

Supporting Information

A Drug-free Strategy to Combat Bacterial Infections with Magnetic Nanoparticles Biosynthesized in the Bacterial Pathogens

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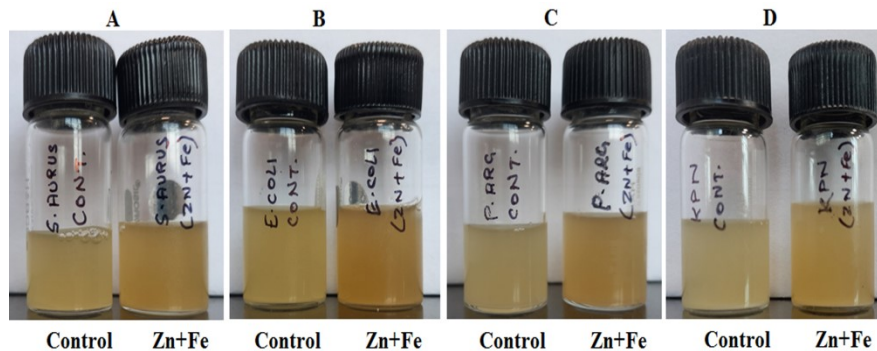


Figure S1: Bacteria treated with/without Fe and Zn. (A) *S. aureus*; (B) *E. coli*; (C) *P. aeruginosa*; (D) *K. pneumoniae*. Untreated bacteria served as control. Color change observed after 36 h treatment.

