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# Play That Funky Music: A Study of Bacterial Contamination of Wind Instruments

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# Play That Funky Music: A Study of Bacterial Contamination of Wind Instruments

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# Play That Funky Music: A Study of Bacterial Contamination of Wind Instruments

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**Abstract:**

Should there be standards to routinely clean instruments and what methods are available for that? It has been found that the bacteria and other microorganisms inside of musical instruments may be the culprit of respiratory infections in musicians (King et al. 2016). This project was divided into two experiments. First, clarinet reeds were introduced to three different species of bacteria. Then mouthpieces of trombonists and clarinetists of Eastern Kentucky University were swabbed and analyzed using a Colony Forming Unit assay. Bacteria was found to live on unused reeds for up to 30 days. Mouthpieces house millions of bacteria, determined by CFU assays. More evidence is needed to support the claim that differing methods of cleaning aid in bacteria removal. Standardization of instrument disinfection needs to be explored to improve the health of instruments and individuals.

**Keywords and Phrases:**

Microbes, *Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes*, Colony Forming Unit, Mouthpiece, Musical Instruments

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| Sample | Colonies | Dilution | Total CFU |
|---|---|---|---|
| CL1 | 0 | 0 | 0 |
| CL2 | 35 | 10 | 350000000000 |
| CL3 | 106 | 6 | 106000000 |
| CL4 | 135 | 9 | 135000000000 |
| CL5 | 94 | 7 | 940000000 |
| CL6 | 0 | 0 | 0 |
| CL7 | 65 | 5 | 6500000 |
| CL8 | 0 | 0 | 0 |
| CL9 | 15 | 6 | 15000000 |
| CL10 | 3 | 7 | 30000000 |
| CL11 | 0 | 0 | 0 |
| CL12 | 0 | 0 | 0 |



| Sample | Colonies | Dilution | Total CFU |
|---|---|---|---|
| TBN 1 | 0 | 0 | 0 |
| TBN 2 | 0 | 0 | 0 |
| TBN 3 | 0 | 0 | 0 |
| TBN 4 | 80 | 4 | 800000 |
| TBN 5 | 0 | 0 | 0 |
| TBN 6 | 61 | 4 | 610000 |
| TBN 7 | 0 | 0 | 0 |
| TBN 8 | 34 | 9 | 34000000000 |
| TBN 9 | 16 | 8 | 1600000000 |

Table 3: Summary of Clarinet Mouthpiece Treated with Dry Cloth Colony Forming Unit Assay

| Sample | Colonies | Dilution | Total CFU |
|---|---|---|---|
| CL1 | 0 | 0 | 0 |
| CL2 | 58 | 9 | 58000000000 |
| CL3 | 125 | 7 | 1250000000 |
| CL4 | 122 | 9 | 122000000000 |
| CL5 | 0 | 0 | 0 |
| CL6 | 50 | 2 | 5000 |
| CL7 | 0 | 0 | 0 |
| CL8 | 68 | 8 | 0 |
| CL9 | 0 | 0 | 0 |
| CL10 | 0 | 0 | 0 |
| CL11 | 171 | 7 | 1710000000 |
| CL12 | 0 | 0 | 0 |

Table 4: Summary of Trombone Mouthpiece Treated with Dry Cloth Colony Forming Unit Assay

| Sample | Colonies | Dilution | Total CFU |
|---|---|---|---|
| TBN 1 | 0 | 0 | 0 |
| TBN 2 | 194 | 5 | 19400000 |
| TBN 3 | 0 | 0 | 0 |
| TBN 4 | 167 | 4 | 1670000 |
| TBN 5 | 0 | 0 | 0 |
| TBN 6 | 102 | 2 | 10200 |
| TBN 7 | 0 | 0 | 0 |
| TBN 8 | 143 | 6 | 143000000 |
| TBN 9 | 76 | 3 | 76000 |

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## Introduction:

