Antibacterial effects of Tungsten nanoparticles on the *Escherichia coli* strains isolated from catheterized urinary tract infection (UTI) cases and *Staphylococcus aureus*

Muhammad Ali Syed[1], Umair Manzoor[2], Ismat Shah[3], S. Habib Ali Bukhari[1]

[1]*Department of Biosciences, COMSATS Institute of Information Technology Park Road, Chak Shahzad, Islamabad, Pakistan;*
[2]*Department of Physics, COMSATS Institute of Information Technology Park Road, Chak Shahzad, Islamabad, Pakistan;*
[3]*Department of Physics, University of Delaware, USA*

—— SUMMARY ——

A significant proportion of all incidents of nosocomial infections in acute-care hospitals is due to contaminated catheters. Alternative strategies e.g. antibiotics as well as surface modifications have been devised in an attempt to reduce the incidence of catheter-associated urinary tract infections (CAUTI), but most have proven unsuccessful. Therefore, the race to identify such substances which can combat pathogenic bacteria is ongoing in order to improve the quality of health care. Novel technologies such as the potential use of antiseptic or antimicrobial coatings on catheters hold promise for reducing these infections in the fight against antimicrobial resistance. In this study, the bactericidal activity of newly synthesized tungsten-nanoparticles was tested on clinical multiple drug resistant *Escherichia coli* isolates from UTI patients with indwelling catheters and *Staphylococcus aureus* reference strain. The results suggest that the particles tested in this study certainly mediate the inhibition of bacterial growth. We believe that the fabrication of W-NPs on catheters could possibly prevent them from being contaminated by pathogens and hence provide continuous protection of the site. This study is the first of its type testing the antibacterial effects of W-NPs on clinical bacterial isolate from catheterized human UTI case.

KEY WORDS: Antimicrobial, Catheters, Urology/urological engineering, Nanoparticles

INTRODUCTION

Urinary tract infections (UTI) are the leading cause of nosocomial infections, with an incidence of about 40% of all nosocomial infection cases. Out of these 40%, a large proportion, *i.e.* 80%, involve catheter-associated urinary tract infections (CAUTI) (Stamm 1991). Since urinary stents or catheters are routinely used in urological practice and despite advances in design and materials used, UTIs remain one of the major complications due to the contamination of such indwelling devices (Dickinson and Bisno 1989).

The complications of long-term catheter-associated bacteriuria generally fall into two categories. The first includes symptomatic UTIs observed during short-term catheterization, i.e. fever, bacteremia, and acute pyelonephritis (Warren *et al.* 1987, Muder *et al.* 1992). Some of these episodes may lead to serious consequences and even death. The second category is often associated with long-term catheterization which may lead to obstruction, urinary tract stones (Mobley and Warren 1987), local periurinary infections, chronic pyelonephritis and with prolonged use of a catheter, bladder cancer (Warren *et al.,* 1994, Locke *et al.,* 1985).

The problem is further complicated due to the formation of bacterial biofilms on the surface of

—————
Corresponding author
Dr. S. Habib Ali Bukhari
Assistant Professor
Department of Biosciences
Comsats Institute of Information Technology
Park Road, Islamabad - Pakistan
E-mail: habib@comsats.edu.pk

catheters (Gilbert *et al.,* 1990). Biofilms in one way or other are involved in up to 60% of human infections (Spoering and Lewis 2001). Two main reasons have been implicated for the resilient nature of biofilm bacteria to common antibiotic therapy. Firstly bacterial outer layers coated with certain polysaccharides act as a barrier and protect the infecting bacterial cells from humoral and cellular host defense systems as well as from the action of antibiotics. Secondly the biofilm bacteria are either extremely slow-growing or not growing at all. Due to the stated reasons the concentrations of antibiotics needed to kill bacteria in the sessile phase are often much higher than those required for bacteria in the planktonic phase (Ishada *et al.,* 1998). In addition, the complex and diverse environment of biofilm may potentially provide an opportunity to allow the movement of drug resistance cassette(s) in association with mobile elements such as integrons from one organism to another. Furthermore, the possession of various virulence factors and toxins by biofilm associated bacteria such as *E. coli* has a proven role in inter species biofilm formation (Muder *et al.,* 1992).

