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Article

Bacterial contamination on New York City transit and the effectiveness of hand hygiene

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SUMMARY

The New York City transit system is the largest in North America, with 1.4 million daily riders. In the Metropolitan Transit Authority (MTA), the M15 bus line and the 6 train had the highest ridership in 2023. As shown by the SARS-CoV-2 pandemic and influenza outbreaks, public transportation poses a risk for infection transmission, with most transmission occurring through handrails and other frequently touched surfaces. The purpose of this study was to evaluate bacterial contamination on the M15 bus and 6 train and assess the efficacy of soap/water versus hand sanitizer at preventing bacterial contamination. We predicted that the 6-train would have more overall bacterial growth than the M15. Within each transit line, we predicted that untreated swabs would show the most bacterial growth and that both soap/water and the hand-sanitizer treatments would equally decrease bacterial growth. We swabbed the entryways of the M15 and 6-train for five consecutive days using untreated swabs or swabs prepared with soap/water or hand sanitizer. Samples were plated and incubated for 72 hours. For plates with growth, we evaluated the growth area using ImageJ and used chromogenic agar to look for the presence of coliforms. We found bacterial contamination on both the M15 and 6 train. The M15 bus showed greater growth, with increase in contamination over the week. Soap/water swabs showed less contamination than untreated swabs, but hand sanitizer was the most effective at preventing contamination. This research supports the use of hand sanitizers in preventing bacterial contamination when using public transportation.

INTRODUCTION

The New York City (NYC) transit system is the largest in North America and one of the largest in the world, with 1.4 million daily riders (1). Of the transit lines, the M15 bus line and the 6 train subway line had the highest ridership in 2023 (1). As shown during the SARS-CoV-2 pandemic and during influenza outbreaks, public transportation poses a risk for infection transmission, and it is known that other pathogens, like bacteria, are also transmitted through public transit (2-4). Pathogens are transmitted both through airborne measures (like coughing or sneezing) or when passengers touch objects such as handrails, and then other passengers touch the handrails and contract those pathogens (5).

In a prior study, investigators found that the five most common taxa of organisms in the NYC transit system were: *Pseudomonas stutzeri*, *Stenotrophomonas maltophilia*, *Enterobacter cloacae*, *Acinetobacter radioresistens*, and *Acinetobacter nosocomialis*, all of which are Gram negative opportunistic bacteria (6). In most people, these bacteria are harmless, however they can cause serious infections in people with weak immune systems (like people who have diabetes or cancer) and all of these bacteria showed some degree of antibiotic resistance (6). The use of surface modification to prevent contamination, such as anti-adhesive surfaces with antimicrobial substances or modified biologically active metals has been previously explored (7). While this can be an effective means of preventing bacterial contamination, it can increase the use of antimicrobials, particularly antibiotics. The overuse of antibiotics increases the risk of creating more antibiotic-resistant organisms (8). In fact, antibiotic overuse has contributed to a global public health problem where organisms gain increased resistance to readily available antibiotics, causing more severe infections, more complications and increased morbidity and mortality (9).

Focusing on simple prevention strategies such as hand-washing and the use of hand sanitizers has been shown to be effective in preventing disease transmission (10). To our knowledge, the effectiveness of hand-washing and hand sanitizer in decreasing bacterial contamination in the NYC M15 and 6 train transit lines, has not been specifically investigated. Therefore, in this study, we aimed to evaluate the degree and type of bacterial contamination on the M15 bus and 6 train, and to assess the efficacy of soap/water versus hand sanitizer at preventing contamination. Additionally, we used selective agar to look for a particular group of bacteria called coliforms, which are responsible for a wide range of illnesses (11).

We predicted that untreated swabs would have the most bacterial growth, but that soap/water swabs would have equivalent growth as hand sanitizer swabs. We also predicted that the 6 train would have more bacterial growth than the M15 bus, based on the higher volume of passengers transported by the subway relative to the bus lines (6). We also predicted that contamination would increase from Monday to Friday, given the increase in ridership over the week (6). We found that untreated swabs had the greatest proportion of plates with growth overall and the greatest number of plates of coliform bacteria, followed by soap/water plates, while hand sanitized plates had no coliform growth. M15 had more plates with growth than the 6 train. Monday had the least amount of growth on both M15/6 train, and Friday had the most. Our research supports the use of hand sanitizers in preventing bacterial contamination when using public transportation.

