AN ASSESSMENT ON THE TOTAL VIABLE COUNT OF ESCHERICHIA COLI IN SELECTED DRINKS OF CANTEENS AT A SCHOOL IN BATAAN

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ABSTRACT

The numerous recorded waterborne cases in and out of Bataan urged the study to determine the total viable count (TVC) of E. coli and coliforms in selected drinks served in the five canteen concessionaires of a school in Bataan. As noted, such tests may serve as an index for the safety and quality of the drinks. A total of five samples were collected and properly preserved to be then analyzed at a regional Department of Science and Technology of the Republic of the Philippines. The total coliform count of the samples ranged from being an estimated of less than 10 up to 1.8x103 total coliform colonies per 100 ml while E. coli counts ranged from an estimated of less than 10 up to an estimated less than 2.5x102 colonies per 100 ml. All the sampled drinks possessed both total coliform counts and E. coli counts that failed to meet the standards set by the Department of Health of the Philippines and were labeled as non-compliant. In this regard, there is an evident E. coli contamination across the canteens of the tested school, while there was no clear cause of contamination. It is speculated that unsanitary handling, contaminated ice, or environmental factors might have affected the samples. If left unchecked, this contamination may lead to a potential health hazard to the students, notably in the case of a diarrheal outbreak. Thus, it is suggested that the school takes precautionary measures to ensure the safety of the students and prevent any future risks.

Keywords: E. coli, total coliform, Drinks, School canteens, Bataan

INTRODUCTION

The Philippines has been tackling problems with the accessibility of people to clean and potable water. This concern is the result of issues brought about by the country's growing population, rapid industrialization, irrigation requirement and urbanization which is often present in rural areas. Several water-related infrastructure investments have been made to meet the ever-growing demand for clean water, however, waterborne diseases cannot be reduced unless people are provided with enough knowledge on their water will be contaminated (Onichandran, et.al., 2014).

A report released by the Department of Health, Philippines (DOH) on May 31, 2019, showed that 15,056 cases of waterborne diseases were recorded between January and May earlier this year; 6,130 cases of which were of acute bloody diarrhea (DOH, 2019). The same report explains that water contamination causes waterborne illnesses which give gastrointestinal symptoms such as diarrhea, abdominal pain, nausea, and vomiting. Waterborne diseases in general are dangerous to human lives. A commonly known bacteria such as Escherichia coli (E. coli) is recognized to cause the mentioned illness in the report (Brazier, 2017). Several studies from different parts of the globe argue that contaminated water is what causes these gastrointestinal infections, leading

to diarrhea (Rossi, Beilke, & Fer, 2017), (Ding, et al., 2017) and (Ahmed, Nasreen, & Parveen, 2009).

Food and water may be contaminated through cross contamination especially when an environment with numerous interpersonal relations occur, which is why the school is a prime example for these contaminations; students gather at a singular area to purchase and consume food and water during their breaks (Nhlapo, Lues, & Groenewald, 2014). In the case of the presence of a contaminated batch of drinks, an outbreak of disease is likely to happen. Young students, especially those in the primary education, are more prone to these waterborne diseases due to their weak immune system (Rossi, Beilke, & Fer, 2017). Currently, literature on water contamination and beverage analysis in schools in Bataan is not evident.

