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Prevalence of *Staphylococcus aureus* and *Salmonella enterica* in ready-to-eat sushi and sashimi

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Abstract. *Staphylococcus aureus* food poisoning and *Salmonellosis* outbreaks have been associated with two popular ready-to-eat items: sushi and sashimi. Thus this study aims to determine the prevalence of *S. aureus* and *S. enterica* in sushi and sashimi in Malaysia. Sushi (149) and sashimi (51) were collected from 14 retail outlets comprising supermarkets, hypermarkets, restaurants and open-air night markets. Bacterial isolation was carried out using Baird-Parker and CHROMagar *Salmonella* Plus selective media. The food pathogens isolated from microbiological media were then confirmed by molecular analysis. The results confirmed an overall *S. aureus* and *S. enterica* contamination of 42% (84/200) in the sushi and sashimi samples. Regarding prevalence of the individual pathogens involved, *S. aureus* was detected in 26% (52/200) and *S. enterica* in 16% (32/200) of the contaminated samples. This study demonstrates a high occurrence rate of *S. aureus* and *S. enterica* in sushi and sashimi foods in Malaysia, and warrants the necessity to monitor the microbiological process of RTE foods to ensure food safety for consumers.

INTRODUCTION

Sushi and sashimi, synonymous with Japanese cuisine, are offered not only in Japanese restaurants but are also especially popular retail foods in Malaysian supermarkets and hypermarkets (Md Akhir *et al.*, 2011/2012). Both sushi and sashimi are considered permissible ("halal") foods by the Muslims. Thus, these foods are eaten by the Malays, Chinese and Indians that comprise the multi-ethnic Malaysian population. The distinct features of sushi and sashimi are their manual preparation using bare hands by chefs to produce cold rolled sticky rice combined with other ingredients (sushi) or fresh raw fish or seafood in slivers (sashimi) for direct consumption with no further cooking. The level of contamination of these two foods with foodborne pathogens may be high due to the preparation techniques (Food and Environmental Hygiene Department HKSAR, 2000), thus stringent food safety measures should be considered to prevent food poisoning.

Several studies have reported that consumption of sushi and sashimi are associated with microbiological hazards including *S. aureus* (Food and Environmental Hygiene Department HKSAR, 2000; Hammad *et al.*, 2012) and *Salmonella* (Barralet *et al.*, 2004). *S. aureus*, a common bacterium in human and animal normal flora, is generally used as an indicator of food contamination. It is frequently isolated from different food origins such as bovine milk, fresh meat, and infant foods (Kadariya *et al.*, 2014). On the other hand, *Salmonella* spp. are found in various foods, mainly in poultry, Ready To Eat (RTE) meat, and egg-related products (Carrasco *et al.*, 2012).

To date, the prevalence of *S. aureus* and *S. enterica* in retailed sushi and sashimi samples in Malaysia has not been studied. *Campylobacter* spp. isolates were detected in sushi in Malaysia (Tan *et al.*, 2008) and the

β-lactamase gene *bla*SHV in bacteria was isolated from a small sample size of only 22 sushi samples (Cheong *et al*., 2014). Hence, the objective of this study is to determine the prevalence of *S. aureus* and *S. enterica* in retail sushi and sashimi samples in Malaysia.

MATERIALS AND METHODS

Sample collection

Klang Valley, a modern urban area composing of the capital city, Kuala Lumpur and neighboring suburbs was selected as the study area because of the availability of many different retail outlets offering varied choices of RTE foods. A total of 200 sushi (n=149) and sashimi (n=51) samples were collected between August and December 2014 from four different types of retail outlets – supermarkets (78), hypermarkets (63), restaurants (41) and open-air night markets (18). Sushi samples consisted of rice with various toppings - marine fishes, fish roe, squid, octopus, jellyfish, edible seaweed, scallop, egg, crab stick, cherry shrimp, prawn and clam. Sashimi samples consisted only of seafood – slivers of salmon, tuna, yellow tail, squid and scallop. All the food samples were transported in a portable cooler at a temperature of 4°C to the laboratory and analyzed immediately.