### *Music and Man:*

Correlation between upper respiratory disorders and playing a wind instrument has been observed in different settings around the world. Ailments such as acute illnesses, bronchitis, and chronic respiratory diseases such as asthma have been detected more frequent in the individuals who play instruments as a hobby or as a profession. Though there is no one reason why this correlation exists, many researchers believe it may be attributed to the repetitive exposure to respiratory irritants from the instrument (Thibaud et al. 2019). These irritants include bacteria, dust, and fungi. Bacteria are found in astonishing amounts throughout all parts of the instrument, so much that it warrants routine disinfection of the whole instrument (Glass et al, 2010). It is imperative for musicians to know what irritants they may potentially be exposing themselves to. Wind instrumentalists depend on the health of their lungs to play their instrument; weakened lungs can negatively impact the sound produced by

the instrument. The results of a study done on Turkish military members supports the potential exposure to respiratory irritants can decrease lung function (Deniz et al. 2006). Nonsmoker military musicians and nonsmoker military members who were not in the military band were compared. Military members who did not play instruments had an increased lung volume when compared to their musical counterparts (Deniz et al. 2006). Even though the results of the military study may raise concern to wind instrumentalists, simply routine cleaning of the instrument could prevent the exposure to these irritants.

Bacteria can both be beneficial and harmful to the human body. Microorganisms live all throughout our body. They reside on the surface of our skin and aid in the digestion of food in our digestive system. Symbiotic bacteria help keep our internal environment constant, whereas an imbalance of bacteria causes infection (Thibaud et al. 2019). Not all bacteria can help humans though, bacteria such as the *Streptococcus* species are responsible for diseases such as strep throat and certain types of pneumonia. A mutated variant of *Staphylococcus aureus* (*S. aureus*) is responsible for methicillin-resistant infections of the skin, but not all *S. aureus* are methicillin resistant. In extreme cases, untreated infections of the mutated type of bacteria can lead to life threatening situations such as sepsis, which is the invasion of bacteria in the blood. All these microorganisms exist all around us; on the palms of your hands, on the desk your computer is on, and internal surfaces of musical instruments.

Respiratory infections are something that most people have experienced from catching the influenza virus to having a sinus infection, some of these infections can cause greater issues and can lead to hospitalizations. Though all people can get sick, there has been a rise in recorded cases of Hypersensitivity Pneumonitis (HP) among instrumentalists (Banzhoff et al. 2017). HP is an inflammation of the lungs and bronchi, caused by repeat exposure to inhaled irritants (Davidson et al. 2019). HP, also known as “Bagpipe Lung,” has unfortunately claimed the life of a 61-year old instrumentalist, where the mold and other microorganisms existing in his bagpipe led to his infection and untimely death in 2016 (Murphy, 2016). Similarly, cases of HP have been documented from other instruments as well. In a case study reviewing HP, two patients who performed on two different instruments were studied. A trombonist and bassoonist were both hospitalized for HP infections (Møller et al. 2016). Both individuals had MRIs that showed inflammation and excess fluid in the lungs with seemingly no explanation. The trombone and the bassoon were both swabbed to identify if there were any possible pathogens or microbes that could cause irritation. Both cases had fungi and atypical mycobacterium cultured from the instrument, which lead to the diagnosis of HP. Professionals in the field believe that HP is underdiagnosed among musicians. Due to the similarities the symptoms have with other respiratory illnesses (Møller, 2016).

Alongside the risk of HP, there is a correlation between the development of asthma and performing with a wind instrument (Okoshi et al. 2017). It was found that musicians who had not been diagnosed with asthma prior to playing an

instrument were more likely to develop the condition at some point in their lives (Okoshi et al. 2017). Though this study did ignore factors such as environment and other lifestyle choices, this correlation does lead to a question of if neglected instruments could be the cause of asthma in musicians. The combination of patterns of illness in musicians opens the door for further research into microbiome of wind instruments.

Certain instruments can also harbor more bacteria than others. In a 1969 study, it was recorded that woodwind reeds alone harbor countless pathogenic bacteria (Bryan, 1969). In the same study it was found that small woodwind mouthpieces contained uncountable amounts of bacteria and that brass instruments were the cleanest of wind instruments, though they still harbored millions of pathogenic bacteria. Bryan did look at different sanitary measures for instruments; however, most of the recorded procedures took about 20-30 minutes to complete. In a classroom setting, disinfecting mouthpieces using Bryan's chemical method would be near impossible as teachers need all the time they can get for rehearsals and taking half an hour out of a rehearsal would not be feasible for disinfecting instruments. The methods discussed in Bryan's study did discuss instrument-safe compounds for disinfecting such as soap and water, rubbing alcohol and Lysol being amount the few tested (1969). In a more recent study, trumpet mouthpieces were successfully disinfected using a steaming apparatus (Moore & Millar, 2020). There is great potential for disinfecting mouthpieces and expelling millions of pathogens from potentially harming

musicians, but there is nothing in place to help guide these individuals in successfully cleaning their instruments.

