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A COMPARATIVE STUDY OF CONVENTIONAL ANTIBIOTIC SENSITIVITY TESTING AGAINST SILVER NANOPARTICLES AMONG DIABETIC FOOT ULCER PATIENTS

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ABSTRACT

Often hailed as a revolutionary new technology, Nanotechnology has the potential to impact almost every area of the society. One area in nanotechnology that holds promising great benefits for the society in future and is in the realm of medicine is Nanomedicine.Among which comes our core of the study Silver Nanoparticles which has shown great affirmation in terms of biomedical applications, not only due to their large surface area to volume ratio, but also because they exhibit different biomedical activities.Antibacterial efficacy of Silver nanoparticles synthesized from the extract of guava fruit (Psidium guajava) were tested against multi drug resistant strains of Gram negative bacteria isolated from diabetic foot ulcer patients by agar well diffusion method. Antibacterial activities of the silver nanoparticles were compared with the conventionally used antibiotics. Silver nano particles showed maximum inhibitionagainst MDR strains Pseudomonas aeruginosa,E.coli, Klebsiella pneumoniae,Proteus mirabilis and Acinetobacter baumannii.The aim of this invitro study is to examine the efficacy of silver nanoparticles in treating bacterial infections from diabetic foot ulcers patients.The findings implicating that the silvernanoparticles have effective antibacterial actions than the commonly used antibiotics.We conclude, silver nanoparticles are now considered a viable alternative to antibiotics and seem to have a high potential to solve the problem of the emergence of bacterial multidrug resistance on diabetic foot ulcer infections.

Keywords – *Diabetic foot ulcer, Antibacterial activity, Antibiotics, Silver nanoparticles*

1. INTRODUCTION

The emergence of bacterial resistance to antimicrobial drugs has become a serious problem in health care due to abuse of commonly available antibiotics. It is a result of evolutionary processes occurring in the course of an antibiotic treatment. Such resistant bacteria survivein this medical treatmentand pass this trait to future generations.[1, 2]Due to the outbreak of the infectious diseases caused by different pathogenic bacteria and the development of antibiotic resistance the pharmaceutical companies and researchers are searching for new antibacterial agents.[3]

Nanotechnology is an emerging scienceand growing particularly in developing new materials at nanoscale levels.[4]Metal nanoparticles (NPs) (e.g., silver, gold, platinum, etc.) have been the subject of ample research interest in the last few years, due to their exciting size-dependent electrical, optical, physical, and chemical properties. In particular, colloidal silver nanoparticles (AgNPs) have attracted extreme attention for research in the field of microbiology. [5]Sincethe properties of the AgNPs depend significantly on their size and morphology, intensive investigations have focused on the control of such parameters, using different preparation methods.[6]

In the present scenario,nanoscale materials have emerged as novel antimicrobial agents owing to their high surface area to volume ratio and the unique chemical and physical properties.[3]Among different types of available nanomaterials, nanosilver is proved to be most effective material which has good antimicrobial properties against bacteria, viruses and other eukaryotic microorganisms.[7]Nanosilver can be found even in clothing, food containers, wound dressings, ointments, implant coatings, and many other items.[11]

Several mechanisms have been projected to explain the inhibitory effect of silver nanoparticles on bacteria. But it is assumed that the high affinity of silver towards sulfur and phosphorus is the key element of the antimicrobial effect. Due to the abundance of sulfur-containing proteins on the bacterial cell membrane, silver nanoparticles can react with sulfur-containing amino acids inside or outside the cell membrane, which in turn affects bacterial cell viability. It was also suggested that silver ions (particularly Ag^+) released from silver nanoparticles can interact with phosphorus moieties in DNA, resulting in inactivation of DNA replication, or can react with sulphur containing proteins, leading to the inhibition of enzyme functions.[9,10]

The general understanding is that Ag nanoparticle of typically less than 20 nm diameters get attached to sulfur-containing proteins of bacterial cell membranes leading to greater permeability of the membrane, which causes the death of the bacteria.[11]Hence the current study aimed to determine the efficacy of Silver nanoparticles against microorganisms which grow on diabetic foot ulcers.

2. MAREIALS AND METHODS

2.1 Sample collection

Patients were randomly selected and we obtained informed consent after explaining the aim of research. Swabs were taken from the base of the ulcer and deep wounds. A total of 60 diabetic foot ulcer samples were collected from diabetic patients. The specimens were processed in the microbiology laboratory using thioglycollate broth and incubated at 37°C for 24 hours. Bacterial growths were gram stained and culturedon blood agar, Mac Conkey agar and they were incubated under aerobic conditions at 37°C for 48 hours.

