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Prevalence of *Campylobacter* spp. in retailed ready-to-eat sushi

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Abstract: The prevalence of *Campylobacter* spp. in retailed sushi were examined using the techniques of polymerase chain reaction (PCR) in combination with most probable number (MPN) to quantify the bacteria in 150 samples obtained from three supermarkets. The average prevalence of *Campylobacter* spp. in retailed sushi was 26.6% with 32%, 16% and 32% from supermarket I, II and III, respectively. *Campylobacter jejuni* was found to be the predominant species in retailed sushi with 82.49% of all *Campylobacter* spp. positive samples. *Campylobacter coli* was not detected in all samples. The maximum MPN number of *Campylobacter* spp. in retailed sushi purchased from supermarket I, II and III ranged from 3.6-11.0 MPN/g, 9.4->1100 MPN/g and 27-1100 MPN/g, respectively. The isolation of *C. jejuni* from a variety of ready-to-eat retail sushi may indicate that these products can act as possible vehicles for the dissemination of food-borne campylobacteriosis.

Keywords: ***Campylobacter*, sushi, supermarket, polymerase chain reaction**

INTRODUCTION

Campylobacter is a genus of Gram-negative, motile bacteria with a rod-like appearance (Snelling *et al.*, 2005; Schrotz-King *et al.*, 2006). Many species of *Campylobacter* have been implicated in human diseases, with *C. jejuni* accounting for approximately 90% of the human isolates. *C. coli* are also commonly found. *Campylobacters* are a major cause of diarrhoeal illness in humans, and are generally regarded as the most common bacterial cause of gastroenteritis worldwide (Tam *et al.*, 2003; Skanseng *et el.*, 2006).

Campylobacters are widely distributed and exist in most warm-blooded domestic, agricultural livestock and wild animals. They are prevalent in livestock such as poultry, cattle, pigs, sheep, ostriches and shellfish, and in pets, including cats and dogs (Altekruse *et al.*, 1999). The main route of transmission is generally believed to be foodborne, *via* undercooked meats and meat products, as well as raw or contaminated milk. In both developed and developing countries, they cause more cases of diarrhoea than *Salmonella* spp. (Altekruse *et al.*, 1999; Schrotz-King *et. al.*, 2006; Zorman *et al.*, 2006). In the United States, it is estimated that 1% of the population is diagnosed with campylobacteriosis every year, and with many cases going unreported, up to 0.5% of the general population may unknowingly harbor *Campylobacter* in their gut annually (Jain *et al.*, 2005).

Campylobacter requires a fastidious condition to grow. It grows best in microaerophilic environment, ideally 5% O_2, and 10% CO_2, and 85% N_2 (Altekruse *et al.*, 1999). Survival of *C. jejuni* outside the gut is poor, and replication does not occur readily as they will enter the viable but non-culturable (VBNC) state under adverse conditions (Altekruse *et al.*, 1999). Due to the difficulty in culturing this microorganism, conventional methods used to detect Campylobacter may be tedious as biochemical tests are required prior to culturing of Campylobacter. Therefore, an alternative method, using polymerase chain reaction was developed to detect Campylobacter.

The sample of choice in this study is a Japanese food called sushi, which is not only popular among the Japanese but has also gained popularity worldwide. Sushi is usually vinegared rice combined with other ingredients such as fish and seafood as the toppings. Most of the toppings are raw and uncooked which heightens the risk of bacteria contamination. To our knowledge, studies on the prevalence of *Campylobacter* spp. in retailed sushi have not done in Malaysia, and this is a premier study. Therefore, the objective was to determine the prevalence of *Campylobacter* spp. in retailed sushi from supermarkets in selected areas in Kuala Lumpur, Malaysia. The data collected can also be used as important information in risk assessment of sushi consumption in Malaysia.

