

## Comparative Study of Antibiofilm Activity of Copper Oxide and Iron Oxide Nanoparticles Against Multidrug Resistant Biofilm Forming Uropathogens

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**Abstract** The effectiveness of the metal oxide nanoparticles viz. CuO and Fe<sub>2</sub>O<sub>3</sub> as antibacterial agents against multidrug resistant biofilm forming bacteria was evaluated. CuO nanoparticles were also experimented for antibiofilm and time kill assay. The CuO displayed maximum antibacterial activity with zone of inhibition of (22 ± 1) mm against methicillin resistant *Staphylococcus aureus* (MRSA) followed by *Escherichia coli* (18 ± 1) mm. The Fe<sub>2</sub>O<sub>3</sub> showed the zone of inhibition against MRSA of (14 ± 1) mm followed by *E. coli* (12 ± 1) mm. CuO proved to be more toxic than Fe<sub>2</sub>O<sub>3</sub> nanoparticles showing significantly high antibacterial activity and found to possess dose dependent antibiofilm properties.

**Keywords** Biofilm · Uropathogens · Nanoparticles ·  
CuO · Fe<sub>2</sub>O<sub>3</sub>

Biofilm can be defined as the microbial-derived sessile communities characterized by the cells that remain attached to any surface. Quorum sensing among the biofilm forming organisms plays significant role in the formation of biofilm [4, 6]. Urinary catheters when inserted into the human body parts, may readily acquire biofilm. Eradication of the biofilm is very hard and cause many chronic infections. Recent research work reveals that use of quorum sensing inhibitors could be useful for restricting biofilm formation [3, 5]. Nanoparticles have good antibacterial

activity and they could be used as an effective bactericidal agent [8, 10, 11]. Considering the above facts, experiment was conducted for detection of biofilm producing uropathogens and antibacterial/antibiofilm activity of iron oxide and copper oxide nanoparticles against multidrug resistant biofilm forming uropathogens was investigated.

A total of 50 samples and 213 isolates were screened for the biofilm production. All the clinical samples were collected from hospitalized female patients with urinary catheter inserted for more than 2 days from government hospitals in Sonitpur (Tezpur) (Lat. 26.63°N Long. 92.8°E) and Dibrugarh (Lat. 27.48°N Long. 95°E) districts of Assam, India.

The biofilm formation was analyzed by different standard methods [9]. Table 1 represented biofilm formation by uropathogens isolated from urine of normal and hospitalised females with urinary catheter inserted. Among 168 clinical samples maximum 88 isolates (52.4 %) of uropathogens were detected as biofilm former in Microtitre plate method. Among different biofilm formers *Escherichia coli* detected as maximum biofilm producer with 24 isolates (80 %) followed by *Proteus mirabilis*, 16 isolates (72.73 %); *Staphylococcus aureus*, 16 isolates (57.1 %); *Enterococcus faecalis*, 14 isolates (53.8 %); *Pseudomonas* species 14 isolates (43.8 %) and *Staphylococcus epidermidis* 4 isolates (40 %).

Antibiotic resistance of different kinds of uropathogens was studied by Kirby–Bauer disc diffusion method on MHA plates, which revealed that resistance was higher in biofilm formers than in non-biofilm formers. Three antibiotics namely penicillin, ampicillin and piperacillin were found to be resistant equally in both biofilm formers (100 %) and non-biofilm formers (100 %). Among Gram-positive biofilm formers, oxacillin (82.35 %) and erythromycin (70.6 %) were found to be most resistant antibiotics.

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**Table 1** Biofilm formation by uropathogens isolated from urine of hospitalised females with urinary catheter inserted

Methods	Biofilm formers			
	Total biofilm formers	Strong	Moderate	Weak
Microtitre plate method	88 (52.4 %)	10 (11.36 %)	22 (25 %)	56 (63 %)
Pellicle assay	49 (29.17 %)	13 (26.53 %)	16 (32.65 %)	20 (40.81 %)
Tube method	57 (33.93 %)	16 (28.07 %)	20 (35.08 %)	21 (36.84 %)
Congo red agar method	21 (12.5 %) <sup>a</sup>	–	–	–
Coverslip assay	57 (33.93 %)	16 (28.07 %)	20 (35.08 %)	21 (36.84 %)