The recent advances in research on nanoparticles and the use of such particles in various biomedical applications (Manzoor and Kim 2009, Manzoor and Kim 2006, Sun *et al.,* 2005, Feng *et al.,* 2000) has encouraged finding new solutions to old problems such as developing new antibacterial coating material for indwelling devices. In this study the antibacterial activity of newly synthesized tungsten-nanoparticles (W-NPs) was tested on multiple drug resistant clinical Gram negative *Escherichia coli* (*E. coli*) strains isolated from UTI patients with indwelling catheters and Gram positive *Staphylococcus aureus* (*S. aureus*) reference strain (ATCC 6538).

EXPERIMENTAL PART

Materials, Bacterial strains and Culture conditions

W-NPs were suspended into 2.5 mM polyvinyl pyrrolidinone (PVP). PVP solution used during the study was of analytical grade and purchased from MP Biomedical, Inc France. The nanoparticles in the PVP solution were sonicated for 10 minutes to get them dispersed. The *E. coli* iso-

lates were obtained from hospitalized patients with indwelling catheters suffering from UTIs and were subcultured on MacConckey's agar for confirmation and isolation of colonies. Further confirmation was made by performing Gram staining and various biochemical tests including API 20 test kits. Bacteria were stored in nutrient broth containing 20% glycerol at -20°C for future use. The *S. aureus* reference strain ATCC 6538 was used as a representative member of Gram positive bacteria.

Preparation of Tungsten Nanoparticles

W NPs were deposited on hollow copper (Cu) substrate by sputtering. The distance between the target and substrate was approximately 12 cm and the temperature of the Cu substrate was maintained at liquid nitrogen temperature by a continuous flow of liquid nitrogen into the hollow Cu substrate throughout the deposition process. The W target (99.99%, diameter 150 mm) was sputtered with plasma of argon (99.99%) and the base pressure in the plasma chamber was 400 mTorr during the sputtering process. Before sputtering, the chamber was evacuated to less than
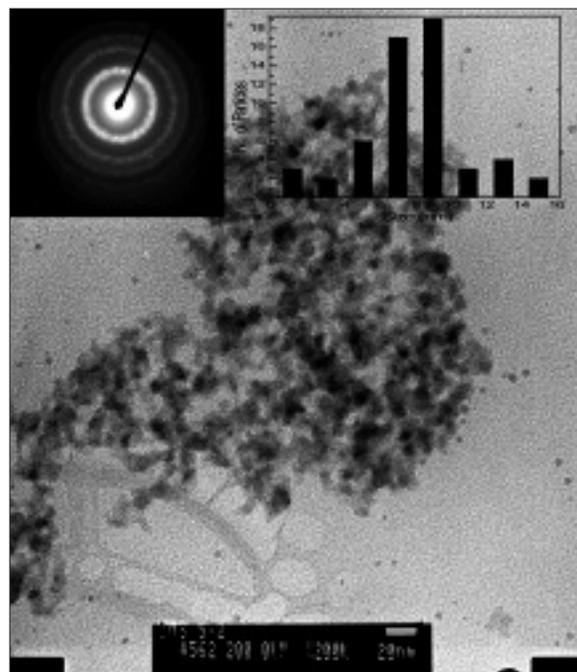

FIGURE 1 - *TEM image of discrete W-NPs with average diameter of 8.1 ± 2.8nm. Inset shows diffraction patterns of W-NPs suggesting that these particles are crystalline in nature.*

1* 10^{-6} mTorr, and argon gas was introduced to maintain the desired vacuum. Black color W NPs (expected purity same as the W target i.e. 99.99%) were deposited on liquid nitrogen cooled Cu substrate which was carefully removed to do further analysis.

