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Prevalence and antimicrobial resistance of *Escherichia coli* isolated from Ramadan bazaar in Kuching, Sarawak

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ABSTRACT

E. coli contamination is not in compliance with food safety international standards. As such, this study assessed the occurrence of *E. coli* contamination. The study also investigated multiple antibiotic resistance indices among *E. coli* isolates. A total of 194 food samples from six categories (beverages, sweet cakes, cooked meat/fish, cooked rice, cooked vegetables, and noodles) were analyzed to determine the *E. coli* count using a standard method. The results showed that 20% of the samples were contaminated with *E. coli.* Cooked rice showed the highest mean value of 3.785 ± 0.000 Log CFU/g. While noodles (2.344 ± 0.791 Log CFU/g) and beverages (2.002± 0.504 Log CFU/ml) have shown the most unsatisfactory level of *E. coli* among food categories. The multiple antibiotic resistance index of *E. coli* isolates in this study ranged from 0.12 to 0.59, and ampicillin showed the most resistance antibiotics. *E. coli* indicates the lack of good food handlers and hygiene practices, whereby consuming contaminated food may pose a risk of food poisoning.

INTRODUCTION

The Ramadan bazaar is a food bazaar set up for breaking fast during the fasting month before Eid-al Fitr. Ramadan bazaars are one example of street food that is only available during the month of Ramadan and offers a variety of dishes, from drinks and side dishes to heavy meals. Street foods are ready-to-eat foods and beverages prepared and/or sold by vendors and hawkers, especially in streets and other similar public places [1]. In contrast, ready-to-eat foods are defined as foods that include any food (including beverages) consumed in its raw state or any food handled, processed, mixed, cooked, or otherwise prepared to a form during which it is commonly consumed without further processing [2]. In Malaysia, this seasonal street food is becoming an increasing threat to public health every year due to improper handling. The

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Ministry of Health Malaysia recorded an increase in food poisoning cases during Ramadan compared to other months [3]. Most food handlers in the Ramadan bazaar do not practice good hygiene and violate various offenses under the Malaysian Food Hygiene Regulations 2009 [4].

Microbial contamination is a major possible health hazard related to food poisoning, and one of the common food-borne pathogens associated with street foods is *E. coli* [5]. This bacterium is considered an indicator bacterium for fecal contamination [6]. Food contamination with *E. coli* is closely related to a lack of hygiene in food handling and poor access to clean water [7]. Kashima et al. [8] reported a large-scale food poisoning outbreak involving approximately 3000 elementary and junior high school students in Saitama, Japan, caused by *Escherichia coli* serotype O7:H4 in 2020.

The microbiological quality of some street food, such as sweet cakes, fried snacks, sandwiches, fruit juice, cooked rice, cooked meat, and salad sold on the streets of various countries, is contaminated with *E. coli [5,7,9-12]*. Additionally, several studies also revealed the presence of the *E. coli* toxin and multiple antibiotic resistance in various types of street foods [6,13,14]. However, the information on the microbiological safety and quality of foods sold at Ramadan bazaars in Malaysia could be much better. To the best of our knowledge, this is the first study to report a prevalence of *E. coli* and antibiotic resistance profile in various food samples from Ramadan bazaar in Kuching, Sarawak, specifically.

MATERIALS AND METHODS

Sample collection

A total of 194 samples of different food categories were obtained from Ramadan bazaars in three locations in Kuching, comprising the northern and southern parts of Kuching and Padawan. The food categories included beverages, noodles, sweet cakes/ snacks (*kuih*), cooked rice, cooked meat/fish, and cooked vegetables. All samples were serially numbered, transported to the laboratory in a cool box, and maintained at 4°C. The samples were examined immediately, kept in a refrigerator, and tested within 24 hours of sampling.

Determination of *E. coli*

E. coli counts for the food samples were analyzed by using AOAC International Official Method 991.14:2002 (3M™ Petrifilm™ Plate). 25 g of food samples was added to 225 ml Butterfield's buffered phosphate diluent (10^{-1}) and homogenized. A triplicate sample of 1 ml was placed onto the film's center. A series of dilutions were performed up to 10^{-3} and incubated at 37°C for 48 ± 4 hours. Reference cultures used for establishing acceptable performance of the medium were *Enterobacter aerogenes* ATCC 13048 and *E. coli* ATCC 11775. *E. coli c*ontamination levels were then compared to standard guidelines [15].

