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FULL PAPER

Assessment of bacteriological quality and *Escherichia coli* O157: H7 in ready-to-eat street foods

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Abstract

Ready-to-eat (RTE) street foods are usually prepared well in advance (4-8 hrs) before the sale. Owning to minimal cooking procedures, cross-contaminations, and abused holding temperature and time, there is a high probability for deteriorating microbiological quality in these foods. Thus, this study was aimed to assess the bacteriological quality of RTE foods sampled from night markets and street stalls based on the guidelines provided by the Public Health Laboratory Service. A total of fifty samples (category 2, 3 and 4) were evaluated for aerobic colony count, total coliform, and *E. coli*. The pathogenic strain *E. coli* O157: H7 were examined using the multiplex PCR technique. It was noticed that category 3 and 4 RTE street foods were largely unsatisfactory for the coliform (>10^4 CFU/mL) and *E. coli* count (>100 CFU/mL). In opposite, category 2 RTE street foods were unsatisfactory for the aerobic colony count (>10^5 CFU/mL) and *E. coli* (>100 CFU/mL). *However, there was no E. coli* O157: H7 or Shiga-toxin producing bacteria reported in this study. The statistical analysis showed that in overall, category 3 and 4 RTE street foods were significantly at worsening bacteriological quality compared to category 2. Strict legal enforcement and amending the existing rules are needed to improve the quality of RTE street foods sold in the night markets and street stalls.

1. Introduction

Street vending is a mode of goods and foods being traded to the public with the traders normally have no permanent premises to present. Hence the wares are usually traded in a temporary static structure or mobile unit in the street or pavement. Street foods vending, on the other hand, are conspicuously gaining fast acceptance among the people of all sociodemographic as the foods traded here are vastly economical besides a surplus of selections are offered (Alami, 2016). In some countries, street food vending has not only gained popularity among the locals but also had bound substantial influx of tourist and food junkies, thus bettering the socioeconomic status of both urban and rural populaces (Quee *et al*., 2010; Alimi, 2016).

In Malaysia, street food vending concepts are widely commercialized by the night markets and street stalls. Night markets are the traditional street markets selling household goods, clothes, and many other authentic Malaysian foods. These premises are bound temporary and are usually located in a highly-populated area to boost sales. Therefore, the basic sanitation and utility at these sites are often reported lacking (Abdul-Mutalib *et al*., 2012). For instances, night markets normally operate at the high populous and congested residential area and on the other hand, street stalls commonly located at the major street corners, busy train and bus stations and school compounds (Akinbode *et al*., 2011). The type of foods sold here is mostly the pre-cooked and ready-to-eat (RTE) which are largely available for immediate consumption at the point of sale (Khater *et al*., 2013). Therefore, the preparation of these foods is made well in advance (4 – 8 hrs) and it is relatively simple as some RTE street foods only require minimal cooking (heating) procedures while the others are not at all. Owning to such preparation procedures and the absence of basic cleanliness at these premises, the RTE street foods sold here have markedly cast doubt on the bacteriological quality.

When foods are prepared hours before the actual trading takes place and it is being displayed in an open

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environment without a proper incubation, there is a high possibility for the bacterial contamination to occur. Studies have indicated that the improper holding temperature and longer holding time in street vending had cause major food poisoning events and foodborne outbreaks (Adesiyun and Balbirsingh, 1996; Gitahi *et al*., 2012; Birgen *et al*., 2020). Furthermore, the knowledge, attitude, and hygienic practice among the food handlers in the street stalls and night markets are always dubious and questionable. Nee *et al*. (2011) had reported that the food handlers scored absurdly poor for the knowledge on food storage and preparation temperature and on the other hand, Woh *et al*. (2016) had reported that majority of the food handlers have no knowledge on foodborne pathogens and the symptoms it may possess should the infections happen (Nee and Sani, 2011; Woh *et al*., 2016). In another study, even though the food handlers were reported of having good knowledge in the bacterial cross-contamination, the high bacterial occurrence was still recorded in the cooking utensils; knives (*S. aureus* - 75.4%, total coliform - 66.4%, and *E. coli* - 6.7%), chopping board (*S. aureus* and total coliform - > 70% and *E. coli* - 9.7%) and dish plates (*S. aureus* - 69.4%, total coliform - 23.9%, and *E. coli* - 3.0%) (Ain *et al*., 2018). Such findings can positively correlate to the potential bacteriological hazards and it may be attributed to the RTE street foods sold in the night markets and street stalls in Malaysia. Nawawee *et al*. (2019) had shown that the street vended beverages in Kuala Lumpur were mostly unsatisfactory for bacteriological quality however the analysis of RTE street foods sold in night markets and street stalls in Malaysia is still inadequate (Nawawee *et al*., 2019).

