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MICROBIOLOGICAL EVALUATION ON RAW MATERIALS AND FOOD CONTACT SURFACES OF 'KEROPOK LEKOR' PREMISES IN KUALA NERUS, TERENGGANU AND THEIR PREVALENCE OF ANTIBIOTIC RESISTANT BACTERIA

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ABSTRACT

'Keropok lekor' is a popular street food and widely available in Terengganu. However, there is limited study on the microbiological status of sources of contamination from 'keropok lekor' premises in Terengganu. Microbiological quality of raw materials and food contact surfaces were determined by Total Plate Count, coliform count, *Staphylococcus* spp. and *Vibrio* spp. count. The presumptive of *Escherichia coli*, *Staphylococcus aureus* and *Vibrio* spp. were further identified by phenotypic identification such as IMViC tests and API20 NE identification system. The antibiotic resistance of the identified bacteria was determined using disc diffusion method. *Escherichia coli* and *Vibrio* were predominant microorganisms isolated from raw materials, whereas *Staphylococcus* was the predominant microorganism on food contact surfaces. The boiling process of 'keropok lekor' at 100°C was significantly ($p < 0.05$) reduced the Total Plate Counts, coliform, *Staphylococcus* spp. and *Vibrio* spp. count for safe consumption. The identified antibiotic resistance of *E. coli*, *S. aureus* and *Vibrio* spp. showed that the bacteria were within controllable emergence strains, whereby the present antibiotics used are able to suppress the growth. Regular monitoring programme is essential to further improve the microbiological quality of raw materials and food contact surfaces.

Key words: 'keropok lekor', raw materials, food contact surfaces, microbiological evaluation, antibiotic resistance

INTRODUCTION

In Malaysia, 'keropok lekor' is one of the traditional processed fish products and the popular street foods in Terengganu. There are 115 'keropok lekor' premises registered with the Terengganu Health State Department (Ministry of Foreign Affairs (FoSIM) Domestic, 2014). 'Keropok lekor' is in the form of sausage which is chewy and can be consumed either boiled or fried. The main ingredients of 'keropok lekor' are minced freshwater fishes, water, sago flour or/and tapioca flour, salt, monosodium glutamate (MSG), sugar and flavouring. The 'keropok lekor' production involves five stages, which comprises of mincing, mixing the minced fish with other ingredients, kneading and moulding the dough, cooking and cooling the product before packaging (Wan-Md-Hatta, 2015).

Despite the processing conditions, raw material quality and sanitation practices of 'keropok lekor' premises are also the main factors that affect the quality of the 'keropok lekor' produced. Fresh fish usually cause less microbial contamination compared to chill-stored or frozen-stored fish and different types of fish may have different initial microbial loads. Besides, the quality of tap water may affect the contamination level of ready-to-eat food like 'keropok lekor' (Gram *et al.,* 2007;

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Kumar & Anand, 1998). Moreover, a mechanized production process greatly reduces the direct surfaces and hands contact during the handling of this kind of processing, which offers a better control on sanitation, provided the machines are in good sanitary condition (Philip, 2015).

'Keropok lekor' is known as the ready-to-eat foods which usually consumed upon purchase, without further processing. However, it is a perishable product with a short shelf life at ambient temperature and turned organoleptically unacceptable in a few days (Nor-Khaizura *et al*., 2009). In fact, most of the 'keropok lekor' premises in Kuala Terengganu are small and medium enterprises (SMEs) without MeSTI or GMP certification (Philip, 2015). In order to assure the quality assurance of ready-to-eat foods, Food and Agriculture Organisation of United Nations had encouraged all countries to take initiative to create and develop microbiological database for the food industry on microbiological level of food which can be used as the basic regulations and references for food quality assurance to protect consumers (FAO/ WHO, 2002).

There are urgent needs to establish the microbiological quality of ready-to-eat foods in Malaysia. Previous studies showed that each processing stage of 'keropok lekor' had a different microbiological profile and microbial counts except for *Vibrio* spp. count (Nor Khaizura *et al.,* 2010; Tang *et al.,* 2014). Nevertheless, it is important to investigate the microbiological status of the fish product industry in Kuala Nerus because it is one of the largest 'keropok lekor' producer in Terengganu (Ministry of Foreign Affairs (FoSIM) Domestic, 2014). In addition, no study has ever reported on the antibiotic resistance of pathogenic bacteria strains isolated from 'keropok lekor'. It is known that any ready-to-eat foods that contaminated by antibiotic resistant bacteria may have high potential as vehicle to cause foodborne diseases. Therefore, the determination of antibiotic resistance among the predominant bacteria found in 'keropok lekor' is important for determining whether the emergence of new antibiotic resistance become prevalent in local food products.

