S 2.1. Bacteria preparation

Experimental

Bactericidal experiments using NSs were performed on Gram-negative-*Escherichia coli* (*E. coli*- ATCC 25922) (DH5α), Gram-positive-*S.aureus* (SA-29213 ATCC, partially antibiotic-resistant) and wild-type antibiotic-metal-resistant bacteria wtKARBJ1. We selected the model organisms of *E.coli*, *S.aureus*, and wtKARBJ1 bacteria as they were intrinsically resistant to almost all clinically relevant antimicrobial agents and have the capacity to accumulate and transfer resistant genes, primarily through horizontal gene transfer. [1–5] Studies were conducted using different water sources, namely, nutrient media, tap water, kitchen wastewater, and river water. These water samples were chosen to mimic different water ecosystems: nutrient broth represents the pre-enrichment food source for microorganism to thrive in water, tap water (collected in IITK campus) represents the main source of drinking water in the community that is supplied after primary water treatment, kitchen wastewater (collected in IITK campus) represents domestic wastewater that contains pollutants such as food particles, oil, salts, soap, detergents, metals, grit, and sand, and finally, river water (collected from the Ganges) represents the direct and natural source of potable water. Each of the water samples were tested using basic characterization methods for their turbidity, colour, odour, total dissolved solids, and pH. To remove the effect of contaminants on the experiments, all the above water samples were filtered and autoclaved before preparing stock cultures, and then the bacterial strains that were grown on an LB agar plate were added to them. A fresh colony was inoculated in test tubes containing 5 ml LB nutrient media. The test tubes containing bacteria were kept in a cell culture environment (5 % CO_2, 37 °C) under shaking condition at 250 rpm for 16 h. After inoculation, the bacterial culture solution containing approximately 5 x 10^8 CFU ml^{-1} were grown separately in conical flasks containing LB growth medium. For collecting bacterial strains, the stock culture was centrifuged (3000 rpm for 15 min) and the pellets were washed three times with sterilized PBS solution (pH 7.4) to remove the residual LB growth medium. The bacterial suspension was dispersed separately in the aforementioned four types of water in test tubes. Starting bacterial concentrations of OD_{600} = 1 and 1.5 were used for all the microbiological studies. Before performing any experiments, all the glasswares were sterilized in an autoclave.

S 2.2. Inhibitory effect of FATCNS on bacteria

Experimental

Bacterial concentration was adjusted using LB to the desired density, OD_{600} (1 and 1.5). Further (10 ml) aliquots of this bacterial suspension were prepared in different test tubes. To investigate the MIC of NSs and NPs, different concentrations of NSs (20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300 μg ml^{-1}) and NPs (200, 400, 600, 800, 1000, 1200, 1400, 1600, 1800, 2000, 2200, 2400, 2600, 2800, 3000,3200,3400,3600 μg ml^{-1}) were added into the above test tubes and the final volume was maintained at 10 ml. Test tubes containing LB (10 ml) with only bacteria were used as controls. All the control and treated culture suspensions were incubated in a shaking incubator at 37 °C and 200 rpm. After incubation, the MIC was determined by measuring the absorbance of a bacterial suspension at OD_{600}.

