

Investigation of everyday locations for antibiotic-resistant bacteria in Cambridge, Massachusetts

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SUMMARY

As a society we have become dependent on the use of antibiotics for the treatment of bacterial infections. However, bacteria can evolve in ways of reducing the effectiveness of antibiotics and become antibiotic-resistant. The main objective of our project was to determine if any of the bacteria collected from various locations in Cambridge, Massachusetts grew in the presence of an antibiotic. To test our hypothesis, we collected bacterial samples from five different everyday trafficked locations in Cambridge, MA. These locations included the Harvard MBTA subway station (T-station), Fresh Pond Park water fountain, the button of a traffic light, a Cambridge resident's sneaker bottom and cell phone. Then, we asked if any of these bacteria would grow in the presence or absence of ampicillin. We observed an increase in the growth of bacterial colonies in samples obtained from the Harvard T-station, water fountain, traffic light, bottom of the shoe, and cell phone screen. However, no colonies were present in the antibiotic dish except for the bacterial sample obtained from the Harvard T-Station sample, it grew bacterial colonies in the presence of ampicillin.

INTRODUCTION

Bacteria are found almost everywhere, from thermal vents in the ocean to the human digestive tract (1). Today, there are more and more microbes that are becoming resistant to ampicillin (2). We need to be cautious what children and young adults touch in order to avoid diarrheal diseases (3). Many studies have shown that the risk of contracting an infectious illness is reduced when people are consistent with washing their hands in public settings. There is then less of a risk of contracting an infectious illness (4).

Our society is dependent on the use of antibiotics for the treatment of bacterial infections, such as ear infections, strep throat and meningitis (5). Recently, there has been a lot of media attention around antibiotic-resistant bacteria. Antibiotic resistance occurs when bacteria genetically change in a way that reduces the effectiveness of antibiotics designed to cure or prevent infections (5). Once resistance has been acquired, bacteria survive and continue to multiply, even when the antibiotic treatment is administered, causing more harm (6). For our study, we wanted to understand what this meant and how to test for it, since certain bacteria can cause

sepsis and other horrid diseases (5). Therefore, our main objective was to determine if any of the bacteria collected from various locations in Cambridge, Massachusetts, grew in the presence of an antibiotic. We asked, "If we collect bacteria from five different commonly traffic locations in Cambridge, will any of them have an antibiotic resistant response when exposed to the common antibiotic ampicillin?" We decided to use ampicillin because it is a penicillin used to treat a wide variety of diseases such as ear infections, stomach infections, bladder infections and even meningitis, which can be deadly (7). It is also the most commonly used antibiotic treatment, usually from a prescription by a doctor. We hypothesized that there will not be any antibiotic-resistant responses.

The five locations in Cambridge that we chose were: a push-button for a street cross-walk, a water fountain in Fresh Pond Park, a cell phone screen, the bottom of a sneaker that had walked around Cambridge, and an electric subway escalator railing from The Massachusetts Bay Transportation Authority, also commonly known as T-station, at Harvard Square (Harvard T-Station). After collecting samples from these five locations, we tested the ability of the bacteria to grow in the presence of ampicillin. Our results showed that most of the bacteria samples were not resistant to the ampicillin. However, one sample did contain some antibiotic-resistant bacteria. With our study, we hope to make the public more aware of the potential risk of contracting a disease from bacteria lurking right in our own neighborhood.

RESULTS

We employed multiple controls in this experiment to ensure that there was no bacterial contamination of the plates, water, or antibiotic used for the bacterial growth assay. Our first control consisted of a petri dish with just agar and no bacterial sample. This control informed us that our agar plates were not contaminated with any previous bacteria and that what we saw in our dishes were the actual bacteria from the original source. Our second control consisted of a petri dish with agar and the sterile water we boiled without any bacterial sample. Our third control was a petri dish containing agar and the ampicillin antibiotic only. This control indicated to us that the antibiotic alone did not induce any type of bacteria growth. After 24 hours of incubation, we observed that no bacterial colonies were present in any of our three controls (**Figure 1**).

After a 24-hour incubation period, we observed more than 250 colonies grew in the absence of ampicillin from the

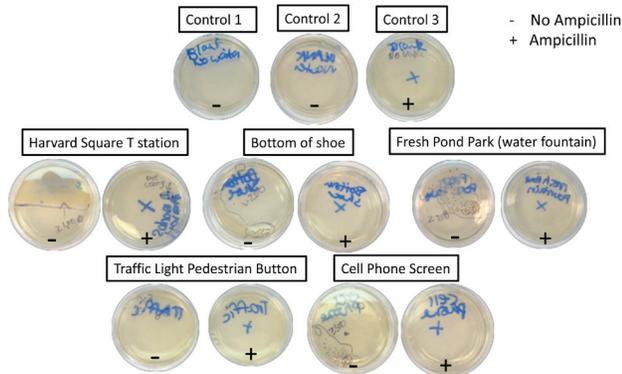


Figure 1. Representative images of samples after 24-hour incubation. Samples were incubated for 24 hours at 37°C in petri dishes with LB Agar made with (+) or without (-) ampicillin.

sample we scraped off of the bottom of the shoe (**Figures 1 and 2A**). These colonies were white and opaque, with a circular form and a convex elevation; its margin was entire with a smooth surface (**Table 1**). No bacterial colonies grew in the presence of ampicillin (**Figures 1 and 2B**).