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MATERIALS AND METHODS

Sample collection

A total of 150 samples were collected from three selected supermarkets in Kuala Lumpur from July to October in the year 2007. Five types of sushi with different toppings were collected from each supermarket. The five different toppings chosen were uncooked salmon, uncooked crab egg, cooked octopus, cooked eel, and cooked omelet.

Enumeration with most probable number (MPN) technique

Each sample was cut into small pieces, then a 10 g portion of sample was stomached with 90 ml of Bolton Enrichment Broth Base (Merck, Germany) supplemented with Bolton Supplement (Merck, Germany) and 5% lysed horse blood in a stomacher for 60 sec. Dilutions of 1:100 and 1:1000 were prepared from the stomached fluid in triplicate following three-tubes MPN format. All MPN tubes were incubated in anaerobic jar under microaerophilic conditions produced using Anerocult C system (Merck, Germany) at 37°C for 48 hours. According to the MPN technique, turbid tubes would be considered as positive. However, in order to have more conclusive results, all turbid tubes were subjected to PCR detection for the presence of *Campylobacter* spp, *C. jejuni* and *C. coli*.

All PCR-positive tubes were preceded to plating on *Campylobacter* modified charcoal-cefoperazone-deoxycholate blood-free selective agar (mCCDA) (Merck, Germany) to recover *Campylobacter* spp. isolates. Approximately 0.2 ml of enrichment broth were plated on mCCDA and incubated for 48 hours at 37°C under microaerophilic conditions as described previously (Chai *et al.*, 2007). Presumptive colonies grown on the plates with colony morphology consistent with *Campylobacter* spp. were inoculated into Brain Heart Infusion Broth (BHIB) (Conda, Spain) and incubated for 48 hours at 37°C under microaerophilic conditions for further identification using polymerase chain reaction.

PCR detection of Campylobacter *spp.*

DNA was extracted using the boiled-cell method. Five hundred micro-litres of the broth from the turbid tubes were subjected to centrifugation at 12,000 rpm for 3 min in order to pellet the bacterial cells. The pellet was then resuspended in 400 µl of sterile distilled water, and boiled for 10 min followed by freezing at -20°C for 10 min. It was then centrifuged at 10,000 rpm for 5 min and the supernatant was then kept at -20°C for use in PCR. For the identification of *Campylobacter* spp. from the selective agar plates, a single and well isolated colony was picked up and resuspended in 400 µl of sterile distilled water and DNA was also extracted using the boiled-cell method.

DNA from boiled lysates was first subjected to PCR detection for *Campylobacter* spp. All *Campylobacter* spp. positive boiled lysates were then subjected to PCR detection for *C. jejuni* and *C. coli*. All PCR was performed in 25 µl of reaction mixture containing 1X PCR buffer, 0.2 mM of dNTPs mix, 0.4 µM of each primer and 2 µl of DNA boiled-lysate. The final concentration of $MgCl_2$ and *Taq* DNA polymerase (Vivantis Technologies, Malaysia) as well as primer sequences used for three PCR assays are as summarized in Table 1. All three PCR were subjected to the initial denaturing at 94°C for 2 min. This was followed by amplification cycles of denaturation at 94°C for 1 min, annealing

Table 1: PCR primers and conditions for detection of *Campylobacter* spp., *C. jejuni* and *C. coli*

Targeted species	Targeted gene and primers used	Sequence 5'-3'	Amount of Taq (U)	$MgCl_2$ conc. (mM)	Tm (°C)	Targeted size (bp)
Campylobacter spp. (genus)	16S ribosomal RNA		0.625	2.5	55	816
	C412F	GGATGACACTTTTCGGAGC				
	C1288R	CATTGTAGCACGTGTGTC				
C. jejuni	*hipO* gene		0.625	2.5	66	735
	HIP400F	GAAGAGGGTTTGGGTGGTG				
	HIP1134R	AGCTAGCTTCG-CATAATAACTTG				
C. coli	ceuE gene	ATGAAAAAATATT-TAGTTTTTGCA	0.5	3.0	57	894
		ATTTTATTATTTGTAGCAGC				

at specific temperature for each primers pair (Table 1) for 1 min, and extension at 72°C for 1 min. The final extension step was set at 72°C for 5 min. All assays were performed with Tpersonal Thermocycler (Biometra, Germany). PCR primers for *Campylobacter* spp., *C. jejuni* were used according to Linton *et al.* (1997) and Nayak *et al.* (2005) for *C. coli* (Table 1). All oligonucleotides used were synthesized by Research Biolabs, Singapore.

