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Research Article

Evaluation of microbiological quality in fresh sushi samples

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ABSTRACT

In this study, it is aimed to determine the microbiological quality of 60 different raw salmon finger sushi (maki rolls) samples randomly supplied from consumption points (sushi bar, sushi buffet, hotel, restaurant etc.) serving fresh sushi. The counts of *Escherichia coli*, coagulase-positive *Staphylococcus aureus*, *Vibrio paraheamolyticus* and *Salmonella* spp. presence (+/-) were examined in these samples for the detection of hygienic quality and pathogenic microorganisms. Twenty-nine of 29/60 (48.3%) samples were found to be positive for these microorganisms. *Salmonella* spp. presence was detected in 2/60 samples (3.3%) while coagulase-positive *S. aureus* was found in 10/60 samples (16.7%) with the highest level of 4.84 log CFU/g. *E. coli* was detected in 15/60 samples (25.0%), *V. paraheamolyticus* was determined in 13/60 samples (21.7%) with a level of 2.90 log CFU/g and 2.82 log CFU/g, respectively. Based on the current findings, it is considered that microorganisms determined in the samples pose a risk to public health, authorities should mandate to conduct routine inspections more frequently and businesses selling sushi should be included in the annual sampling plan. It will be beneficial for healthy sushi production that it should be paid attention to hygienic production during the all manufacturing, storage and service stages and points of sale and relevant staff should be ensured to routinely receive necessary food safety training to minimize the risk of foodborne disease outbreaks.

Taze suşi örneklerinde mikrobiyolojik kalitenin değerlendirilmesi

ÖZ

Bu çalışmada; taze suşi servisi yapan rastgele seçilmiş tüketim noktalarından (sushi bar, sushi büfe, otel, restorant vs.) temin edilen toplamda 60 adet çeşitli çiğ somon parmak suşi (maki rolls) örneklerinin mikrobiyolojik kalitelerinin belirlenmesi amaçlanmıştır. Örneklerde hijyenik kalitenin ve patojen mikroorganizmaların tespit edilmesine yönelik *Escherichia coli*, koagulaz pozitif *Staphylococcus aureus*, *Vibrio paraheamolyticus* sayıları ile *Salmonella* spp. (+/-) varlığı araştırılmıştır. İncelenen mikroorganizmalar yönünden 29/60 (%48,3) örneğin pozitif olduğu görülmüştür. 2/60 örnekte (%3,3) *Salmonella* spp. varlığı tespit edilirken, koagulaz pozitif *S. aureus*'un 10/60 (%16,7) örnekte ve en yüksek 4.84 log KOB/g, *E. coli*'nin 15/60 (%25,0) *örnekte ve V. paraheamolyticus*'un 13/60 örnekte (%21,7) sırasıyla ortalama 2.90 log KOB/g ile 2.82 log KOB/g olduğu saptanmıştır. Mevcut bulgulara dayanarak, örneklerde tespit edilen mikroorganizmaların halk sağlığı açısından riskler taşıdığı, yetkili otoritelerin daha sık rutin denetimler yapması ve suşi satışı yapan işletmelerin yıllık numune planına dahil edilmesi gerektiği düşünülmektedir. Gıda kaynaklı hastalık salgınları riskini en aza indirgemek için suşi üretim ve satış yerlerinde tüm üretim, muhafaza ve servis aşamalarında hijyenik üretime önem verilmesi ve ilgili personellerin rutin olarak gerekli gıda güvenliği eğitimini almalarının sağlanması sağlıklı suşi üretimi açısından faydalı olacaktır.

INTRODUCTION

Sushi is a traditional Japanese food, consisting of raw or cooked seafood in combination with eggs, vegetables and cold cooked rice prepared with vinegar and condiments that is shaped into bite-sized pieces, formed into a roll and often wrapped in seaweed (nori) (1, 2). Sushi is known to be a method to preserve fish in Southeast Asia in previous years (3). With its low-fat content, intense nutrients and delicious to consume, sushi, which is rich in high-quality protein and an excellent source of omega-3 fatty acids, is a popular product consumed all over the world. It has become a popular food particularly in our country in the past 15 years and adopted by a wide population (4). The high level of diversity in raw materials is based on manufacturer choices and local preferences. It is prepared with raw farmed salmon, tuna, and halibut, whiting, cooked scampi, large freshwater prawns, and raw vegetables such as cucumber, spring onion (scallions), and avocado (5, 6). It is a popular ready-to-eat (RTE) food preferred by consumers and offered cold at 4 °C with a sell-by date of 2 to 3 days after production which is served directly to human consumption without cooking or other processes to eliminate or reduce microorganisms to an acceptable level by manufacturers or businesses (6). Intrinsic factors (high water activity, near-neutral pH) of fish meat and raw fish products provide a favorable condition for microorganisms to develop and can cause spoilage within the whole process from the moment of catching of fish to consume in the presence of extrinsic