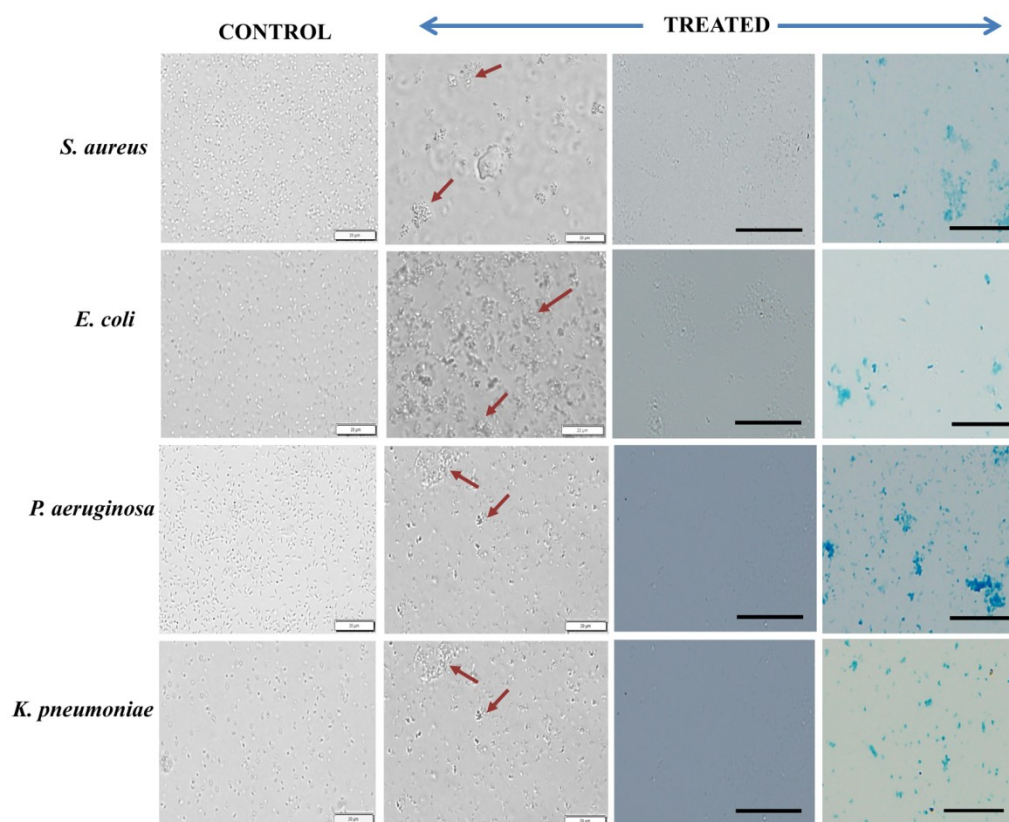


Figure S2: Bacterial response to Fe and Zn. Bright field images of untreated (1st column) and treated bacteria (2nd column). Arrows show bacterial aggregates. FeCl_2 and FeCl_2 + zinc gluconate treated bacteria stained with Perl's Prussian blue (3rd and 4th column); Scale bar represents 20 μM .

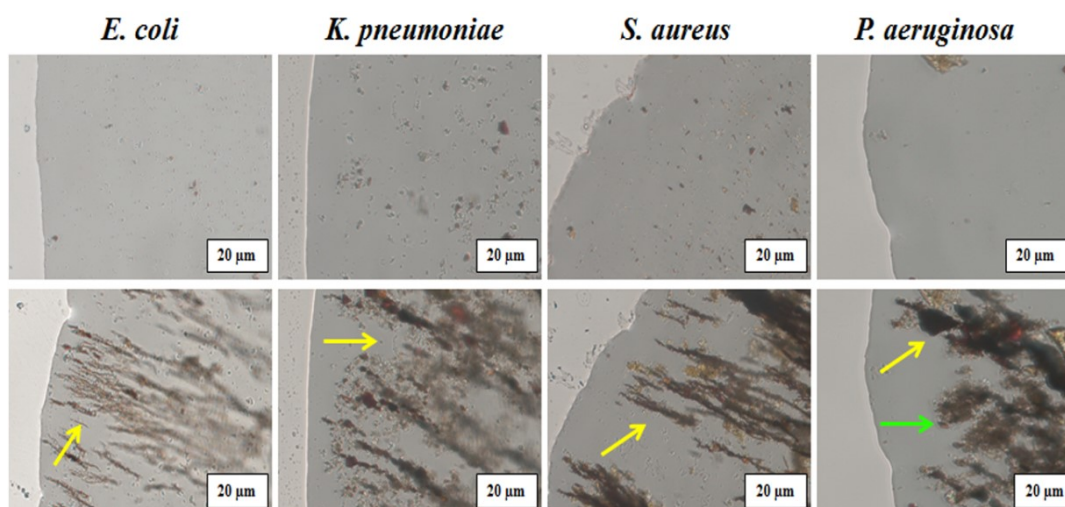


Figure S3: Magnetic-field induced migration of MNPs. Lysates of bacteria treated with Fe & Zn. Upper panel: Microscopic images of the periphery of a drop of lysate. Lower panel: Images of magnet-induced aggregation of nanoparticles. Yellow arrow represents magnetic nanoparticles and green arrow represents cell debris.

M1-M6

Magnetotaxis Videos

M1: Movie of untreated *S. aureus*.

M2: Movie of *S. aureus* treated with $\text{FeCl}_2 + \text{Zn}$ gluconate.

M3: Movie of untreated *E. coli*.

M4: Movie of *E. coli* treated with $\text{FeCl}_2 + \text{Zn}$ gluconate.

M5: Movie of untreated *P. aeruginosa*.

M6: Movie of *P. aeruginosa* treated with $\text{FeCl}_2 + \text{Zn}$ gluconate.

