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Original research article

Deterrence in metabolic and biofilms forming activity of *Candida* species by mycogenic silver nanoparticles

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ABSTRACT

Candida, a commensal and opportunistic fungal pathogen has been typically known for its biofilm forming ability and device-associated hospital acquired infections in human. The study aimed at exploring the *in vitro* anti-biofilm and anti-metabolic activity of AgNPs against *C. albicans* ($n=2$), *C. tropicalis* ($n=2$) and *C. parapsilosis* ($n=2$) isolated from urine samples. Broth dilution method revealed greater than 50% inhibition at 100 ppm against Ag NPs in 24 h. An overall reduction of 55–86% in biomass (crystal violet staining assay) and 20–73% in metabolic activity (XTT assay) was observed in 24 h old biofilms. However, *C. albicans* proved to be more susceptible to AgNPs compared to *C. tropicalis* and *C. parapsilosis*. Scanning Electron Microscopy revealed patchy growth and deterrence in biofilm biomass when Ag NPs were coated on urinary catheter. Furthermore, viable cell counts of *Candida* were significantly reduced on AgNPs coated catheter compared to control.

Introduction

An about 60–80% of human infections occur as a consequence of biofilms formation by pathogenic microorganisms (Harriott et al., 2010; Seneviratne et al., 2008). *C. albicans* belongs to the group of commensal fungi that are generally present asymptomatically on skin, oral cavity, vaginal and gastrointestinal tracts (Brown et al., 2012). Strains of *Candida* become pathogen due to evolving resistance to antimicrobials and changing micro-environmental conditions. Pathogenic *Candida* is associated with mucosal to invasive systemic life threatening infections such as *Candida* septicemia (Nobile and Johnson, 2015). *Candida* spp. are characterized based on their virulence factors with diverse attributes. The most prominent virulence factors are the cell surface adhesins and invasions. Additionally, the phenotypic switching, biofilm forming ability along with production of different hydrolytic enzymes are involved in candidiasis (Deepa et al., 2015). The highly structured biofilms of *Candida* spp. are composed of multiple cell types enclosed in exo-polymeric substances. Attachment and colonization to the biotic surfaces such as mucosal linings as well as the abiotic medical implants provide the basis for the development of complex multicellular biofilm (Uppuluri et al., 2010).

Infections caused by biofilm forming *Candida* spp. are difficult to treat due to greater (up to 1000 fold) resistance to drugs compared to planktonic cells (Mah and O'Toole, 2001). Most of the proteins identified in the *Candida* biofilm matrix are suggested to be the hydrolyzing enzymes (Zarnowski et al., 2014). Among these, extracellularly secreted enzymes, aspartyl proteinases are of vital importance. S-aspartyl proteases activity has been directly related to the number of SAP genes in pathogenic species of *Candida* (Staniszewska et al., 2012). The enzyme not only involves in acquisition of essential nitrogen for growth, but it also provides means for enhanced fungal colonization, and penetration through impaired host barriers. Besides, the fungal proteinases may help evading host defenses through direct degradation of host molecules associated with an intracellular lysosomal enzyme and activation of complement system (Naglik et al., 2003).

Candida biofilms have been primarily studied on abiotic surfaces of medical devices such as stents, shunts, oral dentures and implants, pacemakers, endotracheal tubes and other indwelling catheters (Harriott et al., 2010; Seneviratne et al., 2008). Recent reports have even indicated catheter associated biofilm infections that are >50% (approx.) due to different bacterial and fungi strains (Mermel et al., 2009; Nobile and Johnson, 2015). Current remedial measures including different anti-fungal physical and chemicals methods proved to be having limited efficacy due to developing

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resistance among species of *Candida*. Besides, use of high dose of antifungal agents proved to be damaging vital human organs like kidney, liver etc. Medical implants associated with *Candida* infections have been reported to cause mortality rates of up to 30%. Moreover, the biofilm associated with medical devices not only complicate the treatment and removal but it also make them costly and hazardous (Andes et al., 2012; Nobile and Johnson, 2015; Peres-Bota et al., 2004). Thus, for the deterrence of *Candida* biofilm infections, it is essentially important to device cheap and sustainable procedures and practices.

