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Individual and Combined Efficacy of Silver Nanoparticles and Different Antibiotics against Multidrug-Resistant Clinical Isolates

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Abstract*: Multi-drug resistance is a growing problem in the treatment of infectious diseases and the widespread use of broad-spectrum antibiotics has produced antibiotic resistance for many human bacterial pathogens. Advances in nanotechnology have opened new horizons in nanomedicine, allowing the synthesis of nanoparticles that can be assembled into complex architectures. Among them, silver nanoparticles (AgNPs) have been extensively studied because of their good electrical conductivity, as well as their potential. In this research work, total 80 multidrug resistant clinical isolates (23 isolates of Acinetobacter, 31 isolates of E.coli and 26 isolates of Klebsiella pneumonia) were tested with antibiotics, AgNps and AgNp-antibiotic conjugates. The conjugates were prepared by impregnating 30 µl of AgNps in sterile plain disks and the antibiotic disks separately. The antibiotics tested were Cefotaxime, Ceftazidime, Amikacin, Ciprofloxacin, Piperacillin – tazobactam and Amoxicillin- clavulanic acid. Majority of isolates of E.coli and Klebsiella showed zone enhancement with both AgNp and AgNp – antibiotic conjugates. While fewer numbers of isolates of Acinetobacter showed zone enhancement, one of the mechanisms of resistance to cephalosporins and fluoroquinolones in Acinetobacter are point mutations. AgNp' s are not able to act on genetic level to overcome the point mutations. Hence the silver nanoparticles can be used in synergism with various antibiotics as a newer mode of treatment for multi drug resistant clinical isolates.*

Keywords: *Antibiotics, Nanotechnology, Silver nanoparticles, Acinetobacter, E.coli, Klebsiella pneumonia, Conjugates, Mutation*

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I. Introduction

The worldwide ever-increasing of bacterial resistance to the conventional medical antimicrobial agents is currently one of the most serious health crises for modern medicine [1-3]. This has serious negative impacts such as decreases of the effectiveness of existing treatments, and causes higher morbidity and mortality rates in patients with infections caused by multidrug-resistant (MDR) bacteria [1,4]. This growing resistance of pathogenic bacterial strains to traditional antibacterial treatments has encouraged alternate strategies to control infections.

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 [5]. In recent years, silver nanoparticles (AgNPs) particularly attractive for the production of a new class of antimicrobials [6-8] opening up a completely new way to combat a wide range of bacterial pathogens. Silver has always been used against various diseases; in the past it found use as an antiseptic and antimicrobial against Gram-positive and Gram-negative bacteria [9, 10] due to its low cytotoxicity [11].

Further, the addition of silver acted synergistically with antibiotics in treating infections caused by clinically most difficult-to-treat Gram-negative bacteria such as *Acinetobacter baumannii, Enterobacter cloacae, Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa* [12]. Thus, the combination of antibiotics and silver nanoparticles could increase the antibiotic' s efficacy against resistant pathogens. The synergistic effect and enhanced activity of antibiotics combined with Silver nanoparticles may be explained by the concurrent impact of silver on numerous bacterial structures and metabolic processes. This multi level mode of action makes Silver nanoparticles able to destroy or inhibit the growth of pathogenic microorganisms, including highly resistant bacterial strains (from a few mg/mL to several tens of mg/L) [13]. Moreover, nanoparticle– antibiotic conjugates lower the amount of both agents in the dosage, which reduces noxiousness and increases antimicrobial properties. Additionally, due to this conjugation, the concentrations of antibiotics were increased at the place of antibiotic– microbe contact and thus expedited the binding between microbes and antibiotics [14].

The present research work was undertaken to study the individual and combined antibacterial activity of silver nanoparticles and conventional antibiotics against multi drug resistant clinical isolates of *Acinetobacter, Eschereschia coli and Klebsiella pneumonia.*

II. Materials and Methods

Preparation of Inoculum

In the present study, 80 multidrug resistant isolates from clinical samples were tested. This included 23 isolates of *Acinetobacte*r, 31 isolates of *E.coli* and 26 isolates of *Klebsiella pneumonia.* These pathogens were isolated from sputum (15), urine (28), blood (10), endotracheal secretions (33) and pleural fluid (14). All these isolates were resistant to four classes of drugs, i.e. floroquinolones, aminoglycosides, β lactam – β lactamase inhibitor and cephalosporins. The specific antibiotics used were Cefotaxime and Ceftazidime (Cephalosporin), Amikacin (Aminogycoside), Ciprofloxacin (Fluoroquinolone), Piperacillin – tazobactam and Amoxicillin-clavulanic acid (extended spectrum penicillin and β lactamase inhibitor).