RESULTS

Results for the prevalence of *Campylobacter* spp. are summarized in Table 2. Out of the 150 samples examined, the prevalence of *Campylobacter* spp. was found to be 32% for both supermarket I and supermarket III, while supermarket II had a lower prevalence of 16%. Prevalence of *C. jejuni* in supermarket I, II, and III were found to be 24%, 16%, and 26%, respectively whereas *C. coli* was not detected in all three supermarkets. Figure 1 is a representative of the gel image for polymerase chain reaction detection of the *Campylobacter* spp. (816 bp), *C. jejuni* (735 bp), and *C. coli* (894 bp).

The maximum MPN number of *Campylobacter* spp. from various types of retail sushi ranged from 3.6-11.0 MPN/g for Supermarket I, 9.4->1100.0 MPN/g for Supermarket II and 27.0-1100.0 MPN/g for Supermarket III as shown in Table 3. Supermarket II revealed the broadest range of MPN number of pathogens in its retailed sushi, while sushi from Supermarket I was the least contaminated. Recovery rate of *Campylobacter* spp. from campylobacter specific PCR positive samples was only 35.61% (47/132). The number of MPN-PCR positive samples from which *Campylobacter* spp., *C. jejuni* and *C. coli* were recovered via plating on mCCDA plates are summarized in Table 4.

DISCUSSION

In this study, 150 samples from three supermarkets in Kuala Lumpur, Malaysia were examined for the presence of *Campylobacter* spp., *C. jejuni*, and *C. coli* in a period of 4 months from July to October 2007. *Campylobacter* spp. was found to be present in 26.67% of retailed sushi tested. Of all the *Campylobacter* spp. positive samples, 82.49% were found to contain *C. jejuni*. *C. coli* were not detected in all samples. A study on retailed foods in Ireland by Whyte *et al.* (2004) reported a prevalence of 0% for campylobacter in ready to eat food such as sandwiches, salads, and cheeses. However, they did detect campylobacters in poultries (ranged from 37.5% to 49.9%), raw

Table 2: Prevalence of *Campylobacter* spp., *C. jejuni* and *C. coli* in five types of sushi samples studied from three supermarkets

Supermarket	Samples	Prevalence (%)		
		Campylobacter spp.	*C. jejuni*	*C. coli*
I	A	30	20	0
	B	40	20	0
	C	40	40	0
	D	10	0	0
	E	40	40	0
	Average	32	24	0
II	A	20	20	0
	B	10	10	0
	C	20	20	0
	D	20	20	0
	E	10	10	0
	Average	16	16	0
III	A	40	20	0
	B	20	20	0
	C	30	30	0
	D	40	40	0
	E	30	20	0
	Average	32	26	0

