Green Synthesis Using Klebsiella pneumoniae as well as its Execution onto Textiles for Microbe Resistance

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Abstract. Microorganisms like bacteria and yeast and their use in the combination of nanoparticles is a basically a recent phenomenon. These microorganisms lessen the toxicity by decreasing the ions of metal or through the creation of complexes which are insoluble along with the metal’s ions (as metal sulfides) as colloidal elements. The present study focuses on bio-synthesis of silver nanoparticles by the bacterium Klebsiella pneumoniae. The reduction of AgNO$_3$ to Ag nanoparticles was due to the extracellular production of the enzyme nitrate reductase by the K. pneumoniae into the medium. The particles were characterized by SEM. The biosynthesized Ag nanoparticles were padded on the fabrics of cotton by cure method of dry pad. The preliminary antimicrobial activity was performed by disc diffusion method against Escherichia coli and Staphylococcus aureus. The antimicrobial property of the treated fabrics were confirmed qualitatively and quantitatively by parallel streak (AATCC - 147) and challenge test (AATCC -100) respectively. SEM results revealed the form of the nanoparticles were in range of 50 - 60 nm. An area of inhibition of 7 mm as well as 5 mm were found against S.aureus and E.coli respectively by disc diffusion test. Parallel streak method also confirmed the antimicrobial activity. The results of the challenge test revealed the fabrics treated with biosynthesized nanoparticles depicted 100% minimum point in comparison with S.aureus and 99% reduction against E.coli.

Keywords: Antibacterial, Silver nanoparticles, Cotton, Biosynthesis

1. Introduction
Nanotechnology lets us have the capability to design the characteristics of materials by restraining their size, moreover, it has led the study toward a various potential utilizations for nanomaterials. The minimization of materials dimension has given away effects on the physical characteristics that might be hugely different from the relative bulk stuff. Nanoscale materials utility and its forms, generally covering from 1 to 100 nanometers (nm), is an emerging avenue of nanoscience as well as nanotechnology. Nanomaterials might give away answers to the challenges technology as well as environment.[1-5]. The term “green nanotechnology” has ignited the interest. In the global attempts to lessen created dangerous left over, “green” chemistry as well as chemical processes are increasingly combining along with modern growth in the technology and industry.[6-7] The growth of biologically gives rise to experimental processes for the creation of nanoparticles is growing into a significant part of nanotechnology [6-10].

The need for bioactive or antimicrobial and UV-protecting textiles has been raised because of the growth in awareness of health and hygiene. The emergence of nano science as well as technology, a
latest avenue has grown into the proximity finishing of textile. Nanocoating surface of textiles and he overing is one way to access creation of intensely prompt surfaces to take up blocking of UV, antimicrobial as well as selfcleaning charaeristics. The antimicrobial charaeristics are pushed forward by nano-silve$^{11,12}$. Despite silver nitrate being generally utilized as antibacterial agent $^{[11-15]}$, its staining charateristics $^{13}$ was a way forward for nano-silver.

With the intense requisition of “green” synthesis of nano particles processes$^{14}$, nano particle synthesis’s area has lately grown fresh avenues. Nanoparticles of silver are usually of some use in fields like biological labeling, photonics, photography, catalysis, optoelectronics and surface-improved detection of Raman scattering (SERS) $^{[16-20]}$. The present study exploits the antimicrobial activity of silver nanoparticles in developing a novel approach for antimicrobial finishing of textiles. In this work, nanoparticles of silver (Ag NPs) were prepared through green synthesis base which have advantages over traditional ways incorporating chemical agents in connection with toxicity of environment. Silver nanoparticles were characterised by UV- Visible Spectroscopy and by Scanning electron microscope (SEM) analysis $^{[21-25]}$. An broader research was executed to measure effectiveness of antimicrobial of the silver nanoparticle treated cotton fabric by taking up a test of good standard ways as well as results are elaborated in the paper.

2. The Materials as well as Methods

2.1. The Materials
The reagent grade chemicals silver nitrate, nutrient agar and Muller-Hinton broth (MHB) was obtained through Hi-media, Mumbai in India. Fabric with specification as in table 1 was bought through National Textile Corporation Limited, Coimbatore, Tamil Nadu, in India.

| Type | Fre finishing | Warp count | Weft count | Ends per inch | Picks | Width |
|------|--------------|------------|------------|---------------|-------|-------|
| 100% waves cotton fehre | Elearched | 20 E | 20 K | 54 | 4C | 122 CMS |

2.2. Bacterial Strains
The strain Klebsiella pneumonia MTCC 4352 was utilized for the combination of silver nano particles. Escherichia coli MTCC 25922 (Gram negative) and Staphylococcus aureus MTCC 25923 (Gram positive) were used for this study to determine the antimicrobial activity of silver nanoparticles for these two are strains of reference utilized for antimicrobial susceptibility testing as per AATCC formula $^{[26-28]}$. Strains have been maintained by culturing through nutrient agar medium and incubating aerobically at 37$^0$C overnight.

