

# Microbiological Quality and Antimicrobial Susceptibility Profile of Isolates from Beverages Sold at Ramadan Bazaar in Kuching, Sarawak

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**Abstract** Ramadan marks an entire month of fasting by Muslims and is also famous for its festive food bazaar, where people look forward to indulging in their favourite food and beverages. The microbiological safety of food and drink being sold at Ramadan bazaar is one of the important aspects that should be of concern. Thus, the present study was conducted to investigate the microbial quality specifically to beverages sold at Ramadan Bazaar in Kuching, Sarawak and to determine the antimicrobial susceptibility profile of the isolates. Using standard method, 34 beverage samples were analysed for Aerobic Plate Count, coliform, *Escherichia coli*, *Salmonella* spp., and *Staphylococcus aureus*. All samples were positive for coliform. *E. coli*, and *S. aureus* except for *Salmonella* spp. *E. coli* isolates were resistant to ampicillin, amoxicillin/clavulanic, cefoxitin and tetracycline, while *S. aureus* isolates were resistant towards ampicillin and penicillin. Hence, further studies need to assess the main source of bacterial contamination in beverages sold at Ramadan bazaars in terms of hygiene and sanitation of vendors.

**Keywords:** Antimicrobial susceptibility profile, beverages, microbiological quality, Ramadan bazaar, Sarawak.

## Introduction

Malaysia Ramadan bazaar is a special occasion which will be held during the Ramadan month every year. Muslims observe a complete fast from dawn to sunset each day throughout the month and tend to buy food from Ramadan bazaar for breaking fast. Every state in Malaysia will have several designated places where stalls sell various delicacies for breaking fast. Besides cooked rice and sweet cakes, beverages are one of the most sold dishes at the bazaar. There are several categories of beverages including juice, milk based drinks and cordial based drinks that are sold at Ramadan bazaar. For example, *cendol*, *teh tarik cincau*, *air batu campur* (ABC), sugarcane juice, coconut juice and many more. Ramadan bazaar food, as well as street-vended food are not only appreciated for their unique flavors and the role they play within the society's cultural and social heritage, but they have also become important and essential for maintaining the nutritional status of the populations [38].

Food and beverages sold at Ramadan bazaar and street vendors share very similar characteristics: they are inexpensive, convenient and attractive. The common practice of food handlers preparing their food at home in the morning and then selling them at Ramadan bazaar in the afternoon raise doubt about food safety. Moreover, food handlers at Ramadan bazaar are usually seasonal traders and less

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experienced in handling food. According to Mat Zin *et al.* [20] and Rezaeigolestani *et al.* [26], the main risk factor associated with the occurrence of food-borne diseases during Ramadan is inappropriate storage and handling of cooked food products. In Malaysia, many cases of food poisoning involving the Ramadan bazaar are reported by the media almost every year and most cases are reported in Peninsular Malaysia compared to Sabah and Sarawak. For example, the last Ramadan in 2022, a total of 93 food poisoning cases due to Ramadan bazaar in Terengganu within just 11 days since the first day of Ramadan began on April 3, and two clusters of food poisoning related to Ramadan bazaar in Sabah were reported between April 3 and May 1. According to the most common reports of non-compliance by the food handlers at the Ramadan bazaar are not attending the basic food handler course, failing to conform to a neat and complete dress code and unhygienic food handling [9, 31].

The Food Safety and Quality Unit of Kuching Health Department has issued 10 notices under the Food Hygiene Regulations against Ramadan bazaar food handlers for several offenses including not wearing brightly colored aprons, not wearing proper shoes and having long nails [30]. While, the Miri Health Department issued 38 compounds to Ramadan bazaar stall proprietors and their food handlers for various offenses under Food Hygiene Regulations after inspecting 207 stalls and checking 429 food handlers [36]. In Limbang, a Ramadan bazaar food handler was fined for the charge of selling food contaminated with pathogenic bacteria; 'Coagulase Positive Staphylococci' [37].

