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Abstract

The Gram-positive bacterium, *Staphylococcus aureus* can survive in indoor environments in the community, such as schools and homes, contributing to public health concerns related to human exposure and transmission. While convenient methods that do not require refrigeration or surface wetting have been described for identification of environmental *S. aureus*, these methods currently only provide a positive or negative result. Therefore, the goal of this project was to adapt and validate a dry collection method to provide quantification of *S. aureus* from indoor environmental samples comparing culture-based and culture-independent approaches, and then apply this method to environmental surface samples from local schools. For this project, *S. aureus* ATCC43300 was inoculated onto autoclaved Swiffer cloths. Then, *S. aureus* colonies were extracted from the cloths in 100ml of 1x solution phosphate buffered saline (PBS). The PBS extract was concentrated by vacuum filtration, and colony forming units (CFUs) enumerated on CHROMagar staph agar. *S. aureus* was successfully enumerated from experimentally-inoculated cloths. The findings from this work demonstrate that *S. aureus* can be recovered and quantified from dry cloth surface samples. This work also displays that the culture independent method was optimum for extraction efficiency and ease of use. This work highlights the importance of methodological development for *S. aureus* exposure assessment from indoor community environments.

Introduction

Staphylococcus aureus or *S. aureus* is a pathogen that contributes to about 500,000 clinical infections yearly (Tong, 2015). According to Tong (2015), S. *aureus* is a leading cause of bacteremia and infective endocarditis as well as osteoarticular, skin and soft tissue and pleuropulmonary infections (p. 603). According to the Centers for Disease Control (2016), about one in three (33%) people carry staph in their nose, usually without any illness, with two in 100 people carrying MRSA, its methicillin resistant form. Although *S. aureus* is a common pathogen, staph infections can become deadly once the bacteria enters the bloodstream or heart (Merck, 2017). In addition, *S aureus* has been linked to asthma exacerbations, making it a public health threat (Merck, 2017). Knowing that *S. aureus* is prevalent in many indoor environments such as schools and homes, it is best to understand the exact amount of bacteria present within the environment studied.

Currently, there are no published data that examine dry collection, or using autoclaved Swiffers (Swiffer™, Proctor & Gamble), to collect the Gram-positive microbe from indoor environmental surfaces. Furthermore, adaptions from medium, or wet collection based methods are normally used in health care institutions. However, constraints in the ability to refrigerate or transport can be limited in many areas where laboratory preservation is unavailable; therefore dry collection would be an optimum choice to collect microbes for experimentation. The current medium method is known to detect staph, but only in binary form (yes or no detection signal), which limits knowledge of the quantity of *S. aureus* within local schools or home environments, and which is important for public health practitioners studying its prevalence.

Even in scientific investigations, dry collection methodology can beneficial. Crime scenes can pose unique challenges: Items may be too hefty to be removed from the crime scene,

the items may not be accommodated in a controlled laboratory setting, or there may be some concern about loss of trace evidence during transport (Budowle et al., 2006). When the whole item cannot be recovered, an investigator can remove a portion of the item (this approach includes vacuuming, filtration, and/or water sample collection). An approach that is particularly useful for collecting trace materials is swabbing or wiping materials or surfaces with appropriate sample collection such as swabs.

This experiment examined laboratory quantification to allow an accurate representation of extraction of *S. aureus* from environmental samples. After studying dry and wet collection methods, the best method to extract the microbes from the environment will be discussed. Secondly, the experiment used laboratory methods to study the extraction efficiency from the culture-based and culture-independent methods to determine the most feasible method of *quantifying S. aureus*.

Methods

The experiment compared culture-based (dry collection) and culture-independent (swab) recovery of methicillin-resistant *S. aureus* (MRSA) from dry electrostatic cloths (Swiffer™, Proctor & Gamble), as illustrated in *Figure 1.* Whole, autoclaved dry electrostatic cloths were inoculated with a reference ATCC 43300 strain (MRSA), and concentrations were estimated from a 0.5 McFarland using serial dilution (see below). Identical 1µl aliquots of target dilution steps (1000, 1000, and 10 CFU) were inoculated onto both a Columbia CNA blood agar (control) and the dry cloth (experiment). The dry cloth was placed in a sterile stomacher bag, to which peptone was added (see below). Then, one aliquot of 500µl was preserved for qPCR analysis and a second aliquot of 1000ml was subjected to vacuum filtration to determine the extraction efficiency. Each experiment was repeated three times.

