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Escherichia coli detection in the Water Resources of Two Tertiary Schools in the City of Dasmarinas, Cavite, Philippines

Genevee Banta, MD
College of Medicine-Department of Microbiology and Parasitology
De La Salle Medical and Health Sciences Institute
City of Dasmarinas, Cavite, Philippines

Abstract: *Escherichia coli* commonly contaminates the drinking water in a school setting but its other sources are seldomly reported. This study aims to detect *E. coli* in the water resources of two tertiary schools in the City of Dasmarinas, Cavite, Philippines. The water samples used were the lake of school 1, waste water treatment system of school 2 and the swimming pools. Culture, biochemical and enzyme substrate tests were used in *E.coli* detection of the water samples. Multi-tube fermentation method was done to detect coliforms in the water samples. Culture test was observed for any gas formation and colony growth with greenish metallic sheen appearance. Biochemical tests were done in water samples with characteristic growth in culture test. Enzyme substrate test was performed by adding readycult coliform to the water samples. The water samples of the lake and waste water treatment system had gas formation, green metallic sheen colonies in Eosin-methylene-blue agar and its biochemical test results confirm *E. coli* growth. The water samples of school 2 swimming pool, lake and waste water treatment system had a color change in enzyme substrate assay test. However, the samples that fluoresce in a UV light were the water from the lake and waste water treatment system. Thus, the lake and waste water treatment system are highly infested with coliforms. The two swimming pools are safe to use and are not fecally-contaminated.

Keywords: water contamination, enzyme substrate assay, eosin-methylene-blue agar, diarrhea

1 INTRODUCTION

In the Philippines, the drinking water is a common source of diarrhea due to fecal coliforms [13]. Typhoons increases the risks of fecal contamination in the water supplies [11]. Most of the local studies about water contamination in a school setting are focused on the water from the faucets, drinking fountains and vending machines [14, 20].

Students are most vulnerable to illnesses. Their lifestyle and eating habits contribute in the weakening of their immune system. Their activities expose them to infectious and non-infectious sources of illnesses [14, 16]. Any form of illnesses may affect their physical and mental health [21].

Several studies showed that recreational water resources can cause *E.coli* infection. Probert et al. (2017) assessed the water streams in a city park in Northern California. Its water contamination was due to the presence of deer scat specimens. On the other hand, *E. coli* was also identified in the surface water and watershed. Environmental water, thru drinking and recreational activities, may serve as a reservoir of E.coli. Humans becomes infected after ingestion [3,27]. Odonkor and Ampofo (2013) mentioned that the presence of *E. coli* in water samples was indicative of fecal contamination, not of actual presence of the bacteria in the water.

A study about the *E. coli* helps in the improvement of the water quality in the school and community. Park et al. (2017) discovered that the increased diarrheal cases in school were due to a pipeline disconnection between the autochlorination device and water storage tank and also due to poor filtering process of the water purifier. *E. coli* forms, EPEC and EHEC, in the school's drinking water and rectal swabs of the symptomatic patients were detected. This only shows that evaluation of water supply in the schools is important and can also be used in determining any threat to human health.

Other possible water sources of *E.coli* and diarrhea in the schools are often neglected. Hence, the study aims to detect *Escherichia coli* in the water resources of two tertiary schools in the City of Dasmarinas, Cavite, Philippines. The study will inform the different colleges regarding the importance of regular assessment of water within the campus and also improvement in the water sanitation measures. The parents, the students and the government will be aware of the other possible sources of diarrhea in a school setting.

2 METHODS

The study is a qualitative experimental study which focused on the detection of *Escherichia coli* in four water resources, including the lake of School 1, waste water treatment system of School 2 and the swimming pool of the two tertiary schools. The water resources from the drinking fountain and faucets were not included in the study. The study did not include the level of chlorination, other water-borne pathogens and also other external factors that may affect the water condition.

Permission regarding the conduction of the study were obtained from concerned personnel of chosen schools.

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Surface water specimen were collected after an approval from the schools. The sterile containers were properly labelled according to the following: Specimen A for School 1 swimming pool, Specimen B for School 1 lake, Specimen C for School 2 swimming pool and Specimen D for School 2 waste water treatment system. Surface water were collected from the different water resources. A volume of 500ml water were collected per specimen.