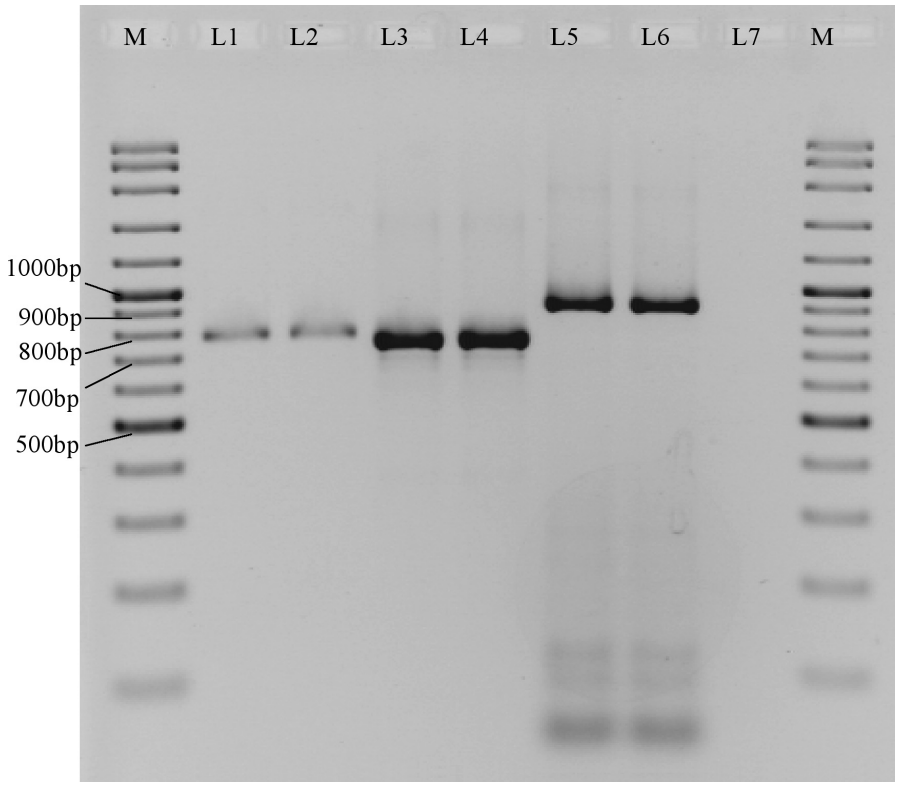

Figure 1: Gel image for PCR detection of *Campylobacter* spp., *C. jejuni*, and *C. coli*. Lane M shows the molecular marker 100bp ladder; L1 and L2 are PCR amplicons specific for *Campylobacter* spp. (genus) at 816bp. L3 and L4 show the PCR amplicons specific for *C. jejuni* at 735bp. L5 and L6 show the PCR amplicons specific for *C. coli* at 894bp. L7 is the negative control

Table 3: The maximum number and median of *Campylobacter* spp., *C. jejuni* and *C. coli* for five types of sushi samples from three supermarkets

Supermarket	Sample	MPN of *Campylobacter* spp. / g		MPN of *C. jejuni* / g		MPN of *C. coli* / g	
		Max	Med	Max	Med	Max	Med
I	A	9.2	<3.0	9.2	<3.0	<3.0	<3.0
	B	3.6	<3.0	3.0	<3.0	<3.0	<3.0
	C	11.0	<3.0	9.2	<3.0	<3.0	<3.0
	D	3.6	<3.0	<3.0	<3.0	<3.0	<3.0
	E	9.4	<3.0	9.4	<3.0	<3.0	<3.0
II	A	210.0	<3.0	210.0	<3.0	<3.0	<3.0
	B	9.4	<3.0	9.4	<3.0	<3.0	<3.0
	C	11.0	<3.0	11.0	<3.0	<3.0	<3.0
	D	>1100.0	<3.0	1100.0	<3.0	<3.0	<3.0
	E	>1100.0	<3.0	>1100.0	<3.0	<3.0	<3.0
III	A	1100.0	<3.0	1100.0	<3.0	<3.0	<3.0
	B	27.0	<3.0	27.0	<3.0	<3.0	<3.0
	C	29.0	<3.0	29.0	<3.0	<3.0	<3.0
	D	35.0	<3.0	35.0	<3.0	<3.0	<3.0
	E	36.0	<3.0	36.0	<3.0	<3.0	<3.0

Table 4: The percentage of positive samples in which *Campylobacter* spp., *C. jejuni* and *C. coli* were recovered by plating