Recent studies have investigated different physical or chemical methods to control *Candida* biofilms. In this context, the role of silver nanoparticles (AgNPs) has been gaining much importance due to their vital role in controlling bacterial and fungal infestation (Kim et al., 2008, 2009; Lateef et al., 2016; Li et al., 2014; Monteiro et al., 2011). Still, these studies were mostly conducted at initial levels for biofilm deterrence during different stages of biofilm growth (Li et al., 2014; Monteiro et al., 2015). In the recent past, the AgNPs anti-biofilm effect was determined but limited data is available regarding the mechanism of action thus needs further evaluation. A detailed mechanistic approach has to be elucidated related to the anti-biofilm properties of AgNPs against test fungal strains. Previously, antifungal action by AgNPs was proposed to be linked with cell membrane disruption (Kim et al., 2009; Lara et al., 2015). However, details on the said process are vague and need further experimental evidences.

Cotemporary growing methods to control pathogenic microbes include the application of metal nanoparticles. Comparatively, the biological synthesized metal nanoparticles are gaining more importance over other physical and chemical due their low cost and eco-friendly nature (Lateef et al., 2016; Naqvi et al., 2014). Immense data has been generated that emphasized the importance of microbes and related bioprocess for development of nanoparticle. Among microbes, filamentous fungi have gained prime importance for metal nanoparticles synthesis because of their certain morphological and physiological features. They are easy to grow and manage thus favoring significant amount of extracellular enzymes production. Large fungal biomass production also neutralize the toxigenic effects of metals and biotransform and bio-accumulate them. The extracellular synthesis of metal nanoparticles by fungi has been reported efficient and require minimum of efforts during downstream processing (Das and Thiagarajan, 2012; Naqvi et al., 2014; Rai et al., 2009; Zhang et al., 2011).

The research work investigated the biofilm deterring ability of biologically synthesized AgNPs against different pathogenic species of *Candida* (*C. albicans*, *C. tropicalis* and *C. parapsilosis*). Similarly, anti-biofilm activity of AgNPs was studied when they were coated on urinary catheter surface.

Materials and methods

Fungal cultures and growth conditions

Clinical isolates ($n = 6$) of *C. albicans* (CA46, CA72), *C. tropicalis* (CP17, CP65) and *C. parapsilosis* (CT28, CT92) were provided by Medical Microbiology and Immunology lab. They were previously collected from different urine samples and used in the study. American Type Culture Collection (ATCC) culture of *C. albicans* (ATCC 24433) was used as a control for comparative biofilm studies. The isolates used in the study showed resistance against fluconazole, amphotericin B and voriconazole. The cultures were refreshed on Sabouraud dextrose agar medium (SDA, Oxoid) at 37 °C for 24–48 h and maintained at 4 °C during storage.

Mycogenic silver nanoparticles synthesis

Mycogenic AgNPs were provided by Microbiology Research Lab, synthesized previously using *Aspergillus flavus*. Most of the nanoparticles were found to be spherical in shape and size ranged between 3 and 80 nm. During nanoparticles synthesis, the color of reaction mixture was changed from light yellow to dark brown which indicated the surface plasmon excitation of AgNPs. Also, the UV–vis spectra of reaction mixture showed a strong surface plasmon response at 400–500 nm, with an increase in intensity with reaction time thus color change was easily investigated by UV-spectrophotometry (Naqvi et al., 2014).

Minimum inhibitory concentrations (MICs) of biogenic Ag NPs against *Candida* spp. biofilms

The Clinical and Laboratory Standards Institute (CLSI, 2013) broth microdilution method was used to determine the *in vitro* minimum inhibitory concentrations (MIC) of AgNPs against *C. albican, C. tropicalis* and *C. parapsilosis*. Different concentrations of AgNPs including 25, 50, 75 and 100 ppm were prepared in deionized water. After incubation, inhibition was evaluated based on lowest concentration causing 50% reduction in turbidity related to the control growth (Jebali et al., 2014).

***Candida* biofilm development and inhibition by Ag NPs treatment**

C. albicans, C. tropicalis and *C. parapsilosis* biofilms were developed in 96 well microtiter plates. Then, 200 μl of *Candida* cell suspension (1.0×10^7 cells/ml) was prepared in Sabouraud dextrose broth (SDB, Oxoid) and supplemented with 10% glucose. For the development of biofilms at intermediate stage, plates containing cell suspension were grown for 24 h at 37 °C and 120 rpm. After 10–15 h, the wells were replaced with fresh medium. After 24 h, wells were washed with 400 μl of PBS (pH 7.2-7.4) to remove non-adherent cells. In the next, step 200 μl solution of AgNPs was prepared in deionized water was added to wells along with 200 μl of fresh medium. The wells were incubated for 24 h after treatment with AgNPs under shaking at 120 rpm at 37 °C. Wells with cell suspension but without AgNPs were designated as growth controls and for sterility purpose wells with sterile media (without cell suspension and AgNPs) were used (Monteiro et al., 2015). The OD was determined at the wavelength of 492 nm using microtiter plate reader (Platos, R492).