2.1 Multi-tube fermentation method

A modified multi-tube fermentation method and its interpretation of most probable number were based on Greenberg et al. (1992). The MTF method is a presumptive test for *E.coli* detection in the water. Each of the water sample, measuring 10ml, is transferred to each of the 5 lactose fermentation tubes containing a double strength lactose broth. The lactose fermentation tubes of water samples were duplicated. All the inoculated lactose fermentation tubes were incubated at 37°C for 24 hours. Gas formation in the tubes indicated a presumptive evidence of coliform organism. No gas formation signified absence of coliform. Gas formation in all of the tubes signified coliform contamination with MPN index >8.0 coliforms per 100ml and further tests were done.

2.2 Culture, biochemical and gram stain tests

The water samples with gas formation in >2 lactose broth tubes were subjected to culture test. The eosin methylene blue (EMB) agar plates and biochemical tests were used to confirm the presence of E.coli in the water while gram staining was used in determining the gram reaction of the colony growth in the culture test. The biochemical tests [8] and gram staining were done in water samples with green metallic sheen colony growth. Selected colonies were processed for biochemical testing such as Indole, methyl red, vogues-proskauer, citrate, triple sugar iron agar (TSI), lysine iron agar (LIA) and urease test. Control positive and negative biochemical tests for *E.coli* were also prepared. All the water samples were duplicated.

2.3 Enzyme substrate assay test

The readycult coliform was used as the enzyme substrate in the study. Each water samples 100ml were transferred to properly labelled sterile bottles. A single readycult coliform vial were added in each of the 100ml water specimen and incubation was done. A yellow color appeared after adding the readycult coliform. All the samples were duplicated. A color change and ability to fluoresce under a ultraviolet light were observed which is interpreted as water contamination of *Escherichia coli.*

3 RESULTS AND DISCUSSION

In the study, fecal coliforms were detected using the multi-tube fermentation method. Multiple-tube fermentation technique is useful in determining the mean density of coliforms in the water sample. It is helpful in evaluating the sanitary quality of a water [24]. Fecal contamination of the water is commonly due to environmental sources such as natural disaster. Water contamination after a typhoon has been reported [6,11]. Poor water sanitation and inappropriate disposal of wastes in the nearby facilities contribute in poor water quality. Intake of unboiled water increases diarrheal cases in rural and urban areas [4]. In rural areas, *E. coli* and fecal coliforms were due to animal manure and poor sanitation [10]. Mixing of the water and sewages in the distribution lines is also a suggested reason for having high coliforms in the water. Lack of knowledge about water contamination increases the risk of diarrhea in a school setting [17]. The study showed that the lake and waste water treatment system have significant level of water contamination based on the MPN index of >8 coliforms/100ml. This is probably due to the presence of few animals and garbages within the vicinity of the area of collection. On the other hand, the swimming pools were safe to use due to low concentration of coliforms (MPN index 0-1.1/100ml).

3.1 Results of culture, biochemical tests and gram stain

The culture test and biochemical testing of the lake and waste water treatment system confirms the fecal contamination of the water. Eosin-Methylene-Blue (EMB) agar is comprised of lactose and sucrose. It is used in isolating gram-negative bacteria. The methylene blue and eosin inhibits the growth of gram-positive bacteria. The gram-negative bacteria use the lactose and produce excessive amounts of acid. It is responsible for the purple color with green metallic sheen [9,23]. The colony growth in the study was a gram negative bacteria based on the gram staining result. A color change in the indole, methyl red and TSI and with gas formation in TSI signified that the colony growth in the EMB agar were *E.coli*. These biochemical findings differentiate *E.coli* from other microbes [5]. The biochemical test findings (Table 1) of this study signified that the colonies that grew in the culture test of lake and waste water treatment system were *E.coli.* The green metallic sheen colony growth (Figure 1) and the biochemical test results of the lake and waste water treatment system confirm *E.coli* detection in the water samples.

