ACT HEALTH PROTECTION SERVICE

MICROBIOLOGICAL QUALITY OF KEBABS MAY – SEPTEMBER 2011

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Prepared by Victoria Wansink, Radomir Krsteski and Simon Rockliff

BACKGROUND/OBJECTIVE

Kebabs are a popular Middle Eastern takeaway fast food. They are a meat dish consisting of thin slices of chicken, beef or lamb (or vegetarian option of falafel) eaten in a roll of flat bread (unleavened) with salad and sauces. The meat is grilled on a vertical rotating skewer in the form of a cone or cylinder. This rotating skewer allows the outer layer of meat to be grilled and carved off in slices.

The preparation and cooking methods used in the production of kebabs have the potential to allow the consumption of undercooked meat and cross contamination issues between raw and prepared ingredients. Due to the takeaway nature of kebabs, they have been categorised as a ready-to-eat food. “Ready-to-Eat” (RTE) food is food that is ordinarily 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.

STANDARDS

The FSANZ Ready-to-Eat (RTE) Guidelines identify four categories of microbiological quality ranging from satisfactory to potentially hazardous. Table 1 details the recommended guideline value. This Table 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[1]

Test	Microbiological Quality (colony forming units per gram (cfu/g))			
	Satisfactory	**Marginal**	**Unsatisfactory**	**Potentially Hazardous**
Indicators				
Escherichia coli	<3	3-100	>100	**
Pathogens				
Coagulase positive staphylococci	$<10^2$	10^2-10^3	10^3-10^4	$\geq 10^4$ SET +ve
Clostridium perfringens	$<10^2$	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

This survey was conducted between May and September 2011. During this period fifty seven samples and fifteen follow-up samples from thirteen ACT retail outlets were collected randomly by Health Protection Service Officers and processed by the Health Protection Service Laboratory. All of the samples were tested for the hygiene indicators, *E.coli* and coagulase positive *Staphylococci,* and the food pathogens *Salmonella* spp. and *Listeria monocytogenes.* Not all samples were tested for *Clostridium perfringens* due to test media

availability. The survey collected multiple samples from single outlets and in general outlets were only tested once.

Marginal results may be re-sampled; this is dependent on resources as these foods are still considered compliant. 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; if the food item is not available other food items may tested. 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 AS 1766.2.5 (modified)
- *Bacillus cereus* AS 5013.2
- Coagulase positive *staphylococci* AS 5013.12.2
- *Escherichia coli* ISO:16649 – 2 (modified)
- *Listeria monocytogenes* AS 1766.2.16.1 (modified)
- *Clostridium perfringens* AS 5013.16 – 2006.

The sample preparation for *Escherichia coli, Clostridium perfringens* and coagulase positive *Staphylococci* consisted of:

- 25g of sample being homogenised with 225mL of 0.1% peptone diluent; with
- subsequent serial dilutions were prepared for use in enumeration.

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

***Clostridium perfringens* enumeration:**
Overlaid pour plates of TSCNE agar using 1ml of 10^{-2} dilution and 10^{-4} were prepared in duplicate and incubated anaerobically at 37ºC/24 h. Typical presumptive *C. perfringens* colonies are black with or without precipitation surrounding the colony. Typical colonies are then confirmed using API 20A biochemical testing kit.

Coagulase positive *staphylococci* enumeration: Spread plates (using a 100µl of each dilution) of Baird Parker medium 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.

***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 cyber green Polymerase Chain Reaction (PCR). 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 cyber green PCR. 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 / DISCUSSION

Escherichia coli

All fifty seven survey samples were tested for *E. coli*. The presence of *E. coli* in RTE foods is undesirable because it indicates that the food has possibly been prepared under poor hygienic conditions. Forty five (78.9%) samples tested in this survey had <3 cfu/g of *E. coli* and met the satisfactory criterion. There were eight (14.0%) samples in the marginal category and four (7.0%) samples in the unsatisfactory category. Re-sample's where taken for all unsatisfactory results. Some re-samples were found satisfactory while other were still unsatisfactory. Where non-compliance remained unresolved further enforcement action was taken with one premise successfully prosecuted. The detection of *E. coli* in foods is not a direct indication that the food is unsafe rather it is an indication of potential problems involving the preparing and handling of foods.

Coagulase positive *Staphylococci*

Coagulase positive *Staphylococci* were not detected in any of the fifty seven samples collected and tested.

Clostridium perfringens enumeration:

Fifty two samples were analysed for *Clostridium perfringens.* All of the samples tested were satisfactory i.e. *Clostridium perfringens* <100 cfu/g.

Salmonella spp.

