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Solar disinfection of turbid hygiene waters in Lexington, KY, USA

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ABSTRACT

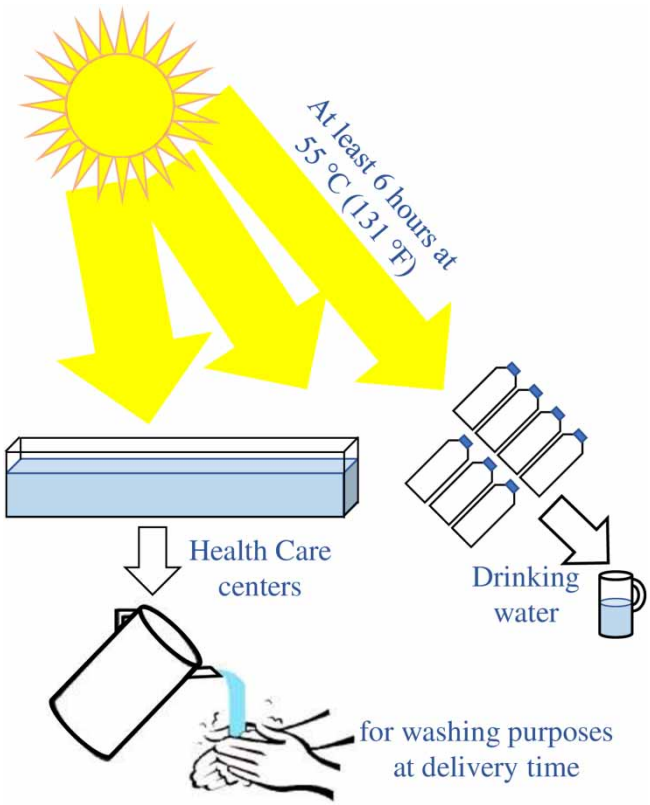
Solar disinfection (SODIS) could be a key to providing a clean, hygiene water for birthing uses, but the recommended climate zone is limited, the microbial indicators are related to gastrointestinal illness and not wound infections. SODIS feasibility was investigated to remove *Escherichia coli* from turbid water at temperatures less than 50 °C in Lexington, KY. Increasing turbidity from 0 to 200 NTU decreased *E. coli* inactivation from 5 to 1 log. With the same experimental protocol, more than 4-log inactivation of *Staphylococcus aureus* and *Staphylococcus epidermidis* (common human-skin microorganisms related to serious post-partum infections of both mother and child) was achieved at different turbidity levels with a maximum, in-bottle temperature of 49.2 °C after 5.5 h. The thermal inactivation of the bacterial indicators was assessed without UV radiation and turbidity in water at 37 and 47 °C. Skin bacteria were inactivated completely after 9.5 h at 47 °C, but only 58% removal happened for thermo-tolerant *E. coli*. These results suggest that SODIS application may be expanded geographically to treat water for hygiene purposes. However, as *E. coli* is also capable of causing wound infections, UV with thermal inactivation may be required to produce safe hygiene water by SODIS outside of recommended latitudes.

Key words: hygiene, solar disinfection, *Staphylococcus aureus*, thermal resistance, turbidity, water

HIGHLIGHTS

- Solar disinfection (SODIS) was applied for the first time to remove *Staphylococcus aureus* from water to provide safe hygiene water for birthing.
- Human skin bacteria are more susceptible to thermal inactivation than gastrointestinal bacteria.
- SODIS protocols need to be updated to evaluate process indicators bacteria for hygiene purposes.
- Consideration of solar disinfection for treating contaminated water for hygiene purposes could have a significant positive impact on saving the lives of women in labour and those of their newborns and decreasing the number of postpartum infections.

GRAPHICAL ABSTRACT



INTRODUCTION

Globally, about 90% of the world's population has access to basic sources of water within a 30-min round trip walk from home. However, for many of these people, water of unreliable quality is the only available source to meet their basic needs, and extra water for hygiene purposes is often lacking. Of the total 1.4 million diarrheal deaths in 2016, 485,000 deaths were attributable to inadequate water. Inadequate hygiene water and behaviors were estimated to be attributable to 165,000 of these diarrheal deaths, and it was estimated that a large portion of these deaths could have been prevented by the provision of adequate, safe supplies of water for hygiene purposes (Prüss-Ustün *et al.* 2019). However, diarrheal diseases are not the only health endpoints for microbes carried in water.

Lack of adequate supplies of hygiene water can impact mothers and newborns. Multiple water-based and water-washed infections were reported in pregnant women attributable to aquatic vectors such as genital schistosomiasis, dracunculiasis, diphyllobothriasis, streptococcus A, and staphylococcus. Most of these water-washed infections occurred due to poor hygiene and infection controls via contaminated hands and surfaces, and unhygienic medical tools (Campbell *et al.* 2015). Results from a prospective cohort study in rural India showed that poor sanitation and lack of hygiene water, such as those associated with conditions of open defecation, cause preterm birth and low birth weight (Padhi *et al.* 2015). Cervical cancer, ectopic pregnancy, and infertility are some health problems related to genital schistosomiasis which can cause anemia, malnutrition, and inflammation in pregnant women. Unhygienic practices during delivery, such as poor hand hygiene in delivery facilities, are correlated by puerperal sepsis caused by streptococcus A, tetanus, hookworm-related anemia, and MRSA (Benova *et al.* 2014; Campbell *et al.* 2015). Poor quality of care in health facilities caused peripartum infections with 4–50% death rates for sub-Saharan Africa and Southeast Asia (World Health Organization 2015).

Many Primary Health Centers in Nigeria and Tanzania do not have access to adequate volumes of high-quality water to support patient care, sanitation, and hygiene purposes (Premium Times 2017a, 2017b). Less than half of healthcare facilities in Tanzania have access to water for acceptable toilets, handwashing, and sterilization. Among these facilities, less than half of them have these services only in the delivery room. Poor clinical conditions and the lack of water can promote bacterial

infections and cause sepsis post-partum. Giving birth under these conditions can have catastrophic consequences, highlighting the need for access to adequate supplies of clean water for hygiene purposes during birthing.

To provide hygiene water, working staff in these facilities, and pregnant women, are expected to buy water from local vendors or bring their own water to the birthing facilities. This water is often delivered in large containers that are not sterile and from providers of unregulated quality. Women in labor come to the centers hauling water from home to save on costs and provide acceptable levels of hygiene, but in emergency situations may not have the time to bring water with them. Water-related infections have a high occurrence rate in birth cases in which pregnant females do not have time to bring their own water. Therefore, they slip out of the facility after delivery without washing their baby because they cannot afford the $15 cost of hygiene water (The Guardian 2017). This has led to poor sanitation and dirty water being the cause of death for one out of 44 women during delivery in Tanzania (Techtimes 2014).

