

A novel imaging system for rapid visualization of bacteria on surfaces

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ABSTRACT

The ability to identify locations that are missed in routine cleaning is important. Visual inspection, ATP bioluminescence systems, and fluorescence or ultraviolet light are monitoring methods that indicate overall cleanliness, but not contamination removal. In this study, we use *Staphylococcus aureus* to evaluate a novel imaging system that provides a rapid, visual confirmation of the presence of bacteria on surfaces at four log concentrations ranging from approximately 4.7×10^9 to 1.8×10^4 CFU/cm². We found that the combination of the illuminator spray and imaging software was able to detect the presence of bacteria on the surfaces and indicate relative concentration by visualizing the contamination as a heat map.

KEYWORDS:

Surface contamination, monitoring, imaging, *Staphylococcus aureus*

INTRODUCTION

Considerable evidence exists regarding the ability of surfaces to act as a reservoir for infectious pathogens, which can pose an infection risk to those who encounter them [1]. In order for a microorganism to present an infection risk in the physical environment, it must be able to both persist in the environment and cause disease once introduced to a susceptible human host. Many human pathogens have been shown to be capable of surviving for long periods of time outside the human host. For example, methicillin-resistant *Staphylococcus aureus* (MRSA) has been shown to survive for up to a year on surfaces such as floors, furniture, dust and *Acinetobacter baumannii* can resist desiccation for as long as eight weeks [2, 3]. Several other pathogens such as vancomycin-resistant *Enterococcus* (VRE), *Clostridium difficile* and gram-negative rods have been shown to be able to survive the harsh environment for varying lengths of time posing an infection risk to patients and staff [4]. Studies have implicated environmental surfaces in the transmission of pathogens [5, 6]. Given the role of environmental surfaces in the transmission of contamination that can either directly or indirectly contribute to healthcare-associated infections, it is important for facilities to implement a cleaning audit program to ensure adherence to the facilities' approved cleaning protocols and identify employees who may require additional training [1, 4].

The most widely used audit tools for cleaning include visual inspections, fluorescent marking, adenosine triphosphate (ATP)

bioluminescence and microbial swabbing. Visual inspections provide a very easy and inexpensive way for quick assessments of cleanliness, but do not allow for a reliable assessment of contamination removal [7]. ATP bioluminescence systems detect the presence of ATP on surfaces (as Relative Light Units, RLU), which correlate to the amount of organic matter present on a surface. A systematic review by Nante *et al* (2017) concluded that ATP bioluminescence testing was a better alternative to visual inspections, but that the limitations of this test must be considered [8]. For example, the benchmarks for the ATP systems vary widely by manufacturer, ranging from 45 RLU to 1000 RLU and the chemical residuals left behind from cleaning interacts with the test causing an artificially high or artificially low reading. Further, since the test is indiscriminate to the source of ATP, the results reflect all sources of ATP including milk, food, human cells, urine and bacteria [9, 10]. The most accurate way to assess the presence of microbial contamination is by way of microbiological swab testing for total aerobic colony counts (ACC) expressed as colony forming units (CFU) per surface area. However, microbial swab testing is more costly, has longer turnaround times, and is often reserved for use during epidemiological investigations.

In this study, we evaluate a novel monitoring technology that offers rapid identification of the presence of bacterial contamination on a surface. This technology uses fluorescence labeling and multi-spectrum imaging. It involves the application of an illuminator spray to the surface, which contains a dye that

binds to bacterial DNA allowing the bioburden to be visualized during the imaging process. The images are captured using a customized, multi-spectrum camera and processed using proprietary software to determine if bacterial contamination is present on a surface along with the relative amounts. The aim of this study is to assess the accuracy of this technology in detecting bacterial cells on a surface.

METHODS

Microbiological methods

Staphylococcus aureus ATCC 43300 was cultured in Tryptic Soy Broth and incubated at $35.0 \pm 1.0^\circ\text{C}$ for 18-24 hours for all experiments. Bacterial counts were serially diluted in Butterfield's Phosphate Buffer. In a sterile biological cabinet, $20 \mu\text{l}$ of an overnight suspension were spread onto 24 individual sterilized stainless steel carriers ($2.54\text{cm} \times 7.62\text{cm}$) in 10^{-1} , 10^{-2} , 10^{-3} , and 10^{-4} dilutions. The initial inoculum count was quantified on 12 carriers using 3M Petrifilm Aerobic Count Plates. Carriers were submerged in Lethen Broth and vortexed for 30 ± 3 seconds prior to dilution and plating.

