"STOP" Study, Sanitize to Omit Pathogens

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By

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Abstract

We are currently living in the age of pandemic, as covid 19 has swept the globe and continues to be an issue to at-risk populations. We, as a society, have had to shift our focus from aggressive treatment of disease toward preventative measures. The objective of our research study was to evaluate the behavior of nursing students in 2019, which would go on to include the graduating classes of 2019 and 2020. Here at SUNY Brockport, nursing students are taught a recommended standard of cleaning their stethoscope with ethanol between every patient to prevent secondary infection from occurring through exposure caused by a contaminated stethoscope. In order to assess whether those measures were carefully followed, we sampled the stethoscopes of 117 students, and evaluated for bacterial growth on three separate solid media meant to encourage growth of distinct groups of bacteria. The identity of the isolated bacteria was also confirmed by staining and microscopic observation. Bacterial contamination was found at all four culture sites with the majority found on the earpieces, and the tubing. *Staphylococcus/Micrococcus* contamination was found most often on the diaphragm. Fecal contamination was also most commonly found on the diaphragm when compared to other portions of the stethoscope. Interestingly, the graduate students had the least amount of contamination while the accelerated students had the most.

Introduction

In 2019 the world watched as healthcare professionals took center stage in the fight against Covid 19, for context the present study was performed before the Covid 19 pandemic. Prior to these events, the general attitude toward secondary infections was in comparison

nonchalant. Healthcare providers were vaguely aware of their role in transmission of secondary infections, however the full extent of their direct contribution to the issue remained unknown. Previously to the pandemic the CDC had published its stance on what was to be recommended practices for how to sterilize hospital surfaces, these guidelines extended to medical equipment and stethoscopes which have been the symbol of medicine for decades. However, the stethoscopes could very well be responsible for the rapid spreading of Covid 19 along with other highly transmissible infections. As stated previously these guidelines were recommended but not always enforced, in the case of best practices professionals would be practicing the idealized behavior though this is not always the case.

The Microscopic Universe in Your Doctor's Office

The human body is full of microbes which are constantly being shed throughout the day. While it is normal to have these microorganisms living on or inside our body, it is important to realize that these organisms come with us everywhere. Anything we touch can cause the transfer of microorganisms; hence I will use this analogy. If you have ever worked with wet paint, you know that it is sticky and will coat anything it encounters. Now imagine that you are fingerpainting and there is bright blue paint all over your hands. You forget to wash your hands and you pick up an apple, now the apple has a blue handprint on it because you just touched it. The trouble is the more things you touch and the longer the paint has to dry then the less that paint will transfer to other things. However, microorganisms, specifically bacteria, have a short procreation time, creating a new cell as often as every 15 minutes. So, imagine now that you have an endless supply of paint dripping from your fingertips and you begin to touch things; the paint on your hands will begin to spread of its own fruition as soon as it has touched an object or surface. So now the entire world is covered in blue paint that endlessly flows and spreads, this is

how microbes work. Now the key here is that if the person had just washed their hands that would have broken the cycle of blue paint from spreading (we will get back to this point).

Different species of microorganisms, including bacteria, viruses and parasites, have evolved to live in different environments. For the purposes of this study, we will focus on bacteria. The human body has evolved alongside bacteria that inhabit it. Some of these bacteria can be beneficial to the body. Housing several different kinds of micro-environments, ranging from basic to acidic, dry to moist or mucosa producing, hot to cold. Depending on the environmental conditions, the organisms that inhabit that space will vary. For instance, the upper respiratory tract and oral cavity often house α-and β-hemolytic streptococci (Davis, 4th edition, 1996). The oral cavity and the upper respiratory tract have their own individual forms of regulation which prevent bacteria from growing despite constant exposure. The upper respiratory system produces mucus to flush out bacteria and while in the mouth salivary glands produce enzymes which breakdown food and kill potential pathogens.

