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Research Article

Prevalence of Foodborne Pathogen in Fresh Sushi at Sushi Take-Out Stores in Hong Kong

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Abstract

Traditional sushi consists of rice and raw fish or seafood which are easily contaminated by microorganisms without heat disinfection and cause human illness. In this project, the presence of four foodborne bacteria including *Vibrio parahaemolyticus* (*V. parahaemolyticus*), *Listeria monocytogenes* (*L. monocytogenes*), *Escherichia coli* O157: H7 (*E. coli* O157: H7) and *Escherichia coli* (*E. coli*) in fresh sushi sold in sushi take-out stores was investigated. 102 fresh sushi (salmon, tuna and shrimp) were brought from take-out stores randomly located in Yau Tsim Mong District in Hong Kong. Raw seafood topping of each sushi was cut into smaller pieces. They were homogenized with peptone water and incubated overnight to make enriched bacterial suspension. Each bacteria suspension was inoculated on four selective medium; Thiosulphate citrate bile salt sucrose (TCBS) agar, *Listeria monocytogenes* selective (LMS) agar, MacConkey (MAC) agar and Sorbitol MacConkey (SMAC) agar. On the next day, targeted isolated colonies on each agar were isolated to undergo different biochemical tests for bacterial identification. 19.61% of sushi were found to have *E. coli* and 2.94% of sushi were found to have *V. parahaemolyticus*, but none of the sushi samples had *L. monocytogenes* and *E. coli* O157:H7. Among the three different types of sushi, 65% of the all *E. coli* positive cases were Salmon sushi, 30% of the all *E. coli* positive sushi were Tuna sushi and 5% of all *E. coli* positive cases were Shrimp sushi. All positive cases of *V. parahaemolyticus* detected sushi was found in Salmon sushi, but not in other sushi. In general, the quality of sushi sold in takeout store in Hong Kong is satisfactory. The detection of bacteria in the sushi may due to the natural occurrence in seafood or the bacterial contamination in the pre-harvesting, transportation, storage and sushi handling stages. Post-harvest regulations and staffs hygiene training are essential to prevent bacterial contamination. The results can be used to raise a concern about sushi quality and the hygiene of the sushi store during processing and prepare for further investigation.

Keywords: *Sushi Take-Out Stores; Microbiological Quality*

Introduction

Sushi is a traditional Japanese ready-to-eat food prepared from raw or cooked fish and cold cooked vinegared rice and served in most Japanese restaurants. As sushi is made by raw seafood which can be easily contaminated with various bacteria, including food-poisoning and hygienic-related bacteria [1]. Food-poisoning bacteria are able to cause gastrointestinal illness, typically with vomiting and diarrhoea. For examples, *Staphylococcus aureus*, *Vibrio cholerae*, *Salmonella species, Shigella species, Clostridium perfringens*. For hygienic-related bacteria, they can act as hygienic indicators, like *E. coli* which can reflect the hygienic quality of food.

Sushi is very popular in Hong Kong. There are many sushi take-out stores in Hong Kong. However, the sushi quality from sushi take-out stores had not been investigated. There is a need to investigate food-poisoning- and hygiene-related bacterial population in sushi sold in

sushi take-out stores and monitor the sushi quality. In recent years, there are some food poisoning cases related to sushi in Hong Kong. In 2015, 33 people had symptoms of food poisoning after eating sushi and sashimi in one Japanese buffet restaurant in Mong Kok [2]. In 2008, there were 20 people got food poisoning after having a meal in some shops of the Japanese chain stores in Wan Chai. Besides, cases of food poisoning after intake sushi are worldwide. In 2016, a man was suffered from high degree fever and got hospitalized after eating over 20 pieces of sashimi sushi in one Japanese store of one sushi chain shops in Singapore [3]. The National Environment Agency (NEA) inspected the raw sushi samples in that shop. In the result, no *Streptococcus* group B (GBS) or other food-poisoning causing microorganisms.

