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Microbiological safety and quality of ceviche, poke, and sushi dishes sold at retail outlets in Orange County, CA

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ABSTRACT

Raw, ready-to-eat (RTE) seafood products, such as ceviche, poke, and sushi, have experienced growing demand globally; however, these products have the potential to be contaminated with foodborne pathogens. The objective of this study was to determine the prevalence of *Escherichiacoli*/coliforms, *Salmonella*, and *Listeria* in ceviche, poke, and sushi dishes sold at the retail level in Orange County, CA, USA. Additional organisms detected during testing were also considered in the results. A total of 105 raw, RTE samples of ceviche, poke, and sushi were collected from restaurants and grocery stores in Orange County, CA. Samples were tested for *Salmonella* and *Listeria* utilizing methods from the Food and Drug Administration (FDA) Bacteriological Analytical Manual (BAM). *E. coli* and total coliforms were enumerated utilizing 3 M Petrifilm plates. Overall, two samples (1.9%) were positive for generic *E. coli,* with a range of 5–35 CFU/g. Coliforms were detected in 85 samples (81%), with a range of 5–1710 CFU/g. The average coliform levels in ceviche samples (259 CFU/g) were significantly higher than the levels in sushi samples (95 CFU/g), according to a Kruskal-Wallis H test followed by the Dunn test ($p < 0.05$). The coliform levels in poke samples (196 CFU/g) were not significantly different from those in ceviche or sushi. All levels of *E. coli* and coliforms were considered acceptable or satisfactory/borderline according to standards for RTE seafood. None of the samples tested positive for *Salmonella* or *Listeria monocytogenes*; however, other microorganisms were detected in 17 samples, including *Listeria* spp., *Proteus mirabilis, Providencia rettgeri,* and *Morganella morganii*. The results of this study are novel in that they present data on the microbiological safety and quality of ceviche, poke, and sushi dishes sold at retail in the United States, as well as provide a comparison across the three categories of raw, RTE seafood.

1. Introduction

Seafood is an important protein source, with global consumption increasing at an annual rate of 3.1% from 1961 to 2017 [1]. However, many consumers have low confidence in their ability to prepare fish at home [2] and one survey found that 34% of respondents acknowledged that they "do not know much" about how to prepare and serve fish [3]. Ready-to-eat (RTE) foods are those that are intended for direct human consumption without the need for additional cooking or processing [4]. Raw, RTE seafood products, including dishes such as ceviche, poke, and sushi, have been growing in popularity due to their convenience and availability at retail

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outlets [5]. These products are also desired by consumers with preferences for natural, healthy, and convenient foods. Ceviche is a Latin American dish, originating from Peru, that contains small cuts of seafood marinated in a citrus juice mixture [6]. The seafood (typically white fish or shrimp) absorbs the marinade, turning opaque, and is then mixed with other ingredients, such as onions, peppers, and seasonings. Poke is a native Hawaiian dish that contains cuts of raw fish (typically tuna, salmon, or yellowtail) mixed with other ingredients such as rice, vegetables, and soy sauce [7]. Sashimi is sliced raw seafood that is served plain without rice, while sushi contains various fillings (e.g., cooked or raw seafood, vegetables, and/or seaweed) served over vinegar-soaked rice [8]. Sushi and sashimi are served with a range of different fish species and are traditionally from Japan, with the term sushi often used to encompass both dishes. The raw, RTE seafood industry has shown consistent growth, with the market size of the sushi restaurants industry in the U.S. expected to increase by 6.3% in 2023 [9].

Despite their increasing popularity, raw, RTE seafood products present health risks due to the potential for foodborne pathogens [10–12]. Some of these pathogens are naturally occurring in the aquatic environment, while others may be introduced through fecal contamination of the environment or during seafood processing and preparation [10,13]. Seafood produced with aquaculture is also at risk for bacterial contamination associated with unsanitary conditions, overcrowding, and/or contaminated feed [13]. With no terminal processing step to inactivate pathogens, raw, RTE seafood is considered to be in the highest risk category for seafood [10]. The term "sushi grade" is often used by retailers to promote the safety and/or quality of raw, RTE seafood products. However, this term is not regulated or defined by the U.S. Food and Drug Administration (FDA) and therefore may be misleading to consumers [14]. Fish consumed raw is recommended to be frozen soon after being caught and remain frozen at −20 °C for at least seven days [15]. This process serves to inactivate eukaryotic parasites but does not effectively inactivate other foodborne pathogens. Mild processing, such as marination, salt, or modified atmosphere packaging, in combination with refrigeration extends the shelf life of RTE seafood, but it does not ensure that the product is free from pathogens [5].

Microbial hazards such as *Salmonella enterica* and *Listeria monocytogenes* have led to outbreaks and/or recalls in the United States associated with minimally processed or raw, RTE seafood in recent years [16–22], despite a zero-tolerance policy for these pathogens in RTE foods [23]. From 2011 to 2022, the Centers for Disease Control and Prevention (CDC) reported five multistate outbreaks of *Salmonella* in the United States associated with raw seafood products, including sushi and poke [16,18,20–22]. *Salmonella enterica* is the leading bacterial cause of foodborne illness in the United States, with over 1 million estimated cases each year [24]. *Salmonella* is commonly found in the intestinal tracts of cold- and warm-blooded animals, such as poultry, cattle, and rodents, and can contaminate food through the fecal-oral route [25]. *Salmonella* contamination has traditionally been associated with raw or undercooked poultry, meat, fresh produce, and dairy products. While fish and shellfish are not common reservoirs for *Salmonella*, cross-contamination in the supply chain can introduce this pathogen into seafood. Without proper cooking, *Salmonella* can cause illness, hospitalization, and/or death in consumers.