Therefore, the current study is aimed to assess the bacteriological quality of RTE foods sampled from night markets and street stalls in Nilai, Negeri Sembilan, Malaysia. The level and the significant occurrence of aerobic bacteria, total coliform, and *E. coli* between the RTE street food categories were evaluated and reported. Besides, the potential occurrence of *E. coli* strain O157: H7 and Shiga-toxin producing bacteria were also assessed using the multiplex PCR technique.

2. Materials and methods

2.1 Sample collections and processing

A total of fifty random RTE street foods was collected from night markets and street stalls located in the Nilai area, Negeri Sembilan. These samples were assigned into categories based on aerobic colony count as suggested by the Public Health Laboratory Service (PHLS) (Gilbert *et al*., 2000). The RTE street food samples collected were category 2 (e.g. cooked hot dogs and sausages), category 3 (e.g. fish and squid gravies) and category 4 (e.g. smoked pork and products) respectively. The sampling period for the current study was from October 2019 to February 2020 and all samples were approximately collected at 8 pm. These samples were collected in original individual packages, tightly sealed, and transported immediately to the laboratory in a sterile container (without ice) to avoid moisture and airborne cross-contamination. Each sample was clearly labelled with sampling ID, date, and location of collections. Upon arrival to the laboratory, 25 g of samples were homogenized in 225 mL of 0.1% peptone water (SIME Scientific, India). Serial dilutions of 1:10 were made from the homogenized solution to assess the total viable count for aerobic bacteria, total coliform, and *Escherichia coli* count (Sair *et al*., 2017). Besides, the samples were also reviewed for the presence of *Escherichia coli* O157: H7 and Shiga-toxin producing bacteria using the multiplex polymerase chain reaction technique.

2.2 Bacteriological analysis

Bacteriological analysis was performed by transferring 0.1 mL of appropriate dilution into the Plate Count Agar (PCA) (Merck Millipore, Germany) for the enumeration of aerobic colony count and chromocult coliform agar (CCA) (Merk Millipore, Germany) for the enumeration of total coliform and *E. coli*. Agar plates were incubated at 37°C for 24 hrs and the bacterial colonies were counted and presented as log CFU/mL (Lange *et al*., 2013; Nawawee *et al*., 2019). Coliform and *E. coli* presence in the CCA was identified with the appearance of purplish-red and blue colonies, respectively. All experiments were performed in triplicate plates. The standards provided by the Public Health Laboratory Service (PHLS) and the Compendium of Microbiological Criteria for Foods were used to evaluate the quality of RTE street foods in this study (Gilbert *et al*., 2000; Food Standards Australia New Zealand, 2016).

2.3 DNA Isolation

Cell boiling method was used to isolate DNA from the overnight incubated homogenized food samples as suggested by Pui *et al*. (2011). Briefly, 1 mL of turbid samples were centrifuged at 18,620 x *g* for 3 mins. The pellet was collected and suspended with 500 µL of double-distilled water (dH_2O). The mixture was vigorously vortexed for 2 mins and boiled at 100°C. After 10 mins, the sample was cooled at -20°C for another 10 mins and centrifuged again at 18,620 x *g* for 3 mins. The supernatant containing DNA is collected into a new sterile tube and used for PCR reaction (Pui *et al*.,

2011).

