

Comparative Anti-bacterial Activity of Differently Capped Silver Nanomaterial on the Carbapenem Sensitive and Resistant Strains of *Acinetobacter baumannii*

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Abstract

Acinetobacter baumannii is an opportunistic Gram-negative pathogen causes pneumonia, respiratory infections, urinary tract infections and emerged as a serious pathogen for the immuno-compromised patients. Its prevalence increases gradually in the clinical setup. Carbapenem are most effective antibiotics against *A. baumannii*. Emergence of resistance of *A. baumannii* against carbapenem will leads to the high mortality and morbidity. Therefore, there is a high time to develop antibiotic alternative drug against carbapenem resistant strain of *A. baumannii*. In present work, we have synthesized and characterized silver nanoparticle (AgNPs) capped with different agents like polyvinylpyrrolidone (PVP), sodium citrate, chitosan, sodium dodecyl sulfate (SDS). Antibacterial effect of these capped AgNPs has been checked on the carbapenem resistant and sensitive strains of *A. baumannii* using UV-Vis Spectrometer. It was found that PVP and sodium citrate capped AgNPs are more effective while chitosan and SDS capped AgNPs shows little effect (only at high concentration) on the carbapenem resistant strain of *A. baumannii*. The result also highlighted the synergism between differentially capped AgNPs with carbapenem. Interestingly, we have found that the synergism between AgNPs and antibiotics is also dependent on the resistance level of pathogen. Result also shows that the total protein content of the both the strains is decrease in the presence of capped AgNPs. Result concludes that PVP capped AgNPs might be suitable replacement of carbapenem or can be used along with carbapenem to cure the infection caused by carbapenem resistant strain of *A. baumannii*.

Keywords: Carbapenem resistance; *Acinetobacter baumannii*; Silver nanomaterial; Sodium-citrate capped silver nanomaterial; PVP-capped silver nanomaterial

Introduction

Acinetobacter is identified as one of the six important and highly drug resistant hospital pathogens by the Infectious Disease Society of America and grouped into ESKAPE pathogen (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species). *A. baumannii* is among the most common pathogens to cause late-onset ventilator-associated pneumonia and the second most common pathogen to cause bloodstream infections acquired in hospital [1,2]. It has acquired a great attention of media because of its eruption among soldiers of Iraq, also known as Iraqibacter. To date, *A. baumannii* has become resistant to almost all antimicrobial agents that are currently available [3]. Carbapenems are the regularly prescribed antibiotics by doctors for the treatment of serious nosocomial infections caused by *A. baumannii*. Report showed that several mechanisms of carbapenem resistance have been described for *A. baumannii*, including loss of outer membrane proteins [4], altered penicillin-binding proteins [5], acquire carbapenemases [6-10], efflux pumps and enhanced metabolism [11,12]. Therefore, it is necessary to develop alternative drug to carbapenem that can be used against *A. baumannii*.

Different approaches have been tried which includes screening of herbal compounds [13] and nanomaterial based approaches [14] etc to find alternative to carbapenem. Silver nanoparticles have shown antimicrobial activity against a wide array of microbes, probably due to their multiple mechanisms of antimicrobial action [15]. Antimicrobial activities of colloid silver particles are influenced by the morphology of the silver particles. Therefore, during synthesis, emphasis should be given to control the size, shape, size distribution and local distribution of silver nanoparticles. There are few reports that initiated the research of silver nanomaterial as an antimicrobial agent for *A. baumannii*

[14,16,17] but no suitable preparation is available for the replacement of carbapenem. Capped silver nanoparticles (like citrate-capped, SDS-capped) have also been synthesised against Gram negative bacteria [14,16,17]. Antimicrobial activities of capped Ag-NPs have been determined only for few bacteria like *Salmonella*, *S. aureus* and *E. coli* [18-20]. In our present study, we have prepared AgNPs with different capping or stabilizing agents to compare their effectiveness against carbapenem resistant strain of *Acinetobacter baumannii*. We have also compared the effect of differentially capped AgNPs on RS307, carbapenem resistant and ATCC, carbapenem sensitive strain of *A. baumannii*. The differentially capped AgNPs have also checked for its synergistic effect with different carbapenems and other antibiotics. The selected AgNPs could be replacement to the carbapenem.

Methods

Preparation of AgNPs and their capping

For the preparation of AgNPs, four capping or stabilizing agents like polyvinylpyrrolidone (PVP), sodium citrate, sodium dodecyl sulphate (SDS) and chitosan were used. All these four different kinds of nanoparticles were prepared by chemical reduction method using published protocols [14,20,21-23] with some modifications.