The World Health Organization [38] defines street-vended food, or its equivalent "street foods", as foods and beverages prepared and sold by vendors on streets and other public places for intermediate consumption or consumption at a later time without further processing or preparation. Meanwhile the Codex Alimentarius Commission [11] defines ready-to-eat foods as foods that include any food (including beverages) consumed in its raw state or any food handled, processed, mixed, cooked or otherwise prepared onto a form during which it's commonly consumed without further processing. WHO [38] realized that street-vended foods may pose significant public health promotion due to the lack of basic infrastructure and services, poor knowledge of street vendors in basic food measures and insufficient public awareness of hazards posed by certain street foods.

Many studies from different countries have demonstrated poor microbiological quality of street-vended food and beverages including Africa [5, 10] Latin America [23], Asia [29, 25, 32] and Malaysia [21, 2]. Collectively, these studies found high microbial counts of food borne bacterial pathogens (i.e., coliform, *E. coli*, *S. aureus*, *Salmonella* spp., and *B. cereus*) in street-vended foods and beverages. The presence of pathogenic bacteria in food poses a high risk to food handlers and consumers, as well as resulting in food poisoning, cholera and dysentery [1, 39, 10]. Moreover, some studies also revealed that bacteria found in beverages were resistant against ampicillin, penicillin, gentamicin, erythromycin, and tetracycline [18, 40].

Therefore, the present study was undertaken to evaluate the microbial quality and antibiotic susceptibility profile of beverages sold at different Ramadan bazaar. The results obtained in this study might be useful for regulatory agencies to take appropriate action in the future. Since there are very limited studies published on the microbiological analysis of food and beverages in the Ramadan bazaar, especially in Sarawak, thus Kuching City, Sarawak was chosen as a research location. As beverages are popular among consumers and potentially contaminated, the present study only focuses on beverages.

## Materials and Methods

### Sample Collection and Preparation

The sampling activities involved six Ramadan bazaar at three different locations in Kuching, Sarawak comprising Kuching North (Ramadan bazaar Satok, Ramadan bazaar Semariang and Ramadan bazaar Mydin Vista Tunku), Kuching South (Ramadan bazaar Stutong) and Padawan (Ramadan bazaar Matang and Ramadan bazaar Kota Sentosa) area. Generally, beverages for sale in Ramadan bazaar were placed in large plastic containers with ice and then pour out into cups of various sizes according to customer requests. A total of 34 samples comprising 12 types of beverages were collected aseptically from April to May 2022 for laboratory analysis. All samples were serially numbered, transported to the laboratory in a cool box, and maintained at 4 °C. The samples were either examined immediately or kept in a refrigerator and tested within 24 hours of sampling. All the samples were performed in accordance with standard. The samples were analysed for aerobic plate count (APC), and total counts of coliform, *E. coli*, *S. aureus* and *Salmonella* spp. to determine the microbial count.

## Microbiological Analysis

A 10-fold serial dilution was performed on the homogenized samples. Then 1 mL of appropriate dilutions were plated in triplicates on selected media. There were three standard methods employed for microbiological analysis used in this study, namely, (1) AOAC International Official Method 990.12:2002 Aerobic Count Plate [6], (2) AOAC International Official Method 991.14:2002 Coliform and *E. coli* Plate [7] and (3) AOAC International Official Method 2003.07 Staph Express Count Plate [8]. The reference cultures used in this study were *E. coli* ATCC 11775, *Enterobacter aerogenes* ATCC 13048, *S. aureus* ATCC 25923 and *S. epidermidis* ATCC 13518 (positive and negative controls) to conform the performance of medium used in the bacterial enumeration. As for the detection and enumeration of *Salmonella* spp., another standard method known as ISO 6579:2002 (E) [17] was used, whereby *Salmonella enterica* subsp. *enterica* serovar Typhimurium ATCC 14028 and *Citrobacter freundii* ATCC 43864 were used as the positive and negative control, respectively. The contamination levels of APC, coliforms, *E. coli*, *S. aureus* and *Salmonella* spp. were then compared to standard guidelines [15].