Most instrument maintenance is taught anecdotally from private instructor to student. Because instrument maintenance is passed down from teacher to student, it allows for variation in the methods and frequency in which an instrument may be cleaned. It also means that students who are unable to have a private instructor due to cost or proximity of a teacher fall between the cracks as it is not guaranteed that their music teacher is an expert in the maintenance of their specific instrument. A study in one school identified that having a pressure steamer in the classroom helped disinfect trumpet mouthpieces after rehearsal (Moore & Millar, 2020). Though using the pressure steamer was successful, it must be considered that this may not be a viable option for all schools. Public schools vary in funding across states and even within cities themselves. Finding the most efficient way to clean wind instruments safely for both the musician and the instrument is still being investigated.

***How Music is Made:***

In wind instruments, sound is made using controlled air that is blown into a mouthpiece. For different types of instruments, the mouthpiece type changes the way the air is vibrated through the instrument, which ultimately produces sound. There are two major classes of wind instruments woodwind and brass wind. Each family of instruments is divided by the manner the sound is produced. Both

divisions of the wind instrument family share one trait in common though, they use air that the musician breathes to produce sound. Just as in regular respiration, the air that is used for wind instruments is taken from the atmosphere. The air is then blown through the horn using a combination of pressure, volume, and aperture of the lips to create sound in the horn.

In general, woodwind instruments use a reed to create a vibration in the air that goes through the instrument. This is what produces the airy, light sound of the woodwind family. This family includes instruments such as the clarinet, saxophone, oboe, and bassoon. Woodwind instruments can be further divided into two subsects, single reed, and double reed instruments. Reeds are generally crafted from sugar cane. In single reed instruments, the reed is ligated to a wooden or plastic mouthpiece. The reed-mouthpiece combination is then inserted into the mouth. The air that is blown into the thin aperture between the reed and mouthpiece is what causes a vibration which produces sound. Instruments that use only a single reed in conjunction with a mouthpiece are the clarinet and saxophone.

Double reeded instruments have two pieces of sugar cane reed that are bound together by wire and resin. The two reeds create a flexible opening which is also placed in the mouth and blown into. Unlike single reed instruments, these instruments do not have a sperate mouthpiece that the reed is bound to. Examples of double reed instruments include the oboe, bassoon, the English horn. Reeds are subjected to much wear in its lifetime and a woodwind

instrumentalist can go through thousands of cane reeds over the course of a professional career.

In the experiment conducted for this paper; only single reed instruments were studied. This is due to the availability and cost of the reed. Single reeds are commercially produced and sold either as individual reeds or in boxes of 10, making them easy to find in an average music store. Double reeds are handmade and only sold individually costing at an average of 30.00$ United States Dollar as a minimum. Specifically, Rico brand 3.0mm thickness B-flat Clarinet reeds were used due to the low cost and the widespread availability of them in the classroom setting.

Brass-wind, more commonly referred to as brass instruments, use only a mouthpiece to generate sound. The lips of the mouth are set in a closed position where the corners of the mouth are pulled shut while leaving the center of the lips loose. Air is blown through an aperture created in the mouth, which causes a vibration of the lips. The mouthpiece is set over top of this aperture. It is what catches the vibrating air and then amplifies it through the rest of the instrument. There are no wooden reeds in brass instruments, only the cup-shaped mouthpiece is used for sound production. The differing sizes of the mouthpiece and instrument is what causes the difference in sound in this diverse family. Brass instruments include the trumpet, trombone, French horn, and tuba. Unlike reeds, there is typically no need to have multiple mouthpieces. Mouthpieces in this family are made from silver or steel and are very durable to wear and tear.