Pre-enrichment

The external surfaces of sample packagings were wiped with 70% (v/v) alcohol. Each sample was aseptically weighed in an analytical balance and 25 g was mixed with 225 mL of buffered peptone water (Oxoid, UK) and homogenized in a Stomacher Bagmixer 400W (Interscience, France). The homogenate was incubated for 16 hours at 37°C with agitation. Isolation for *S. aureus* and *S. enterica* was carried out Baird-Parker agar and CHROMagar *Salmonella* Plus respectively.

Isolation of *Staphylococcus aureus*

One hundred microliters of the pre-enriched homogenate were transferred into 50 mL of trypticase soy broth (Oxoid, UK) containing 7.5% NaCl and incubated at 35°C for 18 h. A loopful of the culture was plated onto Baird-Parker agar supplemented with egg yolk tellurite emulsion (Oxoid, UK) and incubated overnight at 37°C. Three presumptive *S. aureus* colonies (black colonies surrounded by a clear 2–5 mm zone) from each food sample were randomly picked and purified using trypticase soy agar (yellow colonies) and incubated overnight at 37°C for further confirmation by molecular analysis.

Isolation of presumptive *Salmonella* spp.

One hundred microliters of the pre-enriched homogenate were transferred into 10 mL of Rappaport-Vassiliadis Soya broth and incubated at 42°C for 24 h. A loopful of the culture was plated onto CHROMagar *Salmonella* Plus (CHROMagar, Paris, France) and incubated overnight at 37°C. Three presumptive *Salmonella* spp. colonies (mauve) from each food sample were randomly chosen and purified using nutrient agar (smooth colonies) and incubated overnight at 37°C for further confirmation by molecular analysis.

DNA extraction and amplification

Total genomic DNA was prepared using a modified in-house boiling method (Sambrook and Russell, 2001). The identity of food isolates was then confirmed by DNA amplification using polymerase chain reaction (PCR) with primers as described in Table 1. DNA amplification was carried out in a total volume of 25 µL containing TopTaq Master Mix (Qiagen, Germany), 0.2 µM forward primer, 0.2 µM reverse primer, 100 ng DNA template and RNase-free water. The PCR program consisted of an initial denaturation of 5 min at 95°C, followed by 30 cycles of 95°C for 1 min, 55°C for 1 min and 72°C for 1 min, and a final extension of 10 min at 72°C. All PCR products were resolved by electrophoresis in a 1.5% (w/v) agarose gel. *S. aureus* ATCC 29213 and *S. typhimurium* ATCC 13311 were used as controls in PCR assays.

Table 1. Nucleotide sequences of primer pairs for DNA amplification of *Staphylococcus aureus* and *Salmonella enterica* and amplified amplicon sizes

Identification	Primer set	Name	Sequence (5' to 3')	Amplicon size (bp)	References
S. aureus	A	nuc-F nuc-R	GCGATTGATGGTGATACGGTT AGCCAAGCCTTGACGAACTAAAGC	279	Brakstad *et al.*, 1992
Salmonella enterica	B	iroB-F iroB-R	TGCGTATTCTGTTTGTCGGTCC TGCGTATTCTGTTTGTCGGTCC	606	Baumler *et al.*, 1997

RESULTS

Isolation and identification of *Staphylococcus aureus* and *Salmonella enterica*

A total of 180 out of the 200 food samples showed growth on Baird-Parker agar for *S. aureus*. Three typical presumptive *S. aureus* colonies per food sample were randomly selected. Thus, a total of 540 presumptive *S. aureus* isolates were successfully recovered from the 180 food samples using trypticase soy agar. For *Salmonella* spp., 32 out of 200 food samples demonstrated the typical appearance of *Salmonella* spp. with mauve-coloured colonies on CHROMagar *Salmonella* Plus agar. So, a total of 96 presumptive *Salmonella* spp. isolates were successfully recovered using nutrient agar.