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RESULTS

To assess for bacterial contamination on the NYC M15 bus and 6 train transit lines, entryways of the M15 bus and 6 train were swabbed for five consecutive days (Nov. 4 2024-Nov. 8, 2024) with swabs that were untreated or prepared with soap/ water or hand sanitizer, then plated on nutrient agar (NA) and Luria Broth (LB) agar plates and incubated for 72 hours (**Figure 1**). Growth area was evaluated with ImageJ software. Any plate demonstrating growth was then plated onto chromogenic agar (selective agar for coliform growth). Entryways were swabbed because these areas have the most crowding of passengers, with more potential for bacterial contamination (12).

A total of 60 inoculated (20:20:20 unprepped: soap/water: sanitizer) and 10 control plates were collected over 5 days between Monday (11/4/24) and Friday (11/8/24) (**Figure 2**). Unprepped swabs had the greatest number of plates showing any bacterial growth: 100% (10/10) M15 plates and 50% (5/10) plates from the 6 train; followed by soap/water: 60% (6/10) M15 and 70% (7/10) 6 train; and hand sanitizer with 50% (5/10) plates with growth on the M15 and 40% (4/10) plates with growth on the 6 train. None of the control plates showed any growth. There was minimal difference between the type of agar used and the number of plates with bacterial growth. None of the NA plates swabbed with hand-sanitizer showed any growth.

The average percentage area of growth (growth area/total petri dish area) on the petri dish for the plain swab on the M15, was 16.86%, and for the 6 train was 10.4%. Percentage area growth for soap/water was 2.6% for the M15, and 11.8% for the 6 train. For hand sanitizer, it was 0.06% for the M15, as compared to 1.1% for the 6 train (**Figures 3-4**). Growth was present on LB and NA on the same day for each condition except for one condition: none of the hand sanitized NA plates had any bacterial growth, while two of the LB plates showed a small amount of growth (0.06%). This means that LB and NA substrates were essentially equivalent. The amount of bacterial growth was similar between LB and NA for each condition, with an average overall percentage of growth area (for all the positive plates) of 9.8% for the LB plates versus 11.6% for NA.

Untreated swabs showed the greatest number of plates with coliform bacteria (75% (15/20)), followed by soap/water plates (45% (9/20)) while hand sanitized plates had no coliform growth (0/20) (**Figures 5-6**). None of the chromogenic agar plates had a pink color, which indicates *Escherichia coli* growth.

The M15 bus line had more plates with growth (70% (21/30)) as compared to plates from the 6 train (53% (16/30)). Monday had the least amount of growth on both the M15 bus and the 6 train (4/12 plates, average 0.9% plate area), and Friday had the most amount of growth (11/12 plates, average 20.7% plate area).

DISCUSSION

We observed bacterial contamination on both the M15 bus and the 6 train, with the M15 bus showing more bacterial growth overall and increasing contamination over the week. Soap/water swabs showed less contamination overall compared to unprepped swabs, but hand sanitizer was the most effective at preventing contamination, completely preventing coliform contamination. Coliforms are a broad group of bacteria that include pathogenic bacterial species like *Enterobacter spp*. and *E. coli* O157:H7, and they are responsible for a wide range of illnesses (11). Microbiologists tend to monitor coliforms to assess food and drinking water system safety because testing for individual pathogenic species is too complex for routine testing (13). Our results support the use of an alcohol-based hand sanitizer for successful prevention of clinically relevant bacterial transmission, as it showed that this sanitizer prevented all coliform growth in our plated samples.

We predicted that the 6 train would have more bacterial growth than the M15 bus line, based on the higher volume of passengers transported by the subway relative to the bus lines (6). Our result showing that the M15 bus line had greater contamination was surprising. However there are many potential explanations for this result. Post-COVID-19, average daily ridership on the buses increased significantly over subway ridership, at the same time that staffing of bus operators declined, leading to increased bus crowding (14). We swabbed the entryway of the buses and the train, since these areas have been shown to be the most crowded during peak travel times. However, the crowding that we experienced on the buses while collecting the samples during rush hour was much greater than what we experienced at a similar time on the subway, possibly leading to higher bacterial presence.

Alcohol-based hand sanitizer was more effective than soap/water at preventing bacterial contamination in this

Figure 1: Swabbed regions of the M15 bus. The entryway of the M15 (and 6 train, not shown) was swabbed for 30 seconds in the regions shown by the white rectangles with the unprepped swab, the soap/water swab, and the hand-sanitizer swab.