Transmission electron microscopy (TEM) of pure W-NPs (Figure 1) was done with a Jeol JEM-3010 Transmission Electron Microscope. TEM samples were prepared by placing a drop of colloidal W-NPs solution in ethanol on TEM grid and drying at 40°C. TEM image clearly suggested that the particles are discrete and average particle size of W-NPs is 8.1±2.8 nm (at least 40 particles were averaged, measured from TEM image). Diffraction patterns (inset figure) clearly suggested that particles are highly crystalline in nature. XRD results (not shown) also supported the diffraction patterns and only tungsten metal peaks appeared in XRD.

Antibiotic susceptibility

Antimicrobial susceptibility testing was performed on *E. coli* isolates by the disk (Neo-Sensitabs; Oxoid Ltd) diffusion method using a panel of antimicrobial agents. Minimum inhibitory concentration (MIC) of two commonly prescribed antibiotics for the UTIs treatment i.e. cefotaxime sodium and sulfamethoxazole was determined by agar dilution method on Mueller Hinton agar (M H agar).

Assays for antibacterial activity of W-NPs against bacteria

W-NPs were tested for their inhibitory action against *E. coli* isolated from catheterized UTI patients and *S. aureus* reference strain. The following three methods were used for antimicrobial activity determination.

Minimum inhibitory concentration (MIC) determination

Colloidal W-NPs (0.054%, average diameter: 8.1 ± 2.8nm) in solution (stock: 5.0 mg/ml) containing poly-(*N*-vinyl-2-pyrrolidone) (PVP) as a stabilizer (Zordow *et al.,* 2009) were prepared. For the antimicrobial activity measurement, bacterial cultures were routinely incubated at 37°C in Mueller Hinton broth (M H broth) (Oxoid limited). The cells were harvested from log phase culture (OD600nm=0.6) by centrifugation and sub-

sequently washed and suspended into sterile PBS to achieve turbidity equivalent to 0.5 MacFarland (~10^8 CFU/ml). MIC was performed through tube dilution method in tubes containing 1 ml of M H broth.

Different volumes of W-NPs from stock (5mg/ml) suspension were added into Mueller Hinton broth tubes containing 10^8 CFU/ml of bacteria and incubated at 37°C. To establish the antimicrobial activity of W-NPs on the bacterial growth, the minimum inhibitory concentration of W-NPs for *E. coli* & *S. aureus* was determined by visual testing of the bacterial culture solution containing different concentrations of nanoparticles after 24 hours.

Direct spotting on the agar surface

Overnight grown bacterial culture was diluted with PBS to 0.5 McFarland (~10^8 CFU/ml) and spread on the surface M H agar. Different volumes (10, 15, 20 and 25μl) of W-NP suspension (5.0 mg/ml) were spotted on the agar surface. A negative control plate was spotted with the same volumes of PVP solution alone. The plates were then incubated at 37°C for 24 h.

Cup diffusion method

Wells of a diameter of 1.5 mm were dug in the centre of the M H agar plate. These wells were filled with 20 l of W-NPs suspension and incubated at 37°C for 24 h.

Statistical analysis

The experiments were repeated number of times for confirmation. Appropriate data analysis was done and all values of MIC and the zones of inhibition presented in this paper are average values of at least 3 readings with standard deviation.

