Antimicrobial resistance in Neisseria gonorrhoeae is a major issue of public health, and there is a critical need for the development of new gonococcal strategies. In this study, we investigated the effectiveness of antimicrobial blue light (aBL; wavelength, 405 nm), an innovative nonpharmacological approach, for the inactivation of Neisseria gonorrhoeae. Our findings indicated that aBL preferentially inactivated N. gonorrhoeae, including antibiotic-resistant strains, over human vaginal epithelial cells in vitro. Furthermore, no aBL-induced genotoxicity to the vaginal epithelial cells was observed at the radiant exposure used to inactivate N. gonorrhoeae.

405 nm, an innovative nonpharmacological approach, for the inactivation of N. gonorrhoeae. Was involved in aBL inactivation of N. gonorrhoeae. Together, these findings show that aBL represents a potential potent treatment for antibiotic-resistant gonococcal infection.

Keywords. Neisseria gonorrhoeae; gonorrhea; antimicrobial blue light; antibiotic resistance; endogenous porphyrins.

As the use of antibiotics drives the emergence and spread of multidrug-resistant N. gonorrhoeae [3, 12, 13], there is a critical need for the development of new treatment strategies [14, 15]. Antimicrobial blue light (aBL; wavelength, 405 nm), an innovative nonpharmacological therapy, has attracted increasing attention because of its intrinsic antimicrobial properties without the involvement of exogenous photosensitizers [16, 17]. Although the mechanism of action of aBL is still not fully understood, a common hypothesis is that aBL excites naturally occurring endogenous photosensitizers (eg, iron-free porphyrins) within bacterial cells and subsequently leads to the production of cytotoxic oxidative species [16–18].

In contrast to antibiotics, aBL is an innovative approach that is based on the physical properties of light and bacteria. The advantages of aBL over antibiotics include rapid action and equal inactivation effectiveness independent of antibiotic resistance status [16, 17]. It is also currently thought that bacteria are less able to develop resistance to aBL than to traditional antibiotics because of the multiple-target characteristic of aBL [16, 17, 19]. In comparison to traditional antimicrobial photodynamic therapy (aPDT), aBL is appealing in that it inactivates bacteria (ie, it renders bacteria unable to multiply) without the involvement of exogenous photosensitizers [20–24]. In addition, it is well accepted that aBL is much less detrimental to the host cells than germicidal UV irradiation [25, 26].
Although aBL has been reported to be effective in inactivating a range of nosocomial pathogens [20, 27–29], aBL has never been studied as a treatment for gonorrhea [30]. The superficial nature of gonococcal infection (<0.5 mm in depth [31–33]) renders aBL an amendable candidate treatment for this infection (the aBL penetration depth in human tissue is 0.5–1.0 mm [34, 35]). To be potentially transferable to the clinic, aBL should preferentially inactivate N. gonorrhoeae while sparing normal host cells. In this study, we investigated the effectiveness of aBL inactivation of N. gonorrhoeae, the toxicity of aBL to human epithelial cells, and the potential development of gonococcal resistance to aBL. In addition, we identified and quantified aBL-activatable endogenous photosensitizing chromophores in N. gonorrhoeae.

MATERIALS AND METHODS

Blue Light Sources
A light-emitting diode with peak emissions at 405 nm and a full width at half maximum of 20 nm (M405L2; Thorlabs, Newton, NJ) was used for irradiation. The wavelength of 405 nm is the optimal wavelength targeting porphyrins [46]. The irradiance of 60 mW/cm², which was optimized on the basis of the log₁₀ bacterial colony-forming units inactivated per unit radiant exposure of aBL (in J/cm²) in a preliminary study, was used throughout the study. Light irradiance was measured using a PM100D power/energy meter (Thorlabs). The radiant exposure of light (in J/cm²) was calculated as the irradiance (in W/cm²) multiplied by the irradiation time of light (in seconds).

N. gonorrhoeae Strains and Culture Conditions
ATCC 700825 (FA 1090) and 4 clinical N. gonorrhoeae isolates were studied. The clinical isolates were obtained through the CDC and Food and Drug Administration Antibiotic Resistance Isolate Bank. Supplementary Materials 2 shows the antibiotic susceptibilities of the 4 N. gonorrhoeae clinical isolates. Bacteria were routinely grown on gonococcal medium base GC (Remel, Lenexa, KS) agar plates containing GCHI enrichment (Remel) and hemoglobin at 37°C and 5% CO₂.

