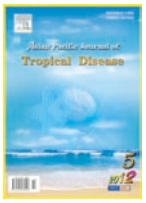
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage:www.elsevier.com/locate/apjtd



Document heading doi: 10.1016/S2222-1808(12)60089-9

In vitro antibacterial potential of metal oxide nanoparticles against antibiotic resistant bacterial pathogens

R Gokulakrishnan[1], S Ravikumar[1*], J Anandha Raj[2]

[1]*School of Marine Sciences, Department of Oceanography and Coastal Area Studies, Alagappa University, Thondi Campus, Thondi – 623 409, Ramanathapuram District, Tamil Nadu, India*

[2]*School of Chemistry, Department of Industrial Chemistry, Alagappa University, Karaikudi–630 003, Sivgangai District, Tamil Nadu, India*

ARTICLE INFO

Article history:
Received 15 July 2012
Received in revised form 2 August 2012
Accepted 28 October 2012
Available online 28 October 2012

Keywords:
Antibacterial activity
Antibiotic resistant bacteria
MBC
MIC
MgO nanoparticle
Time kill assay

ABSTRACT

Objective: To investigate the antibacterial potential of 5 different metal oxide nanoparticles against antibiotic resistant bacterial pathogens *viz*., *Pseudomonas aeruginosa*, *Klebsiella* sp. *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Streptococcus* sp. **Methods:** The antibacterial activity of the five different nanoparticles was assessed by well diffusion method. Different concentrations of the nanoparticles were analyzed by MIC and MBC techniques. Finally the potential MgO nanoparticle was also subjected for the time kill assay method. **Results:** The results reveal that, the MgO nanoparticle showed maximum sensitivity [(16.00±0.53) mm dia] against *Streptococcus pneumoniae* and showed minimum sensitivity against *Klebsiella* sp. [(9.00±0.31) mm dia]. None of the nanoparticles showed sensitivity against the *Streptococcus* sp. The MIC result reveals that, the MgO nanoparticle showed maximum inhibition at a concentration of 10 μg against *Streptococcus pneumoniae*. Moreover, the time kill assay reveals that, the bacterial growth was inhibited from the 2nd h onwards at a concentration of 10 μg. **Conclusions:** It is concluded from the present findings that, the MgO nanoparticle could be used as an alternative antibacterial agent after completing successful *in vivo* trials.

1. Introduction

The infectious diseases are one of the major health problems to the developing and developed countries. During the last decade, various resistant mechanisms have been increased worldwide in bacterial pathogens which lead to failure treatment in human and animal diseases[1,2]. Bacteria are able to adapt rapidly to new environmental conditions such as the presence of antimicrobial molecules and, as a consequence, resistance increases with the antimicrobial use[3,4]. Recently, the metal oxide nanoparticles played a vital role in the novel drug delivery systems[5]. Synthesis of noble nanoparticles has been used as an antibacterial agent, catalysis, environmental and biotechnology is an area of constant interest[6]. Moreover, the biosynthesized and chemically synthesized silver nanoparticles showed various biological activities[5,7,8]. However, studies related with the antibacterial agents from metal oxides nanoparticles against antibiotic resistant bacterial pathogens are poorly understood. In this connection, the present study is made an attempt to find out the antibacterial potential of metal oxide nanoparticles.

*Corresponding author: S Ravikumar, School of Marine Sciences, Department of Oceanography and Coastal Area Studies, Alagappa University, Thondi Campus, Thondi 623 409, Ramanathapuram District, Tamil Nadu, India.
Tel: 04561-243470, 9003306959
E-mail: ravibiotech201321@gmail.com
Foundation Project: Supported by University Grants Commission, New Delhi for financial assistance, Grant No. F.No. 39-563/2010 (SR).

2. Materials and methods

Commercial nanoparticles of Al_2O_3, Fe_3O_4, CeO_2, ZrO and MgO were procured from Sigma Aldrich Company, India. The characteristics of the nanoparticles are presented in Table 1.

Table 1
Properties of nanoparticles.

