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Effect of Nano - Titanium Dioxide with Different Antibiotics against Methicillin-Resistant *Staphylococcus Aureus*

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ABSTRACT

The different investigation has been carried out on the biological activities of titanium dioxide nanoparticle but the effect of this nano product on the antibacterial activity of different antibiotics has not been yet demonstrated. In this study the nano size TiO_2 is synthesized using citric acid and alpha dextrose and the enhancement effect of TiO_2 nanoparticle on the antibacterial activity of different antibiotics was evaluated against Methicillin-resistant Staphylococcus aureus (MRSA). During the present study, different concentrations of nano-scale TiO_2 were tested to find out the best concentration that can have the most effective antibacterial property against the MRSA culture. Disk diffusion method was used to determine the antibacterial activity of these antibiotics in the absence and presence of sub inhibitory concentration of TiO_2 nano particle. A clinical isolate of MRSA, isolated from Intensive Care Unit (ICU) was used as test strain. In the presence of sub-inhibitory concentration of TiO_2 nanoparticle (20 µg/disc) the antibacterial activities of all antibiotics have been increased against test strain with minimum 2 mm to maximum 10mm. The highest increase in inhibitory zone for MRSA was observed against pencillin G and amikacin (each 10 mm). Conversely, in case of nalidixic acid, TiO_2 nanoparticle showed a Synergic effect on the antibacterial activity of this antibiotic against test strain. These results signify that the TiO_2 nanoparticle potentate the antimicrobial action of beta lactums, cephalosporins, aminoglycosides, glycopeptides, macrolids and lincosamides, tetracycline a possible utilization of nano compound in combination effect against MRSA.

Keywords: *Nano - Titanium Oxide, S. Aureus, Drug Resistance, Antibacterial Activity*

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the major nosocomial pathogens responsible for a wide spectrum of infections, including skin and soft tissue infections, pneumonia, bacteraemia, surgical site infections (SSI), catheter related infections [1]. Intensive care unit characteristically has higher rates of infections and increased transmission rates, high antibiotic use and large numbers of vulnerable patients [2]. The emergence of bacterial resistance to antibiotics and its dissemination, however, are major health problems, leading to treatment drawbacks for a large number of drugs [3,4]. Consequently there has been increasing interest in the use of inhibitors of antibiotic resistance for combination therapy [5,6].

Nanostructured materials are attracting a great deal of attention because of their potential of achieving specific processes and selectivity, especially in biological and pharmaceutical applications [7-9]. Gold, silver and copper have been used mostly for the synthesis of stable dispersions of nanoparticles [10,11]. A unique characteristic of these synthesized metal particles is that a change in the absorbance or wave length gives a measure of the particle size, shape and interparticle properties [12]. Nanomaterials are called "a wonder of modern medicine". It is stated that antibiotics kill perhaps a half dozen different disease-causing organisms but nanomaterials can kill some 650 cells [13]. Resistant strains fail to develop if we apply nanoparticles based formulations in their culture media.

The antibacterial activity of TiO_2 has been found to be due to a reaction of the TiO_2 surface with water. On exposure to ultraviolet (UV) irradiation, TiO_2 releases free radicals such as OH, O_2^-, HO_2-, and H_2O_2. This

potent oxidizing power characteristically results in case of bacteria and other organic substances [14-16]. The small nanometer-scale TiO_2 particles impose several effects that govern its antibacterial action we examined the antimicrobial activity of nanostructured titanium dioxide with different antibiotics against MRSA. The different investigation has been carried out on the biological activities of titanium dioxide nanoparticle but the combination effects of this product with different antibiotics have not been demonstrated. The nanocrystalline particles of TiO_2 are synthesized using ultrasonic irradiation, and the particle sizes are controlled using different solvents during the sonication process.

Objectives of the present study are (i) synthesis of nano size titanium dioxide using citric acid and alpha dextrose (ii) analyse the effect of Titanium nanoparticles on the antibacterial activity of different antibiotics against MRSA (iii) estimation of MRSA growth in the presence of TiO_2 nanoparticles have been reported having an extremely good safety profile and no toxicity observed when taken at different nanosize. Taken together, this compound as a highly safe compound may be considered for combination therapy against MRSA, due its potential synergetic effect with important antibiotics such as beta lactums, cephalosporins, aminoglycosides.

2. Materials and Methods

Titanium dioxide particles preparation: In the following, the two step sol-gel preparation method used is described detail. Nanocrystalline titanium dioxide was prepared by employing citric acid route were saturated solution of α-Dextrose used as a surfactant.

