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# The Antimicrobial Activity of Silica Oxide Nanoparticles Against Some Bacteria and Fungi Isolates

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## Abstract

The capability of some bacteria and fungi to resist common antibiotics has been a guide to discover new planning to treat the infections connected to antibiotic resistance in the patients. In this study, the antifungal and antibacterial activity of silica oxide nanoparticles was detected in vitro against some bacterial species (Klebsiella pneumonia, Staphylococcus aureus, Escherichia coli, Proteus mirabilis, and Pseudomonas aeroginosa) and some fungi (Aspergillus terrus, Aspergillus parasiticus, Aspergillus flavus, and Candida albicans) and contrasted with antibacterial antibiotics (Amikacin, Amoxillin, Augmastin, Cefotaxine, Ceftazidime, Ciprofloxacin, Gentamycin, Meropenum, and Tetracycline) and antifungal antibiotics (Amphotericin B and Ketoconazole). Silica oxide suspension was prepared by acetic acid solution. Results showed that concentrations of (10-40) µg/ml of silica oxide have antifungal and antibacterial activity against the tested microorganisms in this work more than antibiotics. In conclusion, Silica oxide relates superior antimicrobial characteristics.

**Keywords:** *Antimicrobial activity, Silica Oxide, Bacteria, Fungi.*

## Introduction

The resistance of antibiotics is one of the most significant health problems. Various reasons have been related to this phenomenon, like excessive use of broad-spectrum antibiotics, low antimicrobial efficiency, obstacle in control and extension the antimicrobial functions, and difficulty in functioning, efficacious circumference. The restricted option of antifungal agents is also one of the most challengeable problems about fungal diseases. Several studies indicate that nanoparticles can be used as effective antimicrobial agents, oxide NPs. Antimicrobial agents are chemical combinations, which have potential to inactivate or inhibit the growth of microorganisms. Metal oxide nanoparticles, well recognized for their highly powerful antimicrobial impact. For example, Panacek et al. reported silver nanoparticle had better antifungal effect against Candida by lesser concentrations [1-5].

The antifungal and antibacterial activity of conventional (bulk) ZnO was reported by Sawai and Yamamoto, and Yoshikawa. Also, it has been demonstrated ZnO nanoparticles possess significant antifungal properties against Penicillium expansum, Botrytis cinerea, and the inhibitory effects increased by its concentrations.[6] ZnO nanoparticles increased intensity of lipid and protein bands in E. coli.[7] Nanomaterials as antibacterial complementary to antibiotics are extremely favorable and are earning large advantages, they might close the gaps where antibiotics considerably defeat.[7] Antimicrobial NM currently in application (i.e. metal, metal oxide, and organic nanoparticles) exhibits a variety of substantial, and modulated chemical. Because the information about the anti-bacterial and anti-fungal effect of SiO2 nanoparticles has not been assessed. So, this

study aimed to visualize the effect of Silica oxide (SiO2) on some bacteria and fungi isolates.

## Materials & Methods

### Bacterial and Fungal Isolates

A total of 5 bacterial isolates, (4 isolates of gram negative bacteria; E. coli, K. aerogenes, Pr. mirabilis and 1 gram positive bacteria, and P. aeruginosa; S. aureus), and 4 fungal isolates (Aspergillus terrus, Asp. parasiticus, Asp. flavus and Candida albicans) isolated from different clinical samples collected from Al-Diwaniyah Teaching hospitals during the period from 10/2016 to 1/2017. Conventional biochemical tests were used to identify all of the isolates.8 The tests were carried at the department of Environment, Science College, University of Al-Qadisiyah.

### Preparations of Bacterial Cells

Bacteria were cultured on a nutrient agar for 24h. By using a sterile loop, 4 to 5 well-isolated colonies were transferred, from an overnight culture, to the tube of sterilized 0.8% saline solutions (10 ml). To adjust the inoculums standard to a 0.5 McFarland, which equals approximately 108 CFU/ml, the inoculums, to avoid clumping of the cells, were emulsified inside the saline tube and for 10 min incubated at 37℃.

