Bioaerosol Effect on Safe Use of Bathroom Appliances for Drinking Water Consumption

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Abstract

Purpose: The purpose of this study was to assess the bioaerosol effects on the use of bathroom appliances (a fountain faucet and a reusable cup) for drinking water consumption.

Methods: A mechanically pressurized hydraulic spray nozzle was used to generate bioaerosols containing non-pathogenic *E. coli*. These bioaerosols became airborne and came in contact with a fountain faucet (NASONI, Inc.) and a reusable cup. 10 mL and 100 mL of water samples from the cup and the faucet stream, respectively, were collected at intervals of 10 secs, 30 secs, 1 min, 2 mins, and 5 mins. A Tryptic Soy Broth (TSB) liquid solution was used to determine whether *E. coli* was present in the water, while the *Colilert* test was conducted to quantify *E. coli* concentrations.

Results: 88 MPN/100 mL – 866 MPN/100 mL of *E. coli* from the aerosol effect was removed from the fountain stream after the faucet was kept open for 10 secs. However, *E. coli* continued to be present in the reusable cup over the sampling period.

Conclusion: The fountain feature of the faucet had a significantly lower risk of microbial contamination from the aerosol effect as compared with the reusable cup.

Introduction

Household bathrooms are one of the most vulnerable locations for bacterial contamination. Regular or ordinary human activities, e.g. toilet flushing, coughing, washing, sneezing, and sweeping floors, can cause microbial contamination in household bathrooms (Kummer and Thielb, 2008). Among these human activities, flushing a toilet has been considered as one of the main contributors to microbial contamination (Aithinne et al., 2018). Toilets in general are designed to dispose human waste by flushing the waste mixed with water, which then turns into sewage. However, flushing the toilet can produce droplet and droplet nuclei bioaerosols that can contaminate surfaces and expose persons by contact or air currents. Studies showed that these bioaerosols contain pathogenic organisms, such as *Escherichia coli (E. coli),* MS2 bacteriophase bacteria, *S marcescens* and enterobacteria, are present in a toilet plume (Johnson et al., 2013; Best et al., 2012). Consequently, the prevalence of bioaerosols can be associated with certain human diseases, such as gastrointestinal illness and infectious disease (Kim et al., 2017; Aithinne et al., 2018).

Studies showed that each flush of the toilet can produce up to 145,000 aerosol particles. Greater than 99% of these aerosol particles are less than 5μm and can remain suspended for minutes to hours (Prussian, 2015). After multiple flushes, *E. coli* and MS2 bacteriophage could persist in the toilet bowl (Prussian, 2015) thus implicating that a toilet may continue to generate bioaerosols and the resulting droplet nuclei could contaminate the environment when settling on surrounding surfaces, such as sink tops, hygiene accessories, faucet openings, showerheads, and cups used on a daily basis. Some of these appliances, such as faucets and cups, are used for drinking water consumption. From a public health standpoint view, it is important to understand whether the aerosol effect impacts the safe use of faucets and cups in the bathroom setting. Such results are useful for public health education and control measures to minimize microbial contamination in the bathroom setting.

Based on the Safety Drinking Water Act, the United States Environmental Protection Agency (US EPA, 2019) has set standards to guard against microbial contamination in drinking water (US EPA, 2019). Indicator organisms, including *E. coli* and fecal coliform, were selected to monitor the safety of drinking water (US EPA, 2019). The presence of indicator organisms

indicates a greater risk that pathogens are present. States are required to test these indicator organisms in drinking water from public water supplies on a regular basis. The objectives of this study were to 1) Determine the presence of *E. coli* in the tap water from the fountain stream of the faucet and the reusable cup created by the aerosol effect, 2) Quantify *E. coli* concentrations in the tap water from the fountain faucet and the reusable cup created by the aerosol effect in a time series, and 3) Determine the rate of decay of *E. coli* in the water samples from the fountain faucet and the reusable cup.

Methods

Two kinds of bathroom appliances, a fountain faucet and a reusable cup for drinking water use, were tested for the aerosol effect. The fountain faucet (NASONI, Inc), which has just been introduced to the market this year, has innovative features. There is a fountain feature at the top of the arch of the faucet's downspout to the water with a lever on the right side to control the fountain stream (Figure 1C). This feature makes easy access to the water. Also, the moderate flow of the faucet can reduce the amount of water consumption. The reusable cup represents a common, traditional method for water use in the bathroom setting.

The fountain faucet was installed in a portable vanity (Figure 1A). The faucet was connected to a drinking water source via the main pipe of the sink fixture in the Environmental Health laboratory on the campus of Old Dominion University (Figure 1B). A confinement area was created for bioaerosol dispersion. As shown in Figure 2, a cardboard box was set on the top of the sink counter. Both the fountain faucet and the reusable cup were located on the countertop inside the confinement area. Also, a reusable cup was located outside the cardboard boundary to serve as a control.

Figure 1.