Other countries share Tanzania's hygiene water problem. An assessment of water supplies for 53 primary health centers in five states in Nigeria showed that only 41.5% utilized a motorized borehole as recommended by the National Primary Health Care Development Agency, while 30% relied on dug wells, 13.5% collected rainwater, and 15% used surface waters (Christian Aid Nigeria Country Programme 2015). The quality of water from the non-recommended sources is quite variable, and when hauled in non-sterile containers, vulnerable to contamination during hauling and storage procedures. Consequently, the development of an affordable, accessible, culturally acceptable, low-tech, in-home, water treatment method that could be applied to water sources with questionable quality can be a powerful tool to decrease a substantial amount of hygiene-related diseases globally. It could be a system where water for birthing is treated continuously, by increasing temperature and UV irradiation via solar exposure of bottles filled with non-potable water (SODIS), which could help fill the gap and reduce the post-partum burden of disease due to bacterial infections from common skin organisms. A system, where water is constantly being brought in and treated days prior to a birthing event, could provide birthing centers, and women without the means to pay for water, another source of water to use during birth.

Solar disinfection (SODIS) has been introduced as a low-cost, low-tech method for improving the quality of contaminated water for potable uses in poor areas in the 1980s (Caslake *et al.* 2004; Kalt *et al.* 2014). Thus far, multiple experiments have been conducted to investigate the effects of different variables on the efficiency of SODIS (Wegelin *et al.* 1994; Yu *et al.* 2012; Asiimwe *et al.* 2013; Dessie *et al.* 2014), but generally for potable uses, which require a different level of treatment than water used for hygiene and cleaning. It may be possible to utilize this simple technique on turbid waters to provide a safely stored quantity of hygiene water for use in birthing centers. However, the indicators used to evaluate the feasibility of treatment, and the primary vectors of inactivation for those indicators, must be evaluated. Thermo-tolerant, gram-negative, coliform bacteria used as process indicators to prevent enteric disease, who are inactivated primarily by UV irradiation, may be an imperfect indicator when designing treatment systems to prevent the transmission of thermally inactivated, gram-positive bacteria.

This study assessed the feasibility of the SODIS method as applied to plastic bottles to improve the microbial quality of turbid water in an area of the world outside the recommended regions, Lexington, KY. This experiment was conducted using bottled water and turbidity was created by adding clay particles in the lab to contain values in the ranges measured in the large Kentucky river that serves as the drinking water source for the city of Lexington where monthly averages of 10 NTU during dry months (August 1997) to 143 NTU during rainy months (March 1997) have been recorded (unpublished data). Multiple turbidity levels 0, 30, 100, and 200 NTU were used to examine the role of turbidity on SODIS efficiency. Research investigated the effectiveness of solar inactivation mechanisms (UV and temperature) to remove potentially pathogenic, human-skin microorganisms, *Staphylococcus aureus* and *Staphylococcus epidermidis*, and compared these with the inactivation of thermo-tolerant indicator bacteria, *E. coli*. Furthermore, strictly thermal inactivation experiments were conducted for S. *epidermidis*, *S. aureus*, and *E. coli* to determine the behavior of these bacteria under time and temperature conditions achievable at this region of the world, 38° N in a humid, subtropical climate.

This study demonstrated the potential of SODIS to treat turbid water for hygiene purposes in small communities, rural areas, and healthcare centers that lack access to safe, reliable, inexpensive, and abundant sources of hygiene water, especially with regard to the possibility for preventing hygiene water-related diseases and deaths in women and their newborns.

METHODS

SODIS experiments: location and times

SODIS experiments were conducted at a laboratory scale. All procedures, except exposing bottles to sunlight, were done in the Environmental Research Training Laboratory (ERTL) at the University of Kentucky, which is located in Lexington, KY, USA with a latitude of 38.04°N and a longitude of 84.51°W. The latitude of Lexington exceeds the most favorable regions (between 15° and 35°N/S latitudes) recommended for solar inactivation for potable water. The outdoor exposures for experiments were conducted in an open courtyard without any shadowing effects from trees.

To evaluate the feasibility of SODIS, two separate experiments were conducted at two different time periods. The first SODIS experiment was done on a semi-cloudy day on 20 April 2017 to inactivate the bacterial process indicator *E. coli* at three turbidity levels. All bottles were exposed to sunlight for 7 h from 9 am to 4 pm. The second SODIS test was performed on a perfectly sunny day on 26 June 2019 to compare bacterial removal in turbid waters with three different types of process indicator bacteria for 6 h of sun exposure, from 11 am to 5 pm. Records of solar insolation were obtained from the University of Kentucky Agricultural Weather Center.

Calculation of rolling averages for UV radiation data

Solar radiations (W/m^2) of each sampling event were recorded every hour by the University of Kentucky Agricultural Weather Center. However, solar intensity had a great deal of variation during the period of our experiment due to cloud coverage. Therefore, rolling averages of solar insolation data were computed by averaging three recorded UV radiation observations between 9 am and 5 pm during two SODIS experiments. For time points 0–5 h, the insolation value at the hour and the next 2 h following was averaged. For the 6-hour time point, the recorded UV radiation data at the last 3 h (4, 5, and 6) were used. SigmaPlot 14 software was used to analyze the rolling averages for both experiments. A paired *t*-test compared the two sets of rolling averages in April 2017 versus June 2019.

Preparing test waters

Turbid water was prepared by adding fine, dried, local clay particles to bottled water. Clay particles were passed through a No. 200 sieve with 0.074 mm hole size and then autoclaved for sterility before being added to water. For the first SODIS experiment, three turbidity levels were considered 0, 30, and 200 Nephelometric Turbidity Units (NTU). For the second SODIS experiment, 0, 30, and 100 NTU were tested. The required amount of clay to prepare turbidity levels of 30, 100, and 200 NTU was determined by trial and error. The weight of clay particles was measured before adding to a specific volume of water and then the resultant turbidity was measured by a HACH portable turbidimeter (Model 2100P) calibrated for accurate measurement with standards in the mid and upper ranges. Clay was added until reaching the desired turbidity levels. For final confirmation of set turbidity levels, the turbidity levels were measured.