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factors (harvest, hunting, transportation, process, storage etc.) (7). It carries some potential health hazards due to possible contamination of pathogenic microorganisms during raw material, process and storage. Monitoring of microbiological quality is also important to prevent food poisoning cases (3, 6, 8, 9). Sushi is consumed similar to other fast foods without additional heat treatment to reduce microbial load. Therefore, forms of raw seafood products prepared in homes, restaurants and sushi bars should be consumed in a shorter time than industrially prepared products (1). Several factors have been stated to be effective in a large number of food poisoning cases reported in relation to sushi consumption (10, 11). The fact that other foodstuffs used in making sushi are prepared in advance for quick service and possible contaminations of hands and other tools and equipment during the process may lead to an increase in the incidence of potential foodborne pathogens (2). Due to the reasons such as fish and other seafood products used in the preparation of the product, other mixtures such as vegetables and rice and also being consumed raw, hygiene of raw material and business, possible presence of pathogenic microorganisms (*Salmonella* spp., *Vibrio parahaemolyticus*, *Staphylococcus aureus*, *Vibrio cholerae*, *Bacillus cereus*, *Listeria monocytogenes*), parasites (Anisakiasis etc.) and viruses (Norovirus) can cause health problems (1, 6, 12, 13). Considering the growing popularity of sushi and other raw seafood dishes especially worldwide, it would be beneficial to raise awareness about the safety of raw seafood products consumption.

In the present study, it was aimed to determine the hygiene level of the products at the consumption points serving fresh sushi (sushi bar, sushi buffet, hotel, restaurant etc.) in Antalya, to evaluate the microbiological quality and preparation conditions and to determine preventive measures to increase food safety in order to provide information about the possible health risks associated with the increased consumption of ready-to-eat sushi.

For this purpose, the samples were examined for *E. coli* as hygiene indicator microorganism, specific food-borne pathogens by the numbers of coagulase-positive *S. aureus* and *V. paraheamolyticus* and presence of *Salmonella spp.*

MATERIAL AND METHOD

Sampling and preparation for analysis

Between June–July 2017, 60 samples of sushi (N=60) obtained from the consumption points serving fresh sushi (sushi bar, sushi buffet, hotel, restaurant etc.) in the province of Antalya on different days were used in this research study. Sushi samples taken into sterile containers under aseptic conditions were placed in polyethylene styrofoam boxes (2-4 °C) containing ice and were delivered to the laboratory in a short time. Sample size and weight were determined as 5.00±0.88 cm and 21.00±1.74 g, respectively. Microbiological analyses were carried out with two replications.

Microbiological analyses

Twenty-five g sample was homogenized in 225 ml MRD (Maximum Recovery Diluent, MRD-Oxoid CM0733, UK) and aliquots of 0.5 ml of prepared dilution were spread-plated onto Chromocult TBX Agar (Oxoid CM0945, UK). Plates were first incubated at 30±1 °C for 4±1h, then at 44±1 °C for 18±2h and growing blue-green colonies were determined as *Escherichia coli*. No confirmation has been made since chromogenic medium was used. The strain *E. coli* ATCC 25922 (ATCC® 25922™, USA) was used as a positive control (14).

Five g sample was homogenized with 45 ml MRD for coagulase-positive *Staphylococcus aureus* (CFU/g) count, aliquots of 0.4-0.3-0.3 ml of this dilution (a total of 1 ml) by adding 5% Egg Yolk Tellurite (Becton-Dickinson, BBL-212357, USA) were spread-plate onto previously prepared Baird Parker Medium Agar (Oxoid CM0275, UK). The plates were incubated at 35±1 ºC for 48h. At the end of the incubation, black-gray shiny colonies with a diameter of 2-3 mm, surrounded by round, convex, smooth, narrow, bright zoned area in plates are considered as possible *S. aureus* colonies and all developing typical colonies were counted. Confirmation of typical colonies was performed by Staphylase (Staphylase Test Kit, Oxoid DR0595, UK) test. Colonies forming a visible agglutination were considered as coagulase-positive *S. aureus*. If the coagulase (staphylase) test result is positive, the number of *S. aureus* was determined as CFU/g by multiplying the counted colonies by the dilution coefficient. The strain *S. aureus* ATCC 25923 (ATCC® 25923™, USA) was used as a positive control (15).

