Supplementary Information

Oxide Anchored Multi-Charged Metal Complexes with Binary Nanoparticles for Stable and Efficient Anti-Bacterial Coatings on Cotton Fabrics

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1. Materials and Methods

All reagents were used as received without further purification, unless otherwise noted. Syntheses and experiments were carried out in clean and oven-dried glassware. Reactions were monitored using silica gel G-60 F254 aluminum TLC and compounds were visualized by short/long-wavelength UV lamps. Column chromatography was done using silica gel 100-200 mesh as a stationary phase.

1.1.1 Molecular Characterization

^{1}H and ^{13}C NMR spectra were recorded in deuterated solvents at 300 K on a 500 MHz (1H) Bruker 109 Advance DPX spectrometer using TMS as an internal standard. Chemical shifts are presented in ppm (δ) along with the corresponding coupling constants (Hz). HRMS data were recorded on a Thermo Scientific Exactive LCMS instrument by electrospray ionization method with ions given in m/z using an Orbitrap analyser. Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectra were obtained using an AXIMA-CFR PLUS (SHIMADZU) MALDI-TOF mass spectrometer using α-cyano-4-hydroxycinnamic acid as the matrix. FT-IR spectra were recorded in the solid state (KBr) using Shimadzu IRPrestige-21 Fourier transform infra-red spectrophotometer.

1.1.2 Absorption Spectroscopy

Electronic absorption spectra in solution were recorded on a Shimadzu UV-2600 Spectrophotometer, using a clean and dry Hellma Analytics 10 mm quartz cuvette. Temperature was regulated using a Shimadzu temperature controller and a blank experiment with the corresponding solvent provided the baseline.

1.1.3 Electron Microscopy and Thermal Characterization

TEM images were obtained using a JEOL JEM F200 microscope built with STEM, EDS, EELS. The samples were dried, redispersed in deionized water using sonication, and drop cast onto carbon-coated copper grids (TED PELLA, INC. 400 mesh and dried at room temperature for 24 h. SEM micrographs and SEM − energy-dispersive X-ray spectroscopy (SEM−EDS) of coated cotton fabrics were acquired using a Zeiss EVO 18 Special Edition scanning electron microscope (Carl Zeiss, Germany) equipped with an EDS detection system (Oxford Instruments, X-Max) with an accelerating voltage of 15 kV. Thermogravimetric analyses (TGA) were performed at a heating rate of 10 °C/min under a nitrogen atmosphere using Shimadzu, DTG-60 equipment.

2. Synthesis

The dibromides **5** and **12** were synthesized from the corresponding commercially available dimethyl 2,2'-bipyridines using a modified procedure reported in literature.[S1]

2.1. Synthesis of Ligand L1

[2,2'-bipyridine]-4,4'-dicarboxylic acid [2]

4,4'-dimethyl-2,2'-bipyridine **[1]** (5 g, 27.14 mmol) is added to conc. H_2SO_4 (125 mL). To the above stirred solution, potassium dichromate (31.934 g, 108.55 mmol) was added in small portions in about 20 min. The mixture was maintained at a temperature of 70-80 ^oC, via occasional cooling using an ice-water bath. The resultant mixture was stirred for 4 h. until the temperature of the mixture fell below 40 ^oC and the color of the solution turned deep green. The reaction mixture was then poured into ice, filtered and washed with cold water until the filtrate turned colourless. The solid thus obtained was dried and refluxed for 4 h. in 50% HNO_3 (100 mL). The solution was then poured over ice and diluted with water (500 mL) and cooled below 5 ^oC. The precipitate formed was filtered and washed with water (cold, 4 x 50 mL), acetone (3 x 15 mL) and dried to afford the desired product as an off-white solid (6.04 g, Yield 91%). The dicarboxylic acid obtained was used in the next step without further purification.

Diethyl[2,2'-bipyridine]-4,4'-dicarboxylate [3]

To a suspension of [2,2'-bipyridine]-4,4'-dicarboxylic acid **[2]** (5.517 g, 22.59 mmol) in EtOH (HPLC grade, 250 mL) taken in round-bottom flask, conc. H_2SO_4 (10 mL) was added in small portions. The mixture was then refluxed for 60 h. EtOH was removed under reduced pressure and ice was added to the mixture. The pH was adjusted to neutral using 2M NaOH. The precipitate formed was filtered, washed with water (5x 100 mL) and dried to get the desired dicarboxylate as an off-white solid (5.929 g, Yield 80%); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.95(s, 2H), 8.87(d, *J* =5 Hz, 2H), 7.91 (d, *J* = 4.5Hz, 2H), 4.48-4.44(q,4H). HRMS: Calculated for $C_{16}H_{16}N_2O_4$ 300.111 found 300.111.

[2,2'-bipyridine]-4,4'-diyldimethanol [4]

A suspension of diethyl[2,2'-bipyridine]-4,4'-dicarboxylate **[3]** (5.9 g, 19.65 mmol) in ethanol (>99.9%, 150 mL) was taken in a round-bottom flask. To this $NaBH_4$ (14.864 g, 392.93 mmol) was added at once. The above suspension was then refluxed with vigorous stirring for 3 h. The mixture was allowed to cool to room temperature and excess $NaBH_4$ was quenched by adding aqueous NH_4Cl (saturated, 150 mL) solution drop-wise until the effervescence ceased. Ethanol was removed under reduced pressure and extracted with ethyl acetate (2 x 250 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to afford the desired product as a white powder (3.65 g, Yield 86 %); ^{1}H NMR (500 MHz, CD_3OD) δ: 8.49 (d, *J* = 5 Hz, 2H), 8.16 (s, 2H), 7.34 (d, *J* = 4.5 Hz, 2H), 4.65 (s, 4H), 4.51 (s, 2H). HRMS: Calculated for $C_{12}H_{12}N_2O_2$ 216.089 found, 217.104.

4,4'-bis(bromomethyl)-2,2'-bipyridine [5]

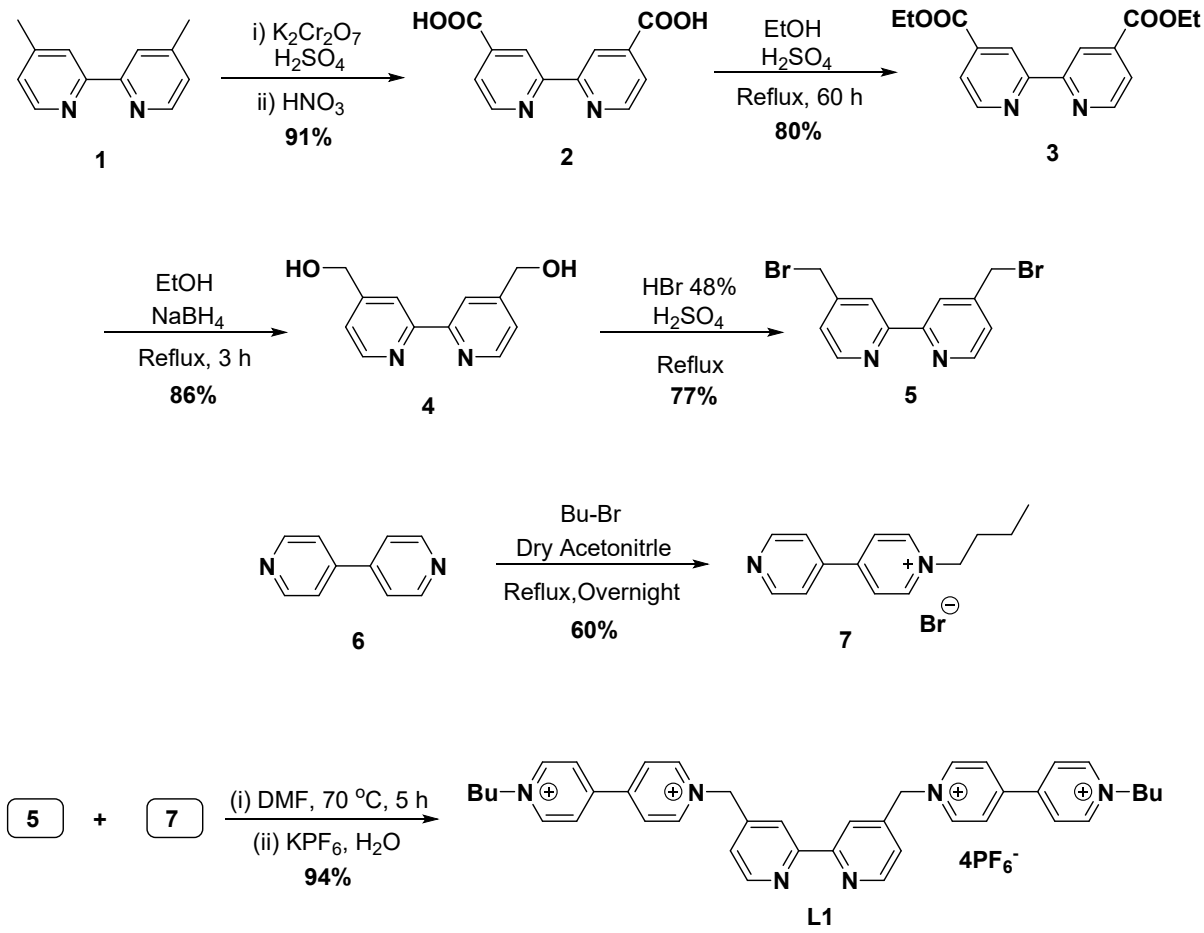
A solution of [2,2'-bipyridine]-4,4'-diyldimethanol **[4]** (3.6 g, 16.65 mmol) in 48% HBr (100 mL) was taken in a round-bottom flask. To the above solution, conc. H_2SO_4 (31 mL) was added and refluxed for 6 h. The resulting solution was allowed to cool under room temperature. To this, water (200 mL) was added and the resulting precipitate was filtered after neutralizing the pH with 2 M NaOH. The precipitate was washed with water (4 x 50 mL) and dried to give a solid. This solid was dissolved in $CHCl_3$ (100 mL) and filtered. The obtained filtrate was dried over anhydrous $NaSO_4$ and the solvent was removed under reduced pressure to give the desired product as an off-white powder (4.41 g, Yield 77 %); ^{1}H NMR (500 MHz, $CDCl_3$): 8.57 (d, *J* = 5 Hz, 2H), 8.28 (s, 2H), 7.33(d, *J* = 3.5 Hz, 2H), 4.44 (s, 4H). HRMS: Calculated for $C_{12}H_{10}Br_2N_2$ 339.921, found 340.929.

1-butyl-[4,4'-bipyridin]-1-ium bromide [7]

The viologen was obtained by the refluxing 4,4'-bipyridine **[6]** with 1-bromobutane in a 3:1 molar ratio in dry acetonitrile overnight. The reaction mixture was precipitated with diethyl ether, filtered, washed and dried under high vacuum to obtain an off-white solid (Yield = 60%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.51 (d, *J* = 6 Hz, 2H), 8.79 (d, *J* = 5 Hz, 2H), 8.33 (d, *J* = 6 Hz, 2H), 7.65 (d, *J* = 4.5 Hz, 2H), 4.95 (t, 2H), 2.02-1.95 (m, 2H), 1.41-1.33 (m, 2H), 0.90 (t, 3H). HRMS: Calculated for $C_{14}H_{17}BrN_2$ 292.057, found 213.139.

1',1'''-([2,2'-bipyridine]-4,4'-diylbis(methylene))bis(1-butyl-[4,4'-bipyridine]-1,1'-diium) [L1]

4,4'-bis(bromomethyl)-2,2'-bipyridine **[5]** (0.6 g, 1.75 mmol) and 1-butyl-[4,4'-bipyridin]-1-ium bromide **[6]** (1.08 g, 3.6 mmol) in DMF were taken in a round-bottom flask. The reaction mixture was heated at 70 ^{o}C for 6 h. After cooling to room temperature, the product was precipitated with saturated KPF_6 solution. The precipitate was filtered, washed and dried under high vacuum to obtain the ligand **L1** as an off-white solid (1.948 g, Yield 94%). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 9.56 (d, *J* = 6 Hz, 4H), 9.38 (d, *J* = 6.5 Hz, 4H), 8.81-8.76 (m, 7H), 8.75 (d, *J* = 5.5 Hz, 3H), 8.61 (s, 2H), 6.09 (s, 4H), 4.69 (t, 4H), 1.98-1.90 (m, 4H), 1.36-1.27 (m, 4H), 0.94 (t, 6H). HRMS: Calculated for $C_{40}H_{44}N_6^{4+}$ 608.361, found 647.361 $[M+K]^+$.



Scheme S1. Synthesis of Ligand **L1**.

