

Titanium dioxide nanoparticles as antibacterial agents against some pathogenic bacteria

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ABSTRACT

Background: Today, many of studies have been done to investigate the efficacy of antimicrobial nanoparticles (NPs) against the pathogens as drug resistance bacteria and metal NPs have been reported with antimicrobial properties. **Materials and Methods:** In the present study, titanium dioxide (TiO₂) NPs with 35 nm size were characterized by X-ray diffraction and Fourier transform infrared then investigates their antibacterial activity, antibiofilm formation, and invasion against various pathogenic bacteria from Gram-positive *Streptococcus pyogenes* and *Staphylococcus aureus* and Gram-negative *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Escherichia coli*, *Proteus vulgaris*, and *Serratia marcescens*. Antimicrobial activity of TiO₂ NPs was examined by disk diffusion assay using dilutions of 500, 250, 125, 62.5, and 31.25 µg/ml also the minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of each isolate is determined. **Results:** TiO₂ NPs show powerful broad-spectrum antibacterial activity against tested bacteria with increase in inhibition zone diameter that is directly proportional with the increase in NP concentration that even exceeded the activity of selected antibiotics. The MIC of TiO₂ NPs ranged from 125 µg/ml to 31.25 µg/ml and the MBC ranged from 125 µg/ml to 500 µg/ml. The metal NPs highly inhibited bacterial biofilm growth and invasion, other studies show that TiO₂ NPs strongly attached to the bacterial cells that contributed to their inhibitory effect on bacterial biofilm growth formation and invasion. We showed that bacterial biofilm growth was reduced at MIC concentrations of TiO₂ NPs compared with another test without the NPs. **Conclusion:** NPs with a suitable concentration are reduced the biofilm growth significantly. It is highly recommended using TiO₂ NPs as an economic alternative antibacterial and antibiofilm agent, especially in treating ectopic infections without taking the risk of developing resistant bacterial strains as with antibiotics.

KEY WORDS: Antibacterial activity, Biofilm, Nanoparticles, Titanium dioxide

INTRODUCTION

Nanobiotechnology, a modern field of nanoscience, utilizes nanobased as systems for different biomedical field and applications. Metal nanoparticles (NPs) have a largely surface area to volume ratio and a high pieces on surface atoms have been studied widely due to the unique physicochemical characteristics including catalytic activity, electronic properties, optical properties, magnetic properties, and antibacterial properties.^[1-3]

As titanium dioxide (TiO₂) NPs, these days have attracting a great deal of interest because it has properties

to achieve highly effect in biological, pharmaceutical applications, purification of environmental source, electronic system, cells of solar energy, photocatalysts, photoelectrodes, and sensors of gas beside American Food and Drug Administration acceptance for using in food technology and drugs, paints pigment, cosmetics, ointments, and toothpaste.^[4] TiO₂ is occurred naturally and has many properties such as high refractive index, absorption of light, free toxicity, stability, and less cost production.^[5] TiO₂ has three crystalline phases: anatase, rutile, and brookite. Thermodynamically, rutile is the more stable shape. Anatase very attracted more interest due to its uses in photovoltaic cells^[6] and photocatalysts^[7] and for its antimicrobial properties. Nowadays, TiO₂ is widely applied as a self-cleaning and self-antiseptic surface covering materials, TiO₂ has a more application

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in purification, the environmental due to its photo has ability to induced superhydrophobicity and antifogging activity.^[8] These specifications have been utilized as antibacteria and remove different materials from air and water, especially harmful organic material, also their capacity as self-cleaning or self-antiseptic surfaces in different medical institutes.^[9] On the other hand, TiO₂ can synthesize by different methods such as biological and chemical process, has been appeared to be biocompatible and appropriate supports for a broad variety of compounds.^[10] The effect of TiO₂ NPs on microorganism is extreme importance due to ability of pathogenic microorganism to attack in ecosystem food chain^[11] and activate free hydroxyl radicals by TiO₂ NPs.^[12] The antimicrobial effect of TiO₂ against fungi and bacteria has been demonstrated^[13] and communicating in modern research. The objective of recent study was used the TiO₂ NPs to study the antibacterial and antibiofilm activity on some Gram-negative (G-ve) and Gram-positive (G+ve) bacteria to anticipate information to improve new antimicrobial disinfecting solutions can be used as effective on bacterial biofilm in science technology.