2.3. Making of the Supernatants
MHB, Muller-Hinton broth has been designed, inoculated as well as sterilized along with a new group of test strain. Flasks of Culture had been prepared by incubating for 24 hrs at 37$^0$C with moving at 150 rpm. When this period gets over, cultures get confluenced at 12,000 rpm for 5 minutes as well as the supernatant was gathered. The supernatants have utilized like beginning suff for combination of nano particles.

2.4. Synthesis of Silver Nanoparticles
Conventional combinaion of silver nano particle extra cellularly, 50 mL aqueous solution of 1 mM silver nitrate ($\text{AgNO}_3$) has been worked along with 50 ml K. pneumonia supernatant solution in a 250
3 mL Erlenmeyer flask (pH adjusted to 8.5). All the mixture was poured into a shaker at 40°C (200 rpm) for 3 days as well as adjusted in the darkness. Restrain experiments have been executed with uninoculated medium, to evaluate bacteria’s performance in the making of nanoparticles.

2.5. Silver Nanoparticles Characterisation

2.5.1. UV. Visible Spectroscopy
The decrease of Ag⁺ ions has been observed through modeling an aliquot (2 ml) of the solution on the gaps of 24 hrs. and calculating the solution’s UV-Vis spectra. Immersion measurements were executed on a Beckmann UV-Visible spectrophotometer. The spectra has been taken into record at the temperature of room utilizing a quartz cuvette of one-centimetre.

2.5.2. SEM Analysis.
T dropcoating bio synthesized silver nano particles solution on carboncoated copper SEM grids (40 µm x 40 µm mesh size) designed the scanning microscopy of electron (SEM) dissection of extra cellular combined silver nano particles. Before keeping them onto a specimen holder Samples were sucked out of water and put under vacuum in desiccators.

2.6. Preliminary Assessment of Antimicrobial Activity by Disc Diffusion Method
AATCC Bacteriostasis agar was prepared, sterilized and dispensed in sterile Petridishes. Overnight broth cultures of test organisms were used as an inoculum. The test organisms were swabbed over the surface of the agar plate (mat culture) using sterile cotton swab. Empty sterilized discs of Whatmann no.1 filter paper (diameter 2.0 cms) were each impregnated with 100 µl of silver nano particles. Discs were kept at agar plates and the plates have been incubated at 37°C, 24 hours. When the incubation is over, the area of inhibition was calculated.

2.7. Coating of Silver Nanoparticle on Cotton Fabric
Silver nanoparticle was executed at 100% fabric of cotton to a soggy stuff of 100% utilizing ‘pad-dry-cure’ formula. The fabric of cotton (size: 30 cm x 30 cm) has been absorbed into solution comprising silver nanoparticle for 10 min afterwards it went through a laboratory padding mangle (RBE Make), that was moving with a speed of 20 rpm at the same time having pressure of 1.5 kgf cm⁻² to do way with solution which is excess. When padding is done, fabric has been airdried and then healed for 5 min at 70°C. Afterwards treated samples have been measured to activity of antimicrobial.

2.8. Assessment of Antimicrobial Activity
Antimicrobial activity was measured by both the test methods, qualitative and quantitative. Given below are the elaborations of test methods utilized for this research.

2.8.1. Qualitative Assessment by Parallel Streak Method (AATCC Test Method 1471988)
Control fabric samples reaed and untreated kept in close touch with AATCC bacteriostasis agar, that has been before only inoculated (Mat culture) with an inoculum of test organisms. The two test organisms termed as; Staphylococcus aureus and Esherichia coli were utilized for the research. When the incubation is complete, a clear zone of consistant growth underneath and along the test’s side stuff marks antibacterial fabric usefulness.

2.8.2. Quantitative Assessment through Reduction Test (AATCC Test Method 100)
The models of the material of test were heaved in a familiar suspension’s concentration of he bacteria as well as decrease in bacterial reaction in good time is calculated. Potential of antimicrobial process is calculated through comparing decrease in concentration of bacteria of the treated sample with that of restrain model manifested as a percentage decrease in he standard time.

\[ \% \text{Reduction} = \frac{A-B}{B} \times 100 \]
Over here A as well as B are the cells which are surviving (CFU/ml) for fasks comprising control (blank cotton fabric) and samples of the test (silver nanoparticles treated cotton fabric) respectively, after 18 hrs of time of contact.

3. Results and Discussion

3.1. Silver Reduction
Silver nanoparticles’s formation through supernatants of the culture of K. pneumonia has been inspected. Look of a yellowish brown color in the activity containers indicated creation of silver nanoparticles [29]. It delineates two conical flasks with the supernatant of K pneumonia before (left flask) and after reaction with Ag (right flask). The silver containing solution before reaction, (left flask) is without color but transforms into a brownish color on fulfillment of the reaction (right fask) Various hydroquinones with the best redox characteristics were stated that could work as electron shuttle in metal reductions [30]. Hence, it was that electron shuttles or other reducing agents delivered by K. pneumonia have the potential of decreasing silver ions to silver nano particles. While, the decrease of silver ions too happens in bacterial cells’ absence. It shows clearly that decreasing agents which are delivered of the K. pneumonia’s cultures are incorporated into reduction method.