While the gastrointestinal tract varies greatly, with reduced numbers of microbes in the stomach, where bacteria are introduced to the GI tract via the food we consume, these bacteria are then controlled by the acidic environment. However, the stomach harbors an abundance of *Helicobacter*, a genus of bacteria known to cause health conditions such as ulcers. In comparison the small intestines are flourishing with bacteria …

> "Further along the jejunum and into the ileum, bacterial populations begin to increase, and at the ileocecal junction they reach levels of 10^6 to 10^8 organisms/ml, with streptococci, lactobacilli, *Bacteroides*, and bifidobacteria predominating." (Davis, 4th edition, 1996)

Again, an imbalance of the microbiome can result in numerous conditions which result in malabsorption of nutrients. The large intestine has anywhere from 10^9 to 10^{11} bacteria/gram of fluid contents, most of them anaerobes (Davis, 4th edition, 1996).

While these locations are mostly internal, the body's first line of defense happens to be its largest organ, the skin. The human skin is a complex ecosystem which produces sweat via (sweat glands) and oil via sebaceous glands in conjunction to vellus hairs which act to maintain homeostasis within the body through lubrication, thermoregulation, and eliminating unwanted microbes including but not limited to bacteria, viruses, and parasites (Davis, 4th edition, 1996). Here Davis compares the variations of the human skin to different types of geography... "Skin regions have been compared to geographic regions of Earth: the desert of the forearm, the cool woods of the scalp, and the tropical forest of the armpit." (Davis, 4th edition, 1996). Human skin consists of 3 layers the epidermis dermis, and subcutaneous, when these layers are compromised through lesions, this leaves the body open to infection. Skin infections or irritation can also occur when the immune system is compromised, in the instance of overzealous cleansing, rashes or chafing. The general rule across the human body is that increased moisture and increased contact with surfaces increase the number of bacteria present. Common genera and species of bacteria found on the skin include but are not limited to, *Staphylococcus epidermidis, Staphylococcus aureus, Micrococcus*, diphtheroid or *Corynebacterium*, which are often found in areas containing acne and or have high numbers of sebaceous glands. In addition, the less common but still present are *Streptococcus* as well as several Gram-negative bacilli (Davis, 4th edition, 1996).

Secondary Infection:

Again, we will go back to the wet paint analogy, now that we have talked about some of the microfauna that lives in the human body and on the skin. These are the types of organisms, if

given the right environment, can grow and flourish (if it can survive it will survive), and spread. This became apparent during the covid-19 pandemic as the public became aware that the virus could survive on surfaces such as cloth, cardboard, countertops, and doorknobs. Though the virus' main form of transmission was through respiratory droplets, health care professionals were more worried than ever about secondary infections. Secondary infections can occur with any form of pathogenic substance. This happens when a patient already has a primary infection, so their immune system becomes weakened or overrun, paving the way for another microorganism to infect the body. The primary infection allows for smaller amounts of the secondary organism to take over and infect the body as the immune system is unable to fight off both organisms at once, normally an unburdened immune system would be able to fight back against such organisms, hence preventing infection.

Vehicles for secondary infection in a health care facility include commonly touched surfaces, hospital equipment, and clothing. The standard practice in most hospitals is to use bleach or disinfecting wipes in the case of large objects or surfaces which are frequently used. While other smaller objects that are numerous and easy to produce are disposable between each user, like rubber gloves for instance, masks, or other kinds of paper coverings that prevent direct contact. In the case of surgical tools, a combination of hot water, UV light, and cleaning chemicals are used to scrub the surface clean between instances of use. UV light and hot water are both highly effective ways of irradiating microbes as both methods act to break down cell membranes and heat denatures DNA. UV light is now being instituted in laboratory environments and in some hospital settings, however the wattage, wavelength and time of exposure are still being studied, along with safe levels radiation exposure in humans. While this

method is effective it is time consuming and cannot be practiced on frequently used equipment in a fast-paced environment when there is not enough equipment to go around.

Another common method of preventing unwanted bacterial growth is using materials which are antimicrobial, such as copper, or the use of fabrics that are tightly woven with higher thread counts to discourage microorganisms from sticking to cloths. Some studies are even exploring the properties of shark skin and insect wings for this reason (Hansen, Crawford & Ivanova, 2013). By far the best and full proof method when it comes to preventing transition of secondary infection from the individual remains vigorous hand washing and or the use of hand sanitizer when washing is not possible or when there is no visible contamination present. For the use of stethoscope disinfection ethanol wipes is the CDC's recommendation as these are accessible, effective, and disposable after usage.