Preparation of Silver nanoparticles

Polyvinylpyrrolidone (PVP) capped AgNP' s were procured. These AgNp' s were produced by the method of chemical reduction of $AgNO_3$. An AgNP suspension of 100 ppm was prepared by dissolving 0.1 mg of chemically synthesized AgNPs in 1 mL autoclaved deionized water. The suspension was sonicated for 20 minutes to avoid deposition of AgNPs. 30 µl of AgNp suspension was used in the study.

Preparation of discs

AgNp suspension was calibrated by automated pipette with sterile tips. The tips were sterilized in the autoclave at 121 º C for 15 mins. 6mm sterile discs were obtained from Himedia pvt ltd. Soon after sonication, 30 µl of this suspension was inoculated immediately in the sterile disks. Antibiotic discs of Ciprofloxacin, Amikacin, Cefotaxime, Piptaz, Augmentin and Ceftazidime were obtained from Himedia pvt ltd. These antibiotic discs were inoculated with 30 µl AgNp suspension to check the synergistic effect of AgNp with the antibiotics.

Procedure

The suspension of all these isolates was prepared by diluting with 0.9% NaCl solution. The inoculum was plated on Muller Hinton agar plates after matching with 0.5 Mcfarlands reagent. 5 disks were placed equidistantly from each other, 15 mm away from the edge of the plate. These 5 disks include the 2 disks of antibiotics, 1 nanoparticle disk and 2 disks of AgNp-antibiotic conjugate, (Image I and II). The plates were kept overnight in the incubator at 37ºC. The zone of inhibition was measured the next day. The mean zone of inhibition, zone enhancement, fold increase and P value were calculated and the reported in results**.** Statistical analysis was performed using IBM Statistical Package for the Social Sciences version 20 (SPSS v20, IBM). A p value of <0.05 was considered significant.

III. Results and Discussion

In current study, the efficacy of antibiotics, Silver nanoparticles, and their combination in terms of zone of inhibition was measured against three different Gram negative bacteria and results of synergistic effect in the form of fold increase are presented in Table 1.

Table 1: Individual and combined efficacy of antibiotics and AgNPs against selected bacteria

AgNps, Antibiotics and AgNp-antibiotic conjugates	**Bacterial isolates (Zone of inhibition (mm) = mean ± 2 S.D)**		
	***Acinetobacter* (n=23)**	***Eschereschia coli*, (n=31)**	***Klebsiella pneumoniae* (n=26)**
AgNp	7.87 ± 6.54	11.32 ± 8.72	11.5 ± 8.12
Ciprofloxacin	6.34 ± 1.96	6.35 ± 1.96	6.65 ± 3.34
Ciprofloxacin + AgNPs	8.26 ± 7.96	12.93 ± 8.86	12.88 ± 11.18
Zone enhancement	6/23 (26%)	23/31 (74%)	17/26 (65%)
Increase in fold area (%)	30.28	103.62	93.68
P value	<0.05 Significant	<0.001 Highly significant	<0.001 Highly significant
Amikacin	6.74 ± 2.96	6.35 ± 2.34	6.46 ± 2.14
Amikacin + AgNPs	8.17 ± 6.64	10.71 ± 9.1	11.61 ± 10.92
Zone enhancement	5/23 (22%)	18/31 (58%)	14/26 (54%)
Increase in fold area (%)	21.22	68.66	79.72
P value	<0.05 Significant	<0.001 highly significant	<0.001 highly significant
Piptaz	6.51 ± 2.82	6.35 ± 1.96	6.65 ± 3.04
Piptaz + AgNPs	10.17 ± 7.96	12.71 ± 10.5	12.5 ± 11.72
Zone enhancement	13/23 (57%)	20/31 (65%)	15/26 (58%)
Increase in fold area (%)	56.22	100.16	90
P value	<0.001 highly significant	<0.001 highly significant	<0.001 highly significant
Cefotaxime	6.56 ± 2.56	6.54 ± 3.1	7 ± 3.76
Cefotaxime+ AgNPs	7.21 ± 6.44	11.52 ± 9.76	12.65 ± 11.12
Zone enhancement	3/23 (13%)	19/31 (61%)	16/26 (62%)
Increase in fold area (%)	9.91	76.12	80.71
P value	0.12, insignificant	<0.001 Highly significant	<0.001 Highly significant
Augmentin	6.52 ± 2.54	6.58 ± 2.92	6.81 ± 3.4
Augmentin + AgNPs	7 ± 5.62	11 ± 10.26	11.88 ± 11.52
Zone enhancement	3/23 (13%)	16/31 (52%)	14/26 (54%)
Increase in fold area (%)	7.36	67.17	74.45
P value	0.11, Insignificant	<0.001, Highly significant	<0.001, Highly significant

Ciprofloxacin + AgNPs for *Eschereschia coli* reported the highest antibacterial activity followed by Ciprofloxacin + AgNp for *Klebsiella pneumonia and* Piptaz + AgNPs for *Eschereschia coli*. The least synergistic antibiotic activity was reported for Augmentin + AgNPs by *Acinetobacter* species.