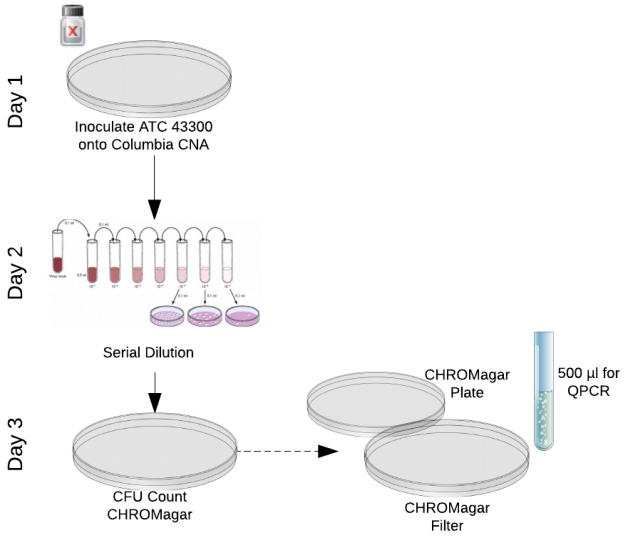

Figure 1. Illustrated protocol for inoculation of the ATC 43 300 strain of *S aureus.* Because a fresh strain of growth is needed, incubation time is at 24 h before inoculation to buffer can occur. Illustrated view of vacuum filtration is not depicted.

For serial dilutions, about 1µl of the incubated growth was placed inside of a standard test tube containing 2750µl of 0.1% Peptone buffer. Using a balanced colorimeter, the sample was diluted until it reached the standard 80% (red) percentile concentration advised by the McFarland scale. For this project, the calculated colony-forming unit (CFU) count of 1000, 100, or 10, serial dilutions are performed using Peptone buffer and pipetting into vials using 1800µl of 1% Peptone buffer and inoculating each dilution with 200µl of the previous dilution mixture. Before each dilution step, each test tube containing a sample was sealed and mixed for 15 seconds on speed 8 of the vortex machine. For PCR, 500 µl of each solution (10^3, 10^2, 10^1) was pipetted into an autoclaved vial. Once the dilutions were reached, the tissue collection hood was cleansed with bleach and ethyl alcohol. All instruments used were also cleansed with alcohol and placed back in their respective locations. The dilutions were placed under the closed hood until preparation of inoculation and testing was to be performed.

In steps where the procedure was to be used with a touched surface, sterility was achieved using sterile nitrile gloves and using the autoclaved cloths by wiping a touched surface within a 10x10 inch section for 30 seconds and then placed into a stomacher bag, which was sealed immediately with tape and labeled according to standard.

Vacuum Filtration

The filtration membrane system consists of one vacuum, filtration device, a glass filter, rubber stopper connection, a glass beaker containing 1000mL of boiling Milli-Q water, a 500 mL beaker containing 1% Peptone Buffer, a clamp for the housing system, tweezers, a lighter, ethanol, and .45um filters. *Figure 2* depicts the methods and table for the vacuum filtration protocol. 100 or 60 mLs of 0.1% of Peptone buffer was added to inoculated Swiffers in

stomacher bags, and the bag shaken gently for 1 minute. After shaking, the Swiffer was disposed into a proper medical waste container, while continuously squeezing out as much buffer as possible into the bag, being careful not to allow any contaminates into the bag. After disinfecting tweezers with ethanol and flame, .45um filters were placed on each filter housing, being careful not to touch any other surface with the filter. Immediately after filter placement, housing was placed and clamped again. For plating, a .1mL of the solution was pipetted onto another CHROMagar plate, using the spreader tool to properly spread mixture onto the plate. To begin filtration, samples were then poured into housing with the valve open to filter. After the entire sample was been filtered through, the valve was closed completely. Using sterile tweezers, the filters were transferred to a CHROMagar plate by grabbing only the dry edge of the filter and rolled onto the plate slowly to avoid air pockets. After labeling each plate, decontamination of the filter housing was achieved by pouring 150mL of boiling Milli-Q water onto the housing and closing the housing, allowing water to pass through completely. After housing returned to room temperature (approximately four minutes), sterile tweezers were used to place another filter onto the housing to begin with the next sample. After all sampling had been completed, plates were incubated at 37°C for about 24 hours.