RESULTS AND DISCUSSION

The *E. coli* isolates used in this study were found to be resistant to both antibiotics *i.e.* Sulphamethoxazole and Cefotaxime. MIC values of antibiotics sulphamethoxazole and cefotaxime sodium against *E. coli* isolates were investigated. The rate of MIC values showed alterations from 2000 g to 10000 g and 800 g to 7000 for sulphamethoxazole and cefotaxime sodium respectively *i.e.* showed variations in terms of resistance.
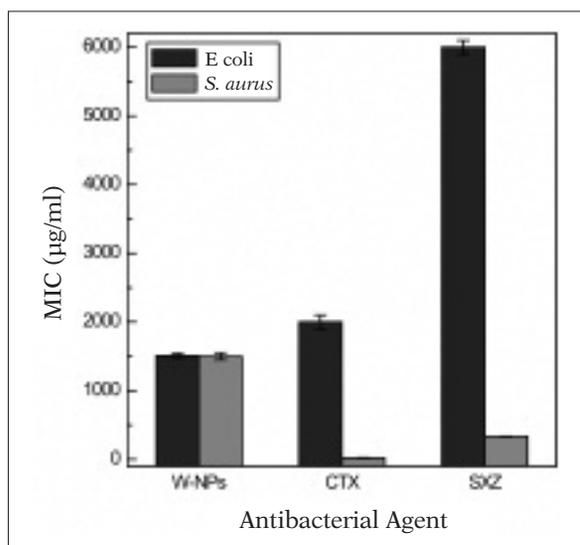
FIGURE 2 - *Comparison of MIC values (µg/ml) of W-NPs, Cefotaxime sodium (CTX), Sulfamethoxazole (SXZ) against E. coli and S. aureus using tube dilution method. W-NPs show similar MIC values against both E coli and S aureus strains.*

Moreover, these isolates also possessed genes to encode various virulence factors such as adhesins *i.e. ag*43, *pap, sfa* and *pic* and toxin *i.e.* hemolysin, in different combinations (Data not shown).

After determining the MIC values, suitable concentration was selected for all isolates in order to do a proper comparison. The *E. coli* isolates found to be resistant to two above mentioned common-

ly prescribed and the MIC values of W-NPs stabilized with PVP were determined and compared with them. The antibacterial activity of cefotaxime sodium and sulphamethaxazole against *E. coli & S. aureus,* when compared with that of W-NPs, showed less average value of MIC. PVP alone did not show any antibacterial activity (results not shown) as expected (Bechert *et al.,* 1999, Bechert *et al.,* 2000). However, the MIC values of these drugs were found to be comparatively less against *S. aureus* compared to what has been observed for *E coli*. MIC values of W-NPs did not vary much having values approximately 1500 µg/ml compared to *E coli* ranging from approximately 2000 to 6000 µg/ml for cefotaxime sodium and sulfamethoxazole respectively (Figure 2).

Determination of antibacterial activity of W-NPs through cup diffusion and direct spotting on the agar surface methods clearly suggested growth inhibition (Table 1).

The development of materials with antibacterial activity has long been the goal of medical science. It is generally believed that materials used in making indwelling devices such as catheters can be of significant importance in controlling UTIs especially among elderly. Since nosocomial pathogens are exposed to fluctuating antibiotic pressure with episodes of varying concentrations of antibiotic concentrations, e.g. due to pharmacokinetics of the antibiotics or biofilm formation, antibiotic resistance can develop.

TABLE 1 - *Determination of antimicrobial effects of W-NPs on different clinical drug resistant isolates of E. coli and on ATCC 6538 S. aureus (average of 4 experiments) by direct spotting on agar surface and cup diffusion method. Inhibition of bacterial growth is measured as diameter of clear zones around the dug applied on the agar plates.*