Two separate solutions were prepared. In first step: titanium nitrate and citric acid are taken in 1:3 and are thoroughly stirred using magnetic stirrer with ammonia solution at 80˚C about 5-6 hrs. Ammonia solution is used to maintain the pH 4 of solution. Finally a gel is formed. In second step, saturated solution of alpha dextrose is added and stirred for 1hr at 120˚C to the spongy type gel of nanoscaled TiO_2 formed. This spongy gel is ignited at a temperature of about 300˚C for 1 hr. At this temperature a combustion process takes place in the spongy gel containing citric acid a result of it we have nanostructured titanium dioxide.

3. X-Ray Diffraction (XRD)

X-ray diffraction Phase identification was carried out by X-Ray powder diffraction at ambient temperature. A Shintag X1 diffractometer with Cu Kα (1.54 A) radiation in θ – θ configuration was used. The patterns were recorded in the 2-70 range at 0.05 step size using 3-s acquisition time per step. The mean particle size was calculated using the Debye–Scherrer Equation 1 in which K is a constant equal to 0.9, λ is the wavelength of the Cu Kα radiation, β is the half peak width of the diffraction peak in radiant and θ is the Braggs angle of (311) plane.

$$\tau = K\lambda/\beta\cos\theta \quad (1)$$

Staphylococcus aureus was isolated from Clinical specimens collected from ICU of Durgabai Deshmukh Hospital and Research Center and Osmania Hospital, Hyderabad, South India. Oxacillin-disc diffusion method was done for identification of methicillin-resistance. This MRSA was used as test strain. Antibiotic susceptibility test was performed for the test strain (MRSA) against 23 antibiotics by disc agar diffusion method (DAD) on Muller-Hinton agar (Himedia, India), according to the guidelines recommended by National Committee for Clinical Laboratory Standards (NCCLS) [17].

4. Disk Diffusion Assay to Evaluate Combined Effects

To determine combined effects, each standard paper disc was further impregnated with sub-inhibitory concentration of titanium dioxide nanoparticle (10 μg/disc). A single colony of test strains were grown overnight in Muller-Hinton broth medium on a rotary shaker (200 rpm) at 35˚C. The inoculums were prepared by diluting the overnight cultures with 0.9% NaCl to a 0.5 McFarland standard and were applied to the plates along with the standard and prepared disks containing of titanium dioxide nanoparticle (10 μg/disc). Clinical isolates of MRSA from our culture collection were used as test strains. After incubation at 37˚C for 24 hrs, the zones of inhibition were measured. The assays were performed in triplicate.

5. Estimation of MRSA Growth in the Presence of Nanocrystalline TiO_2

The 2 mL of the overnight-cultured MRSA was added to 100 mL nutrient broth, containing 0.12% glucose with and without 0.01, 0.5 and 1% nano-TiO_2 and incubate at 30˚C for 24 hrs. Optical density measurements were taken at 600 nm to monitor the bacterial concentration.

6. Results and Discussions

The nano size titanium dioxide is synthesized using citric acid and alpha dextrose. The small nanometer scale TiO_2 particles as seen in the **Figure 1** will impose several effects that govern its antibacterial action. The X-ray diffraction pattern shows cubic peaks of TiO_2, which indicates the nanocrystalline nature of pure nanostructured titanium dioxide and is shown in the **Figure 2**. By comparing the XRD pattern standard JCPS data (432-161) of TiO_2, indicating the prominent peaks corresponding to $2\theta = 27^{\circ}, 39^{\circ}, 48^{\circ}, 55^{\circ}$ and 63° are due to (110), (200), (112),

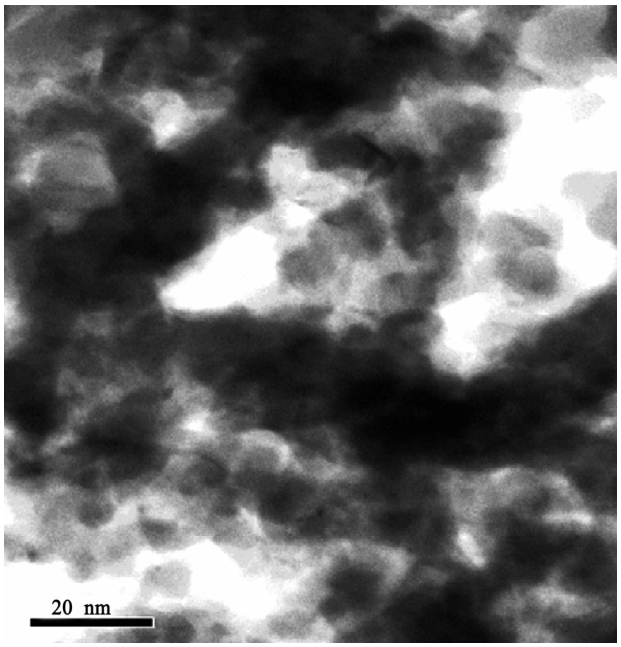

Figure 1. Showing TEM image of TiO_2 nanoparticles.