Notice all the above recommendations fit within the category of preventative tactics for the expulsion of microorganisms. This is because health care has shifted gears as in recent years with the over-prescription of antibiotics and the misuse of medicine bacteria has evolved to become antibiotic resistant and scientists simply cannot keep up with the demand. This combined with higher rates of virus mutations as viruses have taken advantage of a globalized world. Hoping from person to person, from country to country, species to species these mutations become harder to anticipate and creating an effective vaccine can take months if not years. In the instance of both vaccines and antibiotics these methods while effective methods of intervention are often unpredictable when it comes to short- and long-term side effects. It is for this reason that approval of such methods is then delayed longer after completion because these products must be tested for safe human consumption and be government approved by organizations such as the FDA.

Objectives:

The standard practice taught to our nursing students by the faculty at SUNY Brockport was to clean the bell, diaphragm, tubing and earpieces of their personal stethoscopes between patients. This was the expected behavior which was demonstrated and reinforced by our faculty here at the college. The main objective of our study was to evaluate the usage of the idealized behavior that was taught to our students verses the actual behavior which was carried by the students when they were sent off on clinical rotations.

1. Are students consistently cleaning their stethoscopes between patients? In an effort to reduce transition of secondary infection.
2. If bacteria was present on our resulting samples at the end of the study, we must then go through the process of identifying the kinds of bacteria found.
3. To evaluate and compare the behavior between the classes of students studied. Is there a difference in amount and kind of bacteria present between juniors, seniors, graduate students and undergraduate students?
4. Is there any bacteria present that would indicate fecal contamination on the stethoscopes?

Materials and Methods

After conducting an initial literature review of related articles, it became clear that our next step was to get under way with our investigation by forming our research questions and objectives. Then we mapped out our experiment and sent in a proposal for IRB approval with The College at Brockport. Once approved we began gathering the proper materials for taking our samples. We began by gathering test tubes and making saline solution in the lab, this saline

solution was then measured out and put into each test tube using a pipet. Before gathering our samples, we made and then set aside. Three separate media designed to facilitate growth of a particular bacterial group based on dietary needs to mimic the environment which these microorganisms would naturally be found in. These mediums are as follows Mannitol-salt agar (MSA) which grows *Staphylococcus* and *Micrococcus*, brain heart infusion agar (BHI) which is for total contamination and eosin y-methylene blue agar (EMB) which grows fecal contamination.

Sampling

The test tubes were divided up into rows of four, four tubes for each potential student to be in that section of the nursing laboratory that day it is important to mention that the class populations were not mixed, each class consisted of only one student grade level for instance juniors and seniors were not in the same lab. This was important for keeping track of which group of stethoscopes belonged to which class while allowing individuals to remain anonymous. The test tubes were then capped to ensure that they remained sterile, for each anticipated student we had also made three agar plates one of each kind of medium. We then transported to the nursing building next store, where an attendant or nursing faculty member would read off the prewritten script, informing the members of that section's lab about the study, after which they could choose whether they wanted to participate.

Once those who had opted into the experiment were determined, their stethoscopes were collected and placed on a metal rack where they would be carted away into another room that was closed off and hidden from student view, designated for our study. Each stethoscope was given a number which was then written on the four tubes in the corresponding row identifying

that these samples all came from the same stethoscope we did not keep track of which stethoscope belonged to which student, so our results remained anonymous. Each column of tubes was labeled with a Letter indicating the corresponding portion of the stethoscope which had been swabbed. These letters were E for earpieces, T for tubing, B for Bell and D for diaphragm, wearing gloves we then swabbed each section of the stethoscope separately, placing each contaminated swab into the corresponding labeled tube filled with 5ml of saline solution, breaking off the wooden tip of the far end than replacing the cap of each tube.

After each participant's samples were collected, the stethoscopes were returned to the students and the samples were transported back over to the biology building next store for plate inoculation. This was done immediately in order to limit the waiting period between sampling and inoculation. Back in the microbiology lab the plates were labeled with a corresponding stethoscope number, 3 plates one of each medium for each stethoscope, each plate was then divided into 4 separate sections, the sections labeled with a corresponding letter indicating which section of the stethoscope the sample had come from. This meant that in the final sampling we had 3 mediums of growth for each portion of the stethoscope to facilitate growth of all types of bacterial matter. The plates were then stored in an incubator at 37° Celsius for two days to mimic normal human body temperature, after two days the plates were checked for colony growth. Plates that showed no colony growth in all 4 sections were then discarded and their numbers were recorded as a part of the results.

