Appl Nanosci (2011) 1:231–236
DOI 10.1007/s13204-011-0031-2

ORIGINAL ARTICLE

Antibacterial potential of silver nanoparticles against isolated urinary tract infectious bacterial pathogens

Samuel Jacob Inbaneson · Sundaram Ravikumar · Nachiappan Manikandan

Received: 14 July 2011 / Accepted: 8 September 2011 / Published online: 21 September 2011

Abstract The silver nanoparticles were synthesized by chemical reduction method and the nanoparticles were characterized using ultraviolet–visible (UV–Vis) absorption spectroscopy and X-ray diffraction (XRD) studies. The synthesized silver nanoparticles were investigated to evaluate the antibacterial activity against urinary tract infectious (UTIs) bacterial pathogens. Thirty-two bacteria were isolated from mid urine samples of 25 male and 25 female patients from Thondi, Ramanathapuram District, Tamil Nadu, India and identified by conventional methods. *Escherichia coli* was predominant (47%) followed by *Pseudomonas aeruginosa* (22%), *Klebsiella pneumoniae* (19%), *Enterobacter* sp. (6%), *Proteus morganii* (3%) and *Staphylococcus aureus* (3%). The antibacterial activity of silver nanoparticles was evaluated by disc diffusion assay. *P. aeruginosa* showed maximum sensitivity (11 ± 0.58 mm) followed by *Enterobacter* sp. (8 ± 0.49 mm) at a concentration of 20 μg disc^{-1} and the sensitivity was highly comparable with the positive control kanamycin and tetracycline. *K. pneumoniae, E. coli, P. morganii* and *S. aureus* showed no sensitivity against all the tested concentrations of silver nanoparticles. The results provided evidence that, the silver nanoparticles might indeed be the potential sources to treat urinary tract infections caused by *P. aeruginosa* and *Enterobacter* sp.

S. Jacob Inbaneson · S. Ravikumar (✉) · N. Manikandan
Department of Oceanography and Coastal Area Studies, School of Marine Sciences, Alagappa University, Thondi Campus, Ramanathapuram District, Thondi 623 409, Tamil Nadu, India
e-mail: ravibiotech201321@gmail.com

Keywords Antibacterial sensitivity · Disc diffusion assay · Silver nanoparticles · UTI

Introduction

Patients with non-infectious diseases who have to stay in hospital for long period such as heart disease, cancer and other chronic diseases have high risk to get nosocomial infections (Nichollas et al. 1975; Asefzadeh 2005; Saonuam et al. 2008). It has been reported that, 10% hospital patients acquire this infection while staying in hospital (Asefzadeh 2005). The common pathogenic bacteria which include *Escherichia coli, Klebsiella pneumoniae, Haemophilus influenza, Streptococcus pneumoniae* and *Proteus vulgaris* are the major causative agents of nosocomial infections (Saonuam et al. 2008; Nichollas et al. 1975). Generally, nosocomial infections develop in respiratory tract (Nichollas et al. 1975) and urinary tract (Saonuam et al. 2008). Treatment with available antibiotics leads to resistance among pathogenic bacteria which leads to greater threat.

In the recent years, silver nanoparticles have been of great interest and widely investigated because of their applications in many areas such as biotechnology (Gao and Xu 2009), microelectronics (Feldheim and Keating 1998), optics (Taton et al. 2000; Zhang et al. 2005) and biomedicine (Caruthers et al. 2007; Farokhzad and Langer 2006; Sahoo et al. 2007; Kumar et al. 2005). It is well known that silver is a very effective antibacterial agent and also possesses a strong activity against bacteria, fungi and viruses, although the mechanism and the manner of action are still not well known (Sharma et al. 2009). Several studies reported that, the silver and silver nanoparticles may attach to the surface of the cell membrane disturbing permeability

and respiration functions of the cell (Kvitek et al. 2008). It is also possible that silver and silver nanoparticles not only interact with the surface of membrane, but can also penetrate inside the bacteria (Morones et al. 2005). Many researchers also proposed that Ag^+ ions interact with the thiol groups in bacteria proteins, affecting the replication of DNA (Marini et al. 2007). It has also been reported that Ag^+ ions uncouple the respiratory chain from oxidative phosphorylation or collapse the proton-motive force across the cytoplasmic membrane (Holt and Bard 2005). The present study was made an attempt to find out the antibacterial activity of silver nanoparticles against the 6 urinary tract infectious (UTIs) bacterial isolates.