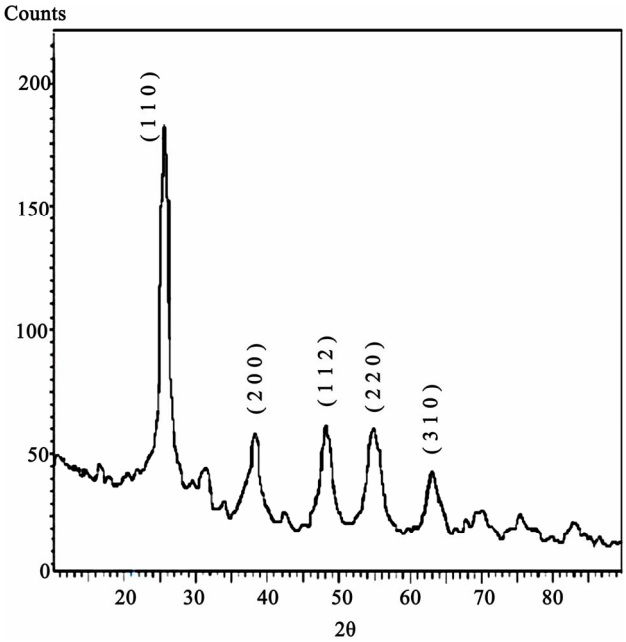

Figure 2. XRD pattern of TiO_2.

(220) and (310) planes which indicates formation of single phase titanium dioxide. The crystalline size of the Titanium dioxide is calculated by using debye-scharrer equation and it was found to be around 20 nm. Titanium dioxide has a very good potential to move into the clinic [18]. In this investigation the effect of TiO_2 nanoparticle on the antibacteria of different antibiotics was investtigated against MRSA using disk diffusion method. The antimicrobial resistance of MRSA against various antibiotics is increased without nano-TiO_2 and decreases with nanoscaled TiO_2. The diameter of inhibition zones (mm) around the different antibiotic discs with TiO_2 and without titanium dioxide nanopatrticles against test strain are shown in [**Table 1**].

The antibacterial activities of all antibiotics have been increased in the presence of nanosize titanium dioxide against test strain. The highest antibacterial activities increases in area were observed for penicillin and amikacin (10 mm) followed by ampicillin and Gentamycin (in each 09 mm), oxacillin, cloxacillin (08 mm), amoxycillin, cephalexin, cefotoxime, ceftazidime, vancomycin, streptomycin (in each 07 mm) erythromycin, clindamycin (06 mm) and tetracyclin (05 mm). The moderate increases in inhibition zone areas for ciprofloxacin, rifampicin, sulphazidime and cotrimoxazole (04 mm).The lowest increase in inhibition zone area against the Chloramphenicol (03 mm) followed by norfloxacin and clarithromycin (02 mm).

Conversely, for nalidixic acid, titanium dioxide nanoparticle shows no effect on the antibacterial activity of this antibiotic against the test strain. It should be pointed out that the titanium dioxide nanoparticle content of 10 μg/disc was chosen to guarantee that the effect produced was due to the combination and not to the effect of the TiO_2 nanoparticle itself. So the effect observed in this condition could be due to the antibiotic-titanium dioxide nanoparticle combination. At the concentration tested, TiO_2 nanoparticle significantly improved antibiotic efficacy against *S. aureus* when combined with beta lactums, cephalosporins, aminoglycosides.

The optical density of the medium was investigated as the number of bacteria after contact with the nano- particles. **Figure 3** shows the growth of MRSA at diff- erent concentrations and the effect of different 0.01, 0.5 and 1% nano-TiO_2 on the in growth and killing of MRSA. As demonstrated by the figure, 0.01% nano-TiO_2 did not have antibacterial efficiency on MRSA but the concentrations of 0.5 and 0.1% nano-TiO_2 inhibited the bacterial growth. Also shows that 0.5% nano-TiO_2 showed 1.9 times decrease the optical density of bacterial cultures as compared to the control. While, in the presence of 1% nano-TiO_2, the optical density of MRSA cultures decreased 4.5 times as compared to the control experiment.

A study states the nano-TiO_2 as a strong and effective bactericidal agent [18]. During the present study, different concentrations of nanosized TiO_2 were tested to find out the best concentration that can have the most effective antibacterial property against the MRSA culture. This is the first report of combination effect of TiO_2 nanoparticles with different antibiotics. Today, TiO_2

Table 1. the comparative activities of various antibiotics and antibiotic with NanosizedTiO_2 against MRSA.