The sample we obtained from the Harvard T-Station's escalator rail grew more than 1,000 bacterial colonies after 24

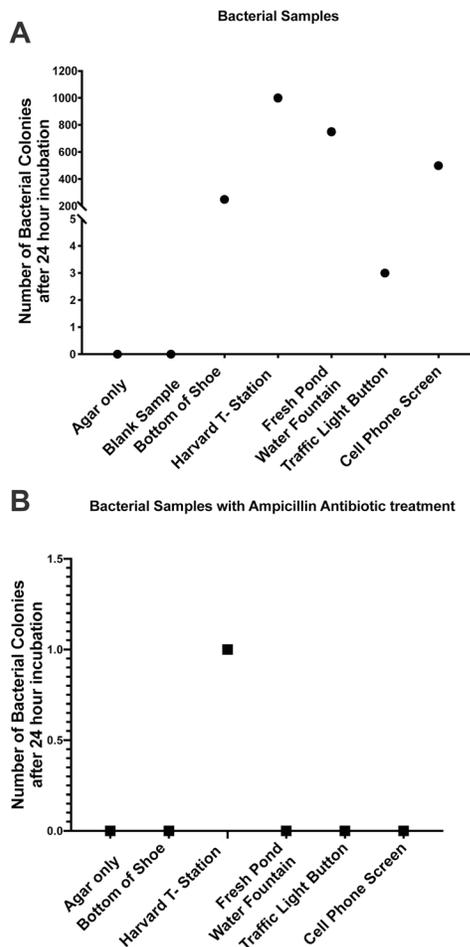


Figure 2: After 24-hour incubation (A) number of bacterial colonies in petri dishes with LB Agar without (-) ampicillin and (B) the number of bacterial colonies in the presence of ampicillin.

hours of incubation (**Figures 1 and 2A**). These colonies were irregular in shape but smooth in their elevation form; although their color was white-yellowish, they were still translucent with a smooth elevation and glistening surface (**Table 1**). We also found a large bacterial colony that grew in the presence of the ampicillin antibiotic, suggesting that these bacteria were resistant to ampicillin (**Figure 2B**). This colony's characteristic was very similar to the bacterial colonies that grew in the petri dish treated with no antibiotic. We repeated the same experiments at a later time, and similar results were obtained (**Figure 3**).

After 24 hours of incubation, we observed over 700 bacterial colonies grown from the sample taken from the water fountain at Fresh Pond Park (**Figures 1 and 2A**), but no colonies were observed in the presence of ampicillin (**Figure**

Table 1. Bacterial Colonies and their Characteristics (see ref. 8 for criteria)

Bacteria Col- lection Sites	Ampicillin	
	-	+
Bottom of Shoe	Characteristics: Form - circular Elevation - convex Margin - entire Surface - smooth Opacity - opaque Chromogenesis - white	None observed
Harvard Square – T-Station's Escalator	Characteristics: Form - irregular Elevation - flat Margin - undulate Surface - glistening Opacity - translucent Chromogenesis - white yellowish	Characteristics: Form - irregular Elevation - flat Margin - undulate Surface - glistening Opacity - translucent Chromogenesis - white yellowish
Fresh Pond Water Foun- tain	Characteristics: Form - irregular Elevation - raised Margin - lobate Surface - glistening Opacity - iridescent Chromogenesis-white yellowish	None observed
Traffic Light Button	Characteristics: Form - circular Elevation - flat Margin - entire Surface - dull Opacity - opaque Chromogenesis- white	None observed
Cell Phone Screen	Characteristics: Form - filamentous Elevation - umbonate Margin - filiform Surface - rugose Opacity - opaque Chromogenesis - white	None observed

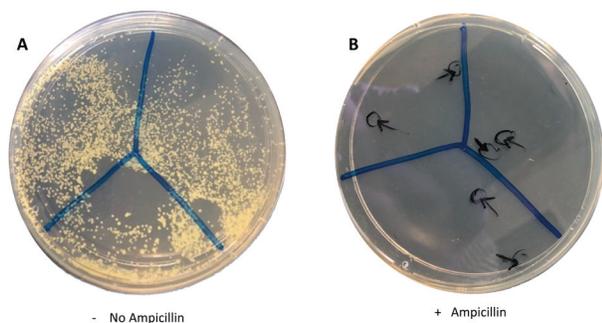


Figure 3: Representative images of samples obtained from Harvard T-Station in August 2019 after a 24-hour incubation at 37°C in petri dishes with LB Agar made with (+) (B) or without (-) (A) ampicillin.