Streaking for Isolation

The plates that did show growth were set aside for further analysis. New plates were made for each one of the observed colonies, these new plates were the same medium as the

originals and were labeled with the stethoscope number and the quadrant letter for which each colony had come from and a small line at the edge of the plate to indicate a starting point. We inoculated the new plates using an inoculation loop which was sterilized under the flame of a Bunsen burner between each colony. First dipping the inoculation loop into a colony from the parent plate, inoculating the new plate, starting at the line marker we had made then turning the plate every few strokes until returning to the starting line. The inoculation loop was only dipped into the parent colony once when streaking for isolation. We then repeated this process for every single bacterial colony that had grown on the parent plates. This was done to ensure that one organism was isolated to each new daughter plate, so that when we conducted the gram stains to identify the species of bacterium present on each portion of the stethoscope, we would only be testing one species of bacterium at a time. Once this process was complete the daughter plates were placed in incubation at 37°C for 2 days.

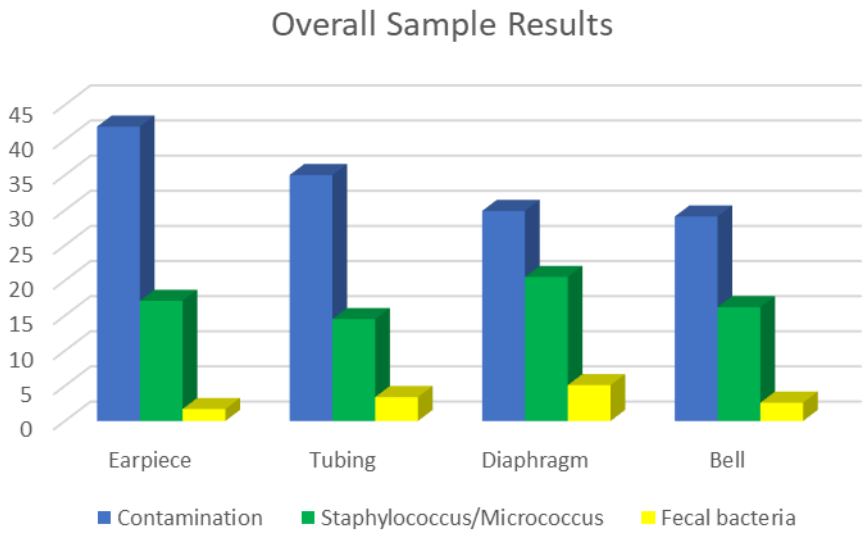
Gram Staining

The next portion of the experiment focused on the identification of the bacteria which had been grown on daughter plates following the streak for isolation. First, we made microscope slides ensuring to sterilize them though the flames of a Bunsen burner to kill any living cells. We then used the dyes crystal violet, and Safranin to gram stain the organisms on the slide. Gram positive bacterium would reflect purple while gram negative was red in color. The other evaluation technique we used was observation, as different species of bacterium are shaped differently. Cocci shows as circular under a microscope, Rods indicate Bacilli and spiral shapes indicate spirillums. It is important to note that each microscope slide was marked with sharpie to indicate which daughter plate the cells had come from. Any slides where the label was illegible or rubbed off were redone to prevent error.

Results

The results were evaluated in several ways, firstly by the presence or absence of bacteria then once each species was identified we documented which type of bacteria was found on each type of plate. Then we analyzed which section of the stethoscope the bacteria had been inoculated from diaphragm. Bell, tubing and earpieces. Next the results were calculated based on which group section the stethoscopes themselves had come from; undergraduates (juniors or seniors), graduates, or the accelerated program students.

