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Optimization of the Antimicrobial Effect of Blue Light on Methicillin-Resistant *Staphylococcus aureus* (MRSA) *In Vitro*

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Abstract

Background and Objective—In previous studies, we showed that irradiation with 405 nm or 470 nm light suppresses up to 92% methicillin-resistant *Staphylococcus aureus* (MRSA) growth *in vitro* and that the remaining bacteria re-colonize. In this study, the aim was to develop a protocol that yields 100% MRSA growth suppression.

Materials and Methods—We cultured 3×10^6 and 5×10^6 CFU/ml USA300 strain of MRSA and then irradiated each plate with varying fluences of 1–60 J/cm² of 405 nm or 470 nm light, either once or twice at 6 hours intervals. Next, we plated 7×10^6 CFU/ml and irradiated it with 45, 50, 55, or 60 J/cm² fluence, once, twice, or thrice at the same 6 hours intervals. In a third experiment, the same culture density was irradiated with 0, 165, 180, 220, or 240 J/cm², either once, twice, or thrice.

Results—Irradiation with either wavelength significantly reduced the bacterial colonies regardless of bacterial density ($P < 0.05$). At 3×10^6 CFU/ml density, nearly 40% and 50% growth of MRSA were suppressed with as little as 3 J/cm² of 405 nm and 470 nm wavelengths, respectively. Moreover, 100% of the colonies were suppressed with a single exposure to 55 or 60 J/cm² of 470 nm light or double treatment with 50, 55, or 60 J/cm² of 405 nm wavelength. At 5×10^6 CFU/ml density, irradiating twice with 50, 55, or 60 J/cm² of either wavelength suppressed bacterial growth completely, lower fluences did not. The denser 7×10^6 CFU/ml culture required higher doses to achieve 100% suppression, either one shot with 220 J/cm² of 470 nm light or two shots of the same dose using 405 nm.

Conclusion—The bactericidal effect of blue light can be optimized to yield 100% bacterial growth suppression, but with relatively high fluences for dense bacterial cultures, such as 7×10^6 CFU/ml.

Keywords

antimicrobial blue light; bacteria suppression; methicillin-resistant *Staphylococcus aureus*; phototherapy

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INTRODUCTION

Since 1993 when the first case of community associated MRSA (CA-MRSA) was reported from a remote part of Western Australia with no hospital facility [1], pandemic strains of CA-MRSA have been found on beaches, computer keyboards, locker rooms, schools, athletic fields, and other locations involving human congregations [2,3]. Estimates suggest that MRSA infection now accounts for 44% of all hospital associated infections in the United States [4], and CA-MRSA has been reported in as many as 92% of persons hospitalized for MRSA infection. With the prevalence rate higher in 2010 compared to 2006, a majority of health care facilities in the United States have now heightened surveillance testing [5]. While the proportion of patients with hospital associated MRSA (HA-MRSA) has decreased, clinical cases of CA-MRSA have been on the rise [5–7], with pulsed-field type USA300 strain often isolated during community outbreaks [8,9].

The main form of treatment to date remains antibiotic therapy, even though 40–50% of *Staphylococcus aureus* isolates are now resistant to methicillin and less than 5% of staphylococci strains are known to be susceptible to penicillin. Moreover, uncontrolled use of antibiotics continues to worsen the situation [10,11], prompting the exploration of alternative treatment approaches. Other therapies currently under investigation include: the use of antibacterial clay [12], combination of honey and antibiotics [6], hyperbaric oxygen [13,14], photodynamic therapy (PDT) [15], and blue light phototherapy [16–20].