2.2 Antibacterial Efficacy by Disk diffusion method

Silver nanoparticles synthesized from the extract of guava fruit(*Psidium guajava*) were tested for their antibacterial efficacy against multi drug resistant strains of Gram negative bacteria on diabetic foot ulcer patients, by following the standard method of Kirby-Bauer Disk Diffusion Susceptibility Test [12]and control was maintained with routinely used antibiotics for comparing the antibacterial efficacy of silver nanoparticles on Gram negative bacteria. Each tested strain were compared with conventionally used antibiotics namely, Cefazoline (30µg), Cefotaxime (30µg),Ceftazidime (30µg), Imipenem (10µg), Amoxycillin/clavulinate (20/10µg), Amikacin (30µg), Gentamicin (10 µg), Ciproploxacin (5µg), Cefazoline (30µg), Cotrimoxazole(25µg). The zones of inhibition were noted and further comparison was done. [3]

2.3 Synthesis of silver nanoparticles

Silvernanoparticles were synthesized from green synthesis method. [13,14] The efficacy of biosynthesized silver nanoparticles were tested against the major pathogens of Diabetic Foot Ulcer (DFU) patients enlisted from SSSMC& RI.

2.4 Antibacterial Activity of Silver Nanoparticles

The antibacterial activity of the synthesized Silver nanoparticles was investigated against *Pseudomonas aeruginosa, E.coli, Klebsiella pneumoniae,Proteus mirabilis,Acinetobacterbaumannii*. All the pure cultures of bacterial strains were sub-cultured in nutrient broth for 24hrs at 37°C. Twenty milliliter of the Mueller Hinton agar medium was poured into a petriplate on a horizontally leveled surface. Each bacterial strain was swabbed uniformly into the individual plates using sterile cotton swabs. Wells of 5mm diameter were made onto each bacterium inoculated agar plates using gel puncture. Using sterile micropipette, different concentrations(20µl (0.005mg), 15µl (0.00375mg),10µl(0.0025mg) and 5µl(0.00125mg) of silver nanoparticle suspension was poured into each of the wells and control was placed at centre. All the plates were incubated at 37°C. After 24 hours incubation, bactericidal activity was determined by the zone of inhibition on the agar plate around the sample loaded wells.[3]All experiments were done in aseptic condition in laminar air flow cabinet. Zones of inhibition for control, SNPs, and antibiotics were compared.

3. RESULTS

Table 1: Bacterial isolation from diabetic foot ulcer patients

Organisms	Number of isolation	Percentage of organisms
Pseudomonas aeruginosa	30	32
E.coli	22	23
Klebsiellapneumoniae	19	20
Proteus mirabilis	9	10
Acinetobacterbaumannii	7	7
Susceptible strains	8	8

Figure 1: Kirby Bauer's Disk Diffusion Technique, Showing drug Resistance pattern against *Pseudomonas aeruginosa*

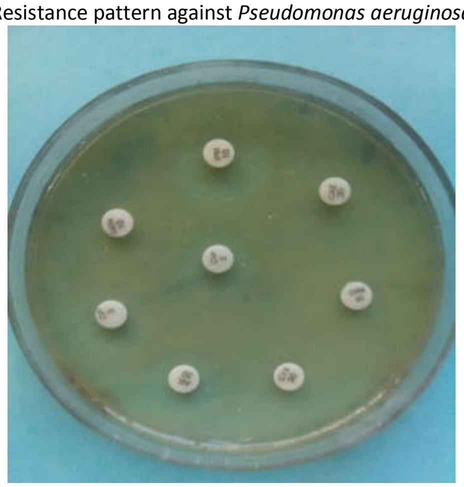


Figure 2: showing the Effects of Ag-NPs on *Pseudomonas aeruginosa*

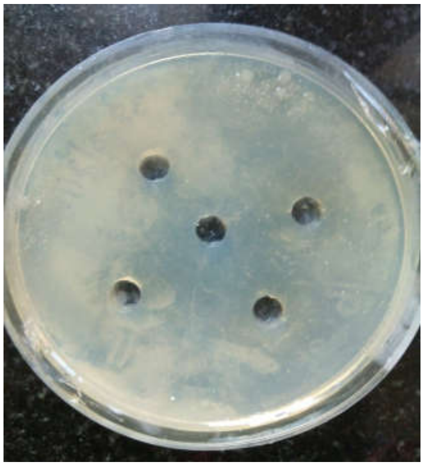


Figure3: Drug resistance pattern against *Proteus mirabilis* by KirbyBauer's Disk Diffusion Technique

