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Original Article

Microbiological assessment of keropok lekor production in Kuala Terengganu and Marang, Malaysia

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Abstract

Keropok lekor is a popular Terengganu heritage traditional snack and its microbiological safety is one of the important aspects should be of concern. Thus, the present study was carried out to assess microbiological status of keropok lekor, and its production premises in Kuala Terengganu and Marang. A total of 136 samples were collected randomly from eight premises (in three replicates) comprising of raw materials, food contact surfaces and ready to eat (RTE). All samples were analysed for aerobic plate count (APC), total coliforms (TC) count, *Escherichia coli* and detection of foodborne pathogens. Results showed that the APC and TC count in raw materials (fish flesh, sago starch, ice, dough and chilli paste) ranged from below the detection limit (< 1.0 $\log_{10}$ CFU/g) to 6.7 $\log_{10}$ CFU/g and 4.6 $\log_{10}$ CFU/g, respectively. While, food contact surfaces have the APC and TC in the range of < 1.0 to 6.4 $\log_{10}$ CFU/cm^2 and < 1.0 to 4.1 $\log_{10}$ CFU/cm^2, respectively. The food handlers hand swabs had APC and TC counts between 2.2 to 6.4 $\log_{10}$ CFU/cm^2 and < 1.0 to 4.4 $\log_{10}$ CFU/cm^2, respectively. RTE keropok lekor and dipping sauce contained APC in 1.8 to 5.5 $\log_{10}$ CFU/g and < 1.0 to 5.1 $\log_{10}$ CFU/g range, respectively. TC was detected as unsatisfactory level (> 1.7 lo g$_{10}$ CFU/g) in three keropok lekor samples. *E. coli* was found in 10.29% of samples and all of them were non-diarrheagenic serotypes. Two RTE keropok lekor and display containers were contaminated with *E. coli*. Coagulase positive staphylococci, *Salmonella* and *Vibrio parahaemolyticus* were detected in four, two and one samples, respectively, with none of them found to have *Vibrio cholerae* and *Listeria monocytogenes*. High prevalence of indicator organisms in food contact surfaces and food handlers hand indicated that hygiene practices were not well implemented. The unsatisfactory levels of presence of APC, TC and *E. coli* in RTE keropok lekor also described cross contamination due to inadequate hygiene practices after cooking process.

Keywords: Cross contamination, Food contact surfaces, Food handlers, Hygiene, Indicator organisms

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Introduction

Keropok lekor is widely produced throughout the districts of Terengganu. It is directly sold in the processing premises and also widely available in hawker stalls, canteens and restaurants. A total of 102 premises of keropok lekor processing in Terengganu had registered with Terengganu Health State Department (Fosim Domestic, 2017) and only five of them had been certified with MeSTI by Ministry of Health Malaysia (FSQP, 2017). They are SMEs with majority of them are categorised as Micro (employees < 5 persons) and Small Enterprise (employees 5 to 74 persons) (SME Corp. Malaysia, 2018). Without any food safety certification, the implementation of hygiene practices during processing of keropok lekor was questionable, thus, it is a need to have better knowledge and information on their processing and final product safety by a scientific study.

The shelf life of keropok lekor at room temperature is known to be short of only one day possibly due to availability of nutrients that promote microbial growth (Embong et al., 1990). The fresh boiled keropok lekor was actually low in microbial count, however, contamination may occur during cooling process and compromised the microbiological safety and quality of this product. Nor-Khaizura et al. (2009) also reported a significant increment of microbial growth in keropok lekor after cooling process, indicated that cross contamination was occurred during the production of keropok lekor.

Nowadays, consumers have become much more aware of food safety as a result of easy accessible information available in mass-media. This awareness may encourage consumers to look for high quality foods, meaning fresh, tasteful and safe foods. The microbiological profile of food product is one of the important surveillance information in educating food manufacturers to produce safe food products as well as consumers in choosing safe foods.

'Indicator microorganisms' term has been used to mean index organisms, that is, indicators of hygiene and sanitation on food products, equipment and environmental surfaces, to indicate final product quality. The most commonly used indicators in food industry are total viable cell or total plate count (TPC), coliform bacteria and fecal coliform / *Escherichia coli*. The examination of food product for indicator microorganisms will provide a simple, reliable, and rapid information about general level of hygiene, processing failure, post-processing contamination and possibility of the presence of foodborne pathogens, in monitoring food production chain (Halkman and Halkman, 2014).