Ciprofloxacin is a 2nd generation fluoroquinolone, Group A drug for *Acinetobacter* species, *Eschereshcia coli* and *Klebsiella pneumonia* (CLSI 2017). Only 26% isolates of *Acinetobacte*r showed zone enhancement whereas the majority of the isolates of *E.coli* (74%) and *Klebsiella* (65%) showed zone enhancement. The fold area increase observed for Ciprofloxacin was highest amongst all the five drugs. Ciprofloxacin with AgNps demonstrated 103.62 % fold increase for *E.coli* that was highest, followed by 93.68 % for *Klebsiella pneumoniae*. Here again the significance of *E.coli* and *Klebsiella pneumoniae* was greater than *Acinetobacter* species. Ciprofloxacin in combination with these AgNPs were found to be most effective in inhibiting bacteria. If bacteria develop resistance to one of them, the other bactericidal agent would kill the bacteria. In synergism, the bactericidal effect is enhanced by interaction between active groups like hydroxyl and amino groups present in these Ciprofloxacin with AgNPs by chelation. As a result, Ciprofloxacin– AgNP conjugate is formed in which an AgNP core is surrounded by Ciprofloxacin molecules. Thus, the antimicrobial concentration is increased at the focal site, which leads to increased destruction of bacteria. Similar kinds of observations were reported by Naqvi et al [14].

Amikacin is an aminoglycoside, Group B drug for *Acinetobacter* species, *Eschereschia coli* and *Klebsiella pneumonia* (CLSI 2017). 22 % isolates of *Acinetobacter* species showed zone enhancement while 58 % isolates of *E.coli* and 54 % of *Klebsiella pneumoniae* reported increase in zone size, these observations were more significant than the *Acinetobacter* species. Here the fold increase reported by *Klebsiella pneumoniae* (79.72 %) was greater than that of *E.coli* (68.66 %). *Acinetobacter* species showed the lowest fold increase of 21.22 %. The antibacterial activity of Amikacin, increased in the presence of AgNPs. The synergistic effect of AgNPs was found to be more prominent than the effect of antibiotics alone. High surface to volume ratio of

AgNPs allows them to interact with antibiotics. Moreover, the presence of active groups such as hydroxyl and amino groups in Amikacin molecules may also enable them to interact with nanosilver by chelation, which may result of the enhancement synergistic effect. It prevents DNA from unwinding. These observations were comparable with study done by Mala et al [15].

Piptaz is a combination antibiotic containing extended spectrum penicillin and β lactamase inhibitor tazobactam, Group B drug for *Acinetobacter* species, *Eschereshcia coli* and *Klebsiella pneumoniae* (CLSI 2017). Piptaz is the only antibiotic in the study that reported substantial synergism for *Acinetobacter* species. Around 57 % isolates of *Acinetobacter* showed zone enhancement with the fold area increase of 56.22 %. The zone enhancement reported for *E.coli* and *Klebsiella pneumoniae* was 65 % and 58 % with fold increase of 100.16 % and 90 % respectively. Thus Piptaz reported very high significance for all the three groups of organisms. Similar kinds of observations were reported by Hafez et al [16].

Cefotaxime is a third generation cephalosporin, Group B drug for *Acinetobacter* species, *Eschereschia coli* and *Klebsiella pneumonia* (CLSI 2017). *Acinetobacter* species showed zone enhancement in only 13 % of its MDR clinical isolates. The fold area increase observed for the same was 9.91 %. Thus the study was statistically insignificant for *Acinetobacter* species. On the other hand, 61 % isolates of *E.coli* and 62 % of *Klebsiella pneumoniae* demonstrated zone enhancement, this was statistically significant and the fold area increase reported was 76.12 % and 80.71 % respectively. Cefotaxime molecules contain active groups like hydroxyl and amino groups, which can easily react with AgNPs and disrupt peptidoglycan in the cell wall. Cefotaxime in combination with positively charged AgNPs both inhibited and disrupted cell-wall synthesis. It has also been proposed that silver ions penetrate the cell intercalate themselves between pyrimidine and purine, and denature the DNA molecule. In synergism, the bactericidal effect is enhanced by interaction between Cefotaxime with AgNPs. As a result, Cefotaxime– AgNP conjugate is formed in which an AgNP core is surrounded by Cefotaxime molecules. Thus, the antimicrobial concentration is increased, which leads to increased destruction of bacteria [17]. Similar types of observations were reported in study done by Hafez et al [16].

