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Short Communication

Assessment of the Prevalence of Presumptive *Bacillus cereus, Vibrio* spp., and Extended-Spectrum β-Lactamase (ESBL)-Producing Enterobacterales in Sushi Products Collected From Swiss Retail Stores

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Abstract

Background: Sushi is a popular Japanese dish made from acidified cooked rice, seafood, and other ingredients. In this study we aimed to assess the microbiological quality of sushi products collected at retail level in Switzerland for the presence of *Bacillus (B.) cereus* group members, extended-spectrum β-lactamase (ESBL)-producing Enterobacterales and *Vibrio* spp. **Methods**: Between January and February 2024, a total of 52 sushi products were randomly collected from five different retail stores and from one restaurant. The collection included boxes of assorted sushi and various sushi snacks from 13 different brands. The products were produced for the most part in Switzerland, Poland, and Germany. **Results**: In a quantitative analysis, one out of 52 products (2%) tested positive for *B. cereus* group members, with a colony count of 100 CFU/g. After enrichment, *B. cereus* group members were isolated from six (11%) of the products. Five products (10%) tested positive for ESBL-producing Enterobacterales. All ESBL-producers were *Serratia fonticola* which harbor a chromosomally encoded *fonA* ESBL gene. *Vibrio* spp. were not detected in any of the 52 products. **Conclusions**: This study attests to a good microbiological quality of the collected samples with regard to the tested parameters.

Keywords: *Bacillus cereus* group members; *Vibrio* spp.; ESBL-E; sushi

1. Introduction

Sushi, a traditional Japanese food, is widely popular and consumed globally. It is prepared using raw or cooked fish, seafood, or vegetables in combination with cold boiled rice seasoned with vinegar [1]. Sushi is available in various forms and varieties, with two of the most common being Nigiri-sushi and Maki-sushi. Nigiri-sushi consists of bite-sized portions of rice topped with raw or cooked fish, whereas Maki-sushi is a roll of rice filled with fish or vegetables, wrapped in a sheet of seaweed (nori) [1]. Sushi is served in sushi bars or restaurants or sold as pre-packed cooled or frozen products in supermarkets. In Switzerland, the per capita consumption of fish and shellfish was approximately 8.44 kilograms in 2024, a figure that has remained stable over the past five years [2]. However, sushi is associated with microbiological hazards due to the presence of foodborne pathogens in raw fish and in cooked rice. Contamination may occur throughout food production by three main routes: fecal contamination (reflected by the presence of Enterobacterales such as *E. coli*), natural occurrence of bacteria in marine environments (e.g., *Vibrio* spp.), or improper handling, storage, and cross-contamination during food processing [1,3–8]. As a ready-to-eat (RTE) product, sushi does not undergo a heat treatment step that could inactivate potential pathogenic bacteria. Thus, the risk of foodborne illnesses is increased, making stringent monitoring to ensure consumer safety crucial [9,10]. Foodborne diseases and product recalls related to sushi have already been reported [11–15], highlighting the importance of identifying and controlling microbial hazards. Among these, bacteria of the *Bacillus (B.) cereus* group pose a significant risk. These ubiquitous, spore-forming bacteria can be found in a wide range of foods, including rice-based dishes such as sushi [9,16–18]. Contamination of food with *B. cereus* may lead to the formation of two types of toxins that cause two distinct syndromes, emesis (vomiting), and diarrhea. The emetic toxin cereulide is produced by certain strains of *B. cereus* during post-cooking survival and growth in rice and other starchy foods, particularly when the food is stored room temperature. Adequate rice acidification and refrigeration is critical to prevent the growth of *B. cereus* in sushi [19,20].

Furthermore, recent studies have shown that raw fish commonly consumed in sushi products may be contaminated with antimicrobial-resistant bacteria such as extended-spectrum beta-lactamase-producing Enterobacterales (ESBL-E) [21–23]. ESBL-E can be transmitted to food products through contamination of the aquatic environment and during processing and handling; therefore, strict hygiene practices are essential to prevent cross-contamination [22].

Vibrio (*V.*) spp., including *V. parahaemolyticus*, *V. vulnificus*, and *V. cholerae*, are gram-negative bacteria naturally occurring in marine environments and are commonly associated with seafood, including raw fish used for making sushi [24]. These pathogens are known to cause gastroen-

teritis and, in some cases, more severe infections such as septicemia [24]. *Vibrio* spp. thrive in warm environments including both the aquatic environment and seafood [24]. Therefore, maintaining the cold chain during all stages of food production is essential to minimize the risk of *Vibrio* infections [24].