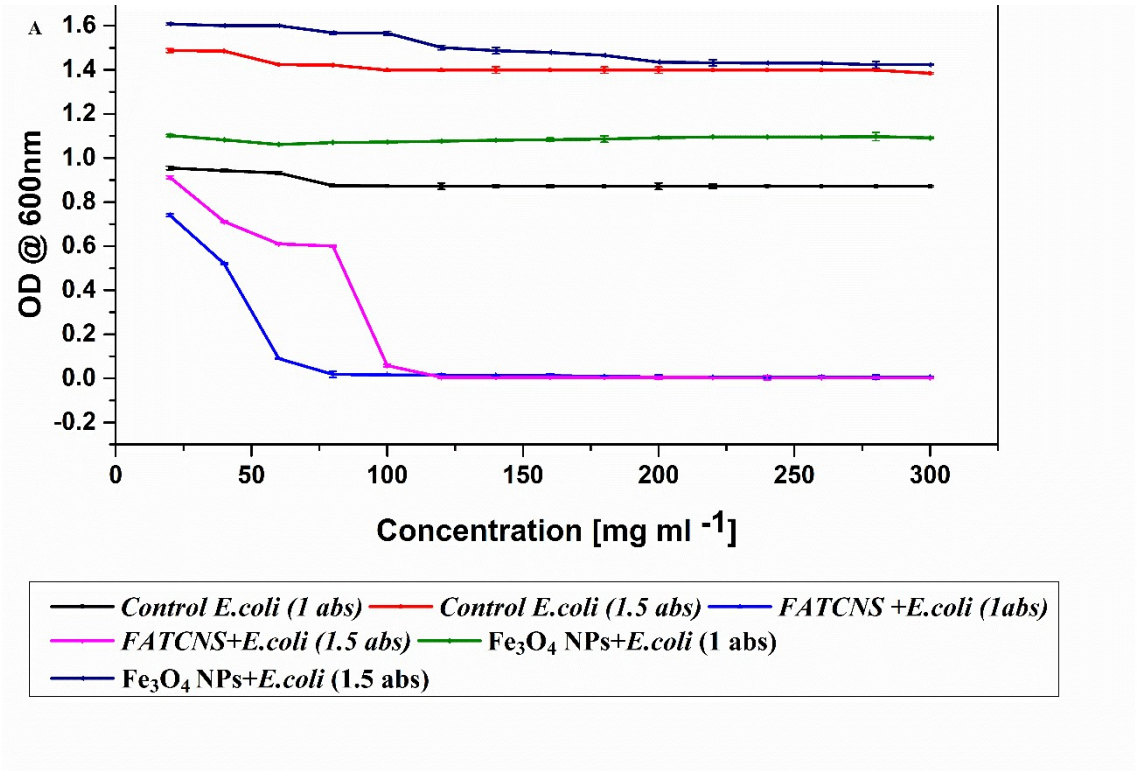
Results

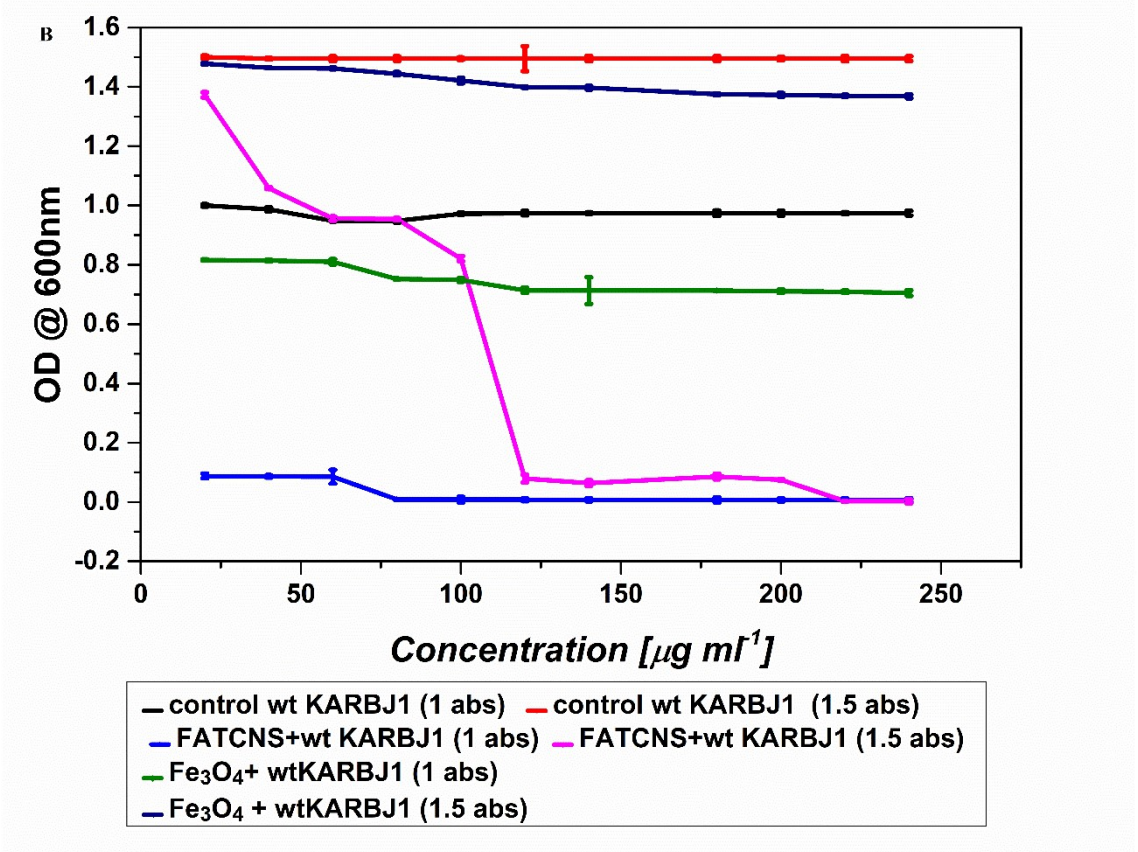
The antibacterial activity of FATCNS is determined by treating different water samples, i.e., LB (turbidity: OD@600nm = 0.2, odour: moldy, TDS: 0.6 mg L^{-1}, pH: 7.2), River Ganges water (turbidity: OD@600nm = 1.08, odour: earthy and mushy, TDS: 0.8 mgL^{-1}, pH: 4.8), tap water (turbidity: OD@600nm = 0.002, odour: NA, TDS: NA, pH: 8.1), and kitchen wastewater (turbidity: OD@600nm = 1.38, odour: fishy, TDS:1.94 mg L^{-1}, pH: 8.1) containing two different concentrations of *E. coli,* wtKARBJ1and *S.aureus*. The minimum inhibitory concentration (MIC) of FATCNS is evaluated by measuring the absorbance at OD_{600} of treated water samples containing bacteria for 16 h under shaking conditions. Similar experiments are conducted to determine the MIC of Fe_3O_4 NPs. The results are presented in SI-2 Figure 1, showing the comparison of a broad range of antibacterial abilities of NSs and NPs towards bacteria in water samples. The OD of the samples treated with NSs decreased rapidly over the first few minutes following treatment and remained unchanged thereafter (as will be discussed later). Consequently, for NSs, only the OD measurements after 6 h post-treatment is presented. The MIC of FATCNS (SI-2 Figure 1) against *E.coli* observed 6 h after treatment with initial OD_{600} = 1 is 60 μg (10 ml^{-1}) and with initial OD_{600} = 1.5 is 100 μg (10 ml^{-1}). The Fe_3O_4 NPs did not significantly affect *E.coli* over 6 h post-treatment (SI-2 Figure 1). However, a decrease in absorbance is observed at the 16^{th} hour. Based on the OD measurements after 