In vitro bactericidal activity of blue light (465 nm) phototherapy on meticillin-susceptible and meticillin-resistant Staphylococcus pseudintermedius

Amy H. Schnedeker ( ), Lynette K. Cole, Gwendolen Lorch ( ), Sandra F. Diaz, John Bonagura and Joshua B. Daniels

Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University Veterinary Medical Center, 601 Vernon L. Tharp St., Columbus, OH 43210, USA. E-mail: Schnedeker.1@osu.edu

Correspondence: Amy H. Schnedeker, Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University Veterinary Medical Center, 601 Vernon L. Tharp St., Columbus, OH 43210, USA. E-mail: Schnedeker.1@osu.edu

Background – Staphylococcus pseudintermedius is the most common cause of bacterial skin infections in dogs. Meticillin-resistant infections have become more common and are challenging to treat. Blue light phototherapy may be an option for treating these infections.

Hypothesis/Objectives – The objective of this study was to measure the in vitro bactericidal activity of 465 nm blue light on meticillin-susceptible Staphylococcus pseudintermedius (MSSP) and meticillin-resistant Staphylococcus pseudintermedius (MRSP). We hypothesized that irradiation with blue light would kill MSSP and MRSP in a dose-dependent fashion in vitro as previously reported for meticillin-resistant Staphylococcus aureus (MRSA).

Methods – In six replicate experiments, each strain [MSSP, n = 1; MRSP ST-71 (KM1381) n = 1; and MRSA (BAA-1680) n = 1] were cultivated on semisolid media, irradiated using a 465 nm blue light phototherapeutic device at the cumulative doses of 56.25, 112.5 and 225 J/cm² and incubated overnight at 35°C. Controls were not irradiated. Colony counts (CC) were performed manually. Descriptive statistics were performed and treatment effects assessed using the Wilcoxon–Mann–Whitney rank-sum test. Bonferroni-corrected rank-sum tests were performed for post hoc analysis when significant differences were identified.

Results – There was a significant decrease in CC with blue light irradiation at all doses for MRSA (P = 0.0006) but not for MSSP (P = 0.131) or MRSP (P = 0.589).

Conclusions – Blue light phototherapy significantly reduced CC of MRSA, but not of MSSP or MRSP. The mechanism for the relative photosensitivity of the MRSA isolate is unknown, but is hypothesized to be due to an increased concentration of porphyrin in S. aureus relative to S. pseudintermedius, which would modulate blue light absorption.

Introduction

Staphylococcus pseudintermedius is a common commensal and opportunistic pathogen of the skin of dogs and is the most common cause of bacterial skin infections. In recent years, meticillin-resistant S. pseudintermedius (MRSP) infections have become much more common. The prevalence in the USA of MRSP in clinical samples from dogs with canine pyoderma ranges from 15.6 to 38.2%. With the emergence of MRSP the number of oral antimicrobial drugs to which bacterial isolates are susceptible is limited, often leading to pharmacotherapeutic choices that have profound adverse effects or are reserved for human infections. Topical antimicrobial therapy has been shown to be effective for treatment of some MRSP infections, but these treatments can be time consuming and inconvenient for owners. There is a need to develop new therapies to treat these infections that are both effective and have minimal adverse effect potential.

Photostimulation is the use of light to activate biological cells or tissues. Therapy with photostimulation is called phototherapy, light therapy or photomodulation. Photostimulation can be performed using light emitting diodes (LEDs) which produce a narrow spectrum of light in an incoherent manner, where the light is randomly spread out once emitted from the light source. LEDs have different depth of penetration based on their wavelength and can affect cellular metabolism by triggering intracellular photobiological reactions. Wavelengths available in commercial LED units include ultraviolet (100–400-nm), blue (400–470-nm), yellow (570–590-nm), red (630–700-nm) and infrared (800–1200-nm). The deepest target of LED light penetration varies. In humans blue light...
targets the epidermis (less than 1 mm), yellow light the papillary dermis (0.5–2 mm), red light the adnexa (2–3 mm) and infrared light (5–10 mm) both the adnexa and reticular dermis. These depth penetrations would be expected to be similar in animal skin.