It is important to define the types of instruments and the ways the sound is produced by them because it is dependent on the air that comes from the musician playing them. Before the air even enters the instrument, it must be breathed in and then blown back out through the instrument. These respiratory cavities are warm and moist with mucous, where both beneficial and harmful bacteria reside. The mouths however, contain more than just mucous and bacteria. It is where the first stage of digestion of food begins, as it is where all food and drink enter the body. This means that everything ranging from the sandwich and soda that you may have had for lunch and whatever bacteria your respiratory tract is housing is expelled through the instrument when played. The reeds and mouthpieces are in direct contact with the body and can be the perfect opportunity for harmful bacteria to attack.

***Pathogens Carried by Wind Instruments:***

As aforementioned, not all microorganisms found in instruments will cause disease in humans (Murphy, 2016). However, there is evidence of disease-causing microorganisms and respiratory irritants that may be responsible for illness in wind musicians (Murphy, 2016). Trombone Lung, another name for Hypersensitivity Pneumonitis (HP) is a suspected underdiagnosed disease that is caused by inhaled fungal and bacterial antigens (Moller et al. 2017). Antigens are molecules that are on the outside of bacteria cells. These molecules interact with human immune cells and are what causes irritation amongst other immune responses (Murphy, 2016). The immune system response is what causes us to

be sick. Up to 18 unique species of bacteria living inside brass-wind instruments (Bridges, 2005). Of these, some of the more recognizable disease-causing bacteria are *Tuberculosis mycobacterium*, the pathogenic agent for tuberculosis, and *Escherichia coli*, a foodborne agent known for causing violent vomiting and diarrhea (Bridges, 2005). Of the 18 species mentioned also includes the species *Streptococcus pyogenes* (*S. pyogenes*) and *Streptococcus pneumoniae* (*S. pneumoniae*), both of which can cause bacterial infections of the upper respiratory tract (Bridges, 2005). Both *S. pyogenes* and *S. pneumoniae* were reported to live on clarinet reeds for at least 24 hours (Marshall & Levy, 2011). *Staphylococcus aureus* (*S. aureus*) was also identified, the superbug antibiotic resistant variant of this species is commonly responsible for Methicillin-Resistant *S. aureus* infection (MRSA). This bacterium lives on our skin naturally and infects open wounds on the skin. If any of these bacteria were to infect the bloodstream, it would cause sepsis, an entire body infection that is responsible for deaths across the globe. *S. aureus* has been found to live for at least two days on clarinet reeds and even longer within the clarinet body itself (Marshall & Levy, 2011).

***Laboratory Analysis of Bacteria:***

The three bacteria that were chosen for the first part of this study were *S. pyogenes*, *S. pneumoniae* and *S. aureus.* There are many reasons why these three were picked. These three species of microorganisms are the most identified in literature that investigate what species of bacteria live in wind instruments.

Alongside the current research backing this topic, these three species were readily available for use in the laboratory at Eastern Kentucky University. For these species, there are also selective and differential medias that are cost effective and are readily available for use in the laboratory.

Differential media is a selective growth material that causes a physical change in the material of the media in the presence of a certain bacteria. This media changes for different species of bacteria as each species is unique and has different biochemical qualities whenever it reacts with different media. Selective media are used to grow a certain species of bacteria while barring the growth of others. The selective material that was used for *S. aureus* was Mannitol Salt Broth. The differential material that was used for *S. pyogenes* and *S. pneumoniae* species was 5% Sheep Blood Agar.

Mannitol Salt Broth is a selective media for *S. aureus*. It is a liquid media in which bacteria are suspended, the bacteria use the contents of the broth to carry out metabolic functions and proliferate. The media contains beef extract which acts as a nutrient source for the bacteria (Sigma-Aldrich, 2023). Mannitol is a carbohydrate source that this species of bacterium can ferment. As *S. aureus* ferments mannitol, it releases an acid byproduct. This is a trait that is specific to this species of bacteria, and Mannitol Salt Broth is a commonly used selective growth media for pathogenic species of *Staphylococci* (Sigma-Aldrich, 2023). As the acid is released into the broth, it interacts with a color changing reagent called Phenol Red. This is the pH indicator in Mannitol Salt Broth, and it changes from red to yellow whenever the pH drops below 6.8 (Sigma-Aldrich, 2023). The

acid byproduct that is released by *S. aureus* drops the pH of the broth below 6.8, which causes the Phenol Red to become a yellow hue. This color change indicates the selective presence of *S. aureus*. Mannitol Salt Broth is easy to make in the lab and is cost effective because only a small amount of the base product can make a liter of broth.