Setup of NASONI fauce

A. NASONI faucet

B. Pipe fixture

C. Fountain & lever

Figure 2.

Setup of confined space for bioaerosol dispersion

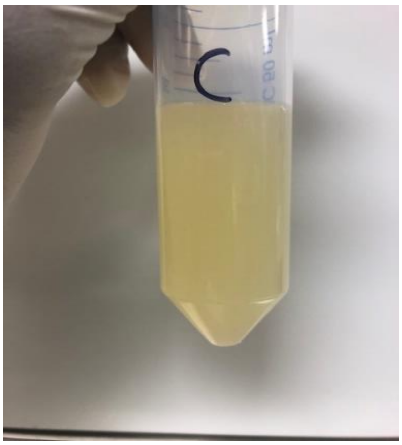
Non-pathogenic *E coli* stock (ATCC Strain 25922) was used to prepare an *E. coli* mixture used to generate bioaerosols. The *E. coli* stock with a concentration range of 1.5 – 2.0 x10^{8} CFU/mL was prepared within one hour of the same day of any experiments conducted to ensure the bacterial concentrations fall within the range. A series of dilutions were conducted to determine a desirable range of *E. coli* concentrations for generating bioaerosols. After conducting the dilution series, an *E. coli* mixture was determined for generating bioaerosols, which included 1-2 mL of *E. coli* stock solution and 20 mL of sterilized tap water. A pressurized hydraulic spray nozzle was used to generate bioaerosols. Manual pumping was used to draw the liquid and force it through the spray nozzle. The technique yielded heterogeneous liquid aerosols with respect to particle size. The 20 mL *E. coli* mixture was pressurized by the manually hydraulic spray nozzle. The bioaerosols then became airborne into the confinement area and were allowed to come into contact with the faucet and the reusable cup.

Prior to the dispersion of bioaerosols, a 100 mL tap sample was collected from the fountain stream of the faucet to ensure no presence of *E. coli*. Immediately after the dispersion of bioaerosols, 100 mL of water was collected from the fountain stream of the faucet to serve as the initial sample to establish the baseline concentration. Simultaneously, 10 mL of water was collected from the reusable cup. The fountain faucet was kept open; 100 mL of water samples were then collected at intervals of 10 secs, 30 secs, 1 min, 2 mins, and 5 mins, respectively. In addition, 10 mL of water samples were collected from the reusable cup at these time points.

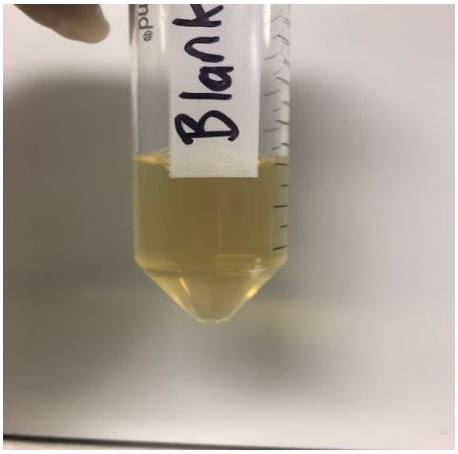
A Tryptic Soy Broth (TSB) solution was used to test the presence of *E. coli*. 10 mL of each water sample was added into 20 mL of TSB solution. The mixture was then incubated at 37ºC for 24 hrs. Turbidity was used to determine whether *E. coli* is present in the water sample (Figure 3). To quantify *E. coli* concentration, the *Colilert* test was used. This method is approved by the US EPA and is included in the Standard Methods for Examination of Water and Wastewater (APHA, 2012). 100 mL of each water sample was added into a sterilized bottle with the *Colilert* reagent (IDEXX, Inc.) Once the reagent was completely resolved, the mixture was poured into a Quanti-Tray, and sealed using a sealer. The tray was incubated at 37ºC for 24hrs and was observed for any presence of fluorescence under an ultraviolet (UV) light. The number of wells with fluorescence were counted (Figure 4) and were used to determine *E. coli* concentrations reported as MPN/100 mL (MPN, most probable number).

Figure 3.

Presence and absence of *E. coli* in TSB



Presence



Absence

Figure 4.

Quantification of *E. coli* using the *Colilert* test

Presence Absence

Quality assurance and control (QA/QC) procedures were included throughout the study. The QA/QC for the *Colilert* test was conducted, according to the manufacturer instructions. Each test included a control to ensure no *E. coli* contamination was outside the cardboard boundary. The countertop and faucet were cleaned with soapy water thoroughly after each experiment. A drinking water sample was taken from the fountain stream before each test to ensure no presence of *E. coli*. Also, a duplicate sample was collected for each water sample.