<sup>a</sup> With the Congo red method differences among the biofilm formers could not be detected, so the distribution of strong, moderate and weak biofilm formers could not be tabulated

**Table 2** Antibacterial activity of nanoparticles against uropathogens

Uropathogens	CuO nanoparticles (mean ± SD) mm	Fe <sub>2</sub> O <sub>3</sub> nanoparticles (mean ± SD) mm	Chloramphenicol (mm)
<i>MRSA</i>	22 ± 1	14 ± 1	23
<i>MRSE</i>	20 ± 1	12 ± 1	22
<i>VRE</i>	21 ± 1	13 ± 1	22
<i>E. coli</i>	18 ± 1	12 ± 1	23
<i>K. pneumoniae</i>	14 ± 1	10 ± 1	23
<i>Pseudomonas</i> sps.	15 ± 1	10 ± 1	23
<i>P. mirabilis</i>	15 ± 1	9 ± 1	22

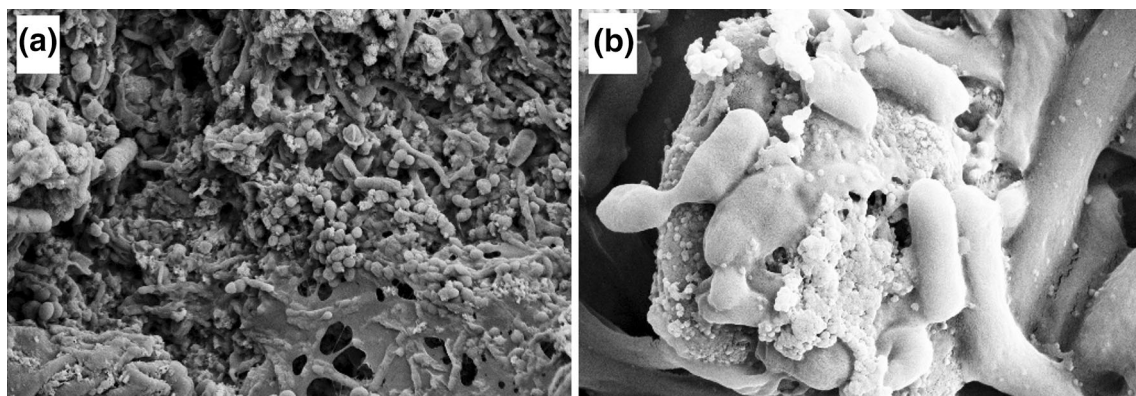
*MRSA* methicillin resistant *S. aureus*, *MRSE* methicillin resistant *S. epidermidis*, *VRE* vancomycin resistant *E. faecalis*