MATERIALS AND METHODS

Bacterial Isolates

The bacterial isolates were obtained from the patients in Teaching Hospital in Hillah, Iraq. All samples were subjected to standard bacteriological processes of culturing on blood and MacConkey's agar plates for 24–48 h at 37°C for isolation and purification. All isolates were confirmed by Vitek 2 compact system (Biomérieux).

Solution and Media

Mueller-Hinton agar and Mueller-Hinton media were obtained from Hi-Media, Mumbai, India. Titanium oxide TiO₂ NPs (35 nm) were supplied from (Zhengzhou Dongyao Nano Materials Co., Ltd., China). Different antibiotic disks ciprofloxacin (CIP) (CIP-10), erythromycin (E-15), gentamicin (CN10), meropenem (ME-5), penicillin (P-10), and tetracycline (TE-30) were purchased from (Bioanalyse, Turkey). TiO₂ was supplied from (Sigma-Aldrich).

Characterization of TiO₂ NPs

The TiO₂ NP was confirmed by characterized these NPs by Fourier transform infrared (FTIR) measurements and reflectance X-ray diffraction measurements (XRD).

Antibacterial Activity of TiO₂ NPs

TiO₂ NPs antimicrobial activity was tested against some human pathogenic bacteria, five G-ve bacteria (*Pseudomonas aeruginosa*, *Serratia marcescens*, *Escherichia coli*, *Acinetobacter baumannii*, and *Proteus vulgaris*) with two G+ve bacteria

(*Streptococcus pyogenes* and *Staphylococcus aureus*) that were maintained on nutrient agar slants. The antimicrobial activity was carried out as described by the Clinical and Laboratory Standards Institute.^[14] Antibiotic sensitivity and TiO₂ NPs against bacteria under study are tested using a disk diffusion assay, with triplicates used in dilutions of concentration of TiO₂ NPs (500, 250, 125, 62.5, and 31.25 µg/ml) in sterile deionized water. The first step, the isolates were incubated for 15 min at room temperature, then incubated at 37°C overnight. Positive results were recorded when the inhibition zone was observed around the well after a period of incubation, and then, the inhibition zone diameter was measured using a digital Vernier caliper.^[15]

Determination the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The bacterial isolates were incubated at 37°C overnight, which were used to prepare 0.5 McFarland. The total of 10 ml tube nutrient broth medium was prepared, and then, each sample was inoculated aseptically with 1 ml of the bacterial suspension (about 10⁸ colony-forming unit/mL). Five dilutions of TiO₂ NPs were prepared (500, 250, 125, 62.5, and 31.25 µg/ml) in sterile deionized water and a negative control (without TiO₂ NPs) was used. Each isolate was tested performed in triplicates using multiplate count. The inoculated plates were incubated at 37°C overnight. The MIC was determined using the spectrophotometry at wavelength 600 nm after incubation period.^[16] Wells showed that no turbidity was cultured on nutrient agar plates and incubated at 37°C overnight. Bacterial colonies growth was checked and the concentration that shows no growth is represented as the MBC.

Effect of NPs on Bacteria Biofilms

Bacteria isolate at the beginning and incubating at 37°C overnight on blood agar plates by taken inoculum from the stock putted in the freeze. The prepared plate was stored at 4°C. For each experiment, only one colony from the plate was inoculated in 10 mL of tryptone soy broth (TSB) then cultured in the incubation for 16 h. The bacteria are suspended in tube of TSB to prepare a concentration of 10.6 bacteria/mL.

