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Article

The effects of different modes of vocalization and food consumption on the level of droplet transmission of bacteria

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SUMMARY

Expulsion of microbes through vocalization represents an important means by which pathogens are transmitted between individuals. Based on the current CDC guideline, a distance of three feet is considered to be sufficient to prevent transmission of microbes. In this study, we specifically focused on droplet transmission of bacteria expelled through the mouth, capturing microorganisms on Petri dishes set at different distances during various activities. Our goal was to assess how the mode of vocalization and the type of food consumed impact the level of bacterial transmission. By measuring the bacterial count in Petri dishes after each activity, we determined that, in fact, transmission of bacteria is significantly impacted by the type of vocal activities and the food consumed prior to vocalization. Additionally, our results are consistent with the CDC three-foot physical distancing guideline for droplet transmission of bacteria. Our study also informs on the types of activities that are associated with greater transmission of oral bacteria, such as speaking loudly and sneezing. Food types that increased bacterial transmission include raw kimchi and apples, whereas consuming raw garlic reduced transmission.

INTRODUCTION

Human expiratory events expel droplets with diameters ranging from 0.6 μm to over 1000 μm at high velocities (1). Respiratory droplets are responsible for and serve as a vehicle for the transmission of microbes despite their small size. Bacteria and viruses are released in different amounts and spread to different distances depending on the type of expiratory activity and the species of microorganism. Pertinent to pathogen transmission, determining which types of expiratory activities (e.g. coughing, talking at different amplitudes, etc.) have the greatest impact on transmission can be valuable for the development of protective measures.

There are two relevant modes of respiratory transmission for expiratory events: droplet transmission and airborne transmission (also known as aerosol transmission). While droplet transmission refers to direct sprays of large microbe-containing droplets generated by close-contact expiratory activities onto a person's mucous membranes, airborne transmission refers to the transfer of easily inhalable microbe-containing particles that remain suspended in air due to their small size (1).

Many previous studies have identified correlations between specific variables and airborne transmission. Asadi *et al.* (2019) evaluated the impact of the amplitude of vocalization on aerosol emission (2). The researchers used an aerodynamic particle sizer to calculate the number and size distribution of particles during various expiratory activities and found that the rate of particle emission was positively correlated with volume during speech. The authors followed up with another study analyzing the effects of phones, which are distinct units of speech sound that have to do with articulation, on particle emission and discovered that airborne transmission could be influenced by language characteristics (3).

A number of studies have also focused on emission of droplets resulting from expiratory activities such as talking and coughing. However, the findings are highly inconsistent due to differences in the instrumentation and methodology used for droplets generation and collection. An early work by Loudon and Roberts (1968) used paper and filter to collect droplet expulsion in speaking, coughing, and singing (4). They found that singing produced fewer droplets than speaking while coughing generated the greatest number of droplets. In contrast, Xie *et al.* (2009) did a similar study using glass slides and an aerosol spectrometer but reported that talking (counting from 1 to 100) released almost 20 times more droplets than a single cough (5).

Another interesting variable is the effects of consuming different types of foods on oral microbial content. Leontiev *et al.* (2018) conducted experiments with thiosulfinates, found in garlic, to study its antimicrobial properties (6). The authors determined that thiosulfinates exhibit significant antimicrobial activities against bacteria and model fungi. The noteworthy antimicrobial properties in garlic led us to question whether consuming garlic before expiration would lower droplet transmission of bacteria. On the other hand, we suspected that consuming kimchi, a probiotic food, would lead to higher transmission. In a study on kimchi, Jung *et al.* (2011) discussed the use of leuconostoc mesenteroides as a starter culture in kimchi fermentation (7). Accordingly, uncooked kimchi contained a high concentration of live bacteria.