This paper aims to assess the prevalence of presumptive *Bacillus cereus*, ESBL-E, and *Vibrio* spp. in sushi products collected from Swiss retail stores, and to generate baseline data for possible Hazard Analysis Critical Control Points (HACCP) adjustments.

2. Materials and Methods

2.1 Analyzed Products

Between January and February 2024, a total of 52 sushi products were randomly purchased from five distinct retail stores (A–E) and from a restaurant in Switzerland (F). Samples were transported in cooler bags and processed immediately upon arrival at the laboratory. If immediate processing was not feasible, samples were stored at –20 °C until analysis. The collection included mixed sushi assortments (n = 29) and different sushi snacks (n = 23) from 13 different brands. The products originated from Switzerland (n = 22), Poland (n = 27), and Germany (n = 1). For two products the country of origin could not be determined (N/A). All products are listed in Table 1.

2.2 Microbiological Analyses

The presence of *B. cereus* group members was analyzed both quantitatively and qualitatively. For quantitative analysis, 10 g of sample was added to 90 mL saline solution (1:10) and 100 µL was spread onto Mossel agar plates (Cereus-Selective-Agar, Merck & Cie, Buchs, Switzerland; Egg Yolk Emulsion, Oxoid, Pratteln, Switzerland). For qualitative analysis, 10 g of the product was enriched in 90 mL peptone water (Bio-Rad Laboratories AG, Cressier, Switzerland) at 37 °C for 24 h. The enrichment was then streaked onto Mossel agar plates. After incubation at 30 °C for 24–48 h, with a detection limit of 100 CFU/g. Presumptive positive colonies (pink colony surrounded by an opaque halo) were identified to species level using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF; Bruker, Bruker Daltonics, Bremen, Germany), according to the manufacturer's instructions (Bruker). Bacterial identification was carried out using the software Flex Control version 3.4., the MALDI Biotyper (MBT) Compass database version 4.1.100, and the MBT Compass BDAL version 12.0 Library. The cut-off score was $\geq$2.0 for identification at species level.

The detection of ESBL-E was performed by enriching 10 g of sample in 90 mL *Enterobacteriaceae* Enrichment (EE)-broth (1:10) (Becton, Dickinson, Heidelberg, Germany). After an incubation at 37 °C for 24 h, the enrichment was streaked onto *Brilliance*^{TM} ESBL agar (Oxoid, ThermoFisher, Pratteln, Switzerland) and incubated again at 37 °C for 24 h. Pink, blue, green or colorless colonies were considered characteristic for ESBL-E and were further analyzed using MALDI-TOF.

The presence of *Vibrio* spp. was analyzed in 10 g of sample according to ISO 21872-1:2017. Enrichment was carried out using alkaline solution peptone water (ASPW) (Bio-Rad Laboratories, Basel, Switzerland) and incubation at 37 °C for 24 h. Thereafter, 1 mL of the enrichment was transferred into two tubes containing 10 mL of ASPW each. One tube was incubated at 37 °C and the other at 42 °C for 24 h. The enrichment cultures were streaked onto Thiosulfate–citrate–bile salts–sucrose (TCBS) agar (Oxoid, Hampshire, UK) and CHROMID® *Vibrio* Identification (VID) agar (bioMérieux, Marcy l'Etoile, France) and incubated at 37 °C for 24 h. Characteristic colonies were further identified using MALDI-TOF.

3. Results and Discussion

In the quantitative analysis, one of 52 products (2%) tested positive for *B. cereus* group members (sample SU42 in Table 2), with a colony count of <100 CFU/g. The product was an organic sushi produced in Switzerland. After enrichment, 6/52 (11%) of the samples harbored colonies phenotypically suspicious for *B. cereus* group members. These colonies were identified by MALDI-TOF as *B. cereus* (n = 1), *B. pumilus* (n = 3), *B. subtilis* (n = 1), and *B. velezensis* (n = 1) (Table 2). Therefore, only *B. cereus* belonged to the *B. cereus* group. Four products originated from Poland (SU8, SU23, SU38, SU41), and two from Switzerland (SU42, SU45). To draw more definitive conclusions, a larger number of products would need to be analyzed. Members of the *B. cereus* group cannot be distinguished by MALDI-TOF, as they share highly similar ribosomal protein profiles. However, MALDI-TOF is sufficient for preliminary species identification. Since four out of five isolates in our study did not belong to the *B. cereus* group, no further identification steps were performed. The results are listed in Table 2.

