ACT HEALTH PROTECTION SERVICE

MICROBIOLOGICAL QUALITY OF SUSHI

September 2011 - December 2011

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BACKGROUND/OBJECTIVE

Sushi is a traditional Japanese dish made by placing a piece of high quality fish on a roll of cold vinegared rice wrapped in dried seaweed. Variations of the dish are common ranging from a small vinegared rice cake topped with a slice of raw fish to a fried tofu bag filled with the vinegared rice and top with a condiment. Most common forms of sushi involves filling the rice with small pieces of meat, fish or vegetable and wrapping it with seaweed to form a roll. Sushi along with sashimi (small pieces of raw fish) is a very popular type of Ready to Eat (RTE) food with a number of outlets in the ACT retailing these products. The preparation, use of raw products and improper food handling practices in the production of sushi and sashimi has the potential for contamination. This survey looked at retailers that specifically serve these types of products.

The sushi survey was undertaken to:

- determine the microbiological status of sushi in the ACT.
- determine the compliance of these products to the Food Standards Australia New Zealand (FSANZ) Guidelines for the Microbiological Examination of RTE Foods.
- compare these results with results from previous surveys conducted in 2003 and 2007.

STANDARDS

Sushi are categorised as a RTE food. The FSANZ Guidelines for the Microbiological Examination RTE Foods as shown in Table 1 are applicable to this food.

Table 1

Test	Microbiological Quality (CFU per gram)			
	Satisfactory	**Marginal**	**Unsatisfactory**	**Potentially Hazardous**
Indicators				
Escherichia coli (E. coli)	<3	3-100	>100	**
Pathogens				
Coagulase positive staphylococci	$<10^2$	10^2-10^3	10^3-10^4	$\geq 10^4$ SET +ve
Bacillus cereus (B. cereus)	$<10^2$	10^2-10^3	10^3-10^4	$\geq 10^4$
Salmonella spp.	not detected in 25g			detected
Listeria monocytogenes	not detected in 25g	detected but $<10^{2\ \#}$		$\geq 10^{2\ \#\#}$

NOTE:

*Pathogenic strains of *E. coli* should be absent.

** Foods with a long shelf life stored under refrigeration should have no *L. monocytogenes* detected in 25g.

The detection of *L. monocytogenes* in ready-to-eat-foods prepared specifically for "at risk" population groups (the elderly, immuno-compromised and infants) should also be considered as potentially hazardous.

SET +ve: Staphylococcus enterotoxin positive.

SURVEY

The sushi survey ran from September to December 2011. During this period a total of 60 samples were collected and processed by the Health Protection Service laboratory. The sushi samples were collected from 10 different establishments. The samples consisted of a wide variety of *meat* sushi including beef, chicken and pork; *fish* sushi including salmon, crab and tuna and *vegetarian* sushi including vegetable, egg and tofu. The samples were tested (Table 1) for a variety of different organisms in line with the RTE food standards.

Where the HPS identifies non-compliance issues in food businesses, corrective actions are addressed through a graduated and proportionate response. Unsatisfactory results are

re-sampled. Marginal results may be re-sampled; this is dependent on resources as these foods are still considered compliant.

MICROBIOLOGICAL METHODS OF ANALYSIS

Samples were tested for the presence of:

- *Escherichia coli* using ISO16649-2 ;
- coagulase positive *Staphylococci* using AS 1766.2.4 (modified);
- *Bacillus cereus* using AS 5013.2;
- *Salmonella* species using AS: 5013.10–2004 (modified);
- *Listeria monocytogenes* using AS 1766.2.16.1 (modified).

The sample preparation for *Escherichia coli,* coagulase positive *Staphylococci* and *Bacillus cereus* consisted of 25g of sample being homogenised with 225mL of 0.1% peptone diluent with subsequent serial dilutions prepared for use in enumeration.

- ***Escherichia coli* enumeration:** Pour plates of 1ml of 10^{-1} dilution were prepared in triplicate on TBX medium and incubated at 37ºC/4 h followed by 44ºC/20 h. Confirmed *E. coli* colonies appear blue/green after incubation.

- **Coagulase positive *Staphylococci* enumeration:** Spread plates (using a 100µl of each dilution) of Baird Parker medium with RPF supplement were prepared in duplicate and incubated at 37°C/48h. Typical black colonies, with a halo of precipitation surrounding the colony were indicative of coagulase activity.

- ***Bacillus cereus* enumeration:** Spread plates (using a 100µl of each dilution) on a solid selective medium containing egg yolk and mannitol. Typical large, pink colonies, with or without lecithinase action were counted and a proportion of the colonies confirmed by a haemolysis test and spore staining. *B. cereus* cells are rods 4-5 µm long and 1-1.5 µm wide and stain red. The cells contain black-stained lipid globules. The spores stain green, are ellipsoidal in shape, central to sub central in position, and do not swell the sporangium.

