Supplementary Information's

Facile Synthesis of CuONPs using *Citrus limon* Juice for Enhancing Antibacterial Activity against Methicillin-resistant *Staphylococcus aureus*, Beta-lactamase and Tetracycline-resistant *Escherichia coli*

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Master mixture (Go Taq® green, 2X):	12.5µl
Nuclease free water	5.5µl
Forward primer	1µl
Reversed primer	1µl
DND template	5µl

Table S1. Preparation of PCR mixture (25µl)

Thermal profile for the amplification: Thermal profiles used for determination of multidrug resistance gene-specific primers were given in the table below.

Table S2. Thermal profile for amplification of the gene used to detect *S. aureus* and *E. coli*

Name of the bacteria	Target gene	Thermal profile	Amplicon size	Reference
S. aureus	*16s rRNA* gene	Initial denaturation at 94˚C for 5mins; followed by 30 cycles of denaturation at 94˚C for 30secs, annealing at 52˚C for 30secs and extension at 72˚C for 30secs. The final extension was conducted at 72˚C for 5mins.	228 bp	[1]
S. aureus	*mec*A gene	Initial denaturation at 94˚C for 5mins; followed by 30 cycles of denaturation at 94˚C for 30secs, annealing at 52˚C for 30secs and extension at 72˚C for 30secs. The final extension was conducted at 72˚C for 5mins.	163 bp	[2]
E. coli	*malB* gene	Initial denaturation at 95˚C for 5mins; followed by 30 cycles of denaturation at 94˚C for 45secs, annealing at 55˚C for 45secs and extension at 72˚C for 1min. The final extension was conducted at 72˚C for 5mins.	585 bp	[3]

Table S3. Thermal profile for the detection of antibiotic resistant gene for *tetA* and *blaTEM-1*

Target gene	Thermal profile	Amplicon size	Reference
***Bla TEM-1* gene**	Initial denaturation at 95˚C for 5mins; followed by 30 cycles of denaturation at 94˚C for 45secs, annealing at 55˚C for 45secs and extension at 72˚C for 1min. The final extension was conducted at 72˚C for 5mins.	643bp	[4]
***tetA* gene**	Initial denaturation at 95˚C for 5mins; followed by 30 cycles of denaturation at 94˚C for 45secs, annealing at 55˚C for 45secs and extension at 72˚C for 1min. The final extension was conducted at 72˚C for 5mins.	577bp	[5]

Table S4. List of antibiotics with their groups, generation, and sensitive (S) or resistant (R) to isolated bacteria

Group of antibiotics	Name of antibiotics	Generation	Name of the isolate	
			S. aureus	***E. coli***
Sulfonamides	Trimethoprim (COT-25)	NGC	R	R
Cephalosporin	Cefuroxime (CXM-30)	2nd	R	R
	Cefotaxime (CTX--30)	3rd	S	R
	Cefixime (CFM-5)	3rd	R	R
Oxazolidinone	Linezolid (LZ-30)	NGC	R	R
Aminoglycoside	Kanamycin (K-30)	NGC	S	S
Quinolones	Ciprofloxacin (CIP-5)	2nd	S	S
	Levofloxacin (LE-5)	3rd	S	S

Macrolides	Erythromycin (E-15)	NGC	R	R
Penicillin	Oxacillin (OX- 30)	2nd	R	R
	Ampicillin (AMP-10)	3rd	R	R
Lincosamides	Clindamycin (CD -2)	NGC	R	R

R= Resistant; S= Sensitive

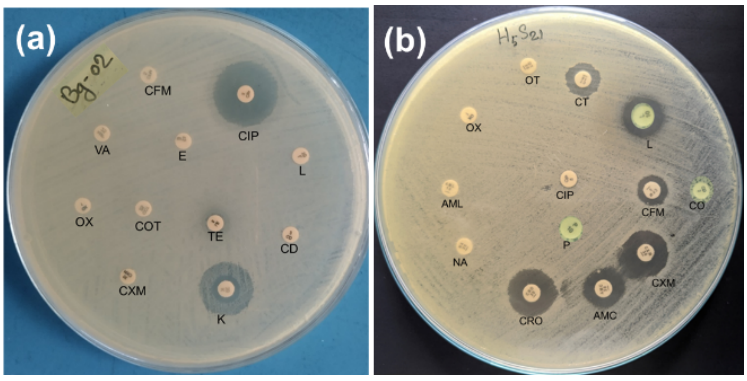

Fig. S1 Determination of MDR pattern using 12 commercially available antibiotics of a) *Staphylococcus aureus* and, b) *Escherichia coli*

