ACT HEALTH PROTECTION SERVICE

MICROBIOLOGICAL QUALITY OF KEBABS & YEROS MAY – JUNE 2014

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BACKGROUND/OBJECTIVE

Kebabs are a popular Middle Eastern takeaway fast food. They are a meat dish consisting of shaved slices of chicken, beef or lamb (or vegetarian option of falafel) eaten in a roll of flat bread (unleavened) with salad and sauce. Yeros (Gyros) is the Greek version of a kebab; it is generally served in a lightly grilled piece of pita, rolled up with various salads and sauces. The meats used are pork, chicken, lamb, beef and are traditionally wrapped up with fried potatoes.

The meat is cooked on a vertical rotating skewer in the form of a cone or cylinder which turns in front of a source of heat. This rotating skewer allows the outer layer of meat to be grilled and carved off in thin 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. The survey of Kebabs/Yeros products was under taken for three main reasons:

1. To determine the bacteriological status of Kebabs/Yeros available on the ACT market.
2. To determine the compliance of these products to Food Standards Australia New Zealand (FSANZ) Guidelines for the Microbiological Examination of Ready-to-Eat (RTE) Foods 2001 (FSANZ RTE Guidelines).
3. To complement and focus audits of high-risk food producing establishments.

STANDARDS

The FSANZ RTE Guidelines identify four categories of microbiological quality ranging from satisfactory to potentially hazardous. Table 1 details the recommended guideline values. 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 (CFU per gram)			
	Satisfactory	**Marginal**	**Unsatisfactory**	**Potentially Hazardous**
Indicators				
Escherichia coli (E. coli)	<3	3-100	>100	*
Pathogens				
Coagulase positive *staphylococci (Staph)*	$<10^2$	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	$\geq 10^4$
Clostridium perfringens (C. perfringens)	$<10^2$	10^2-10^3	10^3-10^4	$\geq 10^4$
Salmonella spp.	not detected in 25g			detected
Listeria monocytogenes (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.
SET +ve: Staphylococcus enterotoxin positive.

SURVEY

This survey was conducted between May and June 2014. During this period forty five samples and three follow-up samples from nine Australian Capital Territory (ACT) retail outlets were collected randomly by Health Protection Service (HPS) Public Health Officers (PHO) and processed by the ACT Government Analytical Laboratory. All of the samples were tested for the hygiene indicator, *E. coli* and the food pathogens coagulase positive *Staphylococci, C. perfringens, B. cereus, Salmonella* and *L. monocytogenes.* The survey collected multiple samples from single outlets and in general outlets were only tested once.

Where the HPS identifies non compliance issues in food businesses, corrective actions are addressed through a graduated and proportionate response. Marginal results may be re-sampled; this is dependent on resources as these foods are still considered compliant. Unsatisfactory results are re-sampled if the food item is still available.

MICROBIOLOGICAL METHOD OF ANALYSIS

Samples were tested for the presence of:

- *Salmonella* species AS 5013.10 – 2009 (modified)
- *B. cereus* AS 5013.2 - 2007
- Coagulase positive *Staphylococci* AS 5013.12 – 2004 (modified)
- *E. coli* AS 5013.19.1– 2012 (modified)
- *L. monocytogenes AS* 5013.24.1- 2009 (modified)
- *C. perfringens* AS 5013.16 – 2006.

The sample preparation for *E. coli, B. cereus, C. perfringens* and coagulase positive *Staphylococci* consisted of:

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

***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.

B. cereus: Spread plates (using a 100µl of 10^{-2} and 10^{-4} dilution, in duplicate) on a solid selective medium containing egg yolk and mannitol (MYP) were incubated at 30°C/24-48h. Typical large, pink colonies, with or without lecithinase action were counted and a proportion of the colonies confirmed by a haemolysis test on Sheep Blood Agar 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.

***C. 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/24h. Typical presumptive *C. perfringens* colonies are black with or without precipitation surrounding the colony. Typical colonies are then confirmed using the API 20A biochemical testing kit.

Coagulase positive S*taphylococci* enumeration: Pour plates of Baird Parker medium using 1ml of 10^{-2} dilution and 10^{-4} 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 found in coagulase positive S*taphylococci*.

***Salmonella* detection:** 25g of sample was weighed out aseptically and homogenised with 225mL buffered peptone water (non-selective enrichment) and incubated at 37°C/20-24h. 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 DuPont BAX Polymerase Chain Reaction (PCR) kit. Selective enrichment broths were inoculated for samples with positive PCR screens using Rappaport-Vassiliadis Soya (RVS) broth incubated at 42ºC/24h and Muller-Kauffmann Tetrathionate-novobiocin broth (MKTTn) incubated at 37ºC/24h. Confirmation testing was carried out using these broths by plating out a loopful onto the selective agars Xylose Lysine Deoxycholate (XLD) and Hektoen. On XLD, *Salmonella* colonies are typically red with a black center and on Hektoen they are green with a black centre due to hydrogen sulphide metabolism. Presumptive colonies were dilution streaked onto non-selective Plate Count Agar and confirmed biochemically using API 20E testing kit and serologically using poly ‘O’ and ‘H’ anti sera.