Name of the Bacteria	Conc. of Fe $\mu\text{g/ml}$	Conc. Of Zn $\mu\text{g/ml}$
<i>E.coli</i> (Control)	0.164 $\pm$ 10	0.198 $\pm$ 2
<i>E.coli</i> (FeCl ₂)	0.179 $\pm$ 7	0.213 $\pm$ 4
<i>E.coli</i> (Fe+Zn)	28.33 $\pm$ 3	0.602 $\pm$ 10
<i>S.aureus</i> (Control)	0.116 $\pm$ 12	0.227 $\pm$ 5
<i>S.aureus</i> (FeCl ₂)	0.169 $\pm$ 6	0.283 $\pm$ 8
<i>S.aureus</i> (Fe+Zn)	12.8 $\pm$ 4	0.512 $\pm$ 7

Table ST1: Estimation of Fe and Zn. IC-PMS analysis of Fe and Zn in the lysates of control and bacteria treated with / without FeCl₂, and zinc gluconate. Results represent values obtained from 3.8×10^8 CFU

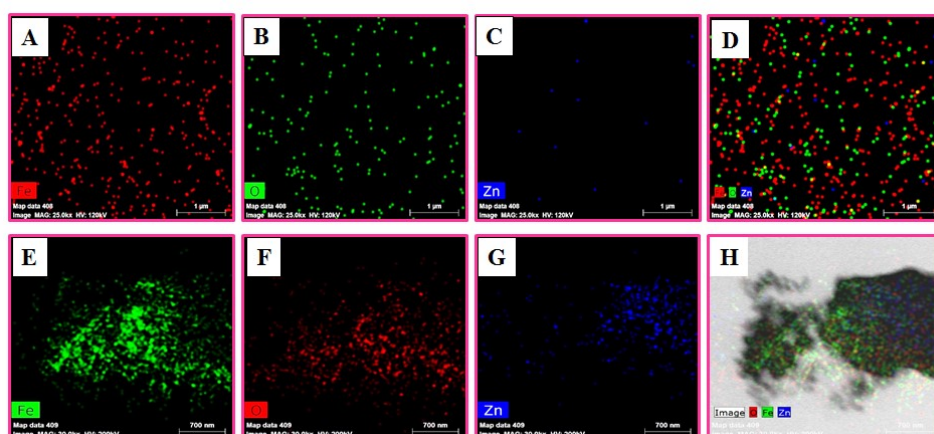


Figure S4: Elemental mapping using TEM. Distribution of iron, oxygen and zinc in treated bacteria. Upper panel represents *S. aureus* (A-C) and Lower panel *E. coli* (E-G). D & H indicate a combination of (A-C) and (E-G) respectively.

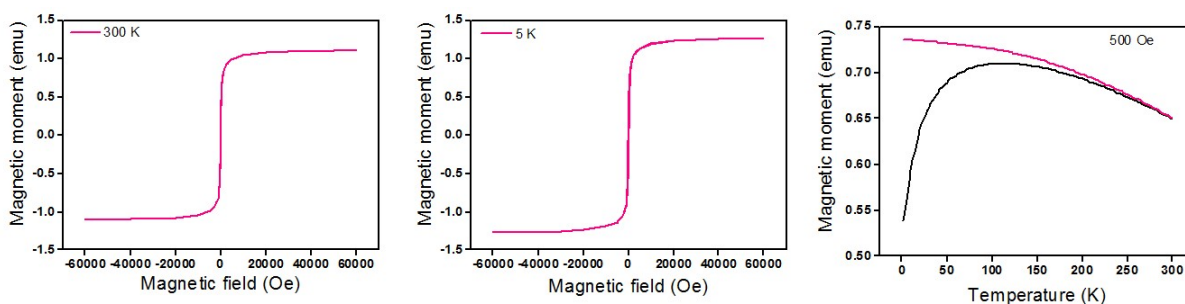


Figure S5: Magnetic measurements of nanoparticles biosynthesized in *E.coli*. Magnetization versus magnetic field measured at (A) 300 K, (B) 5K and (C) Measurements of Temperature Dependence of Magnetization (ZFC/FC curves).