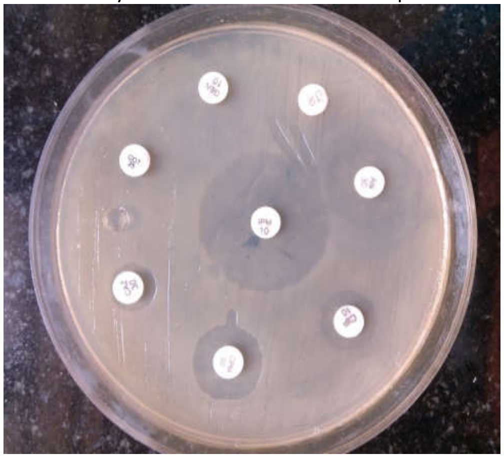


Figure 4: Zone of Inhibition exhibited by the AgNPs against*Proteus mirabilis*

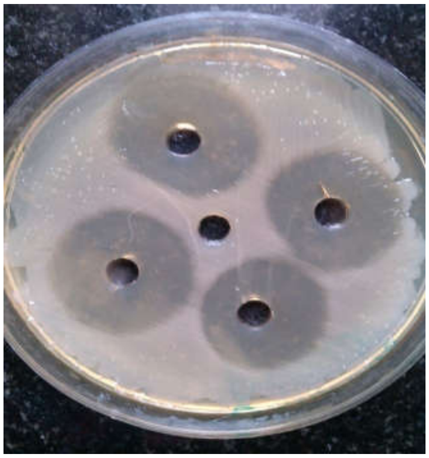


Figure 5: Drug resistance pattern against *E.coli* by Kirby Bauer's Disk Diffusion Technique

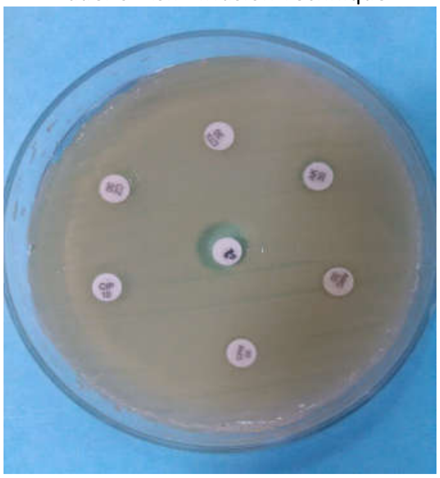


Figure 6: Zone of Inhibition exhibited by the AgNPs against*E.coli*

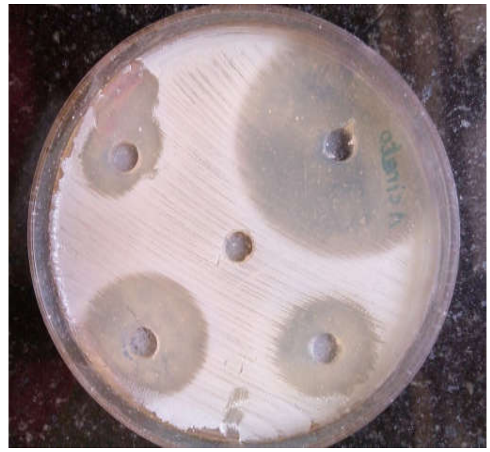


Figure 7: Kirby Bauer's Disk Diffusion Technique, Showing drug Resistance pattern to Acinetobacter *baumannii*

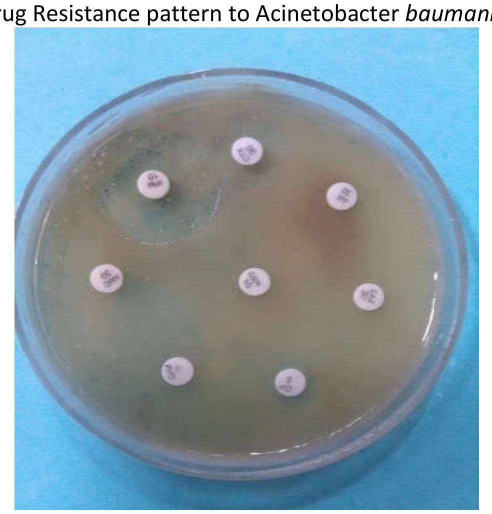


Figure 8: Zone of Inhibition exhibited by the AgNPs against*Acinetobacter baumanni*

