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Research Paper

Antibacterial finish for Hospital dressing using Silver Nanoparticles from ESBL producing *Pseudomonas aeruginosa*

Microbiology

KEYWORDS :

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ABSTRACT

In this study, Pseudomonas aeruginosa was isolated from pus samples and was characterized using phenotypic and genotypic methods. It was confirmed to be an ESBL producer through Double Disc Synergy assay. This was then used to produce silver nanoparticles using two different concentrations of silver nitrae over a period of 24 hrs. The produced nanoparticles were characterized using UV spec analysis, FTIR and SEM. The antibacterial activity of the nanoparticles was checked. These were further coated onto hospital dressing cloth and this cloth was subjected to antibacterial screeing. It had the highest absorbance at 370 nm and the synthesis was maximum when the silver solution was used at 1mM concentraton. FTIR characterization showed that the nanoparticles contained different functional groups.SEM results showed that the nanoparticles were smaller than 1µm in diameter and the smaller particles appeared at 93 nm and the larger ones appeared at 120 nm. These nanoparticles alone and also when coated on hospital dressing cloth showed high antibacterial activity.

Introduction

Nanoparticles are of great scientific interest as they are effectively a bridge between bulk materials and atomic or molecular structures. Silver is a metal which has been proved to possess antibacterial activity [1] and thus has been used for many such applications like to sterilize recycled water to aboard the MIR space station and on the NASA space shuttle [2]. . Silver based topical dressing [3] has been widely used as a treatment for infections in burns, open wounds and chronic ulcers. Silver nitrate is still a common antimicrobial used in the treatment of chronic wounds [4]. Owing to their small size, the total surface area of the nanoparticles [5] is maximized, leading to the highest values of the activity to weight ratio [6]. AgNPs are attractive because they are non-toxic to the human body at low concentrations and have broad-spectrum antibacterial actions [7].

ESBL organisms are those which are capable of producing Exteded Spectrum β- Lactamase enzyme, which render the organism resistant to antibiotics possessing β- lactam rings in their structure and thus is a cause for major concern. Because of their spectrum of activity against oxyiminocephalosporins, these enzymes became to be known as extended spectrum β-lactamases (ESBLs) [8]. ESBLs are often located on plasmids that are transferable from strain to strain and between bacterial species. Although the prevalence of ESBLs is not known, it is clearly increasing, and in many parts of the world 10–40% of strains of *Escherichia coli* and *Klebsiella pneumoniae* express ESBLs.

Materials and Methods

Phenotypic Identification

Pseudomonas aeroginosa isolated from pus samples was collected from a private hospital in Coimbatore. Then the collected samples were brought into the laboratory and sub cultured for further study. The organism was subjected to Gram's staining, culturing and Biochemical characterization.

2.5. Genotypic Identification of the organism

Genotypic identification of the organism was done by 16S rRNA sequencing.After sequencing, the sequence was subjected to BLAST with NCBI after to confirm the genus and species. Based on this sequence a phylogenetic tree was constructed using MEGA4.0 software.

Identification of ESBL organism by Double Disk Synergy Test:

In the Double Disk Synergy Test, Ceftazidime (30 µg) and Co-AmoxyClav (20 µg Amoxicillin + 10 µg Clavulanic acid) discs were used. ESBL production is inferred if the inhibition zone around the test antibiotic disk increases towards the Co-AmoxyClav disk.

The confirmatory test with Ceftazidime was done to confirm the production of ESBL. A zone diameter of 5mm or more shows that the organism is an ESBL producer. Resistance to the third generation cephlosporins is highly suggestive of the presence of ESBL producer [9].

Biosynthesis of Silver nanoparticles

The overnight culture of *Pseudomonas aeroginosa* was centrifuged at 10,000 rpm for 3min. The supernatant was collected and the flask was covered with black paper for preventing the entry of light. Silver nitrate was added at two different concentrations 1mM and 2mM.The flask was kept for continuous shaking for 24hrs at 150rpm for 37$^{oC.}$

Characterization of Silver nanoparticles

UV-Visible spectroscopy analysis.

The synthesized nanoparticles were initially characterized with UV spectrophotometer. The bioreduction was monitored by sampling of 2 ml aliquots and subjecting to spectroscopy. Absorption measurements were carried out on Elico SL159 UV-Visible Spectrophotometer at a resolution of 1 nm. UV-Vis. analysis iswas done in the range of 200nm to 600nm.

FTIR spectroscopy analysis.