Blue light phototherapy has been shown to be a treatment option for bacterial infections. Although the exact mechanism is unknown, blue light is thought to excite intracellular porphyrins and produce cytotoxic reactive oxygen species (ROS). These ROS have an antimicrobial effect on the bacteria, but are not detrimental to the host cells. Blue light phototherapy has been shown to be bactericidal against *Staphylococcus aureus*, with the greatest reduction of bacteria (62%) at the highest dose of 15 J/cm² using 470 nm blue light. Optimal doses of 470 nm blue light phototherapy have been reported to be between 55 and 220 J/cm², resulting in 90.4% phototherapy have been reported to be between 55 and 220 J/cm², resulting in 90.4% – 100% reduction of *S. aureus* with blue light (see below) and incubated overnight at 35°C in ambient air. Plates for the controls were not irradiated prior to incubation. Incubation conditions (time and temperature) were identical for control and treated plates.

**Blue light irradiation**

A 465 nm blue light therapeutic device (MR4 ACTIVet PRO™, Multi Radiance Medical®, Solon, OH, USA) was used for all irradiations (Table 1). The device was clamped approximately 6 mm above the TSA plates for even dispersion of light across the inoculated area (Figure 1). Treatment groups (MSSP, MRSP and MRSA) were irradiated once with the following doses: 56.25 J/cm² (15 min exposure), 112.5 J/cm² (30 min exposure) and 225 J/cm² (60 min exposure). The controls were not irradiated. The three doses were chosen based on a previous study in which irradiation of USA300 MRSA (ATCC BAA-1880) with 470 nm blue light at 55, 110 and 220 J/cm² produced 69–92%, 80–100% and 100% suppression of bacterial growth, respectively. Each dose was repeated in sextuplicate for each isolate. Colony counts (the total numbers of colonies on each individual plate; CC) were performed manually.

### Materials and methods

**Bacterial isolates and culture**

A sequence-typed strain of MRSP ST-71 (KM1381), a sequence-typed strain of USA300 MRSA (BAA-1680) and an untyped clinical MSSP isolate were selected for use as the test isolates. The MRSP ST-71 (KM1381) isolate was obtained from the University of Tennessee College of Veterinary Medicine bacteriology laboratory in Knoxville, TN, USA. The USA300 reference strain of MRSA was obtained from American Type Culture Collection (ATCC BAA-1680). The clinical MSSP isolate was speciated using Matrix Assisted Laser Desorption Ionization Time-Of-Flight (MALDI-TOF) mass spectrometry. A standardized inoculum of 15 μL of approximately 10⁶ cfu/mL of each isolate was spread plated onto 35 mm petri dishes containing tryptic soy agar (TSA). This plate size was chosen to attain uniform irradiation as the blue light was emitted from an opening that was approximately 25 mm in diameter. Briefly, a 0.5 McFarland standard suspension (approximately 10⁶ cfu/mL concentration) was made for each isolate in sterile water. Ten-fold serial dilutions were performed by placing 40 μL of the bacterial suspension in 360 μL of sterile phosphate buffered saline (PBS) to reach a final dilution of approximately 10⁷ cfu/mL. Dilutions were obtained to achieve a range of 10–100 cfu on the 35 mm TSA plates. Plates for the treatment groups (MSSP, MRSP and MRSA isolates) were inoculated with 15 μL of the 10⁶ cfu/mL dilution and spread onto the plates. Control plates for each isolate were spread in the same manner. After inoculation, the treatment group plates were irradiated with blue light (see below) and incubated overnight at 35°C in ambient air. Plates for the controls were not irradiated prior to incubation. Incubation conditions (time and temperature) were identical for control and treated plates.

### Statistical analysis

Descriptive statistics were generated for CC for control and treatment groups at each dose and the data were tested for distribution and normality by visual inspection and with the D’Agostino – Pearson test (MedCalc® for Windows, v15.0; Ostend, Belgium). As data were not normally distributed, the results are displayed as medians, interquartile ranges and ranges (MedCalc® for Windows, v15.0) and percentage reduction for CC.

Data for each of the three doses was also combined for each bacterial isolate and the control and treatment groups were then compared using a Wilcoxon–Mann–Whitney rank-sum test (SPSS IBM v24; Armonk, NY, USA). When a significant difference was identified, Bonferroni-corrected rank-sum tests (SPSS IBM v24) were performed at each dose for post hoc analysis. Statistical significance was set at *P* < 0.05.