Samples	Supermarket I		Supermarket II		Supermarket III	
	No. of positive samples recovered	Percentage (%)	No. of positive samples recovered	Percentage (%)	No. of positive samples recovered	Percentage (%)
A	0/4	0	7/9	77.78	6/15	40
B	0/4	0	3/5	60	4/9	44.44
C	2/12	16.67	1/5	20	4/8	50
D	0/1	0	5/10	50	6/17	35.29
E	0/6	0	3/9	33.33	6/18	33.33
Total	2/27	7.41	19/38	50	26/67	38.81

beef (3.2%), pork (5.1%), lamb (11.8%), shellfish (2.3%), and fresh mushroom (0.9%). Prevalence in this study is comparatively high given the fact that sushi is a type of ready-to-eat food. To date, there is no study of campylobacters on ready-to-eat food carried out in Malaysia, although campylobacters have been reported from salad vegetables (49.67%) (Chai *et al.*, 2007) and poultry (72.6%) (Saleha, 2002).

It was evident from this study that almost equal amounts of campylobacters were detected from all types of sushi having either cooked or uncooked toppings indicating the possibilities for a source of contamination through mishandling of the products used for making the sushi. The most possible source of contamination is cross contamination from other products in the supermarkets such as raw poultry since retailed poultry is always associated with high prevalence of campylobacters. Cross contamination is often due to poor hygiene and sanitation practice of the workers involved. This is supported by the study of Luber *et al.* (2006) where they reported the transfer rate of campylobacter from kitchen utensils or hands to ready-to-eat foods (fried sausages, cucumber slices, and bread) to be in the range of 2.9 to 27.5%. Another possible source of contamination is the seafood used as toppings for sushi. Although seafood may not be the natural reservoir of campybacters, studies have reported their prevalence on shrimps (3.4%) (Adesiyun, 1993), and shellfish (2.3%) (Whyte *et al.*, 2004).

The combined MPN-PCR method used in this study proved to be effective in detecting campylobacters using specific primer pairs as this study reported a high prevalence compared to other studies carried out on ready-to-eat food using conventional plating and biochemical tests. The result shows that only 35.61% of campylobacter specific PCR positive samples were successfully recovered on mCCDA plates. The recovery of *Campylobacter* spp. seems to associate with the number of organisms present in the sample. According to Chai *et al.* (2007), campylobacter may not be isolated from PCR positive samples because they are present in a non-active coccoidal form known as the viable but non-culturable (VBNC) form, and also because of the lack of appropriate methods for recovery of campylobacters. The PCR method is also faster compared to conventional biochemical tests to detect campylobacters especially when a large sample size is considered. The PCR method of detection can be carried out in a few hours after 48 hours of prior enrichment whereas biochemical tests are very tedious especially with the difficulty in culturing the bacteria, and the bacteria being comparatively slow growing with the fastest generation time of approximately one hour even under optimum conditions (Lake *et al.*, 2003). The effectiveness in detection of the organism is especially important when there is an outbreak where the source of contamination has to be determined in the shortest time possible.

However, the PCR method has some disadvantages. For example, false positive results may be obtained by the contamination of DNA (Nierop *et al.*, 2005). Nevertheless, this possibility is minimized by separation of the preparation and amplification in different rooms in the laboratories. The blank negative control included in every PCR also indicates that false positives are unlikely to occur. False negative results may also be obtained due to the inhibitors in food or enrichment medium (Nierop *et al.*, 2005). This possibility is minimized by thorough elimination of enrichment broth during the DNA extraction.

Campylobacter spp. was detected from all three studied locations. This indicates that food products in supermarkets are commonly contaminated with campylobacters. Since sushi is a ready-to-eat product, it can be of high risk to consumers. To

date, campylobacters are reported to cause infection even with doses as low as 800 organisms (Altekruse *et al.*, 1999); which heightens the risk of infection. Therefore, further studies are required to find out the sources of contamination of campylobacters, and the stage at which contamination occurs during the preparation of food. The hygiene practiced in supermarkets must also be closely monitored in an effort to reduce the chances of contamination.

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