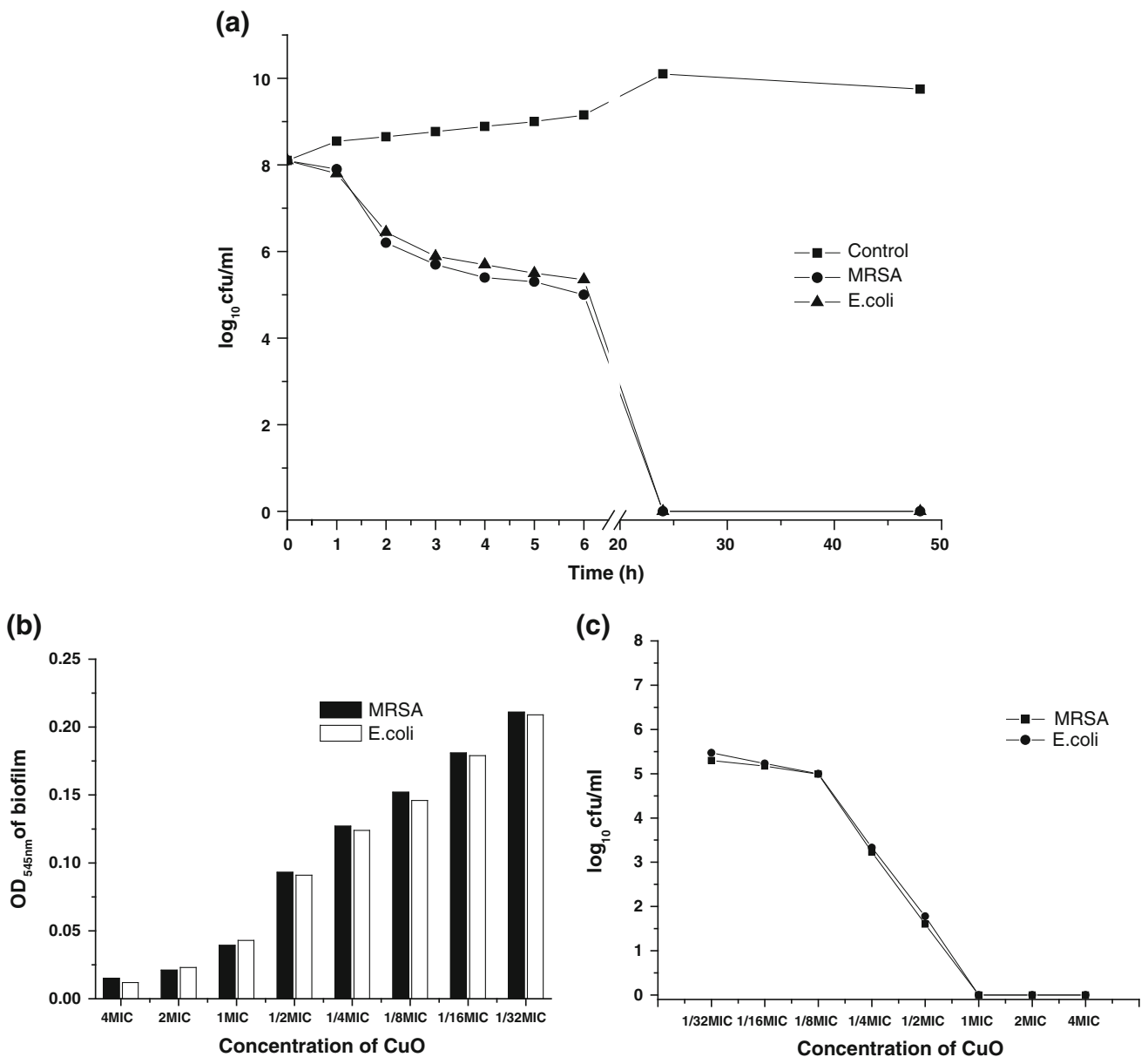
In case of Gram-negative biofilm formers, very high resistance was observed for ceftazidime (79.6 %), cefixime (70.3 %), meropenem (72.22 %), ciprofloxacin (68.5 %) and polymyxin-B (63.5 %). *E. coli* showed maximum resistance to most of the antibiotics.

Antibacterial activity analysis of commercial CuO and Fe<sub>2</sub>O<sub>3</sub> nanoparticles (Sigma Aldrich) of 25–30 nm size were performed by agar well diffusion method as described

by Das et al. [2]. The CuO displayed greater antibacterial activity with zone of inhibition (in mm) against *MRSA* (22 ± 1) followed by *E. coli* (18 ± 1) (Table 2). The Fe<sub>2</sub>O<sub>3</sub> showed the zone of inhibition (14 ± 1) mm against *MRSA* followed by *E. coli* (12 ± 1) mm. Antibiotic chloramphenicol was run as positive control in this experiment where maximum 23 mm zone of inhibition was recorded. MIC for *MRSA* were found to be 30 and 40 µg/ml for CuO and Fe<sub>2</sub>O<sub>3</sub> nanoparticles respectively while for *E. coli*, MIC were found to be 35 and 45 µg/ml for CuO and Fe<sub>2</sub>O<sub>3</sub> nanoparticles respectively.

