

## Blue 470-nm Light Kills Methicillin-Resistant *Staphylococcus aureus* (MRSA) *in Vitro*

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### Abstract

**Background Data:** In a previous study, we showed that 405-nm light photo-destroys methicillin-resistant *Staphylococcus aureus* (MRSA). The 390–420 nm spectral width of the 405-nm superluminescent diode (SLD) source may raise safety concerns in clinical practice, because of the trace of ultraviolet (UV) light within the spectrum. **Objective:** Here we report the effect of a different wavelength of blue light, one that has no trace of UV, on two strains of MRSA—the US-300 strain of CA-MRSA and the IS-853 strain of HA-MRSA—*in vitro*. **Materials and Methods:** We cultured and plated each strain, and then irradiated each plate with 0, 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 25, 30, 35, 40, 45, 50, 55, or 60 J/cm<sup>2</sup> of energy a single time, using a 470-nm SLD phototherapy device. The irradiated specimens were then incubated at 35°C for 24 h. Subsequently, digital images were made and quantified to obtain colony counts and the aggregate area occupied by bacteria. **Results:** Photo-irradiation produced a statistically significant dose-dependent reduction in both the number and the aggregate area of colonies formed by each strain ( $p < 0.001$ ). The higher the dose the more bacteria were killed, but the effect was not linear, and was more impressive at lower doses than at higher doses. Nearly 30% of both strains was killed with as little as 3 J/cm<sup>2</sup> of energy. As much as 90.4% of the US-300 and the IS-853 colonies, respectively, were killed with an energy density of 55 J/cm<sup>2</sup>. This same dose eradicated 91.7% and 94.8% of the aggregate area of the US-300 and the IS-853 strains, respectively. **Conclusion:** At practical dose ranges, 470-nm blue light kills HA-MRSA and CA-MRSA *in vitro*, suggesting that a similar bactericidal effect may be attained in human cases of cutaneous and subcutaneous MRSA infections.

### Introduction

**S**TAPHYLOCOCCI ARE GRAM-POSITIVE BACTERIA that form grape-like clusters upon Gram's staining. They are non-motile, aerobic, and facultatively anaerobic;<sup>1</sup> moreover, they are capable of prolonged survival on a variety of environmental surfaces. *Staphylococcus aureus* (*S. aureus*) is the most virulent of the many staphylococcal species and is responsible for infections ranging from superficial skin and soft tissue infections to those that are systemic and life-threatening.<sup>1</sup> *S. aureus* is part of the normal human flora, colonizing the anterior nares, skin, vagina, axilla, perineum, and oropharynx. These sites of colonization act as reservoirs for future *S. aureus* infections.<sup>1</sup>

In the last few decades, *S. aureus* strains resistant to semi-synthetic penicillins, such as methicillin, have emerged in both nosocomial and community environments.<sup>2,3</sup> Two

prominent strains of methicillin-resistant *S. aureus* (MRSA) have been well studied: hospital-acquired methicillin-resistant *S. aureus* (HA-MRSA) and community-acquired *S. aureus* (CA-MRSA).<sup>2–8</sup> Unlike HA-MRSA, which seems to be limited to clinical settings, CA-MRSA has been found in more common environments, such as computer keyboards,<sup>9</sup> that were never before believed to harbor such deadly bacteria. Moreover, outbreaks of CA-MRSA have been reported in schools, locker rooms, sporting arenas, and other locations in which sports enthusiasts and athletes congregate.<sup>4,10–13</sup> The CA-MRSA strain is clearly distinct from the HA-MRSA strain, and infections with CA-MRSA have been reported in rural and urban settings in individuals with no previous exposure to medical environments. Moreover, the median age for CA-MRSA infection is 23 y,<sup>3</sup> and the corresponding age for HA-MRSA is 68 y.<sup>2,3,8,11</sup> While most individuals who develop *S. aureus* infections do so with their own colonizing

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strains, reports have shown that MRSA may be acquired from other people and fomites.<sup>2,12</sup>

