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The Inhibitory Effect of Metal Oxide Nanoparticles against Poultry Pathogens

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ABSTRACT
The present study was aimed to investigate the antibacterial potential of metal oxide nanoparticles *viz.,* Al_2O_3, Fe_3O_4, CeO_2, ZrO_2, and MgO against poultry pathogens *viz., Klebsiella* sp., *E. coli, Staphylococcus* sp. and *Salmonella* sp. The antibacterial activity of the metal oxide nanoparticles were assessed with well diffusion method. Various concentrations of nanoparticles were analyzed with minimum inhibitory concentration and minimum bactericidal concentration. Moreover, the potential nanoparticle was also tested with time kill assay. The ZrO_2 showed maximum antibacterial activity against *Salmonella* sp. (15 ± 0.44 mm dia) followed by 12 ± 0.35 mm dia. against *E. coli* respectively. The MIC and MBC results revealed that, the ZrO_2 nanoparticles inhibit the bacterial growth at a concentration of 2.5µg/ml against *Salmonella* sp. All the nanoparticles showed activity against all the tested pathogens. The time kill assay reveals that, the growth of the *Salmonella* sp. was inhibited by ZrO_2 from the 1st h onwards. It is concluded from the present study that, the ZrO_2 nanoparticles could be used as an effective antibacterial agent for the management of poultry systems.

Keywords: Antibacterial activity, MIC, MBC, Metal oxide nanoparticles, Poultry pathogens.

INTRODUCTION

Poultry has undergone rapid changes during the past decades due to modern intensive production methods, new breeds, improved bio-security and preventive health measures. Modern production places high demands on proper health, hygiene and management. Production of eggs and broilers has been rising at a rate of 8 to 10% per annum. [1] India is now the world's 5th largest egg producer and the 18th largest producer of broilers. [2] Although poultry production is considered as secondary agricultural production systems and it has an important role in high quality protein. [3] Poultry provide globally important sources of animal protein and are amongst the most intensively reared of all livestock species. Several microbial diseases have been affecting the poultry and it is a major concern, both locally and international levels. The low productivity is mainly due to high mortality, which is caused particularly by bacterial diseases and the mortality has been estimated in the range of 80-90%. Diagnosis, treatment and prevention of diseases are of major importance to increase the productivity. Recently the antibiotics such as tetracycline, penicillins, sulphonamides and streptomycin and dihydrostreptomycins are used for the poultry bacterial diseases. Moreover, the marine plants [4-7] as well as terrestrial plants [8] have been used for the treatment of major bacterial diseases. The routine treatments lead to loss of biodiversity. One of the earliest nanomedicine applications particularly, an antimicrobial agent from metal oxide nanoparticles for the treatment of various microbial diseases is being emerged. However, studies related with the metal oxide nanoparticles against poultry pathogens are too limited. Hence, the present study has been made an attempt to find out the novel antibacterial agents from metal oxide nanoparticles for the disease free poultry management systems.

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MATERIALS AND METHODS

Commercial nanoparticles of Al_2O_3, Fe_3O_4, CeO_2, ZrO_2, and MgO were procured from Sigma Aldrich Company, India. The characteristics of the nanoparticles are presented in Table 1.

Test organisms

Namakkal is the major poultry producer of southern India. About 75% of birds were produced in the Namakkal zone. In the past two years, the poultry sector has grown by 20% and the production of egg is 2.5 crore per day. In the present study the test organisms *viz., Klebsiella* sp., *E. coli, Staphylococcus* sp. and *Salmonella* sp. were obtained from

Veterinary research and College, Mohanur road, Namakkal district, Tamil Nadu, India.

Antibacterial activity

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 pathogenic bacteria *viz., Klebsiella* sp., *E. coli, Staphylococcus* sp. and *Salmonella* sp. The solid medium was gently punctured with the help of cork borer to make a well. Finally the nanoparticle samples (50µg/ml) were added from the stock into each well and incubated for 24 h at 37 ± 2°C. After 24 h of incubation, the zone of inhibition was measured and expressed as millimeter in diameter.

Minimum Inhibitory Concentration (MIC)

Different concentrations (2.5, 5, 10, 15 and 20µg/ml) of chosen nanoparticles were prepared with Dimethyl sulphoxide (DMSO) and mixed with 450µl/ml of nutrient broth and 50µl of 24 h old bacterial inoculum and allowed to grow overnight at 37°C for 48 h. Nutrient broth alone served as negative control. Whole setup in triplicate was incubated at 37°C for 24 h. The MIC was the lowest concentration of the nanoparticles that did not permit any visible growth of bacteria during 24 h of incubation on the basis of turbidity. [9]

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°C for 24 h. MBC was regarded as the lowest concentration that prevents the growth of bacterial colony on this solid media. [9]

Time kill assay

The potential nanoparticle (ZrO_2) which showed maximum antibacterial activity against *Salmonella* sp. was also subjected for time kill assay. The inoculum of *Salmonella* sp. (50µl) at a concentration of 10^8 cells/ml was mixed with 50µl (contains 2.5µg/ml) of ZrO_2 nanoparticle 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; $MgSO_4.7H_2O$-0.20; $FeSo_4$. $7H_2O$-0.01; $MnSO_4.H_2O$-0.007 and H_2O-1000 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). [6]