The microbiological quality of raw materials especially related to pathogens contamination should be of concern, since the possibility for pathogens to transfer to cooked food (cross contamination) is significant (Soares et al., 2012; Tang et al., 2011). Soares et al. (2012) estimated about 40-60% cases of foodborne disease were caused by improper handling practices such as cross contamination of raw materials to end product through the cutting board.

The indigenous pathogens in marine environment such as *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Listeria monocytogenes*, *Salmonella* and *E. coli* are naturally present on live fish (Nilsson and Gram, 2002). Considering that raw fish as the main ingredient in the production of keropok lekor, its quality is an important factor which influences the quality of end product.

Pathogens may access into ready to eat (RTE) keropok lekor from various sources including ingredients, equipment, environment and food handlers due to inappropriate processing conditions. Boiling is one of processing stage in keropok lekor production that reduce microbial level and destroy pathogens, however, recontamination may occur during cooling process. Furthermore, the presence of spoilage bacteria normally restricts the growth of pathogens in fish product through competition, however, heat treatment process such as boiling will eliminate the spoilage bacteria and allows rapid growth of newly introduced pathogenic bacteria in food product (FDA, 2011).

Routine application of sanitation procedure should be capable of eliminating pathogens in food processing plant. Mishandling of food and disregard of hygienic practice by food handlers may enable pathogens to contaminate food products, in some cases, the pathogens may survive and multiply in sufficient numbers to cause illness (Dudeja and Singh, 2017).

Due to the popularity and high consumption of keropok lekor, the microbiological safety of this product is one of an important element should be into account beside their organoleptic qualities in order to prevent health hazards among consumers. Thus, the objectives of this study were to evaluate the microbiological profile of raw materials, food contact surfaces, food handlers hand and RTE keropok lekor taken from eight keropok lekor production premises in Kuala Terengganu and Marang, Terengganu.

Material and Methods

Samples collection

Raw materials (fish flesh, sago starch, ice and chilli paste), dough and RTE (keropok lekor and dipping sauce) samples were collected from eight keropok lekor processing premises in Kuala Terengganu and Marang, Terengganu. Approximately, 250 g of samples were weighed aseptically using a sterile spoon and transferred into a sterile sample collection bag (3M™ plain sample bag). Surface swab of food contact equipment (mixer bowl, mixer hood, chopping board, plastic packaging, display container and freezer internal surface) and food handlers hand were taken using sterile cotton swab (3M™ quick swab). The food contact surfaces were sampled at the time they were clean and ready to use and hand swabs were sampled after food handlers wash their hands. All samples were transported to the laboratory in a box containing small ice cubes to maintain temperature of 1 to 4ºC for analyses within 4 h of sample collection.

Microbiological analyses

Aerobic plate count

Aerobic plate count (APC) for raw materials, RTE and swab samples was tested by using AOAC International (2002) Official Method 990.12: 3M™ Petrifilm™ Aerobic Count Plate (ACP). While, APC for water sample was tested by using the ISO 4833-2 (2013) test method. *Enterobacter aerogenes* ATCC 13048 was used as reference culture to conform the performance of medium used for enumeration.

Coliforms and *Escherichia coli*

Coliforms and *E. coli* in raw materials, RTE and swab samples were enumerated according to AOAC International (2002) Official Method 991.14: 3M™ Petrifilm™ *E. coli* and Coliform Count Plate (ECC). For water sample, coliform and *E. coli* were tested according to Standards Australia (1992) 1766.2.3-1992. *Enterobacter aerogenes* ATCC 13048 and *Escherichia coli* ATCC 11775 were used as control organisms. Isolated *E. coli* cultures were sent to National Public Health Laboratory, Ipoh, Perak for definitive serotyping by using Polymerase Chain Reaction (PCR).

