Original Research Article

Progress in Microbes and Molecular Biology

HH PUBLISHER

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

Yi-Mian Ang[1], Hong-Wai Tham[1*]

[1]Biopharmaceutical Research Unit, Faculty of Pharmacy, SEGi University, Petaling Jaya, Selangor, Malaysia.

**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

***Correspondence:** Hong-Wai Tham, Biopharmaceutical Research Unit, Faculty of Pharmacy, SEGi University, Jalan Teknologi, Kota Damansara, Petaling Jaya, Selangor, 47810, Malaysia; thamhongwai@outlook.my

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

***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]the vending machine has contributed to a revolution in how we buy food and drink. Despite the very obvious benefits associated with this technology, vending machines have not always been welcome by the customers they are intended to serve. Although occasionally blamed for various nonspecific illnesses, there have been very few studies about the microbiology of food and drink served from such machines. The few studies that have been reported have found high total viable counts (TVC).

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.

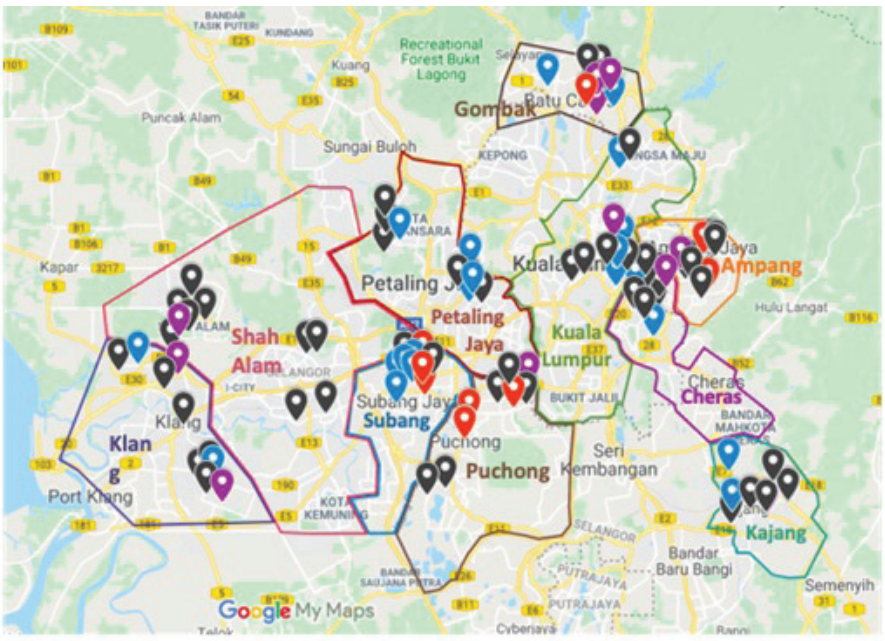


**Figure 1.** Sampling sites of this research project. Red pointers indicate the location of water vending machines detected with *Enterobacter* or *Citrobacter*; Black pointers indicate the presence of *Salmonella* or *Shigella*; Purple pointers indicate the presence of *Klebsiella*; Blue pointers indicate the absence of coliform contamination.

## 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).

## 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).

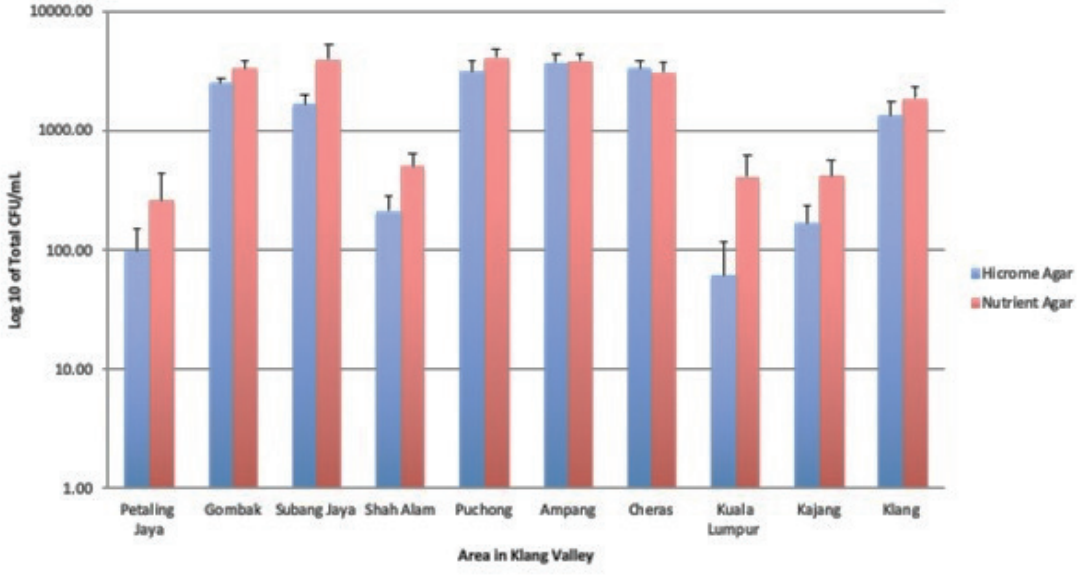


**Figure 2.** Average of total microbiome (measured in CFU/mL) of various samples collected from 10 districts in Klang Valley.

