International Journal of Advance Research in Science and Engineering
Vol. No.10, Issue No. 04, April 2021
www.ijarse.com
IJARSE
ISSN 2319 - 8354

Utility of synthesizedsilver nanoparticles fromfungal extracts of Aspergillus flavus against urinary tract infectious pathogen,E. coli.

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ABSTRACT

Infections in urinary tractsare considered as the most common nosocomial infections among the aged people worldwide. The prevalence of extendedspectrum beta (β) lactamase (ESβL) fabricating*Escherichia coli*in urinary tract infection is growingin most of the partsin the world, particularlyin under developed countries. The patients with in-dwelling urinary catheters remain as the highest risks of receiving infections. The fungal metabolites have the ability to reduce the AgNO3 to positively charged Ag ions which inhibit the growth of microorganisms like bacteria and *Candida albicans*. The antibacterial efficacy of silver compounds have been known to mankind from ancient time. It has been found recently that metallic silver nanoparticles retainnoteworthy antibacterial potentials. Externalalterations of urinary catheters with Ag NPs may prevent the threat of contamination as well as the associated infection. In our presentwork, we studied the antibacterial effect of Ag-NPssynthesized from *Aspergillus flavus*on ESBL producing *Escherichia coli* as well as other pathogenic bacteria isolated from UTI patients. Our current results found the possible utility of Ag-NPs as antimicrobial agents against UTI pathogens in particular to ESβL producing *E. coli*.

Key words: *Urinary tract infections (UTI), ESβL creatingE. coli,Aspergillus flavus, AgNO3, AgNPs.*

INTRODUCTION

Infectionsin urinary tractsare thought to be the most familiar hospital acquired or nosocomial infections that are most natural bacterial infections mostly affecting one hundred fifty million people each year over the world (Stamm and Norrby, 2001). It is one of the significant source of diseases in infants, old females and men of all the age groups. Serious infectionscontainrenal damage in young children, pyelonephritis with sepsis, frequent recurrences, preterm births and other impedimentsproduced by recurrentuse of antimicrobials, high-level antibiotic resistanceand*Clostridium difficile colitis*. Clinically, it is categorized as complicated or uncomplicated disease. UTIs of uncomplicated cases naturally affect the individuals who are elsestrong and have no neurological or structural urinary tractsdeformities, infection like these are distinguished into upper UTIs (pyelonephritis) and lower UTIs (cystitis)) (Foxman 2010, Nielubowicz 2010, Hannan et al. 2012, Hooton 2012). Problematical UTIs are demarcated as UTIs connected with causes that negotiationwith the host defence or urinary tracts,urinary obstruction,including urinary retention caused by renal failure, neurological diseases, immunosuppression, renal transplantation,pregnancy and the occurrence of external bodies likedrainage devices,calculi indwelling catheter (Lichtenberger and Hooton 2008, Levison and Kaye 2013).

Urinary Tract Infections are instigated by togetherGram-positive and Gram-negative bacteria, besidesfew fungi. Bestof the common causative agents for both complicated and uncomplicated UTIs are uropathogenic UPEC;*Escherichia coli*. Agents tangled in uncomplicated UTIs, the UPEC are followed in the prevalence by *Enterococcus faecalis,Klebsiella pneumoniae,* group B;*Pseudomonas aeruginosa,Streptococcus, Staphylococcus saprophyticus, Proteus mirabilis, Candida* spp and *Staphylococcus aureus* (Nielubowicz and Mobley 2010, Foxman 2014, Ronald 2002, Kline et al., 2011).For the complicated UTIs, the order of frequency for causative agents, subsequent UPEC as the most common, that is *K. pneumoniae*, *Enterococcus* sp., *S. aureus*, *Candida* sp., *P. mirabilisP. aeruginosa* and GBS (Jacobsen et al., 2008,Chen et al., 2013, Fisher et al., 2011, Levison and Kaye 2013,). Patient'sgrief from characteristic UTIs are normallydiagnosed with antibioticand these treatment can be in long-term effect modification of the regular micro-biota of the gastrointestinal tract and vagina and in enlargement of multidrug resistant microbes (Kostalioti et al. 2012).Current studies employed sequencing of RNA to analyse directly uropathogens from the women's urine of undergoing symptomatics UTIs.*Klebsiella*