Twenty-five g sample was homogenized with 225 ml alkaline peptone water (APW, Merck 101800, Germany) (containing 3% NaCl), incubated at 35-37 ºC for 18-24h for *Vibrio parahaemolitycus* count. The content taken from the upper part of this enrichment medium using a loop without shaking was spread-plate onto Thiosulfate-Citrate-Bile Salts-Sucrose Agar (TCBS, Hardy Diagnostics, USA) (containing 3% NaCl) and incubated at 35-37 ºC for 18-24h. Round colonies with a diameter of 2-3 mm and green-blue in the middle were evaluated as *V. parahaemolitycus* and confirmation of typical colonies was performed by catalase, oxidase test and API® 20E (BioMerieux, France) biochemical reagent kit (16).

Twenty-five g sample was taken into 225 ml buffered peptone water for *Salmonella* spp. identification and was incubated at 37±1 °C for 16-20h. After incubation, 1 ml of this suspension was transferred into 10 ml of Muller-Kauffmann Tetrathionate-Novobiocin Broth (MKTTn-Oxoid CM1048, UK) for selective enrichment. Tubes were incubated at 37±1 °C for 6-8h. Concordantly, 10 ml of pre-enrichment suspension were transferred into Rappaport Vassiliadis Soy Broth (RVS-Oxoid CM0886, UK) and incubated at 41.5±1 °C for 6-8h. Aliquots of these secondary enrichment broth were then spread-place onto Xylose Lysine Deoxycholate Agar (XLD-Oxoid CM0469, UK) and Brilliant Green Agar-Modified Agar (BGA-Oxoid CM0329, UK) using a loop and typical colonies were examined. Positive *Salmonella* spp. samples with typical colonies were confirmed using a Vidas device (mini VIDAS®, BioMerieux, France) (17).

RESULTS

Numbers of *E. coli*, coagulase-positive *S. aureus*, *V. parahaemolitycus* of 60 analyzed sushi samples were calculated using base-10 logarithms and given as log CFU/g in Table 1. *Salmonella* spp. is presented as absent/present in Table 1.

DISCUSSION

RTE sushi is considered a kind of potentially hazardous food because it contains perishable ingredients. If it is not prepared in a hygienic procedure and stored at low temperature, it may cause food poisoning (2). It is recommended by ICMSF (International Commission on Microbiological Specifications for Foods) that food containing seafood products should be kept at temperatures below or close to 5 °C (8). *E. coli* count in food products, which have been used to indicate direct or indirect fecal contamination, is a method used to indicate the cleanliness in food handling as well as the appropriateness of storage condition (2, 11). According to our study results, *E. coli* number was found to be ranging from ND (not detected the bacteria) and 3.96 log CFU/g in 15/60 samples (25%) and 2.90 log CFU/g on average, and 75% of the samples were

Table 1 Microorganisms analyzed in sushi samples and their numbers (N=60)

Number of microorganisms (log CFU/g)	Indicator and Pathogen Microorganisms Levels (sample-%)			
	Escherichia coli	*Staphylococcus aureus (coagulase positive)*	*Vibrio parahaemolitycus*	*Salmonella* spp. (+/-)
ND	0.0 (75.0%)	0.0 (83.3%)	0.0 (78.3%)	58.0 (96.7%)
1.00<2.00	ND	ND	ND	
2.00<3.00	9.0 (15.0%)	4.0 (6.7%)	9.0 (15.0%)	
3.00<4.00	6.0 (10.0%)	5.0 (8.3%)	4.0 (6.7%)	2.0 (3.3%)
4.00<5.00	ND	1.0 (1.7%)	ND	
5.00≥	ND	ND	ND	
Min.	ND	ND	ND	-
Max.	3.96	4.84	3.86	-
Means	2.90	3.24	2.82	-
TOTAL	15.0(25.0%)	10.0(16.7%)	13.0(21.7%)	2.0 (3.3%)

CFU: Colony Forming Unit, N: Number of samples, ND: Not Detected

found to be negative for this microorganism (Table 1). The recommended limit for good quality foods (2.7 log CFU/g) was exceeded in 9 samples for *E. coli* (18). It is considered that this situation results from possible contamination due to poor hygiene and sanitation in operation, personnel or equipment during production and preservation in businesses, raw material and other reasons (production line design, water, etc.).