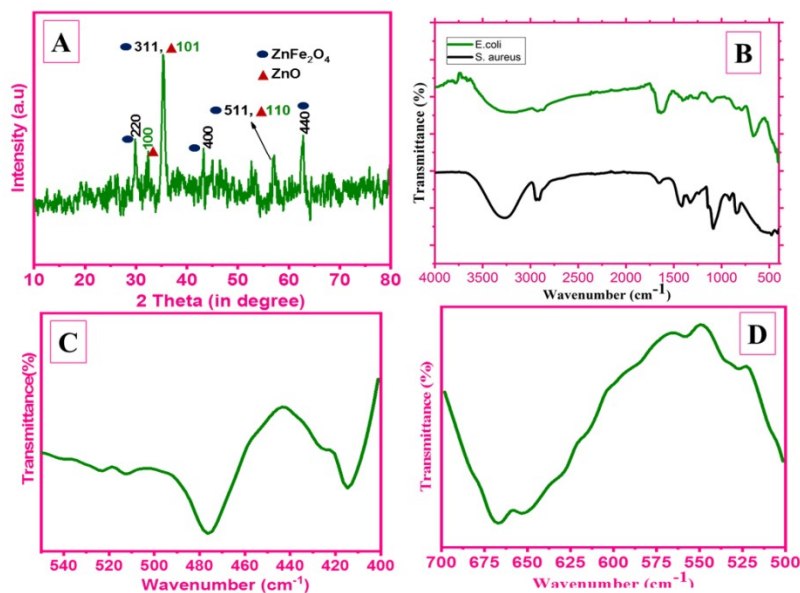


Figure S6: Characterization of Fe and Zn treated bacteria. (A) XRD diffraction pattern of *S. aureus* after calcination. (B) FT-IR of the treated bacteria after lyophilization of *S. aureus* and *E. coli*. (C) Peaks observed in the region from 476-417 cm^{-1} represent Zn-O bond (D). Peaks in the region from 670-550 mode represent Fe-O bonds.

Clinical Specimens			Antimicrobial Susceptibility																												
Sample No.	Infected Tissue	Micro-organism																													
			Cefotaxime	Amikacin	Ceftazidime	Cefepime	Imipenem	Meropenem	Cefoperazone-sulbactam	Gentamycin	Aztreonam	Levofloxacin	Ceftioxone	Tigecycline	Minocycline	Piperaciline + tazobactam	Cotrimoxazole	Eartapenem	Tetracycline	Chloramphenicol	Tobramycin	Netilmicin	Doxycycline	Ciprofloxacin	Clindamycin	Linezolid	Teicoplanin	Erythromycin	Vancomycin	Oxacillin	Colistin
S1	Blood	<i>S. aureus</i>	R	R	R	R	R	R	R														R								
S2	Blood	<i>E. coli</i>	R	R	R	R	R	R	R					S	R			R					S	R		S	S	S	R	R	R
S3	Blood	<i>P. aeruginosa</i>		S		S	R	R	S	S	S		R							S			S								IS
S4	Blood	<i>K. pneumoniae</i>	R	R	R	R	R	R	R					S	R			R					S								
S5	Blood	<i>P. aeruginosa</i>		S		S	R	R	S	S	S		R							S			S								IS
S6	Blood	<i>E. coli</i>	R	R		R	R	R	R	R		R	S		R	R							R								IS
S7	Blood	<i>S. epidermidis</i>	R	R	R	R	R	R	R																				R		
S8	Blood	<i>S. haemolyticus</i>	R	R	R	R	R	R	R															R					R		
S9	Abdomen	<i>E. coli</i>	R	R		R	R	R	R	R		R	S		R	R								R							IS
S10	Muscle	<i>E. coli</i>	R	S		R	S	S	IS	S		R	S		S	S	S						R								IS
S11	Bone	<i>E. coli</i>	R	R		R	R	R	R	R		R	S		R	R							R								

*Where, R= Resistance; S=Susceptibility ; IS=Intermediate susceptibility

Table ST2: Table represents the various infected specimens analyzed and its susceptibility to antibiotics