***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 Fraser broths incubated for 37°C/48h and MOPS BLEB broths 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 DuPont BAX PCR kit. Confirmation testing was performed on samples with positive PCR screens 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. On Oxford and Palcam agars, *Listeria* colonies are typically green and about 1.5 to 2.0 mm in diameter, with a central depression and surrounded by a black halo. Up to ten typical colonies were streaked onto a Sheep blood agar plate using the CAMP test method and incubated for 37°C/24h. Positive CAMP isolates were then inoculated into Rhamnose and Xylose broths and incubated at 37°C for up to five days. A positive reaction usually occurs within 24h to 48h. *L. monocytogenes* is positive for Rhamnose (Yellow) and negative (Blue-green) for Xylose.

RESULTS / DISCUSSION

E. coli

All forty five 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 one (91.1%) samples tested in this survey had <3 cfu/g of *E. coli* and met the satisfactory criterion. There were four (8.89%) samples in the marginal category. Re-samples were taken for one outlet that included the positive *B. cereus* results and were found satisfactory for *E.coli*. 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*

Forty five samples were analysed for Coagulase positive *Staphylococci.* All of the samples tested were satisfactory i.e. <100 cfu/g.

C. perfringens

Forty five samples were analysed for *C. perfringens.* All of the samples tested were satisfactory i.e. <100 cfu/g.

B. cereus

B. cereus was tested for in forty one samples. Thirty nine (95.1%) samples were satisfactory; one (2.4%) was marginal and another (2.4%) potentially hazardous. The premise was re-sampled and the re-samples were found to be satisfactory.

Salmonella

Salmonella was not detected in any of the forty four samples tested. One sample could not be tested due to 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

L. monocytogenes was not detected in any of the forty four samples tested. One sample could not be tested due to insufficient sample size. Foods in which all components have been cooked in the final food preparation should be free of *L. monocytogenes*. The detection of *L. monocytogenes* in such foods indicates the food was inadequately cooked or the food was contaminated post preparation.

Summary

The presence of *E. coli* and *B. cereus* may be due to a variety of factors including low level contamination on salads/vegetables (e.g. tomato, onion), herb/spices used in sauce served on the kebab (e.g. hummus, garlic sauce) and/or contamination during the handling and preparation of kebabs.

The presence of bacteria due to the above factors is not unexpected and would not normally constitute a safety risk provided proper temperature control is maintained after preparing kebab ingredients and the final kebab is not kept for a long period (> 2 hours) at room temperature prior to eating (3).

CONCLUSION

The microbiological quality of the kebabs surveyed in the ACT is quite good. Raw results of analysis are attached at Appendix B. Overall the results show a slight improvement on those found in the previous studies undertaken by ACT Health (Appendix A) and were also better than those obtained by the NSW Food Authority in 2008. *E. coli* and *L. monocytogenes* results have improved on previous surveys. *B. cereus* results could not be compared with previous years' surveys as this was the first time this test has been included. But the one sample with the potentially hazardous count highlighted the need that all future surveys include *B. cereus* testing.

The survey highlighted that there are still some areas of concern in regards to the preparation of kebabs. The detection of low levels of *E. coli* in foods is not a direct indication that the food is unsafe but more an indication of potential problems involving the preparing and handling of foods (3). This may be a result of poor knowledge of good hygiene practices. This was rectified with fact sheets and verbal advice given to businesses at the time of follow-up re-sampling and inspection.

As a high risk food group it would be prudent to conduct this survey again in the near future to ensure food handling practices remain appropriate.

BIBLIOGRAPHY

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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.
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APPENDIX A

COMPARISON TO PREVIOUS SURVEY

Table 1 is a summary of the quality of surveyed Kebabs in the ACT. *E. coli* and *L. monocytogenes* results have improved on previous surveys. We cannot compare *B. cereus* as there are no results from previous years and coagulase positive *Staphylococcus* has not been detected since 2002.