The *Listeria* genus includes 20 species, with *Listeria monocytogenes* being the main species associated with illness in humans [26]. *L. monocytogenes* generally causes self-limiting gastrointestinal infections in healthy individuals. However, it can cause serious complications (e.g., meningitis or septicemia) in immunocompromised individuals, as well as fetal loss in pregnant women [27]. *Listeria* is widespread in the environment and is often found in moist environments, soil, and decaying vegetation. *L. monocytogenes* can survive in unfavorable conditions and is persistent in food processing facilities. The main food vehicles associated with *Listeria* outbreaks are RTE foods, including meat, dairy, and seafood products. Due to the recognized hazards of *Listeria* in raw, RTE foods, the FDA published a revised draft guidance for industry in 2017 on the control of *Listeria monocytogenes* in RTE foods [28]. Several outbreaks of *Listeria* in raw and minimally processed fish have been reported in Europe over the past decade [29–32]. However, the CDC did not reported any multistate outbreaks associated with *Listeria* in seafood in the United States during this timeframe [33].

The microbial status of seafood is heavily reliant on the production environment. Levels of total coliforms are typically used to indicate sanitary conditions in the food processing environment, while the presence of *Escherichiacoli* specifically indicates fecal contamination [34]. *E. coli* and other coliforms may be present due to several factors, including environmental contamination, lack of post-harvest care and/or inadequate hygiene conditions in the processing environment [35].

Despite the potential health risks of ceviche, poke, and sushi, there is a lack of information regarding the microbiological quality and safety of these products sold at the retail level in the United States. Therefore, the goal of this project was to determine the prevalence of *E. coli*/coliforms, *Salmonella,* and *Listeria* in ceviche, poke, and sushi dishes sold at retail outlets in Orange County, CA, USA. Additional organisms detected during testing were also considered in the results. Orange County is a coastal region in Southern California with numerous raw, RTE seafood restaurants. In addition to providing novel data on these high-risk food products, this project is significant because it allows for a direct comparison of the microbiological quality and safety across three different categories of raw, RTE seafood. The hypothesis was that *E. coli*/coliforms, *Salmonella,* and *Listeria* would be detected in all three categories of raw, RTE seafood tested, with a lower proportion of microbial contamination in ceviche due to the acidic nature of the product.

2. Materials and methods

2.1. Microbiological media

All media was prepared as specified by the manufacturer. Lactose Broth (LB), Rappaport Vassiliadis (RV), Tetrathionate (TT), Xylose Lysine Deoxycholate (XLD), Bismuth Sulfite (BS), Lysine Iron Agar (LIA), Triple Sugar Iron (TSI), Oxford Agar (OXA), and Trypticase Soy Agar with 0.6% Yeast Extract (TSAYE) were obtained from Neogen (Heywood, UK). Hektoen Enteric (HE) was obtained from Oxoid (Hants, UK). Buffered Listeria Enrichment Broth (BLEB) was obtained from Becton, Dickinson, and Company (Sparks, MD, USA) and 5% Sheep's Blood Agar was obtained from Thermo Fisher Scientific (Waltham, MA, USA). *E. coli*/Coliform Count plates were

obtained from 3 M Petrifilm (Saint Paul, MN, USA).

2.2. Sample collection

A total of 105 raw, RTE fish samples were purchased from restaurants (n = 54) and grocery store seafood counters (n = 3) in Orange County, CA (Fig. 1). Orange County was selected as the sampling region because it is where Chapman University is located and there are numerous raw, RTE seafood restaurants in this region. Sampling was based on dish type and consisted of 35 samples each of ceviche, poke, and sushi/sashimi (referred to hereafter as 'sushi') containing raw fish. The sample number was pre-determined to allow for statistical comparison of the three categories of raw, RTE seafood. Each sample was associated with a unique dish (i.e., no duplicate samples from the same location) and a maximum of four dishes per location were purchased. The type of fish collected depended on availability and varied based on dish type (Fig. 2). Following collection, samples were transported on ice to the laboratory at Chapman University (Orange, CA). The raw fish from each sample underwent testing within 4 h of purchase for *E. coli*/coliforms, *Salmonella*, and *Listeria*, as described in sections 2.3-2.5 (Fig. 3).

2.3. E. coli/coliform testing

All samples were tested for *E. coli* and total coliforms as described in Levy et al. [36]. Fish samples were mixed in a 1:10 dilution with Butterfield's phosphate buffer in a Stomacher 400 Circulator (Seward, Worthing, England) for 30 s at 230 rpm. Each sample was plated in duplicate by adding 1 mL of the dilution onto an *E. coli*/Coliform Count Petrifilm plate. The plates underwent incubation in stacks of 20 or less at 35 ± 2 °C for 48 ± 2 h. The average *E. coli* and total coliform counts were determined for each sample using a countable range of 15–150. In instances where the number of colonies exceeded the countable range, an estimated count was determined by counting the number of colonies in one square and multiplying by 20, according to the manufacturer's instructions.