Results and Discussion

Table 1 summarizes the presence or absence of *E. coli* in water samples after bioaerosols were dispersed. Results show that the water in the reusable cup on the countertop had *E. coli*. In addition, the turbidity of these samples in the TSB solution in the reusable cup increased from Day 1 to Day 3. This suggests the possible growth of *E. coli*, as the water in the reusable cup was retained providing an ideal condition for *E. coli* to incubate. After dispersing bioaerosols, an initial water sample from the fountain stream of the faucet was collected. Turbidity in the water sample was observed and illustrated that *E. coli* was present in the water. The results show the

dispersion of bioaerosols worked properly to introduce *E. coli* to the fountain faucet. After running the fountain stream for one minute, *E. coli* continued to be present in these water samples. However, no *E. coli* was present in those samples after the fountain stream of the fountain faucet was kept running for two minutes.

These results from the presence and absence test show that the use of the fountain feature of the faucet is safer than the reusable cup. First, the opening of the reusable cup (5 cm in diameter) is significantly wider than the fountain faucet's fountain nozzle (3 mm in diameter). The reusable cup had a significantly higher chance to contract bioaerosols than the fountain nozzle of the fountain faucet does. For example, the water in the reusable cup was contaminated with *E. coli* after only one spray of <25 μL *E. coli* mixture. However, >5 mL of *E. coli* mixture was required to generate bioaerosols accumulated in the fountain nozzle of the fountain faucet to reach the detection level of *E. coli* in the fountain stream of the faucet. Second, once bioaerosols came in contact with both the reusable cup and the fountain faucet, the faucet's fountain feature could remove all bacteria in the tap water from aerosol contamination, while a reusable cup had bacteria propagating in any retained drinking water over time.

Table 1.

Presence and absence of *E. coli* in water samples after bioaerosol dispersion*

	B	C1	C2	F0	F1	F2	F3	F4	F5
Day 1	--	+	+	+	+	--	--	--	--
Day 2	--	+	+	+	+	+	--	--	--
Day 3	--	+	+	+	+	+	--	--	--

*B = blank | C= cup | C1: left on the counter | C2: right on the counter

F0 = 0 min | F1 = 30 seconds | F2 = 1 minute | F3 = 2 minutes | F4 = 3 min | F5 = 5 minutes

+ = *E. coli* presence | -- = *E. coli* absence

Day 1 = Samples incubated for 24 hours; Day 2: Samples incubated for 48 hours; Day 3: Samples incubated for 72 hours

Table 2 shows quantification of *E. coli* concentrations in the water collected from the fountain faucet. *E. coli* concentrations in the non-disposable cup were consistently greater than >1x 10^8 MPM in all the samples. Thus, the *E. coli* concentrations in the water in the reusable up were not tabulated in these tables. F0 samples indicate the initial concentrations of *E. coli* immediately after the dispersion of bioaerosols. As shown in Table 2, after running the fountain stream for 30 seconds, the fountain feature of the fountain faucet completely removed 88 MPN/100 mL of *E. coli* from the fountain stream (F1).

Table 2.

E. coli concentrations in the tap water from the stream of the faucet**

	B	F0	F1	F2	F3
Day 1	--	88	--	--	--
Day 2	--	88	--	--	--
Day 3	--	88	--	--	--

*B = blank | F0 = 0 minute | F1 = 30 seconds | F2 = 1 minute | F3 = 2 minutes

***E. coli* concentration in MPN/100 mL

In another set of the experiment, the fountain feature of the fountain faucet completely removed 866 MPN/100 mL of *E. coli*, after just running the water for 10 seconds (Table 3). To determine a decay rate, three points of measurements are required. Since the fountain feature of the fountain faucet removed *E. coli* within 10 seconds, only one point of *E. coli* concentration was detected. Thus, a decay rate was not determined.

Table 3.

E. coli concentrations in the tap water from the fountain stream of the faucet*,*

	B	F0	F1	F2	F3

Day 1	--	866	--	--	--
Day 2	--	866	--	--	--
Day 3	--	866	--	--	--

*F0 = 0 min | F1 = 10 seconds | F2 = 30 seconds | F3 = one minute

***E. coli* concentration in MPN/100 mL

The NASONI faucet is very efficient at removing *E. coli*. During testing, about 150 ml of water was collected in beakers for each sample. The time to collect each sample took roughly 10 seconds. After 10 seconds passed, there were no *E. coli* after the initial samples were taken. There were a lot of steps made to standardize experimental procedures in order to improve our consistency, concluding that after 10 secs of running water, the fountain can effectively wash away any bacteria trapped in its spout. The results gathered can aid in providing information that could further the understanding of water quality safety in household settings.

Conclusion

The safety of drinking water has been a field of interest that scientists are constantly striving to improve. This investigation enhances our knowledge on how bioaerosols affect the safety of drinking water in household bathrooms and how to possibly prevent their spread or improve hygiene. Simple things such as closing the toilet lid when flushing the toilet and cleaning the bathroom regularly using hygiene products such as soap holders, mouth rinse cups, toothbrush holders, etc. will help prevent the possibility of contracting various harmful bioaerosols.

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