The objectives of this study were to determine the microbiological quality of raw materials and food contact surfaces from different sources of contamination among 'keropok lekor' premises in Kuala Nerus, Terengganu. Further identification of *Escherichia coli* and *Vibrio* using IMViC tests and API20 NE identification system, and confirmation of *S. aureus* was carried out using amplification of *nuc* gene by Polymerase Chain Reaction. Then, the antibiotic resistance of identified *E. coli*, *S. aureus* and *Vibrio* spp. were determined using disc diffusion method.

MATERIALS AND METHODS

Samples

Raw materials, food handlers and swabs from food contact surfaces were collected in August and September 2015 from four different premises in Kuala Nerus, Terengganu which were labelled as A, B, C and D. The sources of possible contamination of each 'keropok lekor' premises consist of raw materials (water, fish or minced fish and fish dough at each processing stage), three food handlers (2 persons involved in kneading and 1 person involved in packaging) and 5 food contact surfaces (mincing machine, mixing machine, basin, preparation table and container) of the premises. The whole experiments were repeated twice for all four premises in order to obtain the average data for statistical analysis.

Food sampling and storage

100 g for each sample unit was obtained by random sampling of collectively four parts of each sample at 25 g. Plastics which were clean, dry, leak-proof, sterile with suitable size were used for sample collection. For the sampling of food contact surfaces, swab method (sterile cotton bud was wetted in 5 mL of 0.1% peptone water) was used (Yousef & Carlstrom, 2003). All samples were kept cooled in ice box at 4°C with a proper label, delivered to the laboratory and examined immediately.

Microbiological analysis of raw materials of 'keropok lekor' processing

Raw materials of 'keropok lekor' processing involved water, raw fish or minced fish and fish dough were tested on the microbiological quality such as Aerobic Plate Count and coliform count. Upon arrival in the laboratory, 25 mL/g of samples were added to each sterile stomacher bags and homogenized for 3 minutes using a stomacher (BagMixer, Interscience, France). Detection and enumeration of Aerobic Plate Count, *Escherichia coli* count, *Staphylococcus* count and *Vibrio* count were conducted according to the methods described by Bacteriological Analytical Manual (Merker, 1998).

Suspensions (1 mL) were drawn out from the homogenized sample and subjected to ten-fold serial dilution. The homogenate aliquots (0.1 mL) from each dilutions were spread on plate count agar (PCA) incubated at 35°C for 24 h for Aerobic Plate Count, Eosin Methylene Blue incubated at 35°C for 24 h for *E. coli* count (Feng *et al*., 2002), Baird Parker Agar supplemented with Tellurite Yolk Egg for *S. aureus* count incubated at 35°C for 48 h (Bennett & Lancette, 2001) and Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) incubated at 35°C for 24 h for *Vibrio* count (Pfeffer & Oliver, 2003). The

procedures for coliform count in water sample was tested using 11-tubes Most Probable Number method (Feng *et al*., 2002). All microbiological media were purchased from Merck, Germany.

Identification of isolated bacteria from raw materials of *'keropok lekor'* processing and determination of antibiotic resistance among identified *E. coli*, *S. aureus* and *Vibrio*

Gram staining, oxidase test and catalase test were done prior to proceeding to specific biochemical tests, such as IMViC tests and API 20NE (Awong-Taylor *et al*., 2008) for the identification of Enterobacteriaceae. However, for identification of *S. aureus*, the presumptive isolates of this organism was further confirmed using amplification of *nuc* gene by Polymerase Chain Reaction method described by Saiful *et al.* (2006). Then, the antibiotic resistance of identified *E. coli*, *S. aureus* and *Vibrio* spp. were determined using disc diffusion method (Clinical and Laboratory Standards Institute, 2009; Balouiri *et al*., 2016).

Microbiological analysis of swab samples of food handlers and food contact surfaces

Sampling of food contact surfaces (palm of food handlers and mincing machine, mixing machine, basin, preparation table and container) were performed by swabbing a delimited area (100 cm^2) according to methods described by Yousef & Carlstrom (2003). Swab head rubbed slowly and thoroughly over an area of 50 cm^2 of sampled area as mentioned earlier. Then, rinsed swab head into 10 mL of 0.1% sterile buffered peptone water (Merck, Germany) and pressed out the excess. Using the same swab head, it was repeated for other 50 cm^2 area. The swab was broken off, while the head was remained. All swab samples were placed in an ice-cooled box and transported to the laboratory for analysis.