Figure 1

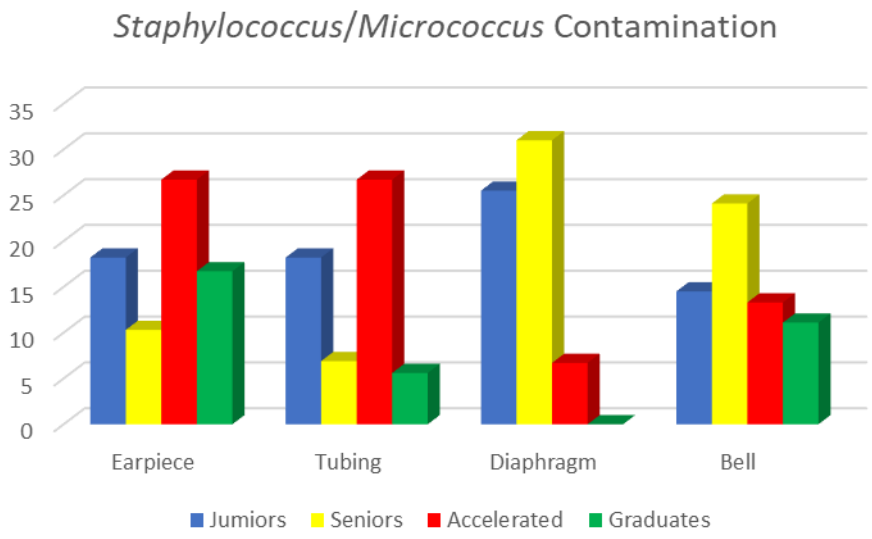


Note: This figure shows visual representation of contaminates on each section of the stethoscope which was evaluated. Overall contamination than *Staphylococcus/Micrococcus* and Fecal bacteria contamination.

In the first portion of the study plates that showed no colony growth were discarded the remaining plates were then evaluated based on the section of the microscope they had been found on and the species present were documented. On the diaphragm was found five different species of bacteria three different kinds of *Staphylococcus* including *hominis, hyicus,* and

carnosus subsp. utilis, Micrococcus cohnii and *Nosocomiicoccus ampullae* were also present. The bell was consistent with the findings on the diaphragm with the addition of *Staphylococcus intermedi*us. On both the tubing and the earpieces was found *Staphylococcus hominis, carnosus subsp. Utilis,* and *aureus* along with *Nosocomiicoccus ampullae* and *Kucvia varians* formerly known as *Micrococcus varians.* These findings are consistent with what we know about microbiome of the skin as S*taphylococcus and Micrococcus* are naturally found on the surface of human skin. These results are consistent with stethoscope to skin contact likely on the surface of the patient and then the device was not properly sanitized after exposure allowing for the transmission of the bacteria.

Figure 2

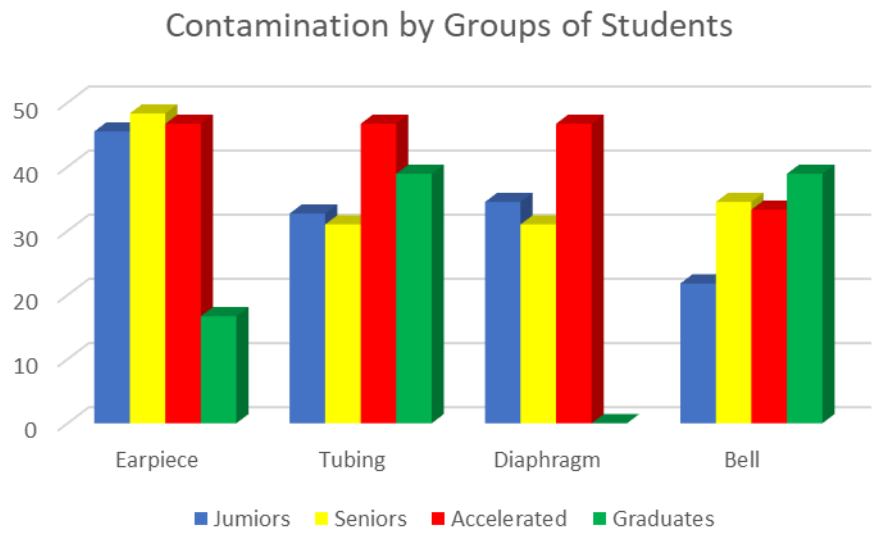


Note: This figure breaks down *Staphylococcus/Micrococcus* contamination via the different grade levels tested (each color represents a different grade level), then via the section of stethoscope.