Human Vaginal Epithelial Cells and Growth Conditions
Human vaginal epithelial cells VK2/E6E7 (ATCC CRL-2616) were used. Cells were cultured in keratinocyte serum-free medium (Gibco, Grand Island, NY) supplemented with 5 ng/mL recombinant epidermal growth factor, 50 μg/mL bovine pituitary extract (Invitrogen, Grand Island, NY), and 100 units/mL each of penicillin and streptomycin (Life Technologies, Grand Island) at 37°C and 5% CO₂. All experiments were performed in the exponential growth phase of VK2/E6E7 cells (48 hours after plating).

aBL Inactivation of N. gonorrhoeae in Suspensions
Overnight N. gonorrhoeae cultures were collected from GC agar plates, washed using phosphate-buffered saline (PBS), and then resuspended in PBS to an OD₆₀₀nm of 0.3 (approximately 10⁸ colony-forming units [CFU]/mL). A 3-mL bacterial suspension was added to a 35-mm-diameter Petri dish prior to aBL exposure. After varying aBL exposures (9, 18, 27, 36, 45, 72, 90, and 108 J/cm²) had been delivered, 30-μL aliquots of the bacterial suspension were collected, and the number of N. gonorrhoeae CFU was measured using a colony-forming assay. The experiment was performed in 3 independent replicates for each condition. In addition, bacterial suspensions without exposure to aBL served as negative controls.

Transmission Electron Microscopy (TEM) of aBL-Induced Morphological Changes to N. gonorrhoeae Cells
To examine aBL-induced morphological changes of N. gonorrhoeae cells, untreated and aBL-treated ATCC 700825 N. gonorrhoeae cells were fixed in 1% paraformaldehyde and 1.25% glutaraldehyde immediately after aBL exposures (9 J/cm² [2.5-minute illumination], 18 J/cm² [5-minute illumination], and 27 J/cm² [7.5-minute illumination]) and stored at 4°C for 2 hours. The N. gonorrhoeae cells were then washed 3 times with 0.1 M sodium cacodylate buffer after centrifugation (at 13 500 × g for 10 minutes) and decanting the fixative. The cell pellets were subsequently processed for TEM.

Evaluation of aBL-Induced Toxicity to Human Vaginal Epithelial Cells
To test the toxicity of aBL to normal vaginal epithelial cells, VK2/E6E7 cells were subjected to aBL exposures of 54, 108, and 162 J/cm². The viability of VK2/E6E7 cells was measured using the MTT assay 24 hours after aBL exposure. The experiment was performed in 6 independent replicates for each condition.

In addition, aBL-induced DNA damage in VK2/E6E7 cells was evaluated using a CometAssay kit (Trevigen, Gaithersburg, MD). The experiments were performed on the basis of the Trevigen standard protocol for single-cell gel electrophoresis. Briefly, aBL-treated and untreated VK2/E6E7 cells were detached using trypsin. The cell pellets were suspended at 37°C and 27 J/cm² [9 J/cm²] and stored at 4°C for 2 hours. The N. gonorrhoeae cells were then washed 3 times with 0.1 M sodium cacodylate buffer after centrifugation (at 13 500 × g for 10 minutes) and decanting the fixative. The cell pellets were subsequently processed for TEM.

Ultraperformance Liquid Chromatography (UPLC) Analysis of Endogenous aBL-Activatable Photosensitizers in N. gonorrhoeae and Human Vaginal Epithelial Cells
UPLC was used to indentify and quantify the endogenous aBL-activatable photosensitizers (porphyrins) in N. gonorrhoeae and VK2/E6E7 cells. To extract the endogenous porphyrins from the cells, overnight cultures were washed using PBS. The cell pellets were collected after centrifugation (at 13 500 × g for 6 minutes), resuspended in 1.0 mL of extraction buffer, and then allowed to stand on ice for 1 hour. The cell pellets were then washed 3 times with 0.1 M sodium cacodylate buffer after centrifugation (at 13 500 × g for 10 minutes) and decanting the fixative. The cell pellets were subsequently processed for TEM.

Evaluation of aBL-Induced DNA Damage in VK2/E6E7 Cells
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were collected, subcultured, and grown overnight for the next cycle. In each cycle, the surviving bacterial cells after aBL exposure was then used throughout the successive cycles.