Multiwells microtiter plate (12 wells) were filled by 1 mL of bacterial suspension under study and let go to grow and better grow under aerobic condition 37°C for 4h. Then, TiO₂ NPs were prepared in different concentrations (500, 250, 125, 62.5, and 31.25 µg/mL), then allowed the biofilms of bacteria to grow for 24 h. Thereafter, washing all wells with sterile distilled water, to remove all unbounded bacteria, dried under room temperature, then stained using 125 µl of crystal violet dye solution 0.1% for about

10–15 min. After the staining, all wells were washed as 3 times using sterile distilled water and then removing the stained using 125 μ l of 30% acetic acid dissolved in water. A new sterile, multiwell microtiter plate with flat-bottomed was prepared with 125 μ l destaining solution in each well. Measuring the absorbance of the destaining solution was measured at 630 nm using the ELISA reader (Stat Fax-2100). Each test was performed in triplicate, and the control (only medium) was used. Classification of bacterial biofilm formation by tissue culture plate method in to three categories: Weak (BF <0.120), Moderat (0.120 > BF \leq 0.240), and Strong (BF >0.240) at OD. value 630 nm.^[17] Data are documented as removing completely and incompletely in the biofilm bacterial growth with presence of TiO₂ nanoparticles and compared with the absence of TiO₂ nanoparticles (control).

RESULTS AND DISCUSSION

FTIR spectra of TiO₂ NPs under test are as shown in Figure 1. Peaks at 3429.55 cm⁻¹ and 3225.09 cm⁻¹ matching to stretching vibration of O-H bond. Peaks showed at 2048.47 cm⁻¹ matching to the C-H stretching vibrations. The peak at 1649.19 cm⁻¹ corresponds to the C = O vibrations. Observation of peaks at 651.96 cm⁻¹, 584.45 cm⁻¹, and 569.02 cm⁻¹ corresponds to Ti-O vibrations.^[18]

Figure 2 shows the XRD patterns of the TiO₂ NPs sample, peaks at 25°, 37°, 47°, 53°, 54°, 63°, and 68° (JCPDS-781510) the observation which confirms to the formation of anatase stage of TiO₂ NPs and corresponds to various crystalline planes. The sharp diffraction patterns showed to the small size of NPs,

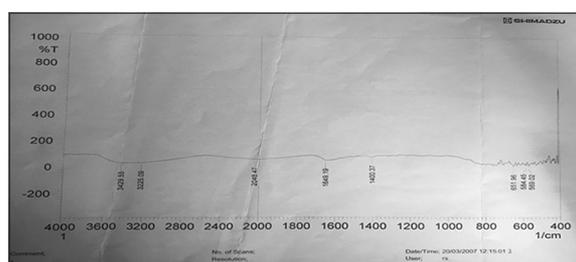


Figure 1: Fourier transform infrared spectra of TiO₂ nanoparticles

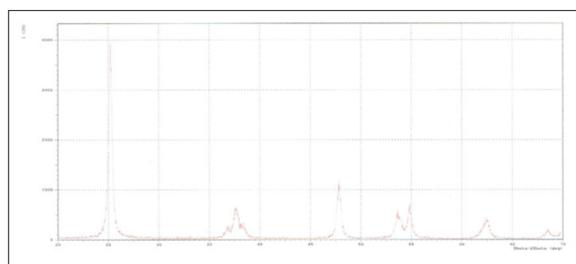


Figure 2: X-ray diffraction of TiO₂ nanoparticles

crystallinity shape, and high purity of the TiO₂ NPs synthesized sample.^[19]