Treatment of *S. aureus* infections has become increasingly difficult, as available medications have had limited success in combating the disease and stemming outbreaks of infection. Efforts have been made to develop new drugs, but even the newest antibiotics have had limited success in controlling the spread of MRSA. Estimates indicate that two billion people carry some strain of *S. aureus* worldwide, and of these 53 million have MRSA, usually in their nasal cavities.<sup>2,6,7</sup> Currently fewer than 5% of *Staphylococcus* strains remain susceptible to penicillin. In response to these increasing virulence factors, new pharmaceutical targets within the bacterial genome have been studied, resulting in the use of semisynthetic penicillinase-resistant penicillins, such as methicillin, in the treatment of these penicillin-resistant isolates. Even then, 40–50% of *S. aureus* isolates remain resistant to methicillin,<sup>1</sup> underscoring the need to find new ways to prevent increased resistance and limit outbreaks of disease.

Blue light phototherapy appears to be a promising alternative approach to eradicating MRSA, given the responses of other bacteria to blue light.<sup>14–17</sup> Papageorgiou et al.<sup>14</sup> have shown that *Propionibacterium acne*, the bacteria that causes acne, responds to blue light. They reported significant improvements in patients with acne vulgaris following treatment with combined blue (415 nm) and red (660 nm) light, and attributed their findings to the potential antibacterial and anti-inflammatory effects of the blue and the red light sources, respectively. In a recent report, Guffey and Wilborn<sup>15</sup> examined the effects of 405-nm and 470-nm light on two common aerobes, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, and the anaerobe *P. acnes*, *in vitro*. They found both wavelengths to be bactericidal, but the kill rate was higher with the 405-nm light source than the 470-nm light source, which had a 90% kill rate for *S. aureus* and a 95.1% kill rate for *P. aeruginosa*. However, neither wavelength was effective on the anaerobe *P. acnes*.<sup>14</sup> More recently, Lipovsky et al.<sup>18</sup> demonstrated that high-intensity broad-spectrum polychromatic light with wavelengths in the range of 400–1000 nm kills bacteria in infected diabetic ulcers.

In our pioneering studies,<sup>19,20</sup> we showed that 405-nm light kills both HA-MRSA and CA-MRSA *in vitro*. The effect was dose-dependent, with maximum eradication rates of 92–94% of each type of bacteria occurring within 8–10 min of irradiation. Our light source had a spectral width of 390–420 nm, with 405-nm peak emission. Therefore it had a trace of ultraviolet (UV) light, to which some of its bactericidal action could be attributed. This amount of UV light may raise safety concerns in certain types of patient care situations, even though it is small, low in intensity, and less than the amount received from several minutes of exposure to sunlight. The UV light can be filtered from the 405-nm light source; however, doing so removes some of the advantages of using commercially available superluminous diodes (SLDs), namely their ubiquity, ease of use, and relatively low cost. To overcome this concern, we sought to assess the potential bactericidal effect of a 470-nm SLD light source on HA-MRSA and CA-MRSA *in vitro*. With a spectral width of 455–485 nm, the energy emitted by this SLD contains no UV light.

## Materials and Methods

### Bacterial culture

We cultured two strains of MRSA. The IS-853 strain was obtained from Winthrop University Medical Center, Mineola, New York, and the US-300 strain was obtained from the New York Medical College, Valhalla, New York. These strains represent HA-MRSA and CA-MRSA, respectively, and were identified by standard identification procedures, including Gram's staining, hemolytic patterns seen on blood agar, and catalase and coagulase production. As detailed in our previous reports,<sup>19,20</sup> each strain was separately diluted to a cell count of  $5 \times 10^6$ /mL in 0.9% normal saline. Then they were volumetrically streaked onto round 35-mm plates of tryptic soy agar before being irradiated with the light source.

