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In vitro antibacterial activity of the metal oxide nanoparticles against urinary tract infectious bacterial pathogens

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ABSTRACT

Objective: To investigate the antibacterial properties of the five metal oxide nanoparticles *viz*., Al_2O_3, Fe_2O_3, CeO_2, ZrO_2 and MgO against urinary tract infectious pathogens *viz*., *Pseudomonas* sp., *Enterobacter* sp., *Klebsiella* sp., *Escherichia coli* (*E. coli*), *Proteus morganii* (*P. morganii*) and *Staphylococcus aureus* (*S. aureus*). **Methods:** The antibacterial activity of the five different nanoparticles was assessed by well diffusion method. Different concentrations of the nanoparticles were analyzed by minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) techniques. Finally, the potential nanoparticle Al_2O_3 which showed maximum antibacterial sensitivity was also subjected for the time kill assay method. **Results:** Among the nanoparticles, Al_2O_3 nanoparticle showed maximum sensitivity (16.00±0.21) mm against *E. coli*. None of the nanoparticles showed activity against *Pseudomonas* sp. The MIC results also revealed that, the Al_2O_3 nanoparticle showed maximum inhibition at the concentration of 5 μg/mL against *E. coli*, followed by 10 μg/mL against *Klebsiella* sp. and *P. morganii*, respectively. Moreover, the time kill assay revealed that, the bacterial growth was maximum inhibited at the concentration of 5 μg/mL from the 2nd h. **Conclusions:** It can be concluded from the present findings that, the Al_2O_3 nanoparticle can be used as an alternative antibacterial agent for the urinary bacterial diseases after completing successful clinical trials.

1. Introduction

The infectious diseases remain one of the greatest challenges to global health. Urinary tract infection (UTI) is the second most common clinical disease and possesses a significant healthcare burden[1]. This infectious disease can alter the urinary system either structurally (complicated UTI) or functionally. About 80 to 90 percent of UTIs are caused by a single type of bacteria. *Escherichia coli* (*E. coli*) is the most common cause of uncomplicated urinary tracts (anatomically normal urinary tract). The diagnosis of UTI is very difficult for the elder people because of the asymptomatic bacteriuria[2]. So there is an urgent need to produce the new antibacterial agents from different sources. The terrestrial plant such as *Phylanthus amarus* and *Parquetina nigrescens* showed potential antibacterial activity against UTI pathogens[3]. Moreover, the marine resources such as mangroves, seaweeds, sponges and sea grasses already showed antibacterial[4-8], antifungal[6], and antiplasmodial[9-13] activities. However, most of the antibacterial agent entered into clinical practice, resistance was reported in at least one bacterial pathogen[14]. During the past decades, the nanoparticles are attracting a great deal in biological and pharmaceutical applications[15-18]. Moreover, the metal oxide nanoparticles have good antibacterial activity and antimicrobial formulations comprising nanoparticles could be used as an effective bactericidal agent[19-24]. Nevertheless, studies related with metal oxide nanoparticles against urinary tract infectious pathogens are too limited. Hence, the present study has been made an attempt to find out the potential nanoparticles against urinary tract infectious pathogens.

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2. 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.

Table 1
Properties of nanoparticles.

Formula	Molecular weight	Form	Particle size (nm) (transmission electron microscope)
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. Isolation of UTI bacterial pathogens

A total of 50 urine samples from 25 male and 25 female patients admitted in the hospitals as UTI problems were collected from different hospitals and laboratory localities along the coastal area of Thondi, Ramanathapuram District, Tamil Nadu, India (Lat. 9° 44′ N and Long. 79° 10′E) in a separate sterile wide mouth bottle. Before collecting a sample, the women were instructed to swab the vulvae and men to retract the foreskin and cleanse the glans penis. Midstream urine was collected in a sterile wide mouthed container. For the isolation of UTI bacterial strains, loop full of urine samples were streaked into the nutrient agar, Mac Conkey agar, blood agar and chocolate agar plates and incubated at (37±2) ℃ for 24 h. Next day individual colonies were selected and identified on the basis of morphological characteristics, gram staining and biochemical characters[25,26].