*pneumoniae*and *Escherichia coli*are considered as the most formal causative agents ofurinary tracts infections (UTIs) in juvenileages (Sitthisarunkul et al. 2019, Moore et al. 2016). Both the species regularlyyield extendedspectrum β-lactamase (ESβL) enzymethatconvenethe resistance towardsβ-lactam antibiotics countingfourthand thirdgenerations'monobactams and cephalosporin(Bradford 2001, Pana and Zaoutis 2018). The amassed isolation of ESβL-producing *E. coliK.* and *pneumoniae*triggering UTI amongkids is of new worldwide concern due to the empirical failure treatmentthat may results in severe clinical difficulties such as prolonged hospitalisation,renal scarring and sepsis in comparison to the infection by nonESβL strains (Moore et al. 2016, Bradford 2001, Pana and Zaoutis 2018, Pitout and Laupland 2008, Tratselas et al 2011, Bee et al 2013, Ozcakar et al 2011, Kim, Yang and Kim 2017, Patwardhan et al 2017 and Kocak et al 2016). Isolation of ESβL +microbes limit the therapeutic routes and the relevant patients regularlyneed parenteral antibiotic's therapies. Preferably, empirical suitable antibiotics should be recommendedinstantly on the demonstration of suspected UTIs before their vulnerabilityresults are obtainable but most empirical routines lack action against ESβL optimisticmicrobial isolates. Therefore, the majority ESβL treatments and diagnoses are often postponed subject to antimicrobial vulnerabilitydocuments.

Most common approach relies on using antibiotics; however, these are only effective against bacterial infection in short-term catheterization (Warren, 2001). Recently, silver nanoparticles (AgNPs) have been of particular interest as they exhibit efficient long-term toxicity to a wide range of various bacteria, yeast, fungi and antibiotic-resistant microorganisms (Rai et al 2012). However, they offer low toxicity to mammalian cells (Kumar and Sujitha 2014, Pollini et al., 2011, Li et al 2009). Based on these distinctive excellent properties of AgNPs, they have been widely used for antimicrobial applications in numerous fields, like fabric, food storage, cosmetics, medicine, and medical devices (Rai et al 2012, Burdusel et al., 2018, Sim et al., 2018). The antimicrobial action of AgNPs depend on their shape and size. The spherical shape with the smallest size exhibits the strongest antimicrobial action against *E. coli* in comparison to the spherical, larger and triangular shape due to the large high-atomic-density and volume to surface ratio facets (Cheon et al., 2019, Raza et al., 2016).

In particular, the AgNPs with spherical particle diameter in the ranges of 1–10 nm have the highest effectiveness for direct interaction with the bacterial cell surface (Pollini et

al., 2011, Morones et al., 2005, Agnihotri et al., 2014). Consequently, variety of AgNPsproduced by synthetic methods including chemical, biological and physical methods, developed in recent years highlightsmonitoring the size, shape, and stability of AgNPs. Among those methods, the chemical method provides colloidal AgNPs with good uniform size distribution and dispersion stability. Unfortunately, it also produces a large amount of chemical waste resulting in environmental pollution (Xu et al., 2020). Biological method based on using varied biological strains, like microbes and parts of plants, has therefore emerged to synthesize AgNPs using a green chemistry approach with simple steps and less toxicity (Majeed et al., 2018). The current study aims with the isolation and confirmation of ESβL producing *Escherichia coli* form UTI suffered patients and to analyses their antibiotic sensitivity pattern against few drugs combined with AgNPs.

