Materials and Methods

Preparation and characterization of CDots. CDots were prepared by the chemical functionalization of small carbon nanoparticle with oligomeric polyethylenimine (PEI, averge molecular weight ~600), thus denoted as PEI_{600}-CDots. The small carbon nanoparticles were harvested from the commercially acquired carbon nanopowders sample (US Research Nanomaterials, Inc.) in procedures similar to those reported previously [26, 43]. Briefly, the carbon nanopowders sample (2 g) was refluxed in concentrated nitric acid (8 M, 200 mL) for 48 h. The reaction mixture was cooled back to room temperature, and centrifuged at 1,000 g to discard the supernatant. The residue was re-dispersed in deionized water, dialyzed in a membrane tubing (molecular weight cut-off ~500) against fresh water for 48 h, and then centrifuged at 1,000 g to retain the supernatant. Upon the removal of water, small carbon nanoparticles were recovered and used for the functionalization with PEI. In the thermally induced functionalization reaction with microwave energy, the small carbon nanoparticles (100 mg) as an aqueous slurry were mixed with PEI_{600} (2 g) and ethanol (2 mL) in a scintillation vial. The resulting mixture was sonicated in an ultrasonic cleaner (VWR 250D) at 40 °C for 1 h, followed by a complete removal of solvent via evaporation for a solid mixture. Separately, a bath of silicon carbide (150 g) in a silica crucible casting dish (about 8 cm in diameter and 2.5 cm in height) was prepared and pre-heated in a conventional microwave oven at 500 W for 3 min. Several rounds of microwave treatments were as follows: (1) the vial containing the solid mixture was immersed in the pre-heated silicon carbide bath for microwave irradiation at 1,000 W for 3 min; (2) the sample vial was taken out of the bath for

being cooled in the ambient, and more PEI_{600} (1 g) and ethanol (2 mL) were added and mixed well, followed by the removal of ethanol, and then microwave irradiation the same as in (1); (3) a repeat of (2) but with the microwave irradiation at 500 W for 8 min; and (4) a repeat of (3). After the microwave treatments, the reaction mixture was cooled to ambient and dispersed in deionized water with vigorous sonication. The resulting aqueous dispersion was centrifuged at 5,000 *g* to collect the supernatant, followed by dialysis (molecular weight cut-off ~1,000) against fresh water for 6 h to obtain the PEI_{600}-CDots in an aqueous solution [44]. The PEI_{600}-CDots were characterized using NMR, microscopy, and optical spectroscopy techniques, from which the results were consistent with those of similarly prepared samples reported previously. The aqueous sample solution was stored in the dark at room temperature and used for antimicrobial experiments described below.

Bacterial strains and the surface plating method. Drug resistant *Enterococcus faecium* strain *BM 46* and *Enterococcus faecalis BM29* were from Dr. Kathariou's laboratory in the Department of Food Science at North Carolina State University. *BM29* and *BM 46* were isolated from animal productions, their antimicrobial susceptibility was determined using the standard agar dilution method, and the information about their susceptibility to tested antibiotics is listed in Table 1.

Table 1. The Multidrug-Resistant and non-resistant *Enterococcus* strains

***Enterococcus* Strains**		**Antibiotics Resistance**
BM46	*Enterococcus faecium (putative)*	resistant to tetracycline (>16ug/mL), erythromycin (>8ug/mL), nalidixic acid (>32mg/mL), and low level ciprofloxacin (<4ug/mL)
BM29	*Enterococcus faecalis*	resistant to tetracycline (>16ug/mL), nalidixic acid (>32ug/mL), low level streptomycin (<64ug/mL) and low level erythromycin (<8ug/mL)

For our experiments, the bacterial cultures were grown in 15 ml brain heart infusion (BHI) broth (Oxoid, UK) by inoculating the broth with a single colony from BHI agar plates, and incubated overnight at 37 ^{o}C with constant agitation at 225 rpm in an Excella E24 incubator shaker (New Brunswick Scientific). Bacterial cultures were centrifuged at 12000 x g (Beckman Coulter Life Sciences, Indianapolis, IN, USA) for 8 minutes and washed twice using phosphate buffer saline (PBS). The pellet was then re-suspended in PBS and prepared for further experiments.

The actual cell numbers in the cultures were determined using the traditional surface plating method. Briefly, cell suspensions were 1:10 serial diluted with PBS, and aliquots of 100 μL of appropriate dilutions were surface plated on BHI agar plates, the plates were then incubated at 37 °C overnight. The colonies were counted and calculated into colonies forming unit per mL (CFU/mL) for the cell concentration in the cell suspensions. .