- ***Salmonella* detection:** 25g of sample was weighed out aseptically and homogenised with 225mL buffered peptone water (non-selective enrichment) and incubated at 37°C/16-20h. Aliquots were then transferred into Brain Heart Infusion broth (BHI) and incubated for 4h. DNA was extracted from 200uL of enriched BHI. This was screened for the presence of salmonella using a BAX System Salmonella PCR Assay. No confirmation testing was performed as there were no samples that screened positive.

- ***Listeria monocytogenes* detection:** 25g of sample was weighed out aseptically and homogenised with 225mL half Fraser broth (selective enrichment) and incubated at 30°C/24h. Aliquots were then transferred into a single tube of Fraser broth incubated for 37°C/48h and MOPS BLEB broth incubated for 37°C/24h. DNA was extracted from 200uL of enriched MOPS BLEB broth. This was screened for the presence of *Listeria monocytogenes* using a BAX system Listeria monocytogenes PCR assay. Confirmation testing was performed using the incubated Fraser broth tubes. A loopful of each positive sample was streaked out onto Oxford and Palcam agar and incubated for 37°C/48h. Up to ten typical colonies on (appear in the form of green colonies about 1.5 to 2.0 mm in diameter,

with a central depression and surrounded by a black halo) Oxford agar and Palcam agar were streaked each onto a CAMP plate (Sheep blood agar) and incubated for 37°C/24h. A positive CAMP isolates are then inoculated in a Rhamnose and Xylose broths and incubated at 37°C for up to five days. A positive reaction usually occurs within 24h to 48h. *Listeria monocytogenes* is positive for Rhamnose (Yellow) and negative (Blue-green) for Xylose.

RESULTS

Escherichia coli

Fifty seven (95.0%) of the 60 samples showed a satisfactory microbiological quality for *E. coli* i.e.<3cfu/g. The remaining 5% of samples (3) were marginal with between 3 and 10 cfu /g of *E. coli*. There were no unsatisfactory *E.coli* results.

The presence of *E. coli* is undesirable as it is indicative of poor hygiene conditions, which have lead to contamination of the product. Ideally *E. coli* should not be detected and as such a level of <3 cfu/g has been given as the satisfactory criteria for this organism. Levels exceeding 100cfu/g are unacceptable and indicate a level of contamination, which may have introduced pathogens or that pathogens, if present in the sushi prior to processing, may have survived.

Coagulase positive *Staphylococci*

Only 50 sushi samples were tested for coagulase positive *Staphylococci.* There was no coagulase positive *Staphylococci* isolated from any of the samples tested in this survey. The results indicate 100% of the samples were satisfactory. Ten samples were not tested due to media quality control issues.

Salmonella spp.

No *Salmonella* were isolated from any of the 60 sushi samples in this study. The results indicate that 100% of the samples were satisfactory.

Listeria monocytogenes

All 60 samples tested were satisfactory i.e. *Listeria monocytogenes* was not detected in 25 grams of the sample.

Bacillus cereus

Fifty eight samples were tested for *B. cereus*. *B. cereus* was isolated from two sushi samples at 50 cfu/ml, this is within the satisfactory range for microbiological quality. The results indicate that 100% of the samples were satisfactory. Two samples were not tested for *Bacillus cereus* as they did not contain any rice.

Table 2

Comparison of 2011 survey with the 2003 and 2007 surveys by percentage

Organism	2011 n=60				2007 n=92				2003 n=55			
	S	M	U	PH	S	M	U	PH	S	M	U	PH
E. coli	95.0	5.0	0.0	NA	92.4	4.3	3.3	NA	85.4	7.3	7.3	NA
Staphylococcus	100	0.0	0.0	0.0	97.4	2.2	0.0	0.0	85.4	9.1	5.5	0
Salmonella spp.	100	NA	NA	0.0	100	NA	NA	0.0	100	NA	NA	0
B. cereus	100	0.0	0.0	0.0	98.9	1.1	0.0	0.0	83.7	10.9	3.6	1.8
L. monocytogenes	100	0	NA	NA	97.8	2.2	NA	NA	87.3	12.7	NA	NA

S – Satisfactory, U – Unsatisfactory, M – Marginal, PH – Potentially Hazardous. NA= Not applicable

Not applicable

DISCUSSION

There has been continual improvement since the last survey in 2007 (See table 2)

- The percentage of satisfactory results for all tests is over 95%, with the majority over 98% for 2011. An improvement since 2007 and 2003.
- The number of unsatisfactory samples has been reduced to zero for all tests. *E. coli* in 2007 had 3.3% unsatisfactory results while *E.coli, Staphylococcus* and *B. cereus* all had unsatisfactory levels in 2003.
- No potentially hazardous samples were detected since 2003.
- Since 2003 *Staphylococcus* and *B. cereus* microbiological quality has improved to 100% satisfactory levels obtained by sushi retailers sampled.
- *Salmonella* has consistently been absent from sushi samples collected in the ACT during the sushi surveys.
- The raw results of analysis are attached at Appendix A.

CONCLUSION

There was a very good level of compliance with the Food Standards Australia New Zealand Guidelines for the Microbiological Examination of Ready-to-Eat Foods December 2001. Overall, the microbiological quality of Sushi surveyed in the ACT has continued to improve over the last eight years.