16 h, the MIC of Fe_3O_4 NPs for *E.coli* with initial OD_{600} = 1 is 1600 μg $ml^{-1}$and with initial OD_{600} = 1.5 was 2200 μg ml^{-1}. The above observations suggest that the antibacterial activity of FATCNS is rapid and effective compared to Fe_3O_4 NPs at both concentrations of *E.coli* studied. It should also be noted that the amount of NSs required to stunt the growth of *E.coli* is significantly lower than the NPs. The antibacterial effect of FATCNS on wtKARBJ1 was rapid and noticeable in all water samples except tap water (please refer to Table SI-2.1). The reduction in turbidity was

detectable in LB after 1 min of FATCNS addition. The MIC of FATCNS for inhibition of wtKARBJ1 with OD_{600} = 1 is 80 µg (10 ml^{-1}) and with initial OD_{600} = 1.5 is 220 µg (10 ml^{-1}). The Fe_3O_4 NPs show inhibition of wtKARBJ1 with 2200 µg ml^{-1} and 2800 µg ml^{-1} to stunt the growth at OD_{600} = 1 and 1.5, respectively after 16 h. The MIC is determined for inhibition of *S.aureus* using FATCNS (SI-2 Figure 1) with OD_{600} = 1 was 20 µg (10 ml^{-1}) and initial OD_{600} = 1.5 was 60 µg (10 ml^{-1}). There is no significant effect on *S.aureus* at the initial 6 h on the growth of bacteria using 3000 µg (10 ml^{-1}) Fe_3O_4 NPs in any water samples. Because of nano size, and numerous adsorption sites on the surface of the multi-layered NSs, the adsorption, the interaction between the cell membrane and NSs, and cellular uptake of NSs rapidly increased. This resulted in cellular death and inhibition of bacterial growth.

Table S 2.1. MIC (µg ml^{-1}) of FATCNS and Fe_3O_4 NPs experimented on *E.coli* in different water samples.