Test bacteria

A non-toxigenic strain of *E. coli* strain P3 (ATCC® BAA- 1428™), used commonly as a surrogate for the inactivation of *Salmonella* and *E. coli* O:157 H:7 in food processing, was used to perform the first SODIS experiment in April 2017. For the second solar experiment in June 2019, *S. epidermidis* (ATCC® 29887™) and *S. aureus* subsp. *aureus* Rosenbach (ATCC® 25923™) were added to *E. coli* strain P3 (ATCC® BAA-1428™) as model process bacteria.

Pure cultures of *E. coli*, *S. epidermidis*, and *S. aureus* were incubated overnight at 37 °C in Bacto™ tryptic soy broth (Difco Laboratories, Becton Dickinson & Co, Sparks, MD) to create bacterial stocks. Then, stock concentrations were determined by serial dilution followed by direct spread-plate culture on plates filled with Difco™ tryptic soy agar (Difco Laboratories, Dickinson & Co, Sparks, MD) overnight at 37 °C. Colony-forming units (CFU) were counted after overnight culture. The required volume of bacterial stock to spike into volumes of test water was calculated to provide the initial bacterial concentration of 10^5 CFU/mL of water for each bacterial strain spiked into test waters.

Procedure of the experiment

The same procedure was used to prepare test bottles for both SODIS experiments. For each turbidity level, 4-L of commercially available, bottled water, 0.5 L (16.9 oz) of purified drinking water in polyethylene terephthalate plastic (PET) bottles measuring 20.32 cm (8 in) tall and 6.35 cm (2.5 in) diameter, were transferred from individual, 0.5 L plastic bottles into an autoclaved mixing jar to prepare a stock for all test bottles, negative controls, and positive controls. For each

turbidity level, one positive control, one negative control, and five replicate test bottles were prepared. For negative controls, one bottle (all bottle labels were removed before use) from each turbidity level was filled by adding 0.5 L of the prepared water prior to spiking with bacterial stocks, then one digital thermometer (TD digital thermometer) was installed through the lid of the bottle to record water temperature every 30 min over the duration of the experiment. Before installing thermometers, 0.1 mL of water from the negative control bottles was inoculated onto three Petri plates filled with tryptic soy agar as a negative control.

After spiking water stocks with the required volume of bacteria, one test bottle for each turbidity condition was filled with 0.5 L of spiked water stock and placed in a refrigerator to serve as a positive control and to quantitate the actual initial concentration (N_0) of bacteria in test bottles that were exposed to sunlight. For each set of experimental conditions, five replicate test bottles, each with a volume of 0.5 L, were utilized per turbidity level to prepare multiple observations for robust statistical results. All test bottles, control or experimental, were filled in two steps. First, after filling three-fourths full of each bottle with spiked, or blank test water, the bottle was shaken for 20 s, and then it was filled up completely.

After preparing bottles, all negative controls and test bottles were transferred to the courtyard outside of the laboratory and placed randomly on cardboard covered with aluminum foil. In the first and second experiments, all bottles were exposed to sunlight for 7 and 6 h, respectively. The ambient temperature and the temperature in negative control bottles were recorded every 30 min during the experiment.

At the end of the experiment, all sample bottles were transferred to the laboratory and processed immediately. Bacterial counting was conducted on tryptic soy agar petri dishes after serial dilution, spread-plate inoculation, and incubation. After spread plating 100 μL of sample, or serial dilution of sample, petri dishes were incubated for 24 h at 37 °C. For each turbidity level, three replicates of each sample, or dilution, were averaged to obtain an accurate count and adjusted by dilution to calculate the number of bacteria per milliliter in each test bottle (N_t). Bacterial inactivation levels were reported by calculating the log reduction of bacteria ($\log(N_t/N_0)$), where N_t is the number of viable CFU/mL found by culture after sun exposure and N_0 is the initial number of CFU/mL before starting the experiment.

Experimental procedure to measure inactivation by temperature, no UV radiation

An assessment of bacterial inactivation due to temperature alone was completed in the lab by measuring the decreasing concentration of spiked bacteria in bottled water during 9.5 h when incubated in two incubators set at two different temperatures. The temperature in the first incubator was 37 °C, which is the incubation temperature for selected model bacteria, and the temperature in the second incubator was 47 °C, which was recorded as the average of the highest achieved temperature for three turbidity levels in the negative control bottles in the second SODIS experiment. Three species of aforementioned indicator bacteria were added to bottled water in a sterile, plastic culture flask to make a final concentration of 10^5 CFU/mL (calculated from stock titer at time = 0). The volume of water in each culture flask was about 50 mL. Before spiking bacteria into each culture flask, the water in the flask had been incubated at the two temperatures, in two incubators, for 1 h to achieve the desired temperature. Then, the required volume of bacterial culture was added to the bottled water contained in the culture flasks to prepare 10^5 CFU/mL. Then, bacterial concentrations were measured by direct plate counting after 0.5, 1.5, 2.5, 5.5, and 9.5 h to assess the effect of temperature on bacterial removal without the effect of UV radiation.

Data analysis

For statistical analysis of two groups, the paired *t*-test was conducted to analyze for differences between two groups of data at different times, point-by-point, if the normality and equal variance testing of the data distribution passed. If the normality or equal variance tests failed, the nonparametric test, Wilcoxon signed-rank test, was used to compare two groups. For pairwise comparison of three groups, the one-way ANOVA with Tukey's pairwise multiple comparison was used to compare the difference of the means in the SigmaPlot version 14 software after the normality and equal variance tests passed.

Experimental conditions

The weather conditions during the first SODIS experiment were partly cloudy, but during the second test, the sky was clear and sunny. Table 1 summarizes temperature information related to those 2 days between 11 pm and 4:30 pm, times both experiments shared.

Table 1 | Summary of temperatures and UV radiation in the two different SODIS experiments

Date	Average UV radiation (11 am–4:30 pm) (W/m^2)	Maximum ambient temperature (11 am–4:30 pm) (°C)	Temperature of negative control (0 NTU bottle)		
			Initial temperature (time = 0 h) (°C)	Mid temperature (time = 2.5 h) (°C)	Final temperature (time = 5.5 h) (°C)
20 April 2017	656	27.2	28.60	33.90	37.4
25 June 2019	848.2	32.0	21.60	38.40	44.20

RESULTS

Temperature variations

The results of a paired *t*-test comparison for the recorded ambient temperatures in the two different SODIS experiments showed significant differences between the means of the ambient temperatures reached in the five replicate bottles (Table 2). The second test event, which was 2 months later in the year than the first event, had a higher average ambient temperature for the day, likely due to the lack of cloud cover and increased solar radiation.