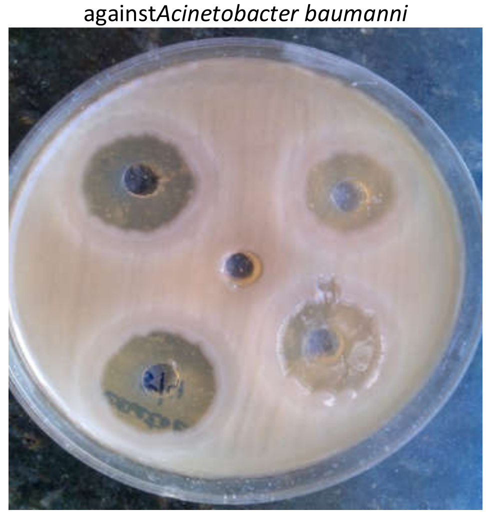


Figure 9: Drug resistance pattern on Kirby Bauer's Disk Diffusion Technique against *Klebsiella pneumonia*

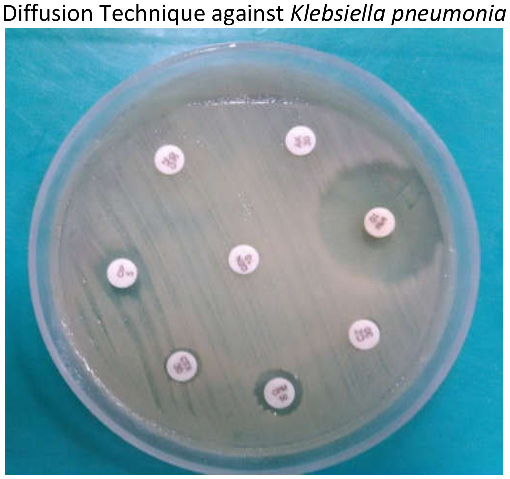
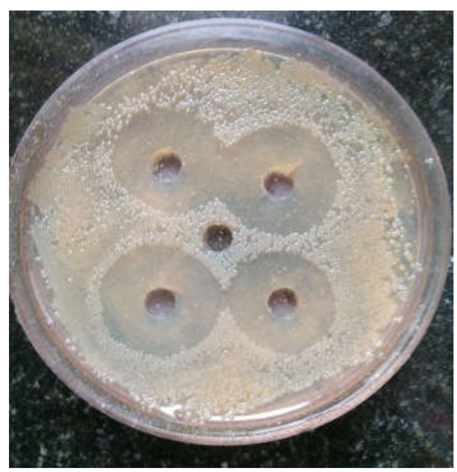


Figure 10: Effects of Ag-NPs on *Klebsiella pneumoniae*



Silver nanoparticles (AgNPs) have emerged as an arch product from the field of nanotechnology. Over the last few years due to its good conductivity, chemical stability,catalytic and antibacterial activity silver has gained much of the interest.The intention of present study was to compare and evaluate the antibacterial effects of conventionally used antibiotics with Ag-NPs against MDR strains recovered from patients with diabetic foot infections.

In our study we have isolated 95 strains of 5 different types of bacteria namely *Pseudomonas aeruginosa* (32%), *E. coli* (23%),*Klebsiella pneumonia* (20%), *Proteus mirabilis* (10%), *Acinetobacter baumannii* (7%) and other susceptible strains(8%)were isolated from 60 diabetic foot ulcer cases. In this isolation, 87were MDR pathogens, remaining8 strains were sensitive tothe commercially used drugs.

These 87MDR strains were tested against synthesized silver nanoparticles. Agnps showed the maximum inhibition zone for all the concentrations(20µl (0.005mg),15µl (0.00375mg),10µl(0.0025mg) and 5µl(0.00125mg)against MDR *Pseudomonas aeruginosa, E.coli, Klebsiella pneumoniae, Proteus mirabilis, Acinetobacter baumannii*. Zone of inhibition of *Pseudomonas aeruginosa* (32%), *E.coli* (23%), *Klebsiella pneumonia* (20%),*Proteus mirabilis* (10%), *Acinetobacter baumannii* (7%) and other susceptible strains (8%) where compared to the routinely used conventional antibiotics such as Cefazoline (30µg), Cefotaxime (30µg), Ceftazidime (30µg), Imipenem (10µg), Amoxycillin/clavulinate (20/10µg), Amikacin (30µg), Gentamicin (10 µg), Ciproploxacin (5µg), Cefazoline (30µg), Cotrimoxazole(25µg) as shown in Table 1 & Figure 1-10.