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Preparation of PVP capped AgNPs: For the preparation of PVP capped silver nanoparticles, 5 ml of 0.01 N AgNO₃ was dissolved in 10 ml ethylene glycol mono methyl ether and continuously stirred for 2 h at room temperature 40-50°C. After this step, PVP was added to this solution in varying concentrations to maintain different metal to capping agent ratio and different colors were obtained at different amount of PVP by continuous stirring of solution for 30 min. After size characterization of these particles by UV-VIS spectrophotometer, it was found that brownish yellow solution of silver colloidal particles have optimum size between 8 ± 2 nm at 0.1 gm of PVP [14].

Preparation of chitosan capped AgNPs: For the preparation of chitosan capped silver nanoparticles, two mixture solutions were prepared. Mixture one contains 0.25 gm (0.01 M) chitosan (C₆H₁₁O₄N) which was dissolved in 50 ml distilled water in a beaker and then 0.1 M (30 µl) acetic acid solution added to it, acetic acid solution was added into it for dissolving the chitosan polymer and then 5 ml of 0.01 M solution of silver nitrate was added into this and then it was kept in ultrasonic water bath for 10 min to attain homogeneity. Mixture two contains 0.18914 gm (0.1 M) sodium borohydride (NaBH₄) dissolved in 50 ml distilled water. Mixture two was slowly added to solution one with constant stirring at a regular interval so that a uniformly size particles were formed. Initially color of solution was brown indicating the formation of silver nanoparticles, this prepared solution was ten times diluted followed by further addition of solution two. After continuous stirring, it was turned into dark green color indicating that chitosan capped silver nanoparticles were formed [22].

Preparation of SDS capped AgNPs: SDS capped silver nanomaterials were synthesized from silver nitrate, as precursor and sodium borohydride as reducing agent and SDS was stabilizer. 1 ml of 0.01 M AgNO₃ solution was dissolved in 30 ml of 0.002 M NaBH₄ solution which gave light yellow color and then it was kept at 37°C for 30 minutes after that 0.00358 gm (0.4 mM) SDS was added into above prepared solution and allowed for continuous stirring for 30 minutes. It gives dark yellow color that indicates the formation of SDS capped AgNPs [20,21].

Preparation of sodium citrate capped AgNPs: For the preparation of sodium citrate capped AgNPs, 5 ml of 0.01 M solution of silver nitrate was heated in deionized water until it began to boil and then 10 ml solution of sodium citrate dehydrate solution (0.4 mM) was added drop wise to the silver nitrate solution as soon as the boiling commenced. After sometime, the color of the solution turned into grayish yellow, which showed that the reduction of Ag⁺ ions began. This prepared solution was further heated for some time and then the solution was cooled to room temperature and it was stored for further experimental analysis [23].

Characterization of AgNPs

To analyze the size range of formed nanoparticles, wavelength scanning of capped nanoparticles was done using UV-Vis spectrophotometer. These chemically synthesized AgNPs with different capping agents were characterized by UV-Vis spectrophotometer analysis in the range of 200-600 nm. Peaks of surface plasmon resonance were observed and recorded. These chemically synthesized particles were kept at the room temperature to check their stability and activity. Diameter of prepared AgNPs varied according to their capping agents or stabilizing agents.

Growth kinetics

Growth kinetics of two strains (RS307, carbapenem resistant and

ATCC-19606, carbapenem sensitive) of *A. baumannii* in the presence and absence of different capped agents. These two strains are available in our laboratory and their resistant levels are already characterized. Growth kinetics was analyzed at regular interval of time period of 1 hr to check the viability of bacterial cell in presence and absence of four different kinds of silver nanoparticles with the help of UV-Vis spectrophotometer at 605 nm. The growth curves were plotted on obtained by UV-Vis spectrophotometer. Differential growth curve was also plotted to compare the bacterial growth in presence and absence of silver nanoparticles.

Disc diffusion assay

Antibacterial assay of two strains (ATCC and RS307) of *A. baumannii* was performed by disc diffusion assay. Luria agar plates were prepared and discs of carbapenem, imipenem and ampicillin were placed on them. These were taken as control and in other plates antibiotic discs were placed along with different capped nanoparticles in order to find out any synergistic effect. 10 µl of nanoparticle solution was used to check antibacterial effect alone and with different antibiotics.