2.2. Synthesis of Ligand L2

Diethyl[2,2'-bipyridine]-5,5'-dicarboxylate [10]

To a suspension of [2,2'-bipyridine]-5,5'-dicarboxylic acid **9** (2 g, 8.20 mmol) in ethanol (HPLC grade, 250 mL) taken in round-bottom flask, conc. H_2SO_4 (10 mL) was added in small portions. The mixture was then refluxed for 60 h. EtOH was removed under reduced pressure and ice was added to the mixture. The pH was adjusted to neutral using 2M NaOH. The obtained precipitate was filtered, washed with water (5x 100 mL) and dried to get the desired dicarboxylate as an off-white solid (1.68 g, Yield 84%); ^{1}H NMR (500 MHz, CD_3OD) δ: 9.17 (s, 2H), 8.55-8.47 (m, 4H), 4.39-4.33 (q, 4H), 1.34 (t, 6H). HRMS: Calculated for $C_{16}H_{16}N_2O_4$ 300.111, found 300.112.

[2,2'-bipyridine]-5,5'-diyldimethanol [11]

A suspension of diethyl[2,2'-bipyridine]-5,5'-dicarboxylate **[10]** (1.68 g, 5.59 mmol) in ethanol (>99.9%, 150 mL) was taken in a round-bottom flask. To this $NaBH_4$ (14.864 g, 392.93 mmol) was added at once. The above suspension was then refluxed with vigorous stirring for 3 h. The

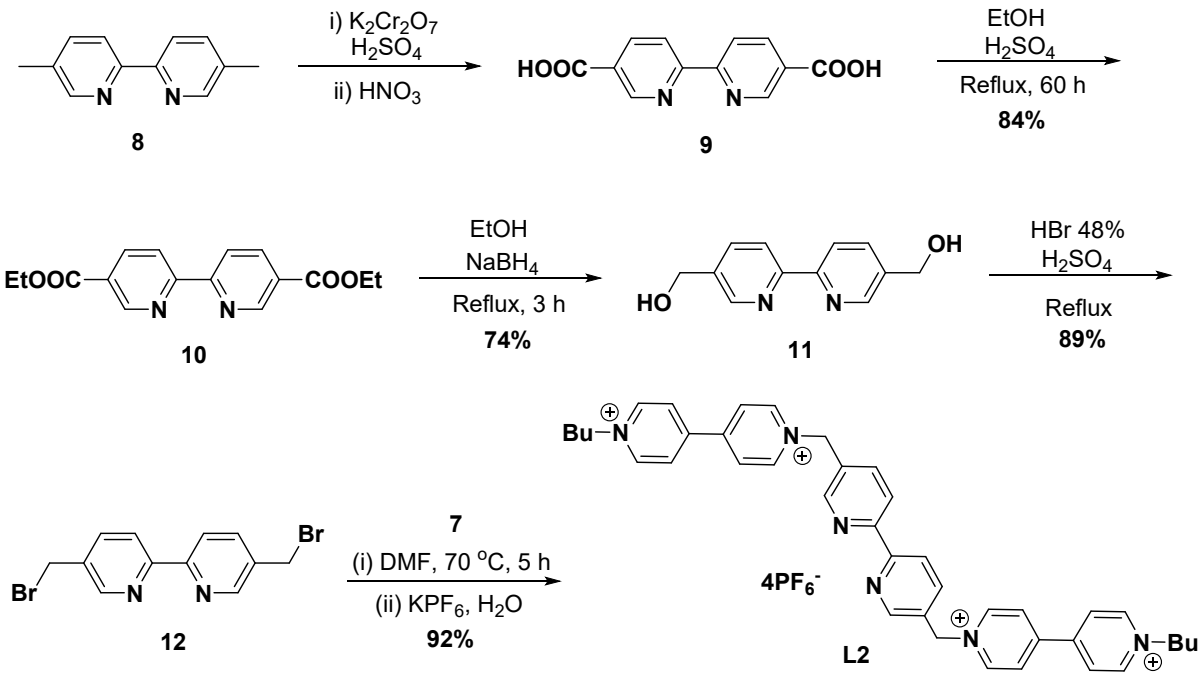
mixture was allowed to cool to room temperature and excess $NaBH_4$ was quenched by adding aqueous NH_4Cl (saturated, 150 mL) solution drop-wise until the effervescence ceased. Ethanol was removed under reduced pressure and extracted with ethyl acetate (2 x 250 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to afford the desired product as a white powder (1.23 g, Yield 74 %); ^{1}H NMR (500 MHz, CD_3OD) δ: 8.49 (s, 2H), 8.18 (d, *J* = 5 Hz, 2H), 7.35(d, *J* = 4.5 Hz, 2H), 4.51 (s, 4H), 3.88 (s, 2H). HRMS: Calculated for $C_{14}H_{14}O_2$ 214.099, found 215.108.

5,5'-bis(bromomethyl)-2,2'-bipyridine [12]

A solution of [2,2'-bipyridine]-5,5'-diyldimethanol **[11]** (1.23 g, 5.74 mmol) in 48% HBr (100 mL) was taken in a round-bottom flask. To the above solution, conc. H_2SO_4 (31 mL) was added and refluxed for 6 h. The resulting solution was allowed to cool under room temperature. To this, water (200 mL) was added and the resulting precipitate was filtered after neutralizing the pH with 2 M NaOH. The precipitate was washed with water (4 x 50 mL) and dried to give a solid. This solid was dissolved in $CHCl_3$ (100 mL) and filtered. The obtained filtrate was dried over anhydrous $NaSO_4$ and the solvent was removed under reduced pressure to give the desired product as an off-white powder (1.091 g, Yield 89%); ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.68 (s, 2H), 8.40 (d, *J* = 8 Hz, 2H), 7.85 (d, J = 7 Hz, 2H), 4.54 (s, 4H). HRMS: Calculated for $C_{14}H_{12}Br_2$ 337.930, found 337.930.

1',1'''-([2,2'-bipyridine]-5,5'-diylbis(methylene))bis(1-butyl-[4,4'-bipyridine]-1,1'-diium) [L2]

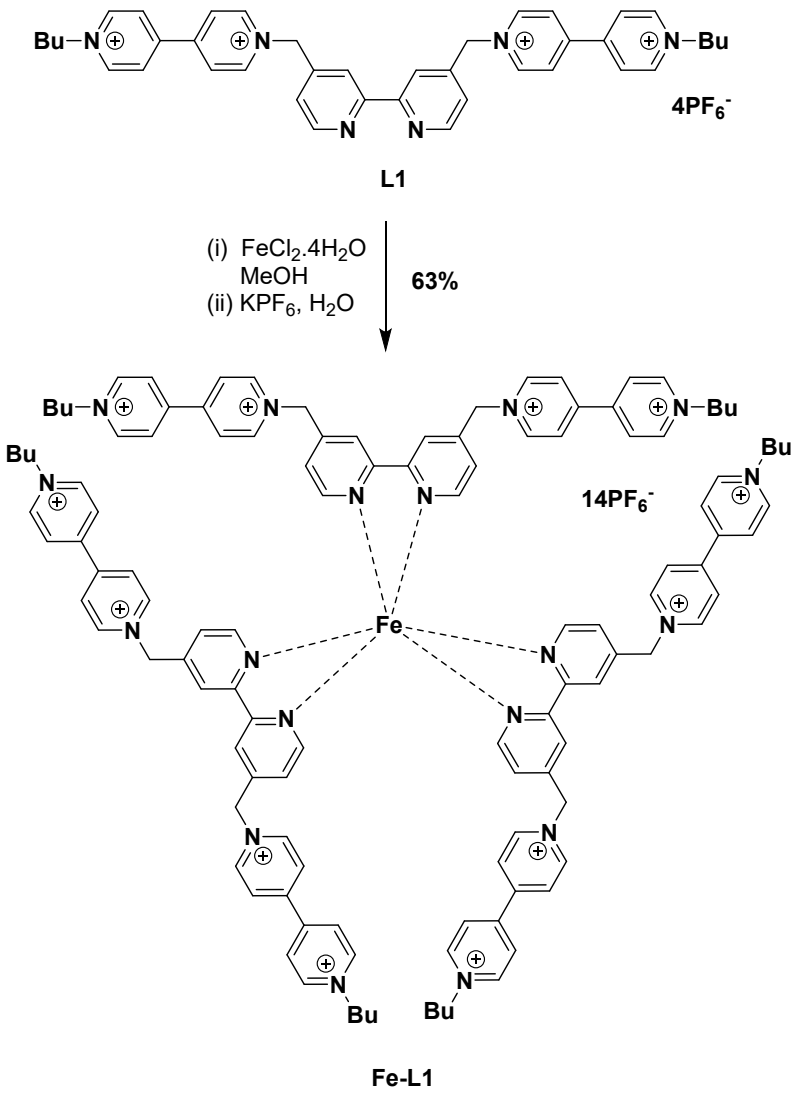
5,5'-bis(bromomethyl)-2,2'-bipyridine **[12]** (1.091 g, 3.21 mmol) and 1-butyl-[4,4'-bipyridin]-1-ium bromide **[7]** (1.975 g, 6.74 mmol) in acetonitrile (5 mL) were heated at 70 ^{o}C for 5 h. The product was precipitated using saturated KPF_6 solution. The precipitate was filtered, and dried under high vacuum to yield the ligand as an off-white solid (1.006 g, Yield 92%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.53 (d, *J* = 5.5 Hz, 4H), 9.35 (d, *J* = 6 Hz, 4H), 8.94 (s, 2H), 8.79-8.70 (m, 8H), 8.44 (d, *J* = 8 Hz, 2H), 8.15 (d, *J* = 7.5 Hz, 2H), 6.04 (s, 4H), 4.67 (t, 4H), 1.98-1.91 (m, 4H), 1.37-1.28 (m, 4H), 0.92 (t, 6H). HRMS: Calculated for $C_{40}H_{44}N_6^{4+}$ 608.361, found 647.460.



Scheme S2. Synthesis of Ligand **L2**.

2.3 Synthesis of Fe complex [Fe-L1]

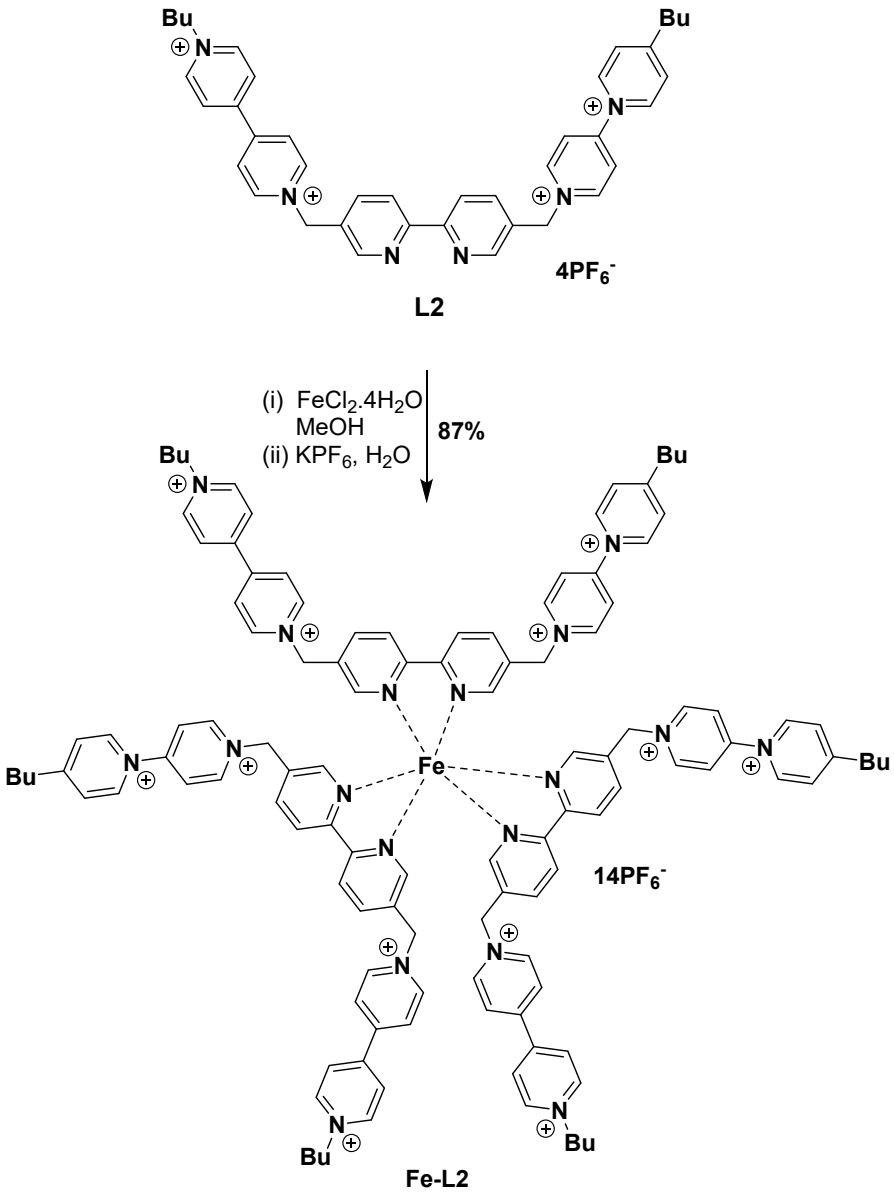
Ligand **L1** (200 mg, 0.168 mmol) dissolved in DMF (1 mL) and Iron(II) Chloride (11.14 mg, 0.06 mmol dissolved in EtOH (20 mL) in a 50 mL round-bottom flask was heated overnight at 80 °C. The crude reaction mixture thus obtained was treated with saturated KPF_6 and the obtained precipitate was filtered, washed and dried under high vacuum to get the desired product as a purple solid (126 mg, Yield 63%). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 9.55 (s, 12H), 9.37 (s, 12H), 8.76 (d, *J* = 30 Hz, 30H), 8.61 (s, 6H), 7.58 (s, 6H), 6.08 (s, 12H), 4.68 (s, 12H), 1.95 (s, 12H), 1.33 (s, 12H), 0.94 (s, 18H).