For Fourier transform infrared (FTIR) spectroscopic measurements, the bio-transformed products present in cell-free filtrate were freeze-dried and diluted with potassium bromide in the ratio of 1:100. FTIR spectrum of samples was recorded on Shimazdu IR Prestige-21 FTIR instrument with a diffuse reflectance mode (DRS-8000) attachment. All measurements were carried out in the range of 400– 4000 cm_1 at a resolution of 4 cm_1.

Scanning Electron Microscopy Analysis

Scanning electron microscopy (SEM) micrographs were obtained using a Hitachi scanning electron microscope (model S-2600 N, Tokyo, Japan) operating in the high-vacuum mode with an acceleration voltage of 20 kV. FTIR and SEM analysis was done at Sophisticated Analytical Instrument Facility (SAIF), STIC Cochin University of Science and Technology, Cochin.

Antimicrobial Activity of Synthesized Nanoparticles

Antibacterial activity of extracted solvent was determined by Agar well diffusion method. The pathogens against which the antibacterial activity was tested were *Escherichia coli, Bacillus subtilis and Klebsiella* sp. The pathogens were swabbed onto separate Muller Hinton agar plates and the synthesized nanoparticles were added (100 ul) into wells cut on the plate using a borer. The plate was incubated at 37oC for 24hrs, after which the the zone of inhibition was measured [10].

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Antimicrobial Activity from Nanoparticles Coated Hospital Dressing Cloth

White colour hospital dressing cloth was cut as squares of 1.5 cm X 1.5 cm dimention and sterilized by autoclaving. The synthesized nanoparticles were coated on the cloth pieces and dried in laminar air flow. After drying the antibacterial activity of the cloth was tested by Kirby Baurer method and the zone of diameter was measured [11].

Results

Phenotypic Identification

The organism was Gram negative rods, which grew well in nutrient agar as well as cetrimide agar with production of yellowish green pigmentation. The biochemical results were as shown below:

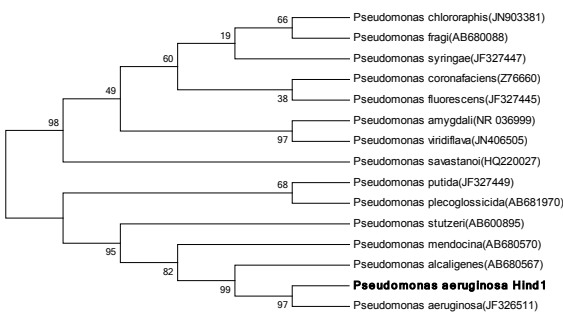
Biochemical test result

INDOLE	MR	VP	CITRATE	OXIDASE	CATALASE
- ve	- ve	- ve	+ ve	+ ve	ve

Genotypic confirmation of the isolate

The organism isolated from pus was confirmed as Pseudomonas aeruginosa by 16s rRNA sequencing done at SynergyScientific Services, Chennai, Tamil Nadu. The seqquences were highly conservative at species level.

Phylogenetic tree for *Pseudomonas aerogenasa*



Through phylogenetic analysis it was found that the 16S rRNA sequence of the organism resembled the most to *Pseudomonas aeruginosa* Hind I

Double disk synergy test

Inhibition zone around the test antibiotic disk was seen to increase towards the Co-AmoxyClav disk. The confirmatory test with Ceftazidime showed a zone diameter more than 5mm, thus showing that the organism is an ESBL producer.

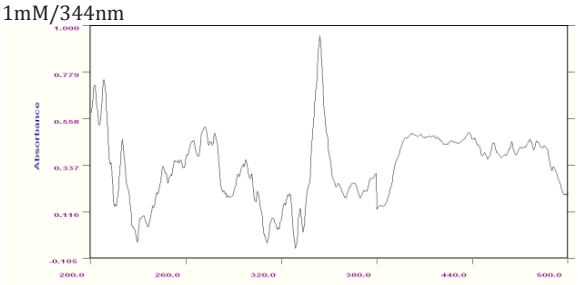
Synthesis of silver Nanoparticles

In the Present study we synthesized the Silver Nanoparticles from ESBL producing organism *Pseudomonas aeruginaosa*. We synthesized the nanoparticles of 1mM and 2mM concentration under a period of 24hrs (fig). The changes of color due to reduction is the preliminary confirmation for nanoparticles.