For both *Streptococci* species, the differential 5% Sheep Blood Agar was used. 5% Sheep Blood Agar is a gelatinous material that is poured into petri dishes. This semisolid agar contains nutrients for the bacteria to live and divide. Both species of *Streptococci* can breakdown erythrocytes, which are red blood cells. This is a process known as hemolysis. There are also different extents of hemolysis, as it can be partial breakdown of cells or complete breakdown of cells. In α-hemolysis the red blood cells that the bacteria are growing directly on top of are partially lysed or broken down. This causes the blood agar to change color from bright red to a yellow brown tone. Complete breakdown of erythrocytes directly underneath and surrounding the colonies of bacteria is known as β-hemolysis. A third type of hemolysis exists and is what is referred to as γ-hemolysis. Deceptive to the name, γ-hemolysis is when no breakdown of blood cells occurs at all. The colonies do not change the color of the media beneath or surrounding them. In this experiment γ-hemolysis was not utilized as a defining test, but α-hemolysis and β-hemolysis were. This is because in the presence of 5% Sheep Blood Agar, *S. pyogenes* and *S. pneumoniae* are β-hemolytic and α-hemolytic respectively.

A second method used in this experiment for analyzing bacteria living inside of instruments was a Colony Forming Unit Assay. This is a common method used for quantifying the number of bacteria present in a sample (Christen & Parker, 2020). Quantifying the bacteria is achieved through a series of small volume dilutions within an aqueous buffer. Starting from a solution containing a sample of bacteria, a set volume is placed into a microcentrifuge tube containing a phosphate buffer solution (PBS). Each time a volume of sample is mixed and then placed into a new container of PBS dilutions (Christen & Parker, 2020). The series of dilutions enables us to quantify bacteria in a sample. Dilutions of PBS are then plated onto tryptic soy agar (TSA) and incubated, this allows us to be able to visually distinguish colonies.

Typically, in lower number dilutions, bacteria are still too great in quantity to distinguish individual colonies from each other. However, as the dilutions increase the number of bacteria colonies decreases, making an inverse relationship between the number of titer and number of bacteria. Small individual “dots” of bacteria appear on the plates. Each is referred to as a Colony Forming Unit (CFU). Physically, this looks like a small round spec on a petri dish. The colony can arise from a single bacterium or a cluster (Christen & Parker, 2020). When there are large masses of bacteria colonies with no distinguishable boundaries, this is known as “too many to count” (TMTC). On the opposite side of the spectrum, when there is no presence of CFUs have no microorganisms (Christen & Parker, 2020).

Calculation of the estimated number of bacteria in CFUs comes from the first dilution where CFUs are countable. This provides a more accurate estimation of bacteria in a sample as it is the fine line between too many bacteria and as we begin to spread the bacteria further and further apart. By convention, this is how microbiologists quantify bacteria (Christen & Parker, 2020). The number of bacteria is calculated by the following equation:

$$Total = CFU_T \times 10^T$$

Where *Total* refers to the total number of bacteria present in the sample, *T* being the number of the first countable dilution and $CFU_T$ refers to the CFU of the first countable titer. In this instance, the number of bacteria that reside inside trombone and clarinet mouthpieces was realized. Based on literature available, a Colony Forming Unit Assay has not been done on any musical instruments.

## Materials and Methodology

This experiment was divided into two halves. The first half of this experiment was directed to look at controlled bacteria growth on clarinet reeds. The second half was to assess whether physically disturbing bacteria within trombone and clarinet mouthpieces with a dry cloth would help eliminate bacteria.

