RESEARCH ARTICLE

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Evaluation of antifungal activity of nanobiosilver particles to treat Candida albicans releated urogenital infections in female rat model

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Abstract

Candida species are the most common encountered agent of fungal infections. Catheter related infections affect over one million patients in Europe and US annually. Candida species infections are responsible for over 63 % of fungal infections in veterinary practice. The aim of this study was to investigate the therapeutic efficacy of nano biosilver particles in urinary catheter related cystitis and vulvo vaginitis of *Candida albicans (C. albicans)*, which is common for human and veterinary medicine is very hard to treat. Thirty healthy adult female Wistar rats were used in the study. The rats were divided into three groups; experimental group with *C. albicans* inoculation and treatment (n = 10) and positive control group *C. albicans* inoculations without treatment (n=10) and negative control group with urinary catheter placed without any pathogen inoculation (n=10). Nanobiosilver was administrated after the establishment of infection. Vulvovaginal and urinary bladder tissues collected at necropsy were process for Gomori staining histopathology and electron microscopy. Rats received inoculation exhibited significant outcomes associated with fungal infections compare to negative control rats. Results of control group were between physiological limits. *C. albicans* was detected in urine samples at 72nd hours after inoculation in experimental group but not observed at 7th day in animals treated with nanobiosilver. In conclusion, due to its safety, efficacy and lack of systemic effects, nanobiosilver could be an excellent alternative for the initial treatment of catheter related candidiasis in veterinary medicine.

Keywords: Nanobiosilver, *Candida albicans*, catheter related infection, treatment.

INTRODUCTION

Candida species are the most common among fungal infections (Achkar and Fries, 2010). *Candida albicans* is the most common isolated infectious agent among Candida species regarding diseases (Wang and Fries, 2011). Dimorphic structured agent can colonize in gastrointestinal system and reproductive path (Sobel 2006). *C. albicans* can be determined as mucocutaneus form which is not life threatening to life-threatening invasive forms with respect to patient's immunologic state or comorbid diseases (Achkar and Fries, 2010). Mucocutaneus *Candidiasis* has two groups; urogenital and non-urogenital forms. Non-urogenital *Candidiasis* usually effects oropharyngeal region in immunosuppressive patients where urogenital *Candidiasis* is responsible for vulvovaginitis in women and balanitis and balanoposthitis in men as well as in dogs and cats (Sobel et al., 1998). In veterinary practice, *Candidiasis* causes urinary tract infections, peritonitis, cutaneous and mucocutaneous infections, gastrointestinal overgrowth, ulcerative glossitis, keratitis (Seyedmousavi et al., 2018). Urinary tract infections are widely seen in hospitalized patients. Catheter-related urinary tract infections are seen over one million patients in Europe and US. *Candida albicans* is one of the most frequent among these infections (Tambyah et al. 2002). Catheters are used in woman of inpatients to provide urine flow and output (Saint et al., 2000). Catheters are suitable for microorganism penetrations and biofilm formation. Numerous microorganisms cause urinary tract infections via catheters. Candida eradication will be very hard if it manages to biofilm formation (Mah et al., 2003).

Candida identification in urine may cause different clinical manifestations (Kauffman et al., 2011). Firstly, agent penetrates to urinary tract via catheter and forms biofilm. Most patients are asymptomatic at this stage. *C.albicans* is excreted with urine in these patients which is called candiduria. Afterwards, it may cause a bladder infection, reach the kidneys with ascendant path and cause pyelonephritis. At this stage clinical symptoms are visible and needs treatment. The worst manifestations is the agent's distributing to whole body using hematogenous paths which is life-threatening. As an alternative, vaginal *Candidiasis* can be seen due to candiduria (Nobile et al., 2008).

Candida albicans was isolated in 89% Australian and US, % 88 Austrian, 84% Italian and 44% Turkish vulvovaginal *Candidiasis* cases (Achkar and Fries, 2010). Most common clinical symptoms are pruritis in vulva and dysuria. Vulvo-vaginal erythema, edema, fissures and dens vaginal discharge are common findings in physical examination (Eckert et al., 1998). Similarly, balanitis cases caused by candidiasis are not specific. Generally local pruritis and dermatitis are seen. Fungal infections of urinary system usually do not cause systemic clinical symptoms. Fever, dysuria and leukocyturia and similar symptoms can be seen but not in all patients with candiduria. Candiduria is usually seen in long time hospitalized patients with catheters (Lisboa et al., 2009).