MIC [µg ml^{-1}]	**Tap water**	**Ganges river water**	**Kitchen wastewater**
E.coli [abs.1,6 h] [FATCNS]	6.00	< 2	16.00
E.coli [abs.1.5, 6 h] [FATCNS]	14.00	2.00	18.00
E.coli [abs.1,16 h] [Fe_3O_4 NPs]	220.00	160.00	180.00
E.coli [abs.1.5,16 h] [Fe_3O_4 NPs]	280.00	240.00	260.00
wtKARBJ1J1 [abs.1,6 h] [FATCNS]	14.00	4.00	8.00
wtKARBJ1J1 [abs.1.5, 6 h] [FATCNS]	22.00	6.00	18.00
wtKARBJ1J1 [abs.1,16 h] [Fe_3O_4 NPs]	260.0	300	300.0
wtKARBJ1J1 [abs.1.5,16 h] [Fe_3O_4 NPs]	220.0	140.0	220.0
Staphylococcus aureus [abs.1, 6 h] [FATCNS]	4.00	< 2.00	4.00
Staphylococcus aureus [abs.1.5, 6 h] [FATCNS]	2.00	4.00	4.00


A
OD @ 600nm
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
0
50
100
150
200
250
300
Concentration [mg ml -1]
Control E.coli (1 abs)
Control E.coli (1.5 abs)
FATCNS +E.coli (1abs)
FATCNS+E.coli (1.5 abs)
Fe3O4 NPs+E.coli (1 abs)
Fe3O4 NPs+E.coli (1.5 abs)


B
OD @ 600nm
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
0
50
100
150
200
250
Concentration [μg ml-1]
control wt KARBJ1 (1 abs)
control wt KARBJ1 (1.5 abs)
FATCNS+wt KARBJ1 (1 abs)
FATCNS+wt KARBJ1 (1.5 abs)
Fe3O4+ wtKARBJ1 (1 abs)
Fe3O4 + wtKARBJ1 (1.5 abs)