MATERIALS AND METHODS

Fungal strainsand their extracts

The airborne fungiwere isolated from the outdoor environments of Sathyabama University campus, Chennai, Tamil Nadu.Highly proliferating fungi such as *Aspergillus niger, Aspergillus flavus* and*Aspergillus fumigatus* were isolated from the mixed culture PDA plates and which were grown in a PDA slant tubes as a pure culture. After 7 to 14 days of incubation at 27 ^{o}C, the all the three *Aspergillus* spp were transferred from the purecultured test pubes to 250ml Erlenmeyer flask which contain 150 ml of Yeast Extract Malt Broth (YEMB). The fungal inoculated flasks were kept in incubator at 27^{o}c for 14 days as in still culture. Over the incubation period, the mycelia free fungal extracts were filtered and stored in refrigerator at 4^{o}c.

Bacterial Strains and Culture condition

Collection of different urine samples were made from UTI infected patients from various hospitals of Hosur. Urine samples were injected on Mac Conkey agar mediated plate and hatched 37°C for 24 hrs. The colonies of sequestered organism were sub cultured on nutrient agar plate and pure culture were achieved in various discerning media such as EMB agar media as well as other Chemicals including antibiotics, buffers and media were procured from Hi Media. Green metallic sheen colored isolates were reflected to be *E. coli* and the probable colonies were biochemically verified for growth on for oxidative/fermentative

degradation of glucose triple sugar iron agar (TSI), urease production, citrate utilization, indol fermentation, lysine iron agar (LIA) tryptophan degradation, glucose degradation (methyl red) and motility test. The *Escherichia coli*isolates were deposited in tryptic soy broths with 15% glycerol at -20 °c. Colony confirmation was performed employing molecular method (PCR). Molecular validation of bacterial clones was dogged according to the 16S rRNA gene regions from *Escherichia coli.*

AgNPs Biosynthesis

Synthesis of AgNPs was organized as designed by Nayak et al., (2022) with some modifications. Fungal filtrates were obtained by separating the fungal biomass from the suspension utilisingthe No.1Whatman filter paper to complete the biosynthesis process. 1mM of concentrated AgNO3 stock solutions were prepared.Addition of 1ml of AgNO3 was made to nine ml of culture filtrates of all the three *Aspergillus* spp separately. Thesolution sample was assumed as the reaction mixtures. Three reaction mixtures were preserved in the dark condition for 72 hours at normal laboratory condition by wrapping with aluminium foil above the test tube to escape photochemical reaction during experiments. After 72h, centrifugation was made tothe samples of reaction mixture at 10,000 rpm for 35min for three times and the pellets were kept for further characterizations.

Biosynthesised AgNPsCharacterization

Biosynthesised silver nanoparticle were visually confirmed by colour changes of the mixture reaction containing tube from yellow colour to golden brown/black. After the centrifugation, pellets were unruffled in powder forms. TheAgNPs powder was again added in 1ml of doubled distilled waters. Synthesised AgNPs in water solutions were examined at diverse instrumental analyser such as UV-Vis Spectrophotometry (range of 350-700nm), Scanning Electron Microscopy analysis (particle size, surface morphologies), Fourier-Transform Infrared (surface dimensions) as well as X-ray Diffraction analysis (metallic nature, crystallinity, cubic structure etc)

Antimicrobial activity of synthesised AgNPs

Antibacterial action of mycosynthsised AgNPs was accomplished by agar disc diffusion methodsin contrast to urinary tract infectious pathogen,*E. coli.*Overnight fresh culture of *E. coli* strain was wiped uniformly onto individual plates separately. The 30µl, 40µl

and 50µl of AgNPs solution permeated disc were placed onto the plate and gestated for 24 h at 37^{o}c. Commercially availableantibiotic discs were used as control. The entire test was done in triplicate. Over the incubation period, different levels of clear inhibition zone were formed around the disc which was measured by zone scale (Hi media).