$$\%\text{age inhibition} = \frac{\text{OD in control} - \text{OD in treatment}}{\text{OD in control}} \times 100$$

Effect of AgNPs on total biofilm biomass

Total biofilm biomass of *C. albicans, C. tropicalis* and *C. parapsilosis* were evaluated after treatment with AgNPs by biomass reduction assay using crystal violet (CV) for staining. The biofilms were developed using 1.0×10^7 cells/ml of cultures as inoculums. After biofilm development and treatment with AgNPs, medium was discarded. Subsequently wells were washed with 400 μl of PBS to remove any non-adherent cells. Fixation of adhered *Candida* cells were achieved by 200 μl of 95% ethanol incubated for 15 min. After 15 min ethanol was removed and wells containing different *Candida* spp. biofilms were dried at room temperature. Consequently 400 μl of 1% w/v CV was applied for 5 mins. Excess strain was removed by washing with deionized water. Crystal violet bound to adhered cells was solubilized by using 33% acetic acid. Afterwards the absorbance was measured using microtiter plate reader at wavelength of 492 nm. The test was performed in duplicate and independently for each sample under investigation (Dhanasekaran et al., 2014).

Effect of AgNPs on biofilms metabolic activity

After the AgNPs treatment and incubation period of the *Candida* spp. cultures initially inoculated cell suspension (1.0×10^7 cells/ml), metabolic activity was assessed by XTT reduction assay to estimate the viable cells. 2, 3-bis [2-Methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide inner salt (XTT) and phenazine methosulfate (PMS) stock solutions were freshly prepared and protected from light. AgNPs and media were removed from each well and biofilms were gently washed twice with PBS. To each well, 90 μl of XTT and 10 μl of PMS were added. The plates were incubated in dark at 37 °C for 1 h. Absorbance at 492 nm were recorded using an automated plate reader (Platos, R492). The test was performed in duplicate, independently for each individual sample (Nett et al., 2011).

Scanning Electron Microscopy (SEM) of biofilm on AgNPs coated catheter

For AgNPs coating, urinary catheter (Uriflo, HSC, Pakistan) made up of plastic was cut into 1 cm x 1 cm pieces. The cut portion was immersed in 5 ml mycogenic AgNPs suspension with concentration of 100 ppm for 24 h under sonication. After 24 h, excess suspension was removed via blotting and coated surface was dried under sterile conditions at 50 °C. The AgNPs coating with some particles release with time in surrounding was determined using Fourier transform infrared spectroscopy (FTIR) analysis (Bruker, Tensor27, Germany). The wavelength (λ) applied for analysis ranged between 4000 and 600 nm. For evaluation of anti-biofilm activity, sterile glass vials containing the coated pieces with *C. albicans, C. tropicalis* and *C. parapsilosis* culture were further incubated for 24 h at 37 °C. Catheter surface without coating served as a control. The biofilm developed on the Ag coated and uncoated catheters surface was analyzed via SEM analysis (JSM5910, JEOL, Japan) (Thomas et al., 2015).

Cell viability on pre-coated catheter surface

Cultivatable cells on the surface of AgNPs coated catheter surface was evaluated using colony forming units (CFUs). The sterile glass vials containing the coated catheters 1 $cm^2 \times 2$ cm^2 was inoculated with 24 h *Candida* spp. cultures and further incubated for 24 h at 37 °C. Catheter surface without coating served as a control. After incubation, biofilms on catheters surface were washed and then transferred into separate eppendorf tubes containing 2 ml of PBS. For the removal of cells from catheter surface, the catheters were transferred to the test tubes containing 1 ml sterile PBS (phosphate buffer saline). The tubes were agitated in sonicator (30s) and vortex (30s) and process repeated 2–3 times. Afterwards dilutions were made and plated on SDA followed by 24 h of incubation at 37 °C, colonies were counted using colony counter (Monteiro et al., 2015).

Statistical analysis

For statistical analysis, SPSS software was utilized (SPSS 16.0, Statistical Package for the Social Sciences, Inc., Chicago, IL, USA). The statistical test two-way ANOVA was used for Biofilm biomass and metabolic activity against Mycogenic Ag NPs. Whereas, *t*-test was used to determine the cell viability on catheter coated surface compared to control group.