### *Clarinet Reed Assay*

To prepare for the reed assay, the clarinet reeds and media had to be prepared. Rico brand B-flat clarinet reeds (n=35, 3.0 thickness) were halved longitudinally using a scalpel. After being halved, they were placed in a 500mL beaker and sealed with aluminum foil. The reed halves were then sterilized in an autoclave. Tryptic Soy Broth (TSB) was made and sterilized in the autoclave. 10mL of TSB was measured and transferred into 12 large test tubes. Colonies of *S. aureus*, *S. pyogenes*, and *S. pneumoniae* were cultured in Tryptic Soy Broth (TSB). Colonies were shaken in a rocker and incubated overnight at 37℃. Test tubes (n=4) were labeled for each of the species. Bacteria (1 mL) from the master colonies were placed in respective labeled TSB tubes. Tubes were shaken in a rocker and incubated overnight at 37℃. Mannitol salt broth (MSB, 10 mL) was placed in 40 large test tubes and then sterilized with the autoclave. After sterilization, the tubes of MSB were placed in the refrigerator. 40 plates of 5% Sheep Blood Agar were obtained and kept in the refrigerator until use.

Empty, sterile, Petri dishes were labeled with the species of bacteria assigned to the reed slice and number of days the reed would be left to dry ranging from 0 to 30 days. 3 sterilized reed halves were placed in labeled Petri dishes using ethanol sterilized tweezers to prevent contamination. On day 0, all reeds were introduced to 10μL of TSA containing indicated bacteria using a micropipette. After being introduced to the bacteria, Petri dishes were closed and left on the lab bench in room temperature conditions.

After the desired length of time had passed, reeds were removed from their Petri dishes and placed in confirmation media to determine presence or absence of bacteria. Reeds that had been introduced to *S. aureus* were placed in the test tubes with MSB using ethanol sterilized tweezers. After the reeds were secured in the MSB test tubes, they were sealed shut using tape, labeled with time the reed dried, then shaken and incubated overnight at 37℃. After reeds were incubated, a change in MSB color and turbidity was recorded. Reeds that were introduced to *S. pyogenes* or *S. pneumoniae* were first placed directly on TSA and were left to incubate at 37°C for 48 hours. The bacteria from that TSA plates with the reeds were then transferred to 5% Sheep Blood Agar and left to incubate at 37°C for 48 hours. The color change of agar was noted for both species were recorded.

### *Mouthpiece Assay*

For the CFU assay, Expedited Institutional Review Board approval for human subjects' non-clinical trial was obtained on September 28th, 2022. Volunteers were sought from the Eastern Kentucky University trombone and clarinet studios. Informed consent packets were handed out to those who were interested, signatures were not obtained from volunteers due to approved exceptions from signed informed consent. Clarinet students (n=13) and trombone students (n=9) volunteered after reviewing the informed consent packet. Swab samples of the inside of the respective mouthpieces were taken weekly from the volunteers after their studio rehearsals in the Foster Music Building at Eastern Kentucky University. Different treatments were issued each week: no treatment (control) and a dry cheese cloth. Swabs were taken after the intervention was administered; volunteers were instructed to use dry cloth to run the cloth through the mouthpiece three times. Sterile swabs were placed inside at beginning of the shank of the mouthpiece. The swab was circled around the internal diameter of the shank of the mouthpiece for 15 seconds. Samples were taken to the New Science Building at Eastern Kentucky University for further analysis.

Once the samples were collected and transported to the New Science Building, swabs were stirred in TSB tubes and incubated at 37℃ overnight. Tubes were labeled with mouthpiece types (clarinet or trombone), number and type of intervention. During the incubation period, the CFU analysis was set up. Phosphate Buffer Solution Stock (PBS) was diluted and sterilized. 900µL of

solution was measured and added to microcentrifuge tubes, nine tubes were created per sample. TSA plates were labeled and divided into thirds using a permanent marker. Labels included identification of the sample and the quantity of dilution in each third. Each sample received 3 TSA plates labeled with dilutions $10^{-2}$ to $10^{-10}$. After the incubation period was over, 100µL of TSA solution was added into the first microcentrifuge tube of PBS. 100µL of solution from the PBS tubes were diluted across the remaining tubes to complete the dilution. After the TSA was diluted across the PBS solutions, 5 10µL drops of each PBS dilution were placed on the designated region of the labeled TSA plates. TSA plates were incubated at 37℃ for 24 hours. Whenever the plates were finished incubating, singular colonies were identified and counted on the plates. If colonies were too great in number (200 or more) or indistinguishable from each other, they were labeled as too many to count (TMTC). Data was recorded and analyzed using Excel.