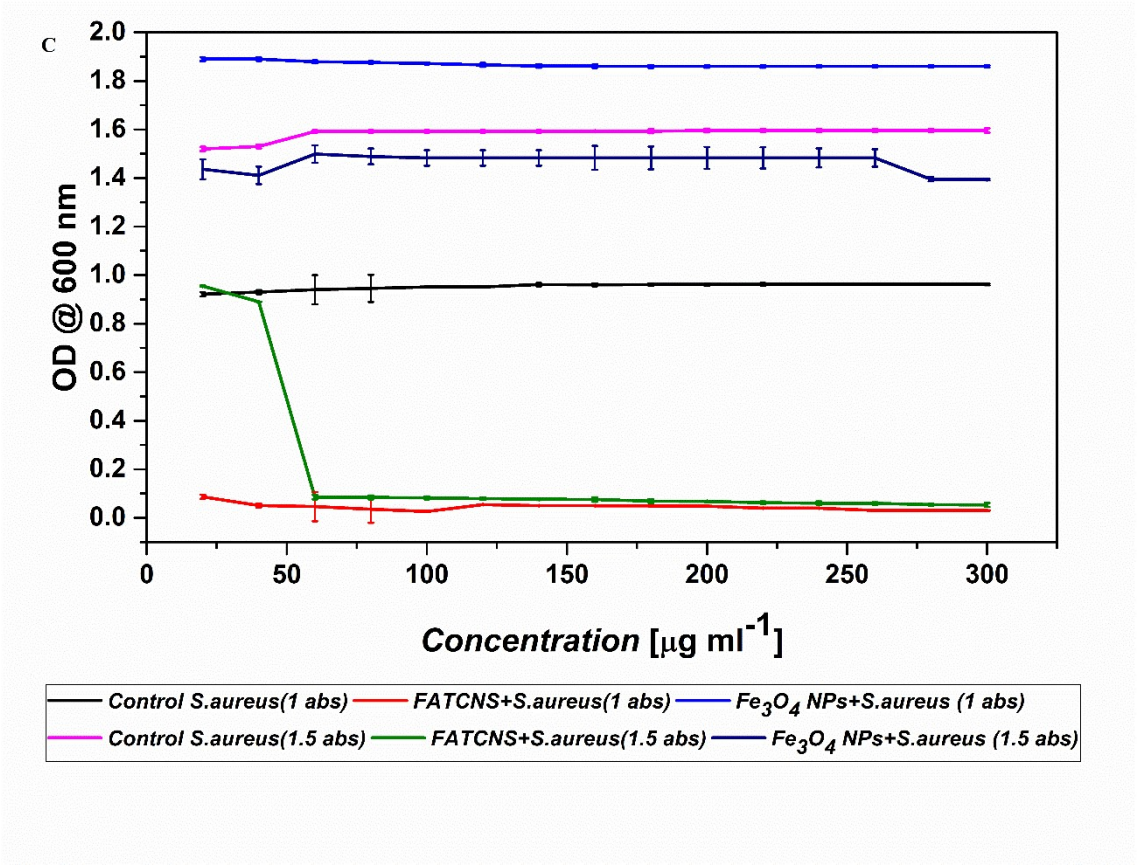


Figure. S 2.1. MIC of FATCNS and Fe_3O_4 NPs experimented on (A) *E.coli* (B) wtKARBJ1 and (C) *S.aureus.* Measurements of absorbance at OD_{600} of LB media were treated with different NS and NPs. Data were recorded for initial OD_{600}=1 and 1.5 after post-treatment hours with NS/NPs.

S 2.3. FATCNS as Bactericides

Experimental

The Gram-negative bacteria *Escherichia coli* (DH5α), the Gram-positive bacteria *S.aureus*, and the antibiotic-metal resistant bacteria wtKARBJ1 were used as model organisms to calculate the minimum inhibitory concentration (MIC) and evaluate the microbial bactericidal concentration (MBC) of the two NAs – FATCNSs and Fe_3O_4 NPs. Before the experiments, the tap water, river water and kitchen wastewater were autoclaved, and the absorbance of the bacterial suspension was maintained at 1 and 1.5 at OD_{600}. For MIC, the standard procedure described in Singh et al.[6] was followed with certain modifications. The methods used for, and the results of, the MIC experiments are discussed above. To study the survival rate of bacteria after the treatment with FATCNS and Fe_3O_4 NPs, the MBC was evaluated using the MIC (±20) of NSs and NPs. NSs and NPs at fixed concentrations were added to the bacterial cultures and

the suspensions were incubated in a shaking incubator for 24 h at 37 °C and 200 rpm. The absorbance of the suspensions was measured and used to calculate the survival rate of bacteria using the following equation.

$$Survival\ rate(\%) = \frac{Absorbance\ of\ treated\ cells}{Absorbance\ of\ control\ cells} \times 100 \quad (1)$$

The MBC determined was used as the optimized dosage to study the bacterial removal and recycling efficacy of FATCNS and Fe_3O_4 NPs in further experiments.

Results

The observed survival rates of bacteria treated with FATCNS and Fe_3O_4 NPs are compared to the control bacteria at 6th h and are presented in SI-2 Figure 2. For both the NAs, the MBC is approximately equal to MIC (For MIC refer above). For an initial bacterial concentration of OD_{600} = 1.5 in LB, the MBCs of FATCNS for *E.coli*, wtKARBJ1 and *S.aureus* are 100 µg (10 ml^{-1}), 220 µg (10 ml^{-1}) and 60 µg (10 ml^{-1}), respectively. The calculated survival rates for *E.coli*, wtKARBJ1 and *S.aureus* are 16 %, 26 % and 4.42 % (which in turn show 84%, 74% and 96% cellular death), respectively. The corresponding values of MBC for Fe_3O_4 NPs are substantially higher and result in significantly lower inhibition even after 16 h of observation. The MBCs of Fe_3O_4 NPs for *E.coli*, wtKARBJ1 and *S.aureus* are 2200 µg ml^{-1}, 2800 µg ml^{-1} and 3000 µg ml^{-1}, respectively, with more than 50 % survival rate at the end of 6 h of observation. For other water samples too, the MBC evaluated for FATCNS is lower than that for NPs.It can be seen from SI-2 Figure 2 that FATCNS show stronger bactericidal ability in river water and tap water when compared to that in kitchen wastewater and LB. To the best of our knowledge, the MBC values of FATCNS reported in this study are much lower than any antimicrobial NAs and functionalized magnetic NPs. For instance, Singh et al. reported that 0.1 mg ml^{-1} of dendritic magnetic NPs are required to inhibit 73 % of *E.coli* at 6 h. [6] In another study, Kawashita et al. [7] reported that the MBC for commercially available antibacterial particles is 800 µg ml^{-1}, while the magnetic NPs do not show any antibacterial activity even at a concentration of 1000 mg ml^{-1}. FATCNS also show MIC lower than that of the NPs in all water samples. The data from previous studies are compared to the present data in Table SI-2.2, where one can see that the FATCNS work more efficiently than other NAs used in water remediation.