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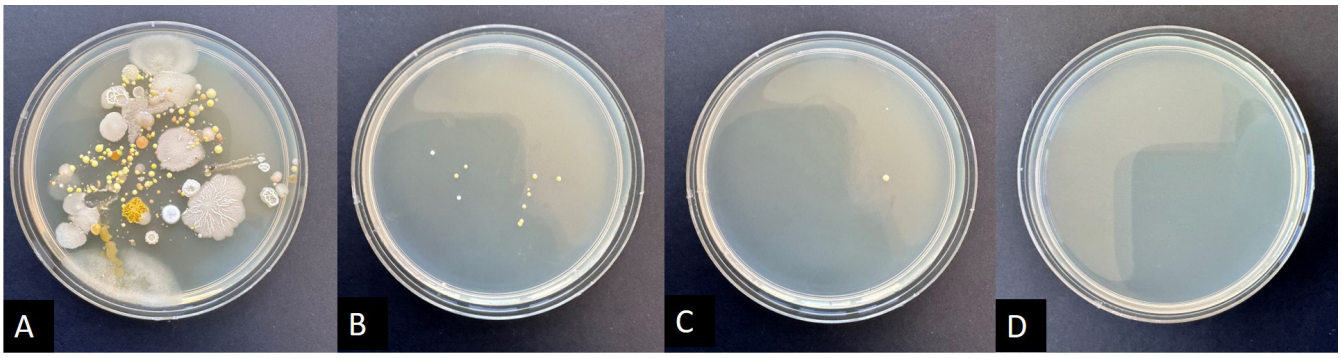

Figure 2: Image of samples on inoculated petri dishes. A sample of inoculated plates from the M15 bus line plated on Luria Broth (LB) on 11/6/24. A) LB agar plated with A) an untreated swab, B) swab treated with soap/water, C) swab treated with hand sanitizer and D) control plate. A) Shows the largest amount of bacterial growth, B) showed a small amount of bacterial growth C) showed a single focus of growth, and D) showed no growth.

experiment. This is likely due to several factors. Soaps are molecules with a hydrophilic head and a hydrophobic (lipophilic) tail, and work against bacteria and viruses by attracting the lipid in their membranes and trapping them in micelles. Then, using water, the trapped bacteria/viruses are washed away. Because we had to agitate the swabs in the soap/water mix and then let the mixture sit on the swab, the bacteria/viruses may have been surrounded in the micelles of the soap, but they weren't washed away. Once plated on the agar plates, the lipid micelle likely broke down, allowing the bacteria to grow. In contrast, alcohol is directly bactericidal, as it dissolves the outer surface of bacteria and viruses and kills them by removing their protective coating (15). This likely explains why the alcohol-based hand sanitizer was more effective than soap/water in this experiment. Likewise, hand sanitizer is a good solution for real-life bus and train ridership, since there are no immediately available bathrooms or hand-washing stations along the NYC MTA bus and subway routes.

There were several limitations of this experiment. There was a limited sample size (12 swabs/day), and the M15 bus and 6 train were sampled only over the course of 5 consecutive days. The swab time was 30 seconds, which is less than the previously reported 3 minutes of swab time for collecting samples; however our results showed that this abbreviated time was still successful in contracting bacteria. We did not have access to polymerase chain reaction (PCR) kits or specialized antibiotic agar plates to do advanced selective testing for different subtypes of bacteria. Also, we saw growth of both bacterial and fungal species on our plates. Using selective plates in the future that inhibit bacterial growth (such as Yeast and Mold (YM) media plates) as well as using plates that contain antifungal compounds to inhibit fungal growth would allow us to look at the growth of fungal and bacterial species separately. We could have also quantified bacteria by calculating colony forming units (CFUs) for each sample. Finally, we only studied soap/water versus one type of alcohol-based hand sanitizer; other studies evaluate other types of soap and sanitizer and compare the relative effectiveness.

Our study showed the efficacy of alcohol-based hand sanitizer over both untreated swabs and swabs prepared with soap/water in both decreasing bacterial transmission and in completely preventing coliform bacterial transmission. Future studies could evaluate the rate of contamination at different sites on the M15 bus and 6 train (i.e., assessment of contamination in the seats versus handrails and doorways)

Figure 3: Example of measured region of growth using Image J. Growth on a Nutrient Agar plate from Wed Nov. 6, 2024 from the M15 bus using an unprepped swab after 72 hours of growth. The green outline shows the area used in ImageJ to calculate the growth area.