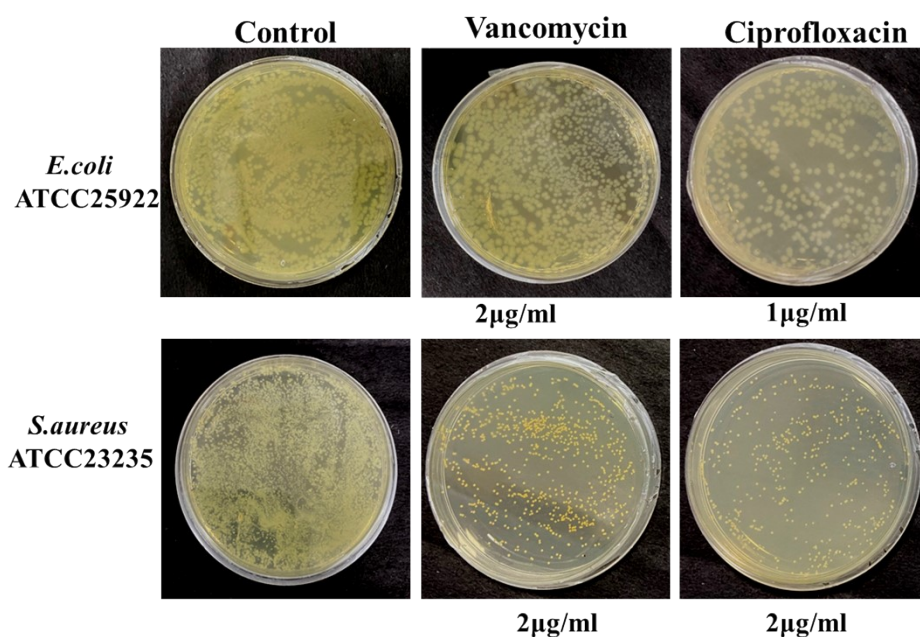


Figure S7: Susceptibility of ATCC strains of *E. coli* and *S. aureus* to vancomycin and ciprofloxacin.

The respective ATCC strain of bacteria was treated with vancomycin/ ciprofloxacin (1-2 µg/mL) for 24 h.