2.4 Multiplex PCR analysis

The DNA for experimental control *E. coli* O157: H7 was obtained from the Faculty of Food Science and Technology, Universiti Putra Malaysia. This DNA sample was used to optimize the multiplex PCR assays as suggested by Kwan *et al*. (2019). Briefly, two assay PCR was performed for each sample; 1st assay for the detection of Shiga-toxin gene using the primers Stx I-F (5'-ATAAATCGCCATTCGTTGACTAC-3'), Stx I-R (5'-AGAACGCCCACTGAGATCATC-3') and Stx II-F (5'-GGCACTGTCTGAAACTGCTCC-3'), Stx II-R (5'-TCGCCAGTTATCTGACATTCTG-3') respectively. The 2nd assay PCR for the detection of *rbfO157* gene and *fliCh7* gene using rbf0157-F (5'-CGGACATCCATGTGATATGG-3'), rbf0157-R (5'-TTGCCTATGTACAGCTAATCC-3') and fliCH7-F (5'-GCGCTGTCGAGTTCTATCGAG-3'), fliCH7-R (5'-CAACGGTGACTTTATCGCCATTCC-3') respectively (Kwan *et al*., 2019). The PCR products were analyzed for the presence of Shiga-toxin, *rbfO157* gene and *fliCh7* gene amplicons in the gel documentation system (UVP, United Kingdom).

2.5 Statistical analysis

The microbial plate counts were presented as mean±standard deviation (SD) of log CFU/mL. The differences in the microbial plate counts between the different RTE street food categories were evaluated using the one-way analysis of variance (ANOVA) using the SPSS version 23.0. Any differences were considered statistically significant with $p < 0.05$ unless stated otherwise.

3. Results

All 50 random RTE street food samples of category 2, 3 and 4 collected from night markets and street stalls were found highly contaminated with aerobic bacteria. Besides that, most of them were also observed with high occurrence for coliform and *E. coli*. The complete result and analysis were summarized in Table 1. For the aerobic colony count, category 4 RTE street foods were found highly contaminated with mean 6.25±0.65 log CFU/mL, followed by category 3 RTE street foods with mean 5.97±0.72 log CFU/mL and lastly category 2 RTE street foods with mean 5.31±0.47 log CFU/mL. Overall, all 50 RTE street foods sampled in this study were showing an average of 5.79±0.72 log CFU/mL for the aerobic bacterial count. The one way-ANOVA analysis shown that the mean occurrence of aerobic bacteria for category 3 and 4 RTE street foods was significantly high compared to the category 2 RTE street foods ($p< 0.05$).

On the other hand, all twenty-two samples of category 3 RTE street foods and all ten samples of category 4 RTE street foods were found highly contaminated by coliform bacteria. This is including the mean 5.83±0.83 log CFU/mL of total coliform evident in category 3 RTE street foods and the mean 6.13±0.73 log CFU/mL in category 4 RTE street foods. For category 2 RTE street foods, 66.7% of them were found contaminated by coliform with the mean occurrence of 3.59±2.66 log CFU/mL. The overall prevalence of total coliform in fifty samples were 88% with an average of 5.09±2.03 log CFU/mL was witnessed. The one-way ANOVA test had shown that category 3 and 4 RTE street foods were significantly contaminated by coliform bacteria compared to the category 2 RTE street foods ($p<0.05$).

For the *E. coli* count, 58% of the samples were found to be contaminated. The high occurrence was noticed in category 4 RTE street foods with 70% of them were contaminated. On average, 4.71±2.57 log CFU/mL of *E. coli* was noticed in category 4 RTE street foods, 4.04±2.87 log CFU/mL in category 3 RTE street foods and 1.86±2.70 CFU/mL in category 2 RTE street foods. The statistical analysis shows that the occurrence of *E. coli* in category 3 and 4 RTE street foods was significantly contaminated compared to the category 2 RTE street foods ($p<0.05$).