Salmonella spp. was not detected in any of the fifty seven samples tested. RTE foods should be free of *Salmonella* spp. as consumption of food containing this pathogen may result in food borne illness.

Listeria monocytogenes

All fifty seven survey samples were analysed for *Listeria monocytogenes*. Fifty five (96.5%) of the samples were satisfactory i.e. *Listeria monocytogenes* was not detected, whereas two (3.5%) samples were positive for *Listeria monocytogenes*. One of the premises was re-sampled. The re-sampled result was negative for *Listeria monocytogenes.* The other premise had ceased trading before further enforcement action could be taken.

CONCLUSION

The microbiological quality of the kebabs surveyed in the ACT is satisfactory but could be improved. Overall the results are comparable to those found in the previous studies undertaken by ACT Health (Appendix A) and the NSW Food Authority. Raw results of analysis are attached at Appendix B. The survey highlighted some areas of concern with the preparation of kebabs. Many of the failures may have been the result of poor knowledge of good hygiene practices. This could be rectified with fact sheets and verbal advice that was given to businesses at the time of follow-up re-sampling and inspection. Where effective

corrective action is confirmed (i.e. compliant re-samples) no further enforcement action is undertaken. Where non-compliance remained unresolved, further enforcement action was taken.

As a high risk food group and due to the results from the survey it would be prudent to conduct this survey in the near future to see if food handling practices have improved or declined.

BIBLIOGRAPHY

1. Guidelines for the microbiological examination of ready-to-eat foods FSANZ Dec 2001
2. Food Safety Survey of Retail Doner Kebabs in NSW, P.Bird, T.Soenario and P. Sutherland, NSW Food Authority 2004.
3. Snapshot survey on the microbiological quality of kebabs, April 2008, NSW Food Authority.
4. Microbiological Quality of Kebab 2002, ACT Health Protection Service.
5. Foodborne Microorganisms of Public Health Significance, AIFST Inc. Food Microbiology Group.

APPENDIX A

COMPARISON TO PREVIOUS SURVEY

Tables 1 is a summary of the quality of surveyed Kebabs in the ACT. *E.coli* results have not improved, whereas coagulase +ve *Staphylococcus* and *Listeria monocytgenes* results have improved on previous survey.

Table 1

%	2002				2011			
	Sat	**Marg**	**Unsat**	**Pot. Haz**	**Sat**	**Marg**	**Unsat**	**Pot. Haz**
E. coli	82.3	15.2	2.5		78.9	14	7.0	
Coagulase +ve Staphylococcus	89.9	10.1	0.0	0.0	100	0.0	0.0	0.0
Cl. perfringens	100	0.0	0.0	0.0	100	0.0	0.0	0.0
***Salmonella* spp.**	100			0.0	100			0.0
Listeria monocytogenes	87.8	12.2			96.5	3.5		0.0

Comparison between the Microbiological Quality indicators
Sat – Satisfactory, Unsat – Unsatisfactory, Marg – Marginal, Pot. Haz – Potentially Hazardous, Not applicable

Appendix B

Assessment: S = satisfactory, M = marginal, U = unsatisfactory and * = estimate count only.

Sample Description	*C. perfringens*	*E. coli*	*L. monocytogenes*	*Salmonella*	*Staphylococci*	Assessment
Chicken Kebab	<50	<3	Absent	Absent	<50	S
Lamb Kebab	<50	<3	Absent	Absent	<50	S
Falafel Kebab	<50	<3	Absent	Absent	<50	S
Beef Kebab	<50	<3	Absent	Absent	<50	S
Chicken kebab(lettuce,tomato, onion, garlic,mayo)	<50	<3	Absent	Absent	<50	S
Beef Kebab (lettuce, tomato, onion, garlic, mayo)	<50	20	Absent	Absent	<50	M
Lamb Kebab (lettuce, tomato, onion, garlic, mayo)	<50	7	Absent	Absent	<50	M
Chicken Kebab (lettuce, tomato, mild)	50	<3	Absent	Absent	<50	S
Beef Kebab (lettuce, tomato, mild)	<50	3	Absent	Absent	<50	M
Beef kebab (lettuce, onion, tomatoes, garlic)	<50	1100	Absent	Absent	<50	U
Chicken kebab (lettuce, onion, tomatoes, garlic)	<50	240	Absent	Absent	<50	U
Lamb kebab (lettuce, onion, tomatoes, garlic)	<50	560	Absent	Absent	<50	U
Lamb	<50	3	Absent	Absent	<50	M
Chicken	<50	<3	Present	Absent	<50	U
Chicken	<50	3	Absent	Absent	<50	M
Falafel	<50	<3	Absent	Absent	<50	S
Zuchinni	<50	<3	Absent	Absent	<50	S
Chicken (all salads, peri peri sauce)	<50	<3	Absent	Absent	<50	S
Lamb (all salads, peri peri sauce)	<50	<3	Absent	Absent	<50	S
Beef (all salads, peri peri sauce)	<50	<3	Absent	Absent	<50	S
Falafel (all salads, sweet chilli sauce)	<50	<3	Absent	Absent	<50	S
Mixed (all salads, garlic mayo)	<50	3*	Absent	Absent	<50	M
Chicken Kebab (yoghurt and garlic)		<3	Absent	Absent	<50	S
Lamb Kebab		<3	Absent	Absent	<50	S