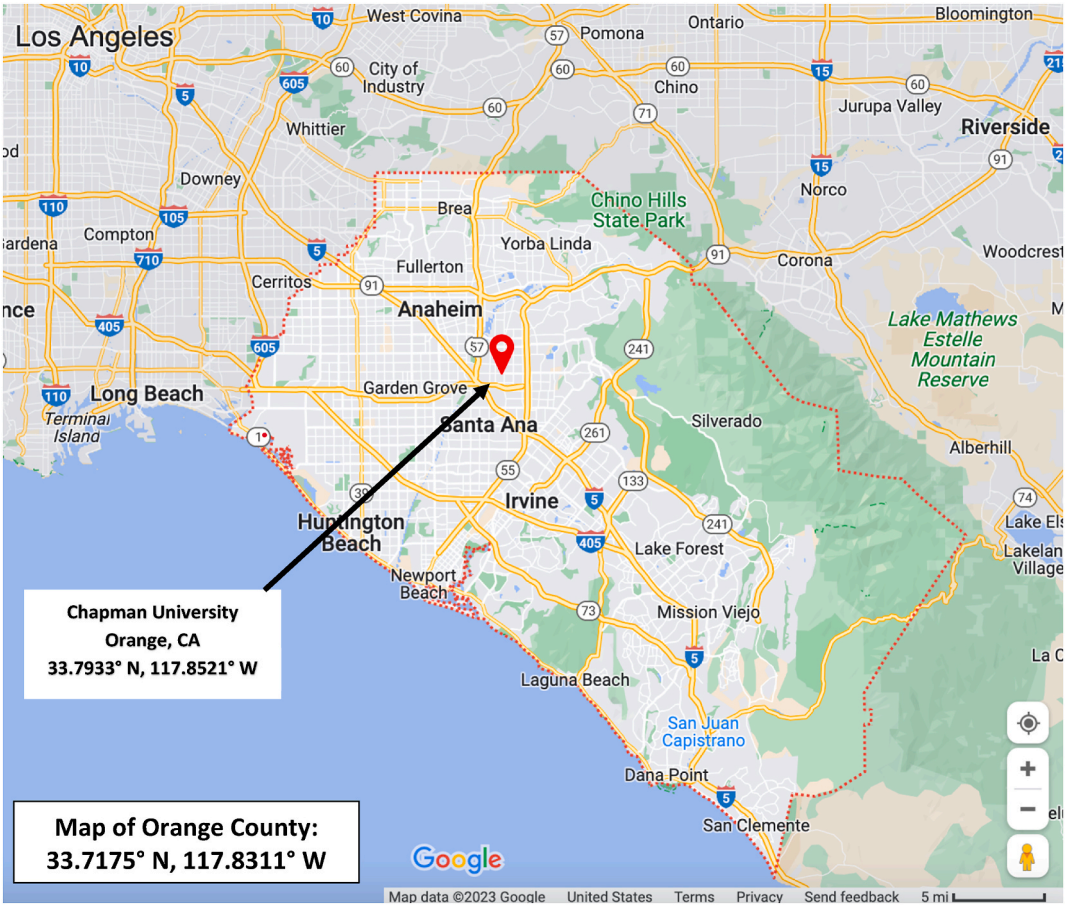

Fig. 1. Geographical map showing the sampling region (Orange County, CA) outlined in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