Antibiotic susceptibility test

The antibiotic susceptibility test was performed using the Kirby-Bauer disc diffusion method using Mueller-Hinton agar [16]. The antibiotic discs tested for *E. coli* were ampicillin (10µg), amoxicillin/clavulanic (20/10µg), chloramphenicol (30µg), cefotaxime (30µg), cefoxitin (30µg), ciprofloxacin (5µg), ceftaroline (5µg), gentamicin (10µg), imipenem (10µg), kanamycin (30µg), levofloxacin (5µg), netilmicin (30µg), ofloxacin (5µg), streptomycin (10µg), tetracycline (30µg), trimethoprim-sulfamethoxazole (25µg) and tobramycin (10µg). The zone of inhibition of growth around each disc was then measured in millimeters, and standards interpreted the zone diameters as sensitive, intermediate, and resistant (Clinical and Laboratory Standards Institute, 2020). The multiple antibiotic resistance (MAR) index for each resistant profile was determined [17].

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Statistical analysis

All statistical analyses were performed using the IBM Statistical Packages for Social Science, SPSS Version 26. The frequencies of positive samples and descriptive statistics such as mean and standard deviation were obtained. Statistically significant differences were confirmed at a 0.05 significance level.

RESULTS AND DISCUSSION

Prevalence of *E. coli* in Ramadan bazaar foods

The findings of this study show *that E. coli* was distributed in all locations and food categories, as shown in Table 1. About 38% (13/34) of the beverage samples were contaminated with *E. coli,* and milk-based drinks and juices were the most contaminated in this study. According to Afreen et al. [18], several risk factors, such as type of beverage, pH, vending site, source of water, washing of utensils, and hand washing habits, showed a significant correlation with *E. coli* contamination. A previous study conducted at Chow Kit, Kuala Lumpur, reported that only one milk-based drink was contaminated by *E. coli*. However, the level of contamination was very high, exceeding log 3 CFU/ml [11].

Various types of noodles are sold at the Ramadan bazaar in Kuching, Sarawak, including local delicacies such as *laksa Sarawak*, *kolo* mee, and *mee jawa*, and these samples were found to be contaminated with *E. coli.* These foods are prone to contamination due to various ingredients added to their preparation. These noodles are served with gravy and other condiments such as meat, sliced spring onion, chilies, bean sprouts, fried onions, and others. This is consistent with a report by Isoni Auad et al. [19] that found foods with mixed ingredients commonly cause outbreaks due to the complexity of the preparation process.

The various condiments added to the food raise the possibility of contamination from inadequate personal hygiene and poor food handling practices. Food handlers usually use their bare hands to put the condiments; thus, their hand hygiene could be better due to the lack of hand washing facilities at Ramadan bazaars. A study in India reported *that E. coli, Staphylococcus aureus, Salmonella* spp., and *Clostridium* were found in street noodles. They reported that the probability of contamination increased greatly due to the poor environment where the foods are prepared and served [20].

Besides beverages and noodles, sweet cakes/snacks, better known as Malay traditional *kuih,* are popular among Ramadan bazaar consumers. Various sweet cakes/snacks sold at Ramadan bazaars are cooked by frying, boiling, or steaming. Since Malay traditional *kuih* mostly uses coconut milk and grated coconut, poor handling practices and storage in producing coconut milk and grated coconut may cause cross-contamination. Nurul et al. [21] proved that *E. coli* was found in fresh coconut milk from wet markets and hypermarkets in western Malaysia. Moreover, post-contamination may occur when the sweet cakes/snacks are filled with unsanitary or bare hands. A similar study in Kuala Lumpur, Malaysia, reported that street sweet cakes and fried snacks were contaminated with *E. coli* and *S. aureus [12].*

Table 1. *E. coli* counts in Ramadan bazaar foods.