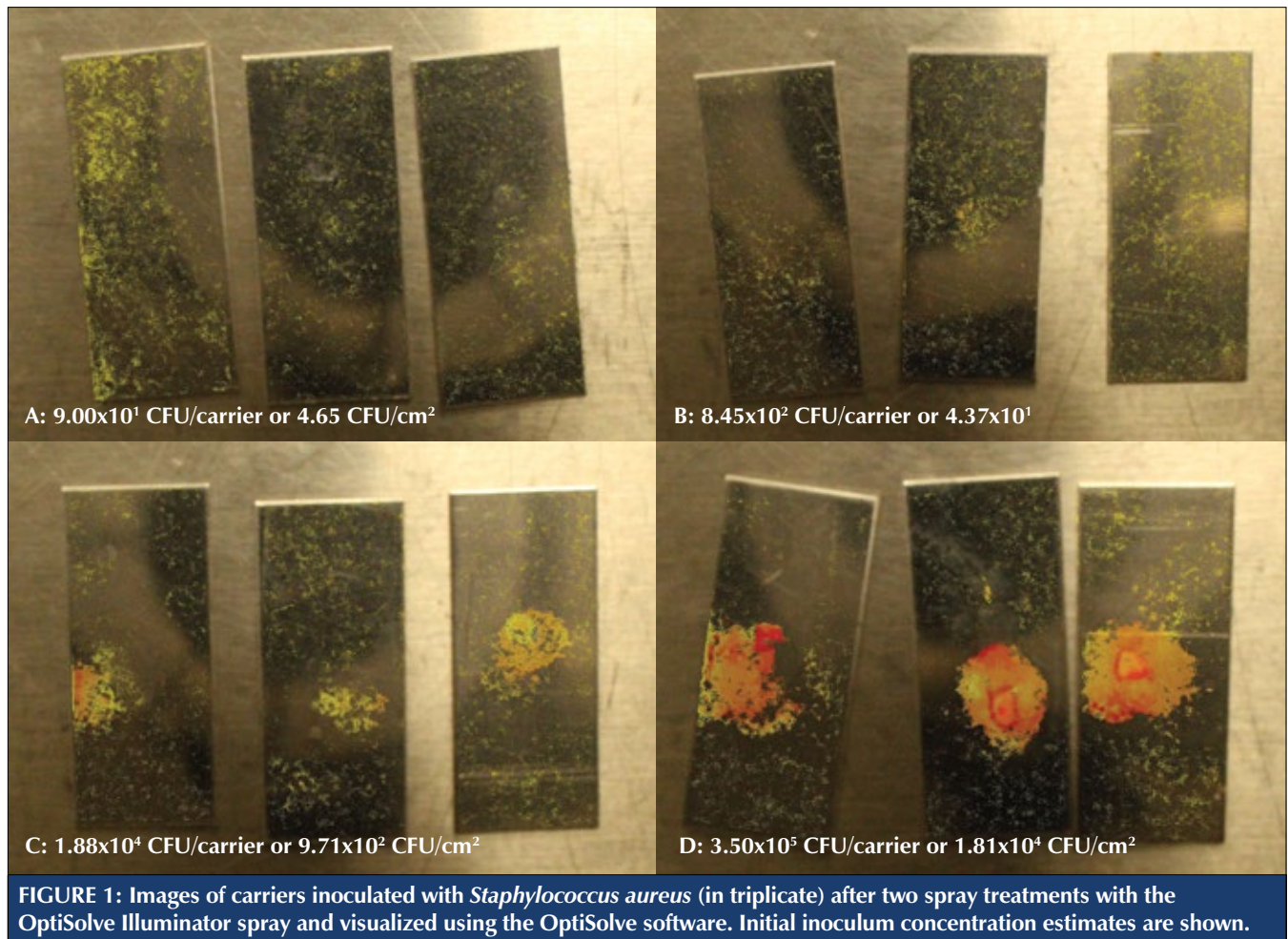
Imaging protocol

The camera was fitted to a tripod, which remained stationary during the imaging protocol.

An initial image sequence was taken of the remaining 12 carriers before application of the illuminator spray using OptiSolve Pathfinder camera (a Canon T6 Rebel fitted with propriety attachments) for baseline images. Each slide was then sprayed with two pumps (approximately 0.1 mL) of the OptiSolve Illuminator via a spray bottle and allowed to dry for 30 seconds. Once dry, each carrier was photographed again using the OptiSolve Pathfinder camera to generate image sequences after the application of the illuminator spray. All photographs were processed using the OptiSolve software which uses an algorithm to generate the final composite image.

RESULTS

Baseline images were taken of all slides after inoculation with *S. aureus*, but prior to the illuminator spray application (not shown). These baseline images were used to help confirm the absence of background noise, but it was very difficult to visualize the actual areas of inoculation. Once the spray was applied, areas of inoculation can be clearly seen at concentrations of 10^4 CFU/carrier or higher (Figure 1, C and D) and is somewhat discernible at 10^2 CFU/carrier followed by 10^1 CFU/carrier (Figure 1, A and B).