There are many research about biofilm formation and medical equipment related infections like urine catheters, vascular stents, cerebrovascular shunts, joint implants. Catheter-related urinary tract infections are

the most common among them and seen as 70% (Kojic and Darouiche, 2004; Dudeck et al., 2011; Siddiq and Darouiche, 2012). Candida biofilm formations are investigated repeatedly in vitro biofilm infection models in medical equipment. The biggest issue here is not achieving a response with antifungal treatment after biofilm formation (Kojic and Darouiche, 2004; Siddiq and Darouiche, 2012).

Using rat models have advantages for usage and give useful information to determine pathogenesis of urinary tract diseases caused by candida species, pharmacokinetics of antifungal drugs and immune response in both human and domestic animals (Dudeck et al., 2011). Besides, plenty of candida models affect kidneys via hematogenous paths due to intravenous candida inoculation. Meaning that this happens with ascendant way (Naglik et al., 2008). Whereas catheter-related candida infections happens with descendant way. Descendent developing urogenital candidiasis is rarely spreadable hematogenous (Wang and Fries, 2011).

There are many treatment options regarding to localization of the infection, form and severity. Most of the antifungals have short and long term side effects. Biosilver particles under 2-100 nm size act as bactericide and fungicide due to increasing area with bacteria and fungus which is much larger than itself. This product can gain antimicrobial feature with the conductivity specialty of silver. Once biosilver particles are over any substance surface, very strong antibacterial effect is provided. Silver ions do not harm any cells because of cell the membrane in body. Consequently, biosilver is considered to be harmless for human, animals, plants, in other words multi cell organisms. Biosilver structure do not change during the process as it does not go into any chemical reaction and works continuously, no need to add more silver into the system. It was reported that it has killed 650 known pathogens in a very short time whether they were mutational or not. Silver (Ag) compounds have a wide spectrum of antimicrobial activity against bacteria, virus and fungus which is called “oligo dynamic activity” (Chen et al., 2006). It was reported that antifungal activity of nano particles against yeast and fungostatic reaction were due to membrane structure effect with a mechanism of electrostatic interaction of oxide particles on fungus cell (Raju and Rajappa, 2011).

In this study, it was aimed to investigate the effects of biosilver compound which does not have a systemic effect in hard to treat urine catheter-related cystitis and vulvovaginitis infections of *C. albicans* that is widely seen in human (especially women) and domestic animals by using rat model.

MATERIALS AND METHODS

Animals

Modeling of urogenital *C. albicans* infection and sample collection was performed at Kırıkkale University Hüseyin Aytemiz Experimental Research and Application Center,Turkey. Thirty Wistar adult female rats with weighing of 300–350 gr. were used in the study. During the study, rats were separated in cages, fed with commercial pellet food and given *ad libitum* water. Animals showing any disease symptoms (nasal discharge, mucosal paleness, hyperemia, apathy etc.) were excluded from the study. The rats were divided into three groups; experimental group with *C.albicans* inoculation and treatment (n=10) and positive control group *C.albicans* inoculations without treatment (n=10) and negative control group with solely urinary catheter placed without any pathogen inoculation (n=10).

Preparation of Urinary Catheter

Single dose of 250 mg/kg cortisone acetate was applied subcutaneously to the rats at the day of urinary catheter placement. Gentamicin 80 mg/kg was also applied subcutaneously twice a day to prevent systemic bacterial infections. In addition, 0.9 mg/ml Penicilin G was added to drinking water. Animals were examined in terms of stress every six hours during the time urinary catheter was placed. Catheter region was controlled in terms of inflammatory and purulent changes.

Insertion of urinary catheter

General anesthesia was given with Xylazine (5 mg/kg) and Ketamine (45 mg/kg) before the administration. Necessary asepsis and antisepsis were performed with surgical scrub from midline to tail. A silicone catheter (Instech Solomon, 3.5 Fr, female luer, round tip, gas sterilized) was placed in urethra and stabilized with 4/0 surgical prolene suture. After urinary catheterization, Elizabeth collar was put on to the rats.

Inoculation of Candida albicans

After the insertion of urinary catheter, *Candida albicans* broth consisting 10^7/mL cells were given via catheter inside the bladder. Furthermore, same doses of inoculum were administered cranial vagina. After inoculation, rats were tracked for monitored for sign of distress for two hours and then placed back to their cages.