Materials and methods

Preparation of silver nanoparticles

The silver nanoparticles were prepared by using chemical reduction method (Fang et al. 2005). 50 ml of 1×10^{-3} M $AgNO_3$ was heated to boiling and 5 ml of 1% tri-sodium citrate was added drop by drop. The solution was mixed vigorously and heated until colour change was evident (pale brown) and stirred until cooled to room temperature. All solutions of reacting materials were prepared in double distilled water. The aqueous solution was air dried up to 3 days so as to enable to obtain powdered form of sliver nanoparticles.

Characterization of synthesized silver nanoparticles

About 1 ml of solution (diluted with 1:20 v/v Milli Q water) was monitored in UV–VIS spectrophotometer (between 300- and 700-nm ranges with 5-nm intervals) with different time intervals (15 min, 30 min, 4 h, 6 h and 8 h). After 8 h of incubation, the solution was centrifuged with 12,000 rpm for 20 min and their pellets were re-dispersed in sterile distilled water. The centrifugation and re-dispersion was repeated three times to ensure the complete separation of nanoparticles. The dried mixture of silver nanoparticles was further analysed with X-ray diffractometer (PAN analytical BV, The Netherlands) operated at a voltage of 40 kV and a current of 30 mA with Cu Kα radiation in a θ–2θ configuration.

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 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. Mid stream 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, MacConkey agar, Blood agar and Chocolate agar plates and incubated at 37 ± 2°C for 24 h. Next day individual colonies were selected and identified on the basis of morphological characteristics, gram staining, and biochemical characters (Thomas 1995; Chess Brough 2000).

Antibacterial sensitivity assay

Disc diffusion assay was performed to determine the antibacterial activity in triplicates (Kim et al. 1995). Overnight culture of UTI pathogens were swabbed over the surface of sterile Mueller–Hinton agar plates using sterile cotton swabs. Discs impregnated with different concentrations of silver nanoparticles (5, 10, 15 and 20 µg $disc^{-1}$) were applied on the solid agar medium by pressing slightly and incubated at 37 ± 2°C for 24 h in triplicates. After incubation, the zone of inhibition was measured and expressed as zone of sensitivity millimetre in diameter.

Results

Out of the 50 midstream urine samples, 32 bacterial isolates were recovered and the biochemical tests revealed that, these isolates belong to 6 species (Table 1). Of these *E. coli* is the predominant one (47%); *Pseudomonas aeruginosa* (22%), *K. pneumonia* (19%), *Enterobacter* sp. (6%), *Proteus morganii* (3%) and *Staphylococcus aureus* (3%) (Fig. 1). The colour intensity of the synthesized silver nanoparticles was increased with increased time duration, and the maximum intensity was observed with 420-nm wavelength (Fig. 2). Further, the results of the X-ray diffraction (XRD) analysis showed 2θ intense values with various degrees (36.20°, 54.56°, 63.11° and 68.72°) and these results correspond to (111), (200), (220) and (311) Bragg's reflection-based (Sathishkumar et al. 2009) silver nanoparticles (Fig. 3). Antibacterial activity of silver nanoparticles is represented in Table 2. The silver nanoparticles showed maximum sensitivity (11 ± 0.58 mm) against *P. aeruginosa* followed by *Enterobacter* sp. (8 ± 0.49 mm) at 20 µg $disc^{-1}$ concentration. The *P. aeruginosa* and *Enterobacter* sp. showed sensitivity against all the tested concentrations (5, 10, 15 and 20 µg $disc^{-1}$). *K. pneumoniae, E. coli, P. morganii* and *S. aureus* showed no sensitivity against all the tested concentrations of silver nanoparticles.