### Results

The median CC and percentage reduction of the treatment groups (MSSP, MRSP, MRSA) after irradiation with 465 nm blue light at 56.25, 112.5 and 225 J/cm² are shown in Table 2. The percentage reduction of CC was greatest for MSSP at all doses, reaching 100% at the two highest doses. In contrast, the percentage reduction of CC for MSSP and MRSP was minimal with the maximum reduction found at the highest dose (225 J/cm²) of 11.7% for MSSP and 21.2% for MRSP.

<table>
<thead>
<tr>
<th>Table 1. Properties of the blue light phototherapeutic device (MR4 ACTIVet PRO™, Multi Radiance Medical®, Solon, OH, USA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of blue LEDs</td>
</tr>
<tr>
<td>Wavelength of blue LEDs (nm)</td>
</tr>
<tr>
<td>Mode</td>
</tr>
<tr>
<td>Average optical output (mW) – each LED</td>
</tr>
<tr>
<td>Power density (mW/cm²) – each LED</td>
</tr>
<tr>
<td>Energy density (J/cm²) – each LED</td>
</tr>
<tr>
<td>Spot size of blue LED (cm²)</td>
</tr>
<tr>
<td>Magnetic Field (mT)</td>
</tr>
<tr>
<td>Irradiation time (s)</td>
</tr>
<tr>
<td>Total dose (J)</td>
</tr>
<tr>
<td>Aperture of device (cm²)</td>
</tr>
<tr>
<td>Energy density at aperture (J/cm²)</td>
</tr>
<tr>
<td>Power density at aperture (mW/cm²)</td>
</tr>
</tbody>
</table>

Energy density at aperture and irradiation times used in the study are in bold.
In two other studies, 470 nm blue light suppressed MSSP with a 93.3% CC reduction at 56.25 J/cm².27 A significant reduction in combined median MRSA CC after treatment (P < 0.0005) for all doses was present. There was no significant reduction in median CC after irradiation for MSSP (P = 0.131) or in median CC for MRSP (P = 0.589) (see Table S1 in Supporting Information).

Figure 1. The 465 nm blue light therapeutic device (MR4 ACTIVet PRO™ device, Multi Radiance Medical™, Solon, OH, USA) irradiating a 35 mm petri dish containing tryptic soy agar plated with a bacterial isolate.

The effects of dose on CC for the treatment groups is shown in Figure 2; there was a significant difference in CC for MRSA between treatment and control groups at each dose (P = 0.006).

A significant reduction in combined median MRSA CC after treatment (P < 0.0005) for all doses was present. There was no significant reduction in median CC after irradiation for MSSP (P = 0.131) or in median CC for MRSP (P = 0.589) (see Table S1 in Supporting Information).

Representative plates are depicted in Supporting Information Figure S1.

Discussion

Blue light 465 nm phototherapy significantly reduced CC for MRSA, but not for MSSP or MRSP. An earlier study using 470 nm blue light phototherapy reported a significant dose-dependent reduction in MRSA colony counts with 90.4% of the colonies killed at a dose of 55 J/cm².14 However, 100% reduction was not obtained, even at the highest dose tested (60 J/cm²).14 Our study had similar results for MRSA with a 93.3% CC reduction at 56.25 J/cm². In two other studies, 470 nm blue light suppressed MRSA at 55 J/cm²; however, to achieve 100% bacterial suppression irradiation had to be performed twice for standard or less dense cultures. Denser cultures, when irradiated once, required the highest dose of 220 J/cm² to achieve 100% kill.12,13 Likewise, in our study, the two highest doses, 112.5 and 225 J/cm² were required to achieve 100% reduction in CC for MRSA.

Although S. pseudintermedius is the most common organism isolated in dogs with bacterial skin infections,1 MRSA infections are an emerging problem in veterinary medicine.2,19,20 As hand-held LED blue light phototherapeutic devices are commercially available, blue light phototherapy may be an effective option for treating MRSA. Based on the results of our study, to achieve 100% kill, treatment durations would need to be at least 30 min, so this therapy would be best suited for localized rather than generalized infections. The effect of factors such as hair and organic debris need to be considered for use in vivo. Clipping the hair and cleaning the skin over the area to be irradiated with alcohol has been shown to increase the depth of penetration of the light when a low-level laser therapy probe was used on the flexor tendon in the horse.21 In an in vivo mouse model of MRSA skin abrasion infections, 415 nm blue light delivered at 108 J/cm² rapidly reduced the bacterial burden, suggesting the use of blue light may be an option for treatment of MRSA skin infections.22