Formula	Molecular weight	Form	Particle size in Transmission electron microscopy (nm)
Al_2O_3	101.96	Powder	<50
Fe_3O_4	231.53	Powder	9-11
CeO_2	172.11	Powder	<25
ZrO_2	123.22	Powder	<100
MgO	40.30	Powder	<30

2.1. Test organisms

Five antibiotic resistant pathogens *viz*., *Pseudomonas aeruginosa*, *Klebsiella* sp., *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Streptococcus* sp. were obtained from Vinayaga Mission hospital, Salem, Tamil Nadu, India.

2.2. Antibacterial assay

The antibacterial activity of the chosen nanoparticles was performed by using well diffusion method. About 20 mL of sterile molten Mueller Hinton agar (HiMedia Laboratories Pvt. Limited, Mumbai, India) was poured into the sterile petriplates. Triplicate plates were swabbed with the overnight culture (10^8 cells/mL) of chosen pathogenic bacteria. Then the solid medium was gently punctured with the help of cork borer to make a well. Finally, the nanoparticle samples (50 μg/mL) were added from the stock into each well and incubated for 24 h at (37±2) ℃ and the antibacterial sensitivity is measured as zone of inhibition in millimeter in diameter.

2.3. Minimum inhibitory concentration (MIC)

Different concentrations (10, 20, 30, 40, 50 and 60 μg/mL) of metal oxide nanoparticles were prepared with dimethyl sulphoxide (DMSO) and mixed with 450 μL of nutrient broth and 50 μL of 24 h old bacterial inoculum and allowed to grow overnight at 37 ℃ for 48 h. Nutrient broth with pathogens alone was served as negative control. Whole setup in triplicate was incubated at 37 ℃ for 24 h. The MIC was the lowest concentration of the synthetic compounds that did not permit any visible growth of bacteria during 24 h of incubation after inoculation examined on the basis of turbidity[5].

2.4 Minimum bactericidal concentration (MBC)

To avoid the possibility of misinterpretations due to the turbidity of insoluble compounds if any, the MBC was determined by sub-culturing the above (MIC) serial dilutions after 24 h in nutrient agar plates using 0.01 mL loop and incubated at 37 ℃ for 24 h. MBC was regarded as the lowest concentration that prevents the growth of bacterial colony on this solid media[5].

2.5 Time kill assay

The potential nanoparticle (MgO) which showed maximum sensitivity against *Streptococcus pneumoniae* was further subjected for time kill assay. The inoculum of *Streptococcus pneumoniae* (50 μL) at the concentration of 10^8 cells/mL was mixed with 50 μL (Contains 10 μg/mL) of chosen nanoparticles and the total volume was made up to 5 mL by using minimal medium (g/L) [Sucrose-10; K_2HPO_4-2.5; KH_2PO_4-2.5; $(NH_4)_2HPO_4$-1; $MgSO_4 \cdot 7H_2O$-0.20; $FeSo_4 \cdot 7H_2O$-0.01; $MnSO_4 \cdot H_2O$-0.007 and H_2O-1 000 mL]. The negative control was maintained without the nanoparticles. The growth of the bacterial species was assessed at every 1 h interval by measuring the optical density at 600 nm by using spectrophotometer (Cyber UV-1, Mecasys Co Ltd)[9].

3. Results

Antibacterial activity of the metal oxide nanoparticles against chosen antibiotic resistant bacterial pathogens was investigated and represented in Table 2. It reveals that, all the nanoparticles showed antibacterial activity against *Pseudomonas aeruginosa* and *Streptococcus pneumoniae*. Of these, MgO nanoparticle showed maximum sensitivity [(16.00 ±0.53) mm dia] against *Streptococcus pneumoniae* and the ZrO_2 nanoparticles showed maximum sensitivity [(12.00±0.51) mm dia] against *Pseudomonas aeruginosa*. The CeO_2 nanoparticles showed minimum sensitivity [(7.00±0.38) mm dia] against *Pseudomonas aeruginosa* and *Streptococcus pneumoniae* [(9.00 ±0.36) mm dia] respectively. Moreover, all the nanoparticles except Al_2O_3 showed sensitivity against *Klebsiella* sp. However, none of the nanoparticles showed sensitivity against *Streptococcus* sp. In the MIC assay reveals that, the MgO nanoparticles showed maximum inhibition of bacterial growth at a concentration of (10 μg) against *Streptococcus pneumoniae*. The CeO_2 nanoparticles showed no inhibition against all the tested pathogens (Table 3). The time kill assay reveals that, the MgO nanoparticle inhibits the bacterial growth from the 2nd h after treatment (Figure 1).