Table 1 Biochemical characterization of isolated bacteria from UTI patients

Characteristics	*Pseudomonas aeruginosa*	*Escherichia coli*	*Klebsiella pneumoniae*	*Enterobacter* sp.	*Proteus morganii*	*Staphylococcus 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	A	A	A	A	−	−
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

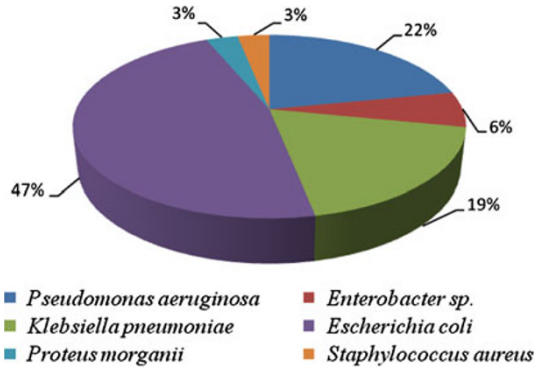

Fig. 1 Percentage occurrence and distribution of bacterial pathogens in UTIs among the patients ($n = 32$)

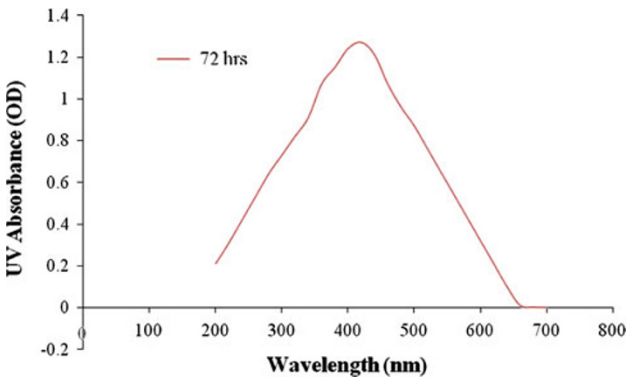

Fig. 2 UV–VIS absorption spectra of the synthesized silver nanoparticles

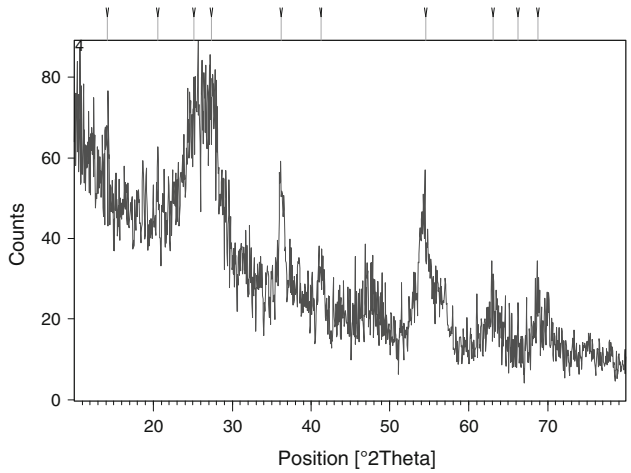

Fig. 3 XRD analysis of the synthesized silver nanoparticles

Discussion

Patients with non-infectious disease who have stay in hospital have high risk to acquire nosocomial infection. It has been reported that, 10% hospital patients acquire this infection while staying in hospital (Asefzadeh 2005). Martinez and Baquero (2002) have reported from their research that, nosocomial infectious bacteria exhibited least susceptibility to antibiotics and some of these bacteria out rightly developed multidrug resistance to these antibiotics. Recently from a 3-year follow-up study in USA, Dowzicky and Park (2008) reported that, UTI bacterial

Table 2 Antimicrobial activity of silver nanoparticles against UTIs pathogens

Test organism	Zone of inhibition (mm)						
	Concentration of silver nanoparticles (μg disc^{-1})				Positive control (20 μg disc^{-1})		Negative control
	5	10	15	20	Kanamycin	Tetracycline	Sterile distilled water
Pseudomonas aeruginosa ($n = 15$)	7.0 ± 0.49	8.0 ± 0.61	9.0 ± 0.65	11 ± 0.58	9 ± 0.69	7 ± 0.75	–
Enterobacter sp. ($n = 7$)	6.0 ± 0.37	6.0 ± 0.52	7.0 ± 0.58	8 ± 0.49	–	8 ± 0.62	–
Klebsiella pneumoniae ($n = 6$)	–	–	–	–	–	–	–
Escherichia coli ($n = 2$)	–	–	–	–	–	–	–
Proteus morganii ($n = 1$)	–	–	–	–	–	7 ± 0.54	–
Staphylococcus aureus ($n = 1$)	–	–	–	–	–	–	–

n Number of isolates, – no sensitivity, ± standard deviation

pathogens have exhibited decreased susceptibility rates to tigecycline over the years. Antibacterial property of silver nanoparticles would be the alternative to overcome the resistance problem.