Results and discussion

The anti-biofouling effect of mycogenic Ag NPs in terms of total biomass and metabolic activity reduction was evaluated against different species of *Candida* (*C. albicans, C. tropicalis* and *C. parapsilosis*) isolated from urine of patients. Urine catheter coated with Ag NPs also indicated reduced biofilm development.

MIC of AgNPs against *Candida* spp. biofilms

Biofilms of different species ($n = 6$) of *Candida* were treated with different conc. (25, 50, 75 and 100 ppm) of AgNPs under *in vitro* condition using broth dilution method. Overall, increase in concentration of AgNPs from 25 to 75 ppm resulted an about 1–30% inhibition of biofilm growth. However, further increase in Ag NPs concentration to 100 ppm resulted in ≥50% inhibition of both *C. albicans* and non-albicans spp. (Fig. 1). Greater inhibition (100%) has been reported even at 0.4 to 3.3 ppm Ag NPs concentration when they were applied against *Candida* spp. under planktonic condition (Monteiro et al., 2014, 2011). Previously, biofilms of different *Candida* species have exhibited resistance to fluconazole (FLC) and amphotericin B (AMB) (Melo et al., 2011). Likewise, AgNPs exhibited comparable anti-fungal activity with AMB, whereas, it was considerably higher by AgNPs compared to FLC against *Candida* spp. (Kim et al., 2008). Differences in MIC of biogenic AgNPs might be due to their polydispersity morphology due to different synthesis procedures. Furthermore, susceptibility of planktonic cells of *Candida* at lower concentration of AgNPs compared to those in biofilm matrix purely reflected the intact and resistant nature of biofilm. Under biofilm mode of living, the toxic effects of chemicals or ions become neutralize or shared by the whole community. Furthermore, varying susceptible to AgNPs by different species of *Candida* also indicated a minor phase variation in cell morphology and physiology that might evolved under varying conditions of host and exposures to different AgNPs concentrations (Reidy et al., 2013).

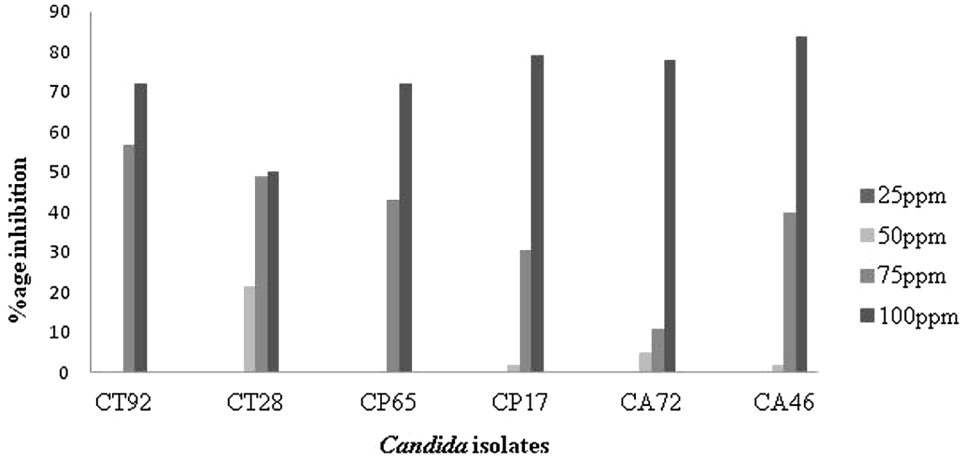

Fig. 1. MIC of AgNPs against *Candida* biofilms.

Effect of AgNPs on total biofilm biomass

Microtiter assay was used to assess total biomass inhibition in different species of *Candida* i.e. *C. albicans, C. tropicalis* and *C. parapsilosis*. A significant reduction (p value < 0.05) in total biofilm biomass was observed in the isolates of *Candida* spp. when treated with 100 ppm AgNPs. About 55–86% reduction in biomass was observed in 24h-old biofilms. The highest reduction of 86% was observed in *C. albicans* CA46 followed by 83% in CA72, 67% in CT92, 62% in CT28 and 57% in CP17. However, 97% inhibition in biomass was observed in *C. albicans* ATCC24433 (control) (Fig. 2). The inhibitory effect of chemically synthesized AgNPs against *C. albicans* and *C. glabrata* adhesion and biofilm formation was observed in previous studies with reduction ranged from 23 to 51.5% (Monteiro et al., 2015, 2014, 2011). It can be speculated that different parameters have implications in anti-biofilm effect. The most critical ones are the method of AgNPs synthesis affecting size and shape. Assay conditions such as incubation time have implications towards AgNPs activity. Furthermore, reduction in biomass may explain the mycogenic AgNPs as fungistatic action against *Candida* biofilms by limiting the production of EPS, an important component of biofilms.