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Variables	N (%)[1]	Mean[2]	±SD
Location			
Kuching North (n=105)	19 (18)	2.191	0.796
Kuching South (n=35)	7 (20)	1.959	0.672
Padawan (n=54)	13 (24)	2.350	0.811
Total (n=194)	39 (20)	2.202	0.694
Food Category			
Beverages (n=34)	13 (38)	2.002	0.504
Noodles (n=33)	9 (27)	2.344	0.791
Sweet cakes/ snacks (n=48)	10 (21)	2.073	0.628
Cooked meat/fish (n=28)	4 (14)	2.364	0.979
Cooked rice (n=31)	1 (3)	3.785	.
Cooked vegetables (n=20)	2 (10)	2.391	0.494
Total (n=194)	39 (20)	2.202	0.694

[1]Number of positive samples/total samples
[2]Mean bacterial counts expressed in Log (CFU/g/ml), SD: standard deviation

Contamination level of *E. coli*

In this study, 39 (20%) of the 194 food samples were positive for *E. coli*, and 27 (69%) of the positive samples exceeded the standard [15], as shown in Figure 1. A previous study showed that only 6% of street food sold at Chow Kit, Kuala Lumpur, exceeded the standard, much lower than this study *[12]*. A study in Mozambique found that 52% of traditional hot and cold foods, sandwiches, fruit, and salad were unsatisfactory for *E. coli [22]*. In Pakistan, 73% of beverage samples sold by street vendors exceeded the standard for *E. coli*, which is slightly higher than this study [18]. Unsatisfactory levels of these hygiene indicator microorganisms found in Ramadan bazaar food indicate contamination post-preparation, as heat treatment can eliminate non-sporulating bacteria *[22]*. Post-preparation contamination may be associated with poor personal hygiene, such as hand washing using poor-quality water. Ramadan bazaar food handlers were observed washing their hands using water stored in buckets, and repeatedly using the same water could cause cross-contamination. It was proved by Berhanu et al. [23], who found that *E. coli* counts from hand rinsing and washing water had a positive and strong relationship.

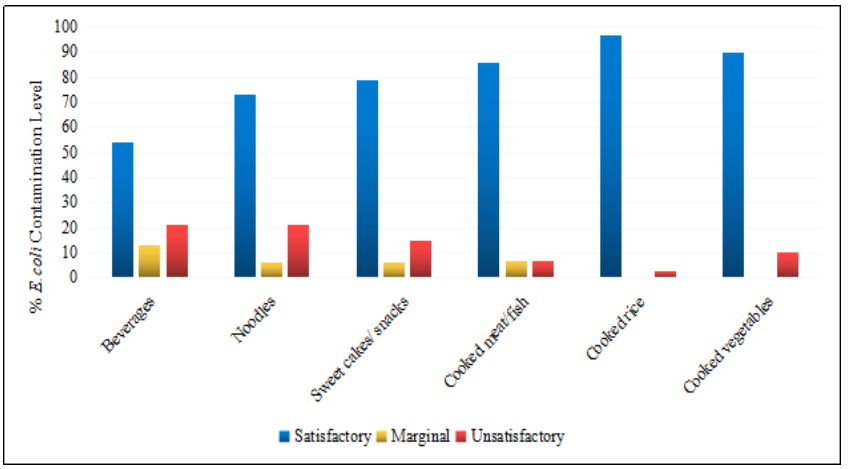

Fig. 1: Percentage distribution of *E. coli* contamination level in Ramadan bazaar foods.

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Antibiotic Resistance (MAR) profile of *E. coli* isolates

Out of 39 *E. coli* isolates, 56% (22/39) were resistant to various types of antibiotics, including ampicillin, amoxicillin/clavulanic, chloramphenicol, gentamicin, tetracycline, and others (Figure 2). As shown in Table 2, the MAR index of *E. coli* isolates in this study ranged between 0.12 and 0.59, which is slightly lower than the MAR index reported by a previous study (0.13 to 0.89) [24]. A previous study reported that 15 antibiotics were tested against 59 *E. coli* isolates from food and water in the streets of Maputo, Mozambique, and showed the highest percentage of resistance towards imipenem and ampicillin [25]. They also found multidrug resistance in 31.3% of the isolates. Siddabathuni (2019) [26] reported that *E. coli* strains showed weak susceptibility to ceftazidime, cefotaxime, cefepime, and ampicillin, and some strains were found to be positive to produce extended-spectrum beta-lactamase (ESBL). Another study by Sabuj et al. [27] reported that *E. coli* isolates recovered from street-fried snacks in Bangladesh were highly resistant to amoxicillin, tetracycline, and chloramphenicol. According to Hassan et al. [28], amoxicillin has a significant resistance level against enteric bacteria, such as *E. coli*. This could be attributed to the usage and cost of antibiotics, as amoxicillin is the most often used antibiotic among low-income individuals.

