ACT HEALTH PROTECTION SERVICE

MICROBIOLOGICAL QUALITY OF FRESH HERBS JULY – OCTOBER 2014

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BACKGROUND/OBJECTIVE

Fresh herbs are highly popular in food preparation because of their flavour properties. These products are often consumed raw as garnish or seasoning in a meal. The way these products are used they could pose a high risk of microbial contamination in "Ready-to-Eat" (RTE) foods.

Fresh herbs are categorised as Ready-to-Eat foods due to its nature of consumption. RTE food is food that is consumed in the same state as that in which it is sold or distributed and does not include nuts in the shell and whole, raw fruits and vegetables that are intended for hulling, peeling or washing by the consumers.

In recent years, several reports have been published regarding food-borne Salmonella outbreaks related to the leafy herbs. In 2006 and 2007 two *Salmonella* related outbreaks were reported in United Kingdom which was caused by the consumption of contaminated fresh basil [2][5]. In the United States (2010-11) parsley and coriander were recalled due to detection of *Salmonella* [4]. In Australia there have been no reported *Salmonella* related food outbreaks associated with fresh herbs; however there have been food recalls throughout Australia in 2011 and 2012 due to the detection of *Listeria monocytogenes (L. monocytogenes)* and *Escherichia coli* (*E. coli*) in vegetable and herb mix [4].

In July 2013, Government of South Australia had conducted a survey to determine the microbiological quality of fresh herbs from the range of retail outlets throughout South Australia. This survey found no target pathogens in the samples tested.

OBJECTIVE

The main objectives of the fresh herbs survey were:

- To examine whether these products are the potential source of the contamination of microbiological organisms such as *E coli*, *Salmonella* and *L. monocytogenes.*

- To assess the microbiological quality of the fresh herbs ready to eat foods based on the Australia and New Zealand Food Standard (FSANZ) Guidelines for the Microbiological Examination of Ready-to-Eat foods (FSANZ RTE Guidelines).

- To identify any link between sample source and microbiological quality by comparing the results of the fresh herb samples collected from different types of outlets in ACT such as supermarkets, Asian shops and farmers markets.

STANDARDS

The FSANZ RTE Guidelines identify four categories of microbiological quality ranging from satisfactory to potentially hazardous. Table 1 details the recommended guideline value. Table 1 not only reflects both the high level of microbiological quality that is achievable for RTE foods in Australia and New Zealand but also indicates the level of contamination that is considered to be a significant risk to the public health.

Table 1

Test	Microbiological Quality (colony forming units per gram (cfu/g))			
	Satisfactory	**Marginal**	**Unsatisfactory**	**Potentially Hazardous**
Indicators				
E. coli	<3	3-100	>100	**
Pathogens				
Salmonella spp.	not detected in 25g			detected
L. 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.

SURVEY

This survey was conducted between July and October 2014. During this period sixty five samples from fourteen ACT retail outlets were collected randomly by Health Protection Service (HPS) Public Health Officers (PHO) and processed by the Australian Capital Territory (ACT) Government Analytical Laboratory. Generally outlets are only sampled once.

Samples were tested for hygiene indicator *E. coli* and food pathogens – *L. monocytogenes and Salmonella*. All sixty five samples were tested for *E. coli* and *L. monocytogenes*. Only sixty four samples were tested for *Salmonella* due to one sample being insufficient in size.

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. Unsatisfactory SPC results are not re-sampled unless pathogens are also isolated.

MICROBIOLOGICAL METHOD OF ANALYSIS

Samples were tested for the presence of:

- *Salmonella* species based on AS 5013.10 – 2009
- *E. coli* based on ISO 16649.2 -2001
- *L. monocytogenes* based on AS 5013.24.1-2009.

The sample preparation for *E. coli* consisted of 25g of sample being homogenised with 225mL of 0.1% peptone diluents.

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

***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 3h.

DNA was extracted from 200uL of enriched BHI. This was screened for the presence of salmonella using a BAX cyber green Polymerase Chain Reaction (PCR). Confirmation tests were not performed as the samples screened negative.

***L. 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 and MOPS BLEB broth which were incubated at 37°C/48h and 37°C/24h respectively. DNA was extracted from 200uL of enriched MOPS BLEB broth. This was screened for the presence of *L. monocytogenes* using a BAX cyber green PCR. Confirmation tests were not performed as the samples screened negative.

RESULTS / DISCUSSION

E. coli

Altogether sixty five survey samples were tested for generic *E. coli*. Fifty nine (90.8%) samples were found to be satisfactory as *E. coli* was not detected. These results had met the satisfactory criteria for this indicator.

The six samples (9.23%) were found to be marginal as *E. coli* were detected within the marginal level (3-100 cfu/g). According to the food FSANZ RTE Guidelines a re-sample is not required if the results are reported marginal. Re-sampling was not conducted in these cases.

The presence of generic *E. coli* in RTE foods is undesirable. Its presence in food indicates the poor sanitation and hygienic conditions which have lead to contamination or inadequate heat treatment. The detection of *E. coli* in RTE food does not reflect that the food is unsafe rather it is an indication of potential problems involving sanitation and poor food handling.