### Photo-irradiation

A Dynatron Solaris<sup>®</sup> 708 device (Dynatronics Corp., Salt Lake City, Utah, USA) fitted with a 470-nm light probe was used to irradiate the bacteria. The 5.0-cm<sup>2</sup> applicator with its cluster of 32 SLDs emits blue light with a peak at 470 nm (spectral width = 455–485 nm), 150 mW average power, and 30 mW cm<sup>-2</sup> irradiance. To minimize thermal radiation, the applicator is cooled by an built-in fan positioned to dissipate any heat produced by the diodes. In preliminary studies, we ascertained that the device did not generate any measurable temperature rise within the range of fluences used in this study. To ensure even irradiation of each plate, we used 5.0-cm<sup>2</sup> culture plates, which were the same size as the surface area of the applicator, which was clamped at a distance of 1–2 mm perpendicularly above each open plate. As each dose was selected, the treatment time was automatically computed by the Solaris device to ensure that the bacteria were irradiated with 0, 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 25, 30, 35, 40, 45, 50, 55, or 60 J/cm<sup>2</sup> of energy fluence. For example, to attain a fluence of 3 J/cm<sup>2</sup>, the Solaris device calculated that 100 sec of irradiation was needed, and the device automatically shut down after 100 sec had elapsed. Each culture was irradiated only once. Afterward, the bacteria were incubated at 35°C for 24 h.

### Quantification of bacterial colonies and data analysis

Standard digital images of plates with bacteria colonies were taken, scanned into the computer, and then the colonies were quantified with Sigma Scan Pro 5 software (Systat Software, Inc., Point Richmond, CA, USA). The colony counts and the aggregate area occupied by the colonies were then automatically computed and subjected to statistical analysis. The experiment was repeated several times; four times with the US-300 strain and five times with the IS-853 strain, to ensure accurate results. Descriptive data were generated, then analysis of variance (ANOVA) was performed with SPSS Version 14 statistical software (SPSS Inc., Chicago, IL, USA) to test the null hypotheses, namely: (1) there were no differences in the colony counts and aggregate area of bacteria at the fluences tested, and (2) the effect of irradiation did not differ between the two strains.

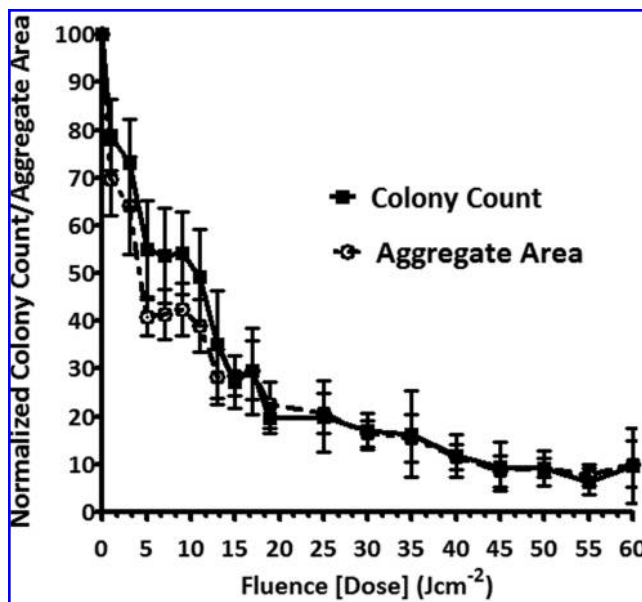


FIG. 1. Effect of 470-nm light on colony count and aggregate colony area of the US-300 strain of MRSA.

**Results**

Photo-irradiation with 470-nm light produced a statistically significant dose-dependent reduction in both the number and the aggregate area of colonies formed by each strain of bacteria ( $p < 0.001$ ; Figs. 1, 2, and 3). Higher dosages resulted in progressively greater eradication of each strain. However, the effect occurred as a non-linear exponentially decreasing curve, with greater dose-to-dose improvement at lower dose ranges than at higher dose ranges. Whereas 50% of each strain was killed with approximately 12 J/cm<sup>2</sup> fluence, up to 35 J/cm<sup>2</sup> fluence was needed to eradicate about 80% of the bacteria. Moreover, 60 J/cm<sup>2</sup> did not kill 100% of the bacteria, as might have been expected from the impressive effects of the lower doses (Table 1 and Figs. 1–3).

On average, 90.4% of the US-300 and the IS-853 colonies, respectively, were killed with a dose of 55 J/cm<sup>2</sup>, which appeared to be the most optimal dose of all doses tested. This same dose eradicated 91.7% and 94.8% of the aggregate area of the US-300 and the IS-853 strains, respectively. There was no statistically significant difference in the effect of 470-nm light on the two strains of bacteria at any dose.