2B). These colonies were white-yellowish and had an irregular, raised form with a glistening and iridescent clarity (**Table 1**).

In the sample obtained from the traffic light, only three colonies grew overnight; no colonies grew in the presence of ampicillin (**Figures 1 and 2**). These colonies were white, circular in form, flat in elevation and had a dull-looking surface (**Table 1**).

We observed that, after an overnight incubation, more than 500 bacterial colonies were present from a sample that we took off of the cell phone screen; no colonies were observed in the presence of ampicillin (**Figures 1 and 2**). These colonies were white and opaque. They were also filamentous in form, and their elevation was umbonate, meaning that although the colonies looked convex, they were also bumpy. Its margin was also different from the bacterial colonies in that it had a filiform shape (**Table 1**).

DISCUSSION

After collecting samples in five commonly trafficked locations, we tested whether or not the bacteria present in these samples would grow in the presence of ampicillin. Without the presence of ampicillin, we observed growth of bacteria colonies obtained from all the samples in our experiment, except for our controls.

The number of bacteria colonies that grew from the sample obtained from the bottom of the shoe was not surprising, since we walk around everywhere and likely pick up a large number of bacteria. However, the samples taken from the electric banister at the metro T-station grew five times the number of bacterial colonies, with an average of more than over 1,000 colonies grown from each sample. The electric handrail is touched every day by many people, which may explain the number of colonies. We also observed that over 700 colonies grew from the sample obtained from the water fountain at Fresh Pond Park. This was not unsurprising, as there are a large number of people in the park every day. Interestingly, the least number of bacterial colonies we observed came from the sample scraped off of the button at the traffic light on Concord Avenue opposite Wheeler Street. This may be because a lower number of people use the

cross street or use the traffic assistance button. We observed that more than 500 bacterial colonies grew overnight from a sample we took off of the cell phone screen. This was not surprising because, although we wash our hands often, our phones are touched no matter if we did wash them or not. Our phones are also placed down on surfaces around everyday places.

Our results showed that most of the bacteria samples were not resistant to ampicillin. However, one of our samples did contain antibiotic-resistant bacteria. We observed the growth of large colonies in the ampicillin plate. This result indicated that the sample taken from the electric subway escalator handrail was resistant to ampicillin. We repeated these experiments at a later time and obtained similar results of bacteria growth in the presence of ampicillin.

We concluded that the subway rail samples would likely have the most amount of hand contact on a daily basis. Therefore, it had the highest chance of containing a bacterium that was resistant to the common ampicillin antibiotic. Another reason, for bacterial growth in the presence of an antibiotic, may be that the colonies that grew in the petri dish were not a strain that can be affected by ampicillin.

Furthermore, testing of various sites in Cambridge and other cities could prove useful in knowing which types of antibiotics can be most valuable in treating everyday bacterial infections, or even more deadly infections. This information would make the public more aware of how to avoid touching public surfaces and reinforce the importance of handwashing.

METHODS

Sample Collection

Sample collections took place in Cambridge, MA in May 2018 and August 2019. Samples were obtained from the Harvard Square-T-station's escalator rail, a water fountain at Fresh Pond Park, a cell phone, the bottom of a shoe, and a traffic light button (on Concord Ave., opposite of Wheeler St.). Sites were evenly swabbed using a sterile 10 μ L inoculating loop and carefully submerged into a 1.5 mL Eppendorf tube containing 1 mL of sterile Molecular Grade Water (G Biosciences Cat. #786-293).

Petri Dish Preparation

Ten grams of LB Broth, Miller (BD Bioscience Cat. #244620) and six grams of agar (ICN Cat. #100262) were added to a final volume of 400 mL of deionized water and then autoclaved. Once cooled to 55°C at room temperature, 10-11 mL of LB solution was poured evenly into each sterile 100 x 20 mm (Corning Cat. #353003) petri dish. Petri dishes were then stored at 4°C until use. For the agar-ampicillin plates, the same procedure was repeated with the exception that we added 5 mL of 10 mg/mL ampicillin (Sigma-Aldrich Cat. #69-53-4).

Plating the Samples

Five hundred μ L of each sample was pipetted onto the

LB Agar petri dish, and another 500 µL of the same sample was pipetted onto an LB Agar petri dish plate containing ampicillin. Plates were then incubated at 37°C overnight. After 24 hours, we removed the samples from the incubator and counted bacterial colonies.

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