SDS PAGE profiling

After growth kinetics and antimicrobial assay, SDS-PAGE protein profiling of ATCC and RS307 cultured in absence and presence of selected AgNPs i.e PVP and sodium citrate were performed. 6 ml sample from each bacterial culture has been taken and centrifuged at 10,000 g for 10 minutes at 4°C. Pellet was resuspended with lysis solution (phosphate buffer saline with pH 7.4 and 50 µl 10% SDS) and vortexed for 5 minutes. This is followed by sonication of all the bacterial samples. Sonicated sample was centrifuged for 5 minutes at 7000 g and supernatant was used for further protein profiling. SDS profiling was performed similar to our published methods [8]. The destained gels were photographed by Bio-Rad Gel Documentation system. It is important to mention here that we have used similar volume approach where same volume of bacterial culture was initially taken for protein isolation because we wanted to monitor the effect of capped AgNPs in the total protein level rather than differential expression of individual proteins which cannot be monitored using SDS-PAGE. Please note that differential expression of individual proteins is not monitored in the present study as treatment leads to the death of the bacteria.

Results and Discussion

Emergence of resistance against *A. baumannii* will lead to the high mortality and morbidity. Therefore there is a high time to develop antibiotic alternative drug against carbapenem resistant strain of *A. baumannii*. In the present study, we have used ATCC and RS307 strain of *A. baumannii*. ATCC is carbapenem sensitive strain with MIC for imipenem i.e carbapenem is 1 µg/ml while RS307 have MIC 64 µg/ml for the imipenem hence used as a carbapenem resistant strain. Here, we have presented the result of antimicrobial activity of AgNPs capped with different capping agents like PVP, sodium citrate, chitosan, SDS on the carbapenem resistant strains of *A. baumannii*. We have also compared effect of differently capped AgNPs on the carbapenem sensitive strain of *A. baumannii*. We have also checked the synergistic effect of capped AgNPs with antibiotics (imipenem, doripenem and ampicillin) using disc diffusion assay.

Characterization of various capped AgNPs by UV-Vis spectrophotometer

UV-Vis spectrophotometer was used for the analysis plasmonic properties of these colloidal and differently capped AgNPs, their synthesis and their size distribution. A peak was observed at 296.65 nm that indicate the presence of NO₃⁻ ions and peak present in the range of 340-540 nm is an indication of agglomerated silver nanoparticles with a wide range of size distribution (Figure 1). Peak observed between 412-431 nm indicates the presence of silver nanoparticle. Peak present in the range of 389-402 nm represent particle size of 4-10 nm while peak around 454 nm represent particle size of 14 nm. Peak observed between 431-412 nm is an indication of average particle size AgNPs. This change was clearly observed in case of chitosan, sodium citrate and PVP capped AgNPs. Capping on the surface of nanomaterials protect them from changes in environmental conditions and prevent aggregation [14]. Aggregation of formed SDS-capped AgNPs might be taken place hence a clear major peak between 340-450 nm was not observed.

Growth kinetics

Growth analyses of RS307 and ATCC strains of *A. baumannii* were performed in the presence and absence of silver nanoparticles capped with different kinds of capping agents. Result of growth analysis of RS307 shows that treatment of RS307 with PVP and sodium-citrate capped AgNPs, result into significant decrease in the absorbance of

RS307 while chitosan and SDS capped AgNPs treatment result into relatively less decrease or no change in the absorbance (Figure 2). This states that sodium citrate capped and PVP capped AgNPs show good inhibitory effect (Figures 2A and 2B) on RS307, while chitosan capped AgNPs and SDS capped AgNPs (Figures 2C and 2D) are ineffective on RS307. Similarly, growth kinetics of ATCC in the presence and absence of different capped AgNPs shows that there is decrease in the OD in the case of PVP and sodium citrate capped AgNPs (Figure 3). This stated that sodium citrate capped and PVP capped AgNPs show good inhibitory effect (Figures 3A and 3B), while chitosan capped AgNPs and SDS capped AgNPs (Figures 3C and 3D) are ineffective on the carbapenem sensitive strain of *A. baumannii*. The growth kinetics results highlighted that PVP and sodium citrate capped AgNPs are effective therefore could be looked as a future replacement of carbapenem while chitosan and SDS capped AgNPs were ineffective against both the strains of *A. baumannii*. To further validate the effect of chitosan and SDS capped AgNPs, growth analysis of ATCC and RS307 has been performed in the presence of higher concentration of these two-capped AgNPs (Figure 4). Result stated that at higher concentration, chitosan capped AgNPs shows some effect against carbapenem sensitive strain of *A. baumannii* but SDS capped AgNPs are ineffective even to carbapenem sensitive strain. It is also further confirmed that even at high concentration, chitosan-capped and SDS-capped AgNPs showed little effective on the