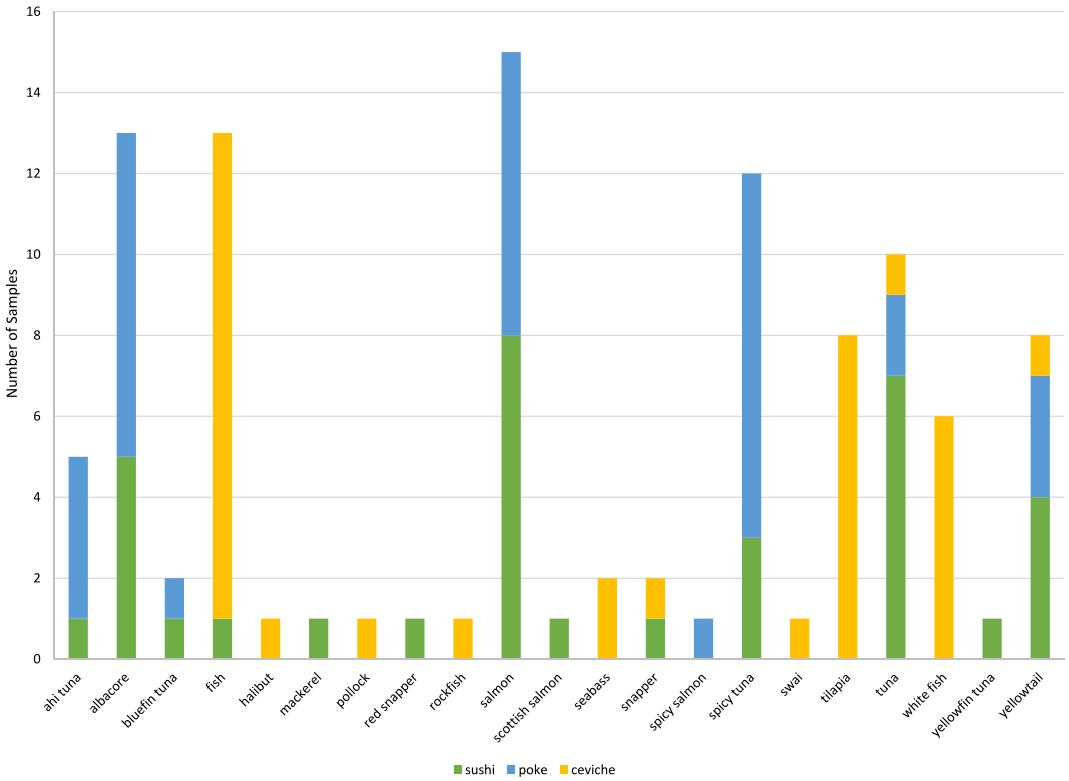

Fig. 2. Summary of raw, RTE fish samples collected for this study, based on menu descriptions of each dish.

2.4. *Salmonella* testing

Samples were tested for *Salmonella* according to the procedures described in the FDA Bacteriological Analytical Manual (BAM) Chapter 5 [37]. Fish samples (25 g) were added to 225 mL of lactose broth and blended for 2 min. The homogenized mixture was held at room temperature for 60 ± 5 min. The pH was monitored and adjusted, if necessary, to pH 6.8 ± 0.2. The samples were then incubated for 24 ± 2 h at 35 ± 2 °C.

After incubation, 0.1 mL of each sample was transferred into a test tube containing 10 mL of RV broth, followed by vortexing and incubation for 24 ± 2 h at 42 ± 0.2 °C. Another 1 mL of each sample was transferred to a test tube with 10 mL of TT broth, followed by vortexing and incubation for 24 ± 2 h at 35 ± 0.2 °C. After incubation, the RV and TT broth samples were vortexed and streaked onto XLD, BS, and HE agar plates. The plates were then inverted and incubated for 24 ± 2 h at 35 ± 2 °C. After 24 h, the plates were examined for typical *Salmonella* growth. Typical colonies if present were selected from each BS, HE and XLD plate and transferred to LIA and TSI slants. In the absence of typical or suspicious *Salmonella* colonies on HE and XLD, atypical *Salmonella* colonies were selected. If typical or suspicious colonies were not present on BS agar after 24 ± 2 h, the plate was incubated an additional 24 ± 2 h. If typical or suspicious colonies were not present after 48 ± 2 h incubation, 2 or more atypical colonies were selected. The LIA and TSI slants were incubated for 24 ± 2 h at 35 ± 2 °C with loosely secured caps to support aerobic conditions. Presumed positive TSI cultures were streaked to HE agar and incubated according to the BAM Chapter 5, Section E.1. Mixed cultures [37]. Samples with typical *Salmonella* growth on HE next underwent testing with the Analytical Profile Index (API) 20E (Biomerieux, Marcy-l'Étoile, France) system, following the manufacturer's instructions. The API 20E system can identify and differentiate members of the family *Enterobacteriaceae*. The results of API test strips were recorded for each presumptive sample, including cases where an organism other than *Salmonella* was detected.

2.5. *Listeria* testing

Fish samples were prepared for *Listeria monocytogenes* testing according to the FDA BAM Chapter 10, subsection D [38]. Fish samples (25 g) were mixed for 30 s at 230 rpm in a Stomacher 400 Circulator with 225 mL BLEB containing pyruvate without selective additives. Samples were incubated at 30 °C for 4 h. Selective supplements (Sigma Aldrich, St. Louis, MO, USA) were then added to the samples as described in the BAM, followed by incubation at 30 °C for an additional 44 h.

At both 24 and 48 h into incubation, the BLEB enrichment was streaked onto an OXA plate. The OXA plates were incubated at 35 °C and were examined for typical *Listeria* colonies after 24 ± 2 h and 48 ± 2 h. Five typical colonies (if present) from OXA were streaked onto TSAYE plates and incubated at 30 °C for 24–48 h. The purified isolates on TSAYE were then confirmed using API *Listeria*