## Antibiotic Susceptibility Test

Identified isolate from each sample were subjected to antibiotic susceptibility testing. The antibiotic susceptibility test was performed using method recommended by the Clinical and Laboratory Standards Institute (CLSI) [12]. The antibiotic discs tested for *E. coli* were ampicillin (10 µg), amoxicillin/clavulanic (20/10 µg), cefotaxime (30 µg), ceftazidime (30 µg), ceftazidime/avibactam (30/15 µg), ceftazidime/meropenem (30/10 µg), ceftazidime/tazobactam (30/15 µg), ceftazidime/vancomycin (30/15 µg), ceftazidime/zinc sulfadiazine (30/15 µg), ceftazidime/zinc sulfadiazine/meropenem (30/15/10 µg), ceftazidime/zinc sulfadiazine/meropenem/vancomycin (30/15/10/15 µg), ceftazidime/zinc sulfadiazine/meropenem/vancomycin/linezolid (30/15/10/15/10 µg), ceftazidime/zinc sulfadiazine/meropenem/vancomycin/linezolid/colistin (30/15/10/15/10/10 µg), ceftazidime/zinc sulfadiazine/meropenem/vancomycin/linezolid/colistin/teicoplanin (30/15/10/15/10/10/30 µg), ceftazidime/zinc sulfadiazine/meropenem/vancomycin/linezolid/colistin/teicoplanin/trimethoprim-sulfamethoxazole (30/15/10/15/10/10/30/25 µg), ceftazidime/zinc sulfadiazine/meropenem/vancomycin/linezolid/colistin/teicoplanin/trimethoprim-sulfamethoxazole/levofloxacin (30/15/10/15/10/10/30/25/5 µg), ceftazidime/zinc sulfadiazine/meropenem/vancomycin/linezolid/colistin/teicoplanin/trimethoprim-sulfamethoxazole/levofloxacin/ciprofloxacin (30/15/10/15/10/10/30/25/5/5 µg), ceftazidime/zinc sulfadiazine/meropenem/vancomycin/linezolid/colistin/teicoplanin/trimethoprim-sulfamethoxazole/levofloxacin/ciprofloxacin/moxifloxacin (30/15/10/15/10/10/30/25/5/5/5 µg), ceftazidime/zinc sulfadiazine/meropenem/vancomycin/linezolid/colistin/teicoplanin/trimethoprim-sulfamethoxazole/levofloxacin/ciprofloxacin/moxifloxacin/ofloxacin (30/15/10/15/10/10/30/25/5/5/5 µg), ceftazidime/zinc sulfadiazine/meropenem/vancomycin/linezolid/colistin/teicoplanin/trimethoprim-sulfamethoxazole/levofloxacin/ciprofloxacin/moxifloxacin/ofloxacin/penicillin G (30/15/10/15/10/10/30/25/5/5/5/10 µg), ceftazidime/zinc sulfadiazine/meropenem/vancomycin/linezolid/colistin/teicoplanin/trimethoprim-sulfamethoxazole/levofloxacin/ciprofloxacin/moxifloxacin/ofloxacin/penicillin G/rifampicin (30/15/10/15/10/10/30/25/5/5/5/10/5 µg), ceftazidime/zinc sulfadiazine/meropenem/vancomycin/linezolid/colistin/teicoplanin/trimethoprim-sulfamethoxazole/levofloxacin/ciprofloxacin/moxifloxacin/ofloxacin/penicillin G/rifampicin/teicoplanin (30/15/10/15/10/10/30/25/5/5/5/10/5/30 µg), ceftazidime/zinc sulfadiazine/meropenem/vancomycin/linezolid/colistin/teicoplanin/trimethoprim-sulfamethoxazole/levofloxacin/ciprofloxacin/moxifloxacin/ofloxacin/penicillin G/rifampicin/teicoplanin/tetracycline (30/15/10/15/10/10/30/25/5/5/5/10/5/30/30 µg), ceftazidime/zinc sulfadiazine/meropenem/vancomycin/linezolid/colistin/teicoplanin/trimethoprim-sulfamethoxazole/levofloxacin/ciprofloxacin/moxifloxacin/ofloxacin/penicillin G/rifampicin/teicoplanin/tetracycline/trimethoprim-sulfamethoxazole (30/15/10/15/10/10/30/25/5/5/5/10/5/30/30/25 µg).