Salmonella

Salmonella was not detected in any of the sixty four samples tested; one sample was not tested due insufficient sample size. RTE foods should be free of *Salmonella* as consumption of food containing this pathogen may result in food borne illness

L. monocytogenes

Total of sixty five survey samples were analysed for *L. monocytogenes* and was not detected in any samples. All sixty five samples (100%) were reported satisfactory.

The detection of *L. monocytogenes* in such foods indicates the food was inadequately prepared or the food was contaminated post preparation. The detection of higher levels (>10^2 cfu/g) of *L. monocytogenes* in RTE foods indicates a failure of food handling controls and is also considered a public health risk.

All RTE foods are tested for the presence of *L. monocytogenes* in 25g. If *L. monocytogenes* is detected PHO will inspect the premises and collect a resample of the food item if available. This re-sample will be tested in a semi-quantitative manner to measure the level of *L. monocytogenes* in the food.

CONCLUSION

The microbiological quality of the fresh herbs surveyed in the ACT was found to be good. No harmful pathogens such as *Salmonella* and *L. monocytogenes* were detected in survey samples. However the survey has found marginal levels of *E coli* in six samples (9.23%). Raw results of the analysis are attached at Appendix A. Although the survey results are compliant to the food guidelines, some of the areas that could be improved are food handling and hygiene practices.

There was no discrepancy in results of the samples collected from different types of retail outlets in ACT. The pathogens such as *Salmonella* and *L. monocytogenes* were absent in all samples. The hygiene indicator organism, *E.coli* was found regardless of where the samples were collected from - such as Asian shops or supermarkets. These results indicate that there is no link between the sample source and microbiological quality of the products.

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Appendix A

Assessment: S = satisfactory, M = marginal, U = unsatisfactory

Sample Description	*E. coli*	*L. monocytogenes*	*Salmonella*	Assessment
Mint	<3	Absent	Absent	S
Dill	<3	Absent	Absent	S
Parsley	<3	Absent	Absent	S
Continental parsley	<3	Absent	Absent	S
Coriander	<3	Absent	Absent	S
Basil	<3	Absent	Absent	S
Mint	<3	Absent	Absent	S
Coriander	<3	Absent	Absent	S
Chives	<3	Absent	Absent	S
Shallots	<3	Absent	Absent	S
Basil	<3	Absent	Absent	S
Mint	<3	Absent	Absent	S
Mint	<3	Absent	Absent	S
Parsley	<3	Absent	Absent	S
Mixed parsley	<3	Absent	Absent	S
Coriander	<3	Absent	Absent	S
Italian basil	<3	Absent	Absent	S
Parsley	<3	Absent	Absent	S
Chives	<3	Absent	Absent	S
Dill	<3	Absent	Absent	S
Basil	<3	Absent	Absent	S
Fish mint	<3	Absent	Absent	S
Dill	<3	Absent	Absent	S
Mint	<3	Absent	Absent	S
Coriander with root	3	Absent	Absent	M
Dill	<3	Absent	Absent	S
Rosemary	<3	Absent	Absent	S
Tarragon	<3	Absent	Absent	S
Chives	<3	Absent	Absent	S
Coriander	<3	Absent	Absent	S
Flat leaf parsley	<3	Absent	Absent	S
Coriander	<3	Absent	Absent	S
Curley parsley	<3	Absent	Absent	S
Lemon grass	<3	Absent	Absent	S
Fenugreek	<3	Absent	Absent	S
Thai basil	<3	Absent	Absent	S
Vietnamese mint	<3	Absent	Absent	S
Fresh coriander	7	Absent	Absent	M
Lemon grass	<3	Absent	Absent	S
Rosemary	<3	Absent	Absent	S
Thyme	<3	Absent	Absent	S

Sample Description	*E. coli*	*L. monocytogenes*	*Salmonella*	Assessment
Sage	<3	Absent	Absent	S
Oregano	<3	Absent	Absent	S
Green basil	<3	Absent	Absent	S
Continental parsley	<3	Absent	Absent	S
Ngo Om	<3	Absent	Absent	S
Vietnamese mint	<3	Absent	Absent	S
Chives	<3	Absent	Absent	S
Coriander	<3	Absent	Absent	S
Tia To	<3	Absent	Absent	S
Mint	100	Absent	Absent	M
Dill	<3	Absent	Absent	S
Coriander	<3	Absent	Absent	S
Chives	<3	Absent	NP	S
Parsley	<3	Absent	Absent	S
Garlic chives	<3	Absent	Absent	S
Vietnamese mint	<3	Absent	Absent	S
Watercress	13	Absent	Absent	M
Dill	<3	Absent	Absent	S
Coriander	<3	Absent	Absent	S
Rosemary	3	Absent	Absent	M
Basil	<3	Absent	Absent	S
Continental parsley	3	Absent	Absent	M
Parsley	<3	Absent	Absent	S
Mint	<3	Absent	Absent	S

NP = Not Performed