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Article

Bacteria and Antibiotic Resistance in School Bathrooms

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SUMMARY

Antibiotics are a class of compounds that can be used to treat patients with bacterial infections. Although these drugs are largely effective, the widespread use of antibiotics has led to the development of antibiotic-resistant bacteria. Since school bathrooms are widely suspected to be unsanitary, we wanted to compare the total amount of bacteria with the amount of bacteria that had ampicillin or streptomycin resistance across different school bathrooms in the Boston area. We hypothesized that because people interact with the faucet, outdoor handle, and indoor handle of the bathroom, based on whether or not they have washed their hands, there would be differences in the quantity of the bacteria presented on these surfaces. Therefore, we predicted certain surfaces of the bathroom would be less sanitary than others. After plating samples with or without antibiotics, we concluded that the only swabbed surface with bacteria was the faucet. As a whole, our results demonstrate that we need to clean our bathroom better in some places more than others. Future research questions could investigate more surfaces in the bathrooms, more bathrooms across Boston, or different times of the day.

INTRODUCTION

Although humans interact with bacteria every day, some strains are pathogenic and can lead to diseases like tuberculosis or cholera. The rise of antibiotic-resistant bacteria is making it harder for doctors to prescribe effective antibiotics (1). The global emergence and spread of extensively drug-resistant bacteria exemplifies the threat that antibiotic resistance presents. Some studies indicate that antibiotic resistance found in clinical settings is closely related to mechanisms found in environmental bacteria (2). This mechanism of antibiotic resistance involves part of the global microbial population and predates the modern selective pressure of clinical human use and abuse of antibiotics (3). Interestingly, bacteria's antibiotic resistance increases healthcare costs by $20 billion in the United States alone (4).

An estimated 15.8 million students attend public schools every day, and each of these students typically use the school restroom at least once a day (5). Therefore, we would expect to find a lot of bacteria in school bathrooms because humans touch bathroom surfaces. We wanted to see how many antibiotic-resistant bacteria live in our school bathrooms. We hypothesized that surfaces with the most human contact prior to hand-washing would be most likely to have antibiotic-resistant bacteria. Each member of our group swabbed the doorknob going into the bathroom, the doorknob going out of the bathroom, and the faucet of their sink in their own school bathrooms, representing three different schools in the Boston area. We could only detect bacteria from the faucets of two schools. In fact, one of the plates not only showed a bacterial lawn, but also showed significant growth when plated on both ampicillin and streptomycin containing plates, suggesting that the bacteria are antibiotic resistant. We chose to use ampicillin because it is an antibiotic that is used to treat different types of infections, including bacterial infections. Similarly, we chose to use streptomycin because it is an antibiotic used to prevent bacterial infection and contamination. Our data hint that while school bathroom faucets should be cleaned properly to prevent the spread of antibiotic-resistant bacteria, in general, school bathroom handles are surprisingly free of bacteria.

RESULTS

Initial visual assessment of the bacterial plates grown from swabbed samples revealed differences in bacterial growth between samples collected from different schools. The plates containing samples taken from the female and male restrooms in school #1 had no bacterial colonies visible on any plates (Figure 1). Although the sample collected from the bathroom faucet (F) at school 3 generated a single bacterial colony on the no-antibiotic plate, none of the other samples taken from this school generated observable bacterial growth (Figure 1-3). In contrast, the samples collected from the bathroom faucet at school 2 produced bacterial lawns that were green and had an odor not only when grown without antibiotics, but also when grown in the presence of ampicillin or streptomycin (Figure 1). Chemicals released from dying bacteria were probably the source of odorant (6). We confirmed that bacteria known not to be resistant to ampicillin and streptomycin would not grow on plates with either antibiotic (Figure 2) and that bacteria transformed with a plasmid conferring antibiotic

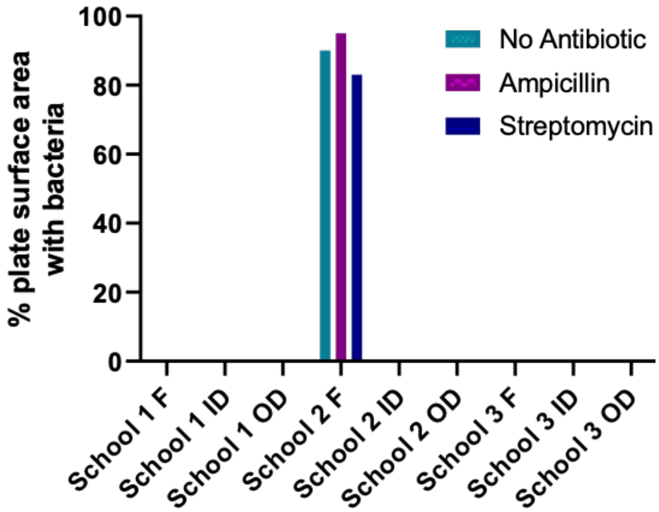

Figure 1: Bacterial growth. Bacterial swabs were collected from three schools around Boston (school 1, 2 & 3), plated without antibiotic, 20 μg/mL ampicillin, or 10 μg/mL streptomycin, and assessed for the percentage of the plate covered in bacterial growth after 24 h incubation.