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. Triplicates plates were swabbed with the overnight culture (10^8 cells/mL) of pathogenic bacteria *viz*., *Pseudomonas* sp., *Enterobacter* sp., *Klebsiella* sp., *E. coli*, *Proteus morganii* (*P. morganii*) and *Staphylococcus aureus* (*S. aureus*). 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) ℃. After 24 h, the zone of inhibition was measured and expressed as millimeter in diameter.

2.3. Minimum inhibitory concentration (MIC)

About 500 μL of different concentrations (2.5, 5, 10, 15 and 20 μg) of chosen 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 alone served as negative control. 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[27].

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 prevented the growth of bacterial colony on this solid media[27].

2.5. Time kill assay

The potential nanoparticle (Al_2O_3) which showed maximum antibacterial activity against *E. coli* was also subjected for time kill assay. The inoculum of *E. coli* (50 μL) at a concentration of (10^8 cells/mL) was mixed with 50 μL (5 μg concentration of Al_2O_3) 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 (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 (Shimadzu, Japan)[6].

3. Results

Out of the 60 midstream urine samples, 45 bacterial strains were isolated and it was identified by using biochemical tests (Table 2). Of these, *Pseudomonas* sp. is the predominant one (38%), followed by *Enterobacter* sp. (22%), *Klebsiella* sp. (18%), *E. coli* (11%), *P. morganii* (7%) and *S. aureus* (4%) (Figure 1). The zone of inhibition of the selected nanoparticles against UTI pathogens was represented in Table 3. It revealed that, the Al_2O_3 nanoparticle showed maximum sensitivity (16.00±0.21) mm against *E. coli* followed by (12.00 ±0.69) mm and (12.00±0.72) mm against *Klebsiella* sp. and *P. morganii*, respectively. The MgO nanoparticle showed maximum sensitivity (13.00±0.64) mm against *E. coli* and showed minimum sensivity (9.00±0.29) mm against *Klebsiella* sp. and *P. morgonii* (6.00±0.61) mm. The Fe_2O_3, Ceo_2 and ZrO_2 showed maximum sensitivity (10.00±0.35) mm against *E. coli*, *P. morganii* (11.00±0.51) mm and *Enterobacter* sp. (12.00± 0.26) mm, respectively. None of the nanoparticles showed sensitivity against *Pseudomonas* sp. The MIC and MBC revealed that, the Al_2O_3 nanoparticle showed sensitivity at the concentration of 5 μg/mL against *E. coli* and *Klebsiella* sp. and *P. morganii* 10 μg/mL, respectively. Moreover, MgO and ZrO_2 nanoparticles showed maximum sensitivity against *E. coli* at the concentration of 10 μg/mL, respectively (Table 4). The effect of Al_2O_3 nanoparticle against *E. coli* was performed with time kill assay. It revealed that, the growth

Table 2
Biochemical characterization of isolated bacteria from UTI patients.

Characteristics		*Pseudomonas* sp.	*E. coli*	*Klebsiella* sp.	*Enterobacter* sp.	*P. morganii*	*S. aureus*
Gram staining		−	−	−	−	−	+
TSI	Slant	K	A	A	A	K	A
	Butt	K	A	A	A	A	A
	GAS	−	G	G	G	G	−
	H_2S	−	−	−	−	−	−
Mannitol		Acid	Acid	Acid	Acid	−	−
Motility		Motile	Motile	Non−motile	Motile	Motile	Motile
Indole test		−	−	−	−	+	−
Methyl red test		+	+	−	−	+	−
V.P. test		−	−	+	+	−	−
Citrate test		−	−	+	−	−	−
Urease test		+	−	+	−	+	−
Oxidase test		+	−	−	−	−	−
Catalase test		+	−	−	−	+	+

+: positive; −: negative; K: alkaline; A: acid; G: gas.

Table 3
Antibacterial activity of chosen 5 nanoparticles against UTI pathogens (mean±SD) (mm) .

Name of the nanoparticles	*Pseudomonas* sp. (*n*=17)	*Enterobacter* sp. (*n*=10)	*Klebsiella* sp. (*n*=8)	*E. coli* (*n*=5)	*P. morganii* (*n*=3)	*S. aureus* (*n*=2)
Al_2O_3	−	−	12.00±0.69	16.00±0.21	12.00±0.72	9.00±0.61
Fe_2O_3	−	7.00±0.23	−	10.00±0.35	−	7.00±0.67
Ceo_2	−	6.00±0.12	6.00±0.74	9.00±0.39	11.00±0.51	8.00±0.24
ZrO_2	−	12.00±0.26	7.00±0.45	10.00±0.59	−	−
MgO	−	−	9.00±0.29	13.00±0.64	6.00±0.61	−

n: number of isolates; −: no sensitivity.