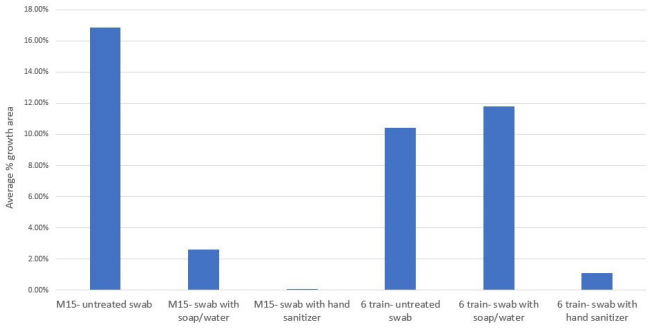

Figure 4: Comparison of M15 bus and 6 train overall bacterial growth. The average growth area from the M15 bus line and the 6 train, as stratified by untreated swab, soap/water swab, and hand-sanitizer swabs.

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and at different times of the day. This study could also be extrapolated to include all of the various subway lines. However, this was a good demonstration showing the utility of a cheap, easily available prevention tool in helping decrease disease transmission

MATERIALS AND METHODS

Using sterile technique (after hand-washing and wearing Nitrile gloves) sterile cotton swabs (McKesson Cotton Tipped Applicator Swabs) were either untreated, or were prepared by inserting them in agitated soap/water, or in Purell alcohol-based hand sanitizer, and then placing them back in their sterile pouch. These were then placed in a Ziploc bag for transport.

Agar plates we used were either NA (Carolina Biological Supply Company) or LB (Carolina Biological Supply Company). Chromogenic media was prepared per manufacturer instructions (Carolina Biological Supply).

For 5 consecutive days beginning on Monday, November 4, 2024, the M15 bus line was boarded during afternoon rush hour and the door entryway was swabbed with each swab for 30 seconds and then the swabs were used to streak the agar plates which were closed and sealed with parafilm (**Figure 1**). Similarly, the 6 train was boarded during afternoon rush hour and the handrails near the entryway were swiped with each swab for 30 seconds, after which each agar plate was plated and closed with parafilm. The control plates were also opened and closed with parafilm at the time the experimental plates were plated. The plates were then placed in a home-made incubator (a covered shoebox container in a cordoned off area in the living room at 25°C). After 72 hours in the incubator, the plates were removed from the incubator and were placed on a black cardstock and photographed (**Figure 2**). The images were uploaded to ImageJ, and the amount of bacterial growth on each plate was quantified by tracing freeform the region of growth on each plate and dividing that area of growth by the total area of the agar plate (**Figure 3**).

Next, growth from the NA or LB plates was used to streak on chromogenic agar (Carolina Biological Supply) and placed in a different home-made incubator for 24 hours. When *E. coli* is grown on this chromogenic agar it produces a pink color. Other coliform bacteria will produce a blue color and non-coliform bacteria will not show any color change. Each plate

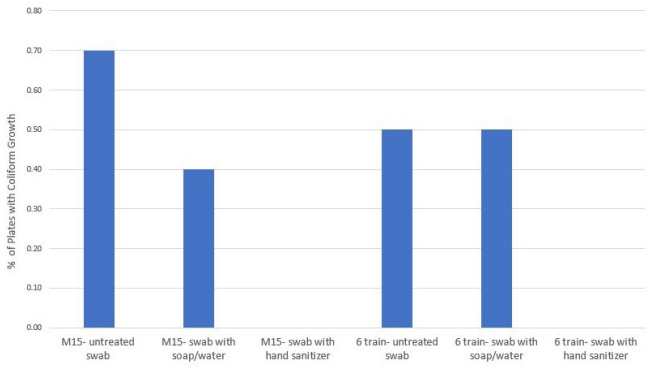

Figure 5: Coliform bacterial growth on the M15 bus and 6 train. Percentage of plates that showed coliform bacterial growth for each condition tested. None of the swabs treated with hand sanitizer showed any coliform bacterial growth.

Figure 6: Coliform bacterial growth. Chromogenic agar plate showing coliform growth from the Wed Nov. 6, 2024 M15 Luria Broth unprepped swab.

was observed after 24 hours and photographed against a black background, with color reaction noted. Bacterial growth was determined as described above.

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