For temperatures attained over time in 0 NTU negative control bottles, there was not a significant difference between temperatures in 0 NTU negative control bottles across the span of time for the two events (Table 2). The data for the 30 NTU comparison failed normality, so a Wilcoxon signed-rank test was applied that showed there is a statistically significant difference between two groups of temperatures for these two experiments (Table 2). The 30 NTU bottles got much hotter on the second SODIS experimental date, likely due to the increased solar insolation and lack of cloud cover.

Results of the paired *t*-test comparisons of temperatures reached in the negative control bottles as compared to the three turbidity levels in April 2017 (0, 30, and 200 NTU) and in June 2019 (0, 30, and 100 NTU) are presented in Table 3.

For recorded temperatures in April 2017, results of paired *t*-test indicated that there was a significant difference between recorded temperatures in 0 and 30 NTU as well as 30 and 200 NTU bottles, at a $P < 0.001$ level of significance. However, the paired *t*-test showed that there was a significant difference in ranks between temperatures reached in 0 and 200 NTU bottles in April 2017 ($P < 0.001$) when the Wilcoxon signed-rank test was considered after failing the normality test. Results of paired *t*-test comparisons for temperatures at 0, 30, and 100 NTU turbidity levels in the second SODIS experiment, June 2019, passed the normality and equal variance tests and showed that there was a statistically significant difference between any temperature for the different turbidities (Table 3).

Figure 1 shows the increase in temperatures over the time of exposure as recorded in the negative control bottles for each turbidity condition and the ambient temperature for the two SODIS test events. As shown in these two graphs, higher temperatures were reached on the second SODIS experiment date (Figure 1(b)), and in-bottle temperatures were greater than ambient.

The maximum recorded temperatures in the first test (April 2017) were 38.5, 39.5, and 42.9 °C at 0, 30, and 200 NTU, respectively. The highest temperature was recorded in the highest turbidity level (200 NTU) at the end of the experiment, indicating greater heating caused by infrared radiation due to the effect of the clay particles. The maximum temperature in 0 NTU bottle lasted for about 1 h between 3 pm and 4 pm. All bottles showed an increase in temperature above ambient, but below 50 °C, which is considered the minimum effective temperature for SODIS.

The maximum for all recorded temperatures occurred during the second SODIS test (June 2019) at 0, 30, and 100 NTU turbidity levels were 44.2, 46.8, and 49.2 °C, respectively. Similar to the first experiment, the highest temperature happened

Table 2 | Paired comparisons of temperatures for two SODIS experiments

Paired *t*-test between temperatures of			Normality test/ equal variance	Wilcoxon signed-rank test	Significant difference
Ambient-April 2017	vs	Ambient-June 2019	Passed/passed	N/A	Yes
0 NTU-April 2017	vs	0 NTU-June 2019	Failed/failed	$P = 0.052$	No
30 NTU-April 2017	vs	30 NTU-June 2019	Failed/failed	$P = 0.009$	Yes

N/A, not applicable since the normality test passed.

Table 3 | Paired *t*-test of comparative differences of recorded temperatures in negative control bottles at 0, 30, 100, and 200 NTU turbidity levels in two experiments

Paired *t*-test comparison of temperatures between				Normality/equal variance	Wilcoxon signed-rank test*	*P*	Significant difference ($P < 0.001$)
April 2017	0	vs	30 NTU	Passed/passed	N/A	<0.001	Yes
	0	vs	200 NTU	Failed/failed	Significant difference	<0.001	Yes
	30	vs	200 NTU	Passed/passed	N/A	<0.001	Yes
June 2019	0	vs	30 NTU	Passed/passed	N/A	<0.001	Yes
	0	vs	100 NTU	Passed/passed	N/A	<0.001	Yes
	30	vs	100 NTU	Passed/passed	N/A	<0.001	Yes

N/A, not applicable.
*Wilcoxon signed-rank test was conducted after failing the normality test.

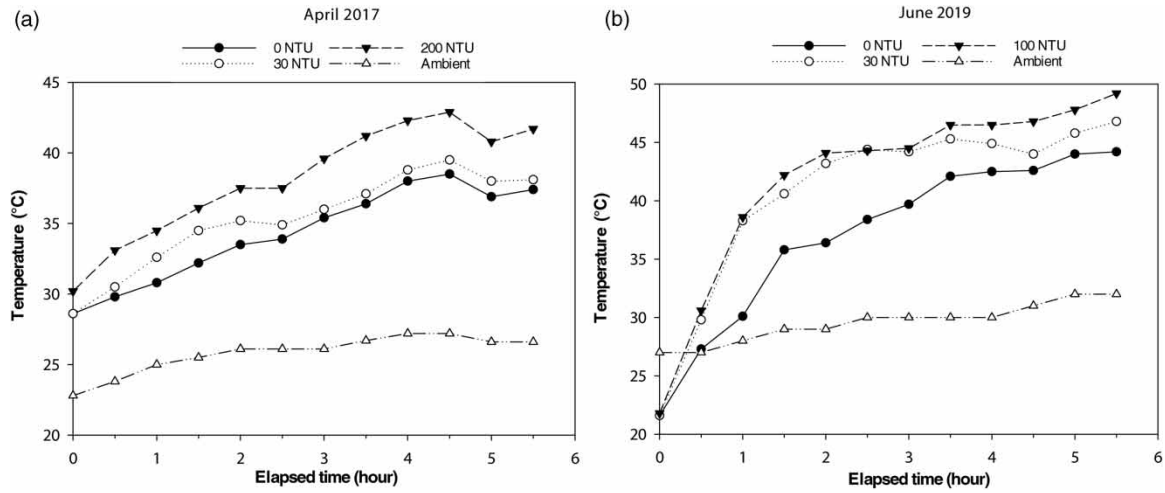


Figure 1 | Temperature changes with time in negative controls and ambient in two SODIS tests: (a) results for the first experiment in April 2017 and (b) results for the second experiment in June 2019.

in the bottle with the highest turbidity level (100 NTU), which verified our previous observation related to the relationship between temperature and turbidity noted prior. The maximum temperature reached in the 0 NTU bottle lasted for about 1 h at the end of the experiment.

Variation of UV radiation

Insolation values for UV radiation were recorded every hour and obtained from the University of Kentucky Weather Centre. Table 4 summarizes the average, maximum, and minimum values of UV radiation between 11 am and 4 pm in two experiments.

Statistical analysis with paired *t*-tests showed a significant difference ($P < 0.001$) between the rolling averages of UV radiation in two SODIS tests (Table 4). Although there was not a significant difference between temperatures reached in the 0 NTU negative control bottles for the two SODIS experiments as shown earlier in Table 2, the UV radiation levels were significantly different.