Out of the 117 samples which were obtained and tested the stethoscopes belonged to 18 graduate students, 29 seniors, 55 juniors, and 15 students belonging to the accelerated program.

Overall, contamination was most often found on the earpieces with an occurrence rate of 41.9% of students. 35% of students had contamination present on the tubing and the bell and diaphragm were 29.1% (bell) and 29.9 % (on the diaphragm). Based on our data the earpieces were least likely to be sanitized while the bell and diaphragm were almost equal in their rate of contamination as they are most often cleaned together. This is equivalent to at least 60% of students following through with the sanitation behavior. However, it is impossible to tell if this behavior is carried out each time or if it had just happened close to a time when testing for contamination. *Staphylococcus* and *Micrococcus* were most often found on the diaphragm when compared to the other portions of the stethoscope which had been tested. Fecal contamination was also tested for and was found to be present on all areas of the stethoscope but never more than 5%. 6 individuals had fecal bacteria contamination on the diaphragm, 3 on the bell, 4 on the tubing, and 2 on the earpieces out of a total number of 117 students tested.

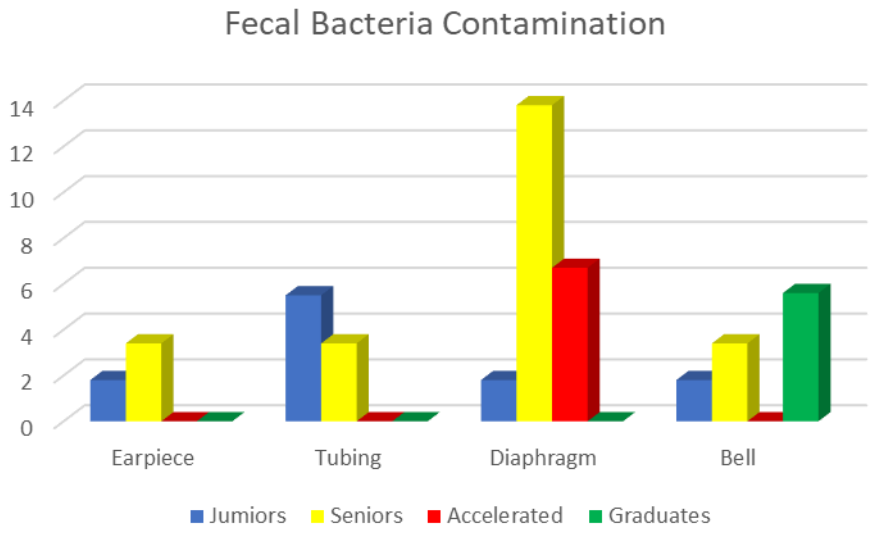
Figure 3



Note: This figure shows the overall contamination broken up via groups of students marked by different colors, then via the different parts of the stethoscope.

Contamination based on groups will be discussed not in percentages but based on the fractions of individuals who showed contamination. This is to prevent inconsistencies from occurring due to the total number of individuals in each group being varied, skewing the percentages. Meaning if one individual out of a smaller group shows contamination their percentage will be counted higher because the whole number is lower in comparison to another group with a larger total number. In the juniors the earpieces were the most contaminated region with approximately less than half of the specimens showing contamination (25/55), *Staphylococcus* was most often found on the diaphragm and fecal bacteria was only found in one sample for the diaphragm, the bell and the earpieces, then on 3 of the tubing samples. Like the juniors the seniors also showed the most contamination on the earpieces *Staphylococcus* was found on several specimens mostly on the bell and the diaphragm and fecal bacteria was found in 4/29 cases on the diaphragm, then only in one sample for each the bell, tubing, and earpieces. In the accelerated students we found almost half of them had contamination present, *Staphylococcus* was most often found on the earpieces and fecal bacteria was only found on one diaphragm sample. While the graduates had zero contamination on the diaphragm, a little less than half of the bell and tubing and only 3 specimens showed overall contamination on the earpieces. Gram positive bacterial contamination was found on the bell in 2 out of the 18 individuals, 1 on the tubing and 3 on the earpieces. In graduate students' fecal bacterial contamination was only found to be present once on a bell specimen.

