Coliform contamination on faucet surface of water vending machines in Klang Valley

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Abstract: In Malaysia, water vending machine serves as an alternative to drinking water supply. However, the quality of drinking water obtained from water vending machines may vary due to microorganism contamination which caused by inadequate hygienic practices and routine maintenance of the machines. In this study, 100 water vending machines were randomly selected from 10 districts of Klang Valley. Sterile cotton swabs were used to collect swab samples from all selected subjects, with swab samples collected on the outer surface of water faucets. Samples were sent to laboratory for culture analyses using Nutrient agars (Oxoid) and HiCrome™ Coliform agars (HiMedia Laboratories). The results showed that none of the water vending machine was contaminated by faecal coliform, however, with close to 80% of the subjects were found contaminated by total coliform (eg. Klebsiella, Enterobacter or Citrobacter species). Although the presence of total coliform may not be deleterious to the health of end users, our findings highlights the need for authorities and water vending service providers to set an effective sanitation procedure in maintaining the hygienic level of water vending machines.

Keywords: Vending machine; microorganism; faecal coliform; water; hygienic; Klang Valley Malaysia

Received: 12th April 2020
Accepted: 14th May 2020
Published Online: 20th May 2020

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Citation: Ang YM and Tham H-W. Coliform contamination on faucet surface of water vending machines in Klang Valley. Prog Microbes Mol Biol 2020; 3(1): a0000086. https://doi.org/10.3687/pmmb.a0000086

Introduction

Food and water have never been safer in terms of the incidence of infectious illness. A variety of bacteria, viruses and even parasite are transmitted via food and water, thus causing a rise of cases in recent years[1–13]. A growing population with limited resources of clean water has resulted in 4 billion cases of diarrhoea every year[14]. Waterborne diseases can be caused by various pathogenic agents, including Escherichia, Enterobacter, Shigella, Klebsiella, Campylobacter, Cryptosporidium, Giardia, Salmonella and enteric viruses[15]. Of this, Salmonella, Vibrio, Escherichia, Campylobacter and Citrobacter are among the causative agent of foodborne pathogens as well[16–31].

In Malaysia, quality of drinking water remains as one of the main concerns of consumers. A survey conducted by Aini et al. reported that odour, taste and colour were the major issues with water supply[32] in spite of these, quantity and quality of drinking water is still one of the main concerns of Malaysian consumers today. An exploratory study was undertaken to determine the level of awareness of respondents on water issues, assess their perception on drinking water quality, and identify measures undertaken by households to improve drinking water quality and to determine sustainable water practices. A cross-sectional research design, utilizing a survey was conducted among urban residents of Seremban town. Data showed that each household had a mean of five members, with an average household income of RM3788.00 (US$1000). Unaffordable water filtration systems have resulted in good sales of product water from vending machines[33], which serve as an alternative source of drinking water. Several factors such as hygienic practice of users, routine maintenance by owners, quality control by authorities are crucial in maintaining the quality of drinking water[34].

Faecal coliform is a rod-shaped anaerobic bacterium with no sporulation. Its members consist of various species of bacteria such as Escherichia, Enterobacter, Klebsiella, Salmonella and Shigella. They exist in the faecal materials and intestinal tract of humans or warm-blooded animals and enter the water bodies through...
the waste products. However, they are usually non-pathogenic and can be used as an indicator organisms to indicate the presence of faecal material in water[33,35].

*E. coli* O157:H7, a pathogenic strain of *E. coli*, is pathogenic and causes gastroenteritis, bloody diarrhoea, urinary tract infection (UTI) and haemolytic-uremic syndrome (HUS)[36]. According to United States Environmental Protection Agency, the presence of *E. coli* serves as a good indicator of faecal coliform contamination to evaluate the microbiological quality of water. On the other hand, the presence of other coliforms such as *Klebsiella*, *Salmonella*, or *Shigella* in consumable products also increases the risk of health conditions such as urinary/respiratory infections[37], salmonellosis[16,38–40], and shigellosis[41], respectively.

According to National Water Quality Standards, Ministry of Health Malaysia, a Class I water quality should contain total coliform at the maximum of 100 CFU/mL, with faecal coliform at the maximum of 10 CFU/mL. In view of the importance of drinking water quality in relation to the health of users in public, this study aimed to test the presence of faecal coliform and total coliform contamination on the faucets of water vending machines. The results were also correlated to different districts, brands, status of maintenance and licence.

### Material and Methods

#### Agar Media

Nutrient agar powder (Oxoid) and HiCrome™ Coliform agar (HiMedia Laboratories) were purchased and prepared according to manufacturer’s instructions. All procedures in this section were conducted under aseptic conditions.

#### Sampling Sites

Ten water vending machines were randomly chosen from commercial areas of each of the 10 districts of Klang Valley, including Ampang, Cheras, Gombak, Kajang, Klang, Kuala Lumpur, Puchong, Petaling Jaya, Subang Jaya, and Shah Alam. These areas were selected for their higher population density. In total, 100 sampling sites were identified.

### Sampling Sample

Sample collections were conducted in biological triplicates, using sterile cotton buds collecting microbiome on the outer surface of water faucets at 1 cycle anticlockwise. The cotton buds were then immediately kept in sterile 15 mL falcon tubes pre-filled with 1 mL of pre-sterilised 0.8% NaCl solution. Samples were sent to Biology Laboratories for downstream assays within 5 hours.

### Detection of Total Microbial and Coliform

All samples were spread on either Nutrient agar or HiCrome™ Coliform agar. All agars were incubated (37 °C, 18 hours) and the numbers of colony were documented (in CFU/mL) and analysed. Meanwhile, the colour and morphology of colonies were also observed and recorded. Some colonies were selected for Gram staining for further characterisation.