Figure 2 shows the variations of the rolling averages of the UV radiation during the times of study for two SODIS experimental events. The rolling averages were calculated by computing the average of three following UV data to provide better visual comparison between the UV intensity in two events. The value of the first rolling average for experiment in 2017 is 521.3 W/m^2 as shown in Figure 2. This value was calculated by averaging their UV radiation values at the first hour (284 W/m^2), the second hour (563 W/m^2), and the third hour (717 W/m^2) after starting the experiment (actual hourly UV radiation did not present here). The value of the second rolling average of 641.3 W/m^2 in 2017 was calculated by averaging

Table 4 | Maximum, minimum, and the average values of UV radiation in two experiments and results of paired *t*-test comparison of rolling averages for two SODIS experiments

UV radiation (W/m^2)				Paired *t*-test comparison of rolling averages of UV radiation				
Experiment date	Maximum	Minimum	Average	Comparison of rolling averages between			Normality/equal variance	Significant difference (*P*-value < 0.001)
20 April 2017	733	507	656	2017	vs	2019	Passed/passed	Yes
26 June 2019	962	703	848					

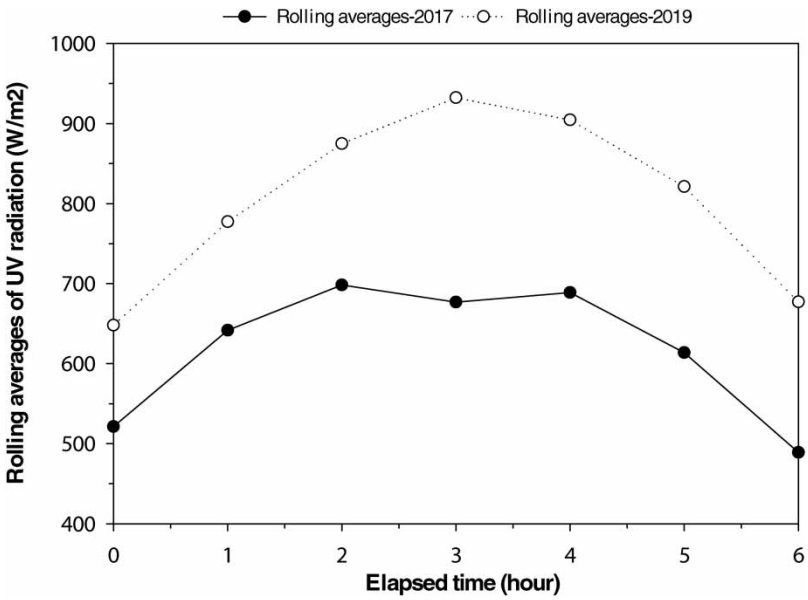


Figure 2 | Rolling averages of UV radiation in two experiments.

the UV radiation at the second hour (563 W/m^2), the third hour (717 W/m^2), and the fourth hour (645 W/m^2) after starting the test. With this procedure, other rolling averages were calculated during the experiments.

Results showed that the second SODIS event held in June had much more UV radiation available for bacterial inactivation. This was also verified by the paired *t*-test as summarized in Table 4. However, as one of the goals of this research project was to see if hygiene water could be produced at lower insolation and final temperatures, the first SODIS event provides an opportunity to explore non-optimal conditions.

Bacterial inactivation efficiency by SODIS

Based on the results of the first SODIS experiment, which had lower insolation and temperatures due to the April test date and cloud cover, the inactivation of the *E. coli* bacteria was impacted negatively as turbidity increased. The highest *E. coli* inactivation was achieved at 0 NTU turbidity levels (over 5.03 logs removal as bacterial counts were below detection), and the lowest inactivation was observed in the 200 NTU bottles with an average of 0.96 ± 0.08 logs reduction (Figure 3).

For 30 and 200 NTU turbidity levels, *E. coli* concentrations decreased by about 1 log of removal (95.31 and 89.04%, respectively). This showed the protective nature of turbidity that helped *E. coli* avoid inactivation by UV radiation. Clay particles reduced the antimicrobial effects of sunlight, changing the wavelength of solar insolation from UV to infrared, which is denoted by the increased temperature as turbidity increased. While UV was clearly effective in the absence of turbidity, the absence of strong UV penetration, combined with lower than pasteurization temperatures achieved, did not inactivate the thermally tolerant *E. coli* bacteria. For this model organism, UV irradiation was the dominant force for inactivation by SODIS.

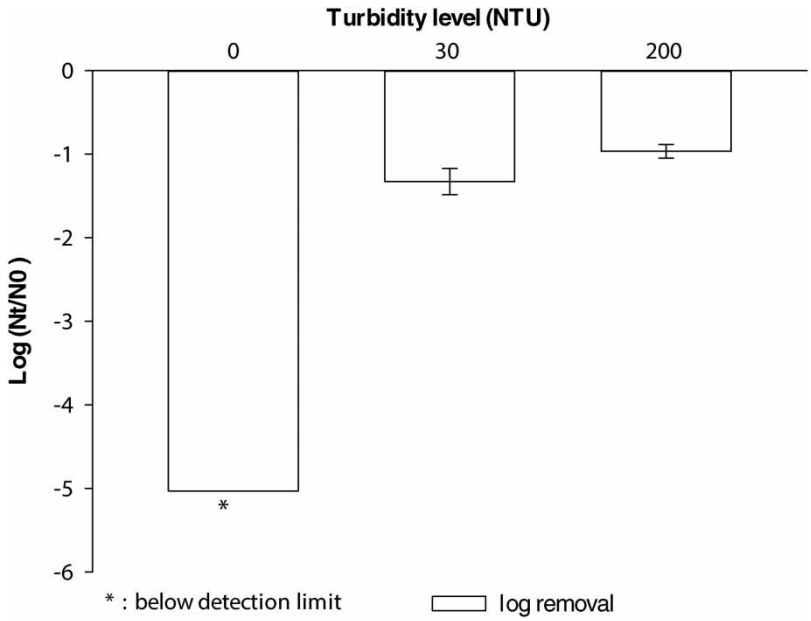


Figure 3 | *E. coli* removal by SODIS at three turbidity levels in April 2017.

Table 5 summarizes the results of Tukey's tests for multiple pairwise comparisons of the mean log removal between turbidity levels in the first SODIS experiment. Results showed significant differences among all turbidity levels ($P < 0.001$). So, while the absolute value of reduction is not the same between water at 30 and 200 NTU, the difference in reduction is statistically significant.