It has been reported that mineral leachates, such as ions of copper, iron, cobalt, nickel, and zinc, from certain varieties of clay have antibacterial properties against *Escherichia coli* and MRSA [12]. These findings support the time tested use of clay for wound care referred to in the 5,000-year-old ancient tablets of Nippur [21] and in the Ebers Papyrus written circa 1,600 BCE where clay was reportedly used as a therapeutic modality for wounds and abscesses as well as gastrointestinal diseases [22]. The ubiquitous and inexpensive nature of clay makes clay therapy a promising alternative to antibiotic treatment for certain cases of MRSA infection; however, the mechanism involved remains unknown and corroborative studies are sparse. Another form of treatment, photodynamic therapy (PDT) has been used beneficially to treat dermatological and ophthalmologic disorders [18,23]. Even though the excitation light used in PDT is itself harmless, particularly in the near infrared (NIR) and visible ranges [23–25], interest in PDT as an antimicrobial agent has waned because available photosensitizers are often non-targeted and known to cause serious side effects.

After our experiments in which we successfully eradicated two strains of MRSA with 405 nm and 470 nm light *in vitro* [16,17], we proposed a paradigm shift in favor of light as antimicrobial therapy for topical cases of MRSA infection. Since then, our group and others have shown that 405 nm, 415 nm, and 470 nm blue light suppress growth in cultures of *Staphylococcus aureus* (both MRSA and Methicillin-sensitive *Staphylococcus aureus* [MSSA]), *E. coli*, and other bacterial pathogens [16,17,19,26–31]. In further studies, we showed that the bactericidal effects of both 405 nm and 470 nm light on MRSA are commensurate, suppressing as much as 92% of standard 5×10^6 CFU/ml cultures [17]. More recently, we found that the remaining bacteria not cleared by 405 nm or 470 nm light can re-colonize and develop into larger viable colonies, and that a larger percentage of the

bacteria is not eradicated when denser cultures—intended to mimic severe bacterial infection—i.e., 7×10^6 CFU/ml to 12×10^6 CFU/ml, are plated [20]. The latter finding suggests a need to explore the development of a protocol that could yield 100% bacterial suppression.

Thus, this study was conducted as an attempt to optimize the antimicrobial effect of both 405 nm and 470 nm light by exploring the use of a variety of irradiation protocols in order to achieve 100% suppression of MRSA growth *in vitro*. Developing a laboratory-based protocol that results in 100% bacterial suppression could be of immense benefit in the ongoing effort to advance the use of commonly available blue Light emitting diodes (LEDs) to eradicate MRSA infection.

MATERIALS AND METHODS

Bacterial Culture

USA300 strain of CA-MRSA, obtained from ATCC (ATCC® BAA-1680), was used in our experiments because it is the most common isolate in community outbreaks of MRSA [8,9]. The strain was identified by standard identification procedures, including Gram's staining, hemolytic patterns seen on blood agar, catalase and coagulase production, and PCR. As detailed in our previous reports [16,17,20], bacteria cultures were separately diluted to concentrations of 3×10^6 , 5×10^6 , and 7×10^6 CFU/ml, in 0.9% normal saline, to reflect increasing bacterial loads. Then bacteria were volumetrically streaked onto round 35-mm Petri dishes containing tryptic soy agar (TSA) before irradiation with the 405 nm or 470 nm blue light. Control plates were not exposed to blue light.

Photo-irradiation

Plated cultures of bacteria were photo-irradiated with a Dynatron Solaris® 708 device (Dynatronics Corp., Salt Lake City, Utah, USA) fitted either with a 405 nm or 470 nm light probe. The 5.0 cm² applicator of the 405 nm probe, with its cluster of 36 LEDs, emits violet-blue light with a peak at 405 nm (spectral width of 390–420 nm), 500 mW average power, and 100 mW cm⁻² irradiance. The power emitted by this probe at a distance of 0.3–0.5 mm from the Petri dishes was 180 mW. We have determined that this average power is attenuated to 81 mW, as measured at the bottom of the petri dish, i.e., after light has passed through the culture medium; thus, indicating that a total of 99 mW is absorbed within the culture. The interchangeable 5.0 cm² 470 nm applicator has a cluster of 32 LEDs, and emits blue light with a spectral width of 455–485 nm; 470 nm peak), 150 mW average power, and 30 mW cm⁻² irradiance. Similarly, at a distance of 0.3–0.5 mm from the culture, i.e., the surface of the culture medium, the average power emitted by the probe is 25 mW. Measurement of the average power at the bottom of the culture medium indicates that this average power is attenuated to 7 mW, suggesting that 18 mW is absorbed within the culture. In order to minimize thermal radiation, the applicator is cooled by a built-in fan positioned to dissipate any heat produced by the diodes. In previous studies, we ascertained that the device did not generate any measurable increase in temperature within the range of fluences studied [16,17] (Table 1).