The zone of growth inhibition around each disc was then measured in millimeters, and the zone diameters were interpreted in according to CLSI standard criteria: sensitive, intermediate, or resistant [12, 14, 35]. The multiple antibiotic resistances (MAR) index was determined according to Zulfakar *et al.* [40].

## Statistical Analysis

All statistical analyses were performed using the IBM Statistical Packages for Social Science, (SPSS) Version 26. The frequencies and descriptive statistics such as mean, standard deviation, maximum and minimum. Bacterial counts (log CFU/mL) were presented as mean  $\pm$  standard deviation (SD). One-way ANOVA was conducted to compare the level of bacterial contamination between the three locations (Kuching North, Kuching South and Padawan). Statistically significant differences were confirmed at a 0.05 significance level.

## Results

### Microbiological Counts

The bacterial counts consisting of APC, coliform, *E. coli* and *S. aureus* counts in the beverages sold at Ramadan bazaar in Kuching are represented in Table 1. Samples from Ramadan bazaar in Padawan showed the highest APC and *E. coli* contamination with the mean value of  $4.846 \pm 0.780$  and  $2.257 \pm 0.252$  log CFU/mL, respectively. Meanwhile, samples from Ramadan bazaar in Kuching South showed the highest coliform contamination with the mean value of  $3.741 \pm 0.446$  log CFU/mL. It was found that *S. aureus* was present in six beverage samples from two Ramadan bazaars, in which Kuching North bazaar demonstrated slightly higher contamination ( $2.156 \pm 0.301$  log CFU/mL) than Padawan bazaar ( $2.108 \pm 0.600$  log CFU/mL). Most importantly, the APC and contamination of coliform, *E. coli* and *S. aureus* in all beverage samples obtained from six Ramadan bazaar was not significantly different among the three different localities investigated in this study ( $p = 0.902$ ,  $p = 0.919$ ,  $p = 0.151$  and  $p = 0.907$ , respectively). Fortunately, *Salmonella* spp. was not detected in all beverage samples in this study.

Compared to standard guidelines [15], 70% (24/34 samples) of the total samples were classified as unsatisfactory with regard to APC contamination. Most samples (97%) were classified as having marginal levels of coliform contamination. For *E. coli* contamination, 62% (21/34 samples) of the total samples were categorized as satisfactory, followed by 15% (5/34 samples) at a marginal level and 23% (8/34 samples) classified as unsatisfactory. For *S. aureus* contamination, 88% (30/34 samples) of the total samples were classified as satisfactory, while the rest were at a marginal level.

**Table 1.** Microbiological counts of APC, coliform, *E. coli* and *S. aureus* in beverages at Ramadan bazaar in Kuching, Sarawak

| Parameter        | Location      | N <sup>1</sup> | Mean <sup>2</sup> | ± SD  | Min   | Max   | p-value |
|------------------|---------------|----------------|-------------------|-------|-------|-------|---------|
| APC              | Kuching North | 17             | 4.711             | 0.916 | 2.732 | 5.301 | 0.902   |
|                  | Kuching South | 5              | 4.702             | 0.676 | 3.954 | 5.380 |         |
|                  | Padawan       | 12             | 4.846             | 0.780 | 3.176 | 6.1   |         |
|                  | Total         | 34             | 4.757             | 0.818 | 2.732 | 6.1   |         |
| Coliform         | Kuching North | 16             | 3.592             | 0.813 | 2.653 | 4.934 | 0.919   |
|                  | Kuching South | 5              | 3.741             | 0.446 | 2.964 | 4.041 |         |
|                  | Padawan       | 12             | 3.596             | 0.715 | 2.230 | 4.663 |         |
|                  | Total         | 33             | 3.616             | 0.716 | 2.230 | 4.934 |         |
| <i>E. coli</i>   | Kuching North | 6              | 1.709             | 0.591 | 1.000 | 2.398 | 0.151   |
|                  | Kuching South | 2              | 2.244             | 0.287 | 2.041 | 2.447 |         |
|                  | Padawan       | 5              | 2.257             | 0.252 | 1.919 | 2.505 |         |
|                  | Total         | 13             | 2.002             | 0.504 | 1.000 | 2.505 |         |
| <i>S. aureus</i> | Kuching North | 3              | 2.156             | 0.301 | 1.845 | 2.447 | 0.907   |
|                  | Kuching South | 0              | .                 | .     | .     | .     |         |
|                  | Padawan       | 3              | 2.108             | 0.600 | 1.415 | 2.462 |         |
|                  | Total         | 6              | 2.132             | 0.426 | 1.415 | 2.462 |         |