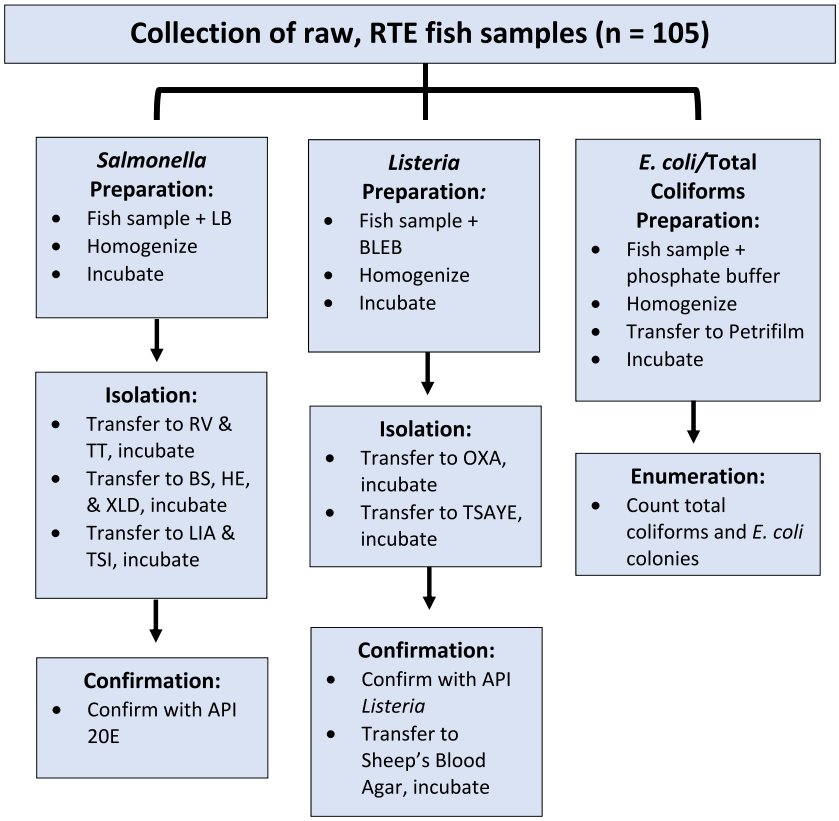

Fig. 3. Experimental design for the testing of raw, RTE seafood samples for *Salmonella*, *Listeria*, and *E. coli*/total coliforms.

(Biomerieux), following the manufacturer's instructions. The isolates also underwent hemolysis testing using 5% Sheep's Blood Agar incubated at 35 °C for 24–48 h.

2.6. Statistical analysis

The number of total coliforms in each sample was statistically compared across all three dish types (i.e., ceviche, poke, and sushi) using a Kruskal-Wallis H test followed by the Dunn test, with a significance level of $p < 0.05$. The results of *E. coli, Salmonella,* and *Listeria* testing did not undergo statistical analysis due to the limited number of detections for these organisms. Statistical analysis was performed using R Studio version 4.0.2 (RStudio, PBC, Boston, MA, USA) [39].

Table 1
Levels of total coliforms and *E. coli* in samples of raw, RTE fish samples (n = 105). Coliforms were detected in 81% of samples and *E. coli* was detected in 1.9% of samples.

Dish Type	Number of Samples Tested	Total Coliforms (CFU/g)			Generic *E. coli* (CFU/g)		
		Mean	Range*	% Positive	Mean	Range**	% Positive
Ceviche	35	259[a]	0–1710	85.7	0.14	0–5	2.86
Poke	35	196[ab]	0–1230	77.1	1.00	0–35	2.86
Sushi	35	95.0[b]	0–1155	80.0	ND[c]	ND	0.00
Overall	105	184	0–1710	81.0	0.38	0–35	1.90

[ab] A different superscript letter in the same column indicates a significant difference, according to a Kruskal-Wallis H test followed by the Dunn test ($p < 0.05$).
[*] Coliform levels of $<10^2$ CFU/g are considered satisfactory and levels of $10^2 \leq 10^4$ CFU/g are considered acceptable for RTE seafood [41].
[**] *E. coli* in levels of <20 CFU/g are considered acceptable and levels of 20 to <100 CFU/g are considered borderline for RTE foods [40].
[c] Not detected.

3. Results and discussion

3.1. Prevalence of E. coli and total coliforms

Out of the 105 raw, RTE fish products tested, two samples (1.9%) were positive for generic *E. coli* (Table 1). The average *E. coli* level across all products was 0.38 CFU/g, with a range of 0–35 CFU/g. The results for individual *E. coli* plate counts can be found in the Supplementary Material. One sample of fish ceviche had *E. coli* at a level of 5 colony forming units (CFU)/g and one sample of spicy tuna poke had *E. coli* at a level of 35 CFU/g. These levels are considered to be satisfactory (<20 CFU/g) or borderline (20 to <100 CFU/g) according to guidelines published by the Health Protection Agency for microbiological safety of ready-to-eat foods [40]. The identification of generic *E. coli* in these seafood dishes indicates the possibility of contamination with animal or human fecal matter. The prevalence of *E. coli* in these samples is slightly lower than previous studies on sushi, ceviche, and other raw, RTE seafood products, which reported *E. coli* detection rates of 4–12% [41–45]. Interestingly, Kim et al. [41] reported the lowest rate of *E. coli* detection in samples collected from online markets (2.0%) compared to samples from fishery markets (6.9%) and hypermarkets (7.5%), whereas Atanassova et al. [43] reported lower rates of *E. coli* detection in frozen retail sushi (4.8%) compared to freshly prepared sushi (19.2%).

Coliforms were detected in 81.0% (85/105) of samples (Table 1), with an average level of 184 CFU/g and a range of 0–1710 CFU/g. The results for individual coliform plate counts can be found in the Supplementary Material. Overall, 66.7% of products sampled had satisfactory levels of coliforms ($<10^2$ CFU/g) and 33.3% were considered acceptable ($10^2 \leq 10^4$ CFU/g) according to evaluation levels for RTE seafood products [41]. Similarly, the majority of raw, RTE seafood products tested in a study in South Korea also had satisfactory or acceptable levels of coliforms, with only 0.5% having unsatisfactory levels ($\geq 10^4$ CFU/g in one squid sample) [41]. The presence of coliforms in raw, RTE seafood indicates environmental contamination in the supply chain, suggesting the need for an increased focus on environmental monitoring.

