed the microbiological quality of 10 commonly consumed food groups from Ecuador's three largest cities, Quito, Guayaquil, and Cuenca, encompassing about 40% of national population. These food groups included both food consumed worldwide and those that are specific to Ecuador or the region, both raw and RTE food, and both animal-originated protein-rich food and plant-based fresh food. The food samples were obtained from outdoor markets, the

predominant venues of food supplies for Ecuadorian consumers. Therefore, this study provided multiangled snapshots of the microbiological quality of food commonly consumed in Ecuador, which can be considered reference points for food safety assessment in Ecuador and the region.

The overall microbiological burdens of high-demand Ecuadorian food (TAM of 0.3 to 7.0 log CFU/g, excluding ground beef and whole chickens, and TC, FC, and EC of nondetectable to 4.11 log CFU/g) are comparable to those found in other countries (22, 46, 49). Samples from Guayaquil showed the highest TAM, TC, and FC levels. Guayaquil is on the Pacific Coast, with significantly higher temperatures compared with the highland cities of Quito and Cuenca. In Guayaquil, ambient temperature varies between 19 to 33°C through the year, whereas temperature ranges of 7 to 21°C are common in Quito and Cuenca. High ambient temperatures are more conducive to the growth and prevalence of foodborne bacteria (56).

Prevalence of *Salmonella* and *L. monocytogenes*. The prevalence of *Salmonella* in raw chicken (in the range from 20 to 60% for each city) was in agreement with findings from other regions (29, 39), but the 20% prevalence of this pathogen in sauces from Quito and Cuenca is higher than the 5% reported in chili sauces from Mexico (23). The key to the microbial safety of sauces is a pH value below 4.5, achieved by the addition of acetic or citric acids (57). Therefore, improper preparation and handling of the sauces probably contributed the most to the prevalence of *Salmonella* in this food type. Ceviche and encebollado samples from Cuenca also showed a high (>20%) prevalence of *Salmonella*. Encebollado and ceviche preparation requires a high level of postcooking ingredient manipulation, and the results may suggest improper hygiene or preparation conditions. The high amount of lemon or lime juice added to ceviche did not prevent the occurrence of the pathogen. Although lime juice can achieve up to 5-log reduction of pathogens (44), the antibacterial effect is diminished on fish and other ingredients of ceviche (31, 44).

The 20 to 40% prevalence of *L. monocytogenes* in cheese from the three cities is higher than the 0.8% reported in fresh cheese from Europe (43) but relatively lower than the 25 to 53% prevalence of the pathogen from the products of different cheesemakers from Mexico (54). The physicochemical composition of cheese is considered conducive to the growth of foodborne pathogens (32). Similarly, the high (>60%) prevalence of *L. monocytogenes* in fruit salad from Cuenca and Guayaquil is higher than the 5.06% prevalence of the pathogen in fresh-cut cantaloupe from Canada (19).

Other multiple potential pathogens and opportunistic pathogens were isolated from multiple foods by analyzing randomly picked colonies, implying higher prevalence in the food samples and indicating the need for improved food production and distribution systems in the country.

Correlations between indicator microbes and foodborne pathogens. The counts of TAM, TC, FC, and EC have been used as food safety indicators for decades (13),

and TAM values below 5 log CFU/g are considered acceptable for RTE food (49). However, the foodborne pathogens *S. flexneri* and *L. monocytogenes* were detected in fruit and fruit salad samples from Cuenca, respectively, despite the absence of TC, FC, and EC and the low TAM counts observed in both types of food. Similarly, fruit from Guayaquil showed no foodborne pathogenic bacteria despite having TAM, TC, FC, and EC levels similar to those of other food types in which foodborne pathogens were detected, such as ceviche. These results are in agreement with previous reports showing that indicator bacteria do not necessarily correlate with the presence of pathogens in food (6, 65).

Other bacteria. Various opportunistic pathogens were prevalent in most food samples. Opportunistic pathogens usually take advantage of the weak defense system in immunocompromised individuals and have caused nosocomial foodborne illnesses (14). The *Enterococcus* spp. detected in most samples are considered opportunistic pathogens, because *E. faecium, E. durans,* and *E. faecalis* are capable of causing bacteremia in immunocompromised people (12, 21). However, *Enterococcus* spp. can produce bacteriocins with application potential for controlling spoilage and pathogenic bacteria (30). Further research is needed to evaluate the risk and biocontrol potential of the *Enterococcus* spp. from Ecuador.

K. pneumoniae, also isolated from most food samples, has been associated to various foodborne outbreaks, causing septicemia, liver abscesses, and diarrhea (67). This opportunistic pathogen has frequently been detected in fruit and vegetables from various countries (1), and the presence of this bacteria in ceviche and encebollado can be linked to the production of the scombroid toxin histamine in fish (5). Similarly, *P. sanguinis* has been associated with bacteremia and meningitis in immunocompromised individuals, and the presence of this bacteria in the RTE dishes from Ecuador may result from cross-contamination with refrigerated food such as cheese (42). *P. sanguinis* was also present in cheese samples analyzed in this study. Likewise, the prevalence of *S. sciuri* in ground beef from Ecuador is in agreement with previous reports showing the prevalence of this bacterium in retail beef (27, 36). *S. sciuri* is mostly considered harmless but has been associated to wound infections, urinary tract infection, endophthalmitis, and other symptoms in immunocompromised people (16, 47). Similarly, the presence of the foodborne pathogen *S. flexneri* in fruit, salsa, and encebollado samples suggests poor sanitation and lack of clean water (38).

Among the spoilage organisms detected in this study, *Serratia* spp. have been reported to cause decomposition of protein-rich food (25), whereas *Acinetobacter* spp. are considered psychrophilic spoilage organisms (9). However, *Serratia* spp. can also cause pneumonia (26), and various *Acinetobacter* spp. have been associated with diarrhea in immunocompromised individuals (17). The presence of both bacteria in food from Ecuador probably resulted from cross-contamination by protein-rich and refrigerated ingredients such as meat and cheese. Further

research is needed to assess the food safety risks of the *Serratia* spp. and *Acinetobacter* spp. detected in bolon, juice, and other food from Ecuador.

B. amyloliquefaciens, detected in most food samples from this study, has been associated to food spoilage *(53)* and food biocontrol, because this microorganism can produce the bacteriocin BaCf3, which is capable of inhibiting pathogens such as *S. enterica* serovar Typhimurium and *Clostridium perfringens (10)*. In this study, the high prevalence of *B. amyloliquefaciens* coincided with the absence of foodborne pathogens from fresh juice samples squeezed at the street markets. The presence of *Bacillus* spp. in orange juice has been reported previously *(4),* but further research is needed to assess the role of *B. amyloliquefaciens* in the preservation of fruit juice.

Bacillus subtilis (60), B. pumilus (55), and *L. lactis (59)* detected in several samples can also produce antimicrobial compounds. Further research is needed to assess the role of these bacteria as biocontrol agents in food from Ecuador.

In conclusion, the high prevalence of *Salmonella, L. monocytogenes,* and several opportunistic pathogens and TAM, TC, FC, and EC levels suggest inadequate food handling in the sampled street markets. Training programs on good practices are needed to improve the food safety from high-demand food retailers. Further research is needed to characterize the pathogenicity and antimicrobial activity of the bacteria detected and to assess the prevalence of *C. jejuni, S. aureus, Vibrio* spp., and other foodborne pathogens not included in this study.

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SUPPLEMENTAL MATERIAL

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