Infection of Human Vaginal Epithelial Cells by \( N. \text{gonorrhoeae} \)
\( N. \text{gonorrhoeae} \) has the potential to develop resistance to aBL, suspensions of ATCC 700825 in PBS were subjected to 15 repeated cycles of subtherapeutic aBL exposures. In the first cycle, the aBL exposure was adjusted to inactivate approximately 4.0-log\( _{10} \) \( N. \text{gonorrhoeae} \) CFU, and the same aBL exposure was then used throughout the successive cycles. In each cycle, the surviving bacterial cells after aBL exposure were collected, subcultured, and grown overnight for the next cycle of inactivation by aBL.

Infection of Human Vaginal Epithelial Cells by \( N. \text{gonorrhoeae} \)
\( N. \text{gonorrhoeae} \) has evolved the mechanism to invade into human vaginal epithelial cells, to overcome the host defense barrier [48]. To evaluate the effectiveness of aBL inactivation of intracellular \( N. \text{gonorrhoeae} \), VK2/E6E7 cells were seeded into a 35-mm-diameter Petri dish (Transwell, Costar, NY) at a cell density of \( 2 \times 10^5 \) cells/dish. The VK2/E6E7 cells were incubated in 2 mL of keratinocyte serum-free medium for 48 hours at 37°C. The supernatant was then discarded, and a 1-mL suspension of ATCC 700825 in PBS was added. A multiplicity of infection (MOI) of 50 bacteria per VK2/E6E7 cell was used according to the optimized MOI from a previous study [49]. The cocultures of VK2/E6E7 cells and \( N. \text{gonorrhoeae} \) were incubated at 37°C with 5% \( \text{CO}_2 \) for 4 hours. The invasion of \( N. \text{gonorrhoeae} \) cells into VK2/E6E7 cells was examined by confocal microscopy (Olympus, FV 1000-MPE Confocal) [50].

aBL Inactivation of Intracellular \( N. \text{gonorrhoeae} \)
Four identical cultures (A, B, C, and D) of VK2/E6E7 cells infected with \( N. \text{gonorrhoeae} \) were prepared for each experiment. Culture A was treated with aBL followed by incubation with 0.05% saponin for 20 minutes to lyse the VK2/E6E7 cells and liberate the bacteria. The viability of \( N. \text{gonorrhoeae} \) was then determined using a colony-formation assay. Culture B was treated with the same aBL exposures, and the viability of VK2/E6E7 cells was assessed using the MTT assay 24 hours after aBL exposure. Cultures C and D were not exposed to aBL. The viability of \( N. \text{gonorrhoeae} \) in culture C was measured using a colony-formation assay, and the viability of VK2/E6E7 cells in culture D was measured using the MTT assay. aBL exposures of 54 and 108 J/cm² were tested. The experiment was performed in 6 independent replicates for each condition.

Statistical Analysis
Data are presented as means ± standard errors. The differences between different conditions were analyzed using 1-way analysis of variance. \( P \) values of <.05 were considered statistically significant.

RESULTS

Susceptibilities of \( N. \text{gonorrhoeae} \) in Planktonic Suspensions to Inactivation by aBL
To achieve a reduction of 3-log\( _{10} \) \( N. \text{gonorrhoeae} \) CFU in suspensions, aBL exposures from 25 J/cm² (for ATCC 700825) to 59 J/cm² (for strain 199) were used (Figure 1A). To eradicate all bacterial CFU in suspensions (>6-log\( _{10} \) CFU reduction), aBL exposures from 45 J/cm² (for ATCC 700825) to 108 J/cm² (for strain 199) were used (Figure 1A).

TEM after varying aBL exposures revealed aBL morphological changes in \( N. \text{gonorrhoeae} \) cells, such as formation of bulges, breakage of cell walls with cytoplasmic release, loss of outer membranes, and cellular disintegration (Figure 1B). Decreased density of cytoplasm was observed in some cells, accompanied by increased cellular size, suggesting that aBL induced damage to the cell wall of \( N. \text{gonorrhoeae} \).

Toxicity of aBL to Normal Human Vaginal Epithelial Cells
As shown in Figure 1A, an aBL exposure of <108 J/cm² eradicated \( N. \text{gonorrhoeae} \) CFUs in suspensions (>6-log\( _{10} \) CFU reduction). In contrast, study of the cytotoxicity of aBL to normal human vaginal
epithelial cells (VK2/E6E7) showed no statistically significant loss of VK2/E6E7 cell viability when aBL exposures of up to 108 J/cm² were used (P = .67; Figure 2A). Comet assay results exhibited that no aBL-induced DNA damage occurred in the VK2/E6E7 cells at aBL exposures up to 216 J/cm² (Figure 2B).