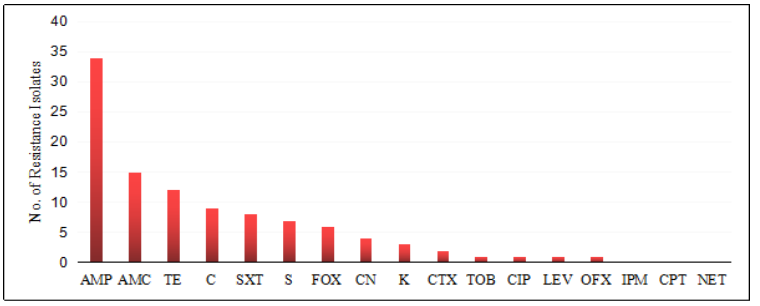

Fig. 2: Number of antibiotic resistance of *E. coli* isolates. AMP:Ampicillin; AMC:Amoxicillin/clavulanic; CTX:Cefotaxime; FOX:Cefoxitin; C:Chloramphenicol; CN:Gentamicin; K:Kanamycin; S:Streptomycin; SXT:Trimethoprim smz; TE:Tetracycline; TOB:Tobramycin; LEV:Levofloxacin; OFX:Ofloxacin; CIP:Ciprofloxacin; CPT:Ceftaroline; IPM:Imipenem; NET:Netilmicin

CONCLUSION

The results of the current study demonstrated that Ramadan bazaar foods sold in Kuching, Sarawak, especially beverages and noodles, are potentially contaminated with *E. coli.* Half of the total isolates showed antibiotic resistance to some common antibiotics. Detecting these pathogenic bacteria in Ramadan bazaar foods could cause potential public health problems for consumers. Thus, this study suggests that food safety awareness programs to educate and improve hygiene should be focused on food handlers and the public. Furthermore, to minimize food poisoning in Ramadan bazaars and other street foods, the '*observe, smell, taste*' campaign by the Ministry of Health Malaysia should be continued comprehensively to various levels of society.

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Table 2. Multiple Antibiotic Resistance (MAR) Index profile of E. coli isolates

Food Category	No. of Isolate	Antibiotic-Resistant Profile	MAR Index
Cooked meat/fish	1	AMP, AMC, C, CIP, CN, K, LEV, OFX, SXT, TE	0.59
Cooked meat/fish	1	AMP, AMC, C, CN, K, S, TE, SXT	0.47
Noodles	1	AMP, C, CN, K, S, TE, SXT, TOB	0.47
Cooked meat/fish	1	AMP, AMC, C, S, SXT, TE	0.35
Cooked vegetables	1	AMP, AMC, C, SXT, TE	0.29
Noodles	2	AMP, AMC , S, SXT, TE	0.29
Beverages	1	AMP, AMC, SXT, TE	0.24
Sweet cakes/ snacks	1	AMP, AMC, CTX, FOX	0.24
Noodles, Beverages	2	AMP, AMC, C, TE	0.24
Cooked meat/fish	1	AMP, FOX, C, S	0.24
Sweet cakes/ snacks, Beverages	2	AMP, AMC, FOX	0.18
Noodles	2	AMP, C, TE	0.18
Noodles, Sweet cakes/ snacks	2	AMP, TE	0.12
Sweet cakes/ snacks	1	AMP, FOX	0.12
Beverages, Sweet cakes/ snacks	2	AMP, AMC	0.12
Beverages	1	AMP, CN	0.12

*AMP:Ampicillin; AMC:*Amoxicillin/clavulanic; CTX:Cefotaxime; FOX:Cefoxitin; C:Chloramphenicol; CN:Gentamicin; K:Kanamycin; S:Streptomycin; SXT:Trimethoprim smz; TE:Tetracycline; TOB:Tobramycin; LEV:Levofloxacin; OFX:Ofloxacin; CIP:Ciprofloxacin

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AUTHOR'S CONTRIBUTION

Nurul Azyla Azmi conceptualized the central research idea, provided the theoretical framework, researched it, and wrote the article. Najihah Mohd Sani and Abu Muslim Ahmad carried out the research. Sharifah Aminah Syed Mohamad, Siti Roha Ab Mutalib, and Fazdlina Sapee designed the research, supervised the research progress, and revised the article.

CONFLICT OF INTEREST STATEMENT

The authors agree that this research was conducted without any self-benefits or commercial or financial conflicts and declare the absence of conflicting interests with the funder.

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