On the other hands, sushi is potentially hazardous food because it supports the rapid and progressive growth of infectious or toxigenic microorganisms. No heat process like cooking is used for the raw seafood toppings on sushi. Cooking and re-heating the food can eliminate ensure the safety in consuming food because most foodborne bacteria and viruses can be killed under high temperature [1]. Parasites and bacteria can survive without heating. Parasite contamination on sushi may also occur if sushi is not frozen in the freezer under proper temperature. Parasites can be killed when it is frozen at -20°C or below for seven days or-35°C for about 20 hours for fish intended for raw consumption will kill parasites [4]. Parasites such as nematodes, cestodes, trematodes and acanthocephalans can be found in marine animals. There is a wide range of parasites can be isolated from fish, but most of them are non-infectious to human. People in high-risk group including the immunocompromised patients, pregnant women and elderly should consume less sushi due to their weakened immunity to prevent the infection of opportunistic pathogens [4]. According to European Food Safety Authority (EFSA), the condition of freezing treatment can be -20ºC for not less than 24 hours, -35ºC for at least 15 hours or at -15°C for at least 96 hours [5]. Since traditional sushi consists of raw or undercooked fish or other seafood which may contain parasites, physical separation of contaminated seafood products and freezing treatment will be applied in order to remove and kill parasites.

Many studies about the bacterial status of sushi were done by researchers worldwide. However, less research investigating the sushi sold from takeaway stores had studied. A study conducted in Germany targeted fresh sushi from sushi bars and frozen sushi from supermarkets. It had found there was a difference in the microbial quality of fresh sushi and frozen sushi. Nevertheless, the microbial quality of the sushi from takeaway stores has been studied, only the supermarket and sushi bars [6]. Limited research about the microbial quality of sushi from takeaway stores had been conducted in Hong Kong. There was only one research about the bacteria quantification of sushi conducted in Hong Kong was focused on sushi sold from takeaway stores. They targeted *Salmonella* species, *Staphylococcus aureus* and *E. coli*, but *V. parahaemolyticus*, *L. monocytogenes* and *E. coli* O157:H7 were not focused [7].

Aim of the Study

The aims of this research project were to detect the *V. parahaemolyticus*, *L. monocytogenes*, *E. coli* and *E. coli* O157: H7 in fresh sushi, to understand food poisoning causing bacteria presenting in sushi and analyze the microbiological status of fresh sushi sold in sushi take-out stores.

Materials and Methods

Sample collection

102 samples of fresh sushi (34 Salmon sushi, 34 Tuna sushi, 34 Shrimp sushi) were randomly collected in sushi takeaway stores of in Yau Tsim Mong District in Hong Kong. There were different sushi types sold in different packages in stores. Traditional nigiri sushi with raw fish/seafood toppings like salmon, tuna and shrimp are representative for investigating sushi quality.

Experimental workflow

The process of identification of bacteria needed four consecutive days. On the first day, sushi was bought in the morning. Each sushi was weighted around 1 gram and the cut part was incubated in 10 mL peptone water (Sigma-Aldrich, UK, #77187 overnight (Figure 1). On the next day, 100ul sushi suspension was added into 9.9 ml saline for dilution. 10 ul diluted sushi suspension was culture on Macconkey

(MAC) agar (Thermo Fisher Scientific, UK, #CM0115), Sorbitol Macconkey agar (SMAC) (Thermo Fisher Scientific, UK, #CM0813B), Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar (Thermo Fisher Scientific, UK, #CM0333), and *Listeria monocytogenes* selective (LMS) agar (Biomerieux, USA). All agars were incubated overnight in 5% CO_2 at 35°C (Figure 2). The growth of target colonies on various agars was identified on the third day. For MAC agar, lactose fermented pink colonies were looking for. Colourless colonies were looking for in SMAC agars, green colonies were looking for in TCBS agars and blue colonies were looking for in LMS agars. All these target colonies would be done by different biochemical tests, e.g. oxidase, TSI agar, indole, 8% salt tolerance, blood agar (BA) (Figure 3).

On the last day, the results of some biochemical tests were read and undergone further biochemical tests if necessary (Table 1). The table showed the expected results of the various targeted organism. For *E. coli*, alkaline slant and butt with the gas of TSI, positive indole of red colonies in MAC agar, the gram-negative rod was used to differentiate *E. coli*. Serotyping was used to identify *E. coli* O157: H7. Salt tolerance and oxidase with green colonies were used for *V. parahaemolyticus* and the catalase positive blue colonies with beta-hemolytic would be identified as *L. monocytogenes* (Table 1). Each agar and the biochemical test was accompanied with a positive control to ensure the efficiency of the testing materials and techniques and negative control to prevent contamination.

Day one

Sushi was bought from the take-out store in the morning and brought them to the lab within two hours. All sushi were stored on ice during transportation. 1 gram of the sushi was weighted and incubated with 10 ml peptone water overnight.