Table 1

%	2002				2011				2014			
	Sat	**Marg**	**Unsat**	**Pot. Haz**	**Sat**	**Marg**	**Unsat**	**Pot. Haz**	**Sat**	**Marg**	**Unsat**	**Pot. Haz**
E. coli	82.3	15.2	2.5		78.9	14	7.0		91.1	8.89	0.0	
Coagulase postive Staphylococcus	89.9	10.1	0.0	0.0	100	0.0	0.0	0.0	100	0.0	0.0	0.0
C. perfringens	100	0.0	0.0	0.0	100	0.0	0.0	0.0	100	0.0	0.0	0.0
***Salmonella*.**	100			0.0	100			0.0	100			
L. monocytogenes	87.8	12.2			96.5	3.5		0.0	100			
B. cereus	NA	NA	NA	NA	NA	NA	NA	NA	95.1	2.4	0.0	2.4

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

APPENDIX B

Sample	C. perfringens	L. monocytogenes	E. coli	Salmonella	Staph	B. cereus	Assessment
Lamb kebab with garlic sauce, salad	<50	Absent	<3	Absent	<50	NP	S
Chicken kebab with tahini sauce, salad	<50	Absent	<3	Absent	<50	NP	S
Kabak kebab with yoghurt sauce, salad	<50	Absent	<3	Absent	<50	NP	S
Falafel kebab with chilli sauce, salad	<50	Absent	<3	Absent	<50	NP	S
Chicken kebab with hummus, salad	<50	Absent	<3	Absent	<50	NP	S
Cooked lamb meat	<50	Absent	<3	Absent	<50	<50	S
Lettuce	<50	NP	<3	NP	<50	<50	S
Onion, Parsley	<50	Absent	7	Absent	<50	<50	M
Beef	<50	Absent	<3	Absent	<50	<50	S
Chicken	<50	Absent	3	Absent	<50	<50	M
Cooked chicken meat	<50	Absent	<3	Absent	<50	<50	S
Cooked beef meat	<50	Absent	<3	Absent	<50	<50	S
Cooked lamb meat	<50	Absent	<3	Absent	<50	<50	S
Falafel	<50	Absent	<3	Absent	<50	<50	S
Tabouli	<50	Absent	<3	Absent	<50	<50	S
Beef, salad, tahini sauce	<50	Absent	<3	Absent	<50	<50	S
Chicken kebab(chicken, tortilla,tomato, lettuce, onion), mild chilli sauce	<50	Absent	3	Absent	<50	<50	M
Lamb, salad, garlic sauce	<50	Absent	<3	Absent	<50	<50	S
Falafel, salad, tabouli, tzatziki	<50	Absent	<3	Absent	<50	<50	S
Chicken, salad, lemon mayonnaise	<50	Absent	<3	Absent	<50	<50	S
Chicken Kebab	<50	Absent	<3	Absent	<50	<50	S
Lamb Kebab	<50	Absent	<3	Absent	<50	<50	S
Beef Kebab (bread, beef, onion, herbs, tomato, lettuce)	<50	Absent	<3	Absent	<50	230000	PH
Falafel	<50	Absent	<3	Absent	<50	100*	M
Tabouli (herbs, tomato, onion)	<50	Absent	3	Absent	<50	<50	M
Lamb Kebab	<50	Absent	<3	Absent	<50	<50	S
Falafel Kebab	<50	Absent	<3	Absent	<50	<50	S
Chicken Kebab	<50	Absent	<3	Absent	<50	<50	S
Chicken kebab (skewer)	<50	Absent	<3	Absent	<50	<50	S
Chicken gozleme	<50	Absent	<3	Absent	<50	<50	S
Falafel kebab	<50	Absent	<3	Absent	<50	<50	S
Mixed Meat Kebab	<50	Absent	<3	Absent	<50	<50	S
Chicken Kebab	<50	Absent	<3	Absent	<50	<50	S

Beef Kebab	<50	Absent	<3	Absent	<50	<50	S
Lamb Kebab	<50	Absent	<3	Absent	<50	<50	S
Chicken Satay Kebab	<50	Absent	<3	Absent	<50	<50	S
Lamb Kebab	<50	Absent	<3	Absent	<50	<50	S
Mixed Kebab	<50	Absent	<3	Absent	<50	<50	S
Falafel Kebab	<50	Absent	<3	Absent	<50	<50	S
Chicken Kebab	<50	Absent	<3	Absent	<50	<50	S
Beef kebab	<50	Absent	<3	Absent	<50	<50	S
Lamb kebab	<50	Absent	<3	Absent	<50	<50	S
Chicken kebab	<50	Absent	<3	Absent	<50	<50	S
Falafel kebab	<50	Absent	<3	Absent	<50	<50	S
Mixed meat Kebab	<50	Absent	<3	Absent	<50	<50	S
Beef meat	*N/A*	*N/A*	*<3*	*N/A*	*N/A*	*<50*	*S*
Tabouli	*N/A*	*N/A*	*<3*	*N/A*	*N/A*	*<50*	*S*
Falafel	*N/A*	*N/A*	*<3*	*N/A*	*N/A*	*<50*	*S*

Italic results are re-samples, * = estimate count only, NP = Not Performed, N/A = Not Applicable.