Though the impact of a broad range of variables on bacterial transmission have been researched, to our knowledge, no previous studies have analyzed the correlation between different modes of vocalization, food types, and the level of droplet transmission of bacteria through the use of Petri dishes. This study explored the effects of different modes of vocalization and food types on the level of droplet transmission of bacteria in an indoor setting in order to

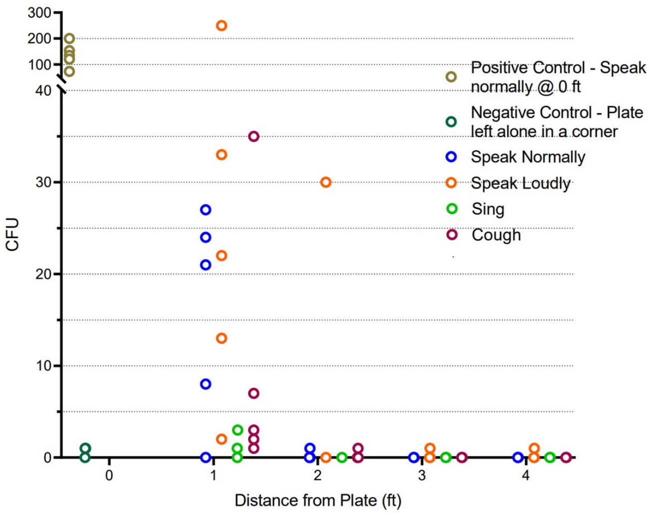

Figure 1. Effects of distance on droplet transmission of bacteria. Each dot represents the number of CFU observed for each expiratory event conducted at distances of 1 to 4 feet from the mounted Petri dish. There are 5 dots for each event, representing the 5 trials. Participant spoke at 60 dB (normal) and 75 dB (loud), sang at 60 dB, and coughed (intermittently) all for 3 minutes. For Positive Control, Participant spoke at 60 dB at 0 feet from the plate. For Negative Control, Petri dishes were left in a vacant room

determine the validity of the Center for Disease Control's (CDC) physical distancing guideline for the prevention of pathogen transmission (3 feet) and whether certain activities promote a higher level of transmission (8). We hypothesized that oral bacterial transmission is not only dependent on the distance between individuals, but also on the mode of vocalization and oral microbial content. By measuring the colony counts collected in Petri dishes after different vocal activities, we found three key results: [1] Consistent with the CDC three-foot physical distancing guideline for the prevention of droplet transmission of bacteria, oral bacteria were not expelled beyond 3 feet under most circumstances. [2] Different vocal modalities influenced the level and distance of bacterial transmission. [3] The type of food consumed prior to expiration could either promote or reduce bacterial transmission.

Taken together, our results suggested that droplet transmission of bacteria occurred as a result of several factors working in synergy: oral microbial content, mode of vocalization, and distance between individuals.

RESULTS

We conducted three different experiments with all data collected from one subject (labelled as "Participant"). The experiments investigated the effects of three parameters on bacterial transmission, which we captured on Petri dishes: 1) distance, 2) mode of vocalization, and 3) oral bacteria composition.

First, we assessed the validity of the CDC's three-foot physical distancing guideline for prevention of microbial transmission. Standing at a distance of 1 to 4 feet from the mounted Petri dish, the participant read a script at 60 dB