**Discussion**

Despite the development of stronger antibiotics to combat MRSA infections, outbreaks of the disease have been on the rise, as effective remedies for common strains remain elusive.<sup>21–23</sup> Of great concern is the frequent outbreaks of CA-MRSA, which accounts for an ever-increasing proportion of MRSA cases seen in the United States and other countries.<sup>2,21–23</sup> CA-MRSA differs from HA-MRSA in its genetic and antibiotic susceptibility profile.<sup>24</sup> Moreover, it is strongly linked with the virulence factor Panton-Valentin leukocidin, a toxin that is associated with an increased risk of invasive disease, as well as skin and subcutaneous tissue

infections. Its staphylococcal cassette chromosome mec type encodes for penicillin-binding protein (PBP2a), which is not inhibited by  $\beta$ -lactam antibiotics.<sup>24</sup> As a result, medications such as the oral cephalosporins, fluoroquinolones, tetracyclines, trimethoprim-sulfamethoxazole, and erythromycin, as well as the semisynthetic forms of penicillin, such as nafcillin, have been minimally effective in combating CA-MRSA infections. Even vancomycin, our last well-proven antibiotic line of defense against the bacterium, is now meeting resistance. MRSA continues to evolve genetically,<sup>25</sup> making it difficult to find an effective pharmacological remedy. Thus an effective treatment must not just eradicate current strains of the bacteria, but future genetic variants as well. The fact that 470-nm blue light eradicates the two genetically different strains of MRSA we tested in a single treatment session indicates that phototherapy may be a viable alternative to drug treatment, and that it also has the potential to kill future variant strains of MRSA. Our statistical analysis showed that both strains were killed with equal efficacy, suggesting a common mechanism for the effects seen on the two strains.

The precise mechanism behind the photo-eradication of MRSA is beyond the scope of this study. However, it is noteworthy that as early as 1930, Gates<sup>26</sup> showed that at increasingly higher fluences, light with wavelengths longer than 400 nm can kill bacteria as does UV light. Irradiation with UV photo-destroys bacteria and other pathogens because the light energy is absorbed by the pyrimidine bases of DNA such as thymidine and cytosine. The absorbed energy opens the bond, allowing the UV-modified base to react with nearby bases, thereby altering the structural conformation of the bases. The photo-irradiated cell dies when the resulting rate of DNA damage exceeds the rate of repair.<sup>26,27</sup> It is possible that blue light photo-damages DNA, resulting in its bactericidal effect on MRSA and other bacte-

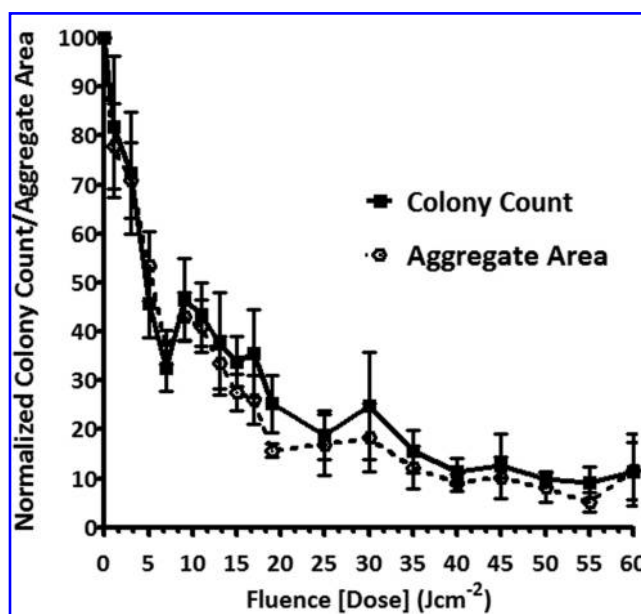


FIG. 2. Effect of 470-nm light on colony count and aggregate colony area of the IS-853 strain of MRSA.