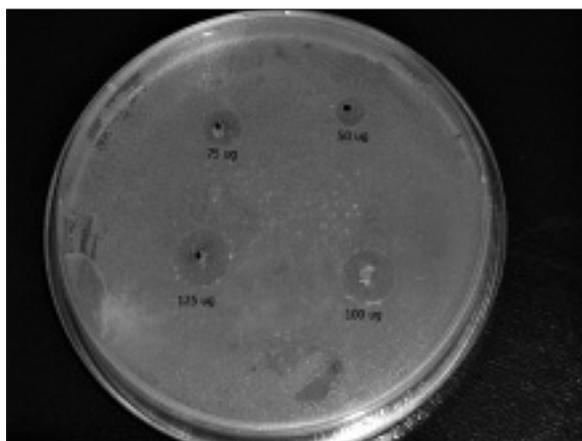|	E. coli		S. aureus	
Isolate Number	Direct spotting method	Cup Diffusion	Direct spotting method	Cup Diffusion
	Zone of Inhibition Diameter (mm)	Ring of Inhibition Diameter (mm)	Zone of Inhibition Diameter (mm)	Ring of Inhibition Diameter (mm)
1	3.4±0.1	2.5±0.08	3.9±0.15	1.9±0.12
2	4.1±0.15	2.0±0.12	4.5±1	2±0.2
3	4.0±0.06	2.0±0.16	3.7±0.29	2.1±0.15
4	4.5±0.1	1.9±0.21	3.5±0.21	2.6±0.05
Average	4.0±0.45	2.1±0.27	3.90±0.43	2.2±0.31

FIGURE 3 - *The W-NPs concentration dependent increase in diameter of zone of inhibition of E. coli cells grown on LB plate. PVP solution containing 50, 75, 100 and 125µg of W-NPs were spotted on the media plates.*

An effective method to prevent the catheter associated infections would be insertion of catheters under aseptic conditions, which is likely to be less feasible in hospital environment. Alternatively, catheters with antibacterial properties seem to be a valuable technique to provide extra protection against the catheter associated infections. Recent study has shown that incorporating effective antibacterial agents into biomaterials is feasible for the production of antibacterial materials, an approach that has been used by many dental materials researchers (Baquero *et al.*, 1998, Blanchemain *et al.*, 2005, Lok *et al.*, 2007).

The prerequisite for selecting any material for coating catheters depends on its effective binding to the surface of Gram negative and Gram positive bacteria and hence mediating their killing (Sondi and Sondi 2004). The possible mode of antimicrobial action by W-NPs could be the inhibition of the microbial processes on the cell surface and within the cell. Previous research involving Ag-NPs demonstrated that they attach the surface of cell membrane, causing the change of membrane permeability, dissipation of the ATP pool and proton motive force, and finally cell death (Lok *et al.*, 2006, Choi *et al.*, 2008, Stoimenov *et al.*, 2002, Cho *et al.*, 2005). However, due to their large size it would be difficult for these particles to cross the bacterial cell wall or cell membrane barrier. The particles of size in the range of 1–10nm could enter the cell based on indirect microscopic evidences (Choi *et al.*, 2008)

and the particles used in this study fall in the same range and possible inhibition by the W-NPs might be attributed to the accumulation of intracellular ROS as proposed for Ag-NPs in a recent report (Choi *et al.*, 2008).

The nanoparticles used in this study are small enough to penetrate the bacterial cell wall (Jones *et al.*, 2008). In contrast to the Silver nanoparticles (Ag-NPs) the MIC values of the W-NPs have been found to be large due to their chemical innertness (Sondi and Sondi 2004, Morones 2005, Lok *et al.*, 2006, Choi *et al.*, 2008, Stoimenov *et al.*, 2002). The enhanced surface to volume ratio of the particles is responsible for the overall mechanism of interference with the cellular machinery whose exact mechanism remains unknown. Small nanoparticles (<10 nm) of the chemically inert compounds such as magnesium oxide and zinc oxide have shown to penetrate *E. coli* cells (Lee *et al.*, 2006) without leading to as much disruption of cell walls as nano-Fe. In this study, W-NPs were taken out of the sputtering chamber after synthesis and they were exposed to air for few days before the antibacterial activities were measured. The chemical interactions between the reactive surfaces of W-NPs and bacterial cells may be responsible for the disruption of cell membranes, and it is possible that it induced reductive decomposition of functional groups in the proteins and lipopolysaccharides of the outer membranes. Alternatively, W-NPs may have been oxidized, leading to oxidative damages via the Fenton reaction (Gromov *et al.*, 2005). The identification of exactly which of the physical and chemical properties of W-NPs are responsible for their antimicrobial activities remains to be established and further experiments are needed.