Table 4
MIC and MBC (μg/mL) of chosen 5 nanoparticles against UTI pathogens.

Name of the nanoparticles	*Pseudomonas* sp.		*Enterobacter* sp.		*Klebsiella* sp.		*E. coli*		*P. morganii*		*S. aureus*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Al_2O_3	−	−	−	−	10	10	5	5	10	20	−	−
Fe_2O_3	−	−	−	−	−	−	15	20	−	−	−	−
Ceo_2	−	−	−	−	−	−	20	20	20	20	−	−
ZrO_2	−	−	20	20	−	−	10	20	−	−	−	−
MgO	−	−	−	−	15	15	10	10	−	−	−	−

−: no activity.

of the pathogen was inhibited from the 2nd h when compared with control (Figure 2).

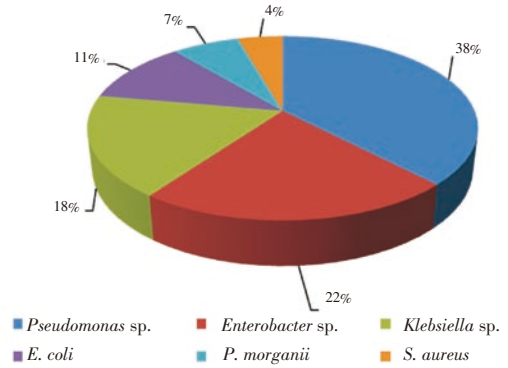

Figure 1. Percentage occurrence and distribution of bacterial pathogens in UTIs among the patients (*n*=45).

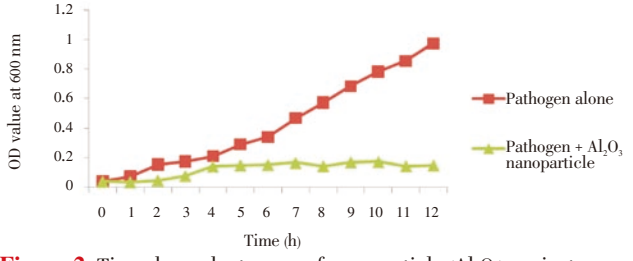

Figure 2. Time dependent assay of nanoparticle (Al_2O_3) against chosen UTI pathogen *E. coli*.

4. Discussion

Nanotechnology is an emerging field and it has been applied in science and technology for the purpose of manufacturing new materials at the nanoscale level[28]. In

the present scenario, the nanoparticles are being emerged as novel antimicrobial agents with unique biological, chemical and physical properties[29,30]. Moreover, the advantages of the metal nanoparticles are less toxicity, heat resistance and suitable for biological application[19,31,32]. All the nanoparticles showed sensitivity against all the pathogens except *Pseudomonas* sp. Of the selected nanoparticles, the Al_2O_3 nanoparticle showed maximum sensitivity against *E. coli*. The MIC result reveals that, the Al_2O_3 nanoparticle showed maximum sensitivity at a concentration of 5 μg/mL against *E. coli*, 10 μg/mL against *Klebsiella* sp. and *P. morganii*, respectively and this activity might be due to the size, surface morphology, particle morphology and structure of the nanoparticles[33] and the possible mechanism for the cell lyses is, the nanoparticles release ions which react with the thiol (-SH) groups of protein present in the cell wall, inactivate the protein and decrease the cell permeability which leads to cellular death[34]. Earlier investigations reveal that, the silver and gold nanoparticles showed various biological properties[35-54]. Moreover, the TiO_2 and CdO, Fe_3O_4 and ZnO nanoparticles showed antibacterial activity against *E. coli*[34,55-57]. Generally, the toxic effects of the Al_2O_3 nanoparticles are time dependent. This oxidative stress in the cell wall might increase the production of lactate dehydrogenase, which is an indicator of cell membrane damage[58]. It is concluded from the present findings that, the Al_2O_3 nanoparticle could be used as an alternative antibacterial agent for the urinary bacterial diseases after completing successful clinical trials.

Conflict of interest statement

We declare that we have no conflict of interest.

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