<sup>1</sup>Number of positive samples

<sup>2</sup>Mean bacterial counts expressed in Log (CFU/mL), SD: standard deviation

### Presence of *E. coli* and *S. aureus* in Beverage Samples

The distribution of bacterial isolates from different types of beverages based on different locations of Ramadan bazaar was presented in Table 2. The result indicated that eight out of 12 beverage types tested in this study were contaminated with *E. coli* and *S. aureus*. In addition, a total of 19 bacteria were isolated consisting of *E. coli* (13 isolates) and *S. aureus* (6 isolates). Sugarcane juice was highly contaminated with both *E. coli* and *S. aureus*. *Teh tarik cincau* was contaminated only with *E. coli* in all three locations. Overall, this study showed that beverages sold in Kuching North and Padawan bazaars mostly contaminated with *E. coli* and *S. aureus* respectively. Interestingly, four types of beverages such as *jagung*, Milo, watermelon and strawberry juice were not contaminated with these two bacteria.

**Table 2.** Distribution and isolate designation of *E. coli* and *S. aureus* according to the different types of beverages

| Type of beverages       | <i>E. coli</i> |               |         | <i>S. aureus</i> |               |         |
|-------------------------|----------------|---------------|---------|------------------|---------------|---------|
|                         | Kuching North  | Kuching South | Padawan | Kuching North    | Kuching South | Padawan |
| <i>Teh tarik cincau</i> | E1             | E8            | E13     | -                | -             | -       |
| Chocolate Milk          | E2             | -             | -       | -                | -             | -       |
| <i>Cendol</i>           | E3             | -             | E10     | -                | -             | -       |
| Sugarcane juice         | E4, E6         | E7            | E9, E12 | S1, S2           | -             | S6      |
| <i>Teh Tarik</i>        | E5             | -             | -       | -                | -             | -       |
| <i>Jagung cincau</i>    | E11            | -             | -       | -                | -             | -       |
| Honeydew shake          | -              | -             | -       | -                | -             | S4      |
| Coconut juice           | -              | -             | -       | S3               | -             | S5      |
| Total                   | 7              | 2             | 4       | 3                | 0             | 3       |

### Antimicrobial Susceptibility Profiles

In this study, 19 isolates of *E. coli* and *S. aureus* were tested for their sensitivity against the commonly prescribed antibiotics according to the CLSI guideline [12]. *E. coli* isolates were 100% sensitive against ceftaroline, ciprofloxacin, imipenem, kanamycin, levofloxacin, netilmicin, ofloxacin, streptomycin and tobramycin as presented in Table 3. Among all tested antibiotics, *E. coli* isolates showed maximum resistance to ampicillin (77%), followed by amoxicillin/clavulanic (31%), cefoxitin (16%) and tetracycline (16%). Five *E. coli* isolates showed a low MAR index (0.12 – 0.18). All *S. aureus* isolates were fully resistant towards ampicillin and penicillin. Isolate S4 showed the highest MAR index of 0.28 with resistance to five tested antibiotics (ampicillin, erythromycin, penicillin, rifampicin and teicoplanin). Other than that, two isolates showed a MAR index of 0.28. Bacterial isolates with a MAR higher than 0.20 were linked to sources with common antibiotic usage [40]. This study also revealed that all *S. aureus* isolates were sensitive to cefoxitin, chloramphenicol, ciprofloxacin, clindamycin, gentamicin, linezolid, moxifloxacin, ofloxacin and trimethoprim-sulfamethoxazole. The MAR index profile of nine isolates from beverages in this study is presented in Table 4.