The presumptive *S. aureus* and *Salmonella* spp. isolated from agar were then confirmed by DNA amplification. *S. aureus* and *S. enterica* isolates showed distinct amplified products of 279 bp (lanes 3 & 4) and 606 bp (lanes 8 & 9), respectively (Figure 1). These amplicons (lanes 3, 4, 8 & 9) showed similar molecular weight with the amplified products of the positive controls, *S. aureus* ATCC 29213 and *S. typhimurium* ATCC 13311 (lanes 2 & 7). *S. typhimurium* ATCC 13311 was used as a negative control for DNA amplification of *S. aureus* and no amplified band was obtained (lane 5). There was no amplicon when *S. aureus* ATCC 29213 was used as a negative control for *S. enterica* (lane 10). After molecular analysis was carried out for all presumptive *S. aureus* and *Salmonella* spp. colonies isolated from selective agar, contamination of *S. aureus* and *S. enterica* was confirmed in 52 and 32 food samples respectively.

Prevalence of *S. aureus* and *Salmonella enterica* contamination in RTE sushi and sashimi

In this study, an overall *S. aureus* and *S. enterica* contamination of 42% (84/200) was confirmed in the sushi and sashimi samples (Figure 2). The absence of contamination with both pathogens was 58% (116/200). Regarding the prevalence of the individual pathogens involved, *S. aureus* was detected in 26% (52/200) and *S. enterica* in 16% (32/200) of the contaminated sushi and sashimi samples (Figure 2). Five of the tested samples were positive for both pathogens in this study (Figure 2). *S. aureus* contamination observed in both sushi (16%) and sashimi (10%) samples were higher when compared with *S. enterica*. *S. aureus* contamination was observed in samples collected from supermarkets (37.2%, 29/78), hypermarkets (20.6%, 13/63) followed by restaurants (17%, 7/41) and open-air markets (16.7%, 3/18). *Salmonella* spp. contamination was highest in the low-end restaurants, 39% (16/41) compared with 11.5%, 9.5% and 5.6% in samples from supermarkets (9/78), hypermarkets (6/63) and open-air markets (1/18) respectively.

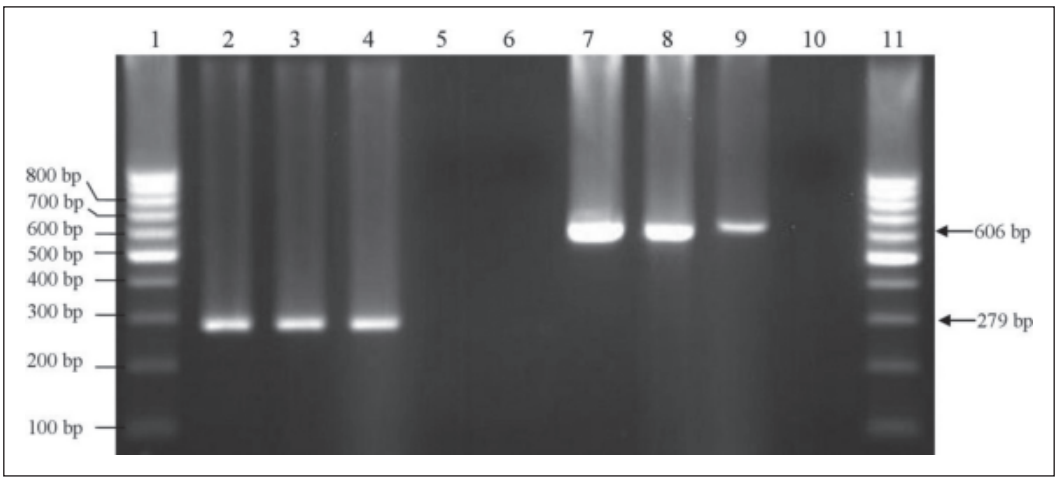

Figure 1. Gel electrophoresis of amplified *Staphylococcus aureus* and *Salmonella enterica* PCR products from food isolates and control bacteria.

Lanes 1 & 11: 100 bp molecular weight marker; lane 2: *S. aureus* ATCC 29213 as a positive control - 279 bp; lanes 3 & 4: amplified products from two *S. aureus* food isolates - 279 bp; lane 5: *S. typhimurium* ATCC 13311 as a negative control – absence of amplified product; lane 6: DNA blank; lane 7: *S. typhimurium* ATCC 13311 as a positive control - 606 bp; lanes 8 & 9: amplified products from two *Salmonella enterica* food isolates - 606 bp; lane 10: *S. aureus* ATCC 29213 as a negative control – absence of amplified product.