### Statistical Analysis

All results and data were analysed using Statistical Package for the Social Sciences (SPSS) software (version 23). Kruskal-Wallis tests and Mann-Whitney U Tests were used in these analyses. Statistical analyses were conducted based on total microbial counts, between brands, maintenance status, licence status of water vending machines.

### Results

#### Sampling Sites

The sampling sites were presented in Figure 1, which was generated using Google My Maps.
Total Microbial Counts of Various Districts in Klang Valley

Total microbiome on the faucet surface of water vending machines were measured using Nutrient agars and HiCrome™ Coliform agars (Figure 2).

Table 1 illustrates the outcome of Kruskal Wallis test between different districts. The difference between total CFU/mL detected on HiCrome™ Coliform agars were significant ($P < 0.05$), while results presented by Nutrient agars showed the opposite ($P > 0.05$).

### Table 1. Correlation between different districts of Klang Valley and total microbial counts detected on Nutrient agars and HiCrome™ Coliform agars.

<table>
<thead>
<tr>
<th>District</th>
<th>HiCrome Coliform agar with SLS (HA)</th>
<th>Nutrient agar (NA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Asymptotic significance</td>
<td>$P$-value</td>
</tr>
<tr>
<td></td>
<td>.015</td>
<td>$&lt; 0.05$</td>
</tr>
<tr>
<td></td>
<td>.112</td>
<td>$&gt; 0.05$</td>
</tr>
</tbody>
</table>

Bacterial Colonies on HiCrome™ Coliform Agars

The number of bacterial colonies tabulated in Table 3 indicates the presence of Klebsiella species (light-pink colonies), Enterobacter or Citrobacter species (salmon-red colonies), or other Gram negative coliform bacterial species (opaque-white colonies).

### Table 2. Correlation between the status of licence during sampling and total microbial counts detected on Nutrient agars and HiCrome™ Coliform agars.

Table 2 shows that the status of licence during sampling does not contribute significantly to the hygienic level of water vending machines ($P > 0.05$).

<table>
<thead>
<tr>
<th>District</th>
<th>Light-pink colonies</th>
<th>Salmon-red colonies</th>
<th>Opaque-white colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampang</td>
<td>1</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Cheras</td>
<td>1</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Gombak</td>
<td>4</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Kajang</td>
<td>1</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Klang</td>
<td>2</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Kuala Lumpur</td>
<td>1</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Petaling Jaya</td>
<td>1</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Puchong</td>
<td>3</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Shah Alam</td>
<td>1</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Subang Jaya</td>
<td>1</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
<td>8</td>
<td>77</td>
</tr>
</tbody>
</table>

Discussion

In 2018, the National Water Service Commission (SPAN) has confirmed the absence of *E. coli* from Malaysia’s water supply, which was also claimed safe for direct consumption. Nevertheless, for the supplier or owner of water vending machines located in various business centres in Klang Valley,
they should maintain a routine maintenance to prevent the filter membranes in RO system become overgrown with microorganisms. The quality control of water vending machines by authorities or service providers is crucial in maintaining the health of end users.

Our study suggested that the level of contamination was not significantly associated with different districts of Klang Valley (Table 1). This conclusion was drawn from the $P$-value given using Nutrient agar as a general growth medium. The low $P$-value from HiCrome™ Coliform agars was deemed less reliable since the agars were supplied with sodium lauryl sulfate (SLS), which suppresses the growth of many microorganisms. This finding was in accordance to the dynamic growth and strong population movement within Klang Valley.

During the study, we noticed that less than half (43%) of the 100 water vending machines received routine maintenance. Nevertheless, the status of maintenance was not found strongly associated with the hygienic level on the surface of water vending machine faucets. Our record shows that, in Shah Alam, 7 out of 10 selected water vending machines had undergone routine maintenance around a week before the day of sampling, whereas only 30–60% of water vending machines had clear indication of routine service from other areas. Nevertheless, statistical analyses have shown that the status of routine maintenance was not strongly associated with the presence of total coliform on the water faucet surface. The same trend was observed for the status of licence issued by respective authorities (Table 2), with only 9 out of 100 subjects were found labelled clearly with licence obtained from authorities.

Despite the standard operating procedure (SOP) set by authorities or service providers, faucets of water vending machines can still be contaminated due to physical contact with hands of user or exposure to fomites on water containers. Routine maintenance with effective sanitisation of all outer and inner surfaces is crucial. We would like to highlight the stressing needs for proper sanitisation on the contact points between users and water vending machines for the persistent hygienic issues.

Although the presence of faecal coliform in water supply may not be harmful, but it is an indication of the presence of faeces. Despite cases of water vending machines contaminated by faecal coliforms (E. coli O157:H7), fortunately, none of the selected subjects in this study was found to harbour faecal coliform (Figure 1, Table 3). However, in accordance to other studies, the presence of other total coliform (eg. Klebsiella, Enterobacter or Citrobacter species) was reported in our findings.

**Conclusion**

In conclusion, our findings report the absence of faecal coliform from the faucet surface of all selected water vending machines in Klang Valley. However, the presence of other total coliforms highlights the importance of a proper and effective sanitisation by authorities and service providers — regardless of the area, licence and maintenance status of the machines.

**Authors Contribution**

The research and manuscript writing were performed by Y-MA. H-WT founded the research project.

**Conflict of Interest**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Funding**

This project was fully funded by SEGi University Research Grant.

**Acknowledgement**

Authors would like to thank the Faculty of Pharmacy (SEGi University) and Research & Innovation Management Centre (RIMC) of SEGi University for their full support in conducting the research project.

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