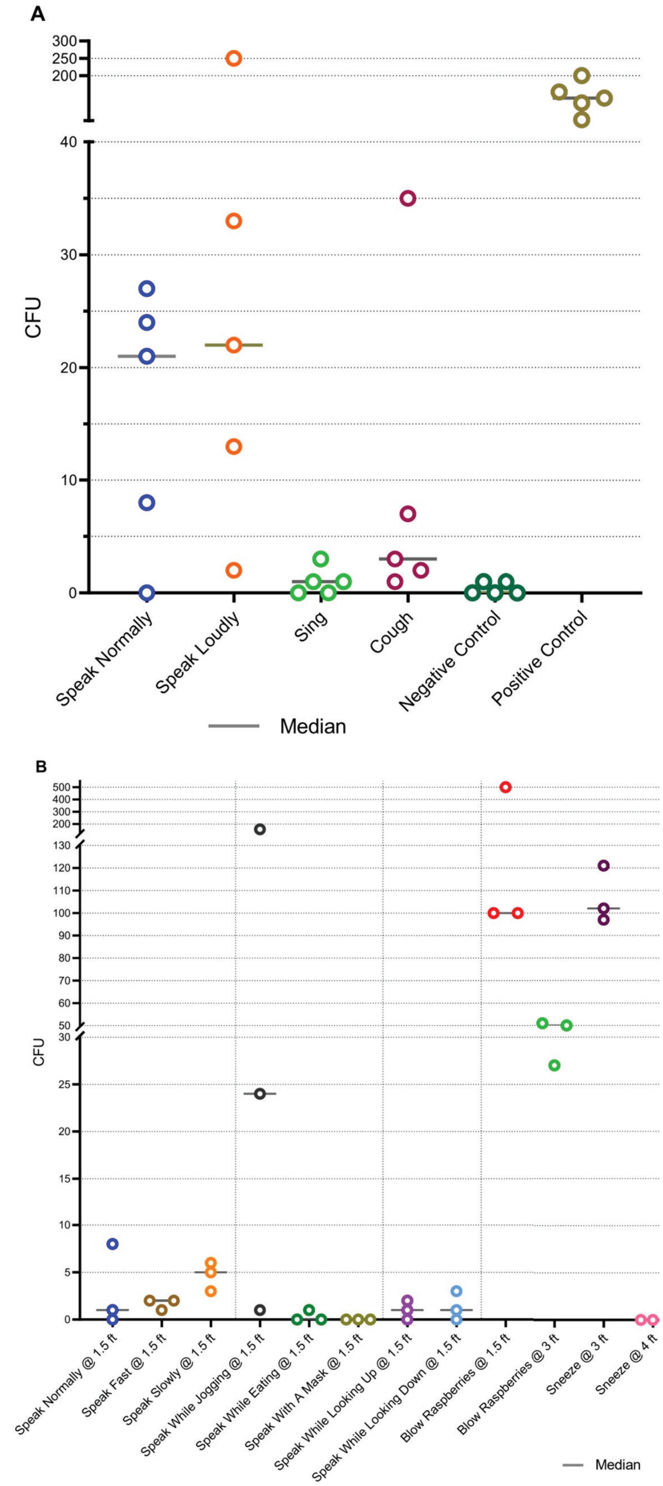

Figure 2. Effects of mode of vocalization on droplet transmission of bacteria. Median value is shown for each expiratory event. (**A**) Each dot represents the number of CFU observed for each expiratory event conducted at 1 foot from the mounted Petri dish. There are 5 data points for each event, representing the 5 trials. (**B**) Each dot represents the number of CFU observed for each expiratory event conducted at 1.5 feet from the mounted Petri dish, except Blow Raspberries and Sneeze. Blow Raspberries was conducted at a distance of 1.5 and 3 feet from the mounted Petri dish, and Sneeze at a distance of 3 and 4 feet. There are 3 data points for each event, representing 3 trials of each.

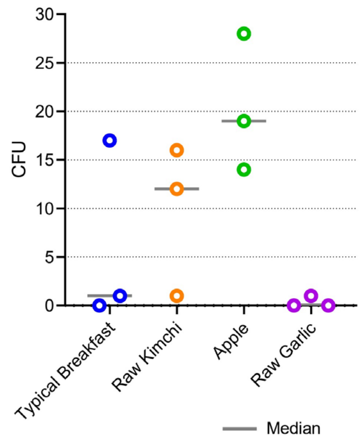

Figure 3. Effects of modification of oral bacteria content with food on droplet transmission of bacteria. Median value is shown for each food type. Each dot represents the number of CFU observed for each type of food consumed. The participant read a script at 1 foot from the mounted Petri dish. There are 3 data points for each food type, representing the 3 trials.