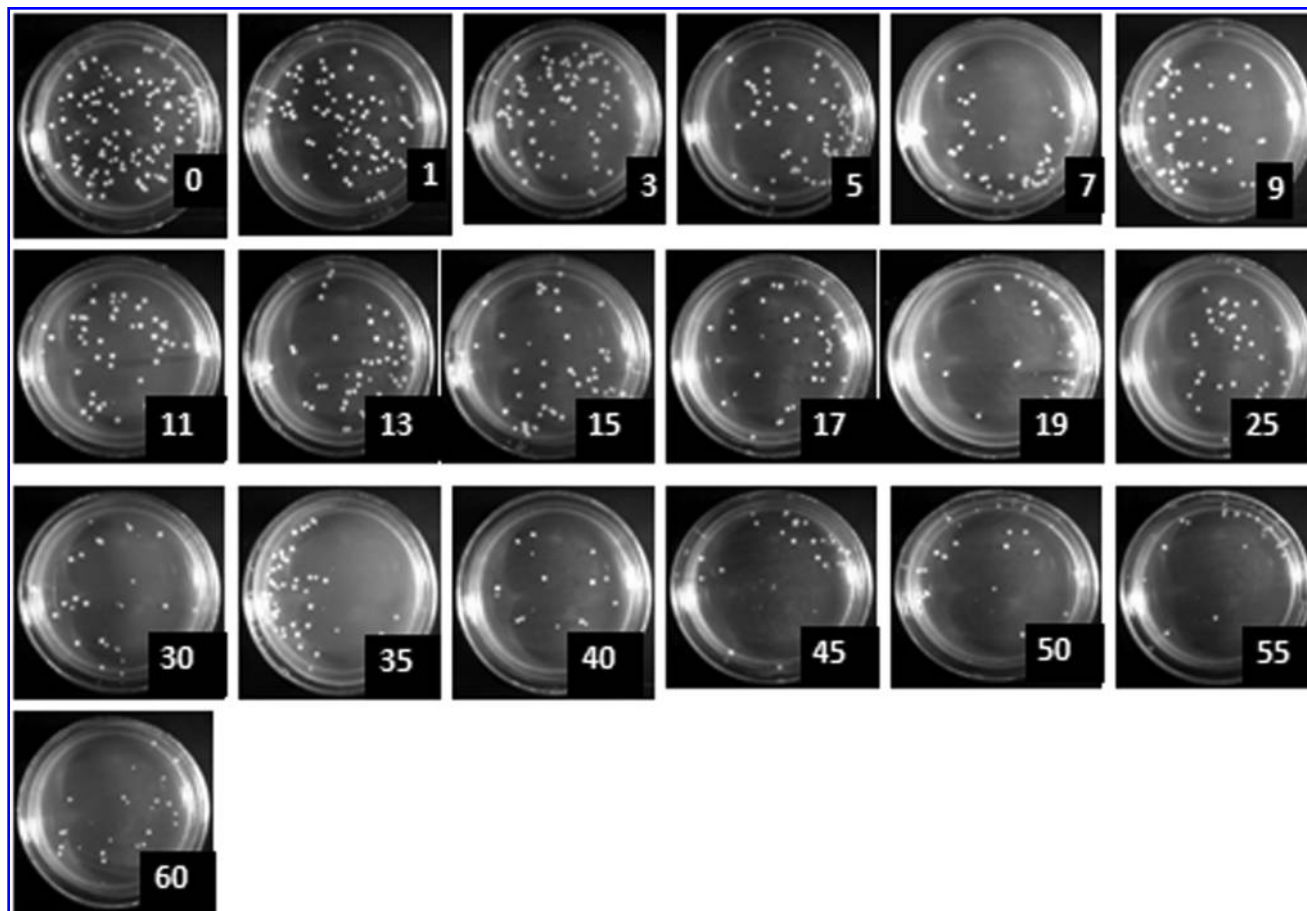


FIG. 3. Representative photographs showing colonies of HA-MRSA (IS-853 strain) irradiated with 470-nm blue light. The number assigned to each photo represents the irradiation dose ( $\text{J}/\text{cm}^2$ ). Panel 0 shows a control plate, that received no irradiation, and the one in the bottom row shows the results of the highest dose tested ( $60 \text{ J}/\text{cm}^2$ ).