Foodborne pathogens detection

Isolation of coagulase positive Staphylococci was done by referring to the ISO 6888-1 (1999) standard protocol with *Staphylococcus aureus* ATCC 25923 and *Staphylococcus epidermidis* ATCC 13518 as positive and negative control, respectively. *Salmonella* spp. was analysed by using the International Standard protocol ISO 6579 (2002), *Salmonella enterica* subsp. *typhimurium* ATCC 14028 was used as positive control and *Citrobacter freundii* ATCC 43864 was used as negative control in this test. International Standard ISO 21872 (2007) was referred in the isolation of *Vibrio parahaemolyticus* and *Vibrio cholerae*, *V. parahaemolyticus* ATCC 17802 and *V. cholerae* (isolate from National Public Health Laboratory) were used as control organisms. *Listeria monocytogenes* in all samples were analysed by using the International Standard protocol ISO 11290-1 (1996), *Listeria monocytogenes* ATCC 13932 and *Rhodococcus equi* ATCC 6939 were used as reference culture.

Statistical analysis

All statistical analyses were performed using the IBM Statistical Package for Social Sciences, SPSS Version 20 for windows (SPSS Inc., Chicago, Il. USA).

Results and Discussion

Indicator microorganisms

The safety of food products is controlled by applying proper hygiene practices throughout the food chain and decontamination interventions inside the processing plants. To optimize hygiene practices and assure product safety, it is of vital importance to identify and evaluate the contamination sources of foods at each processing step, from primary production to the final products at retail (Sofos, 2014). Hygienic level of raw materials and processing environment may reflect the presence of pathogenic bacteria such as *Salmonella* spp. and *Listeria monocytogenes* (Pérez-Rodríguez et al., 2010), which have been associated with several outbreaks.

Table 1 and 2 demonstrate the Aerobic Plate Count (APC) and total coliforms (TC) counts in samples collected from keropok lekor premises in Kuala Terengganu and Marang, respectively. The APC and TC in raw materials (fish flesh, sago starch, ice, dough and chilli paste) range from below the detection limit (<1.0 $\log_{10}$ CFU/g) to 6.7 $\log_{10}$ CFU/g and 4.6 $\log_{10}$ CFU/g, respectively. All fish flesh samples contained APC below the limit recommended by ICMSF (1986) that is < 7 $\log_{10}$ CFU/g. This is in agreement with the previous finding that raw materials of keropok lekor

contained APC of 5 log_{10} CFU/g to 6 log_{10} CFU/g (Lani et al., 2017). There is actually no evidence that APC have given rise to any health hazard, accordingly, the recommended APC limits reflects the present practice of hygiene of keropok lekor is acceptable (ICMSF, 1986). In fact, the total microbial load of fish is often a poor predictor of freshness or its remaining shelf life (Jack and Read, 2008), however, the transportation, freezing, thawing process and storage time of fish have a major influence on the quality of final product (Sampels, 2015).

The presence of TC in fish is indicator of sewage contamination which may also occur during different processing steps such as transport and handling. Moreover, the contamination may also be caused by the water used for washing or icing (Boyd, 1990).

Table 1. Aerobic Plate Count (APC) in samples collected from keropok lekor processing premises in Kuala Terengganu and Marang.