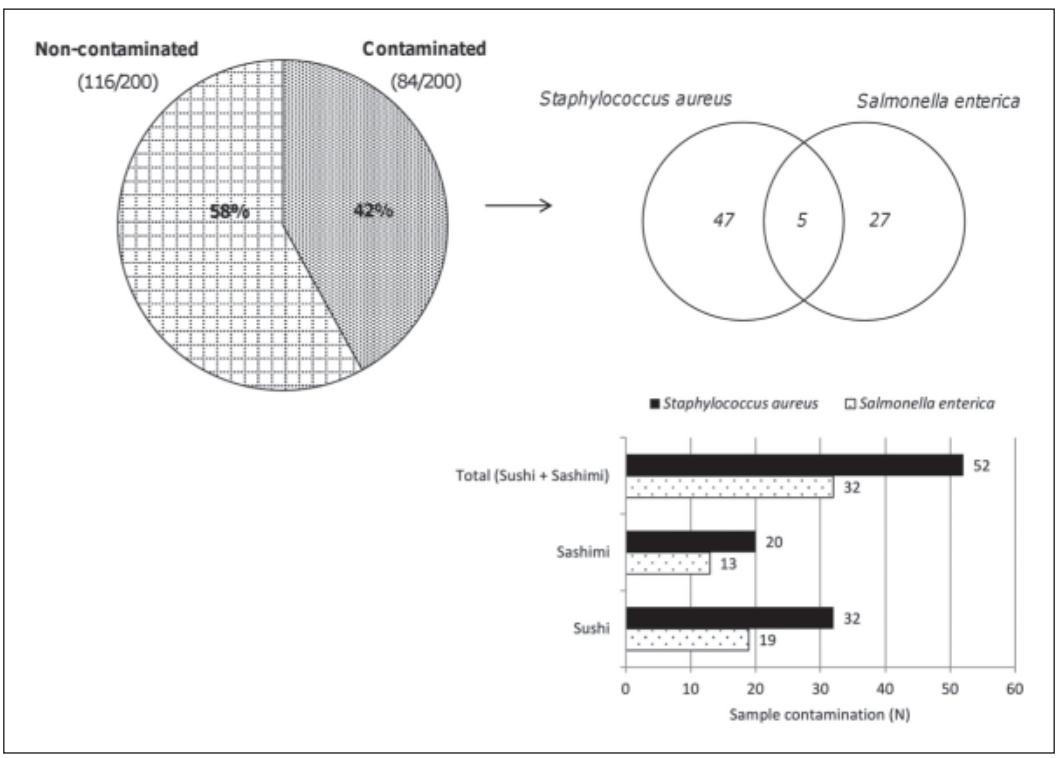

Figure 2. Prevalence of *Staphylococcus aureus* and *Salmonella enterica* contamination in RTE sushi and sashimi.

DISCUSSION

Ready-to-eat foods are considered as high-risk foods because no further cooking is required before consumption. Therefore, the condition of RTE foods is a vital issue as improper handling may result in foodborne poisoning. In this study, the 84 contaminated sushi and sashimi samples showed *S. aureus* and *S. enterica* contamination at 26% and 16% respectively. Both sushi and sashimi exhibit distinct features that may increase the introduction of microbiological hazards – (i) most ingredients are served cold and eaten raw; (ii) cooked ingredients are not reheated before serving; (iii) storage temperature; (iv) preparation involves contact with bare hands (Food and Environmental Hygiene Department HKSAR, 2000).

In this study, the presence of *S. aureus* in 26% (52/200) of the sampled foods may indicate contamination through human contact. *Staphylococcus* is not part of the normal microflora of freshly caught marine and fishes bred in farms (Herrero *et al.*, 2003). *S. aureus* when isolated from food products was reported to be due to the contamination of fishes during capture and/or subsequent unhygienic treatment and processing (Shena *et al.*, 2007), as seafood can be vectors for *S. aureus* which is part of human microflora (Huss *et al*., 2000).