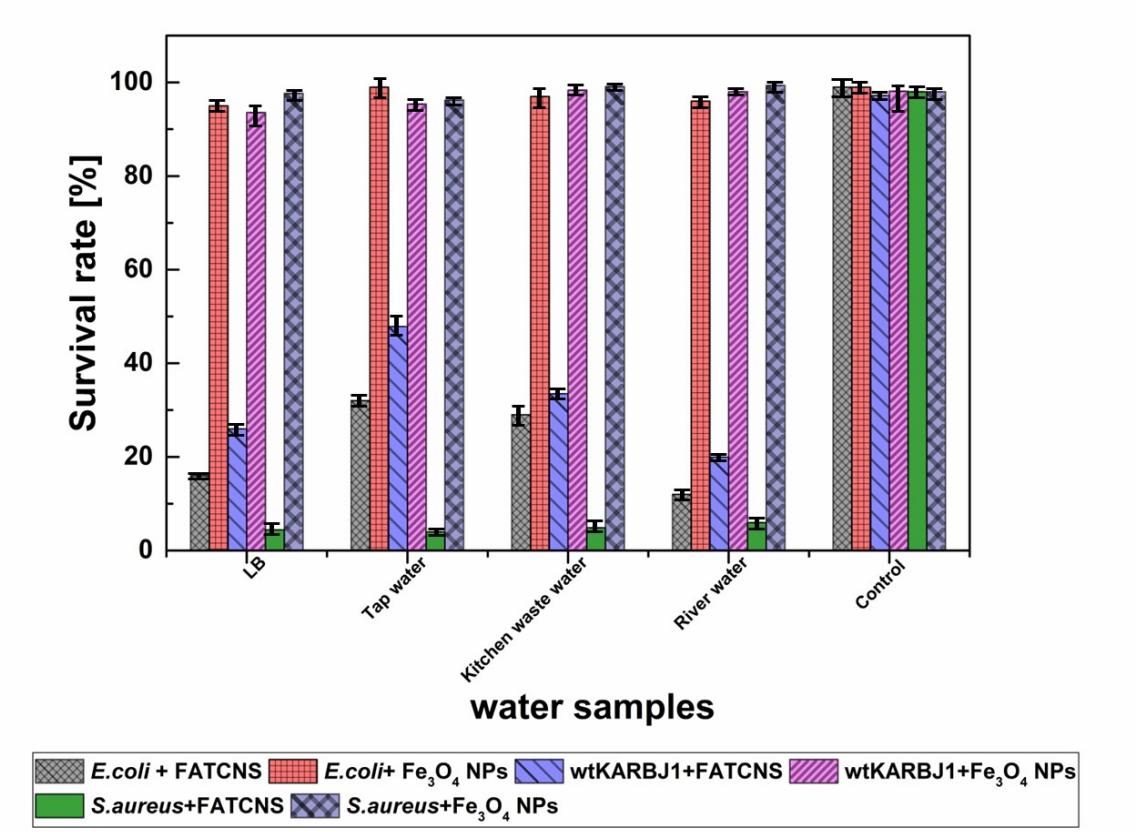


Figure S 2.2. The effect of MBC of FATCNS (concentration less than 250 µg (10 ml^{-1})) and Fe_3O_4 NPs (concentration less than 3200 µg (10 ml^{-1})) on the survival rate of bacteria with initial OD_{600}=1.5 at the end of 6 h at pH 7.4. The survival rate of *S.aureus* is significantly reduced by the NSs compared to the control cells and other cells.

Table S 2.2. Comparison of the bacterial removal efficiency (RE) of FATCNS with that of other NAs.