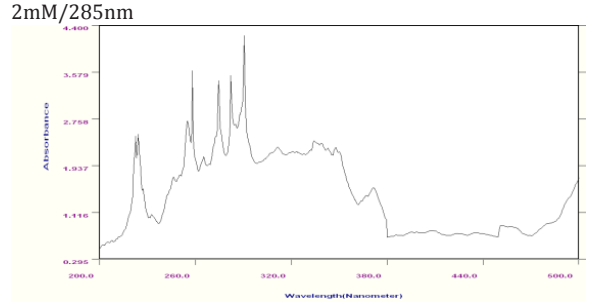
Spectral analysis

The control solution (without silver nitrate solution) showed nil absorption when spectrometry was done in the range of 260 to 500 nm. The samples exposed to the silver nitrate solution shows the wide spectrum in the same range.

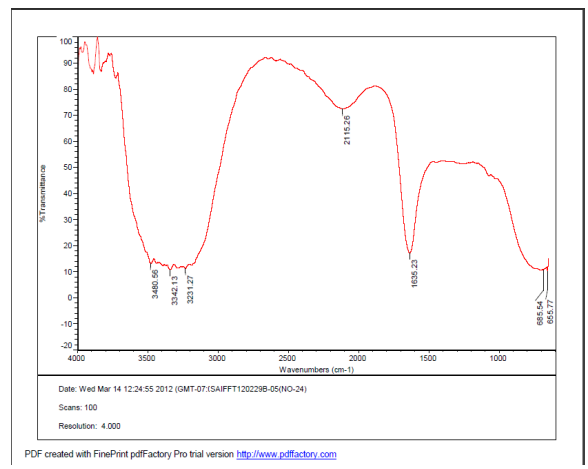
1mM/344nm



2mM/285nm

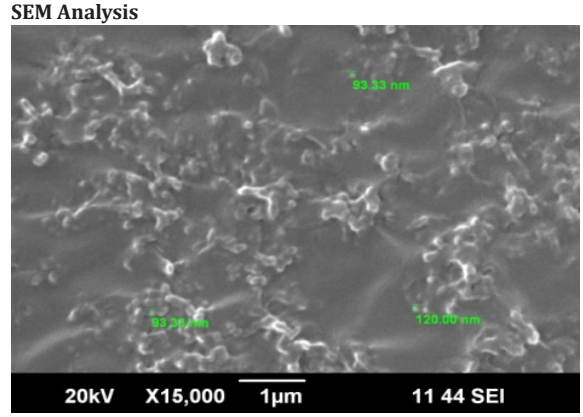


FTIR result



FTIR analysis of isolated silver nanoparticles free from proteins and water soluble compounds was done in this direction. The analysis of IR spectra gives an idea about biomolecules bearing different functionalities which are present in underlying system. Representative spectra Plant extract at 24hrs manifest major IR bands at 3480, 3342, 3231, 2115 cm^{-1} and minor bands at 1635, 685, 655 cm^{-1}

SEM Analysis



The above given is the SEM image for the nanoparticles synthesized by *Pseudomonas* ap. aqueous extract. The synthesized nanoparticles are below 1µm in size. The small particles are appear at 93nm whereas the large particle synthesis occur at 120nm. The SEM image shows cluster of nanoparticles of two different size under 24hrs incubation time.

Antimicrobial Activity of Synthesized Nanoparticles and Nanoparticle coated Hospital Dressing Cloth

The antimicrobial activity of the synthesized nanoparticles and nanoparticles coated cloths against various pathogens- *E.coli, Bacillus sp., Pseudomonas sp*., and *Klebsella sp*. Was checked. the nanoparticles synthesized at 1mM concentration was found to have maximum activity.

Synthesized Nanoparticles				Nanoparticles Coated Hospital Dressing Cloth	
S.No	Organisms	1mM	2mM	Amikacin Disk	1mM AgNo3
1.	Bacillus sp.	15mm	12mm	12mm	35mm
2.	Klebsiella sp.	9mm	5mm	12mm	32mm
3.	E.coli	13mm	4mm	10mm	37mm
4.	Pseudomonas sp.	3mm	2mm	5mm	5mm

Figure: the antibacterial activity of the synthesized nanoparticles at 2 different concentrations

Conclusion

In the present study nanoparticles were employed against various pathogens. Silver nanoparticles were produced using the culture filtrate of ESBL producing *Pseudomonas aeruginosa.* The antibacterial activity of these nanoparticles were tested against various pathogens and high activity was seen. Further these nanoparticles were coated onto hospital dressing cloth and again the antibacterial activity was checked. The nanoparticle coated cloth showed high activity against the various pathogens. This application is sure to benefit the field of health sciences in the coming years. Such nanotechnological solutions needs to be searched for so that the eradication of pathogens may become more successful.

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