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.

| | **Asymptotic significance** | ***P*-value** |
|---|---|---|
| HiCrome Coliform agar with SLS (HA) | .015 | < 0.05 |
| Nutrient agar (NA) | .112 | > 0.05 |

## Correlation Between Microbial Counts and Maintenance Status of Water Vending Machines

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

| | **Asymptotic significance** | ***P*-value** |
|---|---|---|
| HiCrome Coliform agar with SLS (HA) | .464 | > 0.05 |
| Nutrient agar (NA) | .179 | > 0.05 |

**Table 3.** Total number of vending machines with colonies presented in light pink, salmon red, or opaque white.

| **District** | **Total number of vending machines with:** | | |
|---|---|---|---|
| | Light-pink colonies | Salmon-red colonies | Opaque-white colonies |
| Ampang | 1 | 2 | 10 |
| Cheras | 1 | 0 | 8 |
| Gombak | 4 | 1 | 8 |
| Kajang | 1 | 0 | 8 |
| Klang | 2 | 0 | 8 |
| Kuala Lumpur | 1 | 0 | 6 |
| Petaling Jaya | 1 | 0 | 4 |
| Puchong | 3 | 2 | 10 |
| Shah Alam | 1 | 0 | 10 |
| Subang Jaya | 1 | 3 | 5 |
| **Total** | **16** | **8** | **77** |

## 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[42]. This finding was in accordance to the dynamic growth and strong population movement within Klang Valley[43,44].

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[45]. 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[46–48].

Although the presence of faecal coliform in water supply may not be harmful, but it is an indication of the presence of faeces[49]. Despite cases of water vending machines contaminated by faecal coliforms (*E. coli* O157:H7)[50], 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[51,52],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.

## Reference

1. Letchumanan V, Ab Mutalib N-S, Wong SH, *et al.* Determination of antibiotic resistance patterns of *Vibrio parahaemolyticus* from shrimp and shellfish in Selangor, Malaysia. Prog Microb Mol Biol 2019; 2(1): 1–9.
2. Law JW-F, Ab Mutalib N-S, Chan K-G, *et al.* An insight into the isolation, enumeration, and molecular detection of *Listeria monocytogenes* in food. Front Microbiol 2015; 6: 1227.
3. Letchumanan V, Loo K-Y, Law JW-F, *et al. Vibrio parahaemolyticus*: The protagonist of foodborne diseases. Prog Microb Mol Biol 2019; 1(1): 1–8.
4. Lee L-H, Ab Mutalib N-S, Law JW-F, *et al.* Discovery on antibiotic resistance patterns of *Vibrio parahaemolyticus* in Selangor reveals carbapenemase producing *Vibrio parahaemolyticus* in marine and freshwater fish. Front Microbiol 2018; 9: 2513.
5. Letchumanan V, Wong P-C, Goh B-H, *et al.* A review on the characteristics, taxanomy and prevalence of *Listeria monocytogenes*. Prog Microb Mol Biol 2018; 1(1): 1–8.
6. Tan LTH, Lee L-H, and Goh, B-H. The bioprospecting of anti-*Vibrio Streptomyces* species: Prevalence and applications. Prog Microb Mol Biol 2019; 2(1): 1–15.
7. Heng S-P, Letchumanan V, Deng C-Y, *et al. Vibrio vulnificus*: an environmental and clinical burden. Front Microbiol 2017; 8: 997.
8. Learn-Han L, Yoke-Kqueen C, Salleh NA, *et al.* Analysis of *Salmonella Agona* and *Salmonella Weltevreden* in Malaysia by PCR fingerprinting and antibiotic resistance profiling. Antonie Van Leeuwenhoek 2008; 94(3): 377.
9. Yoke Kqueen C, Learn Han L, Noorzaleha A, *et al.* Characterization of multiple antimicrobial resistant *Salmonella enterica* subsp. enterica isolated from indigenous vegetables and poultry in Malaysia. Lett Appl Microbiol 2008; 46(3): 318–324.
10. Tan LTH, Lee L-H, and Goh, B-H. Critical review of fermentation and extraction of anti-*Vibrio* compounds from *Streptomyces*. Prog Microb Mol Biol 2020; 3(1): 1–14.
11. Letchumanan V, Chan K-G and Lee L-H. An insight of traditional plasmid curing in *Vibrio species*. Front Microbiol 2015; 6: 735.
12. Learn-Han L, Yoke-Kqueen C, Salleh NA, *et al.* Analysis of *Salmonella Agona* and *Salmonella Weltevreden* in Malaysia by PCR fingerprinting and antibiotic resistance profiling. Antonie Van Leeuwenhoek 2008; 94(3): 377.
13. Lee L-H and Raghunath P. Vibrionaceae diversity, multidrug resistance and management. Front Microbiol 2018; 9: 563.
14. World Health Organization, UNICEF, Water Supply and Sanitation Collaborative Council, WHO/UNICEF Joint Water Supply and Sanitation Monitoring Programme, editors. Global water supply and sanitation assessment 2000 report. Geneva: New York: World Health Organization; UNICEF; 2000.
15. Nocker A, Burr M, Camper A. Chapter One — Pathogens in Water and