BIBLIOGRAPHY

1. Guidelines for the Microbiological Examination of Ready-to-eat-Foods (December 2001) FSANZ

Appendix A

	E. coli count in food cfu/g	Coagulase Positive *Staphlococcus* cfu/g	*Salmonella* in food P/A in 25g	*L. monocytogenes* in food P/A in 25g	*B. cereus* result cfu/g	Assessment
Chicken Roll	<3	<50	Absent	Absent	<50	S
Salmon Roll	<3	<50	Absent	Absent	<50	S
Pickled Octopus Inari	<3	<50	Absent	Absent	50	S
Scallops Nigirizushi	<3	<50	Absent	Absent	<50	S
Tempura Prawn Roll	<3	<50	Absent	Absent	<50	S
Prawn and Avocado Roll	<3	<50	Absent	Absent	<50	S
Salmon and Avocado roll	<3	<50	Absent	Absent	<50	S
Tofu and Vegetable Roll	<3	<50	Absent	Absent	<50	S
Teriyaki Chicken Roll	<3	<50	Absent	Absent	<50	S
Octopus Salad	<3	<50	Absent	Absent	<50	S
Eel Roll	<3	<50	Absent	Absent	<50	S
Chicken Tempura Roll	<3	<50	Absent	Absent	<50	S
Salmon Roll	<3	<50	Absent	Absent	<50	S
Beef Roll	<3	<50	Absent	Absent	<50	S
Spicy Squid Roll	<3	<50	Absent	Absent	<50	S
Pickled Octopus Inari	<3	<50	Absent	Absent	<50	S
Crispy Chicken Roll	<3	<50	Absent	Absent	<50	S
Spicy Prawn Roll	10	<50	Absent	Absent	<50	M
Tempura Salmon Roll	<3	<50	Absent	Absent	50	S
Tuna Roll	<3	<50	Absent	Absent	<50	S
Spicy Salmon Inari	<3	NT	Absent	Absent	<50	S
Beef Roll	<3	NT	Absent	Absent	<50	S
Crab Roll	<3	NT	Absent	Absent	<50	S
Crispy Chicken Roll	<3	NT	Absent	Absent	<50	S
Salmon Roll	<3	NT	Absent	Absent	<50	S
Tempura Prawn Roll	<3	<50	Absent	Absent	<50	S
Teriyaki Chicken Roll	<3	<50	Absent	Absent	<50	S
Beef Roll	<3	<50	Absent	Absent	<50	S
Tuna Roll	<3	<50	Absent	Absent	<50	S
Salmon Roll	<3	<50	Absent	Absent	<50	S
Tuna Roll	<3	<50	Absent	Absent	<50	S
Teriyaki Roll	<3	<50	Absent	Absent	<50	S
Special Lunch Box	<3	<50	Absent	Absent	<50	S
Rice Paper Roll	<3	<50	Absent	Absent	<50	S
Inari Roll	<3	<50	Absent	Absent	<50	S
Chicken Sushi	<3	<50	Absent	Absent	<50	S
Sashimi	<3	<50	Absent	Absent	NT	S

	E. coli count in food cfu/g	Coagulase Positive *Staphlococcus* cfu/g	*Salmonella* in food P/A in 25g	*L. monocytogenes* in food P/A in 25g	*B. cereus* result cfu/g	Assessment
Egg Sushi	<3	<50	Absent	Absent	<50	S
Inari	<3	<50	Absent	Absent	<50	S
Seaweed Salad	<3	<50	Absent	Absent	NT	S
California Sushi	<3	<50	Absent	Absent	<50	S
Teriyaki Chicken Sushi	<3	<50	Absent	Absent	<50	S
Sushi Gateaux	<3	<50	Absent	Absent	<50	S
Vegetable Sushi	<3	<50	Absent	Absent	<50	S
Spicy Tuna sushi	<3	<50	Absent	Absent	<50	S
Teriyaki Chicken Roll	<3	<50	Absent	Absent	<50	S
Tuna Roll	<3	<50	Absent	Absent	<50	S
Salmon Roll	<3	<50	Absent	Absent	<50	S
Chicken Schnitzel Roll	<3	<50	Absent	Absent	<50	S
Californian Roll	<3	<50	Absent	Absent	<50	S
Avocado Sushi	<3	<50	Absent	Absent	<50	S
Prawn Tempura Sushi	<3	<50	Absent	Absent	<50	S
Tuna Sushi	<3	<50	Absent	Absent	<50	S
Salmon Sushi	<3	<50	Absent	Absent	<50	S
Chicken Sushi	10	<50	Absent	Absent	<50	M
Chicken Teriyaki Sushi	<3	NT	Absent	Absent	<50	S
Salmon Sushi	3	NT	Absent	Absent	<50	M
Beef Sushi	<3	NT	Absent	Absent	<50	S
Chicken Schnitzel Roll	<3	NT	Absent	Absent	<50	S
Vegetable Sushi	<3	NT	Absent	Absent	<50	S

Assessment: S = satisfactory, M = marginal, U = unsatisfactory and * = estimate count only. NT = Not Tested.