As mentioned, there have been numerous recorded cases of waterborne diseases in the Philippines, 147 of these diseases were diarrheal cases in Region 3 (DOH, 2019). There was also another record concerning waterborne diseases. The record presents that in the province of Bataan there were 13,328 cases of diarrhea between 2010 and 2015, however, these cases were not consistent because the years wherein the cases occurred were not specified. It would be that: in 2010 there were 1,806 cases; in 2011 there were 6,057; and in 2014 there were 5,465. The spike in diarrhea during 2014 suggests that rather than the supply of water being the source of contamination, it must be the individual events that introduces bacteria, such as handling, personal hygiene, or contamination from the environment (PEMSEA and Provincial Government of Bataan, 2017). Another study reports that 5,006 confirmed cases of cholera affecting all age groups happened from 2008 to 2013 in the Philippines. The report reasoned that inaccessible water facilities urged the residents to obtain water from contaminated bodies of water such as rivers or streams, and that in severe cases would show symptoms of watery diarrhea and vomiting which could lead to dehydration and in extreme cases, death (Lopez, Macasaet, Ylade, Tayag, & Ali, 2015). One report by the World Health Organization (WHO) presented from January to November in the year of 2013; 28,224 clinically diagnosed typhoid fever cases were recorded in the Philippines, the disease shows symptoms of high fever, abdominal pain, vomiting, and weakness; some older individuals may experience constipation while younger children may have diarrhea (WHO, 2014). The Organization also defined that typhoid fever is more prevalent in undeveloped countries, especially where unsafe drinking water is present. Another study conducted in Iligan city, Philippines determined the presence of pathogenic bacteria in the drinking water in Iligan City Central School and Iligan City North Central School (Nagba, Palangan, Yu, Opena, & Baguio, 2012). The researchers identified E. coli, Salmonella sp., Klebsiella pneumonia, Enterobacter aerogenes, and Klebsiella oxytoca in Iligan City Central School and E. coli, Salmonella sp. and Shigella sp. in Iligan City North Central School; these bacteria particularly, E. coli and Salmonella, are defined in the study to cause diarrheal illnesses and enteric fever (typhoid). Moreover, there was a recorded case of contamination which happened in May 25, 2012 in the province of Bataan; it involves 96 individuals ranging from 2 to 17 years old, all in which have consumed a common food called "dirty ice cream" (National Disaster Risk Reduction and Management Council, 2012) and (Refraccion, 2012). Most of the victims were confined at the Bataan General Hospital in Balanga City.

It is alarming that despite having recorded these cases there is no clear advancement on local water contamination studies in Bataan, especially in the context of schools. International studies argue that in most cases, E. coli is one of the bacteria which causes waterborne diseases (Tan, et al., 2013), (Cabral, 2010) and (Pandey, Kass, Soupir, Biswas, & Singh, 2014). Fecal contamination in the microbiological quality of water generally signals the presence of E. coli (Paraoan, Rivera, & Vital, 2017). The same study also argues that although not all strains of E. coli are harmful, it still is described as an indicator organism for other pathogenic bacteria. Illnesses such as diarrhea, fever, abdominal pain, and even vomiting root from the harmful strains of E. coli; E. coli O157:H7 is one example (Brazier, 2017). This strain of E. coli produces toxin called Shiga toxin, that is commonly grouped as Shiga toxin-producing E. coli (STEC) (Centers for Disease Control and Prevention, U.S. Department of Health & Human Services, 2014).

Water sources may be contaminated with E. coli through a variety of ways, such as an unfunctional sewage system, sewage overflows, and agricultural runoff (Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), 2015). Other ways of E. coli reaching a water source could be through animal or human feces making its way to a river or lake where a population gets their water supply; it could also be just by plain human to human contact, most of the time this is when an infected person does not wash hands after excretion of feces (HealthlinkBC, 2017). E. coli may be transmitted through contaminated water, the individual could acquire these bacteria through drinking, using contaminated ice cubes, swimming, or even eating food washed with the

contaminated water (Davis, 2019). Other potential modes of transmission could be through the environment, one study suggested relaying by insects linked with animal feces; one common example is through houseflies (Lindeberg, Egedal, Phelps, & Hossain, 2017). Housefly larvae are observed to grow in animal feces. In most developed countries, the detection of E. coli is used as a sign for enteric organisms, it is insisted that E. coli may serve as an index on sanitary quality (Ali, Hussain, & Hussain, 2011).

Thus, this study determined the total viable count (TVC) of E. coli and Coliforms in selected drinks served in five canteen concessionaires in a school in Bataan. The study aimed to present the possibility of the presence of harmful strains of E. coli, particularly the serotype 0157. The study only counted for E. coli and coliforms, which is why TVC is an appropriate method as it is a selective method. This method means that medium and conditions such as temperature will all be accustomed for the desired bacteria, in this case E. coli and coliforms (Todar, 2012) and (LibreTexts, 2019). A microbial assessment is greatly affected by the safety and quality of the food or drinks being tested (Illes, Toth, Dunay, Lehota, & Bittsanszky, 2018). Researchers claim that unsatisfactory results may suggest unsanitary procedures by the school canteen staff or unsanitary instruments used when preparing a drink or meal. These results then fuel the urge to conduct interventions among existing procedures to avoid contamination. The study emphasized that objective data resulting from these types of testing shows if there is proper obedience to food safety procedures. Another study also claimed that such testing may ensure safety from the different ways that food may be contaminated (Nhlapo, Lues, & Groenewald, 2014).