Biofilms [Internet]. In: Percival SL, Yates MV, Williams DW, Chalmers RM, Gray NF, editors. Microbiology of Waterborne Diseases (Second Edition). London: Academic Press; 2014 [cited 2020 Jan 10]. page 3–32. Available from: http://www.sciencedirect.com/science/article/pii/B9780124158467000019.
16. Eng S-K, Pusparajah P, Ab Mutalib N-S*, et al. Salmonella*: A review on pathogenesis, epidemiology and antibiotic resistance. Front Life Sci 2015; 8(3): 284–293.
17. Letchumanan V, Chan K-G, Lee L-H. *Vibrio parahaemolyticus*: a review on the pathogenesis, prevalence, and advance molecular identification techniques. Front Microbiol 2014; 5: 705.
18. Kher HL, Krishnan T, Letchumanan V*, et al.* Characterization of quorum sensing genes and N-acyl homoserine lactones in *Citrobacter amalonaticus* strain YG6. Gene 2019; 684: 58–69.
19. Letchumanan V, Ser H-L, Tan W-S*, et al.* Genome sequence of *Vibrio* sp. SALL 6 isolated from shellfish. Prog Microb Mol Biol 2019; 2(1): 1–3.
20. Law JW-F, Ab Mutalib N-S, Chan KG, *et al.* Rapid methods for detection of foodborne bacterial pathogens: principles, applications, advantages and limitations. Front Microbiol 2015; 5: 770.
21. Letchumanan V, Tan W-S, Yin W-F, *et al.* Genome sequence of Vibrio OULL4 isolated from shellfish. Prog Microb Mol Biol 2020; 3(1): 1–3.
22. Letchumanan V, Yin W-F, Lee L-H*, et al.* Prevalence and antimicrobial susceptibility of *Vibrio parahaemolyticus* isolated from retail shrimps in Malaysia. Front Microbiol 2015; 6: 33.
23. Learn-Han L, Yoke-Kqueen C, Shiran M*, et al.* Molecular characterization and antimicrobial resistance profiling of *Salmonella enterica* subsp. enterica isolated from 'Selom'(Oenanthe stolonifera). Inter Food Res J 2009; 16(1): 191–202.
24. Letchumanan V, Pusparajah P, Tan LT-H*, et al.* Occurrence and antibiotic resistance of *Vibrio parahaemolyticus* from shellfish in Selangor, Malaysia. Front Microbiol 2015; 6: 1417.
25. Khoo C-H, Cheah Y-K, Lee L-H*, et al.* Virulotyping of *Salmonella enterica* subsp. enterica isolated from indigenous vegetables and poultry meat in Malaysia using multiplex-PCR. Antonie Van Leeuwenhoek 2009; 96(4): 441.
26. Letchumanan V, Chan K-G, Khan TM*, et al.* Bile sensing: The activation of *Vibrio parahaemolyticus* virulence. Front Microbiol 2017; 8: 728.
27. Cheah Y-K, Salleh NA, Lee L-H*, et al.* Comparison of PCR fingerprinting techniques for the discrimination of *Salmonella enterica* subsp. enterica serovar Weltevreden isolated from indigenous vegetables in Malaysia. World J Microbiol Biotechnol 2008; 24(3): 327.
28. Letchumanan V, Ser H-L, Chan K-G*, et al.* Genome sequence of *Vibrio parahaemolyticus* VP103 strain isolated from shrimp in Malaysia. Front Microbiol 2016; 7: 1496.
29. Tan W-S, Law JW-F, Letchumanan V*, et al.* Decoding the mystery of how bacteria "talk": Among Gram-negative microorganisms. Prog Microb Mol Biol 2019; 2(1): 1–7.
30. Letchumanan V, Ser H-L, Tan W-S*, et al.* Genome sequence of *Vibrio parahaemolyticus* VP152 strain isolated from *Penaeus indicus* in Malaysia. Front Microbiol 2016; 7: 1410.
31. Yano Y, Hamano K, Satomi M*, et al.* Prevalence and antimicrobial susceptibility of *Vibrio* species related to food safety isolated from shrimp cultured at inland ponds in Thailand. Food Cont 2014; 38: 30–36.
32. Aini MS, Fakhrul-Razi A, Mumtazah O, Chen JCM. Malaysian households' drinking water practices: A case study. Int J Sustain Dev World Ecol 2007;14(5):503–510.
33. Gruber JS, Ercumen A, Jr JMC. Coliform Bacteria as indicators of diarrheal risk in household drinking water: Systematic review and meta-analysis. PLOS ONE 2014; 9(9): e107429.