Figure 4



Note: This figure shows overall fecal bacterial contamination broken up via grade and section of the stethoscope the contaminate was found present on.

Discussion

This study took place in Fall 2019 before the Covid 19 pandemic had hit the United States, at the time we had no way of knowing that our investigation into the transmission of secondary infection was about to become one of the main focuses of combating the virus. In fall of 2019 Covid was infiltrating hospitals and infecting those with weekend immune systems targeting vulnerable populations such as the elderly. Those who had mild symptoms became unknowing transmitters for the virus as many assumed they had come down with the common cold and continued their daily activities, therefore exposing more people to the disease. Health care professionals were avenues themselves for transition of the virus, this was proven as Covid 19 swept hospitals before it was discovered to be airborne. We know that the same phenomena occur with bacteria, for example in the instance of MRSA, when a patient is infected contact is

limited and those who enter the premises are required to wear full PPE gear which must then be removed before exiting the room to prevent the bacteria from spreading.

These two examples prove the vitality of the "STOP" Study as our study shows that even in the case of idealized behavior which has been taught to our nursing students that bacterial contamination of stethoscopes is still an issue. Potentially allowing for the transmission of pathogens between patients, then from patients to and from providers. As mentioned previously this study was conducted pre-pandemic, due to the focus over the last 3 years being put on protective measures being used by health care professionals and the public alike. It is imperative that this study be repeated to evaluate whether there has been a significant change in student behavior when data is compared from pre pandemic conditions to post pandemic.

Acknowledging Potential Error

This study took place over the course of three weeks with the help of the nursing department. Samples were collected in batches once the studying groups came into the nursing building for their weekly labs. The department has a hectic schedule with professors teaching classes to multiple grade levels, which all share lab spaces, each specially designed for a simulation purpose. We chose to conduct the study in the laboratory sessions that would have the least interruption to regularly scheduled class activities. This, however, created room for error as the study took place over a series of weeks, students became curious and though informed about the purpose of the study and told not to speak on the topic once their samples had been collected. There is no way of knowing if the students kept their word of not spreading around the department that a study was being done about stethoscopes. As one of the nursing students myself at the time the experimental data was collected my peers were particularly interested in

what I was doing and why I was in the building despite not having class on those days. The other students also became aware of me seemingly sneaking around the department and began to ask questions. There for to reduce potential error and the possibility of a change in behavior due to knowledge being spread about the study, in the future if the study was to be conducted again, I would recommend that all the samples be collected at one time and not over a series of weeks.

Another potential for error what was introduced into the 'STOP' study occurred while collecting samples. Once the IRB statement was read out to the students, those who opted in to participate in the study placed their stethoscopes on a metal rack which would then be wheeled into another room which was shielded from view. During this move I noticed that some of the stethoscopes would clang together as they were transported introducing the possibility of cross contamination. In the event that this study was to be repeated I would recommend a different mode of transport between collection and the lab where the samples were taken. Potentially introducing the use of plastic bins where stethoscopes could be placed for transport one stethoscope for each or perhaps the incorporation of rubber plastic dividers to be placed on the rack between stethoscopes to prevent them from touching.

Conclusion

In conclusion, the results of our experiment indicated contamination across the board, the location of the contaminate was not found to be statistically significant except for in the case of the earpieces which had the highest incidence of contamination overall. *Staphylococcus* and *Micrococcus* were the two most frequently encountered types of bacteria. These findings confirmed our hypothesis as they are organisms which are commonly found across the human skin. Most of the groups that were tested showed less than a 5% instance of fecal contamination

on the stethoscopes regardless of location. We found a correlation between the less time students were spending in the classroom with faculty and staff, the more likely they were to have contaminated stethoscopes. I would like to see a follow-up study done in the future to assess potential changes in behavior from before the pandemic to after the pandemic. As no change in behavior would be an indicator that further innovations need to be put in place to ensure our nursing students are protecting the health of their patients. SUNY Brockport prides itself on shaping the next generation of practicing health care professionals hence it is the school's responsibility to ensure our graduates are following proper procedure as it makes the difference between life and death.

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