When the results were separated by dish type, the greatest rate of coliform detection was found in ceviche (85.7%, 30/35), followed by sushi (80.0%, 28/35), and poke (77.1%, 27/35) (Table 1). Ceviche also had the highest average number of coliforms per sample (259 CFU/g), followed by poke (196 CFU/g), and sushi (95 CFU/g). Fig. 4 shows how the coliform levels compare to the evaluation guidelines for RTE seafood products published by Kim et al. [41]. Among the ceviche samples, 48.6% had satisfactory levels of coliforms ($<10^2$ CFU/g) and 51.4% had acceptable levels ($10^2 \leq 10^4$ CFU/g). In comparison, poke had satisfactory levels in 71.4% of samples and acceptable levels in 28.6% of samples, while sushi had satisfactory levels in 80% of samples and acceptable levels in 20% of samples. The coliform levels in the ceviche samples were found to be significantly higher than the levels detected in the sushi samples, according to a Kruskal-Wallis H test followed by the Dunn test ($p < 0.05$). The coliform levels in poke samples were not significantly different from those in ceviche or sushi samples.

This is the first study to compare coliform levels across the three dish types, and it was expected that ceviche would have lower levels of microorganisms due to the acidic nature of this dish. While sushi and poke can also be made with acidic ingredients (e.g., acidified rice and sauces), the fish in ceviche is soaked in a citrus marinade. However, ceviche often includes other raw ingredients that could introduce coliforms, such as cilantro and tomatoes, both of which were found in the sampled dishes. Levy et al. [36] previously reported that 87.8% of cilantro samples collected from farmers' markets in Southern California contained coliforms, with a range of 0.70–4.08 log CFU/g, while Zoellner et al. [46] reported that supermarket tomatoes had a total coliform range of 0.2–3.7 log CFU/g. Furthermore, the acidic conditions of ceviche are not considered sufficient to reduce the microbial population in the product, with previous research reporting survival of a variety of microorganisms in ceviche [6,47].

An additional contributing factor to the relatively high levels of coliforms in ceviche could be differences in the handling and preparation of ceviche dishes as compared to sushi and poke dishes. Unlike sushi or poke restaurants, where a signification portion of the dishes are raw and RTE, ceviche was typically sold at restaurants that primarily serve heat-treated main dishes. Therefore, it is possible that ceviche may have been exposed to additional sources of cross-contamination and/or different storage conditions as compared to poke and sushi.

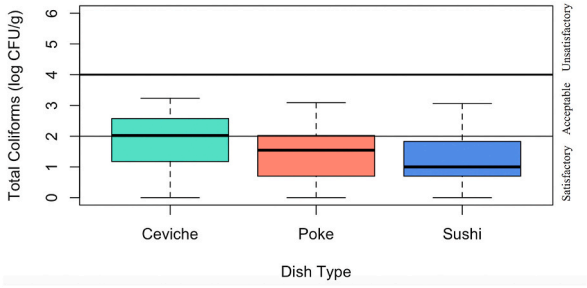

Fig. 4. Boxplot of total coliforms (log CFU/g) separated by the type of dish (i.e., ceviche, poke, or sushi). Samples with no coliforms detected were assigned a value of 0. The box indicates the interquartile range, with the line inside box being the median, and the lower and upper error lines representing the 10th and 90th percentiles, respectively. The circles representing outliers that fall outside the 10th and 90th percentiles. The levels are divided into satisfactory, acceptable, and unacceptable categories according to evaluation criteria for RTE seafood products [41].

3.2. Prevalence of Salmonella and other Gram-negative bacteria

In contrast to the expected results, *Salmonella* was not detected in any of the 105 raw, RTE fish samples tested in this study. The results reported here are similar to several other market studies conducted internationally on sushi, ceviche, and other raw, RTE seafood products, which have reported *Salmonella* detection rates of 0–0.4% [41–45,48–52]. In contrast, a relatively high rate of *Salmonella* detection (16–26%) was reported for sushi/sashimi samples collected in Malaysia [53] and Brazil [54], as well as for ceviche sold in Mexico [55]. Interestingly, a prior study conducted in the United States reported a 2.8% *Salmonella* detection rate in domestic raw seafood and a 10% detection rate in imported, raw seafood [56]. The higher detection rate in imported seafood was attributed to the FDA's HACCP systems and inspections being limited in foreign countries at the time. While fish are not a reservoir for *Salmonella* there have been several outbreaks associated with *Salmonella* contamination of fish [16,18]. These outbreaks indicate that *Salmonella* is an important hazard of concern in raw, RTE fish products.

Although *Salmonella* was not detected in this study, all 105 samples showed atypical growth on one or more of the HE, XLD, and BS agar plates, demonstrating the presence of other microorganisms in these samples. Among these samples, 30 had typical growth on TSI and LIA slants, and 5 showed typical growth after being streaked to HE. These five samples were all associated with poke dishes and tested positive for a variety of other microorganisms based on the results of the API 20E test strips (Table 2), including *Pseudomonas aeruginosa, Proteus mirabilis*, *Providencia rettgeri*, *Providencia stuartii,* and *Morganella morganii.* The presence of these microorganisms is likely due to a combination of fish microbiota and environmental contamination. The poke dishes containing these microorganisms were collected from different restaurants and were labeled as containing albacore (n = 2), spicy tuna (n = 2), and salmon (n = 1).