Contamination with *B. cereus* group members in sushi is closely linked to rice as a component of sushi products. As a ubiquitous occurring and spore-forming bacterium, *B. cereus* can survive cooking, with cooked rice held at room temperature creating favorable conditions for its growth [20]. Notably, the low pH (~pH 4.3) of acidified sushi rice plays a crucial role in effectively inhibiting *B. cereus* growth [20]. According to the guidelines of the U.S. Food and Drug Administration (FDA) and the UK Health Security Agency (UKHSA), *B. cereus* counts below 10^6 CFU/g [25] and 10^5 CFU/g [26], respectively, are generally considered acceptable in RTE foods. The UKHSA further defines counts below 10^3 CFU/g as satisfactory, requiring no action. Counts between 10^3 and 10^5 CFU/g are regarded as borderline, and such foods may pose a potential risk to vulnerable populations [26]. In the Commission Regula-

Table 1. Overview of the sampled sushi products (n = 52), country of origin, and the retail store of purchase.

Sample ID	Product	Produced in	Retail store
SU1	Yorokobi Sushi-Box	Poland	A
SU2	Tanoshii Sushi-Box	Poland	A
SU3	Edogawa Sushi-Box	Poland	A
SU4	Sushi Snack	Switzerland	B
SU5	Best of Salmon Mix	Switzerland	B
SU6	Smoked Salmon Wrap	Switzerland	B
SU7	Sushi Hiroto	Poland	C
SU8	Sushi Set	Poland	D
SU9	Sushi Set	Poland	D
SU10	Sushi Box Joto-Style	Poland	D
SU11	Sushi Wraps	Poland	D
SU12	Sushi with fish and vegetables	Switzerland	E
SU13	Sushi	Switzerland	E
SU14	Sushi	Switzerland	E
SU15	Sushi	Switzerland	F
SU16	Sushi	Switzerland	F
SU17	Sushi Set	Poland	D
SU18	Sushi	N/A	E
SU19	Sushi	N/A	C
SU20	Sushi	Switzerland	B
SU21	Sushi	Germany	A
SU22.1	Edogawa Sushi-Box Nigiri	Poland	A
SU22.2	Edogawa Sushi-Box Sushi	Poland	A
SU23	Yorokobi Sushi-Box	Poland	A
SU24	Tanoshii Sushi-Box	Poland	A
SU25	Sushi Mix Bio	Switzerland	B
SU26.1	Maki Mix raw tuna	Switzerland	B
SU26.2	Maki Mix tuna mousse	Switzerland	B
SU27	Nigiri Salmon	Switzerland	B
SU28.1	Sushi Japanese Style Nigiri	Poland	D
SU28.2	Sushi Japanese Style Hosomaki	Poland	D
SU29.1	Salmon Mix Box Nigiri	Poland	D
SU29.2	Salmon Mix Box Futomaki	Poland	D
SU30	Office Box	Poland	D
SU31	Crunchy cooked salmon	Poland	D
SU32	Sushi Box Nigishi-Style	Poland	D
SU33	Sushi Box Joto-Style	Poland	D
SU34	Sushi Wraps salmon & mango	Poland	D
SU35	Sushi Mix Bio	Switzerland	B
SU36	Smoked Salmon Wrap	Switzerland	B
SU37	Sushi Set Nigrini	Poland	D
SU38	Sushi Set Makis	Poland	D
SU39	Sushi Box Joto-Style	Poland	D
SU40	Salmon Mix Box	Poland	D
SU41	Cooked Salmon Carolina Roll	Poland	D
SU42	Sushi bio	Switzerland	E
SU43	Onigiri Spicy Salmon	Switzerland	E
SU44	Sushi Snack	Switzerland	E
SU45	Uramaki Mix	Switzerland	E
SU46	Smoked Salmon Wrap	Switzerland	E
SU47	Sushi	Switzerland	F
SU48	Sushi	Switzerland	F

N/A, not available.

Table 2. Sushi products with positive results for *Bacillus* spp.

Sample ID	Product	Product produced in	Quantitative detection	Qualitative detection in 10 g	Identification with MALDI-TOF
SU8	Sushi Set	Poland	<100 CFU/g	Positive	*Bacillus pumilus*
SU23	Yorokobi Sushi-Box	Poland	<100 CFU/g	Positive	*Bacillus pumilus*
SU38	Sushi Set Makis	Poland	<100 CFU/g	Positive	*Bacillus pumilus*
SU41	Cooked Salmon Carolina Roll	Poland	<100 CFU/g	Positive	*Bacillus subtilis*
SU42	Sushi bio	Switzerland	100 CFU/g	Positive	*Bacillus cereus*
SU45	Uramaki Mix	Switzerland	<100 CFU/g	Positive	*Bacillus velezensis*

tion (EC) No. 2073/2005, which sets microbiological criteria for foodstuffs, no specific limits are established for *B. cereus* [27]. As only low levels (100 CFU/g) were detected in the sushi samples analyzed in this study, bacterial growth would be required to reach toxin-relevant concentrations [28]. Virulence factors were not further characterized in this study. Thus, maintaining levels below the threshold through strict food safety measures, including adequate refrigeration, controlled acidification, and limiting room temperature exposure, is essential to mitigate the risks associated with *B. cereus*.