With the results from UV-dominated inactivation of thermo-tolerant *E. coli* bacteria in hand, a second SODIS event was planned that would utilize other bacteria that are known to cause post-partum infections related to hygiene and clinical conditions. Results from the second SODIS experiment with three different bacteria were similar to the results of the first SODIS experiment with respect to one of the indicator bacteria (*E. coli*) and for 0 NTU turbidity where removal to below detection limits was seen. However, 5 logs of inactivation (removal to below detection limits) were achieved in bottles containing clear water with 0 NTU turbidity level for all three indicator bacteria (Figure 4), emphasizing the importance of UV irradiation as a primary inactivation mechanism.

Furthermore, in 30 NTU bottles, 5 logs of removal occurred for *E. coli*, which was higher in comparison with the first experiment in 2017 (average 1.32 logs inactivation). This may be related to the higher maximum temperature achieved in the second experiment (46.8 > 39.50 °C), which is also related to the greater UV irradiation on that cloudless day in June. For the human-skin bacteria, *S. aureus* and *S. epidermidis*, an average of 4.95 and 4.83 logs of removal was achieved at 30 NTU level of turbidity, which was below the level of detection. However, when comparing the removals for *S. aureus* and *S. epidermidis* at 100 NTU, there was not a significant difference in the levels of removal as compared with 30 NTU bottles; removal was below detection levels. The number of bacteria was still below the detection limit and at 4.7 and 4.66 logs of removal for *S. aureus* and *S. epidermidis*, respectively. It is apparent that *S. aureus* is sensitive to temperature, so much so that the impact of UV irradiation could not be shown. Others have shown UV to be less effective on gram-positive organisms

Table 5 | One-way ANOVA comparison of *E. coli* removal by SODIS at three turbidity levels in April 2017

Comparison	Normality/equal variance tests	Difference of means (log removal)	Statistical difference ($P < 0.05$)
0 vs 200 NTU	Passed/passed	4.064	Yes (<0.001)
0 vs 30 NTU	Passed/passed	3.701	Yes (<0.001)
30 vs 200 NTU	Passed/passed	0.362	Yes (<0.001)

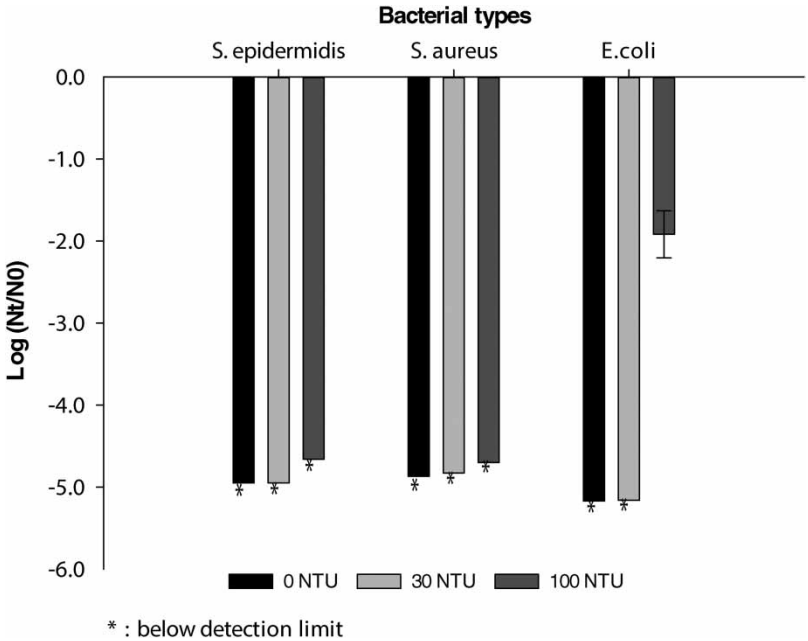


Figure 4 | Bacterial removal by SODIS at three turbidity levels in June 2019.

S. aureus and *Enterococcus faecium* to be more resistant to UV inactivation than *E. coli* (McKinney & Pruden 2012). However, it is clear that for *E. coli*, UV inactivation, not temperature may be the primary factor for disinfection.

The behavior of *E. coli* during SODIS of turbid waters was different than for the skin bacteria. For 100 NTU bottles, an average of 1.92 logs of inactivation was achieved for *E. coli*, which was double that seen in the first experiment (0.9604 log removal at 200 NTU). This increased inactivation happened at higher maximum temperatures in the second experiment (49.2 °C) in comparison with the first experiment (41.7 °C). *E. coli* can be cultured at temperatures of 45 °C and is known to survive at higher temperatures, so the increased temperature did not appear to be the primary causative effect for higher inactivation at lower turbidities, again highlighting the UV sensitivity of *E. coli*. These results for *E. coli* suggest that reaching a 7° higher temperature would not inactivate significantly more bacteria in equally turbid waters. In contrast, for both *S. epidermidis* and *S. aureus* (common human-skin bacteria), 99.997% bacterial inactivation was achieved at all turbidity levels. These results show that the gram-positive skin bacteria are not reacting similarly to the two SODIS inactivation factors (UV irradiation and temperature elevation) as the gram-negative *E. coli*. It appears that the skin organisms may be less impacted by UV irradiation, perhaps due to thick cell walls, and more impacted by raised temperatures, even at temperatures less than 50 °C. To discern the impact that temperature had on the bacteria used for these experiments from UV irradiation, it was decided to eliminate UV irradiation and focus on the impact of temperature of indicator bacteria spiked into clean, bottled water.

Thermal inactivation of selected indicator bacteria

Bacterial inactivation with thermal pasteurization was modeled at lab scale for 9.5 h (Figure 5). The bacterial survivals were different under thermal inactivation. For gram-positive bacteria, *S. epidermidis* and *S. aureus*, significant bacterial reductions happened during the time period of thermal exposure. However, the reduction was generally faster at 47 °C in comparison with 37 °C, especially for skin bacteria.