Interestingly, the percentage CC reductions for MSSP and MRSP were minimal, with the greatest reduction of 11.7 and 21.2% respectively at the highest dose of 225 J/cm². These results were not expected because blue light phototherapy had been effective in vitro against MRSA in previous studies12–14 and in ours. Although the mechanism of action of blue light is not fully understood, it is believed to excite intracellular porphyrins, thus generating the production of cytotoxic ROS that kill the bacteria.10 Because porphyrins are a key factor in absorption of blue light, it is possible that differences in the amount of endogenous porphyrins between bacterial strains would result in differences in blue light absorption and killing of the bacteria. One study compared the difference in absorption of visible light (400–800 nm) of two different strains of S. aureus, one meticillin-susceptible strain (MSSA) and one resistant strain (MRSA).23 The MSSA strain had a maximum reduction in bacterial viability of 99.8% compared to 55.5% for MRSA. Porphyrins were extracted and the relative fluorescence measured using a spectrometer. The MSSA had a higher porphyrin concentration and cytotoxic oxygen radical production compared to MRSA which likely accounted for greater absorption of light and a greater reduction of the bacteria.23 The relative percentage production of porphyrin also has been measured in S. aureus using high performance liquid
To the best of the authors’ knowledge, there are no studies of endogenous porphyrin concentration measurement in MSSP or MRSP.

Photodynamic therapy (PDT) is a treatment option that involves photosensitization of a target using a topical or systemic agent that is activated by light in the presence of oxygen and produces a cytotoxic reaction. PDT can be performed using 5-aminolevulinic acid (5-ALA), a natural amino acid that is the precursor of a strong photosensitizer, protoporphyrin IX within cells. 5-ALA can be administered locally, systemically (intravenous and intraperitoneal) and orally. 5-ALA-PDT combined with a 410 nm LED had an antibacterial effect on MRSA in vitro with a 5 log10-unit decrease in organisms at 50 J/cm2. In the same study, in a mouse model of MRSA-infected wounds, the use of 5-ALA-PDT with a 410 nm LED accelerated wound healing and decreased bacterial counts on the wound surface. 5-ALA-PDT may be an option both in vitro and in vivo for MRSP and MSSP to help increase bacterial kill.

In conclusion, blue light phototherapy significantly reduced CC of MRSA, but not of MSSP or MRSP. The mechanism for the relative photosensitivity of the MRSA isolate is unknown, but is hypothesized to be due to an increased concentration of porphyrin in S. aureus relative to S. pseudintermedius, which would modulate blue light absorption. Further studies are needed to measure the concentration of porphyrins and assess porphyrin relevance in blue light absorption in MSSP and MRSP, as well as to assess the use of PDT combined with blue light for MSSP and MRSP infections.

Acknowledgments

The authors thank Tim Vojt for labelling the figures.

References


**Supporting Information**

Additional Supporting Information may be found in the online version of this article.

**Figure S1.** Representative culture plates of meticillin-susceptible *Staphylococcus pseudintermedius* (MSSP) (a–d), meticillin-resistant *S. pseudintermedius* (MRSP) (KM1381) (e–h) and meticillin-resistant *S. aureus* (MRSA) (BAA-1680) (i–l) irradiated with 56.25, 112.5 and 225 J/cm² 465 nm blue light. Control plates (a,e,i) were not irradiated.

**Table S1.** Combined median, interquartile range and range of colony counts for control groups and treatment groups MSSP meticillin-susceptible *Staphylococcus pseudintermedius*, MRSP meticillin-resistant *S. pseudintermedius* (KM1381) and MRSA meticillin-resistant *Staphylococcus aureus* (BAA-1680) after irradiation with 465 nm blue light.
**Resumen**

**Introducción** – *Staphylococcus pseudintermedius* es la causa más común de infecciones bacterianas de la piel en perros. Las infecciones resistentes a la meticilina se han vuelto más comunes y son difíciles de tratar. La fototerapia con luz azul puede ser una opción para tratar estas infecciones.

**Hipothesi/Objetivos** – El objetivo de este estudio fue medir la actividad bactericida *in vitro* de 465 nm de luz azul sobre *Staphylococcus pseudintermedius* (MSSP) sensible a la meticilina y *Staphylococcus pseudintermedius* resistente a meticilina (MRSP). La hipótesis era que la irradiación con luz azul mataría a MSSP y MRSP de forma dependiente de dosis *in vitro* como se ha descrito anteriormente para *Staphylococcus aureus* resistente a meticilina (MRSA).