RESULTS AND DISCUSSION

In total, 23 fungal species including sterile mycelia were isolated from the outdoor environments of the Sathyabama University campus. Among 23, the highly proliferating three *Aspergillus* sp such as*Aspergillus flavus*,*Aspergillus fumigatus* and*Aspergillus niger*were selected for AgNPs synthesis.Based on the mixture reaction change from yellow colour (control) to yellowish brown (moderate AgNPs) and yellowish black (highly AgNPs) were confirmed as the presence of AgNPs (Fig). Among the three-reaction mixture, the culture filtrate of *Aspergillus flavus* mixed with AgNO3 were turned to deep yellowish block coloured while the culture filtrates of *A. niger* and *A. fumigatus* were mixed with AgNO3 were gives the pale-yellow brown colours. The incidence of colourchange may be caused by the excitation of surface plasmon resonance in the metal silver nanoparticles (Nayak et al., 2018; Zarina and Nanda, 2014a;Nayak et al., 2013Nayak and Anitha, 2014).The synthesised silver nanoparticles powder from the reaction mixture of *Aspergillus flavus* was taken for the instrumental characterization.

UV- Vis analysis

UV-Visible spectroscopy is commonly utilised to confirm the synthesis of AgNPs. Conducting electron start wavering at certain wavelength sort due to surface plasmon resonance (SPR) effects. The aqueous suspension of AgNPs of A. flavus were given the narrow peak at 406 nm which clearly demonstrated the existence of AgNPs in the reaction mixtures (Fig 1).

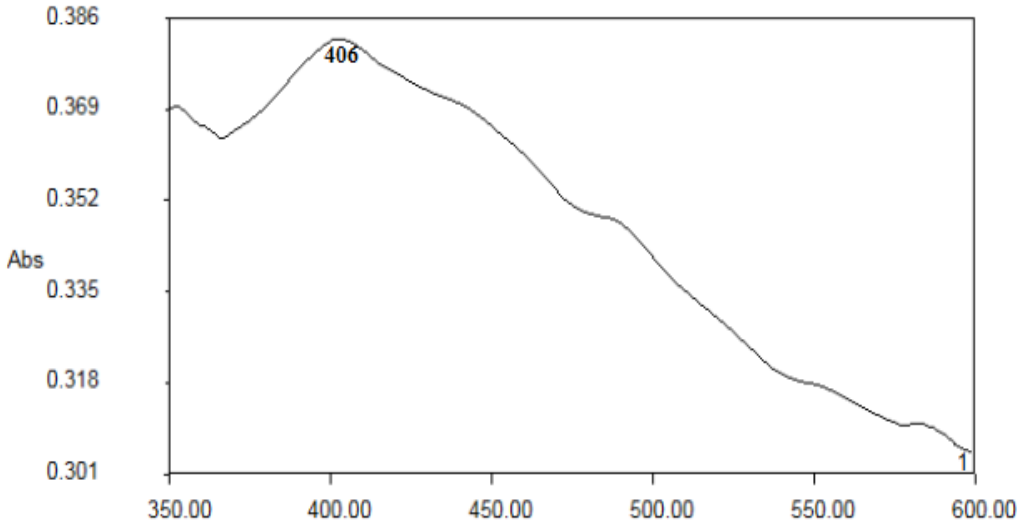


Fig 1:UV-Vis spectroscopy absorption spectra of AgNPs of*Aspergillus flavus*

SEM analysis

Analysis by SEM was done to disclose the size &shape of the nanoparticles (Fig2). SEM images were seen in various magnification ranges from 1 um to 200um which visibly demonstrated the spherical shaped nanoparticles.The average diameter of spherical shaped nanoparticlesis 55nm.