Biosilver synthesis

100 mg silver nitrate (Merk) was dissolved in pure water and diluted to be 100 ppm. 10 ppm pure water solution of ascorbic acid was prepared. 6 g/L of root and body *Cotinus coggygria* plant was boiled for 15 min. Liquid part was vaporized in rotary evaporator and extract was obtained. Afterwards, 100 ml of 100 ppm silver solution, 10 mg cotinus plant extract, 100 ml ascorbic acid 0,1 M solution, 13,4 mg $NaHPO_4$, 180 ml 0,15 molar NaCl solution were mixed in a beaker. The pH of solution was set to 7,8 using NaOH. It was mixed in magnetic mixer in 38.6 $^{\circ}$C for 28 hours using magnetic fish. One ml (3,5 µgr/mL) of biosilver was administrated to bladder via catheter.

Fungal Culture and Urine Analysis

After *C. albicans* inoculation, urine samples were collected via urinary catheter at 72nd hour and 7th day after all study groups and analyzed with using commercial urine dipsticks (Mission® Urinalysis Reagent Strips, ACON Laboratories, Inc. San Diego, USA). Microbiologic analyses were performed in euthanized rats vagina, urinary tissues and urine catheters to obtain *C. albicans* microorganism load. Urine sample, collected in sterile conditions and sent to lab the same day was inoculated in sabauraud dextrose agar (SDA) and blood agar and incubated for three days in 37 ^{0}C. At the end of three days, colonies appeared in SDA and blood agar. Colonies were gram stained. At the same time, a small inoculum from an isolated colony is suspended in 0,5 mL of sheep serum and is incubated at 37 ^{0}C for two hours and investigated in terms of germ tube formation as well.

Tissue Collection

Rats were sacrificed in 4th day after inoculation and rats of experimental group in 7th day of nanobiosilver treatment to determine whether infection modeling were formed and examined the histological changes in tissues. For this reason, rats were euthanized under general anesthesia. Urogenital tissues were collected and transported to the

laboratory immediately for histopathological examination.

Electron Microscope Investigation

Catheters were left for drying at room temperature after removal procedure. They were cut longitudinally under dissection microscope by microtome knife in order to scan its internal surface. Afterwards, catheters had been coated with gold by gold coating machine. The images of internal surfaces were taken by scanning electron microscope for displaying the biofilm formation of *C.albicans* (Jeol SEM).

Histopathologic Examinations

Bladder and urethra was examined histopathologically to determine host response for urinary catheter-related *C. albicans* infection. Taken tissues after euthanasia were fixed in 10% formalin solution and paraffin blocks were prepared for examination. They were stained with methenamine silver and histopathologic changes were determined and *C. albicans* was visualized.

Ethics committee approval

Th study was approved by Kırıkkale University Local Ethics Committee for Animal Experiments with approval number 16/21 on 25th February 2016.

RESULTS

After inoculation, rats were tracked for stress for two hours and taken to their cages. None of any critical stress symptoms was observed. Results of urine analyzes of rats are given in Table 1. Urine and vaginal samples of the experimental and positive control group the colonies of *C. albicans* were appeared as white to cream, shiny, high-convex and S type. Gram blue stain was used on fixed urine and vaginal smears from the colonies. Also, a small inoculum from an isolated colony were suspended in 0.5 mL of sheep serum and were incubated at 37°C for two hours. Small tubes were seen projecting from some of the yeast cells which is characteristic of *C. albicans*. No growth was detected in experimental and control group samples taken seven days after biosilver administration. Although colony formation was specific to *C. albicans* and yeast cells were seen in gram stained samples, small amount of inoculum was taken from colonies.

Table 1. Urine analyzes result. I: 72nd hour after *C. albican*s inoculation, II: 7th day after biosilver administration, III: 72nd hour after catheter application, IV: 7th day after catheter application.

Parameter	Study Group (n=20)		Control Group (n=10)	
	I	II	III	IV
Leucocyte	+/+++	-	-	-/+
Protein	+/++	-	-	-/+
pH	6,0/7,5	6,0/7,0	6,0/7,0	6,0/7,5
Erythrocyte	-/+	-	-	-
Density	1025/1030	1015/1025	1015/1025	1015/1030
Ketone	-	-	-	-
Glucose	-	-	-	-
Bilirubin	-	-	-	-
Urobilinogen	-	-	-	-
Nitrite	-	-	-	-

DISCUSSION

Raju and Rajappa (2011), specified gomori methenamine silver as the most suitable method to determine *C. albicans* in tissues. Gomori methenamin silver, oxidases fungus cell wall and aldehyde groups come off and these aldehyde groups gets into reaction with silver nitrate and the agent is visible (Nassar et al., 2006). In this study, euthanasia was performed under general anesthesia to the animals on 4th day after urogenital tract infection of *C. albicans* and seven days after nanobiosilver treatment. Gomori methenamin silver was used to detect *C. albicans* in urogenital tissues. Especially in the 4th day samples of *C. albicans* formations as dark black brown buds were detected (Figure 1A).