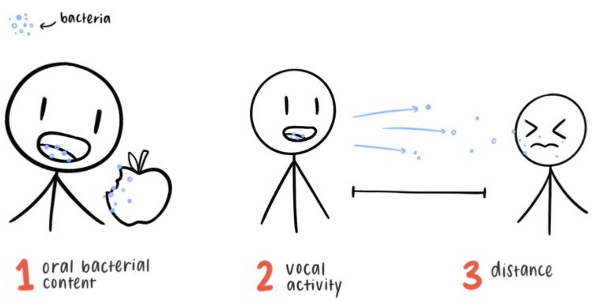

Figure 4. Variables affecting droplet transmission of bacteria. Three components that affect droplet transmission are oral microbial content, expulsion mechanism, and distance.

(a normal talking voice) and also at 75 dB (a loud talking voice), sang at 60 dB, and coughed intermittently, all for three minutes. We repeated the activities 5 times. We then sent the Petri dishes to the laboratory for 72 hours of incubation, after which the lab assistant counted the number of bacterial colonies, measured in colony-forming units (CFU). Consistent with the CDC's three-foot distancing guideline, transmission mostly occurred at 1 foot and rarely beyond 2 feet (**Figure 1**).

We also investigated the impact of expulsion mechanism on oral microbial transmission by comparing the bacterial transmission of each expiratory event at a distance of one foot from the mounted Petri dish (**Figure 2A**). We found that speaking loudly (75 dB, mean of 64 CFU) produced, on average, the greatest level of droplet transmission of bacteria, followed by speaking normally (60 dB, mean of 16 CFU) and coughing (intermittently at around 65 dB, mean of 10 CFU). In contrast, singing (60 dB, mean of 1 CFU) exhibited the lowest level of transmission. Due to the high variability between trials, the median value for Speak Loudly is very close to that of Speak Normally.

Given that our initial findings suggested the mode of vocalization greatly impacts bacterial transmission, we tested twelve additional vocal activities in a separate experiment (**Figure 2B**). The twelve activities were conducted at 1.5 feet from the apparatus with the exceptions of "Blow Raspberries" (1.5 and 3 feet) and "Sneeze" (3 and 4 feet). We noted to our surprise that "Speak Fast" consistently produced fewer colony counts than "Speak Slowly". "Blowing Raspberries" (making a sputtering noise by sticking out the tongue and blowing) and "Sneezing" expelled high loads of oral bacteria at 3 feet. However, "Sneeze" at 4 feet produced zero colony counts. The results for "Sneeze" at 3 feet and "Sneeze" at 4 feet appear to be consistent with the three-feet social distancing guidelines set forth by the CDC. "Speak While Looking Up" and "Speak While Looking Down" did not yield very different results, and the low colony counts resulting from "Speak While Looking Down" contradicted our expectation. Unique to our data was the high variability demonstrated with "Speak While Jogging", with the participant able to expel an oral bacterial load of over 100 CFU in one instance. Lastly, "Speak With A Mask" produced zero CFU for all three trials, and "Speak While Eating" yielded very few colony counts.

In our final experiment, we investigated how differences in oral bacterial content, which were induced through dietary changes, affected microbial transmission. We tested consumption of apples (each carries about one million bacteria) (9), raw garlic (previously associated with antimicrobial properties) (6), raw kimchi (high in leuconostoc content) (7), and a typical breakfast (bagel with cream cheese and oatmeal). Participant waited for an hour after food consumption before reading aloud the script at one foot from the apparatus. Our results indicated that apples and raw kimchi promote oral bacterial transmission while garlic reduced the number of collected bacteria (**Figure 3**).

DISCUSSION

This study sought to determine transmission efficiency of bacteria expelled from the mouth of one subject during vocalization, and the bacteria were captured on chocolate agar plates. Our first experiment investigated the relationship between distance and transmission, and our results indicated that droplet transmission of bacteria for general vocalizations (speaking at 60 dB, speaking at 75 dB, singing at 60 dB, and coughing) does not exceed the CDC's recommended three feet minimum distance to reduce the risk of pathogen transmission. Generally, droplet transmission significantly decreased beyond the one-foot mark (**Figure 1**).

