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INTERNATIONAL PERSPECTIVES

Bacterial Water Quality in Personal Reusable Water Bottles of Undergraduate Medical Students at a Malaysian University

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Abstract **Reusable water bottles are popular nowadays due to the conveniences that they bring. Poor bacterial water quality in these bottles, however, can present risks to consumer health, as the drinking water can become a vehicle for waterborne illnesses. In this study, we aimed to investigate the bacterial water quality and its associated factors in the personal reusable bottles of medical students at our university. A survey, heterotrophic plate count (HPC), and coliform screening were used in this study. Among the 37 water samples, 46% were coliform-positive and 78% had a high HPC of >100 CFU/ml. These results are surprising considering that medical students should generally have a high level of health awareness. In addition, both bottleneck design and bottle material appeared to contribute to poor bacterial water quality, which should warrant further investigation.**

Keywords: bacterial water quality, coliforms, heterotrophic plate count, drinking water, reusable bottles

Introduction

Reusable water bottles are popular nowadays due to the advantages they bring, such as being portable and environmentally friendly. Poor bacterial water quality in personal reusable bottles, however, can present risks to consumer health because the drinking water could become a vehicle for waterborne illnesses. To date, there is no information available about outbreaks or incidences of sickness resulting from the insufficient hygiene of water bottles. This lack of awareness could possibly be due to people not knowing that they can become sick drinking contaminated water from their personal bottles and/or failure to report such cases (Tabaco et al., 2018). Studies are lacking on the bacterial contamination level of drinking water in personal bottles among medical students, who should have a relatively high level of health awareness. In this study, we investigated bacterial water quality and associated factors in personal reusable water bottles of medical students at our university.

Methods

Sampling

The study location was the Universiti Tunku Abdul Rahman (UTAR) Sungai Long campus, Selangor, Malaysia. We have 213 students currently enrolled in the Bachelor of Medicine, Bachelor of Surgery (MBBS) program at our university. In this study, we recruited 37 MBBS students (17%) from this population. Each participant provided informed consent. Data from the participants (demographic information, bottle cleaning behaviors, and bottle information) were collected via a digital questionnaire (Supplemental Survey 1) and water samples were collected from participant bottles. This study was approved by the UTAR Scientific and Ethical Review Committee (Ref. U/SERC/236/2022).

Laboratory Testing

Heterotrophic plate count (HPC) and coliform screening were used to investigate the water quality. HPC was carried out using the standard plate count method, which involved plating 100 µl of the water sample onto plate count agar. Coliform screening was performed by filtering 100 ml of the water sample and culturing the membrane filter on MacConkey agar. Plates were incubated overnight at 37 °C. HPC was expressed in CFU/ml and a coliform (i.e., a pink colony) was noted as either present or absent.

Water Safety Limits

For HPC, the limit for safe consumption was ≤100 CFU/ml (World Health Organization, 2017). Safe drinking water should have no coliforms in 100 ml of the sampled water (Centers for Disease Control and Prevention, 2003; World Health Organization, 2017).

Statistical Analyses

Results were analyzed using SPSS Statistics version 28. We used Fisher's exact test to investigate the association between the occurrence of coliforms and various factors to be investigated. We also used the Mann–Whitney U test and Kruskal–Wallis test to analyze the difference in median HPC under different conditions. A test with a *p*-value <.05 was considered statistically significant.

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TABLE **1**

Descriptive Statistics From Study Participants

Variable	# (%)	Median Heterotrophic Plate Count in CFU/ml (IQR)	Presence of Coliforms # (%)
Sex			
Male	15 (40.5)	4,000 (17,930)	9 (60.0)
Female	22 (59.5)	1,965 (11,493)	8 (36.4)
Bottleneck*			
Yes	27 (73.0)	3,250 (14,630)	16 (59.3)
No	10 (27.0)	1,080 (10,700)	1 (10.0)
Bottle age			
≤3 months	3 (8.1)	2,420 (166,290)	1 (33.3)
>3–6 months	3 (8.1)	7,890 (18,090)	1 (33.3)
>6–12 months	4 (10.8)	1,430 (12,035)	3 (75.0)
>12 months	27 (73.0)	3,250 (13,620)	12 (44.4)
Cleaning method			
With brush and soap	13 (35.1)	4,000 (18,215)	8 (61.5)
With soap	9 (24.3)	2,310 (8,575)	4 (44.4)
With water only	15 (40.5)	3,100 (17,090)	5 (33.3)
Cleaning frequency			
Daily	15 (40.5)	2,420 (15,120)	8 (53.3)
Other	22 (59.5)	3,550 (14,403)	9 (40.9)
Previous cleaning date			
Today	8 (21.6)	1,610 (2,768)	2 (25.0)
<1 week	26 (70.3)	5,945 (17,590)	13 (50.0)
>1 week	3 (8.1)	2,700 (32,200)	2 (66.6)
Water source			
Home	30 (81.1)	3,625 (13,935)	14 (46.7)
University dispenser	2 (5.4)	85,100 (N/A)	0 (0)
Both	5 (13.5)	2,420 (9,145)	3 (60.0)
Bottle material			
Stainless steel	7 (18.9)	40 (3,100)	2 (28.6)
Plastic	30 (81.1)	4,000 (15,308)	15 (50.0)

* A bottleneck was defined operationally as a design in which the user's hand cannot fit into the neck or bottle.