Effect of AgNPs on metabolic activity of *Candida* spp.

The metabolic activity of *C. albicans, C. tropicalis* and *C. parapsilosis* were determined on the basis mitochondrial oxidoreductases activity using colorimetric XTT assay. Metabolic activity was affected by almost 20–73% in 24 h in different species of *Candida* when treated with 100 ppm of AgNPs. Comparatively, there was observed a non-significant difference in enzymatic activities of different *Candida* spp. against 100 ppm AgNPs (Fig. 3). The maximal inhibitory effect of AgNPs was observed in *C. albicans* i.e. 73% in CA72. Reduction in metabolic activity was lower in non albicans. It was 45% in CP17 followed by 33% in CP65, 28% in CT28 and 9% in CT92, whereas, almost 80% reduction in metabolic activity was noted in case of positive control, *C. albicans* ATCC 24433 (Fig. 3). Compared to previous finding, the degree of reduction in metabolic activity was different in the present finding. The difference in reduction of metabolic activity may be due different conc. of AgNPs used (Li et al., 2014). The difference in the inhibitory effect of metabolic activity is thought to be due to variation in synthesis method and concentration used. However, biological synthesis is considered to be safe with reduced toxicity (Naqvi et al., 2014). The varying anti-biofilm activity of AgNPs might also be linked to the variation in different strains of *Candida* species. Further, the inhibition in metabolic activity showed that mycogenic AgNPs may act as fungistatic agent by halting the activity of vital enzyme such as mitochondrial reductases among other enzymes of pathogenic importance.

Biofilm reduction comparison between CV assay and XTT assay

The significant positive relationship between biofilm reduction by CV assay and XTT assay, Pearson's $r = 0.87$, p value < 0.05 was observed. The difference in the degree of inhibition after AgNPs treatment was observed due to the variation in quantification methods employed. i.e.,

CV assay measures bulk biofilm whereas, XTT method detect the metabolically active cells within the biofilms with specificity (Melo et al., 2011). The higher inhibition observed by CV assay compared to the XTT assay is possibly due to the crystal violet staining the biofilms matrix. Furthermore, CV staining assay does not differentiate living cells from dead cells within the biofilms (Monteiro et al., 2015).

AgNPs coating on catheter surface

Catheter surface was coated with the AgNPs and confirmed visually by color change and FTIR analysis (Fig. 4). Change in the peak areas at 2959.77, 1379.17, 1038.84 and 835.32 cm^{-1} that depicted incorporation of AgNPs on the catheter. Major FTIR peaks observed on uncoated catheter surface were at 2929.71, 1273.59, 1072.86 cm^{-1}. Typically, peak at 422.5 cm^{-1} represented the presence of Ag in coated catheter surface, and it was absent in uncoated catheter (Fig. 4). The difference in other peaks on coated catheter surface compared to uncoated might be due to the slight variation i.e. increase or decrease in bond resonance. The FTIR peaks at 1028.14 and 1384.80 cm^{-1} of catheter after AgNPs coating indicated bio-reduction of Ag ions (Abraham et al., 2013; Elavazhagan and Arunachalam, 2011; Hettiarachchi and Wickramarachchi, 2011). The mechanism for the attachment of nanoparticles with solid surfaces was mainly due to the electrostatic forces of mycogenic AgNPs with the polymeric catheter. The stability and degree of the AgNPs coating depends upon different factors such as the solvent used for AgNPs suspension preparation, contact time of surface with the AgNPs suspension and environmental conditions. Furthermore, mycogenic components may act as a stabilizing agent of AgNPs that could help its interaction with catheter surface.