**Results:**

All reeds for days 0, 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 were positive for growth of *S. aureus* in MSB. All reeds for days 0, 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 were positive for growth of *S. pneumoniae* on 5% Sheep Blood Agar. All reeds for days 0, 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 were positive for growth of *S. pyogenes* on 5% Sheep Blood Agar.

For the control sample of clarinet mouthpieces, five of the samples were TMTC throughout all dilutions (Figure 1). Mean CFU of the clarinet control was 40,508,125,000 with a standard deviation of $1.04896 \times 10^{11}$. Control sample of trombone mouthpieces had five TMTC samples throughout all dilutions (Figure 2). Mean CFU of the trombone control was 3,955,712,222 with a standard deviation of 11,279,024,051. The dry cloth clarinet sample was inconclusive. The dry cloth trombone sample had four samples with TMTC throughout all dilutions. Dry cloth intervention of the trombone mouthpieces had a mean of 18,239,577.78 and a standard deviation of 47,214,953.94.

## Discussion

### *Clarinet Reed Assay*

From the results, it suggested that *S. aureus, S. pneumoniae, S. pyogenes* can live on a Rico B-flat Clarinet Cane Reed for at least 30 days. In the context of band rooms across the world, this means that students and musicians are placing bacteria containing devices in their mouth daily for practice and performance. Though all sterilization procedures were followed while working with the reeds, any amount of unsanitary contact with laboratory instruments used to transport the reeds could have caused an accidental contamination, which may have skewed the results and causing positive results unintentionally.

Though the fact of bacteria remaining on reeds for a month at a time may be alarming, it must be kept in mind that not all bacteria are pathogenic. This portion of the experiment did not give insight to the number of bacteria that could exist at one time on a reed. This is important because to cause a pathology in an individual, the bacteria must replicate in the body to a point that overwhelms the body's immune system. If the bacterium is unable to do this, it cannot cause an illness in the person. However, knowing what is going into your body as an instrument is played is important information. Reeds are currently unable to be conventionally sterilized in school and work environments. At most, they can be wiped off with a hand or dry cloth to rid of moisture at the end of use. From there, they are put into a plastic reed guard and then placed into the instrument case.

The reed is a delicate part of the instrument, it can be damaged by the slightest amount of force and can be affected by environmental changes.

One limitation in this part of the experiment is that it did not look at other variations of reeds. Only 3.0mm B-flat clarinet reeds were studied but there are many varieties of reeds for both B-flat clarinets, bass clarinets, and all types of saxophones that range in pitch that use a single reed. Thickness and length could be a factor that contributes to the survival of microorganisms on the reed. This was not determined in this experiment and leaves a gap in the research that other scientists could build on. Another gap that was left behind in this experiment is the double reed instruments such as the oboe, English horn and bassoon. The reason why these instruments were left out of this experiment is because the double reeds necessary for them are too expensive and hard to obtain. Most double reeds are handmade and are used until they are unable to produce sound. Spending 20 to 30 dollars on a reed just to place bacteria on it to see how long it would remain on the reed was not cost-effective for this experiment. Further research would need to be conducted to see if there is a difference in the length of time bacteria can exist on double reeds.

Though this information may be the start of great concern for instrumentalists' health, there is not enough knowledge about this topic to start giving warning against playing instruments. Not all parts of the instruments contain pathogenic bacteria. All wind instruments produce condensate due to the nature of the warm air being pushed through a cool tube of wood or metal. During the COVID-19 pandemic, there was fear that the virus would spread through this condensate

that would leak from the water keys and bells of instruments. However, two studies from years prior helped suggest hat this was not an issue as both woodwinds and brass-winds condensate do not carry pathogenic bacteria (Moberly & Bridges, 2015) (Moberly &Bridges, 2016). In fact, there are physiological benefits that can come from playing an instrument. Students who were diagnosed with asthma prior to learning an instrument experienced improvement in their respiratory cycles after learning to play an instrument (Lucia, 1994). By improving their respiratory cycle, students found that it was easier to breathe after practicing wind instruments. What can be suggested at this point with what has been observed is that more research must be done to determine the true amount of time it takes for bacteria to expire on reeds. Other types of reeds that could be potentially studied include long-lasting plastic reeds. From comparing cane reeds to plastic, it could determine the safest type of reed to use, but it could raise the question of whether a plastic reed would impact the quality of sound produced by the instrument.