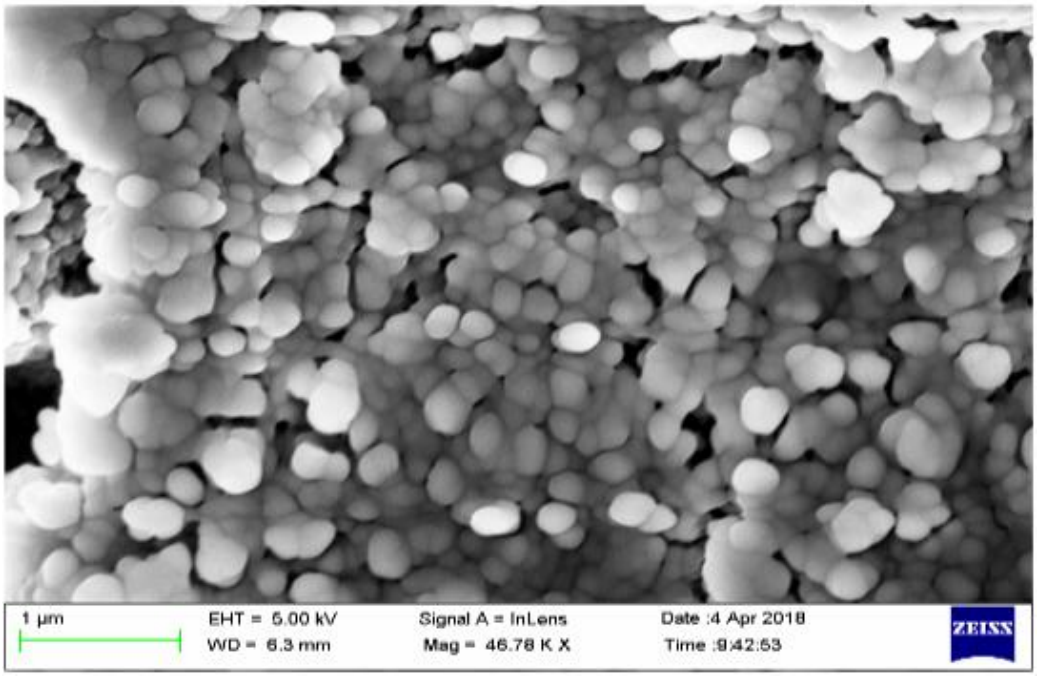


Fig 2: SEM photography of silver nanoparticles of *Aspergillus flavus*

FT-IR analysis

The FTIR spectra of Ag NPs synthesised from reaction mixture of *Aspergillus flavus* sample exhibited the absorption bands at different transmittance such as 3,449.0, 2,851.8, 1,383.8, 2, 033.5, 534.8 and 466.4 (Fig3). Functional groups likeC-O-C, CH3-R, N-H, C-H Stretch, aromatic C-C skeletall vibrations, thioester, -NO3, S-S stretch and COO were perceived at diverse wave records. FTIR readings confirmed that the carbonyl group of peptides of proteins and amino acids have strong similarity to bind metallic ions and therefore they may summarize nanoparticle leading to their steadiness in AgNPs synthesised from *A. flavus* in our current study, that was established by previous reports (Zarina and Nanda, 2014b, Johnson 2014).

Analysis of X-Ray Diffraction

XRD spectra gives an awareness about the crystallinity of nanoparticles. (Fig 4) This technique employed to concludemetallic nature of nanoparticle. X-rays are really electromagnetic radiation with photon energy among the range of 100 eV – 100 KeV. The nanoparticles of *A. flavus*displayed the XRD peaks value at 28, 34, 38, 45, 55, 57 and 78. The present result agreed with the preceding studies completed by the following workers (Majeed et al., 2021; Muthukrishnan et al., 2019b; Nanda and Majeed, 2013; Saravanan and Nanda, 2010).