Scanning electron microscopy of biofilm deterrence

The structure of biofilm (24 h) developed on AgNPs coated and uncoated catheter surface was analyzed by SEM. Without AgNPs

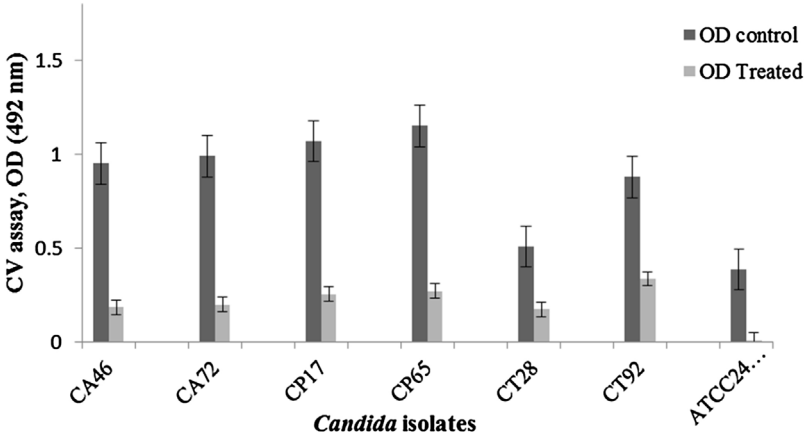

Fig. 2. Total biomass reduction assay of (24 h) *Candida* biofilms by Ag NPs treatment using Crystal violet straining (Mean absorbance at OD = 492 nm, 95% confidence interval).

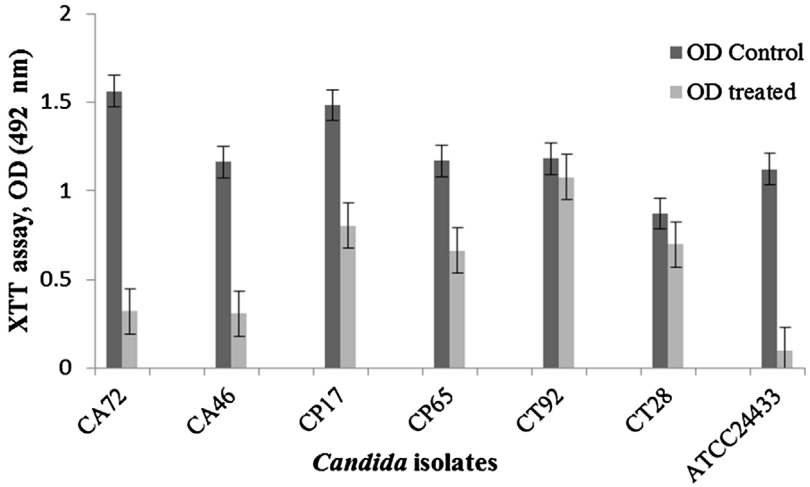

Fig. 3. Metabolic activity of *Candida* biofilms under the effect of Ag NPs using XTT assay.

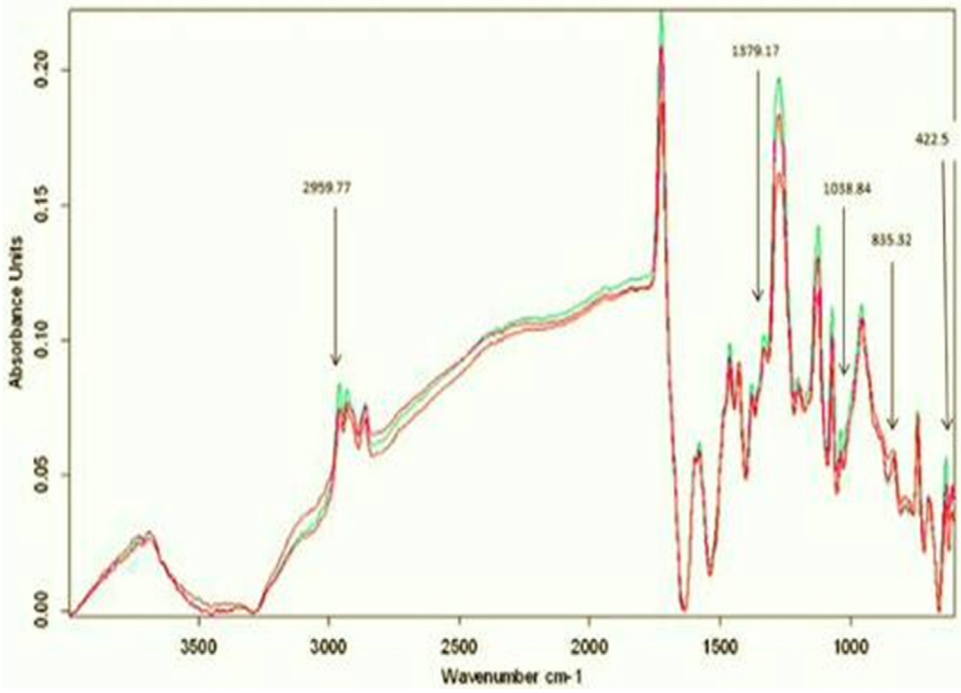

Fig. 4. FTIR spectra of Urine catheter surface with AgNPs with peak at 422.5 cm^{-1} for Ag (Green line) and without Ag NPs coating (red lines) depicting slight shift in the peaks due to variation in bond resonance typically at 2959.77 cm^{-1}, 1379.17 cm^{-1}, 1038.84 cm^{-1}, 835.32 cm^{-1}.