Statistical analysis

All data were presented as mean ± standard deviation. One-way ANOVA of multiple comparisons Tukey's Honest Significant Difference (Tukey's HSD) was used to analyze the significance difference of means at (p<0.05) between sources of contamination and premises respectively.

RESULTS

Microbiological quality of raw materials involved during 'keropok lekor' preparation

Figure 1 shows the Total Plate Count of raw materials from four 'keropok lekor' premises in Kuala Nerus, Terengganu. There was a significant different (p<0.05) of Total Plate Count in raw materials among premises. The minced fish, mixed dough and kneaded dough were the processing steps involved in 'keropok lekor' prior to boiling showed 5 $\log_{10}$ to 6 $\log_{10}$ CFU/g indicating that raw materials involved in 'keropok lekor' contained high population of bacteria. However, the Total Plate Count of boiled dough were significantly reduced (p<0.05) after boiling. This is in agreement with the previous finding that boiling can reduce the Total Plate Count of 'keropok lekor' by 2.66 $\log_{10}$ CFU/g (Nor-Khaizura *et al.,* 2010).

The *Staphylococcus* counts of raw materials from four 'keropok lekor' premises in Kuala Nerus is shown in Figure 2. There was a significant different (p<0.05) between of *Staphylococcus* count in raw materials among 'keropok lekor' premises.

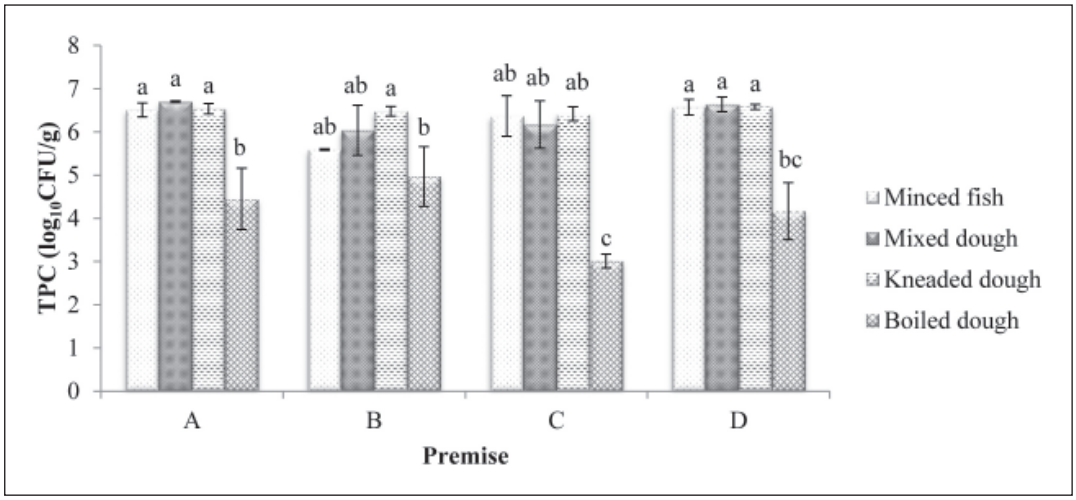

Fig. 1. Total Plate Count of raw materials from four *'keropok lekor'* premises.
Note: values are mean ± standard deviation ($\log_{10}$ CFU/g) of 3 replicates; a-c: Value with the same superscript letter are not significantly different (p > 0.05).

The mean *Staphylococcus* count of minced fish, mixed dough and kneaded dough for all premises were in the range of 4.00 to 4.50 $\log_{10}$ CFU/g, however, after boiling, the *Staphylococcus* counts were significantly reduced around 2 $\log_{10}$ CFU/g. This finding showed a better boiling effect than previous study reported by Nor- Khaizura *et al.,* 2009.

The *Vibrio* count of raw materials from four 'keropok lekor' premises in Kuala Nerus is displayed in Figure 3. There was a significant different ($p<0.05$) between of *Vibrio* count in raw materials among 'keropok lekor' premises. The mean *Vibrio* count of minced fish, mixed dough and kneaded dough for all premises were in the range of 3.00 to 4.00 $\log_{10}$ CFU/g, however, after boiling, the *Vibrio* counts were completely eliminated. Boiling of 'keropok lekor' is effective processing method in eliminating *Vibrio* completely.