To ensure even irradiation of each plate, we used 5.0 cm² Petri dishes, which were the same size as the surface area of the applicator. The applicator 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/cm².

Research Design

Three series of experiments were conducted:

Series one experiments—We investigated one treatment protocol using 405 nm and 470 nm light (Fig. 1, Series 1).

Protocol 1: Cultures plated with 3×10^6 or 5×10^6 CFU/ml MRSA were irradiated with 0, 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 25, 30, 35, 40, 45, 50, 55, or 60 J/cm² once and incubated at 35 °C for 24 hours.

Protocol 2: Cultures plated with 3×10^6 or 5×10^6 CFU/ml MRSA were irradiated with 0, 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 25, 30, 35, 40, 45, 50, 55, or 60 J/cm² twice, with 6 hours of incubation at 35 °C between treatments. Then, plates were incubated for an additional 18 hours; total incubation time being 24 hours.

Series two experiments—One treatment protocol was investigated using 405 nm and 470 nm blue light (Fig. 1, Series 2).

Protocol 3: Plates with 7×10^6 CFU/ml of MRSA were irradiated with 0, 45, 50, 55, and 60 J/cm² either once, twice, or thrice at intervals of 6 hours during which the plates were incubated at 35 °C. Following treatment, plates were incubated until 24 hour after the first irradiation. Thus, twice treated plates were incubated for 18 hours following the second irradiation, and thrice treated plates were incubated for 12 more hours after the third irradiation.

Series three experiments—Again, we studied one treatment protocol in this series of experiments (Fig. 1, Series 3).

Protocol 4: Here we examined the effect of 405 nm and 470 nm irradiation on 7×10^6 CFU/ml MRSA cultures exposed to 0, 165, 180, 220, or 240 J/cm² fluences either twice or thrice at 6 hour intervals. Plates were incubated at 35 °C during the 6 hours between treatments followed by further incubation to ensure 24 hours total incubation time.

Quantification of Bacterial Colonies and Data Analysis

Standard digital images of plates with bacterial colonies were taken, scanned into the computer, and colonies quantified with Sigma Scan Pro 5 software (Systat Software, Inc., Point Richmond, CA, USA). The number of colonies were then automatically computed and subjected to statistical analysis. Each experimental protocol was repeated three times to enhance data accuracy. Descriptive data were generated, and then analysis of variance (ANOVA) was performed with SPSS Version 18 statistical software (SPSS Inc., Chicago, IL,

USA) to determine the effect of fluence on colony counts, and, in particular, to ascertain if any of the optimization protocols resulted in 100% bacterial growth suppression.

RESULTS

General Observation

Consistent with our previous reports, both wavelengths produced a statistically significant reduction in the number of bacterial colonies at each bacterial density tested, progressively with increases in fluence ($P < 0.001$). Moreover, the effect was non-linear even though higher fluences resulted in greater bacterial suppression [16,17,20]. In line with the aforementioned reports, more bacteria were cleared per unit fluence at the lower dose ranges; i.e., doses in the range of 1–11 J/cm², with 5.0 J/cm² dose clearing more than 50% of the colonies in the 3×10^6 CFU/ml and 5×10^6 CFU/ml plates.