Treatment of bacterial cells with PEI_{600}-CDots under visible light illumination. The treatments of cells with PEI_{600}-CDots were performed in 96-well plates. Aliquot of 100 μl of bacteria cells suspension were placed into the wells, and 100 μL PEI_{600}-CDots of various concentrations were added to reach the final concentrations of 0.025, 0.062, 0.12, 0.25 and 0.62 μM PEI_{600}-CDots in the wells. The bacterial cell concentration in each well was around 10^7 -10^8 CFU/mL. The control samples were bacterial suspension with PBS. The plates were placed on Orbital shaker (BT Lab Systems) at 350 rpm and exposed to visible light using a commercial daylight LED lamp (CREE, 17 W, omnidirectional 815 lumens) placed at the distance of ~10 cm

away from the surface of the plate, for 1 h. After the treatment, the viable cell numbers in the treated and the control samples were determined using the traditional plating method described above. The bactericidal effect of PEI_{600}-CDots on these *Enterococcus* strains was evaluated by the logarithmic reduction in the viable cell number in the treated samples comparing to the control samples.

Separately, treatment tests on time-dependence were also performed in 96-well plates. Similarly, aliquots of 100 μl of bacteria cells suspensions were distributed to the wells, and 100 μL PEI_{600}-CDots at desired concentrations were added to the wells to reach the final concentration of CDots at 0.62 μM. The plate was exposed to visible light using the same setting as above for different periods of time ranging from 30 min to 2 h. The bacterial cells without CDots and light treatment were used as the controls. The viable cell numbers in the treated and control samples were determined using the same surface plating method as above. The time-dependent bactericidal effects of PEI_{600}-CDots on these *Enterococcus* strains were evaluated using the logarithmic reduction in viable cell number as described above.

Measurement of Intracellular Reactive Oxygen Species (ROS). The fluorescence probe Dihydrorhodamine 123 (DHR 123) was used to quantify the generation of intracellular ROS in these enterococcus strains upon the treatments with PEI_{600}-CDots using the similar procedure reported previously [22]. After overnight growth, the bacterial cells were collected by centrifugation at 12000 x g for 8 min. The pellets were washed twice with 0.85% NaCl and re-suspended in 5 ml of 0.85% NaCl. The cells were treated with 0.1 mg/mL of PEI_{600}-CDots under visible light illumination as described above. . After the treatment, the cells were collected and washed twice with

800 µl of 0.85% NaCl, and the pellets re-suspended in 350 µl 0.85% NaCl. Aliquot of 200 µl of 10 µM DHR 123 solution were added to the samples and vigorously vortexed. After 40 min incubation in the dark at room temperature, the cells were washed twice and re-suspended in 350 µl of 0.85% NaCl. The fluorescence at 535 nm in each well was measured using SpectraMax M5 Microplate reader, with excitation wavelength of 500 nm. The fluorescence of the treated and control samples were compared to evaluate the levels of generated intracellular ROS upon the CDots treatments.

Transmission Electron Microscopic (TEM) imaging. TEM imaging was performed to examine the difference between PEI_{600}-CDots treated cells and untreated cells using *E. faecium BM 46* cells. To do that, *E. faecium* cells were treated with 1.2 µM PEI_{600}-CDots under visible light illumination for 1 h. The treated cells and the controls were collected in 1.5 ml of microcentrifuge tubes and centrifuged at 9,000 x g for 10 min. After the cells were washed and re-suspended in 100 µl PBS, an equal volume of 4% glutaraldehyde in 0.1M sodium cacodylate buffer, PH 7.4 were added and gently mixed, followed by 10 min centrifugation at 100 x g to concentrate the cells into a pellet (pellets should be between 1 to 2 mm in depth).

The fixed samples were stored at 4^{o}C in the fixative solution overnight and sent to the Microscopy Services Laboratory (MSL) in Department of Pathology and Laboratory Medicine at the University of North Carolina at Chapel Hill, for further processing and imaging. The pellet was rinsed in 0.1 M sodium cacodylate buffer several times, and post-fixed in 1% buffered osmium tetroxide for 1 h at room temperature. Following dehydration with a graded series of ethanol (30%, 50%, 75%, 100%, 100%) and two

changes of propylene oxide, the cell pellet was infiltrated and embedded in PolyBed 812 epoxy resin (Polysciences, Inc., Warrington, PA). Ultrathin sections (70nm) were cut and mounted on copper grids followed by post-staining with 4% uranyl acetate and 0.4% lead citrate. Sections were observed using a JEOL JEM-1230 transmission electron microscope operating at 80kV (JEOL USA, Inc., Peabody, MA) and digital images acquired with a Gatan Orius SC1000 CCD Digital Camera and Gatan Microscopy Suite v3.0 software (Gatan, Inc., Pleasanton, CA).