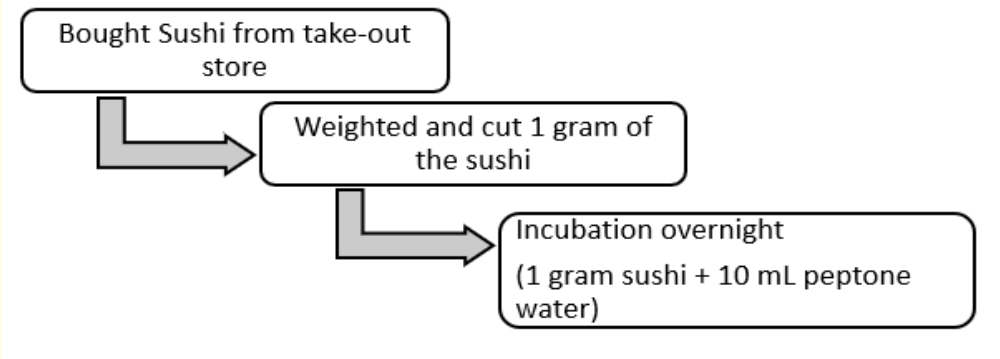

Figure 1: *Sample preparation day one.*

Day two

The culture suspension was diluted with saline and sub-culture on Macconkey (MAC) agar, Sorbitol Macconkey agar (SMAC), thiosulfate citrate bile salts sucrose (TCBS) agar and *Listeria monocytogenes* selective (LMS) agar. All agars were incubated overnight in 5% CO2 at 35°C (Figure 2).

Day three

Different target colonies on various agars were identified as shown in the above figure. For MAC agar, lactose fermented pink colonies were looking for. Colourless colonies were looking for in SMAC agars. Green colonies were looking for in TCBS agars. Blue colonies were looking for in LMS agars. All these target colonies would be done by different biochemical tests, e.g. oxidase, TSI agar, indole, 8% salt tolerance, blood agar (BA) (Figure 3).

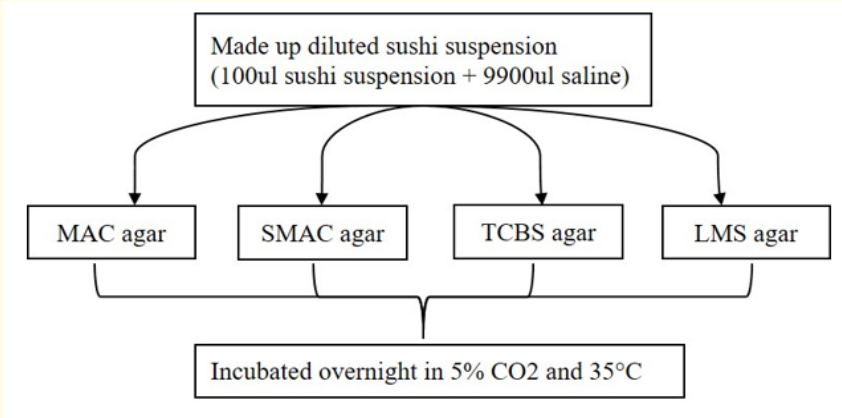

Figure 2: *Sample preparation day two.*

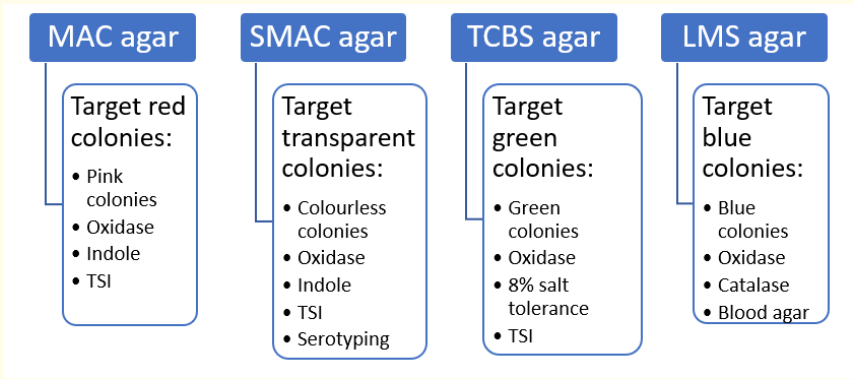

Figure 3: *Sample preparation day three.*

Day four

The results of the biochemical tests for identifying target organisms were listed as above. Each agar and the biochemical test was accompanied with a positive control to ensure the efficiency of the testing materials and techniques and negative control to prevent contamination.