**Table 3.** Antibiotic resistance profiles of the *E. coli* and *S. aureus* isolated from Ramadan bazaar beverages

| Antibiotics                   | No. (%) of <i>E. coli</i> Isolates |              |           | No. (%) of <i>S. aureus</i> Isolates |              |           |
|-------------------------------|------------------------------------|--------------|-----------|--------------------------------------|--------------|-----------|
|                               | Resistance                         | Intermediate | Sensitive | Resistance                           | Intermediate | Sensitive |
| Ampicillin                    | 10 (77)                            | 3 (23)       | 0 (0)     | 6 (100)                              | 0 (0)        | 0 (0)     |
| Amoxicillin/clavulanic        | 4 (31)                             | 3 (23)       | 6 (46)    | NA                                   | NA           | NA        |
| Cefotaxime                    | 0 (0)                              | 3 (23)       | 10 (77)   | NA                                   | NA           | NA        |
| Ceftaroline                   | 0 (0)                              | 0 (0)        | 13 (100)  | 0 (0)                                | 3 (50)       | 3 (50)    |
| Cefoxitin                     | 2 (16)                             | 0 (0)        | 11 (84)   | 0 (0)                                | 0 (0)        | 6 (100)   |
| Chloramphenicol               | 1 (8)                              | 1 (8)        | 11 (84)   | 0 (0)                                | 0 (0)        | 6 (100)   |
| Ciprofloxacin                 | 0 (0)                              | 0 (0)        | 13 (100)  | 0 (0)                                | 0 (0)        | 6 (100)   |
| Clindamycin                   | NA                                 | NA           | NA        | 0 (0)                                | 0 (0)        | 6 (100)   |
| Erythromycin                  | NA                                 | NA           | NA        | 1 (17)                               | 1 (17)       | 4 (66)    |
| Fusidic acid                  | NA                                 | NA           | NA        | 2 (34)                               | 0 (0)        | 4 (66)    |
| Gentamicin                    | 1 (8)                              | 1 (8)        | 11 (84)   | 0 (0)                                | 0 (0)        | 6 (100)   |
| Imipenem                      | 0 (0)                              | 0 (0)        | 13 (100)  | NA                                   | NA           | NA        |
| Kanamycin                     | 0 (0)                              | 0 (0)        | 13 (100)  | 1 (17)                               | 0 (0)        | 5 (83)    |
| Levofloxacin                  | 0 (0)                              | 0 (0)        | 13 (100)  | NA                                   | NA           | NA        |
| Linezolid                     | NA                                 | NA           | NA        | 0 (0)                                | 0 (0)        | 6 (100)   |
| Moxifloxacin                  | NA                                 | NA           | NA        | 0 (0)                                | 0 (0)        | 6 (100)   |
| Netilmicin                    | 0 (0)                              | 0 (0)        | 13 (100)  | NA                                   | NA           | NA        |
| Ofloxacin                     | 0 (0)                              | 0 (0)        | 13 (100)  | 0 (0)                                | 0 (0)        | 6 (100)   |
| Penicillin G                  | NA                                 | NA           | NA        | 6 (100)                              | 0 (0)        | 0 (0)     |
| Rifampicin                    | NA                                 | NA           | NA        | 2 (34)                               | 0 (0)        | 4 (66)    |
| Streptomycin                  | 0 (0)                              | 5 (38)       | 8 (62)    | NA                                   | NA           | NA        |
| Teicoplanin                   | NA                                 | NA           | NA        | 1 (17)                               | 0            | 5 (83)    |
| Tetracycline                  | 2 (16)                             | 0 (0)        | 11 (84)   | 1 (17)                               | 1 (17)       | 4 (66)    |
| Tobramycin                    | 0 (0)                              | 0 (0)        | 13 (100)  | NA                                   | NA           | NA        |
| Trimethoprim-sulfamethoxazole | 1 (8)                              | 0 (0)        | 12 (92)   | 0 (0)                                | 0 (0)        | 6 (100)   |