No.	Sample	Results (Log_{10} CFU/g/ cm^2)							
		Kuala Terengganu				Marang			
		Premise 1	Premise 2	Premise 3	Premise 4	Premise 5	Premise 6	Premise 7	Premise 8
1	**Fish flesh**	5.2 ± 0.07	6.4 ± 0.01	6.1 ± 0.06	5.2 ± 0.01	6.4 ± 0.01	6.2 ± 0.01	6.3 ± 0.09	6.3 ± 0.01
2	**Sago starch**	3.8 ± 0.02	3.8 ± 0.01	4.9 ± 0.05	3.1 ± 0.01	6.1 ± 0.01	5.2 ± 0.03	4.6 ± 0.02	4.2 ± 0.01
3	**Ice**	1.3 ± 0.00	2.0 ± 0.12b	2.3 ± 0.03	2.2 ± 0.01	2.3 ± 0.01	2.5 ± 0.07	3.4 ± 0.03	2.0 ± 0.00
4	**Dough**	6.7 ± 0.01	5.9 ± 0.01	6.1 ± 0.05	3.9 ± 0.01	5.9 ± 0.02	5.3 ± 0.06	6.1 ± 0.02	6.2 ± 0.01
5	**RTE keropok**	4.2 ± 0.00	5.5 ± 0.03	3.5 ± 0.02	3.6 ± 0.01	4.7 ± 0.03	1.8 ± 0.10	2.0 ± 0.00	3.5 ± 0.03
6	**Dipping sauce**	5.1 ± 0.17	< 1.0	< 1.0	< 1.0	4.9 ± 0.01	1.7 ± 0.00	< 1.0	< 1.0
7	**Chilli paste**	6.1 ± 0.02	5.9 ± 0.01	< 1.0	6.0 ± 0.00	6.4 ± 0.01	5.2 ± 0.01	5.0 ± 0.00	< 1.0
Swab									
8	**Sago starch container**	2.9 ± 0.01	6.0 ± 0.01	5.0 ± 0.02	6.4 ± 0.01	4.6 ± 0.04	3.7 ± 0.01	2.7 ± 0.03	6.0 ± 0.01
9	**Freezer internal surface**	2.6 ± 0.02	5.6 ± 0.01	2.9 ± 0.02	6.1 ± 0.00	< 1.0	2.0 ± 0.00	5.6 ± 0.04	< 1.0
10	**Mixer bowl**	3.5 ± 0.01	3.2 ± 0.01	3.4 ± 0.03	3.8 ± 0.02	3.5 ± 0.06	3.7 ± 0.00	3.4 ± 0.06	3.0 ± 0.00
11	**Mixer hood**	3.6 ± 0.01	3.4 ± 0.00	3.5 ± 0.04	3.3 ± 0.02	3.3 ± 0.00	3.5 ± 0.02	3.4 ± 0.06	3.1 ± 0.00
12	**Chopping board**	6.0 ± 0.01	4.1 ± 0.09	5.3 ± 0.01	4.7 ± 0.03	5.0 ± 0.01	5.1 ± 0.01	3.7 ± 0.00	2.5 ± 0.07
13	**Plastic packaging**	1.9 ± 0.03	2.7 ± 0.04	2.5 ± 0.04	1.5 ± 0.02	2.2 ± 0.00	< 1.0	2.0 ± 0.00	< 1.0
14	**RTE keropok display container**	4.2 ± 0.01	6.2 ± 0.00	5.0 ± 0.00	3.8 ± 0.00	4.4 ± 0.03	2.5 ± 0.01	3.5 ± 0.04	< 1.0
15	**Food handler's hand 1**	5.1 ± 0.02	5.0 ± 0.01	6.4 ± 0.01	2.7 ± 0.01	5.8 ± 0.01	4.7 ± 0.02	4.5 ± 0.01	5.1 ± 0.04
16	**Food handler's hand 2**	4.4 ± 0.02	5.2 ± 0.01	3.8 ± 0.03	2.2 ± 0.00	4.0 ± 0.01	2.6 ± 0.01	4.4 ± 0.00	2.7 ± 0.09
17	**Food handler's hand 3**	4.3 ± 0.02	4.6 ± 0.03	3.3 ± 0.02	3.7 ± 0.02	2.8 ± 0.02	2.8 ± 0.01	4.5 ± 0.03	3.0 ± 0.03

*Data expressed as mean ± standard deviation.

*Limit of quantification: 1 Log_{10} CFU/g/cm^2.

Table 2. Total Coliforms (TC) count in samples collected from keropok lekor processing premises in Kuala Terengganu and Marang.