Series One Experiments

Effect of single and double irradiation on 3×10^6 CFU/ml culture—Irradiation of the 3×10^6 CFU/ml culture with either 405 nm or 470 nm wavelength resulted in significant suppression of the MRSA colonies progressively as dose increased. In general, more bacterial growth was suppressed with double irradiation (Figs. 2 and 3). However, with either wavelength, the difference between single or double irradiation at each of the 18 fluences tested was minimal, except at 13, 15, 25, 55, and 60 J/cm² dosages of 405 nm light (Fig. 2) and at 11, 15, 17, 19, and 45 J/cm² dosages of 470 nm (Fig. 3) where bacterial growth suppression were significantly different ($P < 0.05$). Single irradiation with either 55 or 60 J/cm² of 470 nm wavelength resulted in 100% bacterial suppression; 100% suppression was also achieved when the culture plates were twice exposed to either 50, 55, or 60 J/cm² of 405 nm light, or 45, 50, 55, or 60 J/cm² of 470 nm light (Figs. 2 and 3).

Effect of single and double irradiation on 5×10^6 CFU/ml culture—In general, irradiation of the denser 5×10^6 CFU/ml gave a similar result as treatment of the 3×10^6 CFU/ml culture, with double irradiation yielding minimally better results than single irradiation at most of the 18 fluences tested, and with progressively more bacterial growth suppression as fluence increased. However, double irradiation with either 3, 13, 17, 19, 25, 30, or 55 J/cm² of 405 nm light or 470 nm light at 50 or 55 J/cm² fluence resulted in significantly more suppression than single treatment ($P < 0.05$; Figs. 4 and 5). Single irradiation with either wavelength did not result in 100% bacterial suppression at any of the fluences tested. However, double irradiation with 50, 55, or 60 J/cm² of 405 nm light or 55 J/cm² of 470 nm light gave 100% bacterial growth suppression (Figs. 4 and 5).

Series Two Experiments

Effect of single, double, or triple irradiation on 7×10^6 CFU/ml culture—Since 100% MRSA suppression was achieved mainly by exposing the bacteria twice to the higher fluences, we limited series two experiments to testing the effects of multiple irradiation of the bacteria with the high end doses, particularly as we explored the effect of blue light on the denser 7×10^6 CFU/ml culture. Each of the four fluences tested resulted in statistically significant reduction in MRSA colonies ($P < 0.05$). Progressively more bacteria were

suppressed as irradiation was repeated. Triple irradiation gave the best result, particularly at 45 and 50 J/cm² fluences at which there were significant differences between the three modes of treatment (Figs. 6 and 7). A comparison of the four fluences tested, showed no statistically significant differences in suppression of bacterial growth regardless of wavelength. Thus, the outcome of irradiation with 45 J/cm² was as good as the outcome of irradiation at the higher 50, 55, or 60 J/cm² dosages. Notwithstanding the number of irradiation, single, double, or triple, at none of the fluences tested was total bacterial growth suppressed.

Series Three Experiments

Effect of high dose single, double, or triple irradiation on 7×10^6 CFU/ml culture—Since we did not achieve 100% growth suppression of the denser 7×10^6 CFU/ml culture, regardless of the number of times the bacteria were irradiated, we repeated the last experiment with double the amount of each of four fluences used in protocol 3 of series two experiments. With either wavelength, three times irradiation of the bacteria using either 90, 100, 110, or 120 J/cm² at 6 hours intervals did not fully suppress bacterial growth in this dense culture, 7×10^6 CFU/ml (Data not shown). However, single irradiation of the bacteria with 220 J/cm² fluence of 470 nm light totally cleared the bacteria at this same culture density (Table 2), as did two time irradiation with 405 nm light at the same dose repeated at 6 hours interval (Tables 2 and 3). About the same suppression of bacterial growth was achieved using 240 J/cm² (Tables 2 and 3).