(yoghurt and garlic)						
Mix Kebab (yoghurt and garlic)		<3	Absent	Absent	<50	S
Chicken Kebab (garlic and yogurt)		<3	Absent	Absent	<50	S
Doner Kebab (garlic and yogurt)		<3	Absent	Absent	<50	S
Doner kebab	<50	<3	Absent	Absent	<50	S
Chicken kebab	<50	<3	Absent	Absent	<50	S
Mix kebab	<50	<3	Absent	Absent	<50	S
Falafel kebab	<50	<3	Present	Absent	<50	U
Feta cheese Peynirki	<50	<3	Absent	Absent	<50	S
Falafel kebab (all salads)	<50	<3	Absent	Absent	<50	S
Lamb kebab	<50	<3	Absent	Absent	<50	S
Chicken kebab	<50	<3	Absent	Absent	<50	S
Mix kebab	<50	3*	Absent	Absent	<50	M
Chilli dip	<50	80*	Absent	Absent	<50	M
Chicken (mild chilli)	<50	<3	Absent	Absent	<50	S
Chicken (garlic yoghurt)	<50	<3	Absent	Absent	<50	S
Doner (garlic yoghurt)	<50	<3	Absent	Absent	<50	S
Mix (BBQ)	<50	<3	Absent	Absent	<50	S
Falafel (sweet chilli)	<50	3600	Absent	Absent	<50	U
Beef kebab with garlic sauce	<50	<3	Absent	Absent	<50	S
Chicken kebab with sweet chilli sauce	<50	<3	Absent	Absent	<50	S
Lamb kebab	<50	<3	Absent	Absent	<50	S
Falafel kebab	<50	<3	Absent	Absent	<50	S
Mixed (chicken, beef, lamb) kebab	<50	<3	Absent	Absent	<50	S
Mixed kebab	<50	<3	Absent	Absent	<50	S
Chicken Kebab	<50	<3	Absent	Absent	<50	S
Lamb Kebab	<50	<3	Absent	Absent	<50	S
Beef Kebab	<50	<3	Absent	Absent	<50	S
Falafel Kebab	<50	<3	Absent	Absent	<50	S
Mixed Kebab (mild peri peri)	<50	<3	Absent	Absent	<50	S
Falafel Kebab (sweet chilli)	<50	<3	Absent	Absent	<50	S
Lamb Kebab	<50	<3	Absent	Absent	<50	S
Chicken Kebab (garlic)	<50	<3	Absent	Absent	<50	S
Beef Kebab (AB sauce)	<50	<3	Absent	Absent	<50	S
Chicken Kebab	*<50*	*<3*	*Absent*	*Absent*	*<50*	*S*
Lamb Kebab	*<50*	*20*	*Absent*	*Absent*	*<50*	*M*

Falafel Kebab (all salads, hummus, tabouli, sauce)	*<50*	*870*	*Absent*	*Absent*	*<50*	U
Sweet chilli Sauce	*<50*	*<3*	*Absent*	*Absent*	*<50*	S
Tabouli	*<50*	*<3*			*<50*	S
Hummus	*<50*	*6900**	*Absent*	*Absent*	*<50*	U
Zucchini Balls	*<50*	*<3*	*Absent*	*Absent*	*<50*	M
Chicken Kebab		*10*	*Absent*			*M*
Lamb Kebab		*7*	*Absent*			*M*
Beef Kebab		*3*	*Absent*			*M*
Lamb kebab, lettuce, tomato, onion, BBQ sauce		*90*				*M*
Lettuce from front display		*<3*				*S*
Lettuce, parsley, onion, from unopened bags		*<3*				*S*
Chicken Kebab garlic sauce		*<3*	*Absent*	*Absent*	*<50*	*S*
Doner Kebab garlic sauce		*<3*	*Absent*	*Absent*	*<50*	*S*

Results in *italics* are re-samples.