At 37 °C, the temperature the bacterial stocks had been cultured at initially, all bacteria showed a reduction in number at the first time point tested, likely due to death from osmotic shock as bottled water is not as salty and protein-rich as culture broth. *S. epidermidis* and *S. aureus* showed 0.28 and 0.10 log reduction at the first time point of 30 min. *E. coli* had a similar initial reduction as *S. aureus* (0.12 log reduction). Final reductions for the indicator bacteria revealed *S. epidermidis* to be inactivated to a much greater extent as compared to *E. coli* or *S. aureus* (0.82 versus 0.20 and 0.30 log removal, respectively)

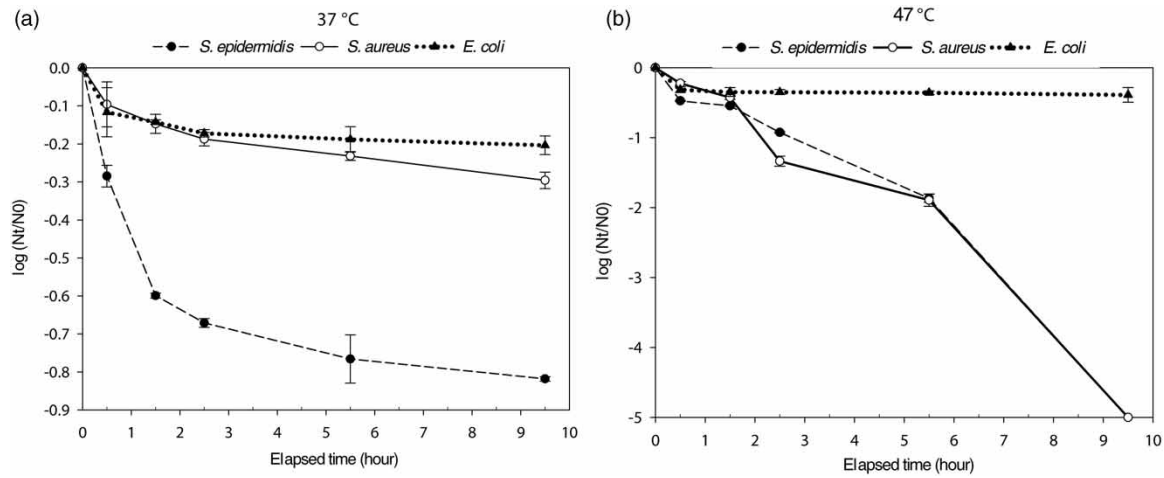


Figure 5 | Bacterial reduction in incubators with time: (a) at 37 °C and (b) at 47 °C.

at 9.5 h. Based on these results, thermal inactivation as the primary cause for the removal of two of three bacteria was not promising at temperatures seen in the first 3 h of the 0 NTU bottles during the SODIS experiments.

However, increasing the temperature to 47 °C demonstrated the differences in the indicator bacteria tolerances to higher temperatures. At 47 °C, the total inactivation of *S. aureus* and *S. epidermidis* happened sometime after 9.5 h. However, *E. coli* did not continue to decline in numbers after the first time point and just showed 0.38 log reduction after 9.5 h, with a very flat curve, which indicates thermotolerance of this strain of indicator *E. coli* bacteria.

DISCUSSION

Based on the observed results from the two different SODIS experiments in this study, SODIS of contaminated water is potentially feasible and effective at temperate latitudes (38°N) like Lexington, KY and also locations with similar latitude and climate outside of the defined favorable regions for SODIS (15–35°N and S). The feasibility of SODIS outside of the recommended regions was reported by Joyce *et al.* (1996) at 1° 29′S in equatorial climates and Davies *et al.* (2009) at 34°S (Joyce *et al.* 1996; Davies *et al.* 2009).

Joyce *et al.* (1996) demonstrated a correlation of *E. coli* inactivation with increasing temperature. Also, it was shown that it would be feasible to apply SODIS to turbid, 200 NTU water with temperatures of 55 °C (Joyce *et al.* 1996). However, our study did not show promising results for *E. coli* at 200 NTU at the lower temperatures we reached. The difference could be related to the location that they conducted their experiment or the time or climate conditions during their experiments. While Joyce *et al.* (1996) emphasized the thermal inactivation of the sun, Davies *et al.* (2009) highlighted the UV inactivation of the sun for the inactivation of *Enterococcus faecalis*, *Clostridium sporogenes* spores, and P22 bacteriophage (Davies *et al.* 2009). Based on their findings, SODIS was determined to be mainly due to UV radiation at a latitude of 34° S, which is in agreement with our observations for *E. coli* in this study. The reverse relationship between turbidity and bacterial inactivation was similar to another previous study that used *E. coli* as a process indicator (McGuigan *et al.* 1998). However, it must be kept in mind that SODIS has two mechanisms for microorganism inactivation, and both are of importance in achieving an acceptable product water.

The limitations of SODIS are related to the conditions of the raw water being treated, the microorganisms that need to be removed, and to the dominant mechanism of disinfection for those microorganisms. In highly turbid waters, where penetration of UV radiation is not achievable, reaching an appropriate temperature for thermal pasteurization is essential, especially based on the weather conditions in temperate latitudes. SODIS of turbid waters in temperate latitudes would require more solar exposure time to allow for the inactivation of organisms, regardless of thermal and UV sensitivities.

Higher inactivation levels were recorded in 30 NTU waters in our current study in comparison with the Dessie *et al.* (2014) study that used fecal coliforms, likely related to the differences in bacterial types, exposure times, and experiment locations of two different studies. As our study showed, skin bacteria were much more sensitive to temperatures at or above 37 °C, with *S.*

epidermidis being the most intolerant. Furthermore, our results supported observations of previous research related to the potential of SODIS to be effective in waters with turbidity levels less than 30 NTU (Rainey & Harding 2005; Dessie *et al.* 2014).

Regarding our current observations, thermo-tolerant indicator bacteria (*E. coli*) survived better at higher turbidity levels (100 and 200 NTU), even though higher temperatures were recorded in these turbid waters during sun exposure. It seems that turbidity gives an opportunity for thermo-tolerant bacteria to avoid UV irradiation and sustain their populations at mildly elevated temperatures for significant periods of time. These observations were in agreement with those of Joyce *et al.*'s (1996) findings about a very small chance of the UV penetration into turbid waters. In turbid waters, clay particles act as a black body and absorb about 99% of the UV radiation before it can affect bacteria. Consequently, thermal inactivation (pasteurization) will be dominant in turbid waters, and effective at inactivating bacteria only if water temperatures reach to 45 °C or more (Joyce *et al.* 1996; McGuigan *et al.* 1998).

The effect of SODIS on *S. aureus* (a pathogenic, often antibiotic-resistant, human-skin microorganism) was investigated for the first time in this study. There is growing awareness of the importance of environmental pathways for transmission of *S. aureus*, and sewage-contaminated waters have the potential to spread this opportunistic pathogen via the water route. This investigation assessed the potential application of SODIS to purify sewage-contaminated surface waters to a point, where they could be used for hygiene purposes in developing countries with water-stressed and insecure healthcare centers.

Other studies have shown that health benefits can be obtained from providing more available water, or water to support handwashing (42–47% reduction in diarrheal morbidity), instead of focusing on the provision of a small amount of water of very high quality for ingestion (Curtis & Cairncross 2003). The provision of solar disinfected surface waters could reduce the health impact of transmitted pathogens and improve the overall days of health in a community, even if that water does not meet potable standard for *E. coli* inactivation. The differential inactivation of known skin pathogens leads to a need to define water quality criteria, and indicator organisms, tailored for hygiene water uses. More research is required, along with clinical studies, but the opportunity to control common wound pathogens in hygiene water is clear.