NA: Not analysed

**Table 4.** Multiple Antibiotic Resistances (MAR) Index profile of bacterial isolates

| Isolate ID | Antibiotic Resistance Profile | MAR Index |
|------------|-------------------------------|-----------|
| E1         | AMP, AMC                      | 0.12      |
| E3         | AMP, AMC, C                   | 0.18      |
| E7         | AMP, AMC, FOX                 | 0.18      |
| E10        | AMP, CN                       | 0.12      |
| E12        | AMP, AMC, SXT                 | 0.18      |
| S1         | AMP, FD, P, RD                | 0.22      |
| S2         | AMP, P, TE                    | 0.17      |
| S3         | AMP, FD, K, P                 | 0.22      |
| S4         | AMP, E, P, RD, TEC            | 0.28      |
| S5, S6     | AMP, P                        | 0.12      |

AMP:Ampicillin; AMC:Amoxicillin/clavulanic; C:Chloramphenicol; CN:Gentamicin; E:Erythromycin; FOX:Cefoxitin; FD:Fusidic acid; K: Kanamycin; P:Penicillin; RD: Rifampicin; SXT:Trimethoprim-sulfamethoxazole; TE:Tetracycline; TEC:Teicoplanin

## Discussion

The Ramadan bazaar is regulated in open area by local authorities. The designated bazaar is commonly situated in an empty space with provided basic infrastructures and facilities such as water supply and drainage facilities. Food handlers are made compulsory to attend food handler training courses as well as vaccination before being allowed to operate at any Ramadan bazaar according to Malaysian Food Hygiene Regulation 2009. In the same regulation, food handler shall maintain a high degree of personal cleanliness, keep food premise clean and ensure preparation, packing and serving food is free from any contamination. In this study, several parameters such as APC, coliform, *E. coli* and *S. aureus* were assessed as an indicator organism for the hygiene practices of food handlers in food preparation [22, 2]. To be specific, the presence of APC will provide substantial information on the microbiological quality of the beverages or foods. Occurrence of coliform or *E. coli* in foods can be associated to contamination of human and animal faeces owing to unhygienic practices during and after production [4, 13].

The present study demonstrated that the most susceptible beverage for microbial contamination of *E. coli* and *S. aureus* was sugarcane juice. Although the Food Safety and Quality Division, Ministry of Health Malaysia has issued guidelines for the sale and preparation of fresh sugarcane juice since 20 to ensure that only safe and clean sugarcane juice is sold to customers, microbial contamination still occurs [16]. Previously, sugarcane juice was sold directly after stalk extraction, but nowadays it is extracted and bottled earlier before being chilled in ice for sale. Improper washing and storage of the sugarcane stalk will be a major cause for the presence of *E. coli* or other bacteria. In the present study, 91% (10/11 samples) and 27% (3/11 samples) of sugarcane juice samples exceeded APC levels and *E. coli* levels, respectively. High count of APC, coliform, *E. coli* and *S. aureus* in sugarcane juice were reported by Afreen *et al.* [3] and Thi *et al.* [32]. Afreen *et al.* [3] reported that all the sugarcane juice samples were found unsatisfactory in comparison to guidelines of APC and *S. aureus*. In addition, up to 80% of the samples also exceeded the guidelines for coliform and *E. coli*. Afreen *et al.* [3] also listed several risk factors that significantly correlated with microbial contamination of their beverage's samples such as source of water, vending site, types of beverages, washing of utensils and hand washing habits. Meanwhile, Thi *et al.* [32] observed a high level of total mesophilic and coliform counts above 5.0 Log CFU/mL and concluded from observational data that food handlers who do not practice good personal hygiene result in high microbial loads in their samples.

On the other hand, the most common *E. coli* contamination was seen in *teh tarik cincau* from all three locations and exceeded the standard value. *Teh tarik cincau* that is made from a strong brew of black tea blended with condensed milk and mixed with *cincau* (grass jelly) can be a source of bacteria contamination if not handled in hygienically. The safety and hygiene of *cincau* storage should also be emphasized as it is added later in the preparation of the *teh tarik cincau*. Mohd Nawawi *et al.* [21] reported only one from nine samples was found to be contaminated with *E. coli* in milk-based drinks, but the level of contamination was very high (> 3.0 Log CFU/mL) and exceeded the standard value. They

also reported 100% of milk-based drink samples were positive for total viable count and coliform, whereas 78% were positive with *S. aureus*. To date, there is no study reported on the microbiological quality of the *teh tarik* sold in Malaysia.