ria, such as *Propionibacterium acne*,<sup>14,17,28</sup> and *Pseudomonas aeruginosa*,<sup>15</sup> even though the peaks of absorption of pyrimidine bases are known to lie outside the blue spectrum; thus other mechanisms may be involved. For example, photodynamic inactivation of the bacteria through excitation of intracellular porphyrins cannot be ruled out, given a recent report that showed that bacterial eradication diminishes with oxygen depletion.<sup>29</sup> Further studies are needed to uncover the precise mechanisms involved in the effects of blue light on bacteria.

As shown in the table, about one-third of the US-300 strain and nearly the same amount of the IS-853 strain was killed with as little as  $3 \text{ J}/\text{cm}^2$  of energy (i.e., 100 sec of irradiation). Similarly, more than 40% and 60% of the respective strains were photo-destroyed at  $7 \text{ J}/\text{cm}^2$ , with the eradication of over 80% of each strain occurring at  $35 \text{ J}/\text{cm}^2$ . These significant levels of photo-destruction at low dosages indicate that irradiation with 470-nm LED light energy may be a practical, inexpensive alternative to treatment with pharmacological agents, particularly in cases involving cutaneous and

TABLE 1. THE BACTERICIDAL EFFECTS OF A SAMPLE OF DOSES ON THE TWO STRAINS OF MRSA TESTED

Dose ( $\text{J}/\text{cm}^2$ )	US-300 MRSA killed $\pm$ SEM (%)		IS-853 MRSA killed $\pm$ SEM (%)	
	Colony count	Aggregate area	Colony count	Aggregate area
0	0	0	0	0
3	$34.1 \pm 6.71$	$29.5 \pm 6.82$	$27.6 \pm 12.51$	$29.1 \pm 7.78$
7	$48.0 \pm 4.26$	$40.9 \pm 9.85$	$67.3 \pm 4.79$	$63.2 \pm 3.54$
11	$61.2 \pm 4.17$	$49.2 \pm 9.39$	$56.4 \pm 6.53$	$58.9 \pm 5.37$
35	$80.7 \pm 7.82$	$80.1 \pm 8.64$	$84.5 \pm 4.38$	$87.8 \pm 4.35$
55	$90.4 \pm 5.60$	$91.7 \pm 2.72$	$90.4 \pm 3.24$	$94.8 \pm 1.96$
60	$90.6 \pm 5.66$	$89.2 \pm 7.98$	$88.5 \pm 5.91$	$88.3 \pm 7.35$

Only doses relevant to the discussion in this paper are shown in this table. The effects of the other doses are shown in Figs. 1 and 2.

subcutaneous MRSA infections that are susceptible to non-invasive types of irradiation.

This study and our previous reports<sup>19,20</sup> on the effects of 405-nm blue light on MRSA were done sequentially and not in parallel. Consequently, our ability to compare findings with 405 and 470 nm wavelengths is limited, but it is noteworthy that both wavelengths killed the two genetically different strains of MRSA in the same non-linear fashion, with impressive eradication of bacteria occurring at lower dose ranges, and higher doses resulting in progressively greater eradication of each strain. But the 470-nm light does so without the attendant dangers of UV irradiation. As noted in the introduction, Guffey and Wilborn<sup>15</sup> showed that 405-nm and 470-nm light photo-destroy two common aerobes, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, *in vitro*, but not anaerobic *P. acnes*. By measuring colony counts alone, they showed that the kill rate was slightly higher with 405-nm wavelength than the 470-nm wavelength. Our results are consistent with this and other reports,<sup>14,17,19,20</sup> which indicate that blue light is bactericidal.

### Conclusion

We conclude that 470-nm light kills HA-MRSA and CA-MRSA *in vitro*, suggesting that a similar effect may be achieved *in vivo* in human cases of MRSA infection, particularly in cutaneous and subcutaneous cases of MRSA infections that are susceptible to non-invasive types of irradiation.

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### Disclosure Statement

No conflicting financial interests exist.

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