In our study the synthesized silver nanoparticles showed maximum zone of inhibition even in the least concentration 5µl when compared with conventionally antibiotics. This current studyclearly shows that the bacterial strains which were resistant to antibiotics were highly susceptible to silver nanoparticles.However, at different concentrations the antibacterial activities of silver nanoparticles were relatively close to each other.

This study evidently confirms that the silver nanoparticles are potential and efficient antibacterial agent against the multidrug resistant bacteria.

4. DISCUSSION

AgNPs are able to physically interact with the cell surface of various bacteria. This is particularly important in the case of Gram-negative bacteria where numerous studies have observed the adhesion and accumulation of AgNPs to the bacterial surface. Many studies have reported that AgNPs can damage cell membranes leading to structural changes, which render bacteria more permeable. This effect is highly influenced by the nanoparticle's size, shape and concentration. [(15)] Several studies have shown that AgNP activity is strongly dependent on the size. [(16,17)] In fact, in our study the bactericidal activity of AgNPs was found to be optimal against MDR *Pseudomonas aeruginosa, E.coli, Klebsiella pneumoniae, Proteus mirabilis, Acinetobacter baumannii* even in the least concentration (5µl).

AgNPs are attractive because they are non-toxic to the human body at low concentrations and have broad- spectrum antibacterial actions. [(18)]Similarly Sondi and Salo pek-Sondi reported that the antimicrobial activity of AgNPs on Gram-negative bacteria depends on the concentration of AgNPs.[(19)]Recently, Danilczuk and co-workers reported that Ag-generated free radicals derived from the surface of AgNPs were responsible for the antimicrobial activity. [(20)]

In our study we proved the antimicrobial activity of silver nano particles against Gram negative bacteria isolated from diabetic foot ulcer patients. Silver nanoparticles have potential applications as antimicrobial agent in clinical use. There is increasing evidence for the use of silver nanoparticles in the treatment of chronic wounds. Wu *et al.*,[(21)] developed silver nanoparticle/bacterial cellulose gel membranes for antibacterial wound dressing and evaluated their use on a variety of chronic nonhealing wounds.

Similar to our study, Mohammad Reza Mohajeri-Tehrani1 *et al.,*[(22)]reported silver nanoparticles condensed thegrowth rate of gram negative diabetic foot microorganisms including*P. aeruginosa, E. coli, Bacillus, Proteus, Klebsiella, Enterobacter, Acinetobacter*and *Citrobacter*.

Similarly Jain *et al.,*[(23)] concluded that silver nanoparticles could have successful therapeutic use as a part of the antimicrobial gel for topical use. A standard antimicrobial sensitivity tests carried out in Muller-Hinton agar plates were used to evaluate the antimicrobial activity of the silver nanoparticles containing gel against bacterial cultures of Escherichia coli (ATCC 117), *Pseudomonas aeruginosa* (ATCC 9027), *Staphylococcus aureus* (ATCC 6538), and *Streptococcus epidermidis* (ATCC 12228). Gram- negative bacteria were killed more effectively (3 log decrease in 5-9 h) than Gram-positive bacteria (3 log decrease in 12 h).

According to Shrivastava*et al*.,[(24)]found the dose dependent silver nanoparticles have obvious activity against gram-negative organisms than the gram- positive organisms.Anitha*et al*.,[(3)]also proved that silver nanoparticles are effective bactericidal agents even in fighting multidrug-resistant against gram negative than the Gram positive organisms.

Morones *et al*., [(25)]corroborate with our findings, they have provedthe antibacterial activityof silver nanoparticles against four types of gram negative bacteria, *E. coli, V. cholera, P. aeruginosa and S. typhus*. Theyalso stressed in their findings that silver nanoparticles attach to the cell membrane and release silver ions, disturbing its function andthe penetration of bacteria.Panacek *et al.,*[(26)] similarly pointed out the high antimicrobial and bactericidal activity ofsilver nanoparticles against gram-negative and also with gram-positive bacteria.

5. CONCLUSION

Nanotechnology is emerging as a rapidly growing field with its application in science and technology for the purpose of manufacturing new materials at the nanoscale level.In our study, silver nanoparticles weresynthesizedfromthe extract ofGuava fruit (*Psidium guajava*) have shown strong antibacterial activity against 5 different human pathogens.AgNPS exhibited good antimicrobial properties than routinely used antibiotics.Finally, we conclude that nanotechnology is an important area of research that deserves all our interest and concentration towards itsimpending application to fight against multidrug-resistant microbes.In future, silver nanoparticles might be used as a substitute antibacterial agent for diabetic foot infections to prevent and treat diabetic foot ulcer.

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