NPs	C_0 [µg ml^{-1}]	T_i [min]	RE [%]	Detection method	Bacteria	Study
MNPs-G1-Mu, MNPs-G2-Mu	625	1080-1440	-	MIC	*S. aureus*	8
Fe_3O_4@PPy NPs	100	10	100	Photothermal sterilization	*Escherichia coli, S. aureus*	9
Green synthesis-S. frutescens CdS nanostructures	0.005−0.05	1440	75	Plate-counting method	*S. aureus, Enterococcus* faecalis, *Pseudomonas aeruginosa, E.coli*	10
Antimicrobial peptide-functionalized	500	0.08	97	Absorbance via UV–vis spectrophotom	*E. coli* (non-pathogenic)	11

MNPs-PDA@Fe_3O_4-AMP				eter		
Green synthesis of *Monsonia burkeana* TiO_2 nanoparticles	50	1440	~100	Plate-counting method	total coliform, *E. coli, S. aureus*, E*nterobacteriaceae*	[12]
Biochar@Fe and biochar@Cu	10,000	60	~100	Plate-counting method	total coliform	[13]
ZnO nanorods	NA	60	100	Plate-counting method	*E.coli*	[14]
Fe_3O_4–SiO_2–NH_2	3000	10	95.03	Plate-counting method	*S. aureus*, *P. aeruginosa, Salmonella*, *B. subtilis*	[15]
G-SPIONs	24–240	15-60	100	Plate-counting method	*Adwardsiella tarda, Aeromonas hydrophila*	[16]
FATCNS	20-100	01	100	Turbidity and plate-count method	*E. coli, S. aureus*, antibiotic-metal resistant bacteria	This study

C_0 and T_i are the concentration and time required for the removal of bacteria

References

(1) Tacconelli, E.; Carrara, E.; Savoldi, A.; Harbarth, S.; Mendelson, M.; Monnet, D. L.; Pulcini, C.; Kahlmeter, G.; Kluytmans, J.; Carmeli, Y.; Ouellette, M.; Outterson, K.; Patel, J.; Cavaleri, M.; Cox, E. M.; Houchens, C. R.; Grayson, M. L.; Hansen, P.; Singh, N.; Theuretzbacher, U.; Magrini, N.; Aboderin, A. O.; Al-Abri, S. S.; Awang Jalil, N.; Benzonana, N.; Bhattacharya, S.; Brink, A. J.; Burkert, F. R.; Cars, O.; Cornaglia, G.; Dyar, O. J.; Friedrich, A. W.; Gales, A. C.; Gandra, S.; Giske, C. G.; Goff, D. A.; Goossens, H.; Gottlieb, T.; Guzman Blanco, M.; Hryniewicz, W.; Kattula, D.; Jinks, T.; Kanj, S. S.; Kerr, L.; Kieny, M. P.; Kim, Y. S.; Kozlov, R. S.; Labarca, J.; Laxminarayan, R.; Leder, K.; Leibovici, L.; Levy-Hara, G.; Littman, J.; Malhotra-Kumar, S.; Manchanda, V.; Moja, L.; Ndoye, B.; Pan, A.; Paterson, D. L.; Paul, M.; Qiu, H.; Ramon-Pardo, P.; Rodríguez-Baño, J.; Sanguinetti, M.; Sengupta, S.; Sharland, M.; Si-Mehand, M.; Silver, L. L.; Song, W.; Steinbakk, M.; Thomsen, J.; Thwaites, G. E.; van der Meer, J. W.; Van Kinh, N.; Vega, S.; Villegas, M. V.; Wechsler-Fördös, A.; Wertheim, H. F. L.; Wesangula, E.; Woodford, N.; Yilmaz, F. O.; Zorzet, A. Discovery, Research, and Development of New Antibiotics: The WHO Priority List of Antibiotic-Resistant Bacteria and Tuberculosis. *Lancet Infect Dis* 2018, ***18*** **(3)**, 318–327.

(2) Ma, L.; Yang, H.; Guan, L.; Liu, X.; Zhang, T. Risks of Antibiotic Resistance Genes and Antimicrobial Resistance under Chlorination Disinfection with Public Health Concerns. *Environ Int* 2022, **158**, 106978.