In our present study, *E. coli* (47%) was found predominant and a similar result was reported by Ravikumar et al. (2010). Despite the availability of antibiotics, UTIs remain the most common bacterial infections in human populations (Phillippon et al. 1989). Urinary tract infections occur more frequently in females (63%) than in males (Schaeffer et al. 2001). Silver is known for its antimicrobial properties and has been used for years in the medical field for antimicrobial applications and even has shown to prevent HIV binding to host cells (Nino-Martinez et al. 2008; Alt et al. 2004; Lee et al. 2007). Additionally, silver has been used in water and air filtration to eliminate microorganisms (Chou et al. 2005).

Previous reports stated that, the silver nanoparticles have potential antibacterial property against *E. coli, S. aureus, Staphylococcus epidermis, Leuconostoc mesenteroides, Bacillus subtilis, Klebsiella mobilis* and *K. pneumonia* (Benn and Westerhoff 2008; Chen and Chiang 2008; Falletta et al. 2008; Hernandez-Sierra et al. 2008; Ingle et al. 2008; Jung et al. 2009; Kim 2007; Kim et al. 2007, 2009; Kvitek et al. 2008; Raffi et al. 2008; Ruparelia et al. 2008; Smetana et al. 2008; Sondi and Salopek-Sondi 2004; Vertelov et al. 2008; Yang et al. 2009; Yoon et al. 2008a, b). It has been shown that silver nanoparticles prepared with a variety of synthetic methods have effective antimicrobial activity (Lok et al. 2006; Baker et al. 2005; Aymonier et al. 2002; Melaiye et al. 2005; Sondi and Salopek-Sondi 2004; Kim et al. 2008; Lee et al. 2008; Alt et al. 2004). In the present study, the silver nanoparticles were prepared by chemical reduction method to assess the antibacterial activity. The results of the UV–VIS absorption showed increasing colour intensity with increased time intervals and this might be due to the production of the silver nanoparticles and the formation of the brownish yellow colour might be due to the excitation of the surface plasmon vibration of the synthesized silver nanoparticles (Krishnaraj et al. 2010). The result of the XRD pattern indicates the presence of sharp bands of Bragg peaks and this might be due to the stabilizing of the synthesized nanoparticles by chemical reducing agents and thus confirming the crystallization of the bioorganic phase occurs on the surface of the silver nanoparticles (Sathishkumar et al. 2009).

Pseudomonas aeruginosa showed maximum sensitivity (11 ± 0.58 mm) at 20 μg disc^{-1} followed by *Enterobacter* sp. (8 ± 0.49 mm). The predominant isolate *E. coli* showed no sensitivity, it might be due to the multidrug resistance nature of *E. coli* developed by point mutations. The mechanism of action may be due to the silver and silver nanoparticles attach to the surface of the cell membrane and disturbing permeability and respiration functions of the cell, moreover, due to the uptake of free silver ions followed by disruption of ATP production and DNA replication. Smaller silver and silver nanoparticles having the large surface area available for interaction would give more bactericidal effect than the larger silver and silver nanoparticles (Kvitek et al. 2008). Normally silver nanoparticles interactions with bacteria are dependent on the size and shape of the nanoparticles (Panacek et al. 2006; Morones et al. 2005; Pal et al. 2007). It is concluded from the present study that, the silver nanoparticles could be used as an effective antibacterial agent for the management of urinary tract infections caused by *P. aeruginosa* and *Enterobacter* sp. after successful completion of in vivo studies and clinical trials.

Acknowledgments The authors are thankful to the authorities of Alagappa University for providing required facilities and also to Indian Council of Medical Research, New Delhi for financial assistance.

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