### *Mouthpiece Assay*

The dry cloth clarinet sample data had been deemed inconclusive. This was due to most samples being TMTC or with very high CFU dilution apart from one mouthpiece. Clarinet six of the dry cloth assay had a CFU dilution of 0, meaning that there were countable colonies from the first dilution (Table 3). Due to the variation of data collected in this sample, this assay was deemed inconclusive.

When the control samples of clarinet and trombone are compared, the trombone mouthpieces have less CFU than the clarinets (Table 1, Table 2). This means that there are less bacteria inside of the trombone mouthpieces. The smaller number of bacteria could be due to the difference in the material the mouthpieces are made from. Clarinet mouthpieces in this trial had a variety of different materials. The mouthpieces are crafted from plastic and wood whereas the trombone mouthpieces are made from silver. Silver inherently has antimicrobial properties, which most likely is the reason why the trombone mouthpieces had less bacteria inside of them. Bacteria can thrive on different materials for varying times. Considering the variation in material for the clarinet mouthpieces, this could be the reason why there was more bacteria in comparison to the control mouthpieces.

Due to the inconclusive dry cloth CFU for the clarinet mouthpieces, a comparison between the control sample and the experimental sample cannot be confidently made. However, this was not the case for the trombone samples. There was a difference between the average number of bacteria between the control and experimental (dry cloth) samples. The average CFU of the experimental sample was lower than that of the control. The difference between the control and experimental sample supports that even simply disturbing the bacteria and removing moisture from the mouthpiece can displace the number of bacteria that exist inside the mouthpiece. Knowing this, further research including different types of interventions is needed to determine the best kind of disinfectant for a mouthpiece.

Limitations of this CFU assay include those of machine and human error. During the time this portion of this experiment was being carried out, the autoclave in the New Science Building at Eastern Kentucky University broke down midway during data collection. PBS was sterilized using the autoclave, but whenever this machine was out of order, the sterilization method had to change. PBS was then hand-sterilized through a double filter syringe. This is not the most effective method of sterilization of the PBS but was the only option at the time. This may have led to contamination of dilutions, which may have caused false TMTC dilutions. Another limitation of CFU assay is that all counting of the CFUs was done by the naked eye. Between individuals, what may look like a colony to one person may not to another. This can lead to variation of CFU counts from person to person or even from day to day in a single individual. More replication of the experiment would lead to determination of the validity of using CFU assay as a quantitative measure for bacteria in mouthpieces.

In recent years, the consideration for the microbiome inside of musical instruments has come to the forefront. This is due to the number of cases of the underdiagnosed HP on the rise as well as musicians developing asthma later in life (Davidson et al. 2019) (Okoshi et al. 2017). Some researchers believe that this can be attributed to the harmful bacteria and other microorganisms that are inhaled through the instrument (Banzhoff et al. 2017). Multiple different species of pathogenic microbes have been documented inside musical instruments, some of which were explored in this work. These pathogens live all throughout the instrument, including in the condensate that the instruments produce. From this

literature, it is suggested that all parts of the instrument should be routinely sanitized for the wellbeing of the musician and the instrument.

Playing an instrument for leisure or professionally should not be discouraged after analyzing the results of this experiment. From this experiment it can be suggested that even the slightest amount of maintenance, such as removing moisture from inside the mouthpiece can alter the number of bacteria that live in them. What can be done now is to encourage the standardization of disinfecting methods for all instruments, as this can prevent the number of people falling ill to upper respiratory infections like HP or developing long term conditions such as asthma. For the musicians, it must be remembered that your health has the potential to be impacted by the internal environment of your instrument. Mouthpieces should be cleaned our at least dried out with a cloth at the end of rehearsals and practice to proactively remove potentially hazardous microbes from the horn.

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