### Fungal Suspension Preparation

Fungi isolates was inoculated on Sabouraud dextrose agar medium, and incubated for 48h in 37 ℃ for Candida albicans and for 7 days at 25℃ C for Aspergillus species, then some colonies of the fungus were dissolved in distilled water, until the stiffness of suspension changed equal to 0.5 MCfarland. The 0.5 MCfarland was prepared according to references.[9]

### Silica Oxide Nanoparticles

Commercially available silicon oxide ($SiO_2$) nanoparticles were purchased from Sky Spring Nanomaterials Inc. The reported, "as manufactured" sizes were 20 nm, non-porous with purity (98.7%). Since the nanoparticles in water were found to be insoluble, in accordance with the factory guidelines, an acetic acid solvent of 5% in distilled water was utilized, and then the concentrations of (10, 20, 30, 40) µg/ml were prepared.

### Antibacterial Susceptibility Testing

Antibacterial susceptibility test of the isolates was carried depending on the CLSI guidelines.[10] The disk diffusion method was used to test the following antibiotics on Oxoid-Mueller–Hinton agar; ampicillin (10µg), Amikacin (30µg), Amoxillin (25µg), Augmastin (10µg), Cefotaxime (30µg), Ceftazidime (30µg), Cetriaxone (30µg), Ciprofloxacins (5µg), Erythromycin (10µg), Gentamicin (10µg), Meropenem (10µg), Tetracycline (30µg), and Vancomycin (30µg) acquired from Bioanalyse/Turkey. In this study antibiotic disks are placed onto an agar plate and each isolate was cultured separately using sterile cotton swabs onto the individual plates. Then the plates are incubated overnight at 37 °C, and the zone of inhibition of bacterial growth is used as a measure of susceptibility.[11] At the same time, 6 mm diameter wells were produced using gel puncture on other Mueller-Hinton agar plates. Each isolate was swabbed evenly onto the individual plate using sterile cotton swabs. A Micropipette was used to pour concentrations of SiO2 nanoparticles solution onto each of four wells on all plates. The different levels of zone of inhibition were measured after incubation at 37℃ for 18h

### Antifungal Activity of $SiO_2$

The antifungal activity of $SiO_2$ nanoparticles was completed in accordance to Devi *et al*. 2014.[12] Antifungal activity was demonstrated using the agar well diffusion assay. A positive control drug (Amphotericin B and Ketoconazole) was also done parallel. The plates were studied for evidence of zone of inhibition, which appears as area around the wells. A metered ruler was used to measure the diameter of such zones of inhibition was. By performing the experiments in triplicates the mean value was calculated.

## Results

The influence, of various antibiotics on bacterial and fungal isolates was scanned. These isolates appeared to have different susceptibility against antibiotics used in this study, as shown in Table (1) & (2).

**Table 1: Zone of inhibition of antibiotics used against bacterial species**

| Bacterial-isolates | Concentration (µg/ml) zone of inhibition (mm) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AK | AX | AMP | AUG | CEF | CTX | CAZ | CTR | CIP | ER | GEN | MRP | TE | VA |
| *E. coli* | 20 | 18 | 0 | 17 | 0 | 0 | 0 | 0 | 19 | 0 | 0 | 18 | 0 | 0 |
| *K.pneumonia* | 0 | 21 | 0 | 0 | 0 | 0 | 20 | 0 | 20 | 0 | 0 | 23 | 18 | 0 |
| *Pr. mirabills* | 18 | 20 | 0 | 0 | 19 | 0 | 22 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Ps.aeroginosa* | 18 | 0 | 0 | 0 | 0 | 0 | 15 | 0 | 21 | 0 | 20 | 20 | 0 | 0 |
| *S. aureus* | 0 | 21 | 0 | 0 | 0 | 20 | 0 | 0 | 0 | 18 | 0 | 0 | 0 | 21 |

AK: Amikacin, AX: Amoxicillin, AMP: Ampicillin AUG: Augmastin, CEF:Cefipime, CTX: Cefotaxime, CAZ: Ceftazidime, CTR: Ceftriaxone ,CIP: Ciprofloxacin, ER: Erythromycin, GEN: Gentamycin, MRP: Meropenem ,TE: Tetracycline and VA: Vancomycin.