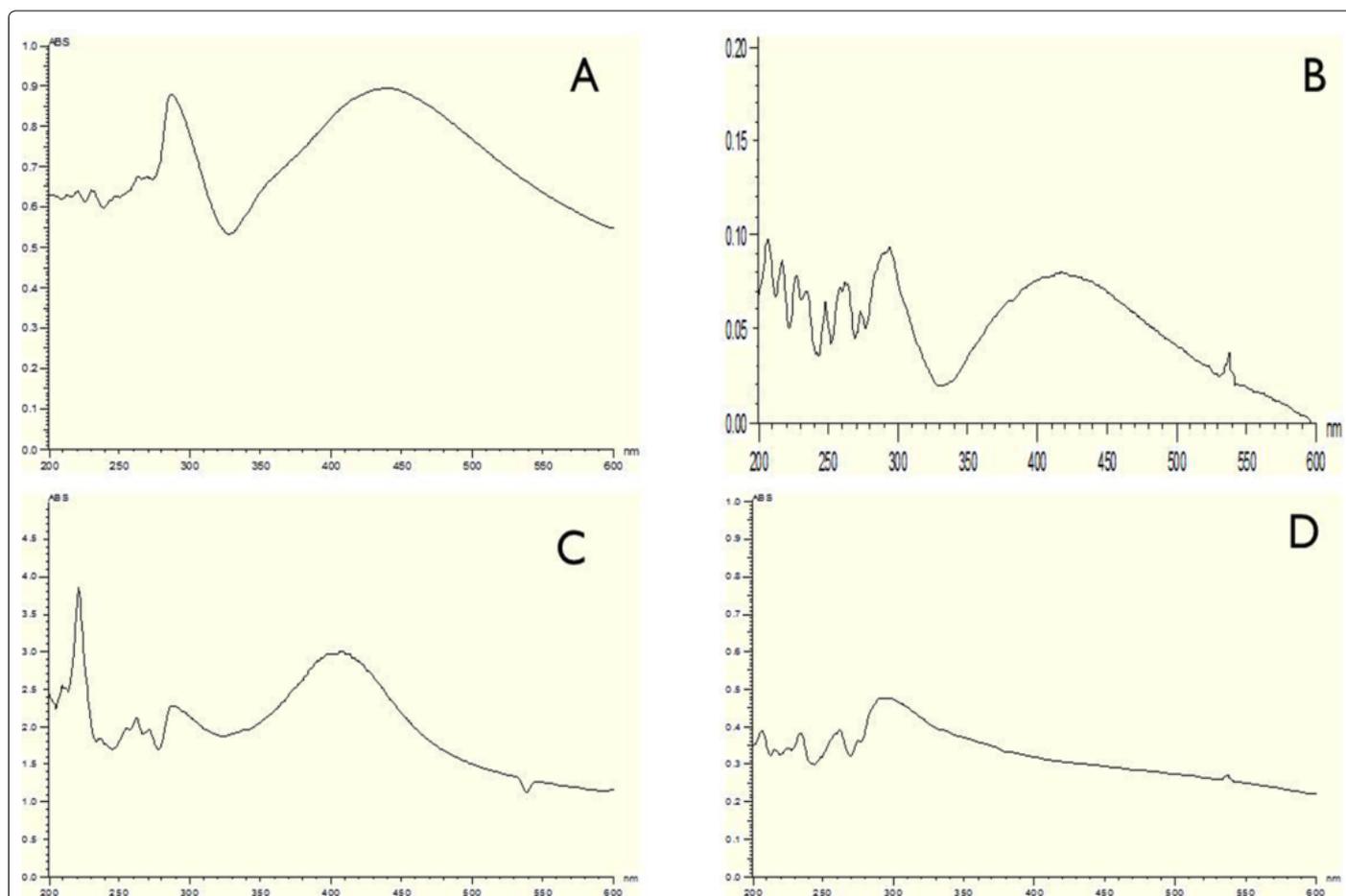


Figure 1: Characterization of AgNPs by UV-VIS spectrophotometer (A)PVP-AgNPs; (B)Na-Citrate-AgNPs(C); Chitosan-AgNPs; (D)SDS-AgNPs. A common peak at 297 nm that indicate the presence of NO₃⁻ ions and peak present in the range of 340-540 nm is an indication of agglomerated silver nanoparticles with a wide range of size distribution.