Scheme S3. Synthesis of the metal complex **Fe-L1**.

2.4 Synthesis of Fe complex [Fe-L2]

Ligand **L2** (200 mg, 0.17 mmol), dissolved in DMF (1 mL) and Iron(II) Chloride (11.135 mg, 0.06 mmol dissolved in EtOH (20 mL) were mixed and heated overnight at 80 °C. After cooling to rt, the mixture thus obtained was treated with saturated KPF_6 solution and the precipitate formed was filtered, washed and dried under high vacuum to get the desired product as a purple solid (173 mg, Yield 87%). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 9.51 (s, 12H), 9.34 (d, *J* = 5 Hz, 2H), 8.94 (s, 6H), 8.77-8.70 (m, 24H), 8.44 (d, *J* = 8 Hz, 6H), 8.15 (d, *J* = 7 Hz, 6H), 6.04 (s, 12H), 4.67 (t, 12H), 1.98-1.91 (m, 12H), 1.36-1.28 (m, 12H), 0.92 (t, 18H).



Scheme S4. Synthesis of the metal complex **Fe-L2**.

3. Cytotoxicity Studies

The viability of L6 cells (skeletal muscle cells) was estimated by means of MTT assay using different amounts (1-50 μg) of the parent ligands (**1**, **8**, **L1** and **L2**) and the metal complexes, **Fe-L1** and **Fe-L2**. The cells, after incubation with the corresponding compound, were washed and MTT (0.5 g L^{-1}), dissolved in DMEM, was added to each well for the estimation of mitochondrial dehydrogenase activity as described previously.[S2] After an additional 90 min of incubation at 37 ^{0}C in a CO_2 incubator, 10% SDS in DMSO was added to each of the wells and the absorbance at 570 nm of solubilized MTT formazan products were measured after 45 min, using a micro-plate reader.

4. Procedure for Coating the Cotton Fabrics

Freshly cut cotton fabric (40 cm x 40 cm) was treated with UV-ozone for 15 min. The metal complex **Fe-L1** or **Fe-L2** (25 mg) was dissolved in acetonitrile (0.5 mL) and was added to TiO_2 sol (2.5 wt%, 100 mL) in water. The mixture was then spray coated (3 layers) using a pressure spray nozzle to the UV-ozone treated fabric with drying cycles in between. After drying the fabric in a hot air oven at 70 ^oC, Ag-Cu binary nanoparticle sol was sprayed (3 layers) using the same pressure spray nozzle with drying cycles in between. The coated fabric was dried for 30 min. in a hot air oven at 80 ^oC. Coatings for control experiments were achieved by eliminating the corresponding components using a similar procedure.

5. Anti-bacterial Studies

5.1 Anti-bacterial Investigations of the Metal Complexes

Bacterial spiking solutions were prepared in Luria Bertiani (LB) medium and taken for test at 18h old stage, and diluted as mentioned below, where the colony forming units (CFUs) were approximately 4.3 x 10^6 and 2.5x 10^5 per millilitre for *E. coli* and *S. aureus* respectively (based on CFU counts of 18h old sample). The two bacterial suspensions (spiking solutions) of *Escherichia coli* and *Staphylococcus aureus* was serially diluted in sterile saline up to 10^3 and 10^4 respectively. Each of these suspensions were again diluted 10x in saline. The undiluted and diluted suspensions were labelled as 0× and 10× respectively. 500 µL of the test samples were added to 10 mL of both 0× and 10× dilutions and incubated at 30 ^oC for one hour at 200 rpm. After incubation, 100 µL of samples were taken from both dilutions and spread plated on Luria Bertiani agar plates and incubated at 37 ^oC for 24 h. After 24h of incubation, the plates were observed for colony formation and counted where applicable. Efficacies were calculated as % reduction of CFUs from the original counts in the spiking solution.

The broad-spectrum anti-bacterial action of the complexes **Fe-L1** and **Fe-L2** against different bacteria were investigated using disc diffusion method. *Bacillus cereus* MTCC 1305 and *Mycobacterium smegmatis* MTCC 993 were chosen as gram-positive models and *Escherichia coli* MTCC 2622*, Klebsiella pneumoniae* MTCC 109*,* and *Pseudomonas aeruginosa* MTCC 2642 were chosen as gram-negative strains. The test bacteria were first inoculated separately in nutrient broth and incubated at 37°C for 18 h. Each of the inoculum was then adjusted to 0.5 McFarland turbidity standards and swabbed into Mueller – Hinton plates. A 10 mg mL^{-1} stock solution of the metal complex **Fe-L1** or **Fe-L2** was prepared in DMSO and 30 µL from the stock solution was loaded onto 6 mm sterile discs. The addition of DMSO alone served as control

and similar solutions prepared from the bipyridines **1** and **8** and the ligands **L1** and **L2** were used for comparison. Sterile discs loaded with the agents were placed on separate plates, pre-inoculated with each tested culture, the plates were incubated for 24 h at 37°C and the zone of inhibition was measured.

5.2 Anti-bacterial Investigations of the Coatings

AATCC 147 Test (Parallel Streaking Method): The test bacteria (*Staphylococcus aureus* and *Escherichia coli*) were prepared in a liquid culture medium. Bacterial spiking solutions were prepared in Luria Bertiani (LB) medium and taken for test at 18h old stage, and was diluted appropriately using sterile saline to get a final concentration of ~ 10^4 CFUs/mL. One loopful of the diluted inoculum suspension was used to streak 5 consecutive evenly spaced parallel streaks onto the solidified LB agar plates. The streaks were intentionally varied in concentration of the bacteria. Anti-bacterial agent-coated strips (25 mm × 50 mm) were carefully placed on the LB agar surface aseptically, over the parallel streaks. A parallel untreated cotton fabric was also tested as control. The contact of the cotton fabric and the inoculated agar was ensured by applying even gentle pressure on the sample. The plates were incubated for 24h at 37 ^oC. Each experiment was repeated in triplicates. Positive controls using Ampicillin- or Penicillin/Streptomycin-coated cotton fabric of the same size were also done.

ASTM E2149-13a Test: This test determines the quantitative anti-microbial activity of a coating by shaking it with a concentrated bacterial suspension for a 1 h contact time. The suspension was then serially diluted before and after contact and cultured. The number of viable organisms from the suspension was determined and the percent reduction was calculated by comparing with controls. In a typical experiment, coated cotton pieces (weight = 1 ± 0.1 g) were added to a bacterial inoculum (50 ± 0.5 mL) and shaken for a 1 h contact time. The bacterial suspensions were then serially diluted and cultured on pre-prepared agar plates. Filtration may be required if any sort of degradation is suspected. The plates were then incubated for 24 h at 37 ^oC. After 24h of incubation, the plates were observed for colony formation and counted where applicable. Efficacies were calculated as % reduction of CFUs from the original counts in the spiking solution, before adding the coatings. Residual bacterial retention on the coatings was tested using agar imprint tests.

5.3 SEM Imaging of Anti-bacterial Action

The morphological alterations in bacterial cell walls caused by the metal complexes **Fe-L1** and **Fe-L2** were monitored by SEM analysis. Strains of *Mycobacterium smegmatis* MTCC 993 and (D-F)

Pseudomonas aeruginosa MTCC 2642 were chosen as representative examples. After the treatments as described Section 5.2 (disc diffusion method), the pellets were washed with phosphate-buffered saline (PBS) and centrifuged (8000 rpm) for 5 min. The pellets were immediately fixed with 2% glutaraldehyde (v/v) at 4 ^{o}C for 12 h. The bacterial suspension was again centrifuged (5000 rpm for 5 min). Then the supernatant was removed and washed three times with PBS to remove any trace of glutaraldehyde. The pellets were then post-fixed in 1% (w/v) osmium tetroxide at 4 ^{o}C for 1 h and washed twice with PBS. The samples were dehydrated in graded ethanol series (30, 50, 70, 80, 95 and 100%) for 10 min each. A few drops of the bacterial suspensions were drop-casted on aluminium foil attached to conductive carbon tape and dried in sterile airflow. The samples were sputtered with gold to avoid charging and analysed under a 10 kV in scanning electron microscope.

5.4 Stability Studies on the Coatings

In order to test the stability of the coatings towards humidity and temperature, the coated cotton fabrics were kept at 90-95% relative humidity (45-50 ^{o}C) or 110 ^{o}C for six hours. The retention of anti-bacterial activity was then investigated using AATCC 147 or ASTM E2149-13a Tests. The stability of the coatings towards washing was investigated under ISO 6330 specifications (2 kg textile with 20 g detergent, 40 ^{o}C, agitation for 35 min). In a typical experiment, 3.7 g of the coated cotton fabric was added to a container with 38 mg detergent and 500 mL water. The system was subjected to vigorous stirring for 35 min at 40 ^{o}C. The fabric was then retrieved and dried in a hot-air oven at 80 ^{o}C. The retention of anti-bacterial activity of the dried fabric was then investigated using AATCC 147 or ASTM E2149-13a Tests. The washing-drying cycle was repeated multiple times (5×). As coated sample, without any treatment, served as controls.

2.1.7.5 Determination of MIC

The broth micro-dilution-based MIC determination experiments were conducted according to EUCAT guidelines. The experiments were conducted in 96 well plates, where each well was supplemented with a different concentration of test compounds **Fe-L1** and **Fe-L2** in a total volume of 200 µL sterile MHB (Muller Hinton Broth) cation-adjusted medium (pH 7.2)[S3]. Diluted test cell cultures (2 µL) of 0.05 OD adjusted (~5x10^{5} CFU/mL) overnight grown cultures of *E. coli*, *S. aureus*, *M. smegmatis, K. pneumonia* were used for the studies. Experiments without the test compounds and without bacterial inoculum served as negative controls. In addition, two known antibiotics (Ampicillin, and Gentamycin) with known MIC were used as positive controls.[S4] The cultures were incubated at 37 °C for 18 h, and then OD_{600} was

measured. The growth inhibition was assessed in comparison with a negative control. The MIC was determined as the test compound concentration that inhibited more than 90% of cell growth based on the OD_{600} measurements. All the experiments were conducted with biological duplicates.

2.1.7.6 Live-Dead Assays

Preparation of Live and Dead Cultures: Live and dead culture assays were performed using a BacLight Kit (Invitrogen, 2004). A turbid overnight grown test cultures in LB medium were collected in microfuge tubes, and centrifuged at 5000 rpm for 5 mins, at 25 °C. The pellets were re-suspended in 0.85% saline, and the above steps were repeated twice to remove completely the media components and other particles in the medium. After washings, the cells were suspended in 300 µL of saline, and were equally distributed in three microfuge tubes. One tube was kept as control. **Fe-L1** and **Fe-L2** at their MIC were added to the remaining two tubes and incubated for 20 mins. After incubation the cells were washed thrice (with saline), and finally re-suspended in 50 µL saline.[S5]

Staining Samples with SYTO 9 and PI: Stock solutions of SYTO 9 and PI were prepared in 1:2 ratios respectively, were 1 µl SYTO 9 and 2 µl of PI were suspended in 497 µL of 0.85% saline in microfuge tubes. The tubes were then covered with aluminum foil, and were stored on ice. Both the control and treated samples were stained by adding 50 µL of the dye solutions. This stained mixture was incubated in the dark for 30 min. at room temperature. The dye-mixed samples were then washed thrice to remove any excess dye. Finally, the stained samples were suspended in 50 µL 0.85% saline and the stained cells were viewed under a florescent microscope under trans luminescence, 515 nm (GFP) and 610 nm (RFP).[S5]

2.1.7.7 Quantification in Live-Dead Assays

The number of viable cells recovered from the MIC-treated samples was estimated from the number Colony Forming Units (CFUs).[S4] The CFUs were estimated using a spot assay agar platting method. 20 µL of samples collected from both overnight (~18 h) incubated control and **Fe-L1** or **Fe-L2** treated wells were serially diluted in 180 µL of sterile 0.85% saline. The serial dilution was done up to 10^8 times. 20 µL samples from 10^8, 10^6, and 10^4 dilutions were spotted onto LB agar plates and were incubated at 37 °C for overnight. The number of viable colonies was estimated and compared with controls.