The quality of RTE street foods was evaluated based on the standards outlined by PHLS and Compendium of Microbiological Criteria for Foods (Gilbert *et al*., 2000; Food Standards Australia New Zealand, 2016). For the

Table 1. Aerobic colony count (ACC), total coliform and *Escherichia coli* count in ready-to-eat foods sampled from night market and street stalls in Nilai, Negeri Sembilan

Food Category	Aerobic colony count (ACC)		Total Coliform		*Escherichia coli*	
	No (%)[1]	Average±SD[2]	No (%)[1]	Average±SD[2]	No (%)[1]	Average±SD[2]
2 (n = 18)	18 (100)	5.31±0.47	12 (66.7)	3.59±2.66	7 (38.9)	1.86±2.70
3 (n = 22)	22 (100)	5.97±0.72*	22 (100)	5.83±0.83*	15 (68.2)	4.04±2.87*
4 (n = 10)	10 (100)	6.25±0.65*	10 (100)	6.13±0.73*	7 (70)	4.71±2.57*
Total (n = 50)	50 (100)	5.79±0.72	44 (88)	5.09±2.03	29 (58)	3.39±2.95

The category 3 and 4 RTE street foods were found significantly contaminated by aerobic bacteria, coliform, and *E. coli* compared to the category 2 RTE street foods. *Significant difference ($p < 0.05$).

[1]Number of positive samples. [2]Mean bacterial count in log CFU/mL. SD: Standard deviation.

aerobic colony count, category 3 and 4 RTE street foods were found in an acceptable range, however, category 2 street foods were in an unsatisfactory range. On the other hand, the total coliform count was found to be unsatisfactory for all categories RTE street foods except for category 2. While the mean occurrence of coliform for category 2 was 3.59±2.66 log CFU/mL, high occurrences were also witnessed, as high as 6.25 log CFU/mL in some of the foods within this category. Besides that, the overall mean 3.39±2.95 log CFU/mL for *E. coli* contamination was recorded, and this shows the RTE street foods are mostly at an unsatisfactory level. Despite this, the assessment for the presence of *E. coli* O157: H7 shows neither the presence of this pathogen nor the Shiga-toxin producing bacteria in all categories of RTE street foods.

4. Discussion

The RTE street foods collected in this study were assigned into respective categories as outlined by the PHLS in their guidelines based on the expected aerobic colony count in foods in reference to the food components and the type of processing it received during the preparation (Gilbert *et al*., 2000). Hence, the category 1 RTE street foods are fully cooked and must be immediately available for sale or consumptions, such as beef burgers and meat pies. Descending the order, category 5 is the raw foods such as raw hams and salami and other fermented meat products. Since the RTE foods sampled in this study were the local street foods, most of them were manually assigned to the respective categories as recommended by the PHLS (Gilbert *et al*., 2000). They were the category 2, 3 and 4 cooked RTE street foods which were exposed to longer holding time and displayed at the improper holding temperature. Category 2 RTE street foods were usually cooked in advance but subjected to minimal handling and storage. Category 3 on the other hand, is also cooked in advance but some human handlings were involved, and finally, the category 4 may contain some components that are not properly cooked. In the current study, fruits and vegetables based RTE street foods were also collected and assigned into appropriate categories (Gilbert *et al*., 2000). As such, there are no category 1 and 5 RTE street foods sampled in the current study as our aim is to examine the bacteriological quality of street foods which were cooked and prepared in advance (4 – 8 hrs) before the sale. The outcome of the analysis was scored against the microbiological quality grades for the RTE foods against the actual aerobic colony count and the presence of coliform bacteria and *E. coli* (Gilbert *et al*., 2000; Food Standards Australia New Zealand, 2016). Hence, the microbiological quality was graded either satisfactory: "the test results indicating good microbiological quality"; acceptable: "an index reflecting a borderline limit of microbiological quality" or unsatisfactory: "further sampling may necessary and that environmental health officers may wish to undertaken a further inspection of the premises concerned to determine whether hygiene practices for food production or handling are adequate or not".