The nonspecific mode of action of nanoparticles against bacteria makes them ideal candidates as antimicrobial agents with less risk of development of bacterial resistance. The results have shown that the particles possess antibacterial properties against bacterial pathogens and fabrication of immobilized nanoparticles on devices such as catheters could possibly prevent the formation of biofilms. One of the main concerns regarding the use of nanoparticles is their toxicity to humans. W-NPs have been reported to be less toxic as compared to silver and many others (Morones *et al.*, 2005 and Lok *et al.*, 2006, Lok *et*

al., 2007). This first report described the idea of using W-NPs as antibacterial agents as an alternative to those with proven toxicity to humans.

REFERENCES

BAQUERO F., NEGRI M. C., MOROSINI M. I., BLAZQUEZ J. (1998). Antibiotics elective environments. *Clin. Inf. Dis.* **27**, 5-11.

BECHERT T., BOSWALD M., LUGAUER S., REGENFUS A., GREIL J., GUGGENBICHLER J.P. (1999). The Erlanger silver catheter: in vitro results for antimicrobial activity. *Infection.* **27** (Suppl 1), 24-29.

BECHERT T., STEINRUCKE P., GUGGENBICHLER J.P. (2000). A new method for screening anti-infective biomaterials. *Nat. Med.* **6**, 1053-1056.

BLANCHEMAIN N., HAULON S., MARTEL B., TRAISNEL M., MORCELLET M., HILDEBRAND H.F. (2005). Vascular PET prostheses surface modification with cyclodextrin coating: development of a new drug delivery system. *Eur. J. Vasc. Endovasc. Surg.* **29**, 628-632.

BRAYNER R., FERRARI-ILIOU R., BRIVOIS N., DJEDIAT S., BENEDETTI M.F., FIE´VET F. (2006). Toxicological impact studies based on Escherichia coli bacteria in ultrafine ZnO nanoparticles colloidal medium. *Nano Lett.* **6**, 866-870.

CHO K.H., PARK J.E., OSAKA T., PARK S.G. (2005). The study of antimicrobial activity and preservative effects of nanosilver ingredient. *Electrochimica Acta.* **51**, 956-960.

CHOI O, DENG KK, KIM NJ, ROSS L JR. SURAMPALLI RY, HU Z. (2008). The inhibitory effects of silver nanoparticles, silver ions, and silver chloride colloids on microbial growth. *Water Res.* **42**, 3066-3074.

DICKINSON G.M., BISNO A.L. (1989). Infections associated with indwelling devices: Infections related to extravascular devices. *Antimicrob Agents Chemother.* **33**, 602-607.

FENG Q.L., WU J.L., CHEN G.Q., CUI F.Z., KIM T. N., KIM J.O. (2000). A mechanistic study of the antibacterial effect of silver ions on Escherichia coli and Staphylococcus aureus. *J. Biomed. Mater. Res.* **52**, 662-668.

GILBERT P., BROWN M.R.W., COLLIER P.J. (1990). Influence of growth rate on susceptibility to antimicrobial agents; biofilms, cell cycle, dormancy, and stringent response. Antimicrob. *Agents Chemother.* **34**, 1865-1868.

ISHIDA H., ISHIDA Y., YUICHI K., OTANI T., SATO K., KOBAYASHI H. (1998). In vitro and in vivo activities of levofloxacin against biofilm producing Pseudomonas aeruginosa. Antimicrob. *Agents Chemother.* **42**, 1641-1645.

JONES N., BINATA R, RANJIT K, MANNA AC. (2008). Antibacterial activity of ZnO nanoparticle suspensions on broad spectrum of microorganisms. FEMS Microbiol. lett. **279**, 71-76.