6. Supporting Figures

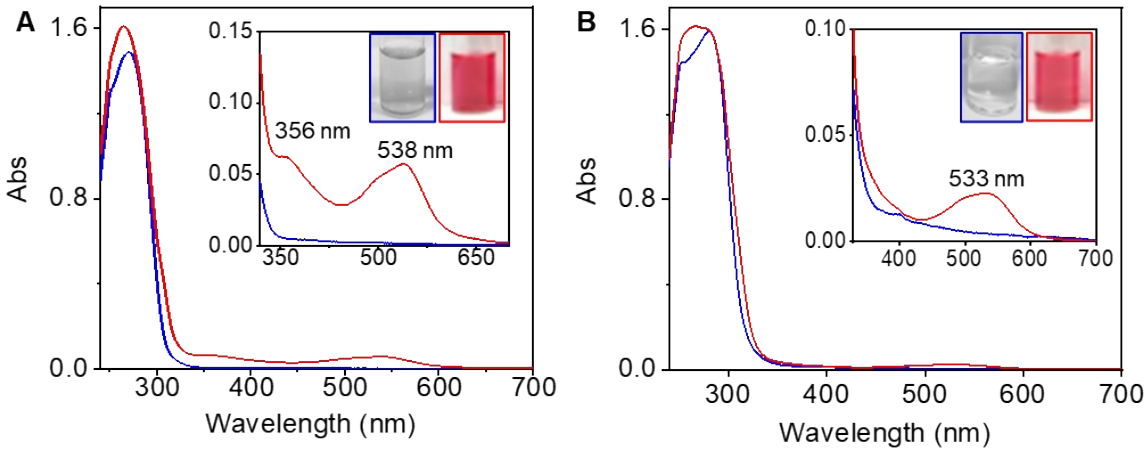


Figure S1. UV-vis absorption spectra of (A) **L1** (blue trace) and **Fe-L1** (red trace) and (B) **L2** (blue trace) and **Fe-L2** (red trace). *c* = 15 μM in MeOH. The blank experiment with the solvent gave the baseline. The zoomed spectra and the photographs of the solutions are shown in the insets.

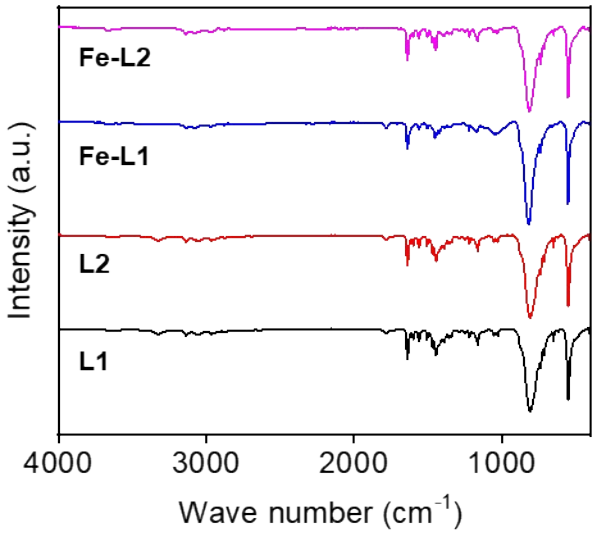


Figure S2. FT-IR (neat, KBr) spectra of **L1** (black trace), **L2** (red trace), **Fe-L1** (blue trace) and (**Fe-L2** (pink trace).

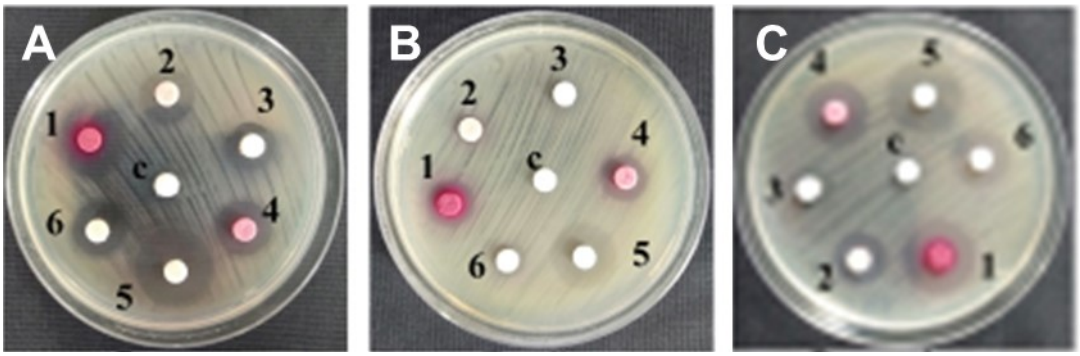


Figure S3. Anti-bacterial activity of the compounds used in this study against (A) *Bacillus cereus* MTCC 1305 and (B) *Escherichia coli* MTCC 2622, and (C) *Pseudomonas aeruginosa* MTCC 2642. The compounds are numbered as: 1: **Fe-L1**, 2: **L1**, 3: bipyridine **1**; 4: **Fe-L2**, 5: bipyridine **8**, and 6: **L2**.

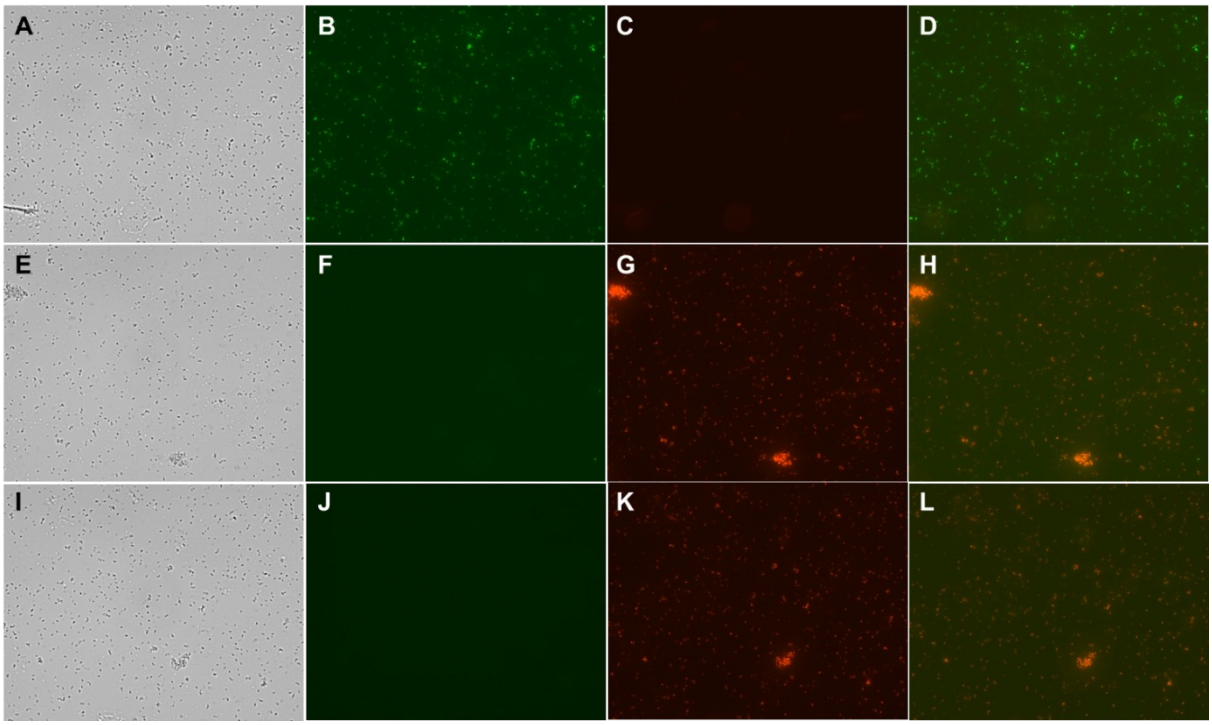


Figure S4. Images of *S.aureus* showing only viable cells with green florescent imaged at 60x zoom in (A) transmittance mode, (B) λ_{ex} 515 (Syto9/Green), (C) λ_{ex} 610 (propidium iodide /red), and (D) 515 nm + 610 nm (Merged). Images of *S.aureus* after treatment with (E-H) **Fe-L1** and (I-L) **Fe-L2** showing predominantly dead cells (red florescence), imaged at 60x zoom in (E,I) transmittance mode, (F,J) 515 nm (Syto9/Green), (G,K) 610 nm (propidium iodine /red), and (H,L) (Merged).

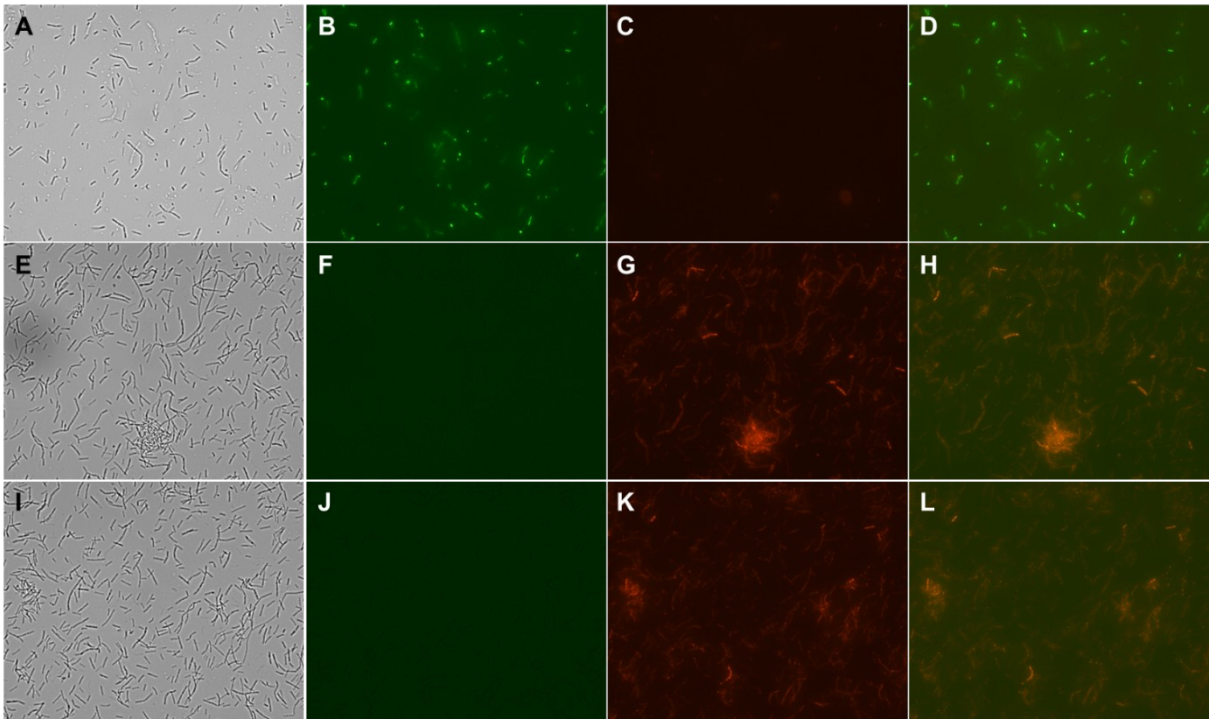


Figure S5. Images of *M.smegmatis* showing only viable cells with green florescent imaged at 60x zoom in (A) transmittance mode, (B) λ_{ex} 515 (Syto9/Green), (C) λ_{ex} 610 (propidium iodide /red), and (D) 515 nm + 610 nm (Merged). Images of *M.smegmatis* after treatment with (E-H) **Fe-L1** and (I-L) **Fe-L2** showing predominantly dead cells (red florescence), imaged at 60x zoom in (E,I) transmittance mode, (F,J) 515 nm (Syto9/Green), (G,K) 610 nm (propidium iodine /red), and (H,L) (Merged).