On this basis, category 2 RTE street foods analyzed in this study were observed with an unsatisfactory level for the mean aerobic colony count. While the acceptable range for the aerobic colony count must be between 10^4 - 10^5 CFU/mL, the outcome from the current study implicating the CFU/mL at $\geq 10^5$ (5.31±0.47 log CFU/mL). Similar findings were also reported across the globe for RTE street foods of the same kind. Average total aerobic bacteria in sausages sampled in Morocco, Greece and Turkey were all reported to be at an unsatisfactory level (Ambrosiadis *et al*., 2004; Erkmen and Bozkurt, 2004; Ed-Dra *et al*., 2017). Foods such as sausages and nuggets generally undergo high processing stages in the food industry to extend their self-life. Moreover, the effect of heat and temperature used for cooking must have substantially reduced the amount of bacterial contamination. Hence, any poor bacteriological quality observed in these foods were merely due to the implication of improper food handling at retails. A study conducted by Sachindra *et al*. (2005), had shown that cooked buffalo sausages were found with a significant decline in the total plate count and coliform when compared to the raw minced meat and stuffed sausages. The same study has also reported that there was no detection of any pathogenic bacteria including the Enterobacteriaceae after the buffalo sausages were fully cooked (Sachindra *et al*., 2005).

While high aerobic colony count was observed in the category 2 RTE street foods, the mean for the total coliform count was registered at marginal (acceptable) although some individual samples were observed at an unsatisfactory level. The Compendium of Microbiological Criteria for Foods was used as a standard to gauge the level of coliform in the street foods (Food Standards Australia New Zealand, 2016). Guidelines provided by the PHLS suggests that the Enterobacteriaceae count must be taken into consideration in place of the conventional total coliform count as an indicator for hygiene and contamination after processing (Gilbert *et al*., 2000). However, Enterobacteriaceae is a large family of Gram-negative comprises of 238 species of facultative anaerobic bacteria. The classification for Enterobacteriaceae is rather complex and implies many exceptional (Octavia and Lan, 2014). Moreover, the criteria listed to test Enterobacteriaceae is not suitable for fruit and vegetable

containing products (Gilbert *et al*., 2000). Hence, the standard for the total coliform count was retained in this study instead of adopting the Enterobacteriaceae count. For the *E. coli* count, 7 out of 18 RTE street foods of category 2 were reported positive. Although the mean occurrence for *E. coli* is likely at an acceptable range, the concentration of *E. coli* in each positive sample was relatively high (> 100 CFU/mL). Hence, for the *E. coli* count, assessing individual sample is therefore obligatory rather than accounting the mean occurrence. On this note, all seven *E. coli* positive samples of category 2 RTE street foods were at an unsatisfactory level. *Escherichia coli* contamination in the street foods and beverages have been reported by many studies in the past and the presence of this bacterium is often used as an important indication of faecal contamination (Eromo *et al*., 2016; Ed-Dra *et al*., 2017; Kwan *et al*., 2019; Nawawee *et al*., 2019). The unsatisfactory level of *E. coli* further emphasizes the deteriorating quality of the street foods being sold in the night markets and street stalls.