Staphylococcus aureus is a microorganism with variable virulence and produces toxin when it is exceeded the level of 5 log CFU/g. Toxin dose of less than 1.0 µg may produce symptoms of intoxication (19). Although *S. aureus* is not part of the normal fish microflora, it is reported that it is possible to have contamination arising from nutrients involved in fish products prepared with personnel and/or other foods during the process (2, 11). In this study, coagulase-positive *S. aureus* was detected ranging from ND and 4.84 log CFU/g in 10/60 samples (16.7%) (Table 1).

According to the results, the presence of a sample with a potential to produce toxin indicates that proper personnel hygiene and other cleaning procedures are disrupted where the relevant sample is provided. *S. aureus* contamination denotes a lack of good hygienic practices by businesses, spoilage after harvest due to diseases in fish used in the preparation of raw materials or sushi or the possibility of cross contamination during raw material and/or process stages (11, 20).

Vibrio parahaemolyticus is a microorganism that is more common in the hot summer months and naturally found in marine environments and constitutes an average of 30% of the total microbial load in seawater. The primary control measure is mostly intended to prevent the proliferation of this organism (8, 21). In our country, there is a legal regulation for the absence of this microorganism in frozen and processed fish products (22). According to our study results, *V. parahaemolyticus* presence was detected in the level of 2.82 log CFU/g on average in 13/60 samples (21.7%), and it was found to be noncompliance with the relevant regulation (Table 1).

Salmonella spp. is a pathogen bacterium causes food poisoning and infection, and it is reported that even <10 CFU *Salmonella* spp. organisms may lead to infection (2). Possible contamination of *Salmonella* spp. in raw materials, food manufacturing, tools and equipment used in the food chain, processing and retailing stages can cause very important health problems for public health. In general, all raw foods including fish, shellfish, fruits, vegetables, poultry, eggs, other foods of animal origin, dairy products and crops are always at risk for non-typhoid *Salmonella* spp. (23). Although there is a close relationship between RTE food consumption and *Salmonellosis*, previous studies have reported that the presence of *Salmonella* spp. in sushi is not very common in general (9). Although outbreaks related to *Salmonella* spp. infected fishery products (fish borne) is not very common, it has been reported that after sushi consumption, 62 people in 11 different locations in The United States of America experienced food poisoning and diagnosed with *Salmonellosis*. In our country, there is no RTE food definition and no legal regulation has been published specifically for these foods. Turkish Food Codex (TFC) (24) and Regulation on Seafood (22) report that *Salmonella* spp. should not be present (0/25 g-ml) in fishery products and processed or frozen fish products. The amount of *Salmonella* spp. in RTE foods has been determined as 0/25 g by the European Commission (25). According to our study results, 2/60 samples (3.3%) were found to be noncompliance with the relevant regulations (Table 1).

A study examining sushi samples taken in winter (December-February) demonstrated that positive sample counts were determined as 11/447 (2.5%) for *E. coli* and 7/447 (16%) for coagulase-positive *staphylococci* while *Salmonella* spp. (0/447) and *V. parahaemolyticus* (0/155) were not detected. In samples taken in summer (June-August), *V. parahaemolyticus* was detected in 1/158 sample, examined samples were negative for *Salmonella*, determined limit was exceeded in 6/404 samples for *E. coli* and 2/404 samples for coagulase-positive *staphylococci* (8). Atanasovva et al. (11) revealed that *E. coli* was detected ranging from 2.0 and 3.3 log CFU/g in 24/125 fresh sushi samples, *Staphylococcus* spp. with high prevalence was detected ranging from 3.8 and 4.7 log CFU/g in all samples and the prevalence was high (125/125), *Salmonella* spp. was found in 1/125 (0.8%) sample and *Vibrio* spp. were not detected in any sample. In several studies, it is shown that *V. parahaemolyticus* contamination in samples may originate from fishery and shellfish products used in the preparation of sushi, *Salmonella* spp. and *S. aureus* contamination may be caused by vegetables, mayonnaise containing raw egg and personnel-related (3, 8).