**Métodos** – En seis experimentos repetidos, cada cepa [MSSP, n = 1; MRSP ST-71 (KM1381) n = 1; y MRSA (BAA-1680) n = 1] se cultivaron en medio semisólido, se irradiaron utilizando un dispositivo fototerapéutico de luz azul de 465 nm a dosis acumuladas de 56,25; 112,5 y 225 J/cm² y se incubaron durante toda la noche a 35°C. Los controles no fueron irradiados. Los recuentos de colonias (CC) se realizaron manualmente. Se realizaron las descripciones estadísticas y se evaluaron los efectos del tratamiento utilizando la prueba de suma de rangos de Wilcoxon-Mann-Whitney. También se realizó una prueba corregida de rangos de Bonferroni para análisis post hoc cuando se identificaron diferencias significativas.

**Resultados** – Hubo una disminución significativa de CC con irradiación de luz azul en todas las dosis para MRSA (P = 0.0006) pero no para MSSP (P = 0.131) o MRSP (P = 0.589).

**Conclusiones** – La fototerapia con luz azul redujo significativamente el CC de MRSA, pero no de MSSP o MRSP. El mecanismo para la fotosensibilidad relativa del aislado de MRSA es desconocido, pero se supone que se debe a un aumento de la concentración de porfirina en *S. aureus* en relación con *S. pseudintermedius*, que modulará la absorción de la luz azul.

**Zusammenfassung**


**Ergebnisse** – Es gab eine signifikante Veränderung der CC bei allen Dosierungen der Blaulichtbestrahlung bei MRSA (P = 0.0006), aber nicht bei MSSP (P = 0.131) oder MRSP (P = 0.589).

**Schlussfolgerungen** – Blaulichtphototherapie reduzierte die CC der MRSA, aber nicht jene von MSSP oder MRSP, signifikant. Der Mechanismus der relativen Photosensibilität der MRSA Isolate ist unbekannt, aber es wird hypothetisiert, dass die Ursache in einer erhöhten Konzentration des Porphyryns im *S. aureus* im Vergleich zum *S. pseudintermedius* liegt, welche die Blaulichtabsorption modulieren könnte.
Blue light therapy for MSSP and MRSP

Resumo
Contexto – O *Staphylococcus pseudintermedius* é o principal causador de infecções bacterianas em cães. As infecções resistentes à meticilina vem se tornando um problema mais comum e de tratamento desafiador. A fototerapia de luz azul pode ser uma opção para o tratamento destas infecções.

Hipótese/Objetivos – O objetivo deste estudo foi mensurar a atividade bactericida *in vitro* de uma luz azul de 465 nm em *Staphylococcus pseudintermedius* suscetíveis à meticilina (MSSP) e *Staphylococcus pseudintermedius* resistentes à meticilina (MRSP). A nossa hipótese é de que a irradiación com a luz azul eliminaría MSSP e MRSP *in vitro*, em um padrão dose-dependente como foi reportado anteriormente para *Staphylococcus aureus* resistente à meticilina (MRSA).

Métodos – Em seis experimentos em duplicata, cada cepa [MSSP, n = 1; MRSP ST-71 (KM1381) n = 1; e MRSA (BAA-1680) n = 1] foi cultivada em meio semissólido, irradiada com luz azul de 465 nm em um aparelho de fototerapia em doses cumulativas de 56,25; 112,5 e 225 J/cm² e incubados durante noite a 35°C. Os controles não foram irradiados. As contagens de colônias (CC) foram realizadas manualmente. Fora realizada estatística descritiva e os efeitos do tratamento foram avaliados utilizando o teste de Wilcoxon-Mann-Whitney rank-sum. Os testes de Bonferroni-corrigidos rank-sum foram realizados para análise post-hoc quando diferenças significativas foram identificadas.

Resultados – Houve uma redução significativa nas CC com a irradiación de luz azul em todas as doses para MRSA (*P* = 0.0006), mas não para MSSP (*P* = 0.131) ou MRSP (*P* = 0.589).

Conclusões – A fototerapia com luz azul reduziu as CC para MRSA, mas não para MSSP ou MRSP. O mecanismo para fotosensibilidade do isolado de MRSA é desconhecido, mas suspeita-se que seja devido à maior concentração de porfirina em *S. aureus*, comparado ao *S. pseudintermedius*, que modular a absorção da luz azul.