We also probed whether different modes of vocalization impact the transmission of bacteria at a one-foot distance from our initial findings (**Figure 2A**). Speaking at 75 dB produced, on average, more CFUs than speaking at 60 dB, which is consistent with the findings by Asadi *et al.* (2019) (2). We observed high variability with speaking at 75 dB, and this may be due to vocal amplitude control. Our participant had a natural low voice that tended to fall despite his effort to raise it. Increasing the number of trials may lead to more consistent results. Singing had the lowest droplet transmission, which matches the findings of Loudon and Roberts (1968), where singing was shown to emit fewer droplets than speaking and coughing (4).

Our results from coughing were harder to interpret. Coughing transmitted very few CFUs compared to speaking at 60 dB in our findings. However, even in seperate studies, both Loudon and Roberts (1967) and Xie *et al.* (2009) showed higher droplet counts from coughing (20 single coughs) compared to talking (counting from 1 to 100). In our experiment, the participant was not asked to contrive a specified number of coughs, but we estimated the number to be on the low side, as the subject took long breaks in between coughs. A lower number of coughs could have accounted for the low colony counts. We will revise our methodology to quantify the number of coughs for future trials.

To supplement our initial experiment, we evaluated the impact of other vocal modalities on transmission (**Figure 2B**). "Speak Fast" and "Speak Slowly" produced a noticeable difference in colony counts, yet interestingly, "Speak Fast" generated fewer CFUs than "Speak Slowly". Although no prior research has been conducted on the effect of the rate of speech on particle emission, one possible interpretation is that speaking fast causes words to lose their enunciation. An experiment by Asadi *et al*. (2020) previously demonstrated that certain phones, or distinct units of speech sound, were associated with higher particle emission (3). Speaking fast may cause specific phones to be blurred or swallowed. Thus, it is conceivable that droplet emission will decrease with faster speech. However, further research must be carried out in order to draw a firmer conclusion.

On average, "Speak While Jogging" in place produced more colony counts than "Speak Normally" while standing in place. A justification may be that a physically taxing activity causes the speaker to exhale large breaths of air, which would likely lead to a higher level of droplet transmission of bacteria. Inconsistent results from "Speak While Jogging" may be solved by increasing the number of trials.

To our surprise, the paired events of "Speak While Looking Up" and "Speak While Looking Down" did not display a significant difference in droplet transmission. We expected that, for example, a mother talking downwards to a child to expel more droplets (onto the child) than the child talking upwards to the mother, because droplets should be affected by gravity. One plausible reason for why this is not the case in our experiment is, that the angle at which our participant spoke while looking up or down (40º) was not steep enough. Re-designing the experiment to account for the potential angle issue may reveal more significant findings.

The colony counts resulting from "Blow Raspberries" at 1.5 feet averaged 233 CFU. We also noted that "Blow Raspberries" at 3 feet yielded, on average, 43 CFU. We feel that it is worthwhile to conduct "Blow Raspberries" at 4 feet to see whether blowing raspberries can exceed the three-foot social distancing guidelines.

We observed a high level of bacterial transmission in "Sneeze" at 3 feet. This is consistent with prior literature, which suggested that sneezing produces up to 40,000 droplets at a speed of 100 m/s (10). However, "Sneeze" at 4 feet did not produce any bacteria, upholding the three-foot CDC guideline. Also consistent with the CDC recommendation, wearing a mask greatly reduced the level of droplet transmission. In our results, "Speak With A Mask" produced zero colony count for all three trials.

We all know not to talk with our mouth full, because not only is it impolite, but it may also result in choking. But does talking while eating affect droplet transmission of bacteria? Our data shows that "Speak While Eating" transmitted very few CFUs, contrary to our expectation. A possible explanation is that talking while eating prevents the speaker from properly enunciating the words, causing specific phones to be blurred or swallowed and potentially leading to a decrease in droplet transmission. In addition, the food may block the emission of droplets out of the mouth.