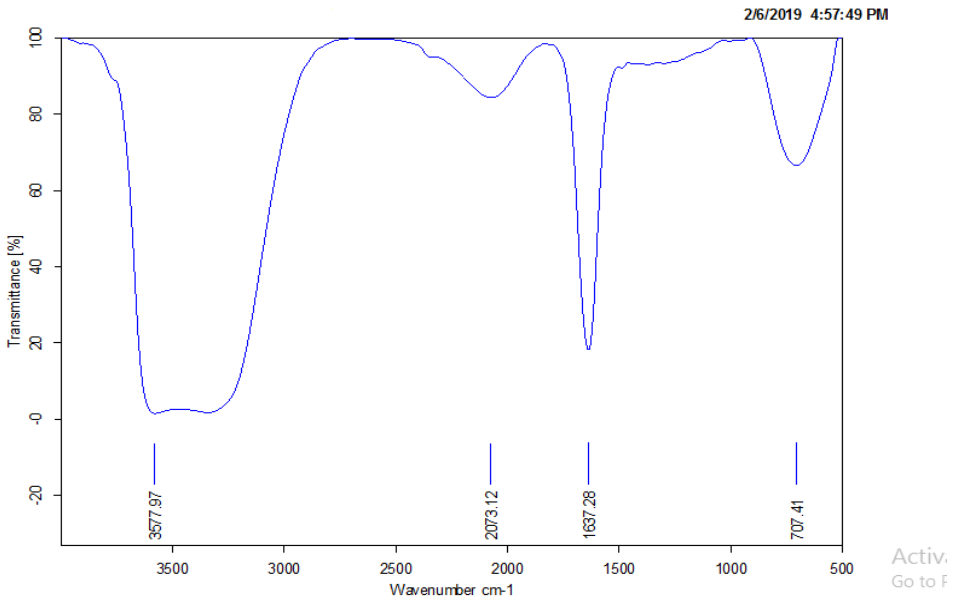


Fig 3: FTIR spectra of silver nanoparticles synthesized from *Aspergillus flavus*

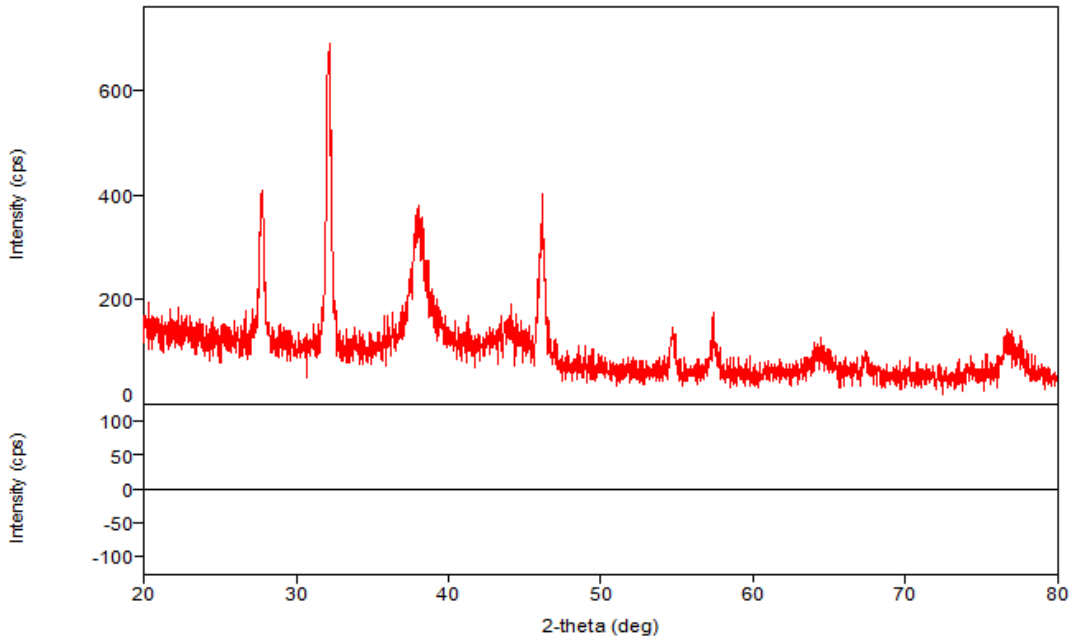
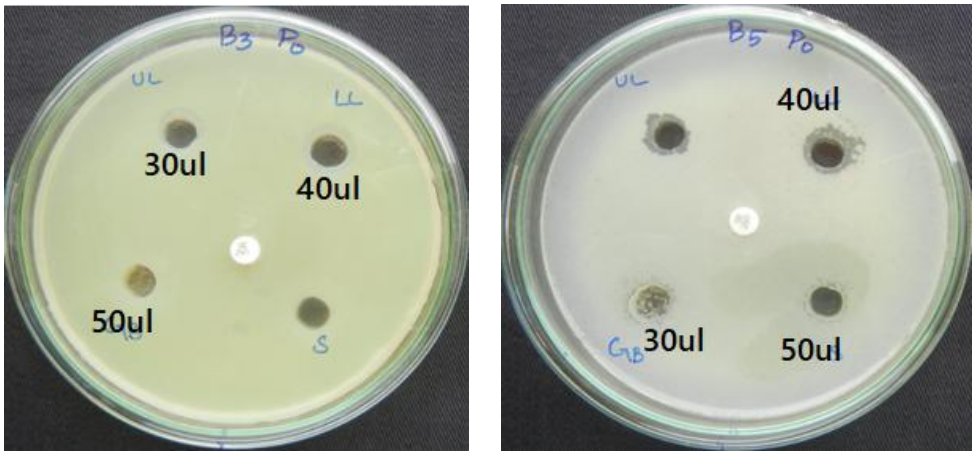