Antibacterial Activity of TiO₂ NPs

TiO₂ NPs shows that powerful broad-spectrum antibacterial activity against multidrug bacteria is tested. The effects of different antibiotics on bacterial isolates were compared. The result in Table 1 showed that the selected antibiotics were not effective against all elected bacterial strains. TiO₂ NPs showed clearly inhibition zone diameter with the increase in NP concentration that even exceeded the activity of selected antibiotics. 500 μ g/ml concentration showed highest zone of inhibition against the test organisms, maximum zone of inhibition of 24 mm appeared against *Streptococcus pyogenes* that showed high sensitivity even at 31.25 μ g/ml. *P. vulgaris* the least sensitive isolate in comparison with the selected antibiotics followed by *E. coli* [Table 2]. TiO₂ NPs cause sudden decline in bacterial cell membrane integrity in addition to the release of reactive oxygen species where superoxide species is generated and contributing in the degradation of biomolecules.^[20]

Minimal residual disease was defined as acquired non-susceptibility to at least one agent among three or more antibacterial antibiotics or categories.^[14]

Table 2 shows that significant increase ($P \leq 0.05$) in all TiO₂ NPs was used against bacterial isolates are used under study. The result has been agreed with the Zhang and Chen^[11] shown that TiO₂ NPs could be inhibited the multidrug-resistant (MDR) bacteria.

MIC and MBC [Table 3] show that the MIC of TiO₂ NPs ranged from 31.25 μ g/ml to 125 μ g/ml and the MBC ranged from 125 μ g/ml to 500 μ g/ml where showed *A. baumannii* and *S. pyogenes* highest sensitivity.^[21]

Antibiofilm Activity of TiO₂ NPs

In vitro experimental data showed the effect of TiO₂ NPs significantly on bacterial biofilm growth. The differences may observe due to the form of NPs, type of bacteria cell wall, and NP concentrations. At a concentration of 500 μ g/mL, TiO₂ NPs showed reduction biofilm growth completely in *P. vulgaris* and *S. aureus* (MBC con.), whereas a concentration of 125 μ g/mL TiO₂ NPs showed reduction biofilm growth completely in *A. baumannii* and *S. pyogenes* (MBC con.) [Table 4].

The biofilm bacterial growth inhibited by TiO₂ NPs is related to specific bacteria isolates or due to growth conditions, G–ve bacteria and G+ve bacteria varied in their capacity to produce their biofilm on the different surface of material. In most studies, the amount of dye (crystal violet) removed from G–ve biofilms was

Table 1: Antibiotic sensitivity for some pathogenic bacterial isolate

Bacterial strain	TE-30 µg/ml	CN-10 µg/ml	ME-5 µg/ml	E-15 µg/ml	CIP-10 µg/ml	P-10 µg/ml
<i>E. coli</i>	I	S	R	R	S	R
<i>P. aeruginosa</i>	I	S	R	R	S	R
<i>P. vulgaris</i>	I	S	R	R	S	R
<i>S. marcescens</i>	I	S	R	R	S	R
<i>A. baumannii</i>	I	S	R	R	S	R
<i>S. aureus</i>	R	S	R	I	R	R
<i>S. pyogenes</i>	S	S	R	S	S	S

I: Intermediate, R: Resistance, S: Sensitive. *E. coli*: *Escherichia coli*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *P. vulgaris*: *Proteus vulgaris*, *A. baumannii*: *Acinetobacter baumannii*, *S. aureus*: *Staphylococcus aureus*, *S. pyogenes*: *Streptococcus pyogenes*

Table 2: Antibacterial activity of TiO₂ NPs against some pathogenic bacteria strain (inhibition zone diameter in mm)

Bacterial strain	TiO ₂				
	31.25 µg/ml	62.5 µg/ml	125 µg/ml	250 µg/ml	500 µg/ml
<i>E. coli</i>	6±1.00000	7.3333±0.76376	7±1.00000	12±2.00000	13±1.00000
<i>P. aeruginosa</i>	10±1.00000	11±1.00000	11.3333±1.25831	13±1.00000	14.3333±0.57735
<i>P. vulgaris</i>	5.3333±0.76376	6.1667±1.04083	8.3333±2.51661	9±1.00000	10.3333±0.76376
<i>S. marcescens</i>	11±1.00000	11±2.64575	11.3333±1.52753	13±0.86603	13±1.00000
<i>A. baumannii</i>	14±1.00000	14.50±0.50000	16.3333±1.25831	18±1.00000	20±1.00000
<i>S. aureus</i>	11±1.00000	12±1.00000	13.3333±1.25831	13±1.00000	13.3333±1.04083
<i>S. pyogenes</i>	20±1.00000	23.33±1.04083	24±1.00000	24.33±0.7637	24±1.00000
LSD (0.05)			1.568		