Representative images of the bacterial colonies grown on agar plates

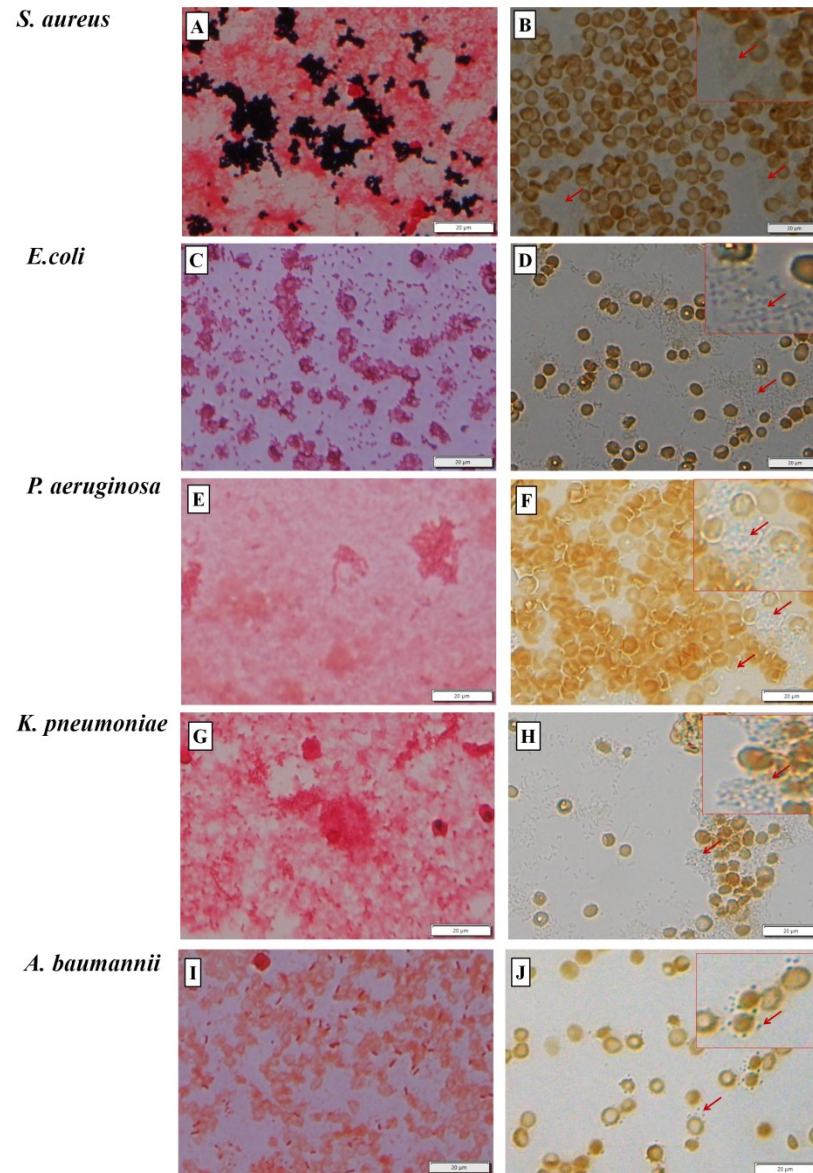


Figure S8: Clinical strains tested for the presence of iron oxide. (A, C, E, G, I). Gram-staining of respective bacteria. (B, D, F, H, J) Blood smear of respective bacterium stained with Prussian blue. Inset shows the magnified image. Arrows indicate blue color obtained after Prussian blue staining. Scale bar represents 20 μm.

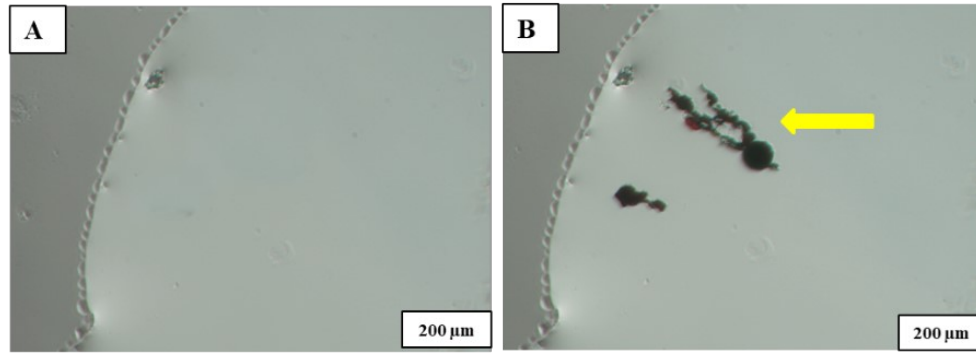


Figure S9: Magnetic-field induced migration of MNPs obtained from *S. epidermidis*: A. Microscopic image of the periphery of a drop of *S. epidermidis* lysate. B. The lysate exposed to magnet. Arrow indicates the alignment of MNPs along the magnetic field lines.