Contrastively, the category 3 and 4 RTE street food samples were majority found to be highly contaminated; coliform count and *E. coli* were both presented at an unsatisfactory level. Category 3 RTE street foods sampled in this study were generally noticed being displayed in open plastic containers and ironically, these foods were also found to be displayed at the temperature "danger zone". On top of that, the lack of dynamism in the night markets had witnessed the food stalls often set adjacent to the butchery shops and other mainstay products such as raw seafood, chicken, and eggs. Pathogens such as *Salmonella* spp. and *E. coli* O157: H7 often reported contaminating the raw meat and carcasses can easily promulgate cross-contamination between the raw and cooked foods in the night market (Thung *et al*., 2016; Kwan *et al*., 2019). Besides that, garbage piles were also conspicuously noticed nearby to these stalls and this can easily expose the foods to pests such as flies, cockroaches, and rodents. Studies have shown the role of pest in food contamination (Bryan *et al*., 1988; Okojie and Isah, 2014). On the other hand, category 3 RTE street foods were mostly cooked and prepared at home or other places well in advance before being transported to the sales locations. As such, bacterial contamination not only can take place at the point of sale but also can occur during storage or transportation. In the past, many incidences of foodborne outbreaks were reported from night markets or street stalls in Malaysia are mostly linked to the contamination that occurs during storage and transportation. In 2014, a large outbreak of *Salmonella* Typhimurium was reported in the night market of the state Terengganu in Malaysia. The case-control study had revealed that the RTE foods were prepared in a different premise was contaminated before transported and sold in the Night market. This finding also revealed a poor hygienic condition of utensils, improper storage of raw material, abuse in the storage temperature and foods were exposed at improper holding temperature and time (Karim *et al*., 2017). Category 4 RTE street foods, on the other hand, usually contain one or two components that were least cooked. As such, a high occurrence of coliform and *E. coli* is mostly anticipated in this food category and hence, this necessitates a thorough bacteriological contamination analysis.

When the RTE street foods were tested for the presence of *E. coli* O157: H7 and Shiga-toxin producing bacteria using the multiplex PCR technique, the outcome was negative for all 50 samples. *Escherichia coli* strains can be either pathogenic or non-pathogenic, and the latter is a normal flora which is commonly found in the intestine of human and animals. Hence, this commensal bacterium is harmless and plays a significant role in food metabolism in human which are often seen as an indicator of faecal contamination in food (Mansan-Almeida *et al*., 2013). On the other hand, the pathogenic strains such as the Enterohemorrhagic *Escherichia coli* (EHEC) serotype O157: H7 causes diarrheal disease and found to be the leading cause of mortality among children age below 5 (Croxen *et al*., 2013). Though there are many types of pathogenic *E. coli*, the EHEC serotype O157: H7 is the most commonly reported in Malaysia (Chang *et al*., 2013; Cheah *et al*., 2015; Kuan *et al*., 2017; Kwan *et al*., 2019). Since the RTE street foods collected from the night market and street stalls in this study were found highly contaminated by *E. coli*, it is vital to test for the presence of O157: H7 strains. Moreover, the serotype O157: H7 is commonly detected in the raw meat and carcasses, hence, it is important to examine if there is any cross-contamination had occurred between the raw meat and the cooked RTE street foods in the current study.

5. Conclusion

In conclusion, the microbial contamination of RTE street foods sampled from the night market and street stalls were reported unsatisfactory. The bacteriological quality was assessed based on the guidelines provided by the PHLS and the Compendium of Microbiological Criteria for Foods found that the category 3 and 4 RTE street foods largely fall into the unsatisfactory range for both total coliform and *E. coli* count. On the other hand, even though the category 2 RTE street foods demonstrated better bacteriological quality for the total coliform, unsatisfactory aerobic colony count was observed. In addition, the *E. coli* count for each positive

sample in this category was also reportedly unsatisfactory. In overall, the category 3 and 4 RTE street foods were significantly shown to possess poor bacteriological quality compared to the category 2 RTE street foods. Though the mean occurrence of *E. coli* in food samples was relatively high, no *E. coli* O157: H7 and Shiga-toxin producing bacteria were detected in this study.

Thus, these findings also suggested that the rules and policies on the street vending and the underlying food safety need to be strictly enforced. The authority concerned especially the Municipal Councils should regularly provide guidelines and conduct training to the food handlers apart from amending the existing laws. Regular inspection and introducing the letter grading system similar to the restaurants in Malaysia may constantly gauge the cleanliness and hygienic level of the street vending stalls. This may significantly improve the bacteriological quality of the RTE street foods sold in the night markets and street stalls in future.

Conflict of interest

The authors declare no conflict of interest.

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