Sl. No.	Antibiotics	Symbol	Inhibition Zone of Antibiotic (mm)	Inhibition Zone of Antibiotic with TiO_2 (20nm) in (mm)	Increased zone size (mm)
1	**B-lactams**				
01.	Penicillin G	P (10U)	34	44	10
02.	Oxacillin	Wx (1 µg)	11	19	08
03.	Cloxacillin	Cx (30 µg)	19	27	08
04.	Ampicillin	A (10 µg)	29	38	09
05.	Amoxycillin	Am (25 µg)	20	26	07
2	**Cephalosporins**				
06.	Cephalexin	Cp (30 µg)	25	32	07
07.	Cefotoxime	CX (30 µg)	24	33	07
08.	Ceftazidime	Ca (30 µg)	16	23	07
3	**Glycopeptides**				
09.	Vancomycin	V (30 µg)	15	22	07
4	**Aminoglycosides**				
10.	Amikacin	Ak (10 µg)	15	25	10
11.	Gentamycin	G (50 µg)	14	24	09
12.	Streptomycin	S (25 µg)	13	19	07
5	**Flouroquinolones**				
13.	Ciprofloxacin	Cf (5 µg)	20	24	04
14.	Norfloxacin	No (10 µg)	15	17	02
6	**Azlides**				
15.	Clarithromycin	Cw (15 µg)	17	19	02
7	**Macrolides**				
16.	Erythromycin	E (15 µg)	15	21	06
8	**Lincosamides**				
17.	Clindamycin	Cl (10 µg)	20	26	06
9	**Sulphonamides**				
18.	Cotrimoxazole	Co (25 µg)	17	21	04
19.	Nalidixicacid	Na (30 µg)	16	16	00
20.	Rifampicin	R (15 µg)	25	29	04
21.	Tetracyclin	T (30 µg)	21	26	05
22.	Sulphazidime	Sz (25 µg)	12	17	04
23.	Chloramphenicol	C (30 µg)	18	21	03

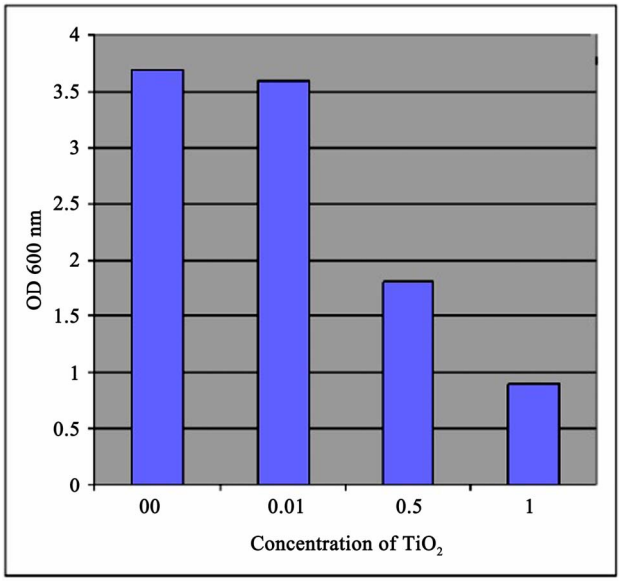

Figure 3. MRSA growth at different concentrations of TiO_2.

nanoparticle are cosmetic ingredient has drawn the attention of scientists because of its extensive pharmaceutical properties. In different phases, clinical trials, no toxicity except mild dehydration was observed when taken at doses as high as g/day and it is reported as an attractive choice for many disease therapies.

Recently some metal nanoparticles have been evaluated for increasing the antibacterial activities of different antibiotics. Several investigations have suggested the possible mechanisms involving the interaction of nanomaterials with the biological molecules. It is believed that microorganisms carry a negative charge while metal oxides carry a positive charge. This creates an "electromagnetic" attraction between the microbe and treated surface. Once the contact is made, the microbe is oxidized and dead instantly. Generally, it is believed that nanomaterials release ions, which react with the thiol group (-SH) of the proteins present on the bacterial surface. Such proteins protrude through the bacterial cell membrane, allowing the transport of nutrients through the cell wall. Nanomaterials inactivate the proteins, decreasing the membrane permeability and eventually causing the cellular death [19]. In this study using disk diffusion assay we showed that the antibacterial activity of

beta lactums, cephalosporins, aminoglycosides, glycolpeptides, erythromycin, clindamycin and tetracycline can be increased by TiO_2 nanoparticles. Therefore, this compound or its future derivatives have a good potential for combination effect against MRSA.

7. Conclusions

The synthesis of nanosize titanium dioxide of 20nm was carried out successfully using citric acid and alpha dextrose as double surfactants. The small nanometer scale TiO_2 particles which impose several effects that govern its antibacterial action. The antibacterial activities at different concentrations of nano-TiO_2 were investigated. The antimicrobial resistance of MRSA against various antibiotics is increased without nano-TiO_2 and decreases with nano-TiO_2. Need the further work to find out the exact reason to for enhancement of activity of antibiotics in presence of TiO_2 nanoparticles.

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