Significant Presence of Nontoxinogenic Coliforms in the Drinking Water Supply of the Mattingly Residential Area of St. Kitts Suggests that the Most Common Cause of Gastroenteritis could be Transmission due to Enteric Viruses in Drinking Water

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Abstract
Safe drinking water is always a challenge in tropical areas. Several outbreaks have been reported in the Caribbean Islands in the past year. In response to foreign students experiencing gastrointestinal problems, an investigation was conducted to determine if there is any fecal contamination that could transmit enteric viruses (e.g. Rota virus and Noro virus), or enterotoxigenic E. coli. Water samples were collected from regions around the Island of St. Kitts. Several coliforms were isolated from one particular residential region of the Island called Mattingly Heights. DNA from these isolates was then extracted and tested by PCR to determine whether any toxigenic E. coli was present. None of the isolates was positive for toxigenic E. coli. A possible explanation, for the presence of E. coli, was provided by a local person overseeing, the water reservoir; supplying the region of Mattingly. The fecal contamination observed, was attributed to monkeys inhabiting the trees surrounding the reservoir, although human fecal contamination cannot be ruled out from our studies. Other pathogens such as Giardia are possibly responsible for some of the gastrointestinal problems experienced by the tourists and the foreign students inhabiting the Island. Monitoring for toxigenic E. coli on an annual basis would ensure that serious bloody diarrhea will not affect the people residing or visiting the Island. This study confirms that viral gastroenteritis, referred to as stomach flu, which is common in the region could be a result of fecal contamination of the water supply as confirmed by the presence of nontoxinogenic coliforms, which are indicators of fecal contamination.

Keywords: E. coli; Fecal contamination; Gastroenteritis; Drinking water

Introduction
This investigation was conducted to address the concerns regarding the quality of water consumed by the local community and foreign students in residential areas where they reside. Such places included Frigate Bay, Mattingly, Trinity Palmetto Point, Saint George and Saint Peter in Basseterre on the island of St. Kitts. The study was conducted to determine the absence or presence of fecal coliforms and E. coli 0157:H7 in household drinking water. The Epidemiology of E. coli 0157:H7 has been conducted in other parts of the world but not in St. Kitts [1]. Although, monthly testing is done for the quality of water supplied in local communities by the St. Kitts Water Services Department, information of the results is not made known to the public. Contaminated water has been associated with diarrhea related diseases, which stimulated a worldwide interest in the knowledge of drinking water quality.

Based on the information that 40% of the population in Latin America and the Caribbean consume water of substandard quality [2], coupled with sporadic gastroenteritis, many students have resorted to buying bottled water and drink only “pure water” or filtered water. Since prior research on the water quality in the selected areas could not be found through a current review of literature, this research is aimed to find out if the extra burden on obtaining safe drinking water by UMHS students is really necessary.

Hypothesis to be tested
The quality of water being consumed by UMHS students in all residential areas near their campus is free from fecal coliforms, and is adequate for oral consumption.

Methods and Materials
Water Collection and testing for physico-chemical properties:

From random residential drinking water faucets during an early sunny afternoon, water was run at a normal rate (roughly 50% of maximum flow), for thirty seconds. Nitrate strips were immersed in stream of water for two seconds. Values of Nitrate were recorded. The pH of water was also recorded. 250 ml of water was filtered into a clean sterile container ensuring that the water did not come into contact with any external object or material. The water was immediately observed for turbidity, color, or odor. Water samples were transported to the lab, kept at moderate to cool temperature coliform enumeration was performed according to EPA procedure. 100 ml of water was filtered using Thermo Scientific Nalgene Analytical Filterware (0.45 microns) attached to a vacuum pump. The filter was aseptically placed on an Endo agar surface (DIFCO, Detroit, Michigan USA). Another 100 ml of the same water sample was similarly filtered and the filter was aseptically placed on an mFC agar plate (DIFCO, Detroit, Michigan, USA). The Endo agar plate culture was incubated at 37°C. The mFC agar was incubated at 44.5°C. All counts were expressed as either total or fecal coliforms per 100 mL of water. All water samples having one or more coliforms per 100 mL were judged to be of poor quality, based on the zero tolerance level for coliforms in water that the EPA
advocates (20). In order to detect \textit{E. coli}, all colonies of coliforms which had characteristic appearance on endo agar and mFC agar were sub cultured on eosin methylene blue (EMB) agar, incubated overnight at 37°C, growth colonies with typical greenish metallic sheen were considered as \textit{E. coli}.

**Characterization of \textit{E. coli} isolates**

All suspected \textit{E. coli} isolates were plated on sheep blood agar plates and incubated overnight at 37°C. Growth of colonies were examined for mucoid appearance and for those with complete hemolysis. Similarly, Sorbitol MacConkey agar was also inoculated with \textit{E. coli} and incubated overnight at 37°C. Colorless or cream-appearing colonies were classified as nonsorbitol fermenters (NSF), and pink colonies were considered sorbitol fermenters (SF).

DNA extraction isolates from water samples from the Mattingly region, which formed pink colonies on sorbitol MacConkey agar plates were transferred to slants, from which they were used to make a suspension in 250 microliters of saline (0.9% sodium chloride) in a Eppendorf tubes. A total of 19 isolates of \textit{E. coli} were assessed for \textit{E. coli} 0157:H7. The tubes were then heated at 92-95°C for 15 min and then left in ice for 5 mins and then centrifuged at 2000 rpm for 5 min in a micro centrifuge. The supernatant was decanted in a separate tube and was shipped to the Laboratory of Dr. Peter Feng at the FDA to perform PCR by a procedure standardized in his laboratory to determine if any of the isolates are \textit{E. coli} 0157:H7 [3].

**Results**

Water from all sources was clear upon visual inspection. No traces of sediment or odor were present. The water tested negative for Nitrate. The pH of water was found to be within normal range 6.5-7.0. No evidence of fecal coliform was found on agar plates for all communities except Mattingly. Water from Mattingly residential faucets showed the presence of coliforms, which were too numerous to count. In addition, the DNA extraction also proved that all isolates were coliforms. However, none of the isolates of \textit{E. coli} indicated the presence of \textit{E. coli} 0157:H7 after a PCR analysis on the DNA extracted performed at the FDA as reported by Cebula et al. [3]. These results indicate fecal contamination but the gastroenteritis was of non-bacterial causes due to the non-toxigenic nature of the coliforms and therefore in all likelihood due to viral causes.

**Discussion**

To confirm our findings, the lab technician, at the St. Kitts Water Services Department who conducts monthly tests on the water quality in St. Kitts communities and residential areas was contacted. He stated that the water from Mattingly residential faucets is derived from an open spring in Old Road, St. Kitts. The water is not filtered (would not serve to remove the coliforms and keep the water safe at all times. Whether such a process will clear the water of viruses, the major cause of gastroenteritis on the Island will be another question to be addressed [4].

**Conclusion**

Students of UMHS who live outside of the Mattingly area could drink water from their home faucets. The numerous coliforms present in the water in Mattingly, is a health concern. Further tests need to be done to examine the drinking water from other residential areas where students live in addition to water fountains on campus, many of which are brand new and were recently installed and appear to provide clean water. Based on these findings we cannot support our hypothesis that all drinking water in residential areas used by UMHS students is safe for consumption. Therefore, students may want to continue purchasing bottled water, using filters or boiling their water to ensure the quality of safe drinking water. Especially for those who reside in Mattingly, given the fact that it rains frequently, students and residents who reside there may benefit from taking extra precaution when consuming water [5].

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**References**