**Table 2: Zone of inhibition of antibiotics used against fungal species**

| Fungal isolates | Concentration (µg/ml)/Zone of inhibition (mm) | |
|---|---|---|
| | Amphotericin B | Ketoconazole |
| *Asp. flavus* | 21 | 28 |
| *Asp. parasiticus* | 23 | 36 |
| *Asp.terrus* | 24 | 25 |
| *Candida albicans* | 22 | 25 |

Antimicrobial materials used in the clinical setting today are beset by significant shortfalls, including weak antimicrobial activities, risk of microbial resistance, difficulty in monitoring, extending the antimicrobial functions, and difficulty in functioning in a dynamic environment.[13]

MIC for nano SiO2 was 10 µg/ml for all tested bacterial species as shown in table (3) and as shown in this table, there is different levels of inhibition zone at all the used concentrations (10, 20, 30, 40) µg/ml. Nanomaterials (NM) may be strategically advantageous as active antibacterial groups since their surface area is exceedingly large relative to their size. Although, only a small dose of the particles is used these nanosized particles may provide high activity. Consequently, NM could serve as an alternative to antibiotics to control bacterial infections.[13]

**Table 3: Zone of inhibition of $SiO_2$ nanoparticles against bacterial species at different concentrations.**

| Bacterial isolates | Concentration (µg/ml)/Zone of inhibition (mm) | | | |
|---|---|---|---|---|
| | 10 | 20 | 30 | 40 |
| *E. coli* | 38 | 34 | 36 | 37 |
| *Klebsiella pneumonia* | 35 | 40 | 50 | 44 |
| *Proteus mirabills* | 40 | 42 | 43 | 45 |
| *Pseudomonas aeroginosa* | 35 | 48 | 39 | 45 |
| *S. aureus* | 34 | 35 | 45 | 37 |

The nanoparticles display antibacterial activity towards pure cultures as well as; Bacillus subtilis, Escherchia coli, Pseudomonas fluorescens, and Staphylococcus.[14] Notably, Metal oxide NPs, Zinc oxide, Magnesium oxide, Titanium dioxide, Silicone dioxide, in laboratory-based studies were proven to be toxic, at concentrations ranging from 10 to 5000 mg/L, to 17 microorganisms (e.g., algae, bacteria, and protozoa).[15]

Some previous studies suggest that cell membrane activity disruption is primarily attributed to the toxicity of metal oxides to bacteria.16 Another contributing factor documented to the toxicity of NPs are dissolved metal ions.[15] Produced by bacteria, extracellular proteins and polypeptides may also lead to the attachment of the metal nanoparticles to bacteria.[17] Although, studies previously have provided conflicting findings as to whether the characteristics of metal oxide nanoparticles such as chemical properties, surface charge, size, and morphology contribute to their toxicity.[18]

Our study showed the amphotericin B and Ketoconazole had antifungal effect but less than nanometal as shown in table (2, 4). According to our knowledge, this is the only study investing the effect of SiO2 nanoparticles on Asp. flavus, Asp. parasiticus, and Asp. terrus. Some studies investigated the effect of SiO2 and other nanoparticles on the yeast, Candida albicans. The antifungal effects of nano ZnO were evaluated against pathogenic fungi (Penicillium expansum and Botrytis cinerea).[19]

**Table 4: Zone of inhibition of SiO2 nanoparticles against fungal species at different concentrations**

| Fungi Isolates | Concentration (µg/ml)/Zone of inhibition (mm) | | | |
|---|---|---|---|---|
| | 10 | 20 | 30 | 40 |
| *Asp. flavus* | 41 | 44 | 45 | 61 |
| *Asp. parasiticus* | 41 | 40 | 45 | 50 |
| *Asp. terrus* | 40 | 41 | 45 | 48 |
| *Candida albicans* | 42 | 43 | 45 | 62 |

It was shown that nano-SiO2 can considerably inhibit the growth of these fungi, in concentrations (10-40) µg/ml, and the highest inhibition zone appeared at the concentration of 40µg/ml. According to this study, nano-SiO2 has the potential of antifungal activity.

There is limited information about this nanoparticle's antifungal effects. Some nanoparticles such as silver increases hydroxyl radicals to induce apoptotic cell death in Candida albicans.20 Although, the antimicrobial effects can influence the shape of the nanoparticle.21 Garcia-Saucedo et al. investigated the Saccharomyces cerevisiae and the toxicity of SiO2 nanoparticles on the yeast, and showed that this nanoparticle is less effective or not effective on this yeast.22 Similar studies on amphotericin B effect on Candida albicans are available.[2]

## Conclusion

The resistance of microorganisms to antibiotics gave as a main strike to the medicinal order. Lately, the usefulness of metal oxide nanoparticles is considered as a possible alternative to antimicrobial agents, where the microorganisms are incapable of improving the resistance versus the nanoparticles. From the results of this research, the antimicrobial activity of silicon oxide against clinical bacteria and fungi has been concluded

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