Note. IQR = interquartile range; N/A = not applicable because the IQR could not be computed due to a limited sample size (two overall samples).

Results and Discussion

Descriptive Statistics

In this study, we investigated eight possible factors that could affect the bacterial water quality (as measured by HPC and detection of coliforms): self-identified sex, bottleneck design, bottle age, cleaning method, cleaning frequency, previous cleaning date, water source, and bottle material. Descriptive statistics are summarized in Table 1.

Coliform Screening

In this study, there was an alarmingly high number of coliform-positive samples (17/37, 46%). According to guidelines from the World Health Organization and the Centers for Disease Control and Prevention, coliforms should be absent or not detectable in 100 ml of the water sample for the water to be considered safe for human consumption.

Coliform bacteria are indicator organisms for fecal contamination in drinking water. Although coliforms might not cause diseases in immunocompetent individuals, the feces that carry the coliforms can contaminate the water with other pathogenic bacteria, such as *Shigella* spp., *Salmonella* spp., and *Vibrio cholerae*.

Out of the eight independent variables investigated, only the bottleneck design had a significant association with the detection of coliforms (p = .010, OR = 13.09), indicating that the odds of having coliforms in bottles with a bottleneck were roughly 13-times higher than in those without a bottleneck. Due to the small mouthpieces of these bottles, it was difficult to clean the inner part of the bottle thoroughly because a hand cannot fit in the bottle, which affects cleaning ease and efficacy (Sun et al., 2017).

Heterotrophic Plate Count

Among the 37 collected samples, the median HPC was 3,100 CFU/ml (interquartile range [IQR] = 14,165), which was 31-fold higher than the safe drinking water limit of 100 CFU/ml. The highest HPC was 167,100 CFU/ml and the lowest was 0 CFU/ml. Disconcertingly, 78% of water samples (29/37) were found to be unsafe to consume because the HPC exceeded the safety limit. This finding was congruent with observations of high HPC in water samples that far exceeded the standards for safe drinking water, as made by Oliphant et al. (2002) of 64% of water bottles (49/76) of elementary students; Liu & Liu (2017) of 97% of bottles (29/30) of 2- to 7-year-olds; and Sun et al. (2017) of 70% of bottles (63/90) from students and staff at a college.

We investigated if any of the eight independent variables contributed to a significant change in HPC. Interestingly, we noted a significant difference in median HPC between bottles made of stainless steel (40 CFU/ml) and plastic (4,000 CFU/ml) (p = .046). One possible explanation for this difference is that the participants were more likely to carry hot water in a stainless-steel bottle, which could lower the bacterial count. The remaining factors did not produce any statistically significant findings.

It is important to acknowledge that consuming water with a high HPC does not necessarily lead to infections, as the heterotrophic bacteria might not be pathogenic. A high HPC, however, can hamper the coliform detection on lactose-based media (Allen et al., 2004), including the MacConkey agar used in this study, which could lead to false-negative results for the coliform screening. Therefore, the prevalence of coliform detection might be underreported in this study.

Limitations

We acknowledge that some of our findings might be susceptible to recall bias (bottle age and previous cleaning date) and social desirability bias (previous cleaning date and cleaning frequency), leading to overreporting or underreporting, which could affect the accuracy of our inferential analyses for these factors. As previously noted, a high HPC can hamper coliform detection on lactose-based media, leading to false-negative results.

Conclusion

According to the results of our survey, 95% of the participants (35/37) were aware that their bottles can act as a microbial reservoir, contaminating their drinking water. Unfortunately, this high level of awareness did not translate into good microbial quality measured in their drinking water, as demonstrated by our finding of 46% of water samples were found to be coliform-positive and 78% had a high HPC >100 CFU/ml. The rate of coliform-positive samples in our study was more than 3-times higher than the rate Oliphant et al. (2002) reported for elementary students of 14% of bottles (11/76). Our study finding is surprising because medical students in general should have a high level of health awareness. In addition, both bottleneck design and bottle material appear to contribute to poor bacterial water quality, which should warrant further investigations.

After we completed this study, we organized a health promotion campaign at our university to raise awareness about the importance of bottle hygiene and clean drinking water. The culture results and plates were shown to our participants. Some of the participants were genuinely surprised that they were drinking water that was heavily contaminated by microorganisms. A possible follow-up study would be to investigate the microbiological water quality of this study's participants after the health promotion campaign. This follow-up study could allow us to measure the effectiveness of our intervention (i.e., the clean water bottle and clean drinking water health promotion campaign).

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