CuO nanoparticles showed remarkable antibacterial activity against both Gram-positive and Gram-negative bacteria. For Gram-positive bacteria, *MRSA* showed maximum activity while *E. coli* showed the highest activity among Gram-negative bacteria against both CuO and Fe<sub>2</sub>O<sub>3</sub> nanoparticles. The variation in the sensitivity or resistance to both Gram-positive and Gram-negative bacteria could be due to the differences in the cell structure, physiology, metabolism, or degree of contact of organisms with nanoparticles. In our experiments, CuO was found to be more effective antibacterial agent than Fe<sub>2</sub>O<sub>3</sub>. Gram-positive bacteria such as *MRSA*, *MRSE* and *VRE* were found to be more sensitive to the CuO nanoparticles which might directly attribute to the greater abundance of amines

**Fig. 1** SEM micrograph of a *MRSA* biofilm, b *E. coli* biofilm



**Fig. 2** Antibiofilm activity of CuO nanoparticles against *MRSA* and *E. coli*. **a** time-kill assay of CuO nanoparticles against *MRSA* and *E. coli*, **b** a plot of sub-MIC value (4MIC to 1/32MIC) of CuO

nanoparticles vs. OD<sub>545 nm</sub> of biofilm, **c** a plot of sub-MIC values of CuO against log<sub>10</sub> cfu/ml

and carboxyl groups on their cell surface and greater affinity of copper towards these groups [7].

Figure 1 represented the SEM images of *MRSA* and *E. coli* biofilm. The effect of CuO nanoparticles was studied with time-kill assay against *MRSA* and *E. coli* as presented in Fig. 2a. *MRSA* and *E. coli* cells were replicating without any hindrance when CuO nanoparticles were not added into the media. But when the cells of *MRSA* and *E. coli* were allowed to grow in the presence of CuO nanoparticles, the growth was found to be slowed down after 1 h of incubation as evident from decreased cfu/ml.

The efficiency of the clinical isolates *MRSA* and *E. coli*, were screened for their ability to form biofilm on the surface of Foley’s catheters and polyurethane catheters in vitro. As presented in Fig. 2b it was found that both of these two organisms were equally capable of forming biofilm on the catheter surfaces as indicated by the increased OD<sub>545nm</sub> of biofilm and was found to be >0.3.

Efficiency of CuO nanoparticles to inhibit biofilm formation at sub-MIC concentrations was evaluated against *MRSA* and *E. coli*. Figure 2b showed the effect of CuO nanoparticles on the biofilm formed by *MRSA* and *E. coli*

and clearly revealed that inhibition of biofilm formation was concentration dependent. From 2MIC to 1/2MIC of CuO nanoparticles, biofilm was almost completely eradicated as the  $OD_{545nm}$  of biofilm was  $<0.1$  and also the cfu/ml was decreased indicating that established biofilm was eradicated. But as soon as the concentration further decreased, removal of the biofilm was also decreased. At the 1/8MIC, 1/16MIC, 1/32MIC, the  $OD_{545nm}$  of biofilm was  $>1$  and  $\geq 2$ , that means the biofilm was not completely eradicated and similar result was also observed when established biofilm was incubated with CuO nanoparticles at sub-MIC values as evident from Fig. 2c.

Antibiofilm activity study of CuO nanoparticles showed that it efficiently reduced the biofilm formation by both MRSA and *E. coli*. It was noticed that when the concentration of the nanoparticles was increased, the inhibition of biofilm was also enhanced which can be attributed directly to the dose dependent activity. Almost all MRSA and *E. coli* biofilm cells died within 4 days of exposure to CuO nanoparticles. However, on the 10th day of cultivation a few persisting biofilm bacteria re-grew to lesser bacterial counts, probably because of low amounts of the antimicrobial (Cu) agent after its absorption by the initially seeded bacteria. Toxicity of copper to the microorganisms is exerted by several parallel mechanisms, which lead to the death of the microorganisms. It seems that the first target site of copper that it damages is the microorganism's envelope. Copper ions also damage nucleic acids. It has been suggested that subsequent to the specific binding of copper to DNA, repeated cyclic redox reactions generate several OH radicals near the binding site causing multiple damage to the nucleic acids. However, it still may be that in some microorganisms, copper oxidative damage to the genetic material may occur through Fenton mechanism [1].

Catheter associated biofilm formation by the multiple drug resistant uropathogens is a great concern and it is very difficult to eradicate biofilm causing serious health related complications to the patients undergoing catheterization. As compared to  $Fe_2O_3$  nanoparticles CuO is found to be highly effective as an antibacterial material against multi-drug resistant biofilm forming uropathogens. CuO

nanoparticles have high potential of absorption, adsorption, penetration and availability which make it an essential antibiofilm agent.

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**Ethical Standards** The present study was approved by the Ethics Committee of Dibrugarh University, Assam, India.

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