Following our characterization of vocalization modes and their influence on bacterial expulsion, we investigated the impact of food consumption prior to bacterial capture and found interesting results (**Figure 3**). There was a notable difference in the number of colony counts produced between Apple and Raw Garlic, where Apple yielded more CFUs than Raw Garlic. This aligns with previous literature, where garlic was shown to possess antimicrobial properties (6). At the same time, apples have been suggested to host approximately 100 million bacterial cells (9). Juices produced by apples may potentially increase oral secretion, which might have led to an increase in droplet transmission. There was also a noticeable difference in the level of droplet transmission between Apple and Typical Breakfast. In our experiment, Typical Breakfast consisted of processed, cooked food (toasted bagel with cream cheese and oatmeal). It is possible that heating up food (bagel and oatmeal) killed the bacteria, thus registering a lower level of droplet transmission of bacteria. Lastly, we noted that Raw Kimchi seemed to promote a higher level of droplet transmission of bacteria on average, as expected. The fermentation process of kimchi involves the addition of various microbes, including *Leuconostoc* and *Lactobacillus* species (7). As a result, it may be inferred that consuming raw kimchi will increase transmission of bacteria. With repeated tests, the significance of our data may rise. We would like to point out that we allowed an hour between food ingestion and experiment. The thought was that an elapse of time would allow the bacteria to grow. On the other hand, larger food residuals could be cleared by saliva. Therefore, it was unclear if waiting an hour promoted more colony counts.

There are two main implications of our work. The first is, that the CDC three-foot physical distancing guideline is appropriate for the prevention of bacterial droplet transmission. Throughout our experiments, the data consistently showed that transmission beyond three feet was negligible or nonexistent. The second implication is, that different modes of vocalization and food consumption have an impact on droplet transmission of bacteria. Taken together, droplet transmission appears to occur as a result of several variables working in synergy: bacteria load in the mouth, expulsion mechanism that propels the bacteria, and distance between individuals (**Figure 4**). In addition, while viruses are generally smaller than most bacteria and likely to transmit greater distances (with some exceptions), the types of activities that affect oral bacterial droplet transmission could provide insight for transmission of pathogens in general, including aerosol transmission.

Our methodology can be applied to the study of COVID-19 airborne transmission. Airborne transmission occurs when microbes from an infected person are dispersed via aerosol particles with smaller diameters than particles produced by droplet transmission. One way to collect airborne particles from expiratory activities such as coughing is through the use of gelatin membrane filters, which are highly efficient in trapping viral particles. The filters can be mounted at distances of 1 to 6 feet from the subject while they talk or cough. Viruses cannot grow in an artificial media like Petri dishes but can be quantified using polymerase chain reaction (11).

For our current study, we recognize that the experiments were performed using only one participant (due to COVID-19 concerns) and that the rate of droplet emission varies by individual and possibly even within the same individual during different activities. As such, our findings need validation with multiple subjects and a greater number of trials. We also acknowledge that bacterial transmission does not imply infection, since the bacterial load required to cause infection varies between pathogens. However, our findings give a perspective on how distance, vocal activity and oral bacterial load are all important determinants of microbial transmission.

Additional topics worthy of investigation are the effects of altitude and temperature on droplet transmission of bacteria. We expect that vocalizing at a higher altitude (5,000 feet) will produce a higher level of droplet transmission due to the effect of altitude on air density, which decreases as altitude increases. Thinner air will exert less drag; therefore, at a higher altitude, droplets will fly farther. Likewise, we expect vocalization at a higher temperature to transmit droplets farther since a higher temperature correlates with lower air density (12).

METHODS

Participants

All parts of the experiment were conducted with one participant, the father of the lead author ("Participant"), due to safety concerns during the COVID-19 pandemic. Participant is a healthy, non-smoking male 57 years of age. All research procedures were approved by the Scientific Review Committee of the Orange County Science and Engineering Fair.