No.	Sample	Results (Log_{10} CFU/g/ cm^2)							
		Kuala Terengganu				Marang			
		Premise 1	Premise 2	Premise 3	Premise 4	Premise 5	Premise 6	Premise 7	Premise 8
1	**Fish flesh**	3.9 ± 0.01	3.2 ± 0.03	2.0 ± 0.04	2.8 ± 0.01	4.6 ± 0.07	1.8 ± 0.06	2.7 ± 0.05	2.5 ± 0.15
2	**Sago starch**	< 1.0	2.1 ± 0.06	2.6 ± 0.02	< 1.0	1.3 ± 0.24	2.6 ± 0.03	< 1.0	< 1.0
3	**Ice**	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
4	**Dough**	3.8 ± 0.01	3.2 ± 0.01	2.0 ± 0.02	2.3 ± 0.02	4.5 ± 0.02	1.6 ± 0.52	2.2 ± 0.16	2.6 ± 0.22
5	**RTE keropok**	4.0 ± 0.01	2.1 ± 0.07	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	2.2 ± 0.15
6	**Dipping sauce**	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
7	**Chilli paste**	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Swab									
8	**Sago starch container**	< 1.0	2.6 ± 0.07	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
9	**Freezer internal surface**	< 1.0	< 1.0	< 1.0	3.3 ± 0.02	< 1.0	< 1.0	< 1.0	< 1.0
10	**Mixer bowl**	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
11	**Mixer hood**	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
12	**Chopping board**	3.2 ± 0.01	2.1 ± 0.02	2.3 ± 0.05	1.1 ± 0.17	1.5 ± 0.15	2.9 ± 0.03	< 1.0	2.5 ± 0.15
13	**Plastic packaging**	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
14	**RTE keropok display container**	3.1 ± 0.00	4.1 ± 0.03	3.5 ± 0.02	< 1.0	3.3 ± 0.06	< 1.0	< 1.0	< 1.0
15	**Food handler's hand 1**	2.9 ± 0.01	2.6 ± 0.01	2.2 ± 0.03	< 1.0	3.1 ± 0.04	< 1.0	2.4 ± 0.10	4.4 ± 0.03
16	**Food handler's hand 2**	< 1.0	1.7 ± 0.05	2.5 ± 0.01	< 1.0	1.6 ± 0.06	< 1.0	< 1.0	< 1.0
17	**Food handler's hand 3**	1.5 ± 0.07	2.1 ± 0.02	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0

*Data expressed as mean ± standard deviation.
*Limit of quantification: 1 Log_{10} CFU/g/cm^2.

TC was not quantified in all ice and chilli paste samples tested. From the observation during sampling process, ice used in the making of keropok lekor in all premises were bought from ice production factories which were known to produce ice form treated and filtered water under controlled condition, this would be the reason of the absence of TC in ice samples. Chilli paste is slurry obtained from grinding chillies, it moist nature provides favourable conditions for growth of microorganisms, thus chemical preservative is always used in prolonging its shelf life. According to Malaysian Food Act 1983 and Regulations in Malaysian Law (2016), benzoic acid is allowed to be present in chilli paste in a maximum level of 1000 mg/kg. The chemical preservative reaction could be the reason of the inhibition of TC in chilli paste.

Flour including sago starch is generally regarded as a microbiologically safe product as it is a low water activity commodity which inhibits growth of microorganisms during storage (ICMSF, 1998). The presence of APC and TC in raw materials may influence the quality of final product produced, however, these microorganisms will be destroyed during processing stages especially in boiling process. An adequate processing conditions and proper hygiene practices will produce safe foods even though indicator microorganisms were found in raw materials used.

Food contact surfaces have the APC and TC in the range of < 1.0 to 6.4 $\log_{10}$ CFU/cm^2 and < 1.0 to 4.1 $\log_{10}$ CFU/cm^2. General standard reference for cleaned food-contact surfaces and hands toward APC and TC are < 1.3 and < 1.0 $\log_{10}$ CFU/cm^2, respectively (Henroid et al., 2004; Sneed et al., 2004; Balzaretti and Marzano, 2013). From all surface swab samples, 91% of them contain APC at unsatisfactory level. Besides that, 16% of all surface swab samples were unsatisfactory in TC count with 78% of them were chopping board swabs. Among several food contact surfaces, chopping boards are notorious for their potential to cross contaminate foods with spoilage and/or disease causing microorganisms (Yemmireddy and Hung, 2017).

Chopping board is required to thoroughly washed and sanitized after each use to avoid the potential risk of microbial cross-contamination in foods. Previous studies show that cleaning with disinfectants such as hypochlorite and quaternary ammonium significantly reduce the number of viable bacteria on contaminated kitchen surfaces and dishcloths, whereas cleaning with detergent and hot water was much less effective (Thormar and Hilmarsson, 2012). The high prevalence of APC and TC on food contact surfaces especially chopping board in this study indicated insufficient cleaning and sanitizing procedures toward kitchen utensils. This finding also could be the sign of possible development of the pathogens persistence on food contact surface, where the pathogens colonized and survived for prolonged period of time in the food processing environment and surface (Larsen et al, 2014). Persistency of pathogens can cause repeated food contamination and an increasing risk of foodborne illness to humans exposed to the contaminated food. According to U.S. Food and Drug Administration (FDA), contaminated surfaces are among the top 5 risk factors contributing to several foodborne outbreaks (FDA, 2000).