S. aureus contamination in sushi and/or sashimi have also been reported worldwide – Germany (Atanassova *et al.*, 2008), Italy (Muscolino *et al.*, 2014), Northern Portugal (Migues *et al*., 2015), Japan (Hammad *et al.*, 2012), Northwest Spain (Vazquez-Sanchez *et al*., 2012) and Korea (Kim *et al.*, 2011). The limitation in this study is that the quantitative contamination level of this pathogen cannot be calculated as the study investigate the prevalence of contamination in sushi and sashimi samples. It is noteworthy that staphylococcal enterotoxins (SEs), the main factor of staphylococcal food poisoning, were also reported in these previous studies. Therefore, the storage of food samples at an incorrect temperature range of between 10–46°C where staphylococcal enterotoxins can be produced is a major hazard to food safety (Schelin *et al.*, 2011). SEs toxins are very stable and resistant to heat, thus re-heating foods contained SEs toxin produced by *S. aureus* even at high temperatures may destroy the bacteria but not the toxins. This is a health risk to consumers who are not aware of the properties of heat stable toxins. In addition, the emergence of methicillin resistant *S. aureus* (MRSA) in fish and fish products has recently been noted (Atyah *et al.*, 2010) and sashimi is a likely vehicle for transmission of MRSA (Hammad *et al.*, 2012). Thus, the entire process from fish capture downstream to processing and storage for RTE foods should be safely monitored because *S. aureus* contamination is associated as an indicator of unhygienic conditions during processing and storage of RTE foods (Tavakoli *et al.*, 2012).

In contrast, *Salmonella* can be present in raw or undercooked foods and it is commonly detected in eggs, egg products and poultry meat. *Salmonella* has also been reported in seafood samples (Amagliani *et al.*, 2012) and salmonellosis outbreaks resulting from sushi consumption have been reported in Hong Kong (Food and Environmental Hygiene Department HKSAR, 2000) and Australia (Barralet *et al*., 2004). Sashimi is a delicacy of solely raw meat or seafood where contamination could have arisen from the natural aquatic environment or during inappropriate storage or preparation/handling. Field laboratory tests by the U.S. Food and Drug Administration on imported and domestic seafood samples over a 9-year period showed *Salmonella* contamination in almost 10% of imported and 2.8% of local raw seafood (Heinitz *et al.*, 2000). Although the investigators also reported that the overall incidence of *Salmonella* contamination in RTE seafood was much lower at 2.6% and 0.47% for imported and domestic seafood respectively, problems in *Salmonella* contamination should be addressed and guidelines implemented to reduce and prevent salmonellosis.

Thus, good hygienic practice in sushi and sashimi preparation is necessary to minimize the risk of contamination. Kitchen surfaces in contact with either food or equipment for food preparation present a substantial risk for cross-contamination (Redmond & Griffith, 2003). As raw materials for sushi and sashimi also pose a potential contamination risk, the original sources of RTE foods can affect the mirobiological safety of the final products. Seafood has been reported to contain pathogens in the natural polluted aquatic environments (Amagliani *et al*., 2012), thus retailers should purchase seafood from reputable sources and preferably accompanied by health certificates from competent authorities of the country of origin to ensure safe products.

This is the first report to show the presence of *S. aureus* and *S. enterica* contamination in RTE foods in Malaysia. Using microbiological media for isolation and confirmation by molecular analysis, *S. aureus* and *S. enterica* pathogens was detected in 39.5% of sushi and sashimi samples. As both *S. aureus* and *S. enterica* contamination was detected in all the samples collected from supermarkets, hypermarkets, restaurants and open-air night markets. This study raises the importance of safe food source and handling in the preparation of RTE food in Malaysia. Consequently, it is also emphasize the importance of implementation and maintenance of Food Safety Management Systems based on seven principles of Hazard Analysis and Critical Control Point (HACCP), that must be a priority of food operators to ensure safety sushi and sashimi to consumers. HACCP food safety program will help to identify where hazards can be reduced on every stage of the food production (from raw material purchasing, receiving, transportation, storage, preparation, handling and serving) and increase food safety standards.

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