Microbiological quality of food contact surfaces involved during 'keropok lekor' preparation

Table 1 shows the Total Plate Count of food contact surfaces from four 'keropok lekor' premises in Kuala Nerus, Terengganu. The mean Total Plate Count of food contact surfaces were not significantly different ($p>0.05$) in the range of 3.48 $\log_{10}$ to 4.04 $\log_{10}$ CFU/cm^2. For premise A, B and C, fish such as sardines and round scad or mixture of both fishes were normally used for 'keropok lekor' production while premise D used minced fish paste. Besides, the level of automated production for each 'keropok

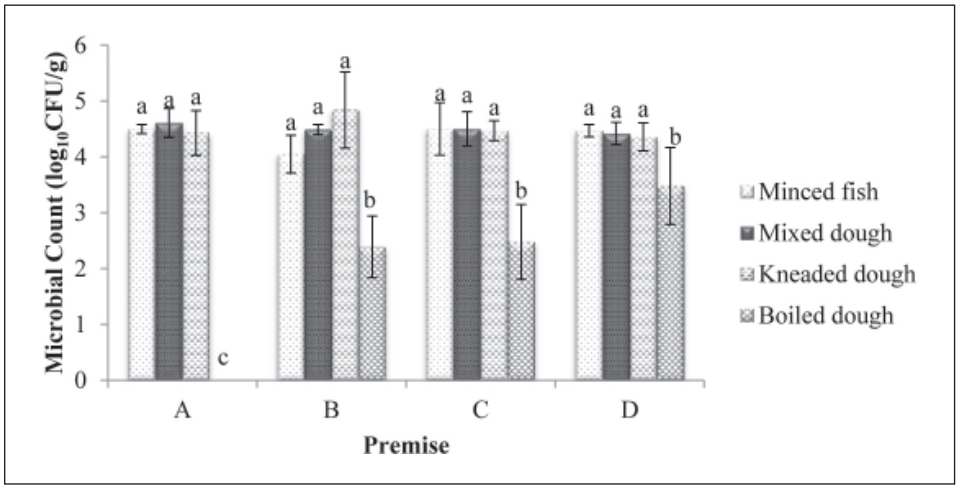

Fig. 2. *Staphylococcus* count of raw materials from four *'keropok lekor'* premises.
Note: values are mean ± standard deviation ($\log_{10}$ CFU/g) of 3 replicates; a-c: Value with the same superscript letter are not significantly different ($p > 0.05$).

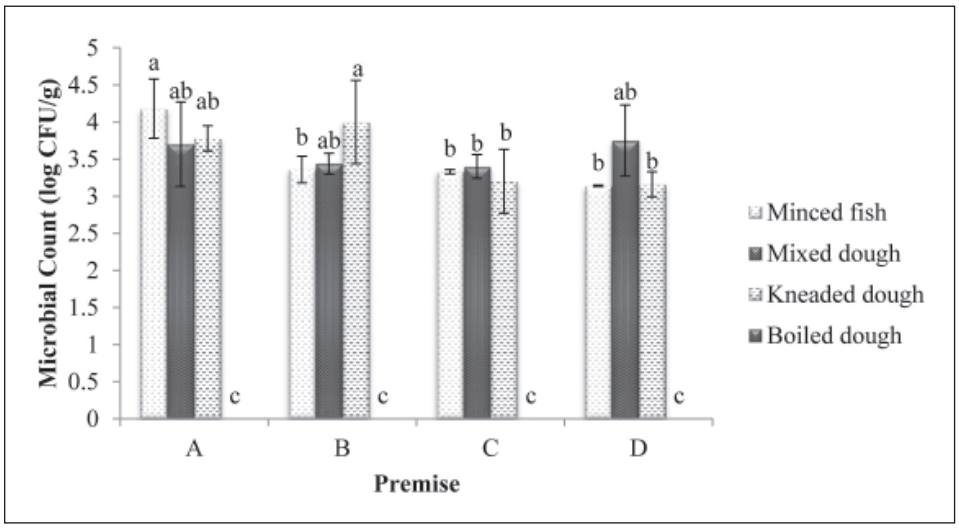

Fig. 3. *Vibrio* count of raw materials from four *'keropok lekor'* premises.
Note: values are mean ± standard deviation ($\log_{10}$ CFU/g) of 3 replicates; a-c: Value with the same superscript letter are not significantly different ($p > 0.05$).

Table 1. Total Plate Count of food contact surfaces from four *'keropok lekor'* premises

Premise	Food contact surface					Mean Total Plate Count (log_{10} CFU/cm^2)
	Mincing machine	Mixing machine	Basin	Preparation table	Container	
A	5.20 ± 0.17	3.72 ± 0.03	3.80 ± 0.26	3.15 ± 0.52	2.47 ± 0.24	3.69[a]
B	3.68 ± 0.76	3.02 ± 0.46	4.26 ± 0.36	4.42 ± 0.19	2.67 ± 0.58	3.61[a]
C	4.14 ± 0.50	4.02 ± 0.86	3.30 ± 0.85	4.72 ± 0.26	4.00 ± 0.70	4.04[a]
D	3.48 ± 0.13	3.47 ± 0.16*	3.70 ± 0.78	4.31 ± 0.04	2.45 ± 0.64	3.48[a]

Note: values are mean ± standard deviation (log_{10} CFU/cm^2) of 3 replicates; a: Value with the same superscript letter within the same column are not significantly different ($p > 0.05$).