In the study, tissue samples taken 7th day after nanobiosilver administration were gomori methenamine silver stained and no *C. albicans* like structures were detected in microscopic examination of histopathologic samples and they seem like tissues of control group (Figure 1 B and C). At the end of the study, urethral catheters placed in control group and catheters of study group taken out at seventh day after nanobiosilver administration were examined by electron microscobe. A film layer was detected in internal side of urethral catheters of control group showing microbial growth. There was no such layer formation in the catheters of nanobiosilver administrated study group animals. Extracellular mucopolysaccharide biofilm formation on the surface of long term used urinary catheters are the major cause of candiduria or bacteriuria in human as well as in domestic pets. Tomm-harsfall proteins, magnesium and calcium ions that are in the urine structure get into that material formation. After placing catheter, there is a rapid biofilm formation that makes a large and rough path on catheter's external and internal surface that enables microorganisms to hold and grow easily (Nicolle, 2014; Rudramurthy et al., 2016). In this study, biofilm formations on the internal surfaces of control group catheters were detected by electron microscope examination (Figure 1D). On the other hand, no biofilm formation was detected in biosilver administrated study group catheters' internal surface (Figure 1E).

Catheters provide a substrate for adherence of microorganisms and proliferation of biofilms. When growing as a biofilm, *Candida spp.* is difficult to eradicate due to inherent drug resistance and immune tolerance. Resistance due to most antifungal and antibacterial agent usage is a major issue today. Improper use of antifungal drugs increases the number of resistant microorganisms (Chen et al., 2006; Shi et al., 2010). Moron et al. (2005) states that this will be threatening in long term as antimicrobials are very few for different species. Nabawy et al. (2014) states that researches concerning antimicrobial metallic particles that are against resistant strains of large surfaced and volume areas are promising. Keuk-Jun et al (2008), made clinical isolations in ATCC cell culture and showed that silver particles are effective against *T. mentagrophytes* and *Candida albicans*, they also stated that particles act by effecting mycelium and further investigations should be conducted in their study concerning antifungal effect of biosilver particles. Bubenik et al. (2007) reported that daily increase of urinary tract infections in catheterized dogs were 27%. Duration of the catheterization leads to resulted in more complicated infections and cost. According to results of this study, contribute with previous study (Bubenik et al., 2007), it was shown that biosilver complex could be effective and alternative in *C. albicans* treatment. Lee et al. (2010)

stated that antifungal inhibitory concentration of nanobiosilver is about 20-30 times less than fluconazole. This gave us the idea that nanobiosilver is much safer than the antifungal drugs as it is used as very little doses. Nanobiosilver particulates have significant antifungal activity due to skin penetration while having lower systemic toxicity than other antifungal agents (Lee et al., 2010).

During recent years nanobiosilver particulates have attracted attention due to its unique physical and chemical properties (Stoimenov et al., 2002; Tak et al., 2015). For this reason, we investigated effectiveness of nanobiosilver complex and positive results were found especially for treatment of *C. albicans* infections.

We concluded that nanobiosilver is an effective chemical agent in treatment of *C. albicans* infections. Nanobiosilver can be an alternative for catheter-related candidiasis in woman and domestic pet species devoting to its safety, effectiveness and not having systemic effects.