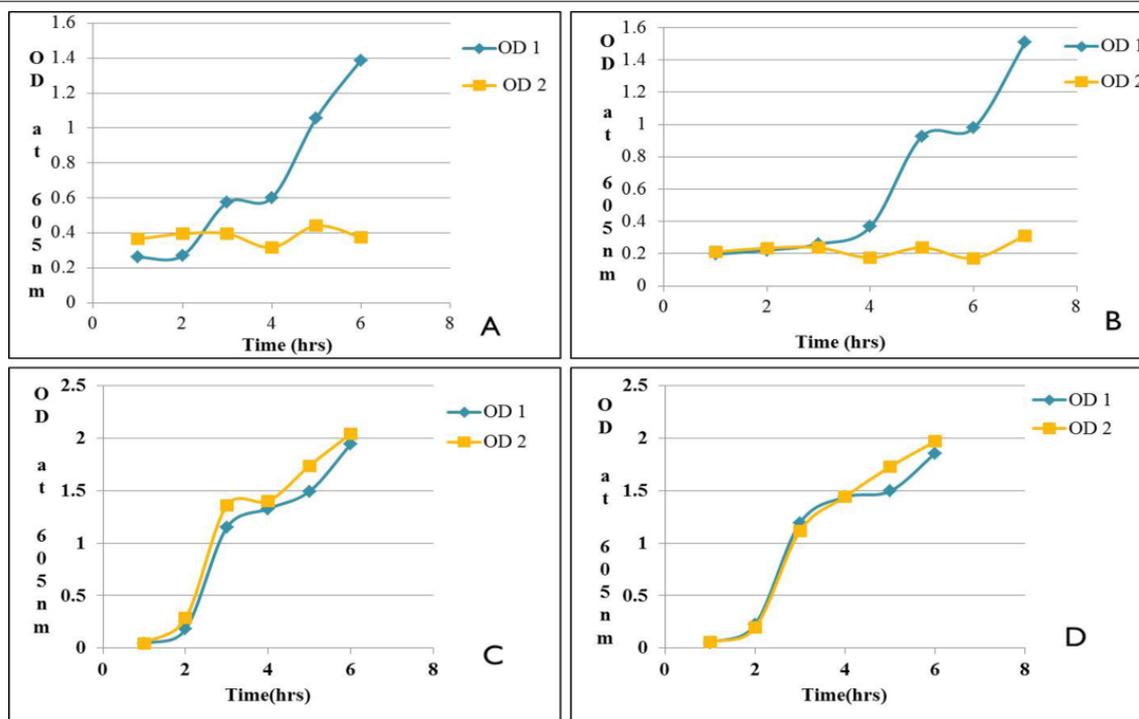


Figure 2: Growth kinetics of RS307 strain of *A. baumannii* in presence and absence of (A) Na-Citrate-AgNPs (B) PVP-AgNPs (c) Chitosan-AgNPs (D) SDS-AgNPs. OD1 represents the absorption peak in absence of AgNPs while OD2 shows absorption peak in presence of capped AgNPs.