P. aeruginosa, which is part of the normal fish microbiota, was potentially identified in two albacore poke dishes purchased from separate restaurants. These API identifications were described as "doubtful profile" and "low discrimination", indicating that additional testing would be needed to confirm the presence of this organism. *P. aeruginosa* is a common spoilage organism in fish and it has developed resistance to a wide range of antibiotics [57]. Under stressful conditions, such as malnutrition or overcrowding, *P. aeruginosa* becomes an opportunistic pathogenic that can cause gill necrosis and other serious illnesses in fish [58]. *P. aeruginosa* is also an opportunistic pathogen in humans that most often infects hospital patients, leading to infections in the blood, lungs (pneumonia), or other areas of the body following surgery [59].

Proteus, *Providencia*, and *Morganella* are closely related genera of Gram-negative bacteria [60]. *P. mirabilis*, an indicator of contamination, was isolated from a spicy tuna poke sample and identified with high confidence by API (very good identification, 99.9% ID). This microorganism is widely distributed in the environment and is commonly found in mammals, with some reports of it in contaminated seafood [61]. *P. rettgeri* was identified with high confidence by API (good identification, 97.4% ID) in an albacore poke sample that also tested positive for *P. aeruginosa* (discussed above). Both of these organisms have been isolated from marine environments and have been detected in the plastisphere of environmental plastic pollution [62,63]. *P. stuartii* was detected with low confidence by API (doubtful profile, 55.6% ID) in a salmon poke sample. The low strength associated with this detection may have been due to the presence of multiple species in the sample. Numerous other significant taxa showed partial matches to this sample, including *P. rettgeri* (16.6% ID), *M. morganii* (16.3% ID), and *Proteus vulgaris* (11.1% ID). *P. rettgeri* and *P. stuartii* are commonly found in water, soil, and animal reservoirs and are opportunistic pathogens associated with urinary tract infections [60].

M. morganii was detected with low confidence (doubtful profile, 99.6% ID) in a spicy tuna poke sample and may be indicative of environmental contamination. This organism is widely distributed in nature and is part of the normal microbiota of the intestinal tracts of humans, mammals, and reptiles [64]. *M. morganii* is an opportunistic pathogen in humans that primarily causes urinary tract infections and post-operative wound infections. *M. morganii* is the most common prolific histamine former in raw fish and plays a key role in rapid histamine formation during storage. High histamine levels cause a foodborne intoxication known as scombroid poisoning, and indicate spoilage associated with storage temperatures greater than 15 °C [65].

Table 2
Microorganisms detected in raw, RTE fish dishes (n = 105) tested in this study. Note: in some cases, one dish tested positive for multiple microorganisms.

Microorganism	API Percent ID	Strength of API Identification	Number of Dishes		
			Ceviche (n = 35)	Poke (n = 35)	Sushi (n = 35)
Salmonella	N/A	N/A	0	0	0
Escherichiacoli	N/A	N/A	1	1	0
Pseudomonas aeruginosa	67.2%	Doubtful profile (n = 1), low discrimination (n = 1)	0	2	0
Proteus mirabilis	99.9%	Very good identification	0	1	0
Providencia rettgeri	97.4%	Good identification	0	1	0
Providencia stuartii	55.6%	Doubtful profile	0	1	0
Morganella morganii	99.6%	Doubtful profile	0	1	0
Listeria monocytogenes	N/A	N/A	0	0	0
Listeria welshimeri	96.8–99.9%	Excellent identification (n = 1), doubtful profile (n = 1)	0	0	2
Listeria spp.	5.6–98.6%	Very good identification (n = 1), good identification (n = 1), acceptable identification (n = 2)	2	1	2
Total Coliforms	N/A	N/A	30	27	28

While the detection of the Gram-negative microorganisms discussed above provides information regarding the microbiological quality and safety of these products, it is important to note that these microorganisms were detected as the result of selective testing for *Salmonella enterica.* Additional research using non-selective methods of detection is warranted in order to better understand the full microbiological profile of these samples. Furthermore, it is possible that *Salmonella* may have gone undetected in the current study and previous studies due to competition from other microorganisms. *Salmonella* is a potential hazard in raw, RTE seafood products and it may enter the seafood supply chain at multiple points, including pre- and post-harvest [13]. Several seafood outbreaks have occurred in the United States associated with raw tuna [16,18,20], which is commonly used in sushi/sashimi and poke dishes. Future studies should consider the use of a higher incubation temperature (43 °C) with TT broth, as recommended in the BAM for foods with a high microbial load [37].

3.3. Prevalence of Listeria spp.

Contrary to the hypothesis of this study, all 105 raw, RTE fish samples collected in this study tested negative for *L. monocytogenes* (Table 2). These results are in agreement with previous studies conducted in Italy and the Czech Republic, which found a 0% detection rate for *L. monocytogenes* in sushi/sashimi products [49,51]. While a few studies conducted outside of the U.S. have reported the presence of *L. monocytogenes* in sushi, poke, and ceviche products [43,66–69], there were no multistate outbreaks reported by the CDC or FDA of *Listeria* in raw, RTE seafood from 2011 to 2021. This is likely due in part to the establishment of *Listeria* as a hazard in all RTE foods and the FDA guidance for the control of *L. monocytogenes* in refrigerated or frozen RTE foods [28].