DISCUSSION

Our results support previous findings that 405 nm and 470 nm light are bactericidal, and indeed, destroy MRSA particularly at higher doses [16,17,20,30,31]. Our finding that 100% bacterial growth suppression was achieved only with single irradiation of 3×10^6 CFU/ml culture but not the denser cultures, clearly shows that repeated irradiation is indeed necessary for effective bacterial growth suppression at relatively low fluences. This is evidenced by the fact that twice exposure to either 50, 55, and 60 J/cm² of 405 nm light or 45, 50, 55, or 60 J/cm² of the 470 nm wavelength completely suppressed the 3×10^6 CFU/ml culture. Moreover, double irradiation at similar doses, i.e., 50, 55, or 60 J/cm² of 405 nm light or 55 J/cm² of 470 nm light suppressed 100% of the bacterial colonies in the standard 5×10^6 CFU/ml culture. Thus, the overall outcome of the first series of experiments in this study is that 55 and 60 J/cm² of 470 nm light completely suppressed the less dense 3×10^6 CFU/ml culture. While either 405 or 470 nm light has the potential to suppress 100% of the standard 5×10^6 CFU/ml culture *in vitro*, irradiation must be done twice to achieve this outcome.

The extent of infection—mild, moderate, or severe—is hard to predict at the beginning of treatment. Therefore, twice irradiation with 55 J/cm² dose may not be a panacea for all MRSA infection, if infection is severe. And this is evidenced by the results of our series two and three experiments which clearly show that with the denser 7×10^6 CFU/ml culture this dose must be quadrupled, and in the case of 405 nm light, applied at least twice, in order to achieve 100% suppression of bacterial growth. Repeated double application of the 55 J/cm²

dose of either wavelength did not result in 100% suppression. Moreover, with either wavelength, even three times irradiation of the bacteria using either 90, 100, 110, or 120 J/cm² at 6 hour intervals did not fully eradicate dense bacterial cultures (7×10^6 CFU/ml). Only when these fluences were quadrupled was a significant suppression of bacterial growth achieved. This finding is consistent with our previous study in which we showed that wavelength and bacterial density influence the bactericidal effect of blue light [20].

One remarkable observation is that double-treatment using 405 nm LED at the fluence of 13 J/cm² (total fluence being 26 J/cm²) resulted in about 85% suppression, and single treatment at 25 J/cm² fluence resulted in about 75% MRSA suppression. Similarly, double-treatment using 470 nm LED at 13 J/cm² fluence (total fluence being 26 J/cm²) resulted in about 89% killing of bacteria, whereas single treatment with 25 J/cm² fluence gave about 75% killing. This finding may be explained by the fact that when treatment is done twice—in this case using 13 J/cm²—the first dose clears some of the bacteria during the time interval between the first and second dose. The second irradiation clears a further amount of bacteria since the underlying layer of MRSA colonies are now exposed to the second dose. Thus, the total amount of bacteria cleared is slightly higher when irradiation is done twice instead of once with a comparable total dose.

The overall outcome of this study is that under our experimental conditions and given our treatment protocol, 100% bacterial suppression is achievable with repeated irradiation using 55 J/cm² for standard cultures or those that are less dense, or 220 J/cm² fluence for those denser than the standard 5×10^6 CFU/ml, for example 7×10^6 CFU/ml. This finding indicates that MRSA growth suppression can be optimized.

Our experiment was designed to mimic practical clinical treatment protocols, such as patient medication which typically occurs at 4, 6, or 12 hour intervals. It is possible that the outcome of our experiment would have been different if a different irradiation regime was used to treat the bacterial colonies. Considering that it will be safer to achieve full MRSA suppression at lower fluences, efforts to explore the possibility of achieving 100% bacterial growth suppression at lower doses using other treatment protocols will be pursued.