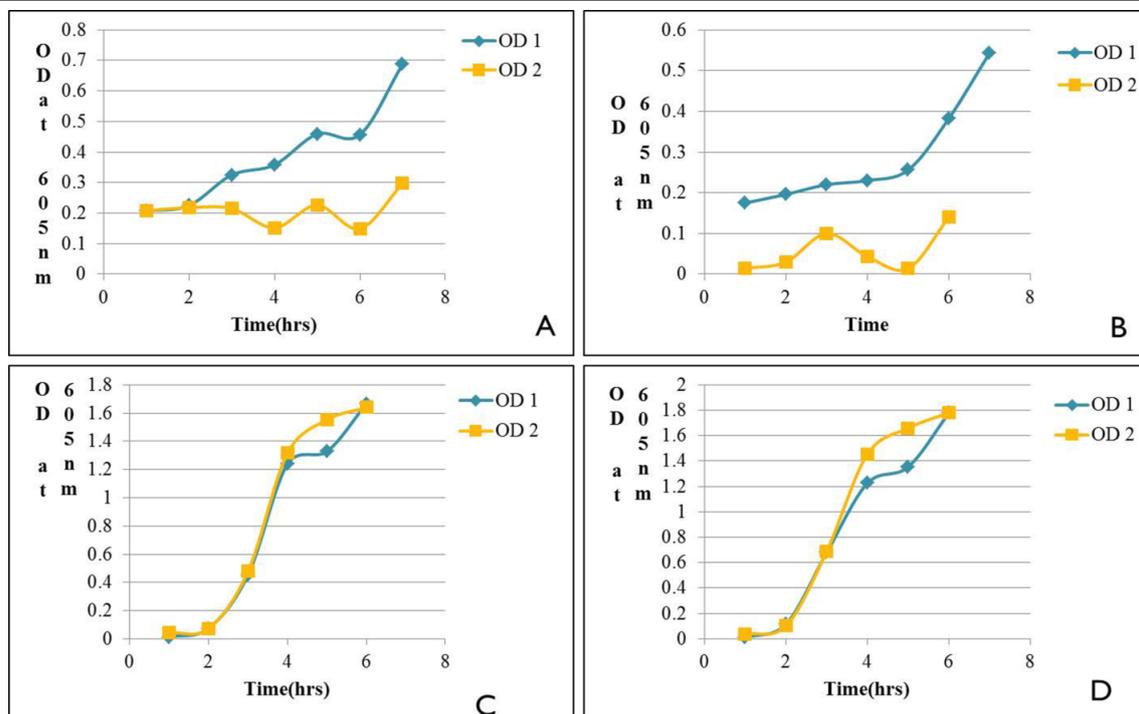


Figure 3: Growth kinetics of ATCC strain of *A. baumannii* in presence and absence of (A) Na-Citrate-AgNPs (B) PVP-AgNPs (c) Chitosan-AgNPs (D) SDS-AgNPs

RS307, carbapenem resistant strain of *A. baumannii*.

Disc diffusion assay

Results of disc diffusion assay of RS307 are presented in Figure 5.

In the present study, imipenem and doripenem have been taken as a representative of carbapenem class of beta-lactam while ampicillin as a representative of non-carabapenem beta-lactam antibiotics. It is clear that in case of control (antibiotics alone), RS307 shows inhibition zone of imipenem and doripenem while resistance against ampicillin

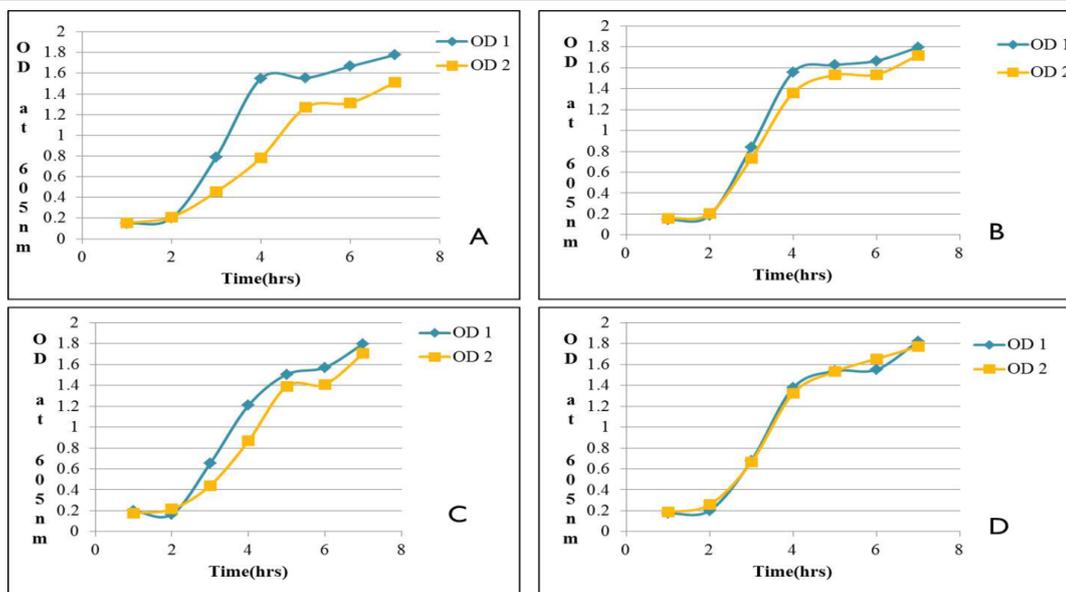


Figure 4: Growth kinetics of ATCC strain of *A. baumannii* in presence and absence of (A) Chitosan-AgNPs (B) SDS-AgNPs and RS307 strain of *A. baumannii* in presence and absence of (C) Chitosan-AgNPs (D) SDS-AgNPs at higher concentration.