(3) Arbuthnott, J. P.; Coleman, D. C.; Azavedo, J. S. de. Staphylococcal Toxins in Human Disease. *Journal of Applied Bacteriology* 1990, **69**, 101S-107S

(4) Frieri, M.; Kumar, K.; Boutin, A. Antibiotic Resistance. *J Infect Public Health* **2017**, *10* (4), 369–378. https://doi.org/10.1016/J.JIPH.2016.08.007.

(5) Pantosti, A.; Sanchini, A.; Monaco, M. Mechanisms of Antibiotic Resistance in Staphylococcus Aureus. *Future Microbiology*, **2007**, **2 (3)**, 323–334.

(6) Singh, S.; Bahadur, D. Highly Efficient and Reusable Dendritic Fe3O4 Magnetic Nanoadsorbent for Inhibition of Bacterial Growth. *Surfaces and Interfaces* 2019, **17**, 100348.

(7) Kawashita, M.; Toda, S.; Kim, H. M.; Kokubo, T.; Masuda, N. Preparation of Antibacterial Silver-Doped Silica Glass Microspheres. *J Biomed Mater Res A* 2003, **66A (2)**, 266–274.

(8) Ekinci, S.; İlter, Z.; Ercan, S.; Çınar, E.; Çakmak, R. Magnetite Nanoparticles Grafted with Murexide-Terminated Polyamidoamine Dendrimers for Removal of Lead (II) from Aqueous Solution: Synthesis, Characterization, Adsorption and Antimicrobial Activity Studies. *Heliyon* 2021, *7* (3), e06600.

(9) Guo, N.; Cang, F.; Wang, Z.; Zhao, T. T.; Song, X. R.; Farris, S.; Li, Y. Y.; Fu, Y. J. Magnetism and NIR Dual-Response Polypyrrole-Coated Fe3O4 Nanoparticles for Bacteria Removal and Inactivation. *Materials Science and Engineering: C* 2021, **126**, 112143.

(10) Munyai, S.; Tetana, Z. N.; Mathipa, M. M.; Ntsendwana, B.; Hintsho-Mbita, N. C. Green Synthesis of Cadmium Sulphide Nanoparticles for the Photodegradation of Malachite Green Dye, Sulfisoxazole and Removal of Bacteria. *Optik (Stuttg)* 2021, **247**, 167851.

(11) Ding, S. Y.; Faraj, Y.; Wei, J.; Wang, W.; Xie, R.; Liu, Z.; Ju, X. J.; Chu, L. Y. Antimicrobial Peptide-Functionalized Magnetic Nanoparticles for Rapid Capture and Removal of Pathogenic Bacteria. *Microchemical Journal* 2020, **159**, 105493.

(12) Ngoepe, N. M.; Mathipa, M. M.; Hintsho-Mbita, N. C. Biosynthesis of Titanium Dioxide Nanoparticles for the Photodegradation of Dyes and Removal of Bacteria. *Optik (Stuttg)* 2020, **224**, 165728.

(13) Priyadarshni, N.; Nath, P.; Nagahanumaiah; Chanda, N. Sustainable Removal of Arsenate, Arsenite and Bacterial Contamination from Water Using Biochar Stabilized Iron and Copper Oxide Nanoparticles and Associated Mechanism of the Remediation Process. *Journal of Water Process Engineering* 2020, **37**, 101495.

(14) Saleh, M.; Gonca, S.; Isik, Z.; Ozay, Y.; Harputlu, E.; Ozdemir, S.; Yalvac, M.; Ocakoglu, K.; Dizge, N. Preparation of ZnO Nanorods or SiO2 Nanoparticles Grafted onto Basalt Ceramic Membrane and the Use for E. Coli Removal from Water. *Ceram Int* 2021, **47 (19)**, 27710–27717.

(15) Zhan, S.; Yang, Y.; Shen, Z.; Shan, J.; Li, Y.; Yang, S.; Zhu, D. Efficient Removal of Pathogenic Bacteria and Viruses by Multifunctional Amine-Modified Magnetic Nanoparticles. *J Hazard Mater* 2014, **274**, 115–123.

(16) Kumar, M.; Gupta, G.; Varghese, T.; Srivastava, P. P.; Gupta, S. Preparation and Characterization of Glucose-Conjugated Super-Paramagnetic Iron Oxide Nanoparticles (G-SPIONs) for Removal of Edwardsiella Tarda and Aeromonas Hydrophila from Water. *Microsc Res Tech* 2022, **85**, 1768-1783.