The food handlers hand swabs were found to have APC and TC count between 2.2 to 6.4 $\log_{10}$ CFU/cm^2 and < 1.0 to 4.4 $\log_{10}$ CFU/cm^2, respectively. The workers washed hands showed 100% unsatisfactory for APC and 46% of them exceeded satisfactory limit in TC. These results suggested that hand washing procedure was not adequately performed by food handlers. In fact, other studies have demonstrated that washing hands efficiently can reduce the microbial load present on hands, surfaces, and in prepared meals (Martinez-tomè et al., 2000; Tessi et al., 2002; Michaels et al., 2004).

Ready to eat (RTE) keropok lekor and dipping sauce taken from all the premises contain APC in 1.8 to 5.5 $\log_{10}$ CFU/g and < 1.0 to 5.1 $\log_{10}$ CFU/g range, respectively. The APC in all keropok lekor samples were in the satisfactory level which were less than 7 $\log_{10}$ CFU/g as described in Table 15, Regulation no. 39 in the Malaysian Law (2016). Dipping sauce is not listed in the interpretation guide in Malaysian Law, but, the maximum 5.1 $\log_{10}$ CFU/g in APC is generally considered as satisfactory. TC was detected in unsatisfactory level in three keropok lekor samples counted 2.1, 2.2 and 4.0 $\log_{10}$ CFU/g, as stated in Table 15, Regulation no. 39, the Malaysian Law (2016), RTE fish and fish products should contain coliforms in less than 1.7 $\log_{10}$ CFU/g. TC was not enumerated in all dipping sauce samples. Dipping sauce is produced by mixing chilli paste with a food grade acetic acid and some sugar. The absence of TC in dipping sauce may be due to its acidic condition and prolonged effect of chemical preservative from chilli paste. The unsatisfactory of APC and TC in food are indicators of low sanitation and could signify unhygienic conditions during food handling and preparation (Maguiat and Fang, 2013).

Fecal microorganisms

There were 14 (10.29%) samples contaminated with *E. coli*. Table 3 presents the counts and serotypes of the 14 *E. coli* isolates. All isolates were non-diarrheagenic serotypes. *E. coli* were detected in two fish flesh samples. ICMSF (1986) suggested that faecal coliforms and *E. coli* are particularly useful as indicators of contamination and mishandling of fish since the organisms are absent at the time of capture except in grossly polluted waters. Moreover, fish should be held at temperatures below those which support growth of *E. coli* or faecal coliforms, thus the presence of *E. coli* indicates contamination, while relatively large numbers suggest temperature abuse in product handling.

Two RTE keropok lekor and two RTE display container swabs were contaminated with *E. coli*, which indicate fecal contamination in RTE keropok and its contact surface. This may have resulted from cross contamination of *E. coli* from raw materials, equipment or food handlers due to inadequate hygiene and sanitation practices during processing of keropok lekor.

Table 3. *Escherichia coli* counts and serotypes in 14 contaminated samples.

No.	Sample	Premise	*E. coli* count (Log_{10} CFU/g/cm^2)	Serotype
EC1	RTE keropok	1	3.7 ± 0.02	Non-diarrheagenic
EC2	Display container	1	3.8 ± 0.02	Non-diarrheagenic
EC3	Hand swab	1	1.7 ± 0.09	Non-diarrheagenic
EC4	Display container	2	4.7 ± 0.03	Non-diarrheagenic
EC5	Hand swab	3	1.6 ± 0.07	Non-diarrheagenic
EC6	Hand swab	3	1.1 ± 0.17	Non-diarrheagenic
EC7	Fish flesh	4	1.2 ± 0.17	Non-diarrheagenic
EC8	Dough	4	1.1 ± 0.02	Non-diarrheagenic
EC9	Chopping board	4	1.1 ± 0.17	Non-diarrheagenic
EC10	Fish flesh	5	1.9 ± 0.03	Non-diarrheagenic
EC11	Dough	7	2.2 ± 0.17	Non-diarrheagenic
EC12	Dough	8	2.6 ± 0.11	Non-diarrheagenic
EC13	RTE keropok	8	2.1 ± 0.17	Non-diarrheagenic
EC14	Hand swab	8	2.7 ± 0.06	Non-diarrheagenic

*Data expressed as mean ± standard deviation.