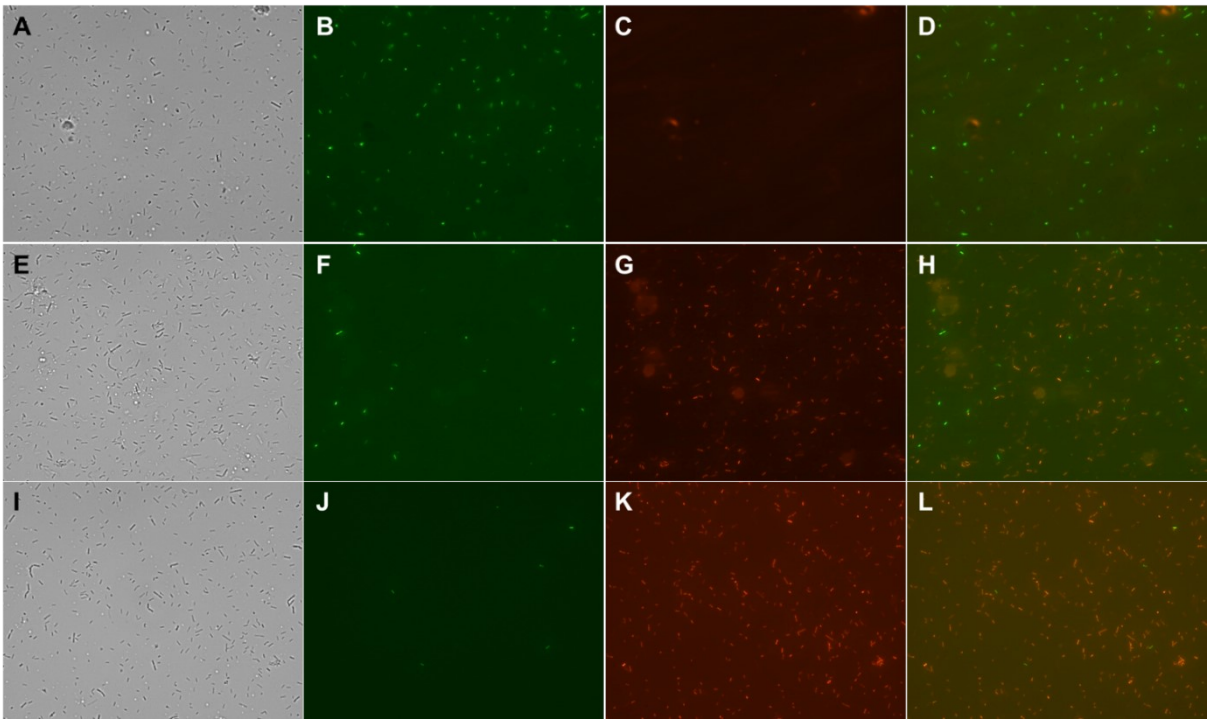


Figure S6. Images of *P. aeruginosa (MTCC 2642)* showing only viable cells with green florescent imaged at 60x zoom in (A) transmittance mode, (B) λ_{ex} 515 (Syto9/Green), (C) λ_{ex} 610 (propidium iodide /red), and (D) 515 nm + 610 nm (Merged). Images of *P. aeruginosa (MTCC 2642)* after treatment with (E-H) **Fe-L1** and (I-L) **Fe-L2** showing predominantly dead cells (red florescence), imaged at 60x zoom in (E,I) transmittance mode, (F,J) 515 nm (Syto9/Green), (G,K) 610 nm (propidium iodine /red), and (H,L) (Merged).

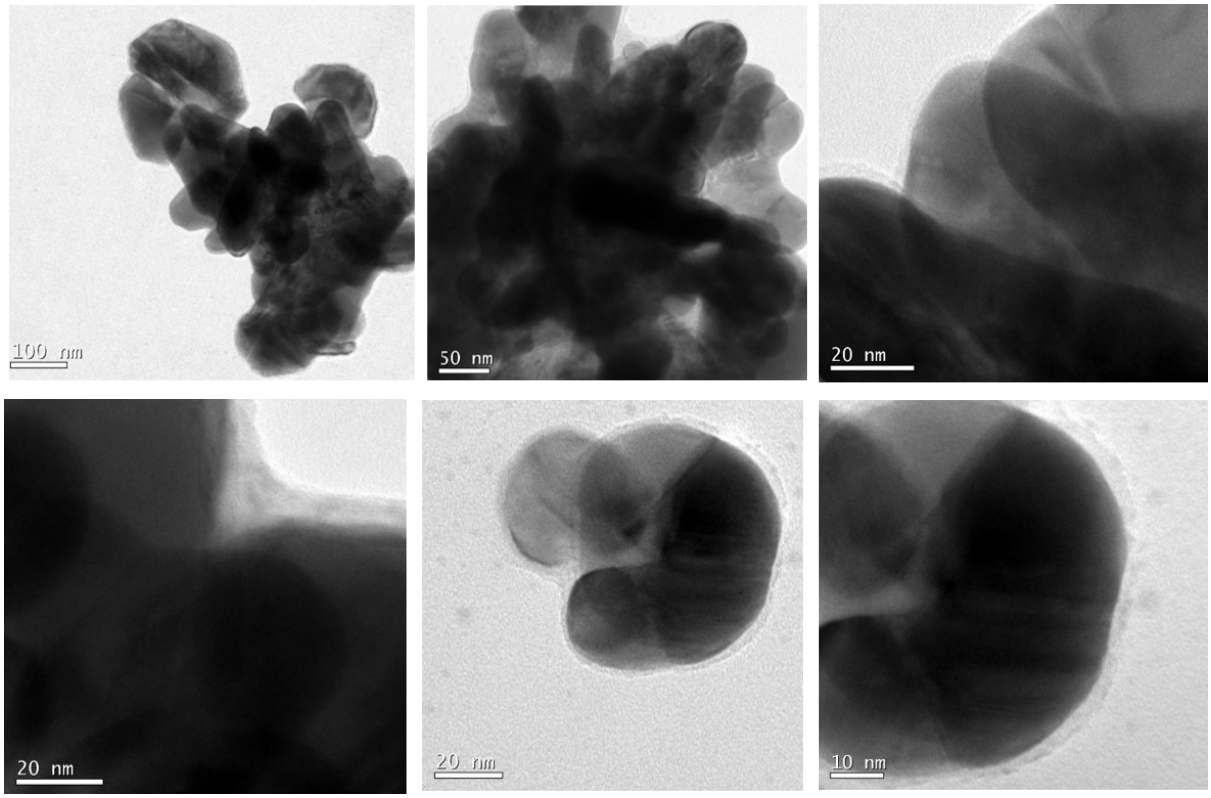


Figure S7. TEM micrographs of Ag-Cu binary nanoparticles.

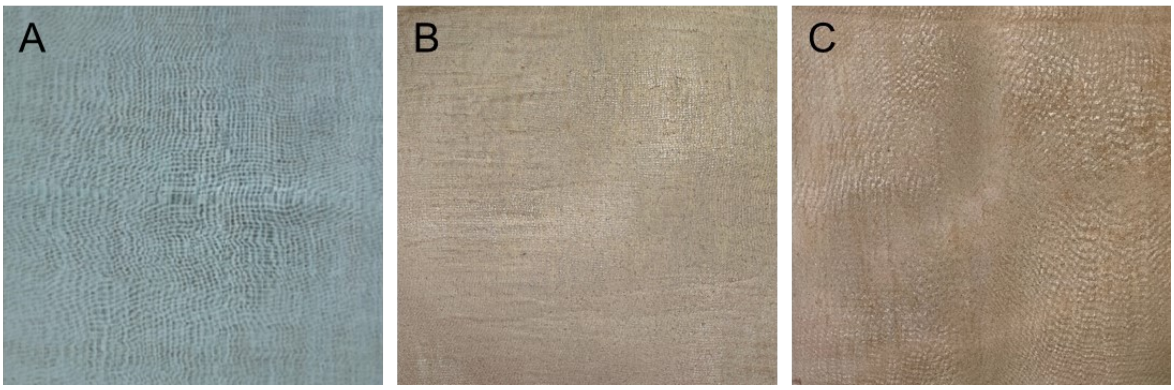


Figure S8. Photographs of (A) pristine cotton fabric, and cotton fabrics coated with (B) **Fe-L1**@TiO2/Ag-Cu and (C) **Fe-L2**@TiO2/Ag-Cu.

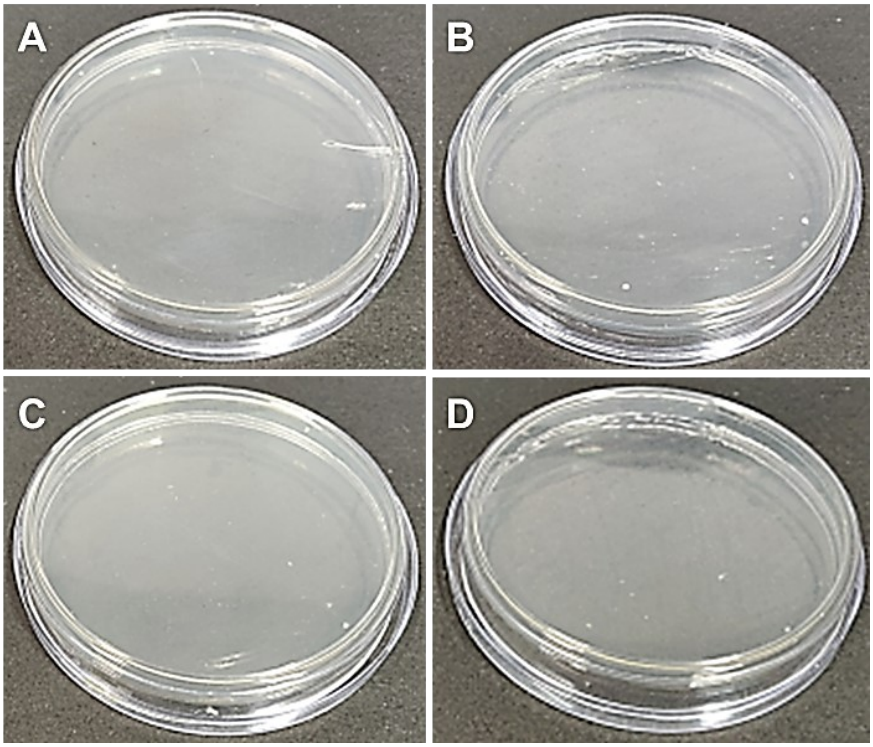


Figure S9. Anti-bacterial activity of **Fe-L1**@TiO2/Ag-Cu against (A) *Staphylococcus aureus* and (B) *Escherichia coli* and **Fe-L2**@TiO2/Ag-Cu against (C) *Staphylococcus aureus* and (D) *Escherichia coli* showing complete disinfection.

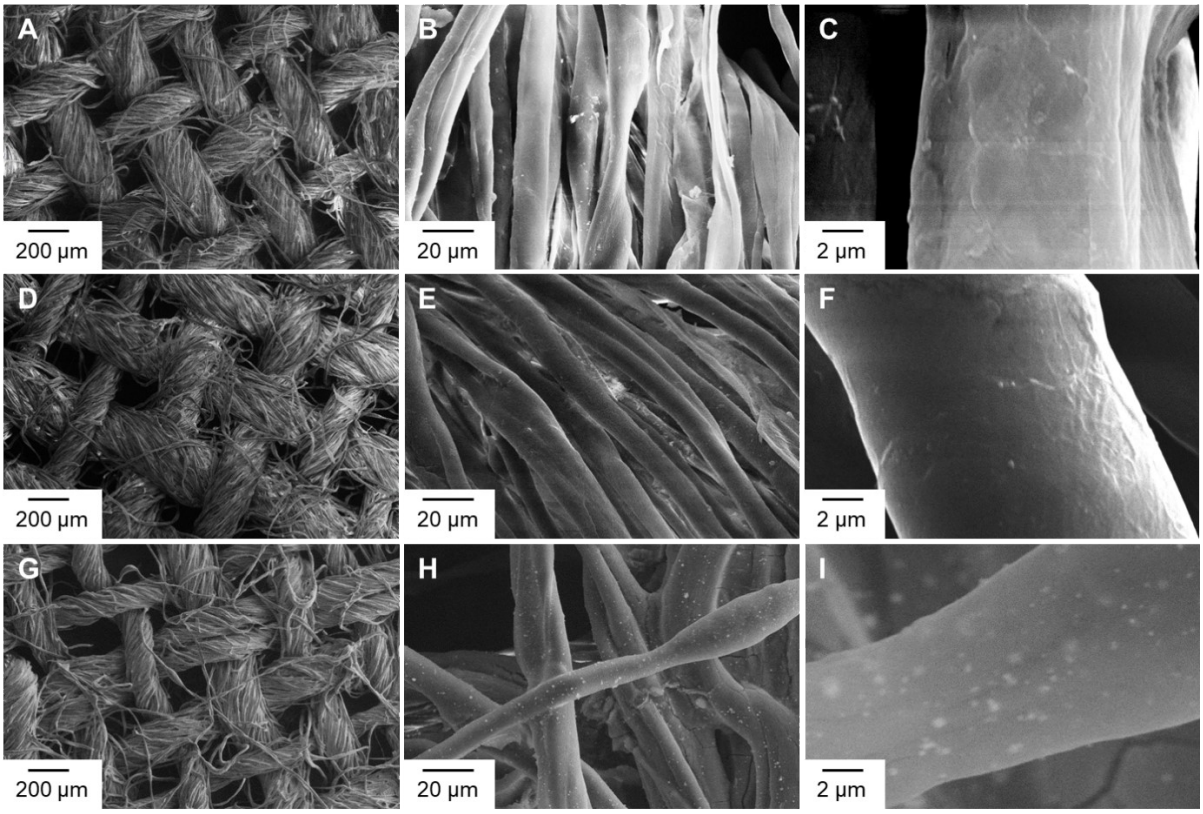


Figure S10. SEM images of (A-C) pristine cotton fabric, (D-F) UV-Ozone treated cotton fabric and (G-I) cotton fabric coated with **Fe-L1**@TiO2/Ag-Cu confirming the modification of the cotton fibers without blocking the pores.