The OptiSolve software indicates greater concentration of the bacteria through a heat map and colour intensity ranging from yellow (lower in concentration) to bright red (higher in concentration). For the lower inoculums (10^1 to 10^2 CFU/carrier), areas of low concentration of bacteria on each carrier was visualized (yellow color). For the higher inoculums (10^4 to 10^5 CFU/carrier), areas of moderate to high concentrations of bacteria was visualized (orange and red in color) with the reddest areas present on the slides with the highest concentration of inoculum (10^5 CFU/carrier), (Figure 1).

DISCUSSION

This is the first study evaluating a technology that uses fluorescence and imaging to assess bacterial contamination on inanimate objects. The ability to monitor the efficacy of cleaning processes is important since people in busy hospital environments can become exposed to infectious microorganisms from contaminated hands, surfaces, or equipment [11]. Further, high-touch surfaces can be easily missed in cleaning, disinfection, and sanitation protocols which is a concern in the case of difficult-to-clean equipment [12].

We evaluated a novel approach that uses fluorescence labelling and multi-spectrum imaging to assess microbial surface contamination. The system works by first spraying the surface with an illuminator spray containing a dye that binds to bacterial DNA, allowing for the visualization of bacteria during the imaging process. The illuminator spray was a clear liquid that was not readily visible to the naked eye once dry, nor did it leave behind any indelible marks on the stainless steel carriers used in this study. Once applied, the sprayed liquid must be allowed to dry before taking the image (approximately 30 seconds). A camera that is customized to emit various spectrums of light while capturing a sequence of images is then used to take the photograph (OptiSolve Pathfinder). The maximum field size for a single-image capture is approximately 21.59cm x 27.94cm, which allows for the imaging of most high-touch surface areas. The images are processed through a proprietary algorithm generating a final, composite image, which portrays the relative quantity of bacteria present in the form of a heat map, ranging from low concentration (yellow) to high concentration (red).

We tested the ability of this technology at four low-bacterial concentrations (10^1 , 10^2 , 10^4 and 10^5 CFU/carrier) on stainless steel surfaces and found that this tool functioned as a semi-quantitative proxy to gauge relative amounts of bioburden. At lower concentrations (10^1 and 10^2 CFU/carrier), the point of inoculation on the stainless-steel carrier is less obvious – but as the inoculum concentration increases from 10^1 to 10^5 CFU/carrier, the relative bacterial concentration can be interpreted from the density and colour of the images (Figure 1, A-D). At higher concentrations of bacteria (10^4 and 10^5 CFU/carrier) areas of red, orange and yellow can be readily seen on each carrier (Figures 1, C and D). We found that the OptiSolve surface imaging technology

could detect the lowest concentration of *S. aureus* tested, 90 CFU/per carrier or 4.65 CFU/cm². Since the threshold for microbial monitoring of high-touch surfaces is ≤ 2.5 CFU/cm² [7], additional testing would be needed to determine the sensitivity of the tool below this level. It is important to note that the camera detects the emission of the fluorescent label, which is assumed to be representative of bacterial cells on the surface. It does not directly detect the cells.

This approach could potentially provide a new, rapid way for approximating the quality of contamination removal from a surface and facilitate precision cleaning processes. However, there are some important limitations that should be taken into consideration. First, the dye used in the illuminator spray does not differentiate between live and dead cells. As such, extracellular DNA, which can be passively released from dead cells or actively released from physiologically active cells, and extracellular DNA that is prominent in a biofilm, is picked up in the imagery. Since the spray is solvent based, it cannot be used on soft, polymer or paint-coated surfaces, and must be wiped away from the surface after the image is captured, limiting the types of surfaces that can be imaged. Also, fluctuations in lighting conditions could impact signal variations and affect the resulting imagery. While we did not evaluate the safety of this product, the label bears a flammable and an irritation warning, suggesting the use of gloves and safety glasses during use.

A limitation of this study is that a pure culture of *S. aureus* was tested without the addition of artificial test soils. Therefore, our results may reflect a higher level of sensitivity than what might be seen in the environment where a variety of types of contamination, including blood, feces or other organic carbon materials are present. However, the purpose of this technology is to monitor surfaces after they have been cleaned and organic materials should have been cleaned from the surface.

The OptiSolve Pathfinder can be used as a training tool, to optimize cleaning protocols, or to identify surface locations that are missed in routine cleaning. Because it is qualitative in nature, it is not recommended to be used to validate disinfection or sterility. However, this novel technology is specific to bacteria and presents a viable alternative for assessing the overall quality of surface disinfection. Additional studies are necessary to determine if disinfectant chemical residuals on surfaces interfere with the illuminator spray, to measure the sensitivity of the technology to other bacteria as well as viruses and spores, and to evaluate the capacity for this technology to detect the impact of environmental cleaning.

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