ESBL-E were detected in 5/52 (10%) of the products (samples SU11, SU29.2, SU35, SU38, and SU40). All ESBL-E were identified as *Serratia (S.) fonticola*, a species that harbors a chromosomally encoded *fonA* ESBL gene. This ubiquitous environmental bacterium has been isolated from a wide range of habitats, including both freshwater and marine environments [29–32]. These intrinsically β-lactam-resistant organisms do not readily transfer antimicrobial resistance (AMR) genes to other bacteria and are not commonly associated with human disease. However, they can spread through non-clinical environments such as water and via non-human hosts, including birds [33–35].

In a Swiss multicenter cross-sectional study involving 1209 hospital employees, the prevalence of gut colonization was 5.4% for ESBL-E [36]. Sushi consumption at least once per month was one of the risk factors that was positively associated with ESBL-E colonization. Other studies have reported the prevalence of ESBL-E in raw fish—and consequently in sushi—ranging from 3% to 63%, depending on the country and type of product [21–23]. Our findings align with these reported prevalence rates and with the species identified in the study of Muscolino *et al*. [17]. Based on previous investigations, *S. fonticola* has been found in plant-based foods, suggesting that plant-derived ingredients represent a likely source of contamination in sushi products and other ready-to-eat foods [37,38]. Currently, no specific microbiological criteria exist in the EU or Swiss regulations for ESBL-E in RTE foods [27], and their detection is generally interpreted as an indicator of environmental or cross-contamination rather than direct risk to the consumer.

Vibrio spp. were not detected in any of the 52 products. This result is consistent with studies from Germany and Austria which report the absence of *Vibrio* spp. in similar products [1,39]. However, other studies from Italy, Japan and South Korea have reported the presence of *Vibrio* spp. in sushi, highlighting the variability of contamination [17,40,41]. *Vibrio* spp. are halophilic and thus frequently contaminate raw seafood, but they may equally occur in saline-free water [17,42]. Temperature is the critical factor influencing the growth rate of *Vibrio* spp., thus, strict adherence to the cold chain control procedure is essential [24]. Notably, climate change may enhance conditions that support the proliferation and persistence of *Vibrio* spp. in aquatic environments, potentially increasing the risk of contamination of seafood [24].

This work represents a preliminary assessment of the microbiological quality of sushi products, limited by sample size and the scope of microbiological characterization. Future research could expand the number of analyzed products and apply molecular methods to further characterize virulence and resistance determinants.

4. Conclusions

Sushi is a globally consumed RTE product but poses a microbiological risk due to its composition of raw fish and cooked rice, both of which may contain foodborne pathogens. The detection of *B. cereus* group members underscores the need for proper rice acidification and refrigeration to prevent bacterial growth and toxin formation. By contrast, the presence of *S. fonticola* is of low concern. Although *Vibrio* spp. were not detected in this study, their potential occurrence in sushi remains a recognized food safety challenge. The relatively small sample size is a limitation of this study.

The microbiological criteria for RTE foods, including sushi, are established in Regulation (EC) No. 2073/2005 (European Commission, 2005), which defines two key food safety parameters: the absence of *Salmonella* spp. in 25 g of sample and specific limits for *Listeria monocytogenes* [27]. No microbiological criteria are specified in this regulation for members of the *B. cereus* group, ESBL-producing Enterobacterales, or *Vibrio* spp. Future amendments to Regulation (EC) No. 2073/2005 may be considered, based the results of this and other studies.

Ensuring sushi safety requires a multifaceted approach, including rigorous monitoring of raw materials, ad-

herence to cold chain protocols, appropriate rice acidification, and stringent hygiene practices throughout processing and distribution.

Availability of Data and Materials

All data are provided as part of this publication.

Author Contributions

KB: Writing – original draft, Formal analysis, Investigation. SC: Writing – review & editing, Investigation. RS: Writing – review & editing, Supervision, Project administration, Conceptualization. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

Given his role as an Editor Board member, Roger Stephan had no involvement in the peer-review of this article and has no access to information regarding its peer-review. Full responsibility for the editorial process for this article was delegated to Corinna Kehrenberg. The authors declare no conflict of interest.

Declaration of AI and AI-Assisted Technologies in the Writing Process

This manuscript was revised with the assistance of ChatGPT (GPT-4o), which was used to improve clarity and rephrase selected sentences during the writing process.

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