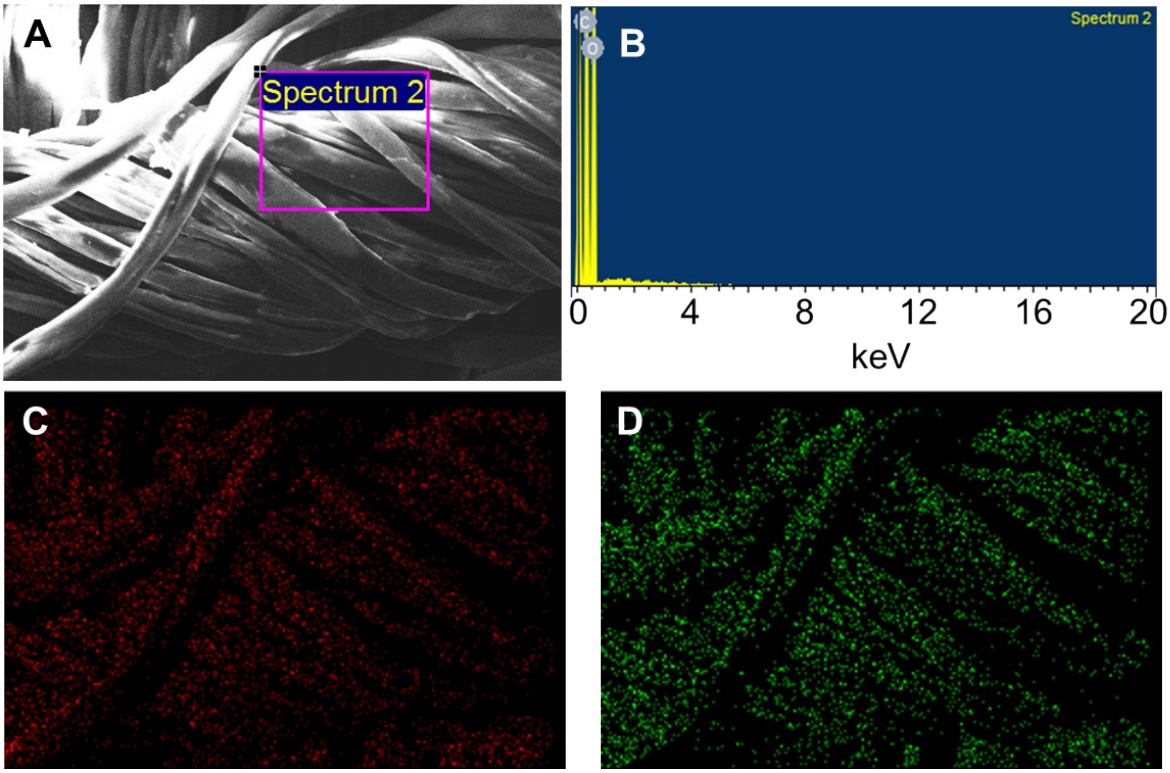


Figure S11. Elemental mapping of pristine cotton fabric using EDS on SEM confirming the presence of carbon (C Ka1) and oxygen (O Ka1). (A) SEM image, (B) EDS spectrum, (C) carbon mapping and (D) oxygen mapping.

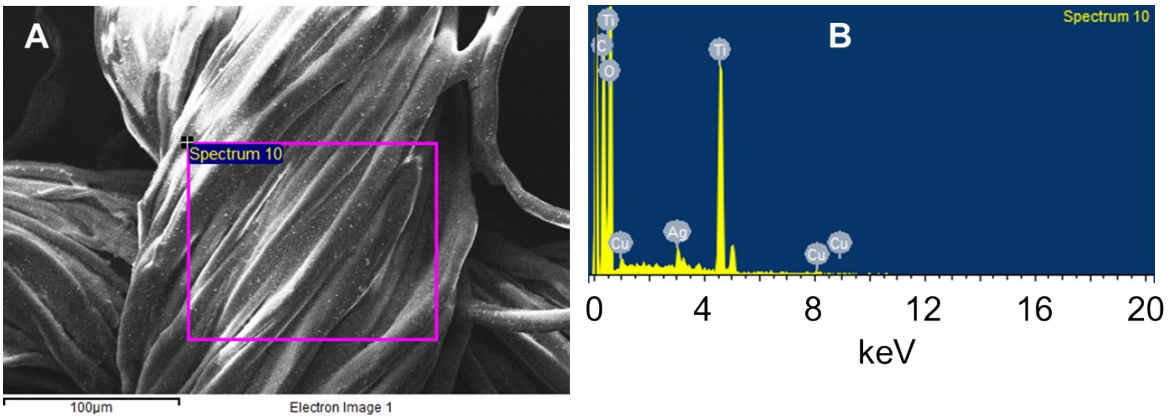


Figure S12. Elemental profile of coated cotton fabric (**Fe-L1**@TiO2/Ag-Cu) using EDS on SEM confirming the presence of carbon, oxygen, titanium, copper and silver. (A) SEM image, and (B) EDS spectrum.

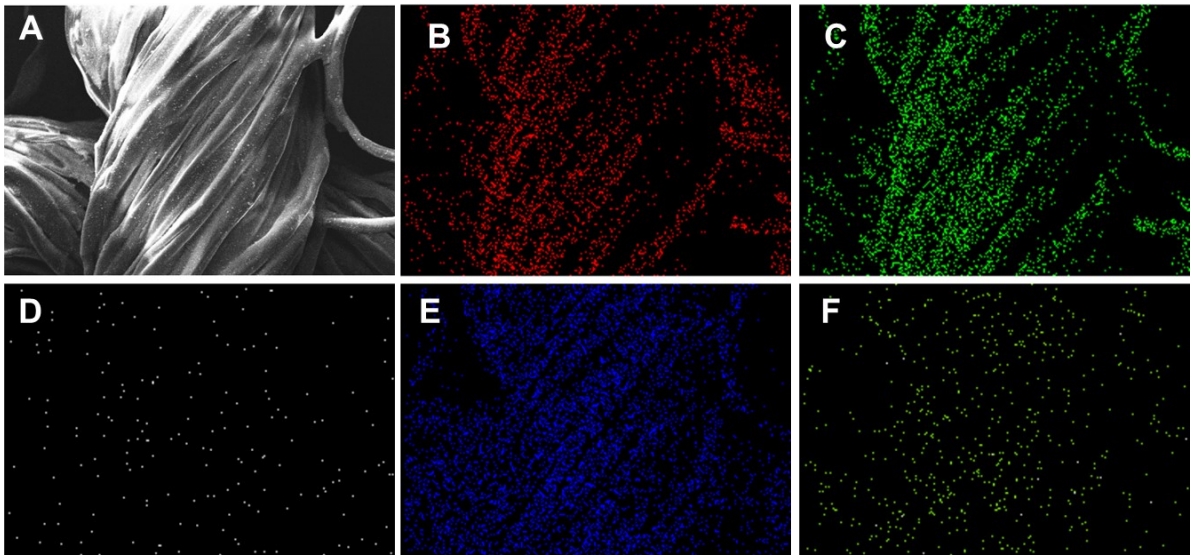


Figure S13. Elemental mapping of coated cotton fabric (**Fe-L1**@TiO2/Ag-Cu) using EDS on SEM confirming the presence of carbon (C Ka1), oxygen (O Ka1), copper (Cu Ka1), titanium (Ti Ka1), and silver (Ag La1). (A) SEM image, (B) carbon, (C) oxygen, (D) copper, (E) titanium, and (F) silver.

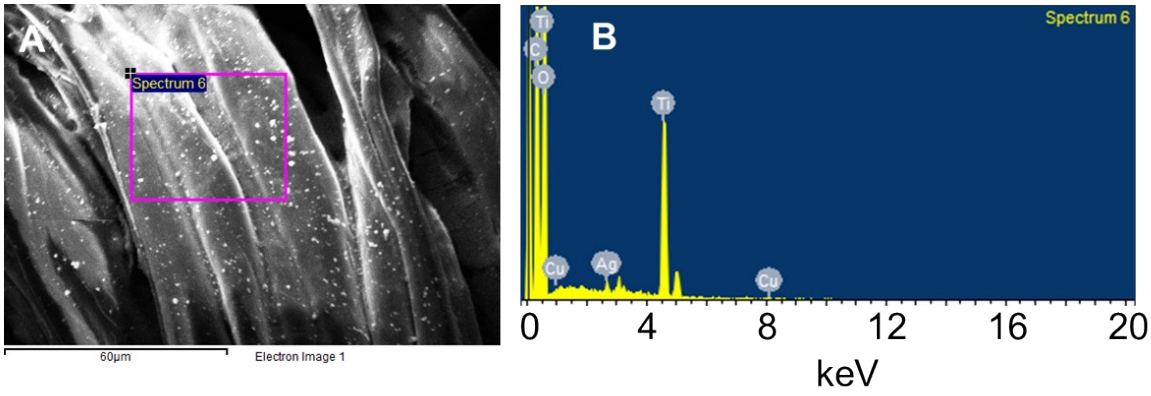


Figure S14. Elemental profile of coated cotton fabric (**Fe-L2**@TiO2/Ag-Cu) using EDS on SEM confirming the presence of carbon, oxygen, titanium, copper and silver. (A) SEM image, and (B) EDS spectrum.

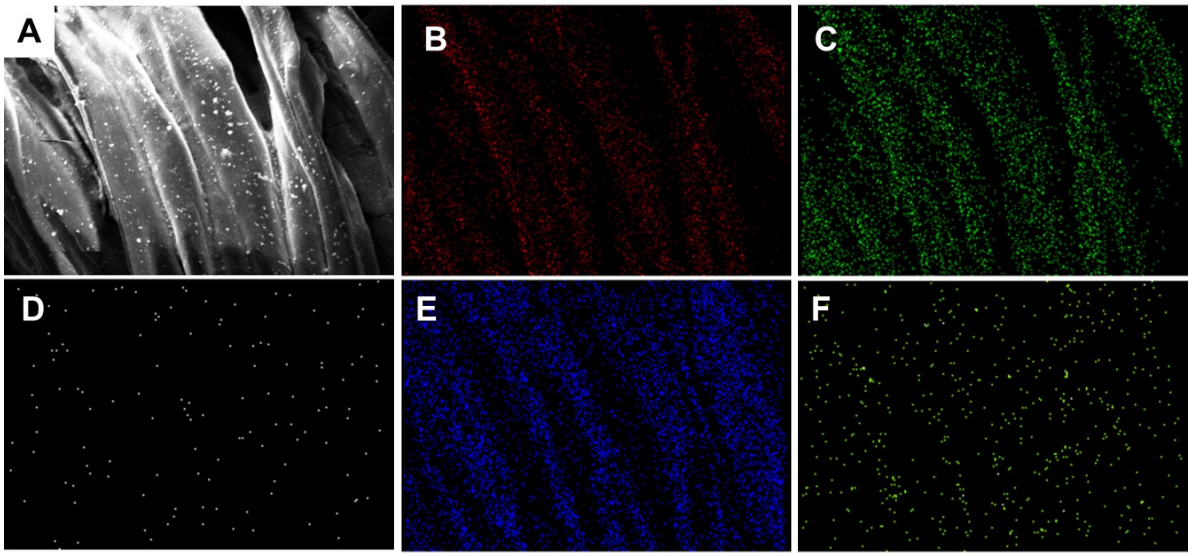


Figure S15. Elemental mapping of coated cotton fabric (**Fe-L2**@TiO2/Ag-Cu) using EDS on SEM confirming the presence of carbon (C Ka1), oxygen (O Ka1), copper (Cu Ka1), titanium (Ti Ka1), and silver (Ag La1). (A) SEM image, (B) carbon, (C) oxygen, (D) copper, (E) titanium, and (F) silver.