Materials

In this study, 140 chocolate agar plates (15x150 mm) from Hardy Diagnostics were purchased. Made of tryptone soya agar and sheep blood agar heated to 60 degrees, these particular Petri dishes were selected due to the higher sensitivity to growth of bacteria on chocolate agar. For measurements, a standard measuring tape and ruler and one roll of painter's tape were used to mark distance increments. Observations were recorded in a lab notebook. An iPhone XS operated as a timer, and the free NIOSH sound level meter app on the phone measured Participant's voice amplitude in decibels (dB). An adjustable music sheet stand from Costco was used to vertically mount Petri dishes. One 9x16 inch piece of cardboard was needed for the mount, and eraser putty from Daiso was used to secure the plates. A print-out of "The Garden" by Shel Silverstein served as a script. Other materials used during the experiments included a disposable mask, cut fruit, and black pepper. 10% Clorox wipes and water wipes were used to sanitize surfaces after the experiment.

Procedure

All experiments were performed in an indoor environment. We ensured that there were no external disturbances (e.g. air conditioner or heater) before testing. Increments from one to four feet from the music stand were clearly marked on the floor with the aid of a measuring tape. The distance to the ground from the participant's chin in centimeters was measured and recorded. One chocolate agar plate was placed vertically on the ledge of the desktop such that the open face was exposed. A small amount of eraser putty as an adhesive was used to secure the back of the Petri dish to the cardboard. The height of the music stand was adjusted so that the ledge where the Petri dish sat was aligned with the distance from the ground to the participant's chin (recorded previously). An iPhone with NIOSH app was placed next to the participant to help regulate Participant's voice amplitude (**Figure 6**). The lid of the dish was taken off as soon as the timer started. At the end of each experiment, the lid was put back on and the Petri dish removed from the cardboard. The top and bottom of the plate were clearly labeled with painter's tape specifying the type of activity, trial number, and distance increment. Then, the plate was tightly sealed with saran wrap and set aside. A new Petri dish was mounted using the same procedures for the next experiment.

Unless explicitly asked to do otherwise, whenever the experiment involved reading "The Garden" by Shel Silverstein aloud, the participant read the script at a "normal conversational pace" (approximately one reading per minute) and read it over again until the timer was up. The participant was not permitted to eat or drink during the experiment but was allowed to rest and drink water between trials. Experiments were conducted in the summer of 2020 over three non-consecutive days to give the participant time to freshen up.

After each day's experiments, all Petri dishes were stacked, taped together with painter's tape, sealed in Ziploc bags, and sent to the UC San Diego laboratory within 24

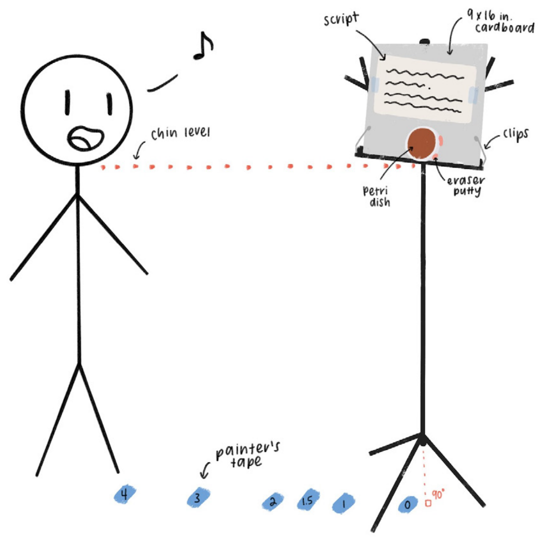

Figure 6. Experimental Apparatus. A 9x16 inch piece of cardboard is secured onto the desktop of the music stand using the built-in clips. A Petri dish is attached to the cardboard using eraser putty at the chin level of Participant. A print-out of "The Garden" by Shel Silverstein is clipped to the cardboard at Participant's eye level. Painter's tape marks the floor at regular intervals of one foot from zero to four feet and a separate mark at one and a half feet. Participant stands at various distances from the apparatus while performing the different types of activities.

hours for incubation at 37 degrees Celsius for 72 hours and subsequent disposal by autoclaving at an external facility. 10% Clorox wipes and water wipes were used to sanitize all surfaces in the immediate vicinity of the experiment at the end of the day.

Effects of Distance on Droplet Transmission of Bacteria

Five trials of each activity at distances of one to four feet were performed.