E. coli: *Escherichia coli*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *P. vulgaris*: *Proteus vulgaris*, *A. baumannii*: *Acinetobacter baumannii*, *S. aureus*: *Staphylococcus aureus*, *S. pyogenes*: *Streptococcus pyogenes*, TiO₂: Titanium dioxide, NPs: Nanoparticles, LSD: Least significant difference

Table 3: MIC and MBC concentration (mg/ml) of TiO₂ NPs for some pathogenic bacteria

Bacterial strain	TiO ₂ NPs MIC	TiO ₂ NPs MBC
<i>E. coli</i>	125	500
<i>P. aeruginosa</i>	62.5	250
<i>P. vulgaris</i>	125	500
<i>S. marcescens</i>	62.5	250
<i>A. baumannii</i>	31.25	125
<i>S. aureus</i>	125	500
<i>S. pyogenes</i>	31.25	125

MIC: Minimal inhibitory concentration, MBC: Minimal bactericidal concentration, TiO₂: Titanium dioxide, NPs: Nanoparticles, *E. coli*: *Escherichia coli*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *P. vulgaris*: *Proteus vulgaris*, *A. baumannii*: *Acinetobacter baumannii*, *S. aureus*: *Staphylococcus aureus*, *S. pyogenes*: *Streptococcus pyogenes*

Table 4: Antibiofilm activity of minimum inhibitory concentration (MIC value in mg/ml) of TiO₂ NPs towered pathogenic bacterial strain

Bacterial strain	Without TiO ₂ NPs	With TiO ₂ NPs
<i>E. coli</i>	Strong	Weak
<i>P. aeruginosa</i>	Strong	None
<i>P. vulgaris</i>	Moderate	None
<i>S. marcescens</i>	Strong	None
<i>A. baumannii</i>	Moderate	None
<i>S. aureus</i>	Moderate	Weak
<i>S. pyogenes</i>	Strong	Weak

E. coli: *Escherichia coli*, *P. aeruginosa*: *Pseudomonas aeruginosa*, *P. vulgaris*: *Proteus vulgaris*, *A. baumannii*: *Acinetobacter baumannii*, *S. aureus*: *Staphylococcus aureus*, *S. pyogenes*: *Streptococcus pyogenes*, TiO₂: Titanium dioxide, NPs: Nanoparticles, MIC: Minimal inhibitory concentration

frequently less than that of G+ve biofilms, that related to the binding of G-ve bacteria is poorly with dye than gram positive bacteria. Among all the pathogenic bacteria, G-ve was more susceptible for inhibited their biofilm than G+ve on these surfaces. The attack

of TiO₂ NPs on sulfhydryl (thiol) or disulfide groups present in the membrane protein results information of more stable S-TiO₂ bond with groups of -SH; thus, the reaction of inhibiting electron transport chain and enzyme catalyzed that are necessary to the formation of bacterial biofilm.^[22]

The inhibition of bacterial biofilm activity occurs during 24 h in G-ve bacteria because to its thinner layer of peptidoglycan in cell wall compared to the G+ve bacteria that mean the inhibition bacterial biofilm increases as a direct relationship with increase the incubation time with exposure to the NPs.^[23] Several studies have believed the germicidal mechanisms of TiO₂ nanoparticles involving release of positively charge ions to reaction medium linked to negative charges thiol group (-SH) of the proteins on the cytoplasmic membrane.^[24] This reaction leads to capture the cell wall and increased permeability beside it causes deform the structure of cellular components such as DNA, ribosomes, and cellular enzymes, caused oxidization and finally death of microbial cell.^[25]