Fig 4: XRD analysis of silver nanoparticles synthesized from *Aspergillus flavus.*

Antimicrobial assay

During the recent study, Agar discs diffusionsmethod was smeared to achieve the antibacterial assay. Antibacterial effect of AgNPs was assayed against UTIs pathogen *E. coli*. Results clearly displayed that the nanoparticles exhibitedgood antibacterial effect in dosedependent fashion (Table 1). Clear zonation observed in all the three concentration like 30 µl, 40 µl and 50 µl (Fig 5). However, the maximum inhibition zone occurred on 50µl disc against *E.coli*. Different mechanisms of action of nanoparticle against bacteria has been conveyed in previous literatures. This paper evaluated the silver nanoparticles synthesized from airborne micro fungi and their efficacy towards the ESβL manufacturing*Escherichia coli* isolated from the urine sample of UTI infected people in and around Hosur, Tamil Nadu. We faced difficulties instruggling these resistant bacteria and prospectiveand effective agents mostly nanoparticles for the treatment of UTIs triggered by multidrug resistant pathogens like *E. coli.*Ceftazidimeantibiotic (Fig 5) did not show any effect against the UTI pathogens at all.



Staphylococcus aureus ***Escherichia coli***

Fig 5: Preventive potency of AgNPs from *Aspergillus flavus* over UTI pathogens

Table 1: Antibacterial assay of AgNPs from fungal extract of*Aspergillus flavus.*

UTI pathogens	AgNPs of fungal extract of *Aspergillus flavus.* **Zone of inhibition in mm**			
	30 µl	**40** µl	**50** µl	**Ceftazidime**
Staphylococcus aureus	10	12	13	-
Escherichia coli	12	14	15	-

CONCLUSION

Fungal metabolites as well as the fungal mediated AgNPshave unique properties in order to prevent the growth of wild and drug resistance bacteria and fungi. In our present study we found that the surface alterations of urinary catheters with the AgNPs inhibited the threat of contaminations as well as the associated infections. Antimicrobial effect of AgNPs synthesized from *Aspergillus flavus* on ESβL producing *Escherichia coli* as well as other pathogenic bacteria isolated from UTI patients was found good in our study. Studies on the same work may give ampule evidence for preventing drug resistance pathogens in future in order to recover from varied dreaded diseases.

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