Positive Control: The participant read aloud "The Garden" by Shel Silverstein for three minutes at a self-regulated "normal" amplitude (60 dB measured with NIOSH) at zero feet from the mounted Petri dish.

Negative Control: The open Petri dish was left in the vacant room for three minutes.

Speak Normally: The participant read aloud "The Garden" for three minutes at a self-regulated "normal" amplitude (60 dB measured with NIOSH) at one foot from the mounted Petri dish. The experiment was repeated at two, three, and four feet from the music stand.

Speak Loudly: Participant read aloud "The Garden" for three minutes at a self-regulated "loud" amplitude (75 dB measured with NIOSH) at one foot from the mounted Petri dish. The experiment was repeated at two, three, and four feet from the music stand.

Sing: Participant sang aloud "Old MacDonald Had a Farm" for three minutes at a "normal singing voice" (around 60 dB) at one foot from the music stand. The experiment was repeated at two, three, and four feet from the music stand.

Cough: Participant simulated coughing at his own pace and without great effort. He was allowed to rest as needed between coughs during the three minutes. The experiment was repeated at two, three, and four feet from the music stand.

Effects of Mode of Vocalization on Droplet Transmission of Bacteria

Three trials of each activity were performed.

Speak Normally: The participant read aloud "The Garden" for three minutes at a self-regulated "normal" amplitude (60 dB measured with NIOSH) and at a "normal conversational pace" (approximately 60 seconds per reading), at one-and-half feet from the mounted Petri dish.

Speak Fast: The participant read aloud "The Garden" for three minutes at a self-regulated "normal" amplitude and at a "fast" pace (approximately 45 seconds per reading), at one-and-half feet from the mounted Petri dish.

Speak Slowly: The participant read aloud "The Garden" for three minutes at a self-regulated "normal" amplitude and at a "slow" pace (approximately 80 seconds per reading), at one-and-half feet from the mounted Petri dish.

Speak While Jogging: The participant read aloud "The Garden" for three minutes at a self-regulated "normal" amplitude while jogging in place at one-and-half feet from the mounted Petri dish.

Speak While Eating: The participant read aloud "The Garden" for three minutes at a self-regulated "normal" amplitude while eating cut fruit at one-and-half feet from the mounted Petri dish.

Speak With a Mask: The participant read aloud "The Garden" for three minutes at a self-regulated "normal" amplitude while wearing a disposable mask at one-and-half feet from the mounted Petri dish.

Speak While Looking Up: The music stand was adjusted to allow Participant to look up at a 40 degrees angle while reading aloud "The Garden" for three minutes at a self-regulated "normal" amplitude. The participant stood at one-and-half feet from the mounted Petri dish.

Speak While Looking Down: The music stand was adjusted to allow Participant to look down at a 40 degrees angle while reading aloud "The Garden" for three minutes at a self-regulated "normal" amplitude. The participant stood at one-and-half feet from the mounted Petri dish.

Blow Raspberries: The participant blew raspberries (making a sputtering noise by sticking out the tongue and blowing) continuously for three minutes at one-and-half feet from the mounted Petri dish. The experiment was repeated at three feet from the music stand.

Sneeze: The participant inhaled black pepper to induce one sneeze for each trial at three feet and at four feet from the mounted Petri dish.

Effects of Modification of Oral Bacteria Content with Food on Droplet Transmission of Bacteria

Three trials of each activity were performed. Before the start of each activity, the participant brushed his teeth, consumed a type of food, and waited for an hour before starting the experiment. Then, the participant read aloud "The Garden" for three minutes at a self-regulated "normal" amplitude at one foot from the mounted Petri dish. The different types of food tested were: a) regular breakfast, consisting of a bagel with cream cheese and oatmeal, b) a small plate of uncooked kimchi, c) one unpeeled apple, and d) one clove of raw garlic.

Statistical Analysis

All graphs were created and data analysis performed in Graphpad Prism 8.4.3. Mean and median values were calculated for each expiratory event. The results of paired t tests were analyzed, but due to the small sample size of one participant, the statistically significant t tests were removed.

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