The antibacterial activity is increase with decreasing the particle size of TiO₂ NPs with increasing the specific surface area and dislocation density. Metal NPs have high surface area, which enhances bactericidal activity than the large size NPs; they realize cytotoxicity to the bacteria.^[26] Recent study used TiO₂ NPs incorporated or coated packaging of food and tools of food preparing has also interest, Chawenqijwaich and Hayata^[27] concluded that the coated film coated by TiO₂ NPs could inhibit and reduce the bacterial contamination completely or incompletely on the surface of different

food products and the risks of bacterial growth can reduce on many fresh-cut products. Inhibition activity of metallic NPs on biofilm formation of bacteria has been importance, as the device-related infections, especially MDR bacteria that cause of many morbidity and mortality in different hospitalized patients.^[28,29] Significant changes in bacterial biofilm after treatment with TiO₂ NPs, NPs caused altered gene expression relating to growth and biofilm formation.^[30] TiO₂ NPs lead to larger reduction of bacterial biofilm formation in the glass surface.^[31]

REFERENCES

- Krolikowska A, Kudelski A, Michota A, Bukowska J. SERS studies on the structure of thioglycolic acid monolayers on silver and gold. *Surf Sci* 2003;532:227-32.
- Crabtree JH, Burchette RJ, Siddiqi RA, Huen IT, Hadnott LL, Fishman A. The efficacy of silver-ion implanted catheters in reducing peritoneal dialysis-related infections. *Perit Dial Int* 2003;23:368-74.
- Catauro M, Raucci MG, De Gaaetano FD, Marotta A. Antibacterial and bioactive silver-containing Na₂O·CaO·2SiO₂ glass prepared by sol-gel method. *J Mater Sci Mater Med* 2004;15:831-7.
- Awati PS, Awate SV, Shah PP, Ramaswamy V. Photocatalytic decomposition of methylene blue using nanocrystalline anatase titania prepared by ultrasonic technique. *Catal Commun* 2003;4:393-400.
- Kamat PV. Photophysical, photochemical and photocatalytic aspects of metal nanoparticles. *J Phys Chem B* 2002;106:7729.
- Fujishima A, Donald A. Titanium dioxide photocatalysis. *J Photochem Photobiol C* 2000;1:1-21.
- Gratzel M. Nanoparticle-sensitized solar cells. *Nature* 2001;414:338-45.
- Fujishima A, Honda K. TiO₂ photoelectrochemistry and photocatalysis. *Nature* 1972;238:37.
- Tojo S, Tachikawa T, Fujitsuka M, Majima T. The photo-absorption and surface feature of nano-structured TiO₂ coatings. *J Phys Chem C* 2008;112:14948.
- Peterson A, Lopez T, Ortiz IE, González RD. Pore structures in an implantable sol-gel titania ceramic device used in controlled drug release applications: A modeling study. *Appl Surf Sci* 2007;253:5767-71.
- Zhang H, Chen G. Potent antibacterial activities of Ag/TiO₂ nanocomposites powders synthesized by a one-pot sol-gel method. *Environ Sci Technol* 2009;34:2905-10.
- Gutierrez FM, Olive PL, Banelos A, Orrantia E, Nino N, Sanchez EM, *et al.* Synthesis, characterization, and evaluation of antimicrobial and cytotoxic effect of silver and titanium nanoparticles. *Nanomedicine* 2010;6:681-8.
- Goerne TM, Lemus MA, Morales VA, López EG, Ocampo PC. Study of bacterial sensitivity to Ag-TiO₂ nanoparticles. *J Nanomed Nanotechnol* 2012;S5:2.
- Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-First Informational, Supplement. CLSI Document M02-A10 and M07-A8. Texas: Clinical and Laboratory Standards Institute; 2012.
- CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 26th ed. CLSI Supplement M100S. Wayne, PA: Clinical and Laboratory Standards Institute; 2016.
- Frey FM, Meyers R. Antibacterial activity of traditional medicinal plants used by Haudenosaunee peoples of New York State. *J Int Soc Complement Med Res* 2010;10:64.
- Christensen GD, Simpson WA, Younger JJ, Baddour LM, Barrett FF, Melton DM. Adherence of coagulase-negative staphylococci to plastic tissue culture plates: A quantitative model for the adherence of staphylococci to medical devices. *J Clin Microbiol* 1985;22:996-1006.
- Hema M, Arasi AY, Tamilselvi P, Anbarasan R. Titanian nanoparticles synthesized by sol-gel technique. *J Chem Sci Trans* 2012;2:239-45.
- Stefanska KS. The influence of addition of a catalyst and chelating agent on the properties of titanium dioxide synthesized via the sol-gel method. *J Sol Gel Sci Technol* 2015;75:264-78.
- Kim SC, Lee DK. Preparation of TiO₂-coated hollow glass beads and their application to the control of algal growth in eutrophic water. *Microchem J* 2005;80:227-32.
- Ahmad R, Sardar M. TiO₂ nanoparticles as an antibacterial agents against *E. coli*. *Int J Innov Res Sci Eng Technol* 2013;2:2319-8753.
- Gabriel MM, Mayo MS, May LL, Simmons RB, Ahearn DG. *In vitro* evaluation of the efficacy of a silver-coated catheter. *Curr Microbiol* 1996;33:1-5.
- Santhosh SM, Natarajan K. Antibiofilm activity of epoxy/Ag-TiO₂ polymer nanocomposite coatings against *Staphylococcus aureus* and *Escherichia coli*. *Coatings* 2015;5:95-114.
- Jayaseelan C, Rahuman AA, Roopan SM, Kirithi AV, Venkatesan J, Kim S, *et al.* Biological approach to synthesize TiO₂ nanoparticles using *Aeromonas hydrophila* and its antibacterial activity. *J Babylon Univ Pure Appl Sci* 2013;25:599.
- Koseki H, Shiraishi K, Asahara T, Tsurumoto T, Shindo H, Baba K, *et al.* Photocatalytic bactericidal action of fluorescent light in a titanium dioxide particle mixture: An *in vitro* study. *Biomed Res* 2009;30:189-92.
- Bhupendra C, Anjana KV, Nidhi A, Upadhyay RV, Mehta RV. Enhanced antibacterial activity of biofunctional Fe₃O₄-Ag core-shell nanostructures. *Nano Res* 2009;2:955-65.
- Chawenqkijwaich C, Hayata Y. Development of TiO₂ powder-coated food packing film and its ability to inactivate *Escherichia coli in vitro* and in actual tests. *Int J Food Microbiol* 2008;123:288-92.
- Del-Pozo J, Crumlish M, Ferguson HM, Turnbull JF. Retrospective cross-sectional study on candidatus arthromitus associated rainbow trout gastroenteritis (RTGE) in the UK. *Aquaculture* 2009;290:22-7.
- Ibrahim KH, Salman JA, Ali FA. Effect of titanium nanoparticles biosynthesis by *Lactobacillus crispatus* on urease, hemolysin and biofilm forming by some bacteria causing recurrent UTI in Iraqi women. *Eur Sci J* 2014;10:324-38.
- Maurer-Jones MA, Gunsolus IL, Murphy CJ, Haynes CL. Toxicity of engineered nanoparticles in the environment. *Anal Chem* 2013;85:3036-49.
- Chorianopoulos NG, Tsoukleris DS, Panagou EZ, Falaras P, Nychas G. Use of titanium dioxide (TiO₂) photocatalysts as alternative means for *Listeria monocytogenes* biofilm disinfection in food processing. *Food Microbiol* 2010;28:164-70.

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