coating, a dense cellular growth of *C. albicans and C. parapsilosis* biofilm were observed and surrounded by exo-polymeric substances (EPS) (Fig. 5A and B). Patchy growth and reduced adherence of *C. albicans* and *C. parapsilosis* biofilm was noticed on catheter surface with AgNPs coating (Fig. 5C and D). SEM analysis depicted AgNPs targeting cell wall causing cell disruption, inhibiting hyphal growth and altered EPS secretion (Fig. 5C and D). Previous data also showed morphological and EPS alternation in *C. albicans* biofilms due to the effect of AgNPs (Lara et al., 2015; Monteiro et al., 2013). AgNPs interaction has also been linked with membrane permeability and changes in the target fungal morphology (Monteiro et al., 2014). In broad-spectrum, AgNPs interact by facilitating its binding to sulfur-containing proteins in biological molecules. Interactions of AgNPs with cellular membrane have been reported causing loss of intracellular contents, inhibition of respiratory enzymes and binding to phosphorus-containing compounds including DNA content thus loss of reproduction (Sule et al., 2009). Our finding is in coherence with the previously findings that showed the effect of AgNPs against *Candida* biofilms. However, incongruence with previous findings as most of the studies depicted the AgNPs effect on preformed biofilms. In our study, adherence of *Candida* cells was evaluated due to pre-coating of catheter surface with AgNPs. These findings provide enough support to use mycogenic AgNPs for reducing device associated infection that are mostly due to biofilm formation. In the recent past, various groups are working on device coating with AgNPs synthesized using different methods as biofilm preventive strategy thereby reducing associated infection (Abdulkareem et al., 2015; Roe et al., 2008; Thomas et al., 2015).

Cell viability of *Candida* on pre coated catheter surface

Viable cell counts in biofilm of *Candida* spp. were significantly reduced (p value < 0.05) on AgNPs coated catheter surface

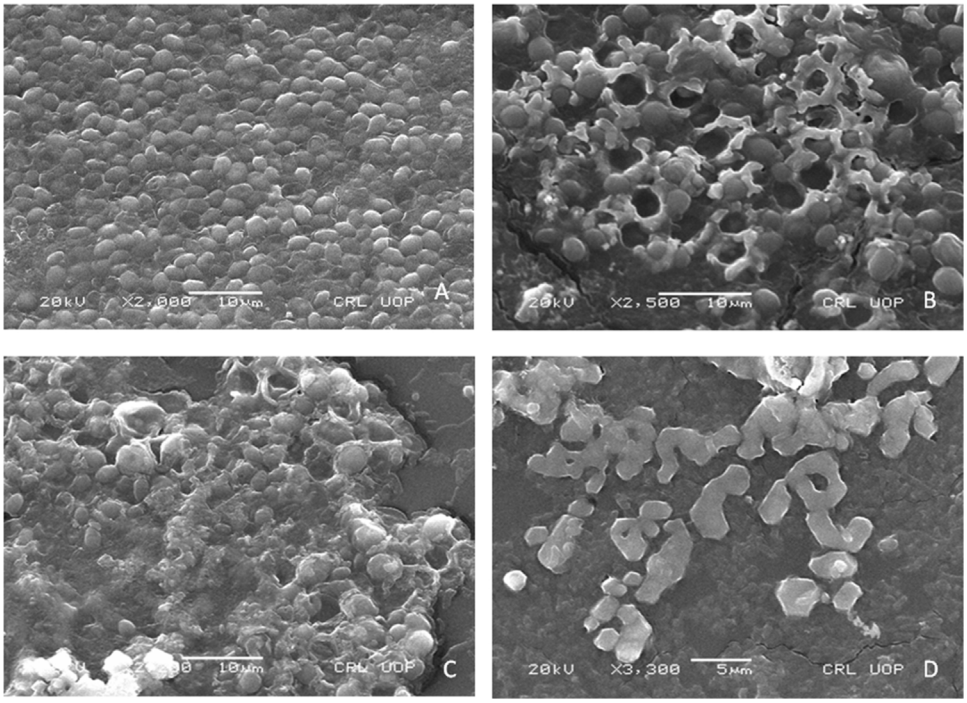

Fig. 5. (A) Scanning electron microscope displayed *C. albicans* biofilm (24 h) on catheter surface (control group) under magnification (×2, 000), (B) Scanning electron microscope displayed *C. parapsilosis* biofilm (24 h) on catheter surface (control group) under magnification (×2, 500), (C) Scanning electron microscope images of *C. albicans* biofilm (24 h) on Ag coated catheter surface (treatment group) under magnification (×2, 200), (D) Scanning electron microscope displayed *C. parapsilosis* biofilm (24 h) on coated catheter surface (treatment group) under magnification (×2, 500).