Table 2. Coliform count of food contact surfaces from four *'keropok lekor'* premises

Premise	Food contact surface					Mean coliform count (log_{10} CFU/cm^2)
	Mincing machine	Mixing machine	Basin	Preparation table	Container	
A	4.56 ± 0.06	3.50 ± 0.24	3.12 ± 0.63	4.11 ± 0.86	2.62 ± 0.68	3.58[a]
B	2.93 ± 0.92	3.20 ± 0.59	3.14 ± 0.63	4.32 ± 0.06	2.32 ± 0.23	3.18[a]
C	3.55 ± 0.85	3.22 ± 0.54	3.66 ± 0.01	3.19 ± 0.64	2.00 ± 0.99	3.12[a]
D	3.36 ± 0.11	3.42 ± 0.11*	3.44 ± 0.04	3.78 ± 0.06	2.61 ± 0.02	3.32[a]

Note: values are mean ± standard deviation (log_{10} CFU/cm^2) of 3 replicates; a: Value with the same superscript letter within the same column are not significantly different ($p > 0.05$).

lekor' premise was different. The level of automated production was ranged from premise C, premise A, premise B and premise D in descending order. The microbiological quality of food contact surfaces were varied among 'keropok lekor' premises.

Table 2 shows the coliform count of food contact surfaces from four *'keropok lekor'* premises in Kuala Nerus, Terengganu. The mean coliform count of food contact surfaces were not significantly different ($p>0.05$) in the range of 4.72 log_{10} to 5.42 log_{10} CFU/cm^2. The mean value of coliform count of mincing machine, mixing machine, basin and preparation table were higher (ranged from 3.34 to 3.85 log_{10} CFU/cm^2) than the container used to put the end product of 2.39 log_{10} CFU/cm^2, but not significantly different ($p>0.05$) among the 'keropok lekor' premises. Although coliform were found in diversified natural environments, positive coliform growth in the food products showed cross-contamination occurs during preparation of 'keropok lekor'.

Identification *Escherichia coli*, *Staphylococcus aureus* and *Vibrio* spp.

There were 34 suspected colonies isolated from four 'keropok lekor' premises, which showed positive green sheen on Eosine-methylene blue agar. For water samples, confirmed fecal coliform such as *E. coli* showed positive growth in Brilliant green bile 2% broth and/or EC broth, when incubated at the elevated 44.5ºC (Yousef & Carlstrom, 2003). Table 3 shows the summary of identified bacteria from suspected *E. coli* isolates from different sources of contamination of four 'keropok lekor' premises. There were 7 (20.6%) positive *E. coli* identified by matching the result with the Identification flow charts of Bergey's Manual of Determinative Bacteriology (Whitman *et al*., 2012). However, there were another 9 types of negative *E. coli* bacteria found from these presumptive bacteria, which belonged to family of Enterobacteriaceae. Results showed that 7 isolates (20.6%) were identified as *Aeromonas* spp., 5 isolates (14.7%) were identified as *Serratia* spp., and *Pseudomonas* spp. respectively, 2 isolates (5.89%) were identified as *Serratia fonticola*, *Proteus vulgaris* and *Klebsiella pneumonia*, respectively and 1 isolate (2.94%) was identified as *Citrobacter diversus*, *Citrobacter freundii* and *Enterobacter* spp. respectively.

There were 29 of suspected *S. aureus* colonies isolated from four 'keropok lekor' premises, which showed shiny black with clear zone on Baird-Parker Agar. The result of genotypic identification of suspected *S. aureus* isolates by Polymerase Chain Reaction (PCR) amplification on *nuc* gene is summarized in Table 4. Positive *S. aureus* produced a single DNA fragments of approximately 278 base

Table 3. Summary of the identified bacteria using IMViC tests from different sources of contamination of four *keropok lekor* premises