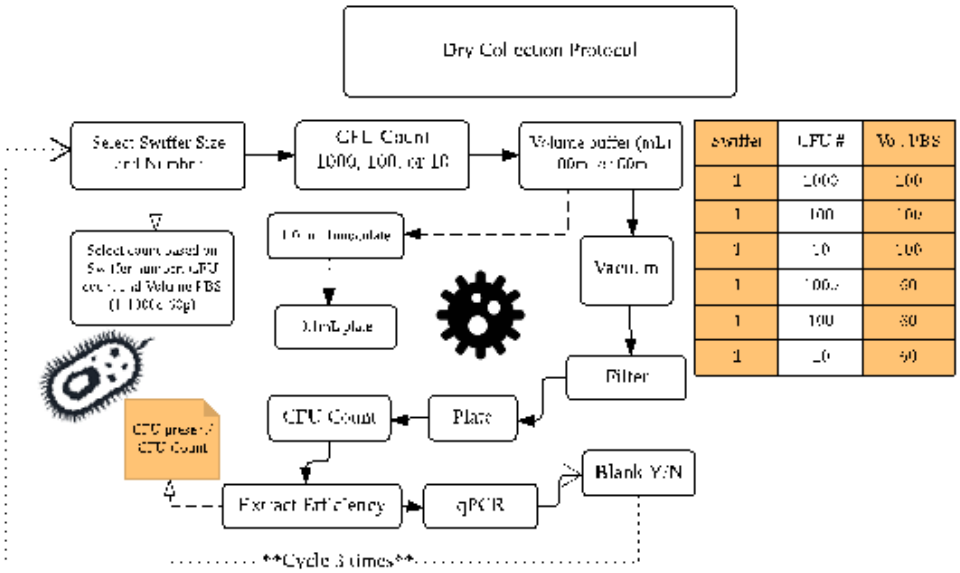

Figure 2. Protocol for filtration

PCR

Quantitative PCR (qPCR) was used to quantify the gene count recovery from inoculated cloths by testing 500µl peptone extract using a protocol developed by? Klotz, Opper, Heeg & Zimmermann (2013). Amplification mixtures contained, in a final volume of 25 µl, PCR buffer (10x) 6 mM $MgCl_2$; 10 mM each dATP, dCTP, and dGTP; 20 mM dUTP; 50 pmol of each primer; 150 nM TaqMan probe; 0.25 µl of AmpErase; 2.5 U of AmpliTaq; and 10µl of template DNA. For the runs, the gene FemB was used alongside the Probe primer with the 301-330 location. With the ABI PRISM 7700 sequence detector, amplification was performed to hold at 50°C for 2 minutes, 95°C for 10 minutes, and completed 45 cycles of 95°C for 15 seconds and 60°C for one minute. Once completed, data were analyzed in the real-time mode. As detected by the ABI PRISM 7700 detector, results were provided as rise in fluorescence signal of the reporter dye. Test results are available 6 hours after isolation of the *S. aureus* strain: DNA extraction

requires about 3 hours; master mixture preparation, depending on the number of samples, requires about 30 minutes; and the TaqMan assay requires 2 hours (Klotz et al., 2013, p.4684).

Results and Discussion

For the results, several rounds of filtration and serial dilutions were run. Normal use of a colorimeter is standard to obtain the optimum amount of CFU's suspended within a solution, though, using a visual standard present within the colorimeter manual can be allowed.

Phase 1

For this phase of the experiment, side-by-side extractions from 11 surfaces from four local schools were compared. As shown in *Table 1*, *S. aureus* was always detected using Swiffers, versus the swab method, which failed to detect *S. aureus* 8/11 (73%) of the time. *Table 1* displays that the swab method may have limitations in detecting *S. aureus* from the environment, or that some microbes may die during transportation and future research can investigate these results further.

		SWAB			
	CFUs	0	20	40	50
SWIFFER	0	0	0	0	0
	5	1	0	0	0
	7	1	0	0	0
	16	1	0	0	0
	25	1	0	0	0
	33	1	1	0	0
	36	1	0	0	0
	43	0	0	1	0
	47	0	0	0	1
	54	2	0	0	0

Table 1. Comparison of Swiffer versus Swab CFU Counts

Phase 2

For the laboratory phase to compare the filtration method versus qPCR, several runs were taken; however, one run was deemed most successful to continue for the real-time PCR phase for comparison. The culture independent method produced results of 304.2982, 30689.21 and 80300.44 gene copies (*Table 2*).

Fem copy per Swiffer				
Dilution	304	30689	80300	Total
10	1	0	0	1
100	0	1	0	1
1000	0	0	1	1
Total	1	1	1	3

Table 2. Fem copy per Swiffer results

Problems during the DNA extraction phase with 1-1000c may have washed microbes during one of the final steps, providing the 80300 result, which corresponds with the 1000 dilution step did not align with the log format which was previously expected. Certain limitations during the post-Swiffer step may include a failed colorimeter to measure the amount of bacteria within the solution, which can mean that all samples will need to be examined visually. The filtration method produced CFU counts of 31, 56 and TNTC after dilution step as shown in *Table 3*. Special care must be made with the filtration procedure as use of large volumes of liquid during vacuuming can cause leakage. Leakage can cause microbes to be lost and contamination of filtration membrane and can be researched in lab-scarce settings where

qPCR may not be available.