Assessing the sensitivity of *S. epidermidis*, *S. aureus*, and *E. coli* to SODIS and pasteurization showed that *E. coli* is more resistant than the two gram-positive, human-skin bacteria. It could be that the thick layer of peptidoglycan in the cell wall around gram-positive staphylococcus species may protect them against the sun's energy, while their adaptation to growing on a cooler skin habitat results in greater thermal susceptibility in comparison with gram-negative *E. coli*. This current result correlates with those published by Boyle *et al.* (2008) from findings in Spain. Their study documented that gram-negative *E. coli* needed more exposure time (more than 1 hour) to be removed in comparison with gram-positive *S. epidermidis*, concurrent with our findings (Boyle *et al.* 2008). However, the shorter time frame that they presented in their research was totally different from the times that we had available for our study, which could be related to the higher solar radiation intensity in their study (1,050 versus 848 W/m^2). Also, our results for bacterial removal without UV radiation in a dark incubator at 47 °C verified that *E. coli* has higher thermal tolerance and was inactivated at a slower rate than the gram-positive bacteria, *S. aureus* and *S. epidermidis*. The thermal resistance of *E. coli* was reported by Spinks *et al.* (2006). However, they showed that *E. coli* is not more thermally resistant than *E. faecalis* and *Shigella sonnei* (Spinks *et al.* 2006). It is suggested that further studies, which isolate the UV inactivation from thermal inactivation during SODIS, and which utilize indicators for the most commonly identified wound pathogens, should be done to assure the utility of SODIS for hygiene water production.

Our SODIS and elevated temperature results showed less exposure time at elevated temperatures were required for removing *S. epidermidis* compared to *E. coli* similar to the findings in Boyle *et al.* (2008). However, research on the emerging wound pathogen, community-acquired, methicillin-resistant, *S. aureus* infections, is lacking. To date, there has not been a published body of research focused on SODIS inactivation of *S. aureus* in water treatment schemes in comparison with *E. coli*. Most published SODIS research studies are interested in microorganisms that cause intestinal infections, not wound infections. However, specific process evaluation and quality indicator bacteria should be determined based on the final use of the water. In much the same way as the meat processing industry has created cocktails of bacteria relevant to different meat processing techniques, the water industry needs to create appropriate process and quality indicator schemes, and standards that are more specific to hygiene water. In this way, the use of SODIS could be expanded and used to augment hygiene water supplies in underserved areas, reducing post-partum infections, and morbidity and mortality rates.

Based on our findings in the current study, at the average water quality conditions in Kentucky surface water sources, like the Kentucky River where during dry summer months the levels of fecal coliforms are consistently less than 200 CFU/100 mL (Nieman & Brion 2003), SODIS could be an effective method of hygiene water treatment for populations lacking access to

piped water during late spring and summer (e.g., homeless and campers) as no less than 95% removal of *E. coli* was seen in 30 NTU water with maximum temperatures not reaching 40 °C in April under cloudy skies. Treatment of this river water at the conditions seen in April would result in the removal of fecal coliform bacteria to levels under 10 CFU/mL, and on hotter days more than 99.999% would be predicted. Even when solar insolation is not strong, as long as temperatures of 47 °C could be reached for over 4 h inside the bottles, regardless of turbidity, then a complete removal of *S. aureus* and *S. epidermidis* could be expected. However, the turbidity of water is critical for the removal of organisms that are thermally tolerant and inactivated primarily by UV irradiation. As such, SODIS for hygiene water would not be recommended for waters with turbidities greater than 30 NTU, even if the turbidity causes a rise in temperature as the incomplete inactivation of *E. coli* at the higher turbidities was demonstrated in this study. SODIS for hygiene water treatment should not be applied when turbidities are too high, or solar insolation levels are too low. However, the results of this study show that SODIS has the potential to be used to create hygiene water outside of the recommended equatorial latitudes if certain limitations are kept in mind.

As to how SODIS might work for those birthing centers lacking hygiene water mentioned prior, we suggest a scheme where raw water, from either low-cost local sources, or that prior patients had brought in with them from their water sources, be continually processed in plastic bottles when the conditions are appropriate, and then stored until needed. The water used to bathe a new mother and child may not be from the water they brought into the clinic, but from a prior patient, so that adequate time is allowed to provide for both UV and thermal inactivation of potential pathogens in hauled water. If cultural fit demands the use of the patient's own home water instead of using SODIS treated water brought in by a prior patient, pre-birth SODIS training and bottle kits can be provided to prospective mothers so that the hygiene water can be pretreated at home and arrive sealed in plastic bottles free from recontamination. The use of raw water sources without the intervention of a water vendor could reduce costs for both the centers and the patients, allowing for birth without economic shame.

CONCLUSIONS

We want to emphasize the potential for the application of inexpensive, low-tech, SODIS treatment to inactivate wound pathogens in raw water, especially skin microorganisms, and produce acceptable hygiene water in non-equatorial regions at certain times of the year. SODIS was demonstrated to be effective in the elimination of *S. aureus* and *S. epidermidis* in turbid waters (30 and 100 NTU) with a maximum ambient temperature of only 25–30 °C. The application of SODIS treatment of hygiene waters is proposed, especially to augment the scarce and insecure supplies available to healthcare centers in regions without access to safe, reliable, piped water sources (African countries) in support of clinical services. Even SODIS purification of harvested rainwater could be an economic solution to water requirements of healthcare centers in poor areas and save days of human life lost to illness or premature mortality. Furthermore, the required exposure time to sunlight should be adjusted based on the target usage of water (drinking, hygiene, and washing) and weather conditions, but appropriate levels of pathogen destruction for hygiene purposes appear to be possible, even in temperate latitudes.

The consideration of fecal coliforms and *E. coli* as a process and quality indicator organism for drinking water is reasonable to prevent diarrhea from water ingestion. However, considering only *E. coli* as a process and quality indicator for processes like SODIS to produce water for hygiene and washing purposes may be over-stringent and underpredict the utility and appropriateness of those technologies, thereby limiting their application due to differences in thermal and UV irradiation sensitivities. More investigations are required to estimate all the design and control parameters for SODIS treatment of water for non-potable usages, as well as to evaluate cultural acceptance and reliance of water provision schemes for the preparation and provision of additional, economical, and alternative sources of purified water in communities under water stress.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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