**Presence and Quantity of Endogenous Porphyrins in N. gonorrhoeae**

The UPLC chromatograms identified the presence of several species of endogenous porphyrins in *N. gonorrhoeae*, including uroporphyrin, 7-carboxylporphyrin, 6-carboxylporphyrin, 5-carboxylporphyrin, and coproporphyrin (Supplementary Materials 1). No PpIX was detected in any of the 5 *N. gonorrhoeae* strains studied. Quantitative analysis revealed that the most-abundant porphyrin species in *N. gonorrhoeae* was coproporphyrin, with concentrations ranging from 7.93 nmol/g (protein weight; in strain 181) to 19.16 nmol/g (protein weight; in ATCC 700825) in the 5 *N. gonorrhoeae* strains (Table 1).

**Presence and Quantity of Endogenous Porphyrins in Human Vaginal Epithelial Cells**

Quantitative analysis by UPLC showed that the total protein concentration of endogenous porphyrins in VK2/E6E7 cells is 0.0176 nmol/g (protein weight; Table 2), which is hundreds of times lower than the total amount of porphyrins in *N. gonorrhoeae* (range, 9.34–22.26 nmol/g [protein weight]; Table 1).

**Role of 1O₂ in aBL Inactivation of N. gonorrhoeae**

In the presence of 10 mM NaN₃, a significant decrease in the aBL-induced reduction in the number of *N. gonorrhoeae* CFU in suspensions was observed (Figure 3). For example, at an aBL exposure of 48 J/cm², the aBL-induced reduction decreased from approximately 7.0 log₁₀ to 2.5 log₁₀ CFU (P < .01).

**Potential Development of Gonococcal Resistance to aBL**

Figure 4 shows the reduction in the log₁₀ ATCC 700825 CFU after 15 successive cycles of subtherapeutic exposure to aBL. Correlation analysis found a weak tendency toward increased susceptibility to aBL among *N. gonorrhoeae* as the number of cycles of aBL exposure increased (correlation coefficient = 0.24). However, the correlation was not statistically significant (P = .40), indicating that development of gonococcal resistance to aBL did not occur after 15 successive cycles of subtherapeutic aBL exposure.
Effect of Bacterium–Host Cell Interaction on the Tolerance of Human Vaginal Epithelial Cells to aBL and the Susceptibility of N. gonorrhoeae to Inactivation by aBL

Confocal microscopy indicated that N. gonorrhoeae cells either adhered to the cell walls or invaded the cytoplasm of the VK2/E6E7 cells in their cocultures (Figure 5A). Both VK2/E6E7 cells and N. gonorrhoeae were stained by SYTO9 (Figure 5A). Figure 5B and 5C display changes in the viability of VK2/E6E7 cells and N. gonorrhoeae, respectively, in their cocultures in response to aBL. Compared with noninfected VK2/E6E7 cells (Figure 2), infected VK2/E6E7 cells were more vulnerable to aBL inactivation. The decrease in viability among infected VK2/E6E7 cells was 11.3% (0.05-log10 CFU) at an exposure of 54 J/cm² (P = .146) and 24.9% (0.12-log10 CFU) at an exposure of 108 J/cm² (P = .0011; Figure 5B).

Under the same aBL exposures, reductions of 4.18- and 5.07-log10 Cell survival fraction (%)

<table>
<thead>
<tr>
<th>aBL exposure (J/cm²)</th>
<th>Positive Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 J/cm²</td>
<td></td>
</tr>
<tr>
<td>54 J/cm²</td>
<td></td>
</tr>
<tr>
<td>108 J/cm²</td>
<td></td>
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<tr>
<td>216 J/cm²</td>
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</tbody>
</table>

**Figure 2.** Toxicity of antimicrobial blue light (aBL) to normal vaginal epithelial cells. A, Change in viability of normal VK2/E6E7 cells in response to aBL (wavelength, 405 nm) at different exposures. Bars, standard errors. P = .3474 for the difference in viability of VK2/E6E7 cells at 0 J/cm² versus 54 J/cm², P = .6676 for the difference between 0 J/cm² and 108 J/cm², and P < .0001 for the difference between 0 J/cm² and 162 J/cm². B, Comet assay images of normal VK2/E6E7 cell cultures exposed to 0 J/cm², 54 J/cm², 108 J/cm², or 216 J/cm² aBL (wavelength, 405 nm). The positive control was treated with H₂O₂, with damaged cellular DNA exhibiting a classic “comet tail.”