Fem copy per Swiffer	Filter CFU Quant.	
	31	56
304	1	0
20689	0	1

Table 3. Filter CFU quantity

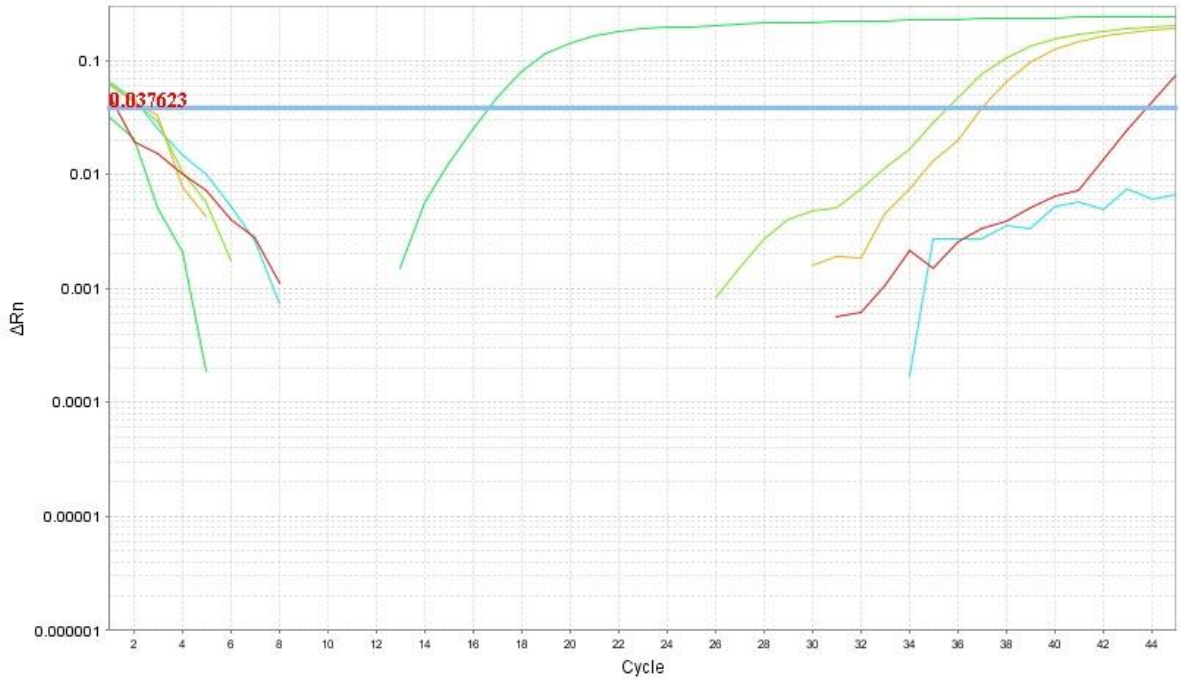

Figure 3. qPCR Results with both controls

When using qPCR, the cycles shown in *Figure 3* depict the time (cycles) taken for the gene to be expressed and amplified by the machine. If the sample contains a large amount of genes, it will be detected more quickly. The positive control is showcased by the bright green and the negative control is showcased with the bright blue line, which concludes that the run is successful per dilution step. *Figure 4* compares gene copies to the CFU dilutions made (1000, 100,10) from the successful run. Because the volume represented for the CFU's are based per

mL, and only 50ul was used for qPCR, generating the x-axis, which is one tenth of the CFU volume. The data in *Figure 4* shows that the culture independent method is comparable to the culture based method, making it a feasible option shown to be much more sensitive at detecting CFU's (genes).

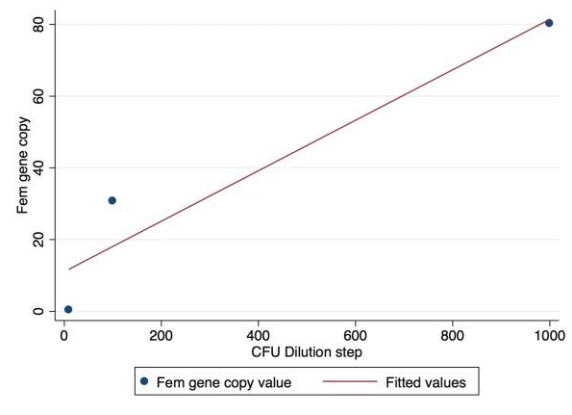

Figure 4. qPCR compared to filtration

Conclusion

Results of the study showed that the dry collection method was more effective at obtaining *S. aureus* in indoor environments versus the wet collection method. The study also found that the filtration method was resource intensive, challenging, and prone to error because of the amount of phosphate buffer used in the method. The filtration method, however, can be optimized for a lab-scarce setting by using a smaller amount of buffer, and recording the extraction given in comparison to the original 100mL volume amount used for the current experiment. Lastly, it was found that the qPCR method was much more sensitive at detecting *S. aureus* and was more feasible in terms of time. Future studies can continue to compare results to the optimized filtration method and compare the costs of each with a large-scale experiment.

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