LEE C., KIM J. Y, LEE W.I., NELSON K.L., YOON J., SEDLAK D.L. (2008). Bactericidal effect of zero-valent iron nanoparticles on Escherichia coli. Environ. *Sci. Technol.* **42**, 4927-4933.

LOCKE J.R., HILL D.E., WALZER Y. (1985). Incidence of squamous cell carcinoma in patients with long-term catheter drainage. *J. Urol.* **133**, 1034-1035.

LOK C.N., CHI-MING H.C.M, CHEN R. HE Q.Y., YU W.Y., SUN H., TAM P.K., JEN-FU C.J. F., CHE C.M. (2007). *J. Biol. Inorg. Chem.* **12**, 527.

LOK C.N., HO C.M., CHEN R., HE QY., YU W. Y., SUN, H.., TAM, P.K.H., CHIU, J.F., CHE, C.M.J. (2006). Proteomic analysis of the mode of antibacerial action of silver nanoparticles. *Proteome Res.* **5**, 916-924.

MANZOOR U., KIM D.K. (2006). Synthesis and enhancement of ultraviolet emission by post-thermal treatment of unique zinc oxide comb-shaped dendritic nanostructures. *Scrip. Mater.* **54**, 807-811.

MANZOOR U., KIM D.K. (2009). Size control of ZnO nanostructures formed in different temperature zones by varying Ar flow rate with tunable optical properties. *Physica E.* **41**, 500-505.

MOBLEY H.L.T., WARREN J.W. (1987). Urease-positive bacteriuria and obstruction of long-term urinary catheters. *J. Clin. Microbiol.* **25**, 2216-2217.

MORONES J.R., ELECHIGUERRA J.L., CAMACHO A., HOLT K., KOURI J.B., RAMIREZ J.T., YACAMAN M.J. (2005). The bactericida effect of silver nanoparticles. *Nanotechnology.* **16**, 2346-53

MUDER R., BRENNEN C., WAGENER M.M, ANGELLA M.G. (1992). Bacteremia in a long-term care facility: a five year prospective study of 163 consecutive episodes. *Clin. Infect. Did.* **14**, 647-654.

SONDI I., SALOPEK-SONDI B. (2004). Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. *J. Colloid Interface Sci.* **275**, 177-182.

SPOERING A.M., LEWIS K. (2001). Biofilm and planktonic cells of Pseudomonas aeruginosa have similar resistance to killing by antimicrobials. *J. Bacteriol.* **183**, 6746-6751.

STAMM W.E. (1991). Catheter-associated urinary tract infections: epidemiology, pathogenesis and prevention. *Am. J. Med.* **91**, 65S-71S.

STOIMENOV P.K., KLINGER R.L., MARCHIN G.L., KLABUNDE K.J. (2002). Metal oxide nanoparticles as bactericidal agents. *Langmuir.* **18**, 6679-6686.

SUN R.W., CHEN R., CHUNG N.P., HO C.M., LIN C.L., CHE C.M. (2005). Silver nanoparticles fabricated in Hepes buffer exhibit cytoprotective activities toward HIV-1 infected cells. *Chem. Comm.* **40**, 5059-5061.

WARREN J.W., DAMRON D., TENNEY J.H., HOOPES J.M., DEFORGE B., MUNCIE H.L. (1987). Fever, bacteremia, and death as complications of bacteri-uria in women with long term urethral catheters. *J. Infect. Dis.* **155**, 1151-1158.

WARREN J.W., MUNCIE H.L., HEBEL J.R. (1994). Hall-Craggs Long-term urethral catheterization increases risk of chronic pyelonephritis and renal inflammation. *J. Am. Geriatr. Soc.* **42**, 1286-1290.

ZODROW K., BRUNET L., MAHENDRA L., LI D. ZHANG A., LI Q, ALVAREZ P.J.J. (2009). Polysulfone ultrafiltration membranes impregnated with silver nanoparticles show improved biofouling resistance and virus removal. *Water Res.* **43**, 715-723.