Table 2
Antibacterial activity of nanoparticles against antibiotic resistant pathogens (mm).

Name of the nanoparticles	*Pseudomonas aeruginosa*	*Klebsiella* sp.	*Streptococcus pneumoniae*	*Staphylococcus aureus*	*Streptococcus* sp.
Al_2O_3	11.00±0.15	–	11.00±0.41	–	–
Fe_2O_3	8.00±0.25	10.00±0.22	13.00±0.12	11.00±0.19	–
Ceo_2	7.00±0.38	7.00±0.16	9.00±0.36	9.00±0.35	–
ZrO_2	12.00±0.51	9.00±0.25	10.00±0.63	–	–
MgO	8.00±0.52	9.00±0.31	16.00±0.53	–	–

– no activity.

Table 3
MIC and MBC of nanoparticles against antibiotic resistant pathogens (μg/mL).

Name of the nanoparticles	*Pseudomonas aeruginosa*		*Klebsiella* sp.		*Streptococcus pneumoniae*		*Staphylococcus aureus*		*Streptococcus* sp.	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Al_2O_3	50	50	–	–	50	50	–	–	–	–
Fe_2O_3	–	–	50	50	30	30	50	50	–	–
Ceo_2	–	–	–	–	–	–	–	–	–	–
ZrO_2	60	60	60	60	40	40	–	–	–	–
MgO	–	–	–	–	10	10	–	–	–	–

'–'no activity.

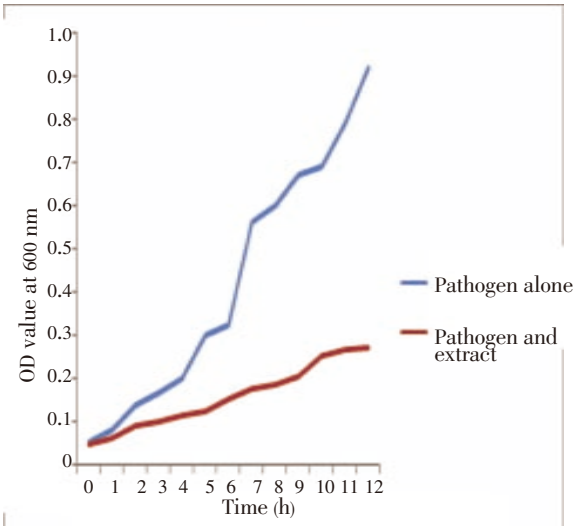


Figure 1. Time kill assay of MgO nanoparticle against antibiotic resistant pathogen *Streptococcus pnemoniae*.

4. Discussion

Antibiotic resistance is the biggest challenge to the medical field for the treatment of infectious diseases. The antimicrobial agents have been categorized according to their mechanism of action[10]. The resistant bacteria spread and infection problems occur not only in the healthcare institutions but in the communities also. The spread of resistant bacteria within the community posse's obvious additional problems for health control[11]. Recently, nanoparticles particularly, Fe_3O_4, ZrO_2 and MgO showed antibacterial activities against ophthalmic pathogens[5]. The results of the antibacterial activity of the present study reveal that, the MgO nanoparticles showed maximum antibacterial activity against *Streptococcus pneumoniae* at the concentration of 10 μg from the 2nd h onwards. Generally, the nanoparticles bind with the thiol (-SH) groups of protein that destroy the cell wall[12]. But in the case of resistance bacteria, the possible mechanism of activity is, the MgO nanoparticles might inhibit the production of β-lactamase enzyme which involved in the drug deactivation process or the nanoparticles block the efflux pump pathway which involved in the drug elimination process[10]. Likewise, the TiO_2, CdO and silver nanoparticles showed excellent antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*[13-16]. This mechanism creates the stress in the cell wall and which produces more lactate dehydrogenase enzymes and leads to damage the cell membrane and the severity depends upon the exposure time[17]. It is concluded from the present study that, the MgO nanoparticle could be used as an effective antibacterial agent for the management of antibiotic resistant bacterial diseases after completing the successful clinical trials.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors are thankful to the authorities of Alagappa University for providing required facilities and also to University Grants Commission, New Delhi for financial assistance and the grant number is: F.No. 39-563/2010 (SR).