In previous studies, we showed that 405 nm and 470 nm blue light suppresses the growth of both community associated (MRSA USA300) and hospital associated MRSA (IS853) strains [17,18], thus demonstrating the versatile nature of blue light to suppress multiple strains of MRSA. In recent works, we demonstrated that blue light suppresses the growth of Gram-negative *Salmonella* bacteria, again indicating the versatility of the antimicrobial effect of blue light irradiation (*manuscript in preparation*). These findings suggest that the mechanisms involved in the antibacterial effects of blue light broadly apply to different strains of bacteria.

Postulated mechanisms of action involve the absorption of this monochromatic light by endogenous chromophores within the bacteria which leads to the production of reactive oxygen species (ROS), and eventual destruction of bacteria membrane and cell death. At present, we are examining the effects of blue light on MRSA genome using DNA profiling

research methodologies such as pulsed field electrophoresis, PCR, and whole genome sequencing to further determine the effects of blue light on the genome of MRSA.

CONCLUSION

Our results warrant the conclusion that: (1) 55 J/cm² of either 405 or 470 nm light has the potential to suppress 100% of standard or less dense cultures of MRSA infection *in vitro*; however, irradiation must be done at least twice to achieve this outcome. (2) With denser cultures, such as 7×10^6 CFU/ml, this dose must be quadrupled and, in the case of 405 nm light, applied at least twice, in order to achieve 100% growth suppression. (3) Under our experimental conditions, 100% bacterial suppression is achievable with repeated irradiation using 55 J/cm² for standard or less dense cultures, or 220 J/cm² fluence for denser cultures.

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REFERENCES

1. Udo EE, Pearman JW, Brubb WB. Genetic analysis of community isolates of methicillin-resistant *Staphylococcus aureus* in Western Australia. J Hosp Infect. 1993; 25:97–108. [PubMed: 7903093]
2. Diep BA, Otto M. The role of virulence determinants in community-associated MRSA pathogenesis. Trends Microbiol. 2008; 16:361–369. [PubMed: 18585915]
3. Graham PL, Lin SX, Larson EL. A US population-based survey of *Staphylococcus aureus* colonization. Ann Intern Med. 2006; 144:318–325. [PubMed: 16520472]
4. Gould DJ, Moralejo D, Drey N, Chudleigh JH. Interventions to improve hand hygiene compliance in patient care. Cochrane Database Syst Rev. 2010; 9:CD005186. [PubMed: 20824842]
5. Jarvis WR, Jarvis AA, Chinn RY. National prevalence of methicillin resistant *Staphylococcus aureus* in inpatients at the United States health care facilities. Am J Infect Control. 2010; 40:194–200. [PubMed: 22440670]
6. Miller LG, Diep BA. Clinical practice: Colonization, fomites, and virulence: Rethinking the pathogenesis of community-associated methicillin-resistant *Staphylococcus aureus* infection. Clin Infect Dis. 2008; 46:752–760. [PubMed: 18220477]
7. Li M, Diep BA, Villaruz AE, Braughton KR, Jiang X, DeLea FR, Chambers HF, Lub Y, Otto M. Evolution of virulence in epidemic community-associated methicillin-resistant *Staphylococcus aureus*. PNAS. 2009; 14:5883–5888. [PubMed: 19293374]
8. Tenover FC, McDougal LK, Goering RV, Killgore G, Projan SJ, Patel JB, Dunman PM. Characterization of a strain of community-associated methicillin-resistant *Staphylococcus aureus* widely disseminated in the United States. J Clin Microbiol. 2006; 1:108–118. [PubMed: 16390957]
9. McCullough AC, Seifried M, Zhao X, Haase J, Kabat WJ, Yogev R, Blumenthal RM, Mukundan D. Higher incidence of perineal community acquired MRSA infections among toddlers. BMC Pediatrics. 2011; 11:96. [PubMed: 22032513]
10. Moellering RC. The growing menace of community-acquired methicillin-resistant *Staphylococcus aureus*. Ann Intern Med. 2006; 144:368–370. [PubMed: 16520479]
11. Okuma K, Iwakawa K, Turnidge JD, Grubb WB, Bell JM, O'Brien FG, Coombs GW, Pearman JW, Tenover FC, Kapi M, Tiensasitorn C, Ito T, Hiramatsu K. Dissemination of new methicillin