In relation, *E. coli* also detected in four hand swabs samples even though all hand swabs were sampled after food handlers wash their hands, these results suggest that hand washing procedure was not adequately performed by food handlers. Tan et al. (2014) also reported the occurrence of *E. coli* (9.41 - 14.12%) on food handler's hand in their study which was comparable with the finding in this study (16.67%). The existing of this fecal bacteria on food handler's hands is likely due to the improper hand washing procedure and from observation done by Tan et al. (2014), some food handlers did not wash their hands before or after processing food.

Pathogens detection

Some samples tested in this study were found to carry coagulase positive Staphylococci (CPS), *Salmonella* and *Vibrio parahaemolyticus*, results were detailed in Table 4.

CPS is known to include *Staphylococcus aureus* as well as *Staphylococcus intermedius*, *Staphylococcus hyicus* and *Staphylococcus delphini* (Normanno et al., 2005). Enterotoxins involved in food poisoning are produced by approximately one-third of *S. aureus* strains (Halpin-Dohnalek and Marth, 1989), in addition, other CPS may also be enterotoxigenic (Vernozy-Rozand et al., 1996). CPS usually originated from humans, animals and environment (Gotz et al., 2006), they may contaminate foods at any stages of preparation (Kennedy et al., 2011).

Table 4. The detection of coagulase positive Staphylococci, *Salmonella* and *Vibrio parahaemolyticus*

Pathogens	Results		
	Sample	Premise	Count / Detection
Coagulase positive Staphylococci	Sago starch	1	2.1 ± 0.03 log_{10} CFU/g
	Hand swab	1	2.6 ± 0.02 log_{10} CFU/cm^2
	Hand swab	1	3.1 ± 0.07 log_{10} CFU/cm^2
	Fish flesh	8	2.4 ± 0.02 log_{10} CFU/g
Salmonella	Fish flesh	2	Detected / 25 g
	Dough	8	Detected / 25 g
Vibrio parahaemolyticus	Fish flesh	3	Detected / 25 g

*Data expressed as mean ± standard deviation.

Staphylococcus as commensal biota in human body is frequently found on anterior nares and skin surfaces (Alves et al., 2014; Argudín et al., 2012). In this study, CPS was detected in hand swabs and raw materials (sago starch and fish flesh) (Table 4). The detection of CPS in hand swabs indicating the least practiced habits toward proper hand washing and the usage of

face mask among food handlers, as reported before by Tan et al. (2013) in their study on food hygiene practices by food handlers in Malaysia. This would facilitate the transmission of CPS into food processing line which include raw materials (as found in this study), food contact equipment and furthermore into RTE foods. This transfer may induce CPS related food poisoning which mainly due to the effect of enterotoxin production by these microorganisms. Tan et al. (2014) reported high prevalence of *S. aureus* (65.88 - 74.12%) among food handlers which is higher than found in this study (8.33%), indicating that they did not maintain good personal hygiene during food handling. It also may be due to the contamination introduced by food handlers through skin lesions, sneezing or coughing (Bischoff et al., 2006). The main reasons for Staphylococcal food poisoning are improper cooling and holding temperature, inadequate personal hygiene, and food handlers as carriers. Therefore, to prevent staphylococcal contamination, food has to be handled in accordance with the good hygiene practice, staff handling food has to be healthy and trained especially in washing hands and wearing adequate hygiene clothing (Ebert, 2018).

Salmonella was detected in one fish flesh and dough sample, while *Vibrio parahaemolyticus* was detected in one fish flesh sample. Biochemical tests done to confirm the presence of both pathogens were showed in Table 5. The *Vibrio parahaemolyticus* isolate showed Gram negative, curved rod cells and motile under microscopic examination.

All *Salmonella* and *V. parahaemolyticus* were detected in raw samples. Fishery products have been recognized as a carrier of foodborne pathogens (Kamat et al., 2002; Upadhyay et al., 2010) including *Salmonella*, as a result of contact with contaminated coastal water and *V. parahaemolyticus*, that is indigenous in marine environment (Nilsson and Gram, 2002; Cho et al., 2016). The presence of these foodborne pathogens is a significant public health issue due to the possibility of pathogens from raw materials to recontaminate cooked food through various agents such as equipment and food handlers. Certainly, the primary cause of cross-contamination is improper or lacking sanitation procedures, temperature controls or the inclusion of improper ingredients. This situation offers a high risk of infection to develop foodborne illness.