The success of this study will provide progression to the literature of water contamination in Bataan. The results obtained could encourage other schools in Bataan to pay adequate attention to their own canteens and assess their food safety standards. The school to be tested will have a basis for possible future interventions in terms of food and drink safety.

The microbial testing conducted was limited to the best-selling drinks of the five canteen concessionaires in a school in Bataan. The study determined the TVC of the bacteria E. coli and coliforms only. The acidity, temperature, oxygen level, and moisture of the samples were not identified during the sampling. The canteens' and school's identity remained anonymous and the microbial results of their best-selling drink were used for research purposes only.

METHODS

Sampling procedure

Authorization to conduct such study was secured from the Student Affairs of the school to ensure the participation of the selected canteen concessionaires. The five selected canteen concessionaires of the school were then informed of their involvement in the study and asked what is their best-selling drink which is not a product of other companies (Water bottles, Soda cans, etc.) and requires physical preparation by a staff. To preserve anonymity of the canteens, they were given an alphabetical name along with their best-selling drink. Appropriate amounts of the best-selling drink were purchased from the five canteen concessionaires on the same day. Samples were stored in sterile polyethylene terephthalate bottles then immediately kept in an insulated container filled with ice for transportation to the Regional Standards and Testing Laboratory of the Department of Science and Technology, Regional Office No. 3. Keeping the samples in a storage with temperatures below 10°C is recommended to preserve most of the physical, chemical, and microbiological characteristics of the sample, ice may be used to cool the sample down before transport (Environmental Protection Agency, Washington, DC. Office of Water, 2010). It is also advised that the maximum transport time of the samples is six hours.

Microbial testing

The Regional laboratory proceeded to testing two days after receiving the samples. The laboratory determined the E. coli and coliform counts using a Solid Chromogenic Medium, Chromogenic Substrate (Pour Plate), according to Chapter 4 / Edition 8 of the Bacteriological Analytical Manual (BAM) online of 2001. According to the manual, the laboratory utilized violet- red bile agar (VRBA) and to identify E. coli, it used 100g 4 methyl-umbelliferyl—D-glucuronide (MUG) per ml in the VRBA overlay to observe for fluorescent colonies under longwave ultraviolet (UV) light. It was then incubated for 18-24h at 35˚C, after which all purple-red colonies that are 0.5mm or larger and surrounded by zone of precipitated bile acids were counted. To validate, select colonies were then confirmed to be producing gas as coliform organisms by incubating at 35˚C in a tube of brilliant green lactose bile (BGLB) broth and examined at 24 and 48h. A gram stain was then performed to exclude gram-positive, lactose-fermenting bacilli, then the number of colonies per gram was determined

by multiplying percentage of tubes confirmed as positive by original VRBA count.

Analysis of data

Upon receiving the results, the total coliform counts were then compared to the Philippine National Standards for Drinking Water of 2017, released by the Philippines' Department of Health thru the Department Administrative Order (DAO) No. 2017-0010 in June 2017. The results of the analysis were compared to the standards based on the membrane filter technique of DAO 2017-0010 since the procedure used a solid chromogenic medium filter.

RESULTS AND DISCUSSION

All samples for the total coliform count have failed to achieve the standards set by the DOH and thus were labeled as non-compliant, as shown in Table 1. Drink C had the most with 1800 total coliform colonies per 100 ml. Drink A and E follows with 730 and 650 total coliform colonies per 100 ml, respectively. The remaining drinks, B and D, both garnered an estimated count of less than 10 total coliform colonies per 100 ml. Since all the drinks tested had the presence of E. coli, as shown in Table 2, it is probable that the total coliforms are fecal coliforms (Washington State Department of Health, 2016). The results shown are most probably due to the same reason how E. coli is transmitted.