compared to uncoated ones. Though, viable cell counts were non-significantly differed in different species. It was maximum i.e. 0.75 $\log_{10}$ in CA46 followed by 0.65 in CT28 and 0.4 in CP65 (Fig. 6). Reduction in the cell viability was reported previously by ammonia stabilized AgNPs against *C. glabarata* (Monteiro et al., 2011). Reduction in viable cell count of oral *C. albicans* biofilms has been observed with AgNPs at 24 h and 48 h (Monteiro et al., 2015). The decline in cell viability may be caused by reduction in adhesion potential of the cells due to altered surface properties like roughness. The data presented by the previous studies and reported in our study showed the efficacy of biologically synthesized AgNPs as an effective anti-biofilm and biofilm preventive agent. The non-significant reduction in cell viability may be attributed to the temporary nature of the AgNPs coating on catheter surface. The release of the AgNPs into to the culture media allows *Candida* cells to adhere to the catheter surface where coating is either ruptured or removed. There is a need to formulate further methods that allow permanent coating on catheter surface thus inhibiting cell adhesion for long period of time. Previous reports also describe the methods of AgNPs coating on catheter surface thus presented antimicrobial potential. Reports indicated that coating was reversible and Ag NPs were washed away in few days. Now the focus is to develop the techniques that could be simple, cheap and sustainable for coating of metal nanoparticles. In addition the concentration of metal nanoparticles for coating should be optimized. The concentration should be non-toxic or less toxicity with greater biocompatibility. Furthermore, such metal nanoparticles should be developed that have broader range of anti-biofilm activity for different pathogenic bacteria forming biofilms on catheters. This will ultimately eliminate the possibility of any microbial growth on catheters. Metal nanoparticles coated would therefore help control of microbial attachment, colonization and at primary level mitigation of infectious microbes (Roe et al., 2008; Thomas et al., 2015).

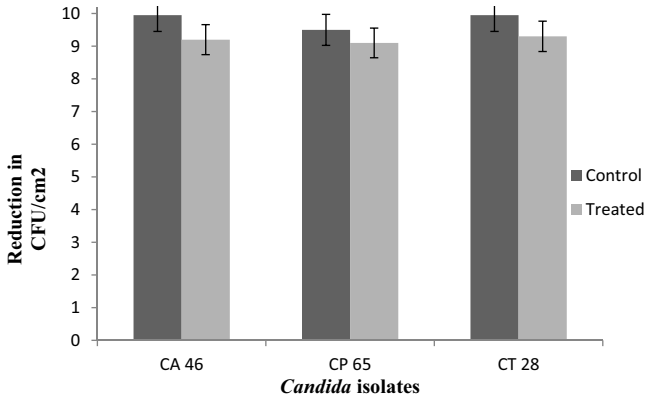

Fig. 6. Colony forming unit/cm^2 for *Candida* biofilms (24 h) on Ag NPs coated catheter surface.

Possible limitations of AgNPs can be addressed in terms of toxicity to human cells. The broad spectrum activity of AgNPs against range of microorganisms may cause imbalance to the normal human microbiota (van den Brule et al., 2016). Toxicological studies comprise an active area of research these days.

Conclusion

The research work provided an understanding on the role of biogenic AgNPs towards control of pathogenic biofilms formed by clinical isolates of both *C. albicans* and non-albicans. The need for toxic stabilizing agents and organic solvents are eliminated during the biological synthesis as stabilizers are biomolecules of protein and carbohydrate. The study also provided also provided some evidence on possible mechanism of action of AgNPs on biofilm control based upon physical and physiological basis. Moreover, the study provided another possible way of controlling catheter related infections by coating it with metal nanoparticles. Further

research work on development of metal nanoparticles coated catheter with more sustainable efficiency is needed in order to make it commercially viable.

Conflict of interests

The authors declare no conflict of interests.

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