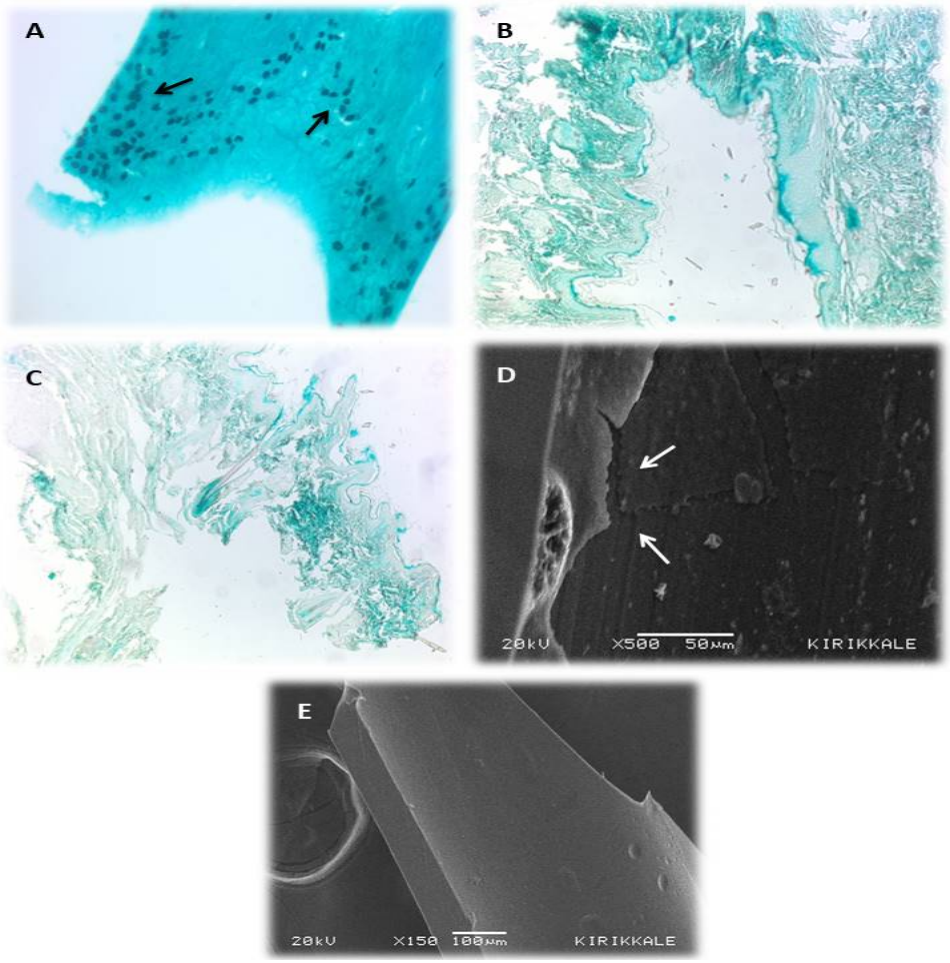

Figure 1. Electron Microscope and Histopathologic results
A. Study group; *C. albicans* infection (gomori methenamin silver stain) (Arrow: *C.albicans*), **B.** Study group; urethra after administration normal appearance gomori methenamin silver stain), **C.** Control group; uretra (gomori methenamin silver stain), **D.** Internal surface of urethral catheter from control group (Arrow: biofilm layer), (Scanning electron microscope), **E.** Internal surface of urethral catheter from study group after biosilver administration (Scanning electron microscope).

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REFERENCES

Achkar JM, Fries BC. 2010. Candida infections of the genitourinary tract. Clin Microbiol Rev, 23, 253-273.

Bubenik LJ, Hosgood GL, Waldron DR and Snow A. 2007. Frequency of urinary tract infection in catheterized dogs and comparison of bacterial culture and susceptibility testing results for catheterized and noncatheterized dogs with urinary tract infections. J Am Vet Med Assoc, 231, 893-899.

Chen CG, Yang YL, Cheng HH, Su L, Huang F, Chen T, Liu T, Su J and Lo J. 2006. Non-lethal C. albicans cph1/cph1 efg1/efg1 transcription factor mutant establiching restricted zone of infection in a Mouse model of systemic infection. Int J Immunopathol Pharmacol, 19, 561-565.

Dudeck MA, Horan TC, Peterson KD, Allen-Bridson K, Morrell G, Anttila A, Pollock A and Edwards R. 2011.

National Healthcare Safety Network report, data summary for 2011, device-associated module. Am J Infect Control, 41, 286-300.

Eckert LO, Hawes SE, Stevens CE. 1998. Vulvovaginal candidiasis: clinical manifestations, risk factors, management algorithm. Obstet Gynecol, 92, 757-765.

Kauffman CA, Fisher JF, Sobel JD and Newman A. 2011. Candida urinary tract infections--diagnosis. Clin Infect Dis, 52, 452-456.

Keuk-Jun K, Sung WS, Moon SK, Choi JS, Kim JG and Lee DG. 2008. Antifungal Effect of Silver Nanoparticles on Dermatophytes. J Microbial Biotechnol, 18, 1482-1484.

Kojic M and Darouiche O. 2004. Candida infections of medical devices. Clin. Microbiol. Rev. 17, 255-267.