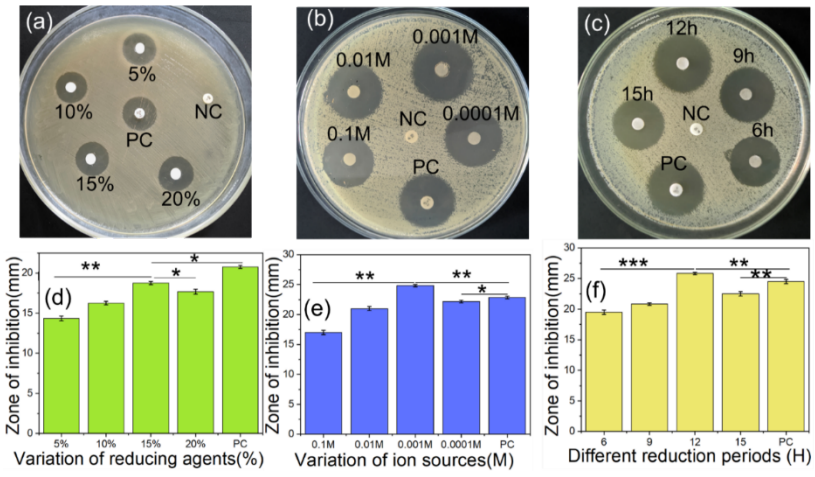

Fig. S2 Visual representation of antibacterial effect of CuONPs based on a) reducing agent concentration, b) concentration of ion source, and c) reduction periods, and respective differences in zone of inhibition size (d – f).

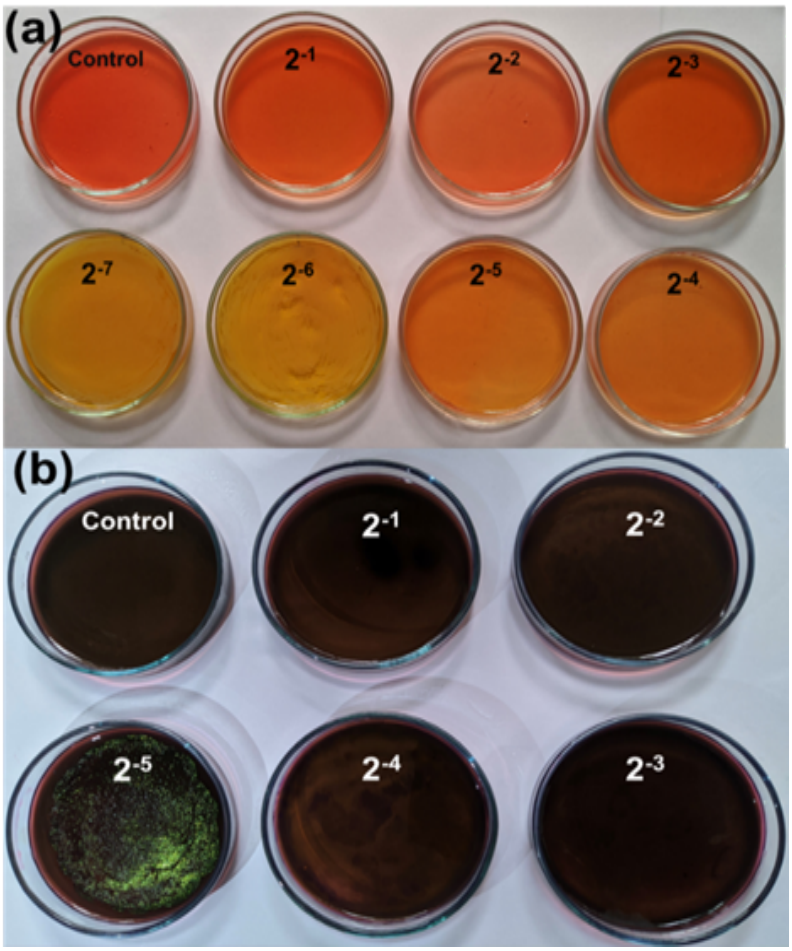

Fig. S3 Determination of MBC against MDR a) *Staphylococcus aureus* and, b) *Escherichia coli*

References

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