34. M.D PRH. Bacteriological, hygienic, and public health aspects of food and drink from vending machines. Crit Rev Environ Control 1992; 22(3–4): 151–167.
35. Pandey S, Singh S, Pani S, Malhosia A. Water quality and pollution status of lararpur reservoir with special reference to bacterial contamination. Nat Preced [Internet] 2009; Available from: https://doi.org/10.1038/npre.2009.3645.1
36. Buckle J. Chapter 7 - Infection [Internet]. In: Buckle J (editor). Clinical Aromatherapy (Third Edition). St. Louis: Churchill Livingstone; 2015 [cited 2020 Jan 8]. page 130–167. Available from: http://www.sciencedirect.com/science/article/pii/B9780702054402000073
37. Chris Brooker. Churchill Livingstone Medical Dictionary (16th Edition). Churchill Livingstone; 2008.
38. Oh J-H, Park M. Recent trends in Salmonella outbreaks and emerging technology for biocontrol of *Salmonella* using phages in foods: A review. J Microbiol Biotechnol 2017;27(12): 2075–88.
39. Ferrari RG, Panzenhagen PHN and Conte-Junior CA. Phenotypic and Genotypic eligible methods for *Salmonella* Typhimurium source tracking. Front Microbiol 2017; 8: 2587.
40. Barco L, Barrucci F, Cortini E, *et al.* Ascertaining the relationship between *Salmonella* Typhimurium and *Salmonella* 4,[5],12:i:- by MLVA and inferring the sources of human salmonellosis due to the two serovars in Italy. Front Microbiol 2015; 6: 301.
41. World Health Organization. WHO | Guidelines for drinking-water quality, 4th edition, incorporating the 1st addendum [Internet]. 2017 [cited 2020 Jan 8]. Available from: http://www.who.int/water_sanitation_health/publications/drinking-water-quality-guidelines-4-including-1st-addendum/en/
42. Bonnet M, Lagier JC, Raoult D, *et al.* Bacterial culture through selective and non-selective conditions: the evolution of culture media in clinical microbiology. New Microbes New Infect 2020; 34: 100622.
43. Chiu Chuen O, Karim MR and Yusoff S. Mode choice between private and public transport in Klang Valley, Malaysia. ScientificWorldJournal 2014; 2014: 394587.
44. Ahmad R, Suzilah I, Najdah WMAW, *et al.* Factors determining dengue outbreak in Malaysia. PLOS ONE 2018;13(2): e0193326.
45. Shi Y, Grech AL and Allman-Farinelli M. Changes in the nutritional quality of products sold in university vending machines since implementation of the health star rating in 2014; an environmental audit. BMC Public Health 2018; 18(1): 1255.
46. Wibuloutai J, Thanomsangad P, Benjawanit K, *et al.* Microbial risk assessment of drinking water filtration dispenser toll machines (DFTMs) in Mahasarakham province of Thailand. Water Supply 2019; 19(5): 1438–45.
47. Hunter PR and Burge SH. Bacteriological quality of drinks from vending machines. J Hyg (Lond) 1986; 97(3): 497–500.
48. Schillinger J and Du Vall Knorr S. Drinking-water quality and issues associated with water vending machines in the city of Los Angeles. J Environ Health 2004; 66(6):25–31, 43; quiz 45–46.
49. Doyle MP and Erickson MC. Closing the Door on the Fecal Coliform Assay. Microbe Mag 2006; 1(4): 162–163.
50. Moniruzzaman M, Akter S, Islam MA, *et al.* Microbiological quality of drinking water from dispensers in roadside restaurants of Bangladesh. Pak J Biol Sci PJBS 2011; 14(2): 142–145.
51. Chaidez C, Rusin P, Naranjo J and Gerba CP. Microbiological quality of water vending machines. Int J Environ Health Res 1999; 9(3): 197–206.
52. Miles SL, Gerba CP, Pepper IL, *et al.* Point-of-Use drinking water devices for assessing microbial contamination in finished water and distribution systems. Environ Sci Technol 2009; 43(5): 1425–1429.