	E. coli	E. coli O157: H7	V. parahaemolyticus	L. monocytogenes
Oxidase	-	-	+	-
Indole	+	+	NA	NA
TSI	a/@	a/@	K/@	NA
Serotyping	NA	Agglutination O157 & H7	NA	NA
8% NaCl tolerance	NA	NA	Growth	NA
Catalase	NA	NA	NA	+
Blood agar	NA	NA	NA	Beta-hemolytic zone

Table 1: *Identification of target organisms.*

Statistical analysis

Statistical analyses were conducted using Graph Pad Prism 8.0 software (GraphPad Software, La Jolla, CA, USA). The prevalence of sushi characteristics was defined by descriptive analysis.

Results

In 102 sushi, pink colonies on MAC agars were found in 97 sushi and they were tested by oxidase, indole and TSI. 20 out of 97 sushi samples were tested to have a negative result in oxidase, positive result in the indole and alkaline slant and butt with the gas of TSI which are the features of typical *E. coli*. These colonies were gram-negative rods. Therefore, *E. coli* was found in 19.61% of total sushi (Figure 4).

66 sushi samples were found to have green colonies on TCBS agar and they were tested by oxidase, 10% salt tolerance and TSI. Only 3 bacterial colonies in the sushi samples were tested to have a positive result in oxidase, red slant and yellow butt with the gas of TSI and have growth in 8% NaCl nutrient broth which are the features of typical *Vibrio parahaemolyticus*. Therefore, *Vibrio parahaemolyticus* was found in 2.94% of total sushi (Figure 4).

No sushi samples were found to have blue colonies on LSM agars nor and transparent colonies on SMAC agars. The strain of *Listeria monocytogenes* acted as a positive control for LSM agars and the strain of *E. coli* O157: H7 acted as a positive control the SMAC agars. Therefore, neither *Listeria monocytogenes* nor *E. coli* O157: H7 was not found in all sushi samples (Figure 4).

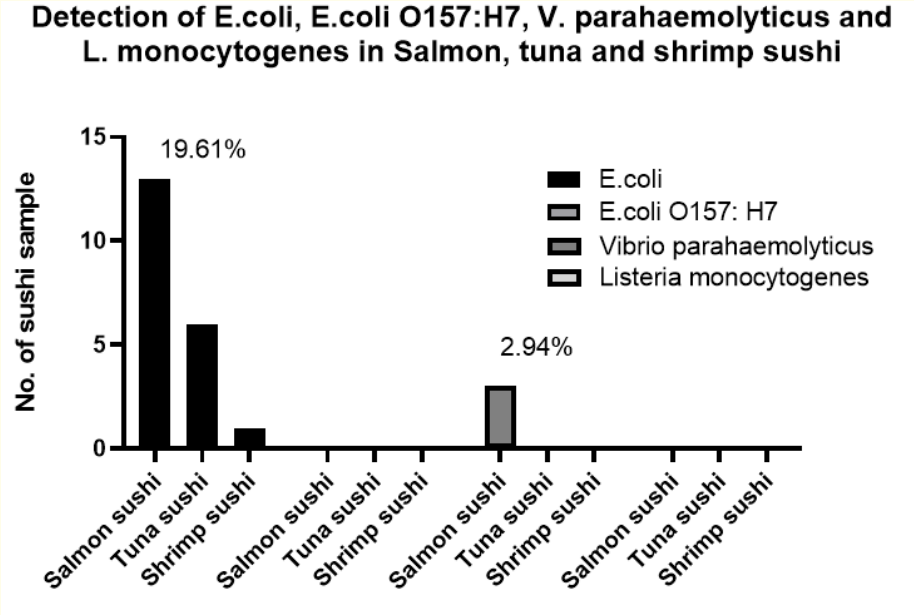

Figure 4: *The detection of E. coli, E. coli O157: H7, V. parahaemolyticus and L. monocytogenes in Salmon, tuna and shrimp sushi. The figure showed that E. coli was found in 19.61% of total sushi. Vibrio parahaemolyticus was found in 2.94% of total sushi. No Listeria monocytogenes and E. coli O157: H7 were found in all sushi.*

By comparing various sushi that was found to have *E. coli*, 13 out of 20 sushi (65%) detected to have *E. coli* were Salmon sushi. 6 out of 20 sushi (30%) found to have *E. coli* were Tuna sushi. Only 1 Shrimp sushi (5%) was found to have *E. coli*. Besides, all 3 positive cases of *Vibrio parahaemolyticus* were detected in Salmon sushi only (Figure 5).