Premise	Sample	Identified bacteria								
		Aeromonas spp.	*C. diversus*	*C. freundii*	*E. coli*	*Enterobacter* spp.	*S. fonticola*	*Serratia* spp.	*P. vulgaris*	*Pseudomonas* spp.
A1	Fish	√	–	–	–	–	–	–	–	–
	Minced fish	√	–	–	–	–	–	–	–	–
	Mixed dough 1	–	–	–	–	–	–	–	√	–
	Mixed dough 2	√	–	–	–	–	–	–	–	–
	Water	–	–	–	√	–	–	–	–	–
A2	Fish	–	√	–		–	–	–	–	–
	Minced fish	–	–	–	√	–	–	–	–	–
	Mixed dough	–	–	–	√	–	–	–	–	–
	Kneaded dough	–	–	√	–	–	–	–	–	–
	Mincing machine	√	–	–	–	–	–	–	–	–
	Water	–	–	–	√	–	–	–	–	–
B1	Fish	–	–	–	–	–	–	–		√
	Mixed dough	–	–	–	–	–	–	–	√	–
	Kneaded dough	–	–	–	–	–	–	–	–	√
	Mincing machine	–	–	–	–	–	√	–	–	–
B2	Minced fish	–	–	–	–	√	–	–	–	–
	Mincing machine	–	–	–	–	–	–	√	–	–
	Container	–	–	–	–	–	√	–	–	–

Premise	Sample	Identified bacteria				
		Aeromonas spp.	*E. coli*	*K. pneumoniae*	*Serratia* spp.	*Pseudomonas* spp.
C1	Kneaded dough	–	–	–	–	√
	Third food handler	–	–	–	–	√
C2	Mixed dough 1	–	√	–	–	–
	Mixed dough 2	√	–	–	–	–
D1	Minced fish	–	–	–	√	–
	Minced dough 1	–	–	–	–	–
	Minced dough 2	–	–	–	√	–
	Mixed dough	–	√	–	–	–
	Second food handler	–	–	–	√	–
	Mixing machine	–	–	–	–	√
	Container	√	–	–	–	–
D2	Minced fish 1	–	√	–	–	–
	Minced fish 2	√	–	–	–	–
	Mixed dough	–	–	√	–	–
	Basin	–	–	√	–	–
	Container	–	–	–	√	–

Note: "–" indicated negative result; "√" indicated the positive result and identified as representive bacteria; A1 = first sampling of premise A; A2 = second sampling of premise A; B1 = first sampling of premise B; B2 = second sampling of premise B; C1 = first sampling of premise C; C2 = second sampling of premise C; D1 = first sampling of premise D; D2 = second sampling of premise D.

pairs. There were 8 (27.6%) positive *S. aureus* DNA was detected, which means there were 72.4% of false positive results identified using the phenotypic identification of biochemical tests. Table 4 shows the summary of phenotypic and genotypic identification of suspected *S. aureus* isolates from sources of contamination of 'keropok lekor' premises.

There were 9 of suspected *Vibrio* spp. colonies isolated from four 'keropok lekor' premises, which showed large, yellow round, smooth, glister and slightly flattened and/or large, blackish green colonies on Thiosulfate-citrate-bile salts-sucrose (TCBS) agar (Pfefer & Oliver, 2003). There were 6 out of 9 suspected isolates (66.7%) were confirmed as *Vibrio* spp., which were *V. algonolyticus*, *V. fluvalis*, *V. parahaemolyticus* and *V. vulnificus*. The other non-*Vibrio* spp. found from the suspected isolates were *Moraxella lacunta*, *Pseudomonas aeruginosa* and *Chromobacteium violaceum*. Table 5 shows the summary of the biochemical tests for identification of suspected *Vibrio* spp. isolates from different sources of contamination of 'keropok lekor' premises.

The antibiotic resistance of identified *Escherichia coli*, *Staphylococcus aureus* and *Vibrio* spp. from 'keropok lekor' premises were tested using Disc Diffusion Method, where colonies suspension of Mc Farland 0.5 turbidity of Mueller-Hinton broth were spread on the Mueller-Hinton Agar (Clinical and Laboratory Standards Institute, 2009). Mueller-Hinton Agar, which is a non-selective, non-

Table 4. Summary of phenotypic and genotypic for identification of suspected *S. aureus* isolates from different sources of contamination of *keropok lekor* premises