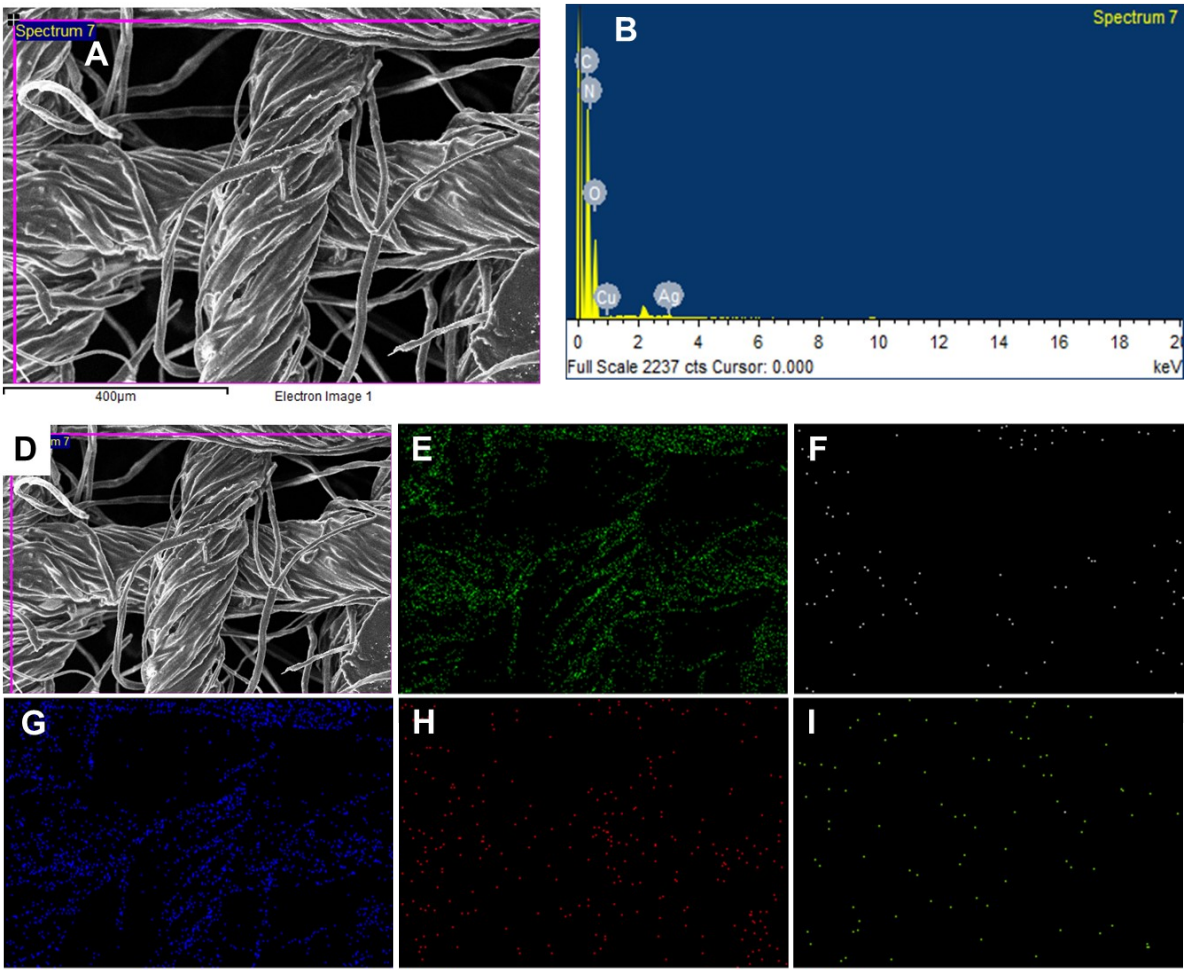


Figure S16. Elemental profile of cotton fabric coated with Ag-Cu binary NPs using EDS on SEM confirming the presence of carbon, oxygen, titanium, copper and silver. (A) SEM image, and (B) EDS spectrum. The corresponding (D) SEM image and elemental mapping of coated cotton fabric (Ag-Cu coated) using EDS on SEM confirming the presence of carbon (C Ka1), nitrogen (N Ka1-2), oxygen (O Ka1), copper (Cu Ka1), and silver (Ag La1). (D) SEM image, (E) carbon, (F) nitrogen (G) oxygen, (H) copper, and (I) silver.

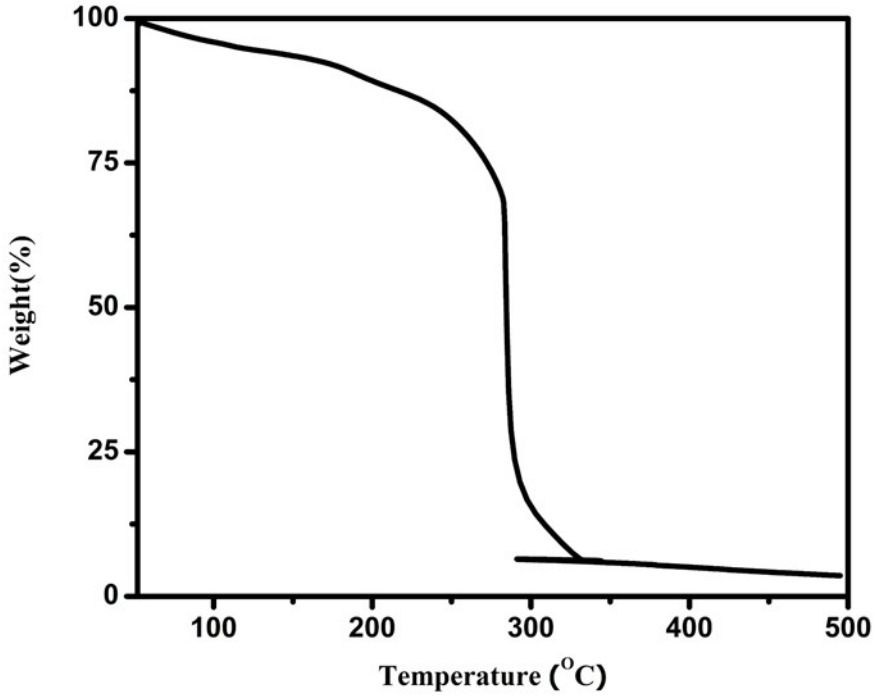


Figure S17. Thermogravimetric analysis of cotton fabric coated with **Fe-L1**@TiO2/Ag-Cu. The residual weight at 500 °C was found to be around 3.5%.

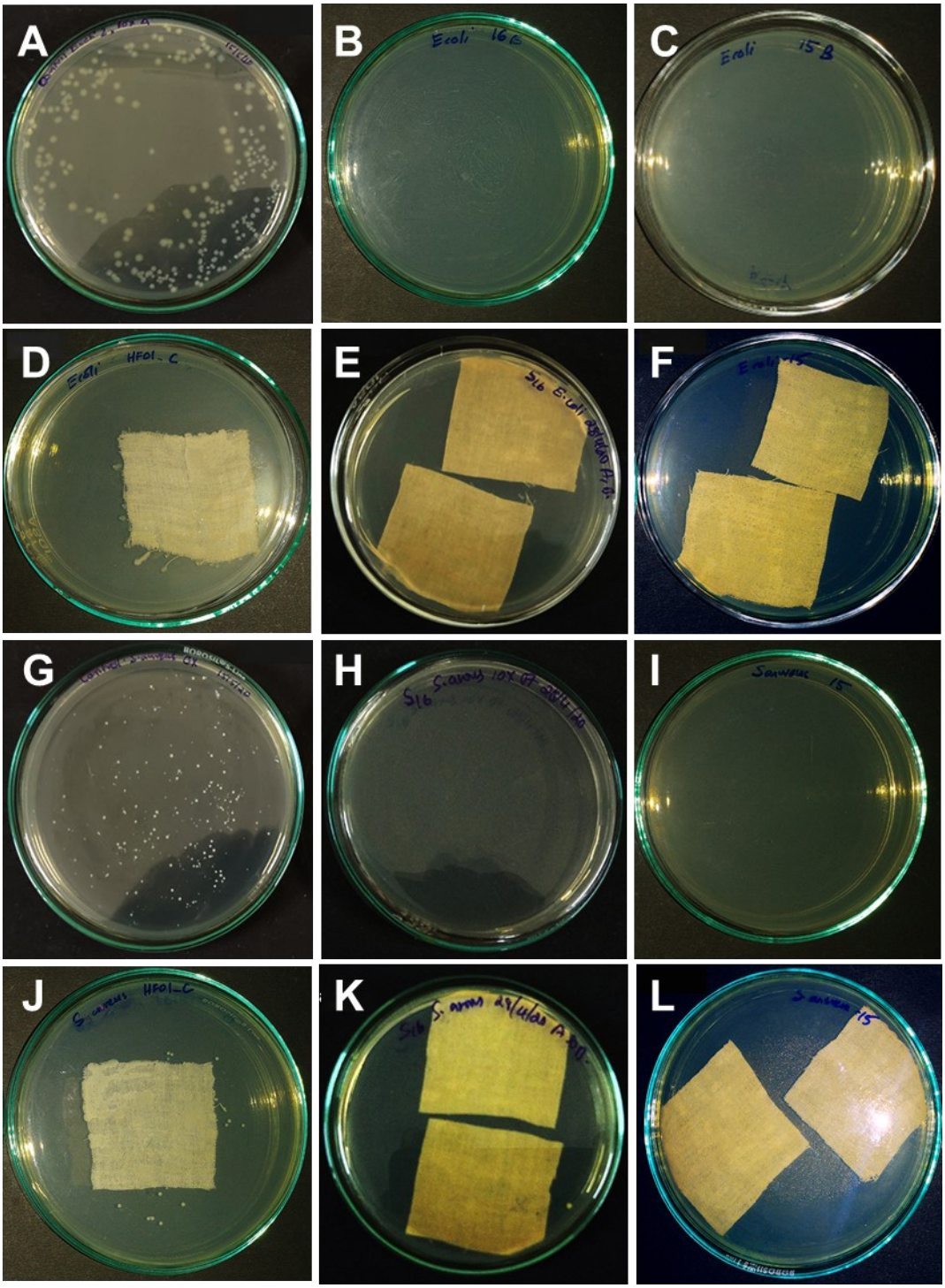


Figure S18. Quantitative adsorption tests using ASTM-E2149-13a specifications on (A-F) *Escherichia coli* and (G-L) *Staphylococcus aureus*. (A,D,G,J) Controls using pristine cotton fabrics and complete disinfection using (B,E,H,K) **Fe-L1**@TiO_2/Ag-Cu- and (C,F,I,L) **Fe-L2**@TiO_2/Ag-Cu-coated cotton fabrics.

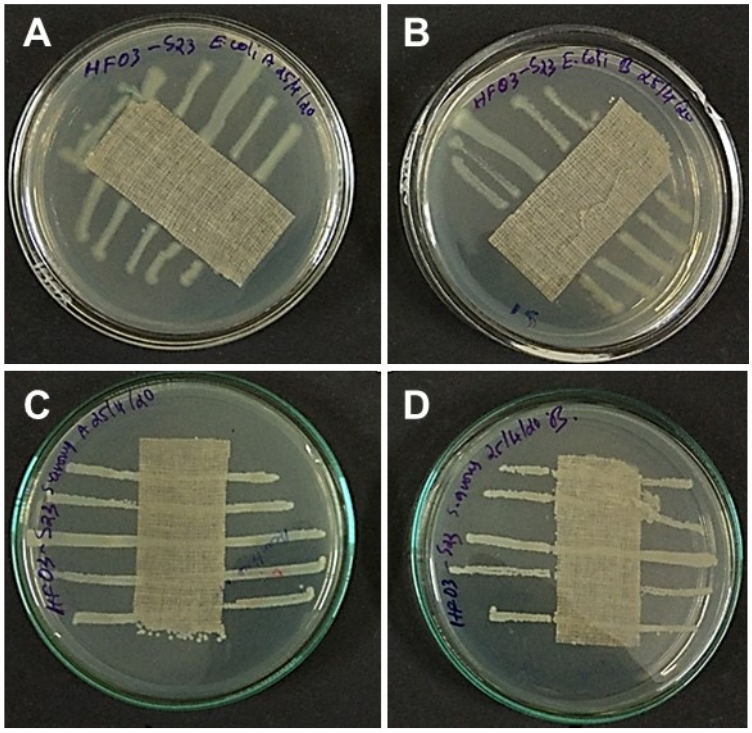


Figure S19. Control experiments using (A,C) pristine cotton fabric and (B,D) low anti-bacterial activity of cotton fabric coated with Ag-Cu bimetallic nanoparticles only, without TiO_2 and the metal complexes against (A,B) *Escherichia coli* and (C,D) *Staphylococcus aureus*, tested under AATCC-147 standards.

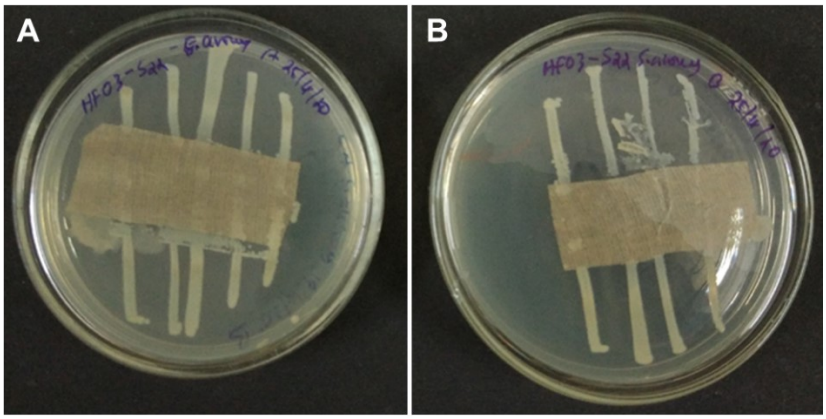


Figure S20. Low anti-bacterial activity of cotton fabric coated with Ag-Cu bimetallic nanoparticles only, using a poly-amino acid base coat against (A) *Escherichia coli* and (B) *Staphylococcus aureus*, tested under AATCC-147 standards.