Lee J, Kim KJ, Sung WS, Kim G, Lee G. 2010. The Silver Nanoparticle (Nano-Ag): a New Model for Antifungal Agents,. 295-308. In: David Pozo Perez (Ed.), Silver Nanoparticles, InTech, Shangai.

Lisboa C, Ferreira A, Resende C and Rodrigues G. 2009. Infectious balonoposthitis: management, clinical and laboratory features. Int J Dermatol, 48, 121-124.

Mah TF, Pitts B, Pellock B, Walker C, Stewart S and O'Toole A. 2003. A genetic basis for Pseudomonas aeruginosa biofilm antibiotic resistance. Nature, 426, 306-310.

Moron B, Soria-Diaz ME, Ault J, Verroios G, Noreen S, Rodriguez-Navarro N, Gil-Seerno A, Oates J, Megias M. and Sousa C. 2005. Low Ph Changes the profile of nodulation factors produced by Rhizobium tropici. Chem Biol, 12, 1029-1040.

Nabawy GA, Hassan AA, El-Ahl Rasha HS, Refai K. 2014. Effect of metal nanoparticles in comparison with Commercial antifungal feed additives on the growth of aspergillus flavus and aflatoxin b 1 production. Journal of Global Biosciences, 3, 954-971.

Naglik JR, Challacombe SJ, Hube B. 2008. Animal models of mucosal Candida infection. FEMS Microbiol Lett, 283, 129-139.

Nassar A, Zapata M, Little JV and Siddiqui TM. 2006. Utility of Gomori Methenamine silver stainig for Pneumocystis jirovecii on Bronchoalveolar Lavage Cytologic Specimens: A Review. Diagn Cytopathol, 34, 719-723.

Nicolle LE. 2014. Catheter associated urinary tract infections. Antimicrobial Resistance & Infection Control, 3,23.

Nobile CJ, Schneider HA, Nett JE Sheppard C, Filler G, Andes R. and Mitchell P. 2008. Complementary adhesin function in C. albicans biofilm formation. Curr Biol, 18, 1017-1024.

Raju SB, Rajappa S. 2011. Isolation and Identification of Candida from the Oral Cavitiy. ISRN Dent, 2011, 1-7.

Rudramurthy GR, Swamy MK, Sinniah UR and Ghasemzadeh A. 2016. Nanoparticles: Alternatives Against Drug- Resistant Pathogenic Microbes. Molecules, 21, 836.

Saint S, Wiese J, Amory JK, Bernstein L, Patel D, Zemencuk K, Bernstein J, Lipsky A and Hofer P. 2000. Are physicians aware of which of their patients have indwelling urinary catheters? Am J Med, 109, 476-480.

Seyedmousavi S, Bosco SD, De Hoog S, Ebel F, Elad D, Gomes R and Pasmans F. 2018. Fungal infections in animals: a patchwork of different situations. Medical mycology, 56, 165-187.

Shi LE, Liangying X, Baochao H, Hongjuan G, Xiaofeng G and Zhenxing T. 2010. Inorganic nano mental oxides used as anti-microorganism agents for pathogen control. 361-368. In: A. Mendez-Vilas (Ed) Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology, Vol I, Formatex, Badajoz.

Siddiq DM, Darouiche RO. 2012. New strategies to prevent catheter associated urinary tract infections. Nat Rev Urol, 9, 305-314.

Sobel JD (2006): The emergence of non-albicans Candida species as causes of invasive candidiasis and candidemia. Curr Infect Dis Rep, 8, 427-433.

Sobel JD, Faro S, Force RW, Foxman B, Ledger J, Nyirjesy R, Reed D and Summers R. 1998. Vulvovaginal candidiasis: epidemiologic, diagnostic and thearapotic considerations. Am J Obstet Gynecol, 178, 203-211.

Stoimenov PK, Klinger RL, Marchin GL and Klabunde S. 2002. Metal oxcide nanoparticles as bactericidal agents. Langmuir, 18, 6679-86.

Tak YK, Pal S, Naoghare PK, Rangasamy S and Song M. 2015. Shape-Dependent Skin Penetration of Silver Nanoparticles: Does It Really Matter ?. Sci Rep, 20, 5:16908.

Tambyah PA, Knasinski V, Maki DG. 2002. The direct costs of nosocomial catheter-associated urinary tract infection in the era of managed care. Infect Control Hosp Epidemiol, 23, 27-31.

Wang X, Fries BC. 2011. A murine model for catheter-associated candiduria. J Med Microbiol, 60, 1523-1529.