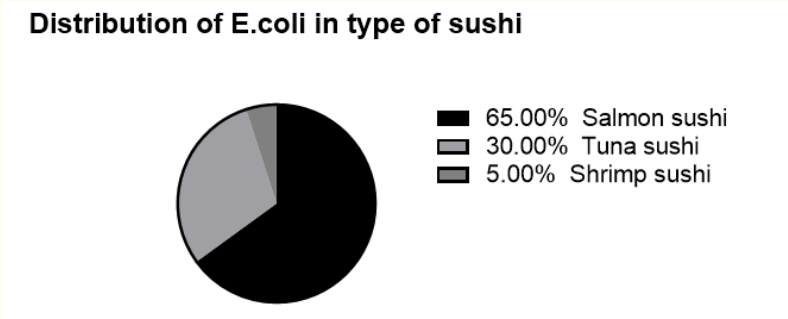

Figure 5: *Distribution of E. coli in sushi.*
65% E. coli was found in Salmon sushi and 30% in Tuna sushi. Only 1 Shrimp sushi sample (5%) was found to have E. coli. All 3 positive cases of Vibrio parahaemolyticus were found in Salmon sushi only.

Discussion

Sushi is considered a potentially hazardous food because it contains raw seafood without cooking and not processed in any heating step before consumption. If sushi is handled improperly, there will be an outbreak of foodborne disease [8]. Atanassova., *et al*. [6] found *E. coli* in 6 of 125 samples of frozen sushi (4.8%) sold in supermarkets in Germany, but they didn't identify the *E. coli* serotype O157: H7. From our study, *E. coli* was found in 19.61% of the total samples. It is suggested that sushi sold in take-out store might have a relatively high amount of *E. coli* contamination compared with sushi sold in supermarkets. Since the detection of *E. coli* can represent the hygiene of sushi, take-out stores might have relatively poor hygiene than supermarkets. Atanassova., *et al*. [6] also detected *Listeria* in 3 of 125 samples of frozen sushi sold in the supermarket, but all isolates were *Listeria welshimeri* but not *Listeria monocytogenes*. Therefore, *Listeria monocytogenes* may not be a common contaminate in sushi sold in both supermarkets and take-out stores. No *Vibrio* species were detected in the same study. The cases of *Vibrio* were low in our study which was similar to other findings. The result revealed that more bacteria contamination found in sushi sold in takeout store which indicates the hygienic standard and conditions. The possible reasons for the differences may be due to the standard rules or protocols between these two types of business. Supermarkets usually have a strict and standardized protocol to process sushi in order to maintain good hygiene of food. The take-out stores especially the non-branching companies may not have sushi proper processing standard due to the different cooking styles among workers. Different workers may have their preferences to follow their own cooking habits. Beside the poor hygiene during the manufacturing of sushi and improper condition for sushi storage, the location of fish harvesting, farmed species, transportation of seafood, post-harvest processing are also factors influencing the sushi quality or other food safety [9].

In our study, *E. coli* and *V. parahaemolyticus* were isolated from most of the sushi samples. The detection of *Vibrio parahaemolyticus* is related to the fish and other sea animals and the detection of *E. coli* can represent the hygiene of the sushi. The occurrence of these bacteria might due to the bacterial contamination during pre-harvesting, harvesting and sushi processing steps. Marine water and harvesting equipment can be the potential sources of pathogen contamination in pre-harvest [10]. Cross-contamination of seawater and seafood and uncleaned harvesting equipment might involve during harvesting. Some pathogenic bacteria are naturally present in the marine (for example, *Vibrio parahaemolyticus*, *Clostridium botulinum* and some *Aeromonas*) and the general environment (for example, *Listeria monocytogenes*). Those marine bacteria can be isolated in fresh fish or other raw material. *Vibrio* bacteria are the most common bacteria found in marine waters where they account for around 30% of all bacteria [11]. Bacteria presenting within the tissue of sea animals can cause infection when they are ingested. *E. coli* can be found in raw seafood if their habitats were having faecal contamination and pollution [10]. Poor personal hygiene of staffs working in the store also contributes to the bacterial contamination. According to Michaels., *et al*. (2004),

poor personal hygiene consists of no handwashing between tasks, lack of handwashing facilities and inefficient hand washing etc. If the chefs or workers who handle the sushi not wearing gloves and not do proper handwashing, a diverse microbial flora on the hand will pollute the food. *E. coli* can act as the hygiene indicator of the sushi.