<table>
<thead>
<tr>
<th>Porphyrin</th>
<th>Strain 174</th>
<th>Percentage of Total Porphyrin Content</th>
<th>Strain 179</th>
<th>Percentage of Total Porphyrin Content</th>
<th>Strain 181</th>
<th>Percentage of Total Porphyrin Content</th>
<th>Strain 199</th>
<th>Percentage of Total Porphyrin Content</th>
<th>Strain ATCC 7007825</th>
<th>Percentage of Total Porphyrin Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uroporphyrin</td>
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<td>0.61</td>
<td>0.08</td>
<td>0.54</td>
<td>0.18</td>
<td>1.94</td>
<td>0.58</td>
<td>3.21</td>
<td>0.37</td>
<td>1.68</td>
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<td>7-Carboxylporphyrin</td>
<td>0.58</td>
<td>5.52</td>
<td>0.70</td>
<td>4.45</td>
<td>0.51</td>
<td>5.44</td>
<td>1.11</td>
<td>6.09</td>
<td>1.22</td>
<td>5.48</td>
</tr>
<tr>
<td>6-Carboxylporphyrin</td>
<td>0.27</td>
<td>2.63</td>
<td>0.41</td>
<td>2.60</td>
<td>0.19</td>
<td>2.06</td>
<td>0.50</td>
<td>2.72</td>
<td>0.41</td>
<td>1.84</td>
</tr>
<tr>
<td>5-Carboxylporphyrin</td>
<td>0.56</td>
<td>5.35</td>
<td>0.46</td>
<td>2.93</td>
<td>0.52</td>
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<td>1.06</td>
<td>5.82</td>
<td>1.10</td>
<td>4.94</td>
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<tr>
<td>Coproporphyrin</td>
<td>8.97</td>
<td>85.89</td>
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<td>7.94</td>
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<td>...</td>
<td>ND</td>
<td>...</td>
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<td>...</td>
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<td>...</td>
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<tr>
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<td>100</td>
<td>15.65</td>
<td>100</td>
<td>9.34</td>
<td>100</td>
<td>18.18</td>
<td>100</td>
<td>22.26</td>
<td>100</td>
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</table>

Abbreviation: ND, not detected.

*Protein weight.
N. gonorrhoeae CFU were observed, respectively (Figure 5C), indicating that the selectivity of aBL inactivation of N. gonorrhoeae over VK2/E6E7 cells still existed in the cocultures.

**DISCUSSION**

In this study, we explored the potential use of aBL for the treatment of gonorrhea, a disease that is becoming untreatable because of increasing antibiotic resistance. Our findings demonstrated that N. gonorrhoeae, regardless of its antibiotic resistance status, was highly susceptible to 405-nm aBL. TEM revealed aBL-induced cellular damage in bacterial cells. Previous studies reported that, in contrast to most other bacterial species, N. gonorrhoeae does not contain superoxide dismutase, which is the major antioxidant defense system in bacteria [36]. This might explain the high susceptibility of N. gonorrhoeae to inactivation by aBL, which is dominantly mediated by singlet oxygen. Unpublished data in our laboratory showed that N. gonorrhoeae is on average 7.32-, 5.87-, and 28.0-fold more susceptible to aBL than Pseudomonas aeruginosa, Acinetobacter baumannii, and Escherichia coli, respectively.

The UPLC analysis in this report ascertained the presence of endogenous porphyrins in N. gonorrhoeae. The most abundant porphyrin species in N. gonorrhoeae was found to be coproporphyrin, which is a highly efficient 1O2 generator [37]. The significant decrease in aBL activity in the presence of the 1O2 quencher NaN3 indicated the participation of 1O2 in inactivating N. gonorrhoeae by aBL. Interestingly, PpIX, which is commonly present in bacteria, was not detected in any of the 5 N. gonorrhoeae isolates studied in this report. In addition, by comparing the data in Figure 1A and Table 1, we found that there was no correlation between the concentrations of endogenous porphyrins and the corresponding aBL susceptibility of the 5 strains of N. gonorrhoeae. This might be due to the variations in the antioxidant properties of different N. gonorrhoeae strains.