3.2. UV-Visible Spectroscopy
Spectroscopy of UV visible characterizes the nanoparticles of silver. The technique introduced above has been significant for the analysis of nanoparticles [19-21]. Fig. 2 elaborates the UV-visible spectra had a record from K. pneumonia the aqueous silver nitrate-culture supernatant reaction medium like an activity of time of reaction. Potent resonance focused at around 430 nm is visible clearly and enhances along the time. Steep decrease in action time, from a few number of days to some minutes, noted for the culture supernatant of K. Pneumonia has been intensely important move toward gaining the aim of growing a fast formula for silver nanoparticle synthesis. Notice of this zenith, designated to a Plasmon of surface, has been properly documented for many metal nano particles along the sizes which has a range from 2 to 100 nm [19,21]. Firm surface plasmon echo of K. pneumonia was focused at ca. 430nm. The time exposed to the ‘K’ pneumonia supernatant, aqueous silver ions had increasingly decreased in solution.

![Figure 1. Silver nanoparticle colloids’ UV-vis spectra of silver.](image)

3.3. Particle Size by SEM Analysis
The silver’s SEM image nano particles combined through the treatment the silver nitrate solution with K. pneumonia’s culture supernatants is depicted in Fig 3. The SEM image clearly shows that the particles were roughly spherical of size moving from 60 to 80 nm.

![SEM image of silver nanoparticles](image)

**Figure 2.** Scanning electron micrograph of silver nitrate solution has a treatment with K. pneumonia’s the culture supernatant for 5 min.

### 3.4. Preliminary Assessment of Antimicrobial Activity by Disc Diffusion Method

Results of formula for disc diffusion against the standard model organisms S. aureus (positive Gram) and E. coli (negative Gram) was labelled in Fig 4. Around the filter paper, there is a clear zone of inhibition, impregnated with silver nanoparticle opposite two of the test species in contrast to the control system that allowed organism to grow.

Silver nanoparticle impregnated filter paper delineated a zone of 20 mm for E. coli as well as 17 mm inhibition for S. quareus this indicate the antimicrobial activity of silver nanoparticles. Similar results were obtained, 17 mm for E.coli and 14 mm for S. aureus of inhibition zone.

### 3.5. Qualitative Assessment by Parallel Streak Method (AATCC Test Method 1471988)

At the time of measuring the antimicrobial reaction of silver nanoparticle form cotton fabric as resulted with similar Streak formula a clear zone of reticence was noted for two the tests species. Fig 4 defined the outcome of matching streak formula.

![Graph showing zone of inhibition](image)

**Figure 3.** Parallel streak method for silver nanoparticle and sulfer nitrate bulk.

In this case, the area of reticence was noted to be 34 mm for E. coli and 30 mm for S. aureus. A zone of 23 mm and 20 mm was obtained for silver nitrate bulk. The clear zone that is observed may be due to the gradual delivery of silver nanoparticle from the fabric on to the medium.

### 3.6. % Reduction Test (AATCC Test Method 100) I’s Quantitative Assessment

In the check to E. coli, S. aureus in broth with AATCC bacteriostasis, gave a vaccination to restrain and inoculated vaccines finished cotton fabric with silver nanoparticle was measured for bacterial proportion declining by cell count. The table. 2 depicted the effect of the percentage reduction tests.
Table 2. Percentage Reduction of both test organisms

| Substrate                        | Organisms | Survival cells (ch/ml) | % reduction |
|----------------------------------|-----------|------------------------|-------------|
| Cotton fabric treated with silver nanoparticle | E. coli   | $7 \times 10^6$        | 100         |
|                                  | S. aureus | $0 \times 10^6$        | 100         |
| Cotton fabric treated with silver nitrate bulk | E. coli   | $7 \times 10^6$        | 42.8        |
|                                  | S. aureus | $4 \times 10^6$        | 37          |

The reduction % for E. coli as well as S. aureus relate number of the bacterial on the respective control panel test of $7 \times 10^6$ per milliliter. In this study the decline % was achieved to 100% for both S. aureus and for E. coli.

4. Conclusion

Here, a low cost method was elaborated by us for decreasing Nanoparticles are made of silver solution. Though many reports on the biological combination of silver nanoparticles was published, The purpose of those reports was to use bacteria and fungi cell masses for combination. Such process is gradual due to the time needed for complete reduction of silver nitrate. UV- vis spectrophotometer and SEM analysis characterizes Silver nanoparticles. Silver nano particles were located within the range of 60-80 nm. Results established that silver nanoparticles depicted maximum reaction at a least concentration, which depicted silver nanoparticles as new antibacterial medium. In future, we are interested to increase the stability of the silver nanoparticle and speed up the reaction for the synthesis. We are also interested to study the antifungal activity of the silver nanoparticles.

5. References

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