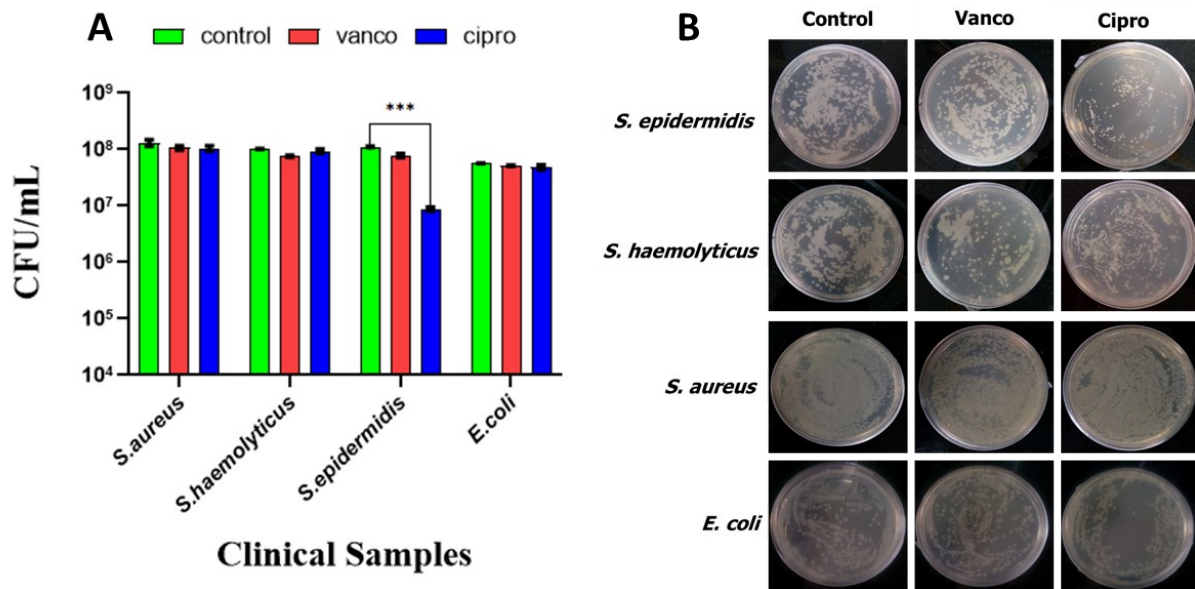


Figure S10: Susceptibility of bacteria (clinical specimens) to vancomycin and ciprofloxacin. The respective bacteria isolated from the infected blood specimens were treated with vancomycin/ ciprofloxacin (2 μg/ml) for 24 h. (A) The number of bacterial colonies obtained after respective treatment. The data represents the mean ± SD obtained from 3 experiments in which N=3 in each group. Two-way ANOVA Bonferroni multiple comparison test where *** represents p-value <0.001 (B) Representative images of the bacterial colonies grown on agar plates.

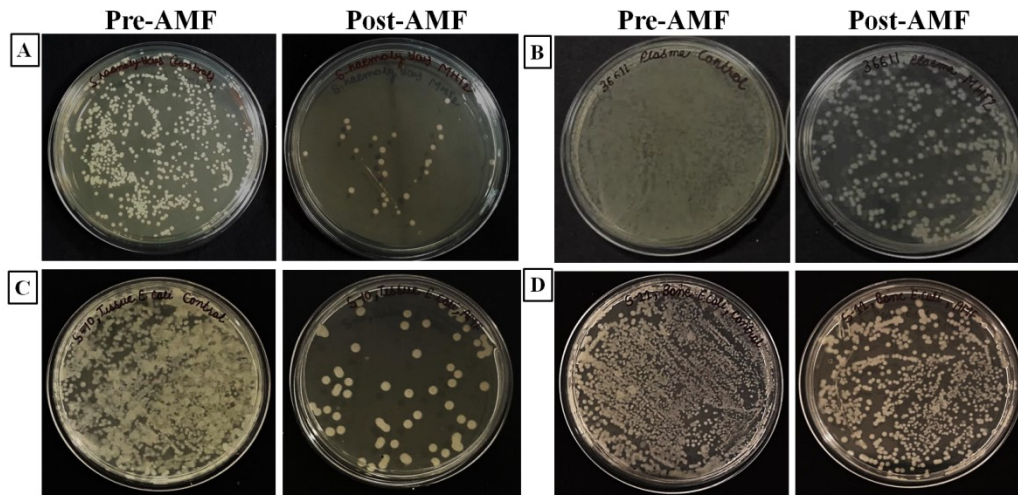


Figure S11: Effect of AMF on infected clinical specimens: Bacterial colonies obtained from pre- and post-AMF exposed samples **A & B** represent *S. haemolyticus* and *E. coli* infected blood samples exposed to AMF (347 KHz, 400 Oe); **C & D** represents *E. coli* infected muscle and bone specimens exposed to AMF at 405 KHz, 400 Oe respectively.