The biosynthesis of photosensitizing porphyrins in bacteria occurs in the pathway of heme synthesis [38]. It is generally believed that the heme synthesis pathway of most bacteria begins with charged glutamyl-tRNA^Glu to form the universal precursor ALA and that porphyrins are formed through a series of conserved enzymatic steps. The PpIX pathway was believed to be the classical pathway. However, the lack of endogenous PpIX and the abundant presence of endogenous coproporphyrin in N. gonorrhoeae indicates that, unlike most other gram-negative bacteria, N. gonorrhoeae synthesizes heme via the coproporphyrin III pathway [39, 40]. In addition to synthesizing its own heme, N. gonorrhoeae is able to internalize and use exogenous heme within host epithelial cells for growth [41].

The comparison of the aBL susceptibility between N. gonorrhoeae and human vaginal epithelial cells suggested that there is a therapeutic window where N. gonorrhoeae could be selectively inactivated while human vaginal epithelial cells are preserved. This can be explained by our UPLC finding that the content of aBL-activatable endogenous photosensitizers in the vaginal epithelial cells is >500-fold lower than that in N. gonorrhoeae.
N. gonorrhoeae has evolved mechanisms to infect a variety of host cells and subvert clearance by the host immune response. Infection of genital mucosa by N. gonorrhoeae involves adherence to and invasion of epithelial cells. Therefore, to be an effective therapeutic agent, aBL must also be able to inactivate intracellular N. gonorrhoeae. This study demonstrated that aBL inactivated N. gonorrhoeae both adherent to and inside the vaginal epithelial cells. Although the infection of the vaginal epithelial cells by N. gonorrhoeae slightly reduced the tolerance of the vaginal epithelial cells to aBL, we showed that there still existed a therapeutic window of aBL for preferentially inactivating N. gonorrhoeae over human vaginal epithelial cells.

The hypothesis that bacteria are unlikely to develop resistance to aBL is supported by the findings in the present study. It is well accepted that photo-oxidative stress reacts with several cellular macromolecules, including proteins, lipids, DNA, and RNA, and subsequently results in cell damage [42]. This multi-target feature of aBL minimizes the potential that bacteria will develop resistance to aBL [16, 17]. We experimentally investigated the potential development of aBL resistance by N. gonorrhoeae by performing 15 successive cycles of subtherapeutic aBL inactivation. No development of aBL resistance by N. gonorrhoeae was observed. This finding is also consistent with that of a recent study showing that 15 repeated sublethal exposures of Staphylococcus aureus suspension did not affect the susceptibility of this organism to 405-nm light [19].

How can aBL be delivered to treat gonorrhea in the clinic? In our ongoing animal study, we have developed a novel polymeric optical fiber to deliver aBL intravaginally to the infection sites in female mice (Supplementary Materials 3). The optical fiber can deliver axially uniform irradiation over a desired length. The fiber emission profile is retained upon deformation or surrounding changes. In a clinical study, the investigators used cylindrical fiber diffusers to deliver light intraurethrally in PDT for urethral condylomata [43]. In another study of aPDT for urinary tract infections in female mice, the authors used a glass cylindrical diffuser to deliver light intravascularly to the infection sites [44]. With the development of waveguide technology, it is now possible to deliver light to almost any anatomical region, via endoscopes, interstitially inserted microneedles, or fiber optics [45].

In conclusion, this study provides novel fundamental information regarding the use of aBL for treating gonorrhea. aBL preferentially inactivates N. gonorrhoeae while preserving the host cells. Endogenous aBL-activatable porphyrins are present in N. gonorrhoeae cells. The development of gonococcal resistance to aBL is unlikely. Further studies are warranted to investigate whether vaginal flora might impact the usefulness of aBL, to assess the effectiveness and safety of aBL therapy for gonococcal infection in animal models, and to explore potential clinical applications.

Supplementary Data

Supplementary materials are available at The Journal of Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and
are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. Y. W., R. F. E., Y. B., K. D. H., Y. H. G., Y. G., J. A. F., and T. D. designed the experiments. Y. W., R. F. E., Y. B., and X. S. G. performed the experiments. Y. W., Y. B., and T. D. analyzed the data and wrote the manuscript. R. F. E., K. D. H., Y. H. G., and J. A. F. reviewed the manuscript.

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Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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