- resistant *Staphylococcus aureus* clones in communities. J Clin Microbiol. 2002; 11:4289–4294. [PubMed: 12409412]
12. Otto CC, Haydel SE. Exchangeable ions are responsible for the *in vitro* antibacterial properties of natural clay mixtures. PLoS ONE. 2013; 8:e64068. [PubMed: 23691149]
 13. Tsuneyoshi I, Boyle III, Kanmura WA, Fujimoto Y. Hyperbaric hypoxia suppresses growth of *Staphylococcus aureus*, including methicillin-resistant strains. J Anesth. 2001; 15:29–32. [PubMed: 14566544]
 14. Turhan V, Sacar S, Uzun G, Sacar M, Yildiz S, Ceran N, Gorur R, Oncul O. Hyperbaric oxygen as adjunctive therapy in experimental mediastinitis. J Surg Res. 2009; 155:111–115. [PubMed: 19181335]
 15. Grinholc M, Kawiak A, Kurlenda J, Graczyk A, Bielawski KP. Photodynamic effect of protoporphyrin diarginate (PPArg2) on methicillin resistant *Staphylococcus aureus* and human dermal fibroblasts. Acta Biochim Pol. 2008; 1:85–90. [PubMed: 18217106]
 16. Enwemeka CS, Williams D, Hollosi S, Yens D, Enwemeka SK. Visible 405nm SLD light photo-destroys methicillin resistant *Staphylococcus aureus* (MRSA) *in vitro*. Lasers Surg Med. 2008; 40:734–737. [PubMed: 19065556]
 17. Enwemeka CS, Williams D, Hollosi S, Enwemeka SK, Hollosi S, Yens D. Blue 470-nm light kills methicillin-resistant (MRSA) *in vitro*. Photomed Laser Surg. 2009; 2:221–226. [PubMed: 19196103]
 18. Enwemeka CS. Antimicrobial blue light: An emerging alternative to antibiotics. Photomed Laser Surg. 2013; 31:1–3. [PubMed: 23234336]
 19. Dai T, Tegos GP, Zhiyentayev T, Mylonakis E, Hamblin MR. Photodynamic therapy methicillin-resistant *Staphylococcus aureus* infection in a mouse skin Abrasion model. Lasers Surg Med. 2010; 42:38. [PubMed: 20077489]
 20. Bumah VV, Masson-Meyers DS, Cashin SE, Enwemeka CS. Wavelength and bacterial density influence the bactericidal effect of blue light on methicillin-resistant *Staphylococcus aureus* (MRSA). Photomed Laser Surg. 2013; 31:547–553. [PubMed: 23621894]
 21. Poebel, A. Ancient Nippur Tablet Tells New Story of Deluge. New York Times; 1913.
 22. Carpenter, S.; Rigaud, M.; Barile, M.; Priest, TJ.; Perez, L.; Ferguson, JB. Possibly Having to Do with Diabetes Mellitus. New York: Bard College Annandale-on-Hudson; 1998. An Interlinear Transliteration and English Translation of Portions of the Ebers Papyrus.
 23. Mitsunaga M, Ogawa M, Kosaka N, Rosenblum LT, Choyke PL, Kobayashi H. Cancer cell-selectivity *in vivo* near infrared photoimmunotherapy targeting specific membrane molecules. Nat Med. 2011; 17:1685–1691. [PubMed: 22057348]
 24. Malik Z, Hanania J, Nitzan Y. Bactericidal effects of photoactivated porphyrins - An alternative approach to antimicrobial drugs. J Photochem Photobiol B. 1990; 5:281–293. [PubMed: 2115912]
 25. Howell-Jones RS, Wilson MJ, Hill KE, Howard AJ, Price PE, Thomas DW. A review of the microbiology, antibiotic usage and resistance in chronic skin wounds. J Antimicrob Chemother. 2005; 55:143–149. [PubMed: 15649989]
 26. Maclean M, MacGregor SJ, Anderson JG, Woolsey. Inactivation of bacterial pathogens following exposure to light from a 405nm light-emitting diode array. Appl Environ Microbiol. 2009; 75:1932–1937. [PubMed: 19201962]
 27. Lipovsky A, Nitzan Y, Friedman H, Lubart R. Sensitivity of *Staphylococcus aureus* strains to broadband visible light. Photochem Photobiol. 2009; 85:255–260. [PubMed: 18764895]
 28. Lipovsky A, Nitzan Y, Gedanken A, Lubart R. Visible light induced killing of bacteria as a function of wavelength: Implication for wound healing. Laser Surg Med. 2010; 42:467–472.
 29. Wasson CJ, Zourelis JL, Aardsma NA, Eells JT, Ganger MT, Schober JM, Skwor TA. Inhibitory effects of 405nm irradiation on *Chlamydia trachomatis* growth and characterization of the ensuing inflammatory response in HeLa cells. BMC Microbiol. 2012; 12:176–186. [PubMed: 22894815]
 30. Maclean M, McKenzie K, Anderson JG, Gettinby G, Mac-Gregor SJ. 405 nm light technology for the inactivation of pathogens and its potential role for environmental disinfection and infection control. J Hosp Infect. 2014; 88:1–11. [PubMed: 25066049]
 31. Guffey JS, Wilborn J. *In vitro* bactericidal effects of 405-nm and 470-nm blue light. Photomed Laser Surg. 2006; 24:684–688. [PubMed: 17199466]