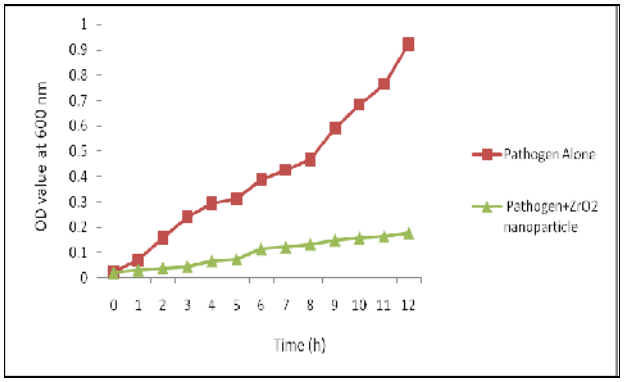

Fig. 1: Time kill assay of ZrO_2 nanoparticle against *Salmonella* sp.

RESULTS

The antibacterial activity of the metal oxide nanoparticles was evaluated and it represented in Table 2. It reveals that, the ZrO_2 nanoparticles showed maximum sensitivity (15 ± 0.44 mm dia) against *Salmonella* sp. and *E. coli* (12 ± 0.35 mm dia) respectively. The MIC and MBC results reveal that, the ZrO_2 nanoparticles showed maximum sensitivity at a concentration of 2.5µg/ml against *Salmonella* sp. and *E. coli* (5µg/ml). Moreover, the Al_2O_3 and MgO nanoparticles showed sensitivity against *Salmonella* sp. and *E. coli* at a concentration of (5µg/ml). All the nanoparticles showed sensitivity against all the tested pathogens (Table 3). The effect of ZrO_2 nanoparticles against *Salmonella* sp. was also performed with time kill assay. It reveals that, the growth of the pathogens was inhibited from the 1st h onwards (Fig. 1).

Table 1: Properties of nanoparticles

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

Table 2: Antibacterial activity of nanoparticles against poultry pathogens

Name of the nanoparticles	Zone of inhibition (mm dia)			
	Klebsiella sp.	*E. coli*	*Staphylococcus* sp.	*Salmonella* sp.
Al_2O_3	6 ± 0.39	10 ± 0.41	7 ± 0.26	8 ± 0.32
Fe_3O_4	8 ± 0.24	8 ± 0.32	6 ± 0.13	9 ± 0.20
Ceo_2	8 ± 0.15	9 ± 0.54	6 ± 0.47	10 ± 0.11
ZrO_2	9 ± 0.33	12 ± 0.35	8 ± 0.25	15 ± 0.44
MgO	6 ± 0.46	7 ± 0.12	10 ± 0.11	11 ± 0.51

Values are in 'mm' in diameter; mean ± SD

Table 3: MIC and MBC of nanoparticles against poultry pathogens

Name of the nanoparticles	Concentration (µg/ml)							
	Klebsiella sp.		*E. coli*		*Staphylococcus* sp.		*Salmonella* sp.	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Al_2O_3	20	20	5	5	15	15	20	20
Fe_3O_4	15	15	15	15	10	10	20	20
Ceo_2	15	15	10	10	10	10	10	10
ZrO_2	15	15	5	5	15	15	2.5	2.5
MgO	20	20	20	20	10	10	5	5

DISCUSSION

The poultry sector contributes a major role in the agriculture industry worldwide. The high mortality is mainly caused due to mismanagement, lack of fresh water, supplementary feed, predators and microbial diseases. [10-11] Of these, bacterial pathogens play an important role in causing respiratory disease in domestic poultry species. Disease outbreaks in agriculture as an important limiting factor in production and trade. [11] The present findings revealed that, the nanoparticles such as Al_2O_3, Fe_3O_4, CeO_2, ZrO_2, and MgO showed antibacterial activity against all the tested pathogens. Of these the ZrO_2 nanoparticle showed maximum inhibition against *Salmonella* sp. Moreover, the MIC and MBC suggested that, the ZrO_2 nanoparticle showed antibacterial activity against *Salmonella* sp at a concentration of 5µg/ml. The time kill assay revels that, the bacterial growth was inhibited from the 1st h up to 12th h. The possible mechanism of action is, the metal nanoparticles are carrying the positive charges and the microbes are having the negative charges which create the electromagnetic attraction between the nanoparticles and the microbes. When the attraction is made,

the microbes get oxidized and die instantly. [12] Generally, the nano materials release ions, which react with the thiol groups (-SH) of the proteins present on the bacterial cell surface which leads to cell lysis. [13] Earlier investigations reveal that, the TiO_2 and CdO nanoparticles showed antibacterial activity against *E. coli.* [12-13] Moreover, the silver nanoparticles showed antibacterial activity against *E. coli* and *S. aureus.* [14-16] Generally, the effects of the ZrO_2 nanoparticles are time dependent. The oxidative stress in the cell wall which increases the production of lactate dehydrogenase, which is an indicator of cell membrane damage. [17]

It is concluded from the present study that, the ZrO_2 nanoparticles could be used as an effective antibacterial agent for the management of poultry systems.

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