However, four types of beverages such as *jagung*, Milo, watermelon and strawberry juice were not contaminated with either *E. coli*, *S. aureus* or both in this study. Previously, there were also studies that reported the absence of certain types of bacteria in fruit juice, cordial-based and milk-based drinks [21, 34]. The possible reasons for this could be the good quality water used for dilution as well as prevailing hygienic conditions related to washing of hands and utensils. Furthermore, adequate peeling times of fruits and appropriate storage of peeled fruits also contribute to reduce probability of microbial contamination in fruit juice [28]. In this study, *Salmonella* spp. were absent in any Ramadan bazaar beverage samples. The result was consistent with previous study by Paul *et al.* [24], who observed *Salmonella* spp. in food samples but not in beverage samples. Another study conducted by Sharma *et al.* [28] reported that no *Salmonella* spp. was found in samples of orange juice, mixed juice and sugarcane juice from Kamla Nagar, India and was associated with lower total bacterial counts of water sources in the area. In contrast with Afreen *et al.* [3], reported that *Salmonella* spp. found in beverages and was significantly associated with the distance between stalls and bins.

In the report by Thi *et al.*, [32], their study about food safety status of sugarcane juice collected from different locations such as schools, hospitals and markets was conducted without ice to avoid the interference of ice contamination. However, the beverage samples in this study were already mixed with ice during sampling and provided a possible source of additional bacterial contamination. Although ice manufacturers have obtained a license to sell ice from the Ministry of Health, Malaysia, it could be another contributor to microbial contamination if the ice is not handled properly. At the Ramadan bazaar, it was found that ice cubes are stored in large plastic bags and put into large containers, or it is put directly into the container before being mixed with the beverages. The cleanliness of the ice container is also questionable. Furthermore, the majority of food handlers at Ramadan bazaar washed their hands with water in a bucket, since the water source is quite far from the stall. Repeated use of the same water to wash hands and utensils can contribute to the source of cross-contamination [3]. In addition, using a cup as an ice scoop can also pose a risk of contamination by contact with the unhygienic food handler's hand. A study in Thailand has proven unsatisfactory coliform counts in ice sold at food and drink premises. They also reported that no premises kept their ice container above 60 cm and no food handlers wash their hands properly before preparing, cooking, or handling food and ice [33].

In the current study, antibiotic resistance profiles of bacteria isolated from beverages were also determined. Approximately 58% (11/19) of the isolates showed the prevalence of multidrug-resistant strain, while 84% (16/19) of the isolates were resistant to ampicillin. This in accordance with Uddin *et al.* [34], who reported that 84% of *S. aureus* isolates and 95% of *E. coli* isolates were resistant to ampicillin. The antimicrobial pattern of *S. aureus* and *E. coli* isolates against ampicillin are also in agreement with Sabuj *et al.* [27]. In the present study, the *S. aureus* isolates were also completely resistant to penicillin indicating that these isolates produce beta-lactamase, an enzyme that deactivates penicillin and other closely related antibiotics [18]. In general, all isolates show the highest level of susceptibility towards ceftaroline, ciprofloxacin, clindamycin, imipenem, levofloxacin, linezolid, moxifloxacin, netilmicin, ofloxacin, streptomycin and tobramycin. However, the susceptibility of both *S. aureus* and *E. coli* isolates to ciprofloxacin and ofloxacin suggested these antimicrobials as the drug of choice for the treatment of food borne illness in the study area. Results from this study show that beverage samples were contaminated with a various of bacteria with some resistant to commonly used antibiotics and therefore represent a risk to public health.

## Conclusions

The high APC counts found in different types of beverages in this study clearly demonstrated that beverages sold at Ramadan bazaar in Kuching, Sarawak were contaminated with various harmful microorganisms. This finding is also supported by the presence of *E. coli* and *S. aureus*, which proved the poor personal hygiene and improper handling practices among food handlers. Thus, it is recommended that the Malaysian Food Hygiene Regulation 2009 needs to be comprehensively enforced to the Ramadan bazaar food handlers. The relevant authorities should plan the framework of food safety training programs and evaluate the root cause of contamination more effectively.

## Conflict of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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