In 38 samples obtained from consumption points such as restaurant, sushi bar, *Salmonella* spp. and *V. parahaemolyticus* were not found, coagulase-positive *staphylococci* was detected ranging from 2.00 and 3.60 log CFU/g in 16/38 samples (42.11%) and found at an unacceptable level in 6 samples (3). Liang et al. (2) reported in packaged sushi samples taken from 120 different consumption points that *E. coli* and *S. aureus* counts were 1.0 and 2.3 log CFU/g on average, respectively and *Salmonella* spp. was not detected in any samples. Wong and Cheung (21) indicated that the number of *E. coli* was found as 20/102 (19.6%) in 102 fresh sushi samples, *V. parahaemolyticus* was detected in only 3/102 samples (2.9%), the presence of *E. coli* and *V. parahaemolyticus* results from inadequate hygiene practices in pre-harvest, harvest and sushi processing stages and possible bacterial contamination in fishery and other aquaculture products. Coagulase-positive *staphylococci*, *V. parahaemolyticus* and *Salmonella* spp. could not be detected in 36 samples taken from several restaurants, whereas positive *E. coli* count was found as 2/36 (5.6%) (7). *Salmonella* spp. was not detected in 28 sushi samples obtained from 7 different businesses whereas *E. coli* was found in 3.6% of samples (9). *E. coli* was detected in 38/50 fresh sushi samples taken from sushi bars and *S. aureus* were found in 11/50 samples (26). *Staphylococcus* spp. was determined ranging from 3.3 and 3.8 log CFU/g in samples obtained from 20 different retail points serving sushi, *S. aureus* count was found less than 2 log CFU/g on average (27).

According to the study results, it is considered that different findings may proceed from microbial contaminations to be occurred pre-harvest during and post-harvest in fishery and other seafood products used in making sushi (21), operational and personnel hygiene during the entire process, possible contaminations during preservation of final product sushi and other raw materials and deficiencies in cold storage applications.

CONCLUSION

In order to maintain the quality of food products, they must be kept at low temperatures. United States Food and Drug Administration (FDA) that in order to eliminate the possible parasite presence in raw seafood used in sushi, it is necessary to wait for 7 days below -20 °C or for 15h below -35 °C. According to EC (28) and TFC (24), in order to kill other parasites other than Trematodes in fishery products to be consumed raw, freezing process should be applied not less than 24h at -20 °C or less than 15h at -35 °C, and there is a regulation to preserve fresh fishery products at a temperature close to melting point. Due to the high potential of food poisoning of raw seafood products, they should be transported under temperature control in order to reduce microbial development and should be kept at ≤5 °C until the process in the businesses (21). It is initially necessary to choose suitable raw materials for microbial quality to make a healthy sushi. It should be taken into consideration that sushi rice is not a good substrate for microbiological development because of its low pH (<4.6), however; it might contribute to inhibiting the development of lactic acid bacteria since it contains fermented carbohydrates (1, 4, 6). As sushi is highly sensitive to deviations from optimal storage temperature, positive correlations between microorganism counts and storage temperatures may lead to loss of quality during sell-by date (29). Careful selection of other nutrients used in making sushi, maintenance of cold chain during preparation processes and storage, compliance with hygiene and sanitation rules in personnel, equipment and service (3, 11) should be adopted as the basic principles in order to maintain the microbiological quality in the final product. In addition, businesses ensuring their personnel to receive hygiene training on a regular basis and increased frequency of inspections by authorities will provide a significant contribution to minimize sushi-related food poisoning. GMP (Good Manufacturing Practice) or HACCP (Hazard Analysis and Critical Control Point) food safety control systems incorporating into the manufacturing process by businesses will help strengthen the food safety control. The fact that consumers behave sensitively during transportation and storage of ready-prepared sushi, prefer places making good quality sushi and care about shelf life, people with chronic conditions such as immune system insufficiency, pregnancy and cancer should be more careful about sushi consumption due to their risks will contribute to healthy sushi consumption.

CONFLICTS OF INTEREST

We declare that there are no conflicts of interest among the authors of the article.

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