Although *L. monocytogenes* was not detected in this study, all samples showed growth on OXA and 6.7% (7/105) of the samples tested were found to contain *Listeria* spp. (Table 2). A total of 17 samples showed typical *Listeria* growth on OXA and underwent further testing for beta hemolysis and API *Listeria.* The results of the API *Listeria* tests showed detection of *Listeria* spp. in seven samples. *Listeria* was identified to the genus level in five samples and *L. welshimeri* was identified in two samples. Hemolysis was observed for two samples, with a slightly cleared zone on 5% Sheep's Blood Agar: (1) an *L. welshimeri* sample that had excellent identification with API (99.9% ID) and (2) a *Listeria* spp. sample with API matches to *Listeria seeligeri* (94% ID) and *Listeria ivanovii* (5.6% ID). A slightly cleared zone is consistent with the expected results for *L. seeligeri*, but not for *L. welshimeri,* indicating the possibility of other *Listeria* species being present in the sample [38].

Within the subcategories of raw, RTE dishes, sushi products had the highest rate of contamination with *Listeria* spp. (4/35, 11.4%), followed by ceviche (2/35, 5.7%) and poke (1/35, 2.9%). The sushi samples were labeled as yellowtail sashimi (n = 1), tuna nigiri (n = 1) and salmon sashimi (n = 2); the two ceviche samples were labeled as containing "fish"; and the poke sample was labeled as containing salmon. The yellowtail sashimi and one of the salmon sashimi dishes that tested positive for *Listeria* in the current study were collected from the same restaurant, while the other samples were from different locations. In comparison, a study in Germany noted a lower rate (2.8%) of *Listeria* spp. detection in sushi samples [43], while a study conducted in Peru reported a high rate (75%) of *Listeria* spp. detection in ceviche samples [69].

Listeria spp. are widespread in the environment and their presence in these raw, RTE foods indicates pre- or post-harvest contamination in the supply chain [28]. Contamination may have occurred during processing, as *Listeria* spp. are problematic and persistent in food processing facilities. For example, one study found that 27.3% of environmental samples in fish processing facilities were contaminated with *Listeria* spp. [70]. Environmental monitoring of processing facilities requires Good Manufacturing Practices (GMPs) and a Hazard Analysis and Critical Control Points [71] plan to control and limit contamination from *Listeria* spp. within the facility [72]. While *L. monocytogenes* was not detected in the current study, the detection of *Listeria* spp. in these products is concerning because it indicates the potential risk of contamination with *L. monocytogenes* [73].

3.4. Dishes with multiple bacterial species

Across all dish types, there were eight samples in which two or more types of microorganisms were detected. Of these samples, five were poke samples, two were ceviche, and one was sushi. For example, the spicy tuna poke sample that tested positive for *P. mirabilis* was also found to contain generic *E. coli* (35 CFU/g) and coliforms (80 CFU/g). The albacore poke sample that tested positive for *P. rettgeri* and *P. aeruginosa* was also positive for coliforms (555 CFU/g). The spicy tuna poke sample that tested positive for *M. morganii* contained coliforms (1040 CFU/g) and the salmon poke sample that tested positive for *P. stuartii* also contained coliforms (35 CFU/g). Four additional samples were positive for both *Listeria* spp. and coliforms: a yellowtail sashimi sample with 5 CFU/g coliforms, a salmon poke sample with 1030 CFU/g coliforms, and two fish ceviche dishes with 25–45 CFU/g coliforms. The presence of multiple organisms in a food product may be due to a single source of contamination or multiple sources of contamination. For example, the combination of *Listeria* and coliforms in a single dish indicates the possibility of environmental and/or processing contamination.

4. Conclusions

To the authors' knowledge, this study presents the first comparison of the microbiological safety and quality of ceviche, poke, and sushi dishes sold at retail in the United States. Overall, low levels of microbiological contamination were detected in all three product categories based on the tests performed. Coliforms were detected in most samples (81%) and *E. coli* was detected in two samples (1.9%); however, all levels of coliforms and *E. coli* were within acceptable or borderline ranges. The coliform levels in ceviche were significantly higher than those in sushi, which may be due to differences in the handling and storage of these products and/or the presence of other raw ingredients. None of the samples tested positive for *Salmonella* or *Listeria monocytogenes*. However, 12 samples

contained other microorganisms, including *Listeria* spp., *P. mirabilis, P. rettgeri,* and *M. morganii*. While the other microorganisms detected are not major health concerns, they include opportunistic pathogens, spoilage organisms, and/or indicators for *L. monocytogenes*. Importantly, microbial contamination of ceviche, poke, and sushi in the supply chain may lead to reduced quality and safety of these raw, RTE seafood dishes. Careful monitoring of factors such as storage temperature and shelf life is essential to reduce the potential for further microbial growth. Additional research on this topic is recommended using larger sample sizes and a wider range of tests, including genomics and/or proteomics, to more accurately determine the microbial composition of these products.

Author contribution statement

Grace E. Marquis: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Samantha M. Covaia, Amanda M. Tabb, Courtney J. Kitch: Performed the experiments.

Rosalee Hellberg: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data will be made available on request.

Declaration of competing interest

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

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Appendix A. Supplementary data

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