Premise	Sample	Phenotypic identification					Genotypic identification *nuc* gene
		BPA Agar	Gram staining	Catalase test	MSA Agar	Coagulase test	
A1	Minced dough	Black/clear zone	+/cocci and rods	+/–	+/–	–	–
	Mixed dough	Black/clear zone	+/cocci and rods	+/–	+/–	–	–
A2	Second food handler	Black	+/cocci and rods	+/–	+	–	–
	Mixing machine	Black	+/cocci and rods	+	+	–	–
B1	Kneaded dough	Black/clear zone	+/cocci and rods	+/–	+/–	–	–
	Boiled dough	Black/clear zone	+/cocci	+/–	+	–	–
	Mixing machine	Black/clear zone	+/cocci	+	+	+	+
	Basin	Black/clear zone	+/cocci	+	+	+	+
	Preparation table	Black/clear zone	+/cocci	+	+	+	+
B2	Minced fish	Black/clear zone	+/cocci and rods	+/–	+	–	–
	Boiled dough	Black/clear zone	+/cocci and rods	+/–	+	–	–
	First food handler	Black/clear zone	+/cocci	+	+	+/–	–
	Third food handler	Black/clear zone	+/cocci and rods	+/–	+	–	–
	Container	Black/clear zone	+/cocci and rods	+	+	–	–
C1	Mixed dough	Black/clear zone	+/cocci	+	+	+	+
	Boiled dough	Black/clear zone	+/cocci and rods	+/–	+	–	–
	First food handler	Black/clear zone	+/cocci	+	+	+	+
	Third food handler	Black/clear zone	+/cocci	+	+/–	+	–
	Mixing machine	Black/clear zone	+/cocci	+	+	+	+
C2	Minced dough	Black	+/cocci	+	+	–	–
	Kneaded dough	Black/clear zone	+/cocci	+	+	+	–
	First food handler	Black	+/cocci	+	+/–	–	–
	Mixing machine	Black	+/cocci and rods	+	+	–	–
D1	Kneaded dough	Black	+/cocci	+	+	+	–
	Preparation table	Black/clear zone	+/cocci	+	+	+	+
	Container	Black/clear zone	+/cocci	+	+	+/–	+
D2	Third food handler	Black/clear zone	+/cocci	+/–	+	+	–
	Basin	Black/clear zone	+/cocci	+	+	+	–
	Preparation table	Black	+/cocci	+	+	+	–

Notes: "+" indicated a positive result; "–" indicated a negative result; "+/–" indicated false positive result.

Table 5. Summary of the biochemical tests for identification of suspected *Vibrio* spp. isolates from different sources of contamination of *Keropok lekor* premises

Premise	Sample	TCBS Agar	Gram staining	Oxidase test	Salinity test	Identification of genus using API® 20 NE (% ID)	Comment
A1	Minced fish	green/yellow	-/curved rods	+	+	*Vibrio vulnificus* (46.2%)	Good identification to the genus
	Mixed dough	black/yellow	-/rods	+	+	*Vibrio parahaemolyticus* (83.9%)	Acceptable identification
	Kneaded dough	yellow	-/short rods	+	+	*Moraxella lacunata* (85.8%)	Acceptable identification
A2	Kneaded dough	green	-/short rods	+	+	*Pseudomonas pseudomallei*	Possibility of *Pseudo. pseudomallei*
B2	Fish	green/yellow	-/ rods	+	+	*Chromobacterium violaceum* (99.4%)	Very good identification
	Minced fish	green	-/curved rods	+	+	*Vibrio fluvialis*	Possibility of *Vibrio fluvialis*
	Kneaded dough 1	green/yellow	-/short rods	+	+	*Vibrio alginolyticus* (99.4%)	Very good identification
	Kneaded dough 2	green	-/curved rods	+	+	*Vibrio parahaemolyticus* (95%)	Good identification
D2	Mixed dough	green/yellow	-/curved rods	+	+	*Vibrio parahaemolyticus* (83.9%)	Acceptable identification

Note: A1 = first sampling of premise A; B2 = second sampling of premise B; D2 = second sampling of premise D.

differential medium and contains starch, which is known to absorb toxins released from bacteria, so that the result were not interfered by toxin reaction on antibiotics. Table 6 shows the number and percentage of overall antibiotic resistance patterns of *E. coli*, *S. aureus* and *Vibrio* spp. isolated from 'keropok lekor' premises.

DISCUSSION

There were different microbial profiles of Total Plate Count, coliform count, *Staphylococcus* spp. count and *Vibrio* spp. count in different sources of contamination from raw materials and food contact surfaces of 'keropok lekor' premises in Kuala Nerus, Terengganu.