Food storage under low temperature can preserve food. Deep-freezing can kill microorganisms. According to the Food and Drug Administration of United State, sushi served with raw seafood have to be frozen at or less than -20°C for 7 days or less than -35°C for 15 hours in order to destroy parasites. Also, during transportation, the temperature is under control because the raw fish materials are potentially hazardous foods. Once the raw fish materials arrive at the shops/restaurants, they are refrigerated at or below 5°C. If raw seafood has not been stored properly under low temperature, most bacteria become active and grow rapidly on the sushi. However, *L. monocytogenes* is psychrophilic which is able to grow at refrigerator temperature. It is environmental and opportunistic pathogens which may be isolated from the unclean refrigerator and cause listeriosis, especially in high-risk populations like pregnant women, infants, elderly and immunocompromised patients [12]. *L. monocytogenes* may be isolated from contaminated gloves of the workers. It may be also found in the drains in the slicing and packaging area both before and during processing. Machines or environments in the skinning, brining, slicing and packaging areas may isolate *L. monocytogenes* [13]. Washing water with poor microbial quality and without sanitizing can deposit more microbes. Packaging might also cause microbial contamination. Fresh sushi sold in takeaway stores can be packed in a box which contains six to eight sushi with different toppings. Bacteria that naturally found in different seafood toppings might contaminate each other. Sushi can also be packed individually in a plastic bag [14]. Sushi processing in the stores also can give many chances for cross-contamination. Sushi with raw fish/seafood does not require the heating procedure. Contamination to sushi due to carry over from the raw seafood or during processing is expectable. The heating process is crucial for preserving food by killing nearly all bacteria. However, no heating step is required in making sushi in order to obtain the fresh texture, integrity and the tastes of fish and sushi rolls. Marine bacteria in raw fish or environmental bacteria can grow and replicate in sushi. Fortunately, no *Listeria monocytogenes* were found in our study. It is suggested that temperature control and storage in take-out stores is satisfactory.

In order to eliminate the bacteria contamination and improve the bacterial quality of the sushi, post-harvest regulations are important. Since many bacteria are naturally occurring, preventing bacterial contamination during pre-harvesting is difficult. The water quality of the coast for harvested seafood should be monitored and protected clean. After harvesting the seafood, they should be frozen or stored under very low temperature to slow down the bacterial growth. For the sushi processing in the stores, the place and workers should have good hygiene. Workers should keep the place clean enough and sanitized every day. Staffs processing sushi should wear disposable gloves and change the gloves in between them handling raw seafood and doing other tasks. All workers in sushi stores should have good hygiene techniques and stick to the food handling guidelines [15].

In this study, we cannot directly conclude the quality of the sushi sold in take-out store because the bacteria detected might not be at a high level that can cause illness to people. Some bacteria are naturally occurring in the seafood and not contribute to foodborne disease to human in a low amount. Most strains of *E. coli* are harmless to human, except EHEC. The infective dose of EHEC is only 50 CFU per gram [16] and the infective dose of *Vibrio parahaemolyticus* is more than 10^5 CFU per gram [11]. Moreover, not all stain of *Vibrio parahaemolyticus* are pathogenic. Only the strains which can produce a characteristic haemolytic reaction on Wagatsuma agar medium can cause illness [17]. Although *E. coli* and *V. parahaemolyticus* were detected in the sushi, they might not cause any harm to consumers. Thus, the detection of bacteria does not mean to have the unsatisfied quality of sushi unless quantitative assay was done and known to exceed the acceptable limit. On the other hand, other bacteria which are not targeted in this study may interfere with the e selective identification and isolation of the targeted bacteria (Doyle, 2001). Rapid detection methods of pathogen are recommended to use in the future study in order to save time, increase sensitivity for low amount of bacteria, improve detection limit and reduce human errors.

Conclusion

More *E. coli* were found in sushi sold in take-out store. More salmon sushi isolated *E. coli* than other types of sushi, like tuna and shrimp sushi. Some *V. parahaemolyticus* was found in salmon sushi. None of the *Listeria monocytogenes* and *Escherichia coli* O157: H7 was isolated

in sushi. Hygiene of the sushi stores has raised a concern. A further quantitative study of bacteria on sushi sold in takeout store will be necessary to reflect the sushi quality and investigate the hygiene of the stores based on the microbiological guidelines for ready-to-eat food [18] from the Centre for Food Safety. Interviewing the store workers also helps to understand more the causation between the presence of bacteria on sushi and the processing of sushi in the stores.

Acknowledgements

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