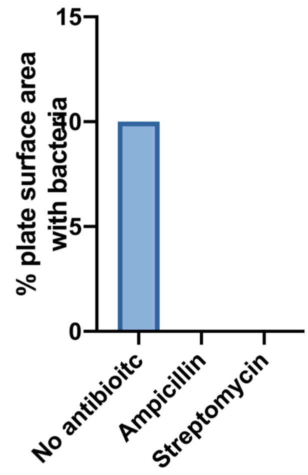

Figure 2: E. coli growth. E. coli with no known resistance to antibiotics were grown on plates without antibiotic, 20 μg/mL ampicillin, or 10 μg/mL streptomycin, and the percentage of the plate covered in bacterial growth was calculated after 24 h incubation.

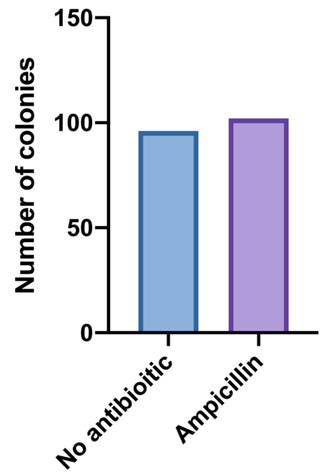

Figure 3: E. coli growth with ampicillin resistance. E. coli transformed with a plasmid known to confer antibiotic resistance to ampicillin were grown on plates without antibiotic or 20 μg/mL ampicillin, and colonies were counted after 24 h incubation.

resistance to ampicillin and streptomycin would grow on both no antibiotic and antibiotic-containing plates.

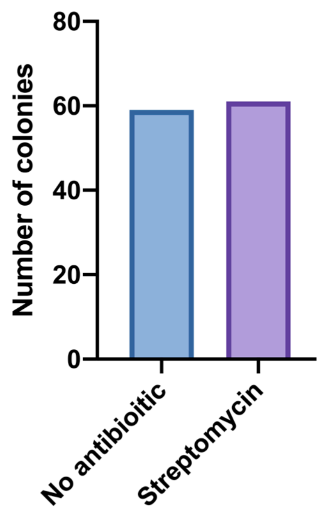

Figure 4: E. coli growth with streptomycin resistance. E. coli transformed with a plasmid known to confer antibiotic resistance to streptomycin were grown on plates without antibiotic or 10 μg/mL streptomycin, and colonies were counted after 24 h incubation.

DISCUSSION

In the experiment, we found that only 1 school had major growth that was in the faucet. Additionally, another school had little growth in the faucet. The remaining school and the rest of the surfaces for the two previously mentioned schools were clean.

Our experiment found that there was no bacterial growth on the door knobs on either side of bathroom doors, regardless of if samples were plated on soft agar with streptomycin, ampicillin, or no antibiotics. For three of the four samples of faucets, there was also little to no growth. However, there were antibiotic resistant bacteria isolated from the faucet of one bathroom.

Essentially, our data suggested that, despite our initial hypothesis that significant differences would be found between F, OD, and ID samples, the most dramatic differences were actually observed between samples collected from different schools on faucet samples alone. These results indicate that faucets may be a place that can harbor lots of bacteria. Moreover, a considerable amount of the bacteria on bathroom faucets may be antibiotic resistant, as suggested by their ability to grow on plates with streptomycin and ampicillin. These data highlight the importance of cleaning bathroom faucets in schools.

Future experiments should control for the time of sample collection. It is possible that bacterial growth is dependent on the time the bathroom was last cleaned. Another improvement that could be made to our experiment would be to incubate the swabs as soon as samples were collected to ensure that no bacteria died while the samples were kept at room temperature. Future research could use the same experimental method to sample other public places. This work could reveal if dangerous bacterial species inhabit public areas. Ultimately, we have identified that bacteria resistant to streptomycin and ampicillin exist in school bathrooms, suggesting the prevalence of these strains in widely used environments.

MATERIALS AND METHODS

Sample Collection

Each experimenter was provided with three swabs and three sample collection tubes to swab each of the three locations. First, we swabbed using a sterile cotton swab three times. Following collection, we placed the swab into a sterile

Eppendorf tube containing 500 uL phosphate-buffered saline (PBS) for storage. After collecting all samples, we refrigerated the tubes at 2-5ºC for storage until platting. Two of our sample sets came from female student bathrooms, and one from male student bathroom.

Plating Samples

Tryptic soy agar plates were prepared and supplemented with 20 μg/mL ampicillin, 10 μg/mL streptomycin, or no antibiotic. Each group member swabbed the inside door knobs of their school restroom (ID), the outside of the doorknob of their school restrooms (OD), and the faucet of their school restroom (F). Samples from these swabs were suspended in PBS as above, spread on these plates, and incubated for 16 h at 37ºC. Throughout our experiment, we followed sterile procedures when handling samples and limiting our samples' exposure to air to avoid contamination. Escherichia coli transformed with a plasmid known to confer resistance to ampicillin and streptomycin were plated on ampicillin and streptomycin plates, respectively, to confirm antibiotic resistant bacteria could grow on the plates (positive control). Additionally, a plate without antibiotic and without sample was grown and shown to not be contaminated (negative control).

Plate Analysis

We did not count the individual colonies because some plates had lawns; therefore, we calculated the ratio of the surface area of the bacterial growth and compared it to the total surface area of the plate. This ratio was calculated by drawing bacterial growth on graph paper and calculating the area of the plate covered by bacteria

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