Table 6. Number and percentage of overall antibiotic resistance patterns of *E. coli*, *S. aureus* and *Vibrio* spp. isolated from *keropok lekor* premises

Antibiotic disc	Total number of isolates	Resistant, N (%)	Intermediate, N (%)	Susceptible, N (%)
Chloramphenicol, 30 µg (C30)	21	11 (52.4 %)	3 (14.3 %)	7 (33.3 %)
Gentamycin, 120 µg (CN120)	15	–	2 (13.3 %)	13 (86.7 %)
Cephalothin, 30 µg (KF30)	7	3 (42.9 %)	1 (14.3 %)	3 (42.9%)
Naladixic acid, 30 µg (NA30)	13	3 (23.1 %)	–	10 (76.9 %)
Sulfamethoxazole, 23.75 µg (SXT)	21	5 (23.8 %)	1 (4.76 %)	15 (71.4 %)
Tetracycline, 30 µg (TE30)	21	1 (4.76 %)	–	20 (85.2%)
Penicillin, 10 µg (P10)	8	2 (25 %)	–	6 (75 %)
Vancomycin, 30 µg (VA30)	8	–	6 (75 %)	2 (25 %)
Ampicillin, 10 µg (AM10)	8	2 (25 %)	–	6 (75 %)
Erythromycin 15 µg (E15)	6	5 (83.3 %)	–	1 (16.7 %)

Note: N = number; "–" indicated no isolate for the respective properties.

Generally, raw materials and in-process food samples contained higher microbial load. According to Sachindra *et al.* (2005), cooking process of boiling 'keropok lekor' at 100ºC for 10 minutes can reduce microbial levels, although high initial microbial load and cross contamination took place. However, the initial microbial load in 'keropok lekor' may affect the heat requirement for cooking. If the raw materials contain high microbial load, longer time is needed to boil 'keropok lekor' to reach sufficient low level microbial count. Decimal reduction time, D-value and temperature resistance coefficient, z-value depend on temperature, but in a limited temperature region (van Doornmalen & Kopinga, 2009).

All the *E. coli* were found in raw materials and food samples. Most of the *S. aureus* were found on raw materials and food contact surfaces. *E. coli*, *S. aureus* and *Vibrio* spp. identified were not found in the end product of boiled dough, indicating the 'keropok lekor' produced in those premises was considered safe for consumption based on the criteria established by the Food Standards Australia New Zealand (2016) and Food and Drug Administration, 2011. Some of the *E. coli* isolates found in 'keropok lekor' industry showed resistance to antibiotics of cephalothin at concentration of 30 µg (KF30). This finding is similar to the reports by World Organisation for Animal Health (2003) where *E. coli* isolated from milk and bovine samples also showed similar resistance to cephalothin.

Antibiotic resistance of most of the *S. aureus* found in 'keropok lekor' industry showed intermediate to vancomycin 30 µg (VA30) and resistance to chloramphenicol 30 µg (C30), which confirmed the presence of vancomycin-intermediate *S. aureus* (VISA) (Gardete & Tomasz, 2014). For antibiotic susceptibility of *V. alginolyticus*, *V. fluvialis*, *V. parahaemolyticus* and *V. vulnificus*, chloramphenicol 30 µg (C30), erythromycin 15 µg (E15) and sulfamethoxazole 23.75 µg (SXT) showed weak inhibitory effect towards these *Vibrio* spp. strains found in 'keropok lekor' industry. These results were contradicted with the finding by Lagana *et al.* (2010) where *Vibrio* spp. isolated from aquaculture farms showed susceptible to chloramphenicol at 30 µg concentration.

The antibiotic resistance of *E. coli*, *S. aureus* and *Vibrio* spp. from a variety of sources of contamination among four 'keropok lekor' premises were different because the bacteria were isolated from different premises and processing conditions. The result of antibiotic resistance of *E. coli*, *S. aureus* and *Vibrio* spp. isolated from 'keropok lekor' premises showed that those food pathogens were controllable emergence strains, whereby 50% of the bacteria were susceptible to the present antibiotics, which were able to suppress the growth or eliminate bacteria successfully. This finding highlights the prevalence of antibiotic resistant among isolated bacteria in 'keropok lekor' premises in Kuala Nerus, Terengganu is still limited and the emergence of antibiotic resistant bacteria is low. This finding would increase the confidence of public on the safety measure in 'keropok lekor' industry in Kuala Nerus, Terengganu.

CONCLUSION

In conclusion, the microbiological data presented in this study highlights that the occurrence and bacterial counts (Total Plate Count, *Staphylococcus* and *Vibrio*) are within satisfactory range according to the regulatory standard. The established practice developed by Small Medium

Industries of 'keropok lekor' in Kuala Nerus, Terengganu is sufficient to produce safe 'keropok lekor' for consumption. Boiling is the important steps that proven to reduce coliform, *Staphylococcus* and *Vibrio* sufficiently in both raw materials and food contact surfaces to safe levels. The sanitation during food processing conditions is very crucial to ensure the quality of end product.

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