Table 1: Total Coliform count of the selected school canteen drinks

Sample	Total Coliform (colonies per 100 mL)	Remarks	Standard Value (DOH DAO No. 2017-0010) (colonies per 100 mL)
A	7.3×10^2 cfu/g	Non – compliant	MFT:<1
B	Estimated less than 10 cfu/mL	Non - compliant	MFT:<1
C	1.8×10^3 cfu/mL	Non – compliant	MFT:<1
D	Estimated less than 10 cfu/mL	Non – compliant	MFT:<1
E	6.5×10^2 cfu/mL	Non – compliant	MFT:<1

*MFT: Membrane Filter Technique

As for the E. coli count compared to the DOH standards, all drinks are labeled as non-standard due to the presence of E. coli. Only drink A had more than 10 colonies per 100 ml with 250, as shown in Table 2. The rest of the drinks had an estimated count of less than 10. As stated, E. coli roots from Total coliforms, specifically a fecal coliform group. It is probable that the fecal coliform groups in Table 1 caused the presence of E. coli. The spike in Drink A may be due to the reason that it required more ice in preparation or simply poorer handling among the rest.

Table 2: E. coli count of the selected school canteen drinks

Sample	E. Coli (colonies per 100 mL)	Remarks	Standard Value (DOH DAO No. 2017-0010) (colonies per 100 mL)
A	Estimated less than 2.5×10^2	Non – compliant	MFT:<1
B	Estimated less than 10	Non - compliant	MFT:<1
C	Estimated less than 10	Non – compliant	MFT:<1
D	Estimated less than 10	Non – compliant	MFT:<1
E	Estimated less than 10	Non – compliant	MFT:<1

*MFT: Membrane Filter Technique

It is speculated that these non-compliant results may be caused by unsanitary handling or the use of unsanitary equipment when preparing the purchased drink (Illes, Toth, Dunay, Lehota, & Bittsanszky, 2018). In this case, it was visibly observable during the sampling that most of the canteen staff failed to wash their hands before preparing the purchased drink, however the equipment used were washed thoroughly beforehand; this was a cause for concern as mentioned that human to human contact is one of the modes of transmission of E. coli (HealthlinkBC, 2017). Another notable observance was that only drinks B and D did not use ice in preparing their drink and coincidentally these canteens also garnered the least coliform counts. It is then suspected that the ice used by the canteens may be one of the concerns as E. coli may be transmitted through consumption of contaminated ice too (Davis, 2019). Lastly, four out of the five canteens were located outside the school's main building, it could be that environmental factors have affected the handling of the staff and caused the contamination (Lindeberg, Egedal, Phelps, & Hossain, 2017).

CONCLUSION

In general, the study found that the school had an evident case of E. coli contamination across their canteens. There was no clear cause for such contamination, but it is speculated that unsanitary handling, contaminated ice, or environmental factors affected the samples. If brought to extreme cases, the school is at risk of a potential diarrheal outbreak among its students. The school may take precautionary measures to assess the contamination before such outbreak happens. It is suggested that the management pursue new policies regarding the issue, especially for those canteens located outside the main building of the school.

RECOMMENDATIONS

The study only tested for the presence of E. coli, but it did not test for Enterotoxigenic E. coli, a harmful type of E. coli which is a major cause of diarrheal diseases. Therefore, such tests are recommended for the upcoming researchers in the field. It may also be recommended to find clearer causes for such contaminations in schools through either an assessment on the knowledge of Good Manufacturing Practices (GMP) of the canteen staff or a surface swab test on the food contact surfaces. After which, proper interventions or sanitary procedures may be produced. The study could also go beyond testing the best-selling drink for each canteen; maybe even testing several drinks or test multiple schools around Bataan. Finally, it is a must to deliver safe and reliable drinking water to customers 24 hours a day, 365 days a year. If the water supply becomes contaminated, consumers can become seriously ill. As such, it is recommended to conduct a regular testing of the water and ice used in the preparation of the drinks sold in the school canteen.

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