Table 1. Biochemical testing results in the water samples of the lake and waste water treatment system before and after incubation

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Biochemical tests	Lake				Waste water treatment syste			
	Before incubation		After incubation		Before incubation		After incubati	
	A	B	A	B	A	B	A	B
Indole	Y	Y	R	R	Y	Y	R	R
Methyl red	Y	Y	R	R	Y	Y	R	R
Vogues-Proskauer	Y	Y	Y	Y	Y	Y	Y	Y
Citrate	G	G	G	G	G	G	G	G
TSI	R +/- gas	R +/- gas	A/A + gas	A/A +gas	R +/- gas	R +/- gas	A/A +gas	A/A + ga:
LIA	P	P	P	P	P	P	P	P
Urease	Y	Y	Y	Y	Y	Y	Y	Y

*Legend: G =green; P =purple; R =red; Y =yellow
*+/- gas = with or without gas formation
*+ gas = with gas gas formation
*A/A=yellow slant and butt

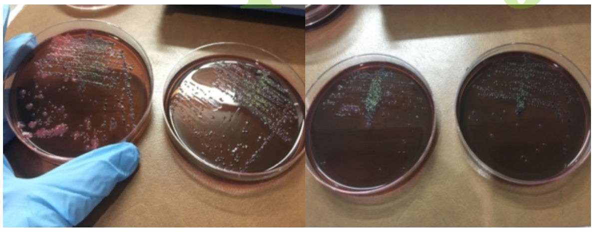

Figure 1. Green metallic sheen colony growth in the culture of the lake water (left) and waste water treatment system (right)

3.2 Result of Enzyme substrate assay test

Enzyme substrate coliform assay such as Colilert can be used in identifying coliform contamination of the water [1]. The substrate assay test indirectly approximates the *E.coli* in the water[2]. Enzyme assay is more cost-effective and rapid water analysis test than multi-tube fermentation method [1,25]. Enzymatic detection of *Escherichi coli* and other coliforms provide a rapid result than the conventional method. The result were detected for at least 4 hours [15]. In the study, the results of enzyme substrate assay test were already detected after 16-18 hours of incubation. This test is shorter than 3-4 days' processing of culture and biochemical testing.

Aside from that, readycult coliform is an enzyme substrate that is comprised of chromogenic and fluorigenic substrates. The readycult coliform substrate has the fluorogenic enzyme substrate, 4-methyl-umbelliferyl-Beta-D-glucuronide (MUG), that reacted with the released beta-glucuronidase of *E.coli*. These results in the release of 4-methylumbelliferone, which caused the color change and also for the water to fluoresce under the UV light [26]. These findings were observed in the water samples of the lake and waste water treatment system (Table 2). Hence, these sources of water are contaminated with *E.coli*. The area of collection of the waste water treatment system was observed near the garbage collection of the school. The nearby garbage collection might have contributed in the contamination of the water. Meanwhile, the lake is surrounded by trees and has no regular cleaning or decontamination. Such condition contributed in the *E. coli* infestation. On the other hand, the chromogenic enzyme substrate (galactopyranoside) of readycult coliform may also react with other coliforms in the water samples. These reactions will change the color of the water but will not emit a blue light using a UV light. This finding was observed in School 2 swimming pool. Aside from *E.coli,* other fecal-borne bacteria are most probably present in the swimming pool of school 2. Given this data, enzyme substrate assay test can be used as alternative method in assessing the water for *E.coli* and other coliforms.

Table 2. Enzymatic detection of *Escherichia coli* in the water samples of the lake and waste water treatment system using enzyme substrate assay

Specimen code	Water samples	Color prior to incubation	Color after 24 hours of incubation	Fluoresces under UV light
A	School 1 swimming pool	Yellow	Yellow	Did not fluoresc
B	School 1 lake	Yellow	Blue-green	Fluoresced
C	School 2 swimming pool	Yellow	Green	Did not fluoresc
D	School 2 waste water treatment system	Yellow	Blue-green	Fluoresced

4. CONCLUSION

The lake and the waste water treatment system are the most infested water with coliforms since it has the highest MPN index. On the other hand, School 2 swimming pool has very low level of coliforms based on MPN index while School 1 swimming pool has no coliforms. Students should take necessary precaution when using different water sources in their campuses.

The water samples from the lake and waste water treatment system are contaminated with *E. coli* based on the culture, biochemical testing and enzyme substrate assay. Since *E.coli* is detected in the lake and waste water treatment system, these sources of water are most probably contaminated also with other fecal borne bacteria. Enzyme substrate assay test is a useful and rapid alternative tool for assessing the water for the presence of *E.coli* and other fecal-borne bacteria.

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