Augmentin is a combination consisting of amoxicillin, a β lactam antibiotic, and Potassium clavulanate, aβ lactamase inhibitor. It is a Group B drug for *Acinetobacter* species, *Eschereshcia coli* and *Klebsiella pneumoniae* (CLSI 2017). In the entire study, Augmentin – AgNp reported the lowest fold area increase (7.36 %) for *Acinetobacter* species. The synergism was seen only in 3/23 (13 %) of its isolates. Thus study was statistically insignificant for *Acinetobacter* isolates. On the other hand, it was significant for *E.coli* and *Klebsiella pneumoniae.* 52 % isolates of E.coli and 54 % of *Klebsiella pneumoniae* exhibited zone enhancement and fold increase area was 67.17 % and 74.45 % for the same. These results were comparable with the study done by Maha Abd El Fattah et al [18], They stated that the mechanism of synergism with Augmentin is that firstly, Augmentin – AgNPs complex are formed then they interacts more strongly with the bacterial cells and causes more Ag+ release, thus creating a temporal high concentration of Ag+ near the bacteria cell wall that leads to growth inhibition of the bacteria.

Over all, *Acinetobacter* species did not report considerable significant changes in the zone size even after conjugation. It is likely that the AgNps were not able to overcome the resistance of these species. Franci et al [19] suggested that the action of AgNps on *Acinetobacter* is mainly due to alteration in cell wall and cytoplasm. Thus it is not possible for AgNp' s to overcome the mutational modes of resistance. In present study, it is possible that the maximum number of *Acinetobacter* isolates had mutations, thus were not able to exhibit enhancement in zone size. Franci et al [19] also reported notable combined effect of AgNPs and antibiotics against *E.coli*, *P. aeruginosa* and *K. pneumonia* [19]. In current study, strong synergistic effects were shown for most of the antibiotics combined with silver nanoparticles at very low concentrations of both antibiotics and silver nanoparticles. A very low amount of silver is needed for effective antibacterial action of the antibiotics, which represents an important finding for potential medical applications due to the negligible cytotoxic effect of silver nanoparticles towards human cells at low concentration levels. Several studies [19-22] have also shown that AgNP activity is strongly dependent on the size. In present research work, the AgNps were procured through chemical synthesis and size of nanoparticles was between 40 – 50 nm. The results would have been better with biological synthesized smaller nanoparticles.

IV. Conclusion

Acinetobacter reported low significant zone enhancement in comparison to the other two isolates. One of the mechanisms of resistance to cephalosporins and fluoroquinolones in *Acinetobacter* are point mutations in the bacterial targets gyrA and parC topoisomerase enzymes. It is possible that AgNp' s are not able to act on the genetic level to overcome the point mutations. While the other pathogens reported good results for all the drugs tested. The present study used 30 µl of AgNps, studies should be done using different concentrations of AgNps and antibiotics. It is likely that higher concentrations of AgNps might work for *Acinetobacter species*. The

potential toxicity of AgNp is lower than the antibiotics. The synergistic action of antimicrobial agents can reduce the need for high dosages and minimize side effects.

Hence in near future, silver nanoparticles can be used in synergism with various antibiotics as a newer mode of treatment for multi drug resistant clinical isolates. The low cost and low toxicity prove to be boon to the patients. More research needs to be done with different combination of antibiotics and AgNps to combat the resistance in *Acinetobacter* species.