Fig. 1.
Research Design.

Fig. 2. Comparative effect of single and double treatment of 405 nm blue light on MRSA USA300, 3×10^6 CFU/ml [$*P < 0.05$].

Fig. 3.
Comparative effect of single and double treatment of 470 nm blue light on MRSA USA300, 3×10^6 CFU/ml [$*P < 0.05$].

Fig. 4.
Comparative effect of single and double treatments of 405 nm blue light on MRSA USA300, 5×10^6 CFU/ml [$*P < 0.05$].

Fig. 5.
Comparative effect of single and double treatments of 470 nm blue light on MRSA USA300, 5×10^6 CFU/ml [$*P < 0.05$].

Fig. 6.
Comparative effect of single, double, and triple treatments of 405 nm Blue light on MRSA USA300, 7×10^6 CFU/ml [$*P < 0.05$].

Fig. 7.
Comparative effect of single, double, and triple treatments of 470 nm Blue light on MRSA USA300, 7×10^6 CFU/ml.

TABLE 1

Properties of Blue LEDs Used for the Experiments

Probe wavelength	405 nm	470 nm
Spectral width	390–420 nm	455–485 nm
LED clusters	36 LEDs	32 LEDs
Surface area of Applicator	5 cm ²	5 cm ²
Average power	500 mW	150 mW
Irradiance	100 mW/cm ²	30 mW/cm ²
Power emitted by probe at a distance of 0.3–0.5 cm	180 mW	25 mW
Power absorbed by culture	99 mW	18 mW

TABLE 2Effect of High Fluences of 470 nm Blue Light on USA300 MRSA (7×10^6 CFU/ml)

Fluence (J/cm ²)	Single irradiation (colony count, %survival)	Double irradiation (colony count, %survival)	Triple irradiation (colony count, %survival)
0	100	100	100
220	0	0	0
240	1	0	0

TABLE 3Effect of High Fluences of 405 nm Blue Light on MRSA USA300 (7×10^6 CFU/ml)

Fluence (J/cm ²)	Single irradiation (colony count, %survival)	Double irradiation (colony count, %survival)	Triple irradiation (colony count, %survival)
0	100	100	100
220	1.67	0	0
240	2.67	0	0