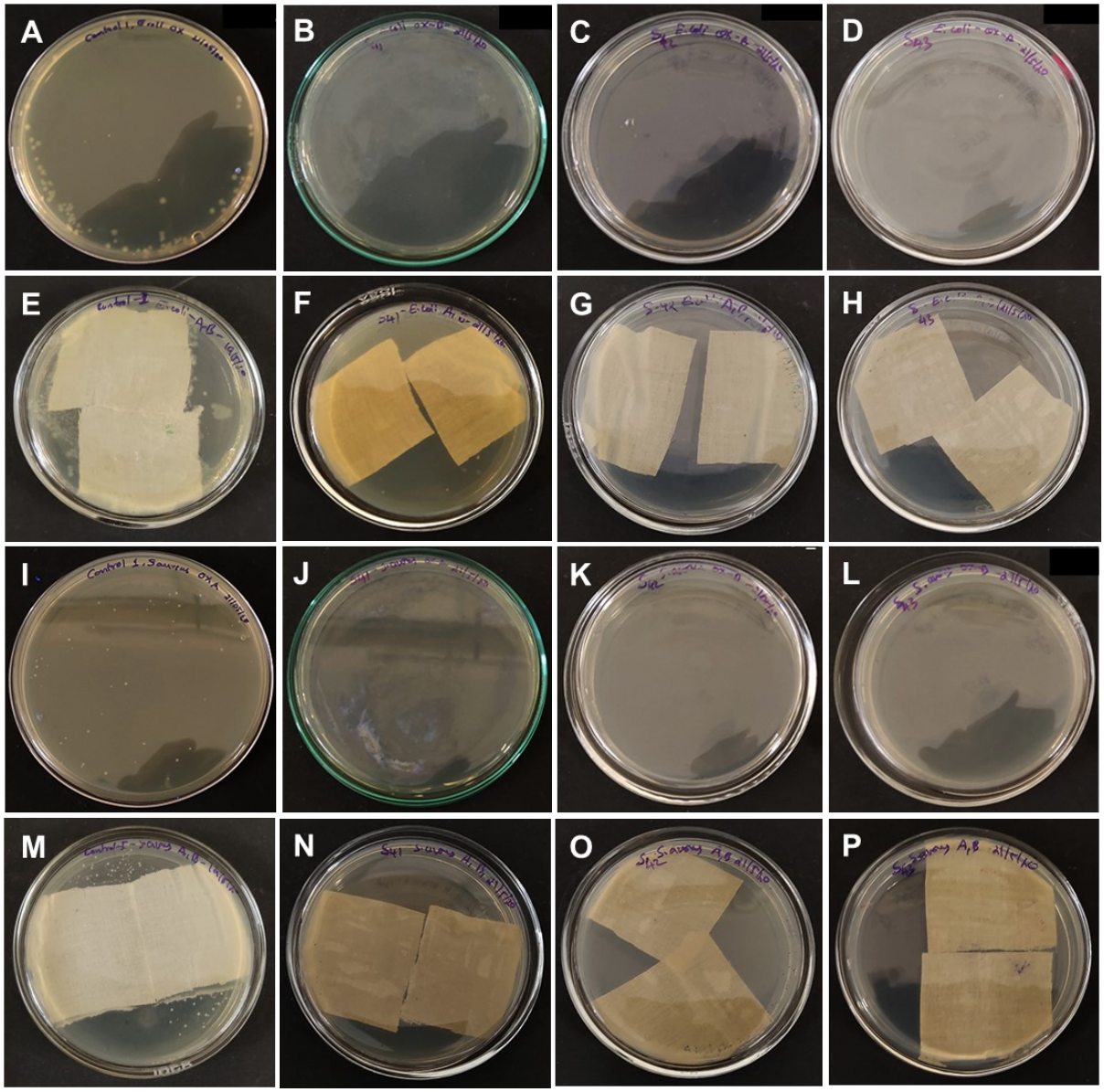


Figure S21. Quantitative adsorption tests using ASTM-E2149-13a specifications on (A-H) *Escherichia coli* and (I-P) *Staphylococcus aureus*. (A,E,I,M) Controls using pristine cotton fabrics, complete disinfection using (B,F) as-coated cotton fabrics with **Fe-L1**@TiO_2/Ag-Cu, (J,N) as-coated cotton fabrics with **Fe-L2**@TiO_2/Ag-Cu, (C,G) **Fe-L1**@TiO_2/Ag-Cu- and (K,O) **Fe-L2**@TiO_2/Ag-Cu-coated cotton fabrics after exposure to high temperature (>110 ^oC) for 6 h, (D,H) **Fe-L1**@TiO_2/Ag-Cu- and (L,P) **Fe-L2**@TiO_2/Ag-Cu-coated cotton fabrics after exposure to humid conditions (90-95% RH, 45-50 ^oC) for 6 h. These results confirm the stability of the anti-bacterial coatings.

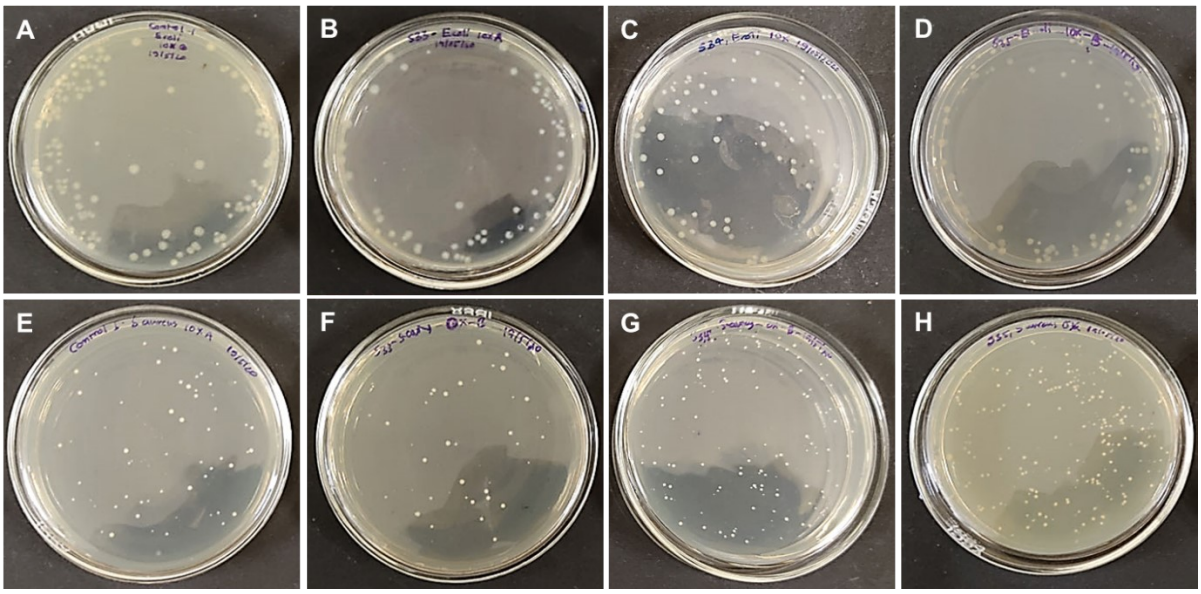


Figure S22. Quantitative adsorption tests using ASTM-E2149-13a specifications on (A-D) *Escherichia coli* and (E-H) *Staphylococcus aureus*. (A,E) Controls using pristine cotton fabrics, and low anti-bacterial activity using (B,F) as-coated cotton fabrics with Ag-Cu bimetallic nanoparticles only, without TiO_2 and the metal complexes, (C,G) Ag-Cu-coated cotton fabrics after exposure to high temperature (>110 ^{o}C) for 6 h, (D,H) Ag-Cu-coated cotton fabrics after exposure to humid conditions (90-95% RH, 45-50 ^{o}C) for 6 h.

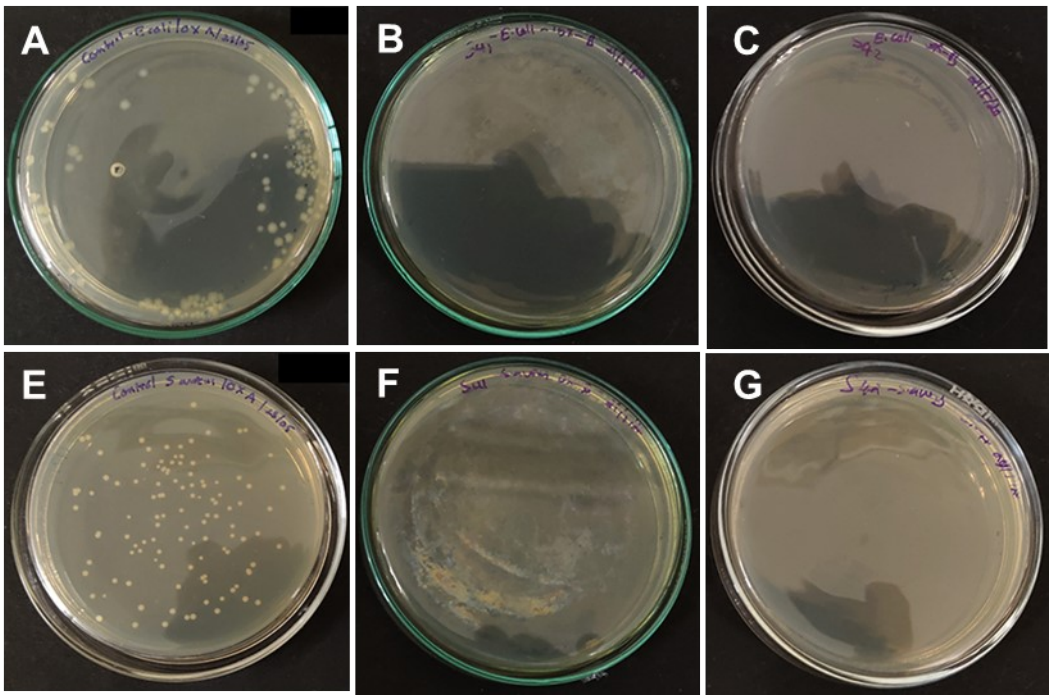


Figure S23. Quantitative adsorption tests using ASTM-E2149-13a specifications on (A-C) *Escherichia coli* and (E-G) *Staphylococcus aureus*. (A,E) Controls using pristine cotton fabrics, and complete disinfection using cotton fabrics coated with (B,F) **Fe-L1**@TiO_2/Ag-Cu, and (C,G) **Fe-L2**@TiO_2/Ag-Cu, after multiple (5×) washing cycles under ISO 6330 specifications (2 kg textile with 20 g detergent, 40 ^oC for 35 min). Conditions used: Fabric weight: 3.7 g, 38 mg detergent in 500 mL water and agitated for 35 min at 40 ^oC. These results confirm the stability of the anti-bacterial coatings towards washing.

7. Supporting Tables

Table S1. Comparison of MIC values of **Fe-L1** and **Fe-L2** with Ampicillin and Gentamycin

No	Bacteria	MIC (µg/mL)			
		Fe-L1	**Fe-L2**	**Ampicillin**	**Gentamycin**
1	*E.coli*	4.3	8.4	>8.0*	4.0
2	*K.pnemoniae* MTCC 109	4.3	8.4	2.0	2.0
3	*M.smegmatis* MTCC 993	4.3	8.4	>8.0*	2.0
4	*S.aureus*	4.3	8.4	>8.0*	4.0

Table S2: CFU profile showing 80-90% growth reduction at MIC and 20-40% growth reduction at ½ MIC concentrations, against various bacterial strains, treated with **Fe-L1**, **Fe-L2** and Gentamycin (MIC).

No	Bacteria	CFU/mL					
		Control	Fe-L1 treated		Fe-L2 treated		Gentamycin Treated
			at ½ MIC	at MIC	at ½ MIC	at MIC	at MIC
1.	*E.coli*	2.33×10^9	5×10^6	$\leq 10^2$	1.28×10^9	$\leq 10^2$	$\leq 10^2$
2.	*K.pnemoniae*	2.48×10^9	1.73×10^7	$\leq 10^2$	3×10^6	$\leq 10^2$	$\leq 10^2$
3.	*M.smegmatis*	3.8×10^8	3.4×10^7	$\leq 10^2$	4.8×10^8	$\leq 10^4$	$\leq 10^2$
4.	*S.aureus*	1.8×10^9	6.8×10^8	$\leq 10^4$	6.8×10^8	$\leq 10^4$	$\leq 10^2$

Table S3. Elemental composition of the coatings obtained via SEM-EDS

Element	Amount (Atomic%)	
	FeL1@TiO_2/Ag-Cu	**FeL2**@TiO_2/Ag-Cu
C K	48.91	50.08
O K	50.74	49.58
Ti K	0.02	0.02
Cu K	0.16	0.16
Ag L	0.17	0.16

8. References

S1. S. Shankar, M. Lahav, and M. E. van der Boom, *J. Am. Chem. Soc.* 2015, **137**, 4050-4053.

S2. T. Mosmann, *J. Immunol. Methods*, 1983, 65, 55-63.

S3. The European Committee on Antimicrobial Susceptibility Testing. Routine and extended internal quality control for MIC determination and disk diffusion as recommended by EUCAST. Version 13.2, 2023. http://www.eucast.org.

S4. M. Balouiri, M. Sadiki, and S. K. Ibnsouda, *J. Pharm. Anal.*, 2016, **6**, 71-79

S5. J. Robertson, C. Mcgoverin, F. Vanholsbeeck, S. Swift, and S. Hope, *Front. Microbiol*, 2019, **10**, 1-13.