References

[1]. Panacek, A. et al. Strong and Nonspecific Synergistic Antibacterial Efficiency of Antibiotics Combined with Silver Nanoparticles at Very Low Concentrations Showing No Cytotoxic Effect. Molecules 2016;21.
[2]. Ling, L. L. et al. A new antibiotic kills pathogens without detectable resistance. Nature 2015;517: 455– +.
[3]. Lu, J. et al. Inhibition of bacterial thioredoxin reductase: an antibiotic mechanism targeting bacteria lacking glutathione. Faseb J 2013;27:1394– 1403.
[4]. Thangamani, S., Younis, W. & Seleem, M. N. Repurposing Clinical Molecule Ebselen to Combat Drug Resistant Pathogens. Plos One 2015;10.
[5]. Rai, M.K.; Deshmukh, S.D.; Ingle, A.P.; Gade, A.K. Silver nanoparticles: The powerful nanoweapon against multidrug-resistant bacteria. J. Appl. Microbiol. 2012;112:841– 852.
[6]. Dos Santos, C.A.; Seckler, M.M.; Ingle, A.P.; Gupta, I.; Galdiero, S.; Galdiero, M.; Gade, A.; Rai, M. Silver nanoparticles: Therapeutical uses, toxicity, and safety issues. J. Pharm. Sci. 2014;103:1931– 1944.
[7]. Rai, M.; Kon, K.; Ingle, A.; Duran, N.; Galdiero, S.; Galdiero, M. Broad-spectrum bioactivities of silver nanoparticles: The emerging trends and future prospects. Appl. Microbiol. Biotechnol. 2014;98:1951– 1961.
[8]. Chernousova, S.; Epple, M. Silver as antibacterial agent: Ion, nanoparticle, and metal. Angew. Chem. Int. Ed. Engl. 2013;52:1636– 1653.
[9]. Lazar, V. Quorum sensing in biofilms—How to destroy the bacterial citadels or their cohesion/power? Anaerobe 2011;17:280– 285.
[10]. Taraszkiewicz, A.; Fila, G.; Grinholc, M.; Nakonieczna, J. Innovative strategies to overcome biofilm resistance. Biomed. Res. Int. 2013;150653.
[11]. Biel, M.A.; Sievert, C.; Usacheva, M.; Teichert, M.; Balcom, J. Antimicrobial photodynamic therapy treatment of chronic recurrent sinusitis biofilms. Int. Forum Allergy Rhinol. 2011;1:329– 334.
[12]. Zou L, Wang J, Gao Y et al. Synergistic antibacterial activity of silver with antibiotics correlating with the upregulation of the ROS production | Scientific Reports 8, Article Number: 11131 (2018).
[13]. Panáček A, Smékalová M, Večeřová R, Bogdanová K, Röderová M, Kolář M, et al. Silver nanoparticles strongly enhance and restore bactericidal activity of inactive antibiotics against multiresistant Enterobacteriaceae. Colloids Surfaces B Biointerfaces [Internet].
[14]. Naqvi SZH, Kiran U, Ali MI, Jamal A, Hameed A, Ahmed S, et al. Combined efficacy of biologically synthesized silver nanoparticles and different antibiotics against multidrug-resistant bacteria. Int J Nanomedicine. 2013;8:3187– 95.
[15]. Mala R, Annie Aglin , Ruby Celsia , Geerthika , Kiruthika , VazagaPriya , Srinivasa Kumar. Foley catheters functionalised with a synergistic combination of antibiotics and silver nanoparticles resist biofilm formation. IET Nanobiotechnol. 2017;11(5):612-620.
[16]. Hafez EHA, Ahmed EA, Abbas HS, Salah RA, Din E. Efficacy of Antibiotics Combined with Biosynthesized Silver Nanoparticles on some Pathogenic Bacteria. Int J Sci Res. 2017;6(1):1294– 303.
[17]. Hassan MHA, Ismail MA, Moharram AM, Shoreit A. Synergistic Effect of Biogenic Silver-nanoparticles with β. lactam Cefotaxime against Resistant Staphylococcus arlettae AUMC b-163 Isolated from T3A Pharmaceutical Cleanroom, Assiut, Egypt. Am J Microbiol Res 2016;4(5):132–7.
[18]. Maha Abd El Fattah, Nanis Gamal, Fatma Ibrahim, Gamal Mohamed, Perihan Saleh. Investigation of the Efficacy of Synthesized Silver and Zinc Oxide Nanoparticles against Multi-Drug Resistant Gram Negative Bacterial Clinical Isolates. Archives of clinical microbiology 2017;8(5):67.
[19]. Franci G, Falanga A, Galdiero S, Palomba L, Rai M, Morelli G, et al. Silver nanoparticles as potential antibacterial agents. Molecules. 2015;20(5):8856–74.
[20]. Das SK, Das AR, Guha AK, Nagrale DT, Gaikwad AP, Sharma L, et al. A study on biosynthesis of iron nanoparticles by Pleurotus sp. Veg Crop Res Bull 2013;8(1):5–19.
[21]. Ge L, Li Q, Wang M, Ouyang J, Li X, Xing MMQ. Nanosilver particles in medical applications: Synthesis, performance, and toxicity. Int J Nanomedicine. 2014;9(1):2399–407.
[22]. Amin BM, Anima N, Nadu T, Nadu T. Biosynthesis and effect of silver nanoparticles on the efficacy. 2014;(1):86–9.

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