References

[1] Gottlieb T, Nimmo GR. Antibiotic resistance is an emerging threat to public health: an urgent call to action at the Antimicrobial Resistance Summit 2011. *Med J Aus* 2011; **194**(6): 281-283.

[2] WHO, the world health report 2007-A safer future: global public health security in the 21st century. [Online] Available from: http://www.who.int/whr/2007/en/index.html [Accessed on 25 February 2009]

[3] Falagas ME, Bliziotis IA. Pan drug-resistant Gram-negative bacteria: the dawn of the post-antibiotic era? *Int J Antimicrob Agen* 2007; **29**: 630-636.

[4] Jansen WT, van der Bruggen JT, Verhoef J, Fluit AC. Bacterial resistance: a sensitive issue complexity of the challenge and containment strategy in Europe. *Drug Resist Updat* 2006; **9**: 123-133.

[5] Ravikumar S, Gokulakrishnan R, Selvanathan K, Selvam S. Antibacterial activity of metal oxide nanoparticles against ophthalmic pathogens. *Int J Phar Res Dev* 2011; **3**(5): 122-127.

[6] Sharma Virender K, Yngard Ria A, Yekaterina L. Silver nanoparticles: Green synthesis and their antimicrobial activities. *Adv Coll Inter Sci* 2009; **145**: 83-96.

[7] Jacob Inbaneson S, Ravikumar S, Manikandan N. Antibacterial potential of silver nanoparticles against isolated urinary tract infectious bacterial pathogens. *Appl Nanosci* 2011; **1**: 231-236.

[8] Gnanadesigan M, Anand M, Ravikumar S, Maruthupandy M, Vijayakumar V, Selvam S, et al. Biosynthesis of silver nanoparticles by using mangrove plant extract and their potential mosquito larvicidal property. *Asi Paci J Trop Med* 2011; **4**(10): 799-803.

[9] Ravikumar S, Ramanathan G, Subhakaran M, Jacob Inbaneson S. Antimicrobial compounds from marine halophytes for silkworm disease treatment. *Int J Med Medi Sci* 2009; **5**: 184-191.

[10] Jayaraman R. Antibiotic resistance: an overview of mechanisms and a paradigm shift. *Curr Sci* 2009; **96**(11): 1475-1484.

[11] McGowan JE Jr. Economic impact of antimicrobial resistance. *Emerg Infect Dis* 2001; **7**: 286-292.

[12] Hosseinkhani P, Zand AM, Imani S, Rezayi M, Rezaei Zarchi S. Determining the antibacterial effect of ZnO nanoparticle against the pathogenic bacterium, *Shigella dysenteriae* (type 1). *Int J Nano Dim* 2011; **1**(4): 279-285.

[13] Zhang H, Chen G. Potent antibacterial activities of Ag/TiO_2 nanocomposite powders synthesized by a one-pot sol-gel method. *Environ Sci Technol* 2009; **43**(8): 2905-2910.

[14] Kim H, Ryu JH, Beuchat LR. Effectiveness of disinfectants in killing *Enterobacter sakazakii* in suspension, dried on the surface of stainless steel, and in a biofilm. *Appl Environ Microbiol* 2007; **73**: 1256-1265.

[15] Rezaei-Zarchi S, Aisha J, Madiha JG, Safieh S, Fatemeh BF, Abdolmajid BM, et al. Comparative study of antimicrobial activities of TiO_2 and CdO nanoparticles against the pathogenic strain of *Escherichia coli*. *Ir J Pathol* 2010; **5**(2): 83-89.

[16] Martinez-Castanon GA, Nino-Martinez N, Martinez-Gutierrez F, Martinez Mendoza JR, Ruiz F. Synthesis and antibacterial activity of silver nanoparticles with different sizes. *J Nanopar Res* 2008; **10**: 1343-1348.

[17] Weisheng L, Yue-wern H, Xiao-Dong Z, Yinfa M. Toxicity of cerium oxide nanoparticles in human lung cancer cells. *Int J Toxicol* 2006; **25**(6): 451-457.