Under Malaysian Food Regulations 1985, Regulation no. 39, all RTE foods contaminated with pathogenic microorganisms cannot be sold to the public (Malaysian Law, 2016). Thus, besides developing health issue, cross-contamination of pathogens from raw materials or food contact surfaces to RTE keropok lekor may also cause the manufacturers to be punished under legislative provision of Malaysia.

Vibrio cholerae and *Listeria monocytogenes* were not detected in all samples tested. The absence of these pathogens in this study may not reflect their absence in keropok lekor processing line, since only eight raw fish samples analysed. Furthermore *V. cholerae* and *L. monocytogenes* have been associated with seafood and seafood related products for causing food poisoning (Ottaviani et al., 2009; Kramarenko et al., 2016). In 2009, cholera outbreak was also reported in Terengganu with 185 cases and one of the suspected carrier of *V. cholerae* is keropok lekor (The Star Online, 2009). The Health Minister of Malaysia had reported more serious issue related to this outbreak, that is some strain of *V. cholerae* isolated from infected patients were resistant toward commonly used antibiotics in hospital (Utusan Malaysia, 2009). The El Tor O1 *V. cholerae* which is responsible for the cholera outbreak in Terengganu in 2009, were found resistant to ampicillin, trimethoprim/ sulfamethoxazole, erythromycin, and tetracycline which were commonly used in cholera treatment.

This antibiotic resistant strain had developed difficulties and the medical practitioners have to find other antibiotics to be used and it is time consuming resulted the recovery process among patients to be delayed. The relevance of *V. cholerae* and other *Vibrio* with keropok lekor is due to the nature of raw fish flesh which is the main ingredient of keropok lekor. Thus, good hygiene practice is crucial in the whole processing line of keropok lekor production to prevent the spread of *Vibrio* into environment and finished products. Until this present study, no other outbreak of cholera was reported in Terengganu. This is a good sign of an improvement in the management of sanitation practices by food industries related to fish and fish products. The undesirable scenarios attributed from food poisoning should be avoided by inhibiting pathogens from disseminating in food and environment by implementation of good hygiene practices among food producers and consumers as well.

Table 5. Biochemical confirmation tests for *Salmonella* and *Vibrio parahaemolyticus* isolates.

Tests	Results		
	Salmonella		*Vibrio parahaemolyticus*
	Fish flesh (premise 2)	Dough (premise 8)	Fish flesh (premise 3)
TSI	K/A, gas + , H_2S +	K/A, gas + , H_2S +	K/A, gas - , H_2S -
Urea hydrolysis	-	-	NA
Lysine decarboxylation	+	+	+
β-galactosidase reaction	-	-	-
Indole reaction	-	-	+
O-antigens	+	+	NA
H-antigens	+	+	NA
Vi-antigens	-	-	NA
Oxidase	NA		+
Ornithine decarboxylase	NA		+
Growth in 0% NaCl	NA		-
Growth in 2% NaCl	NA		+
Growth in 6% NaCl	NA		+
Growth in 8% NaCl	NA		+
Growth in 10% NaCl	NA		-

* K/A = alkaline slant (red)/acid butt (yellow) indicate lactose negative, sucrose negative, glucose positive.
* + = positive reaction; - = negative reaction.
* NA = not applicable.

Conclusion

General level of hygiene in keropok lekor processing need to be improved due to high prevalence of indicator organisms in food contact surfaces and also food handlers' hands. APC, TC and *E. coli* were also found in unsatisfactory levels in RTE keropok lekor, suggested that cross contamination had taken place, most probably due to inadequate hygiene practice after cooking. Keropok lekor industry in Terengganu is in the category of small or medium scale, poor infrastructure and employee turnover may contribute to challenges for the industry to implement the appropriate food handling procedures.

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Contribution of Authors

Hamat HW: Conducting the research and investigation process, specifically data and evidence collection and acquisition of the financial support for the project.
Lani MN: Responsibility for supervising research and principal investigator
Hamzah Y: Co-supervisor and coordinator of research activities leading to this publication
Alias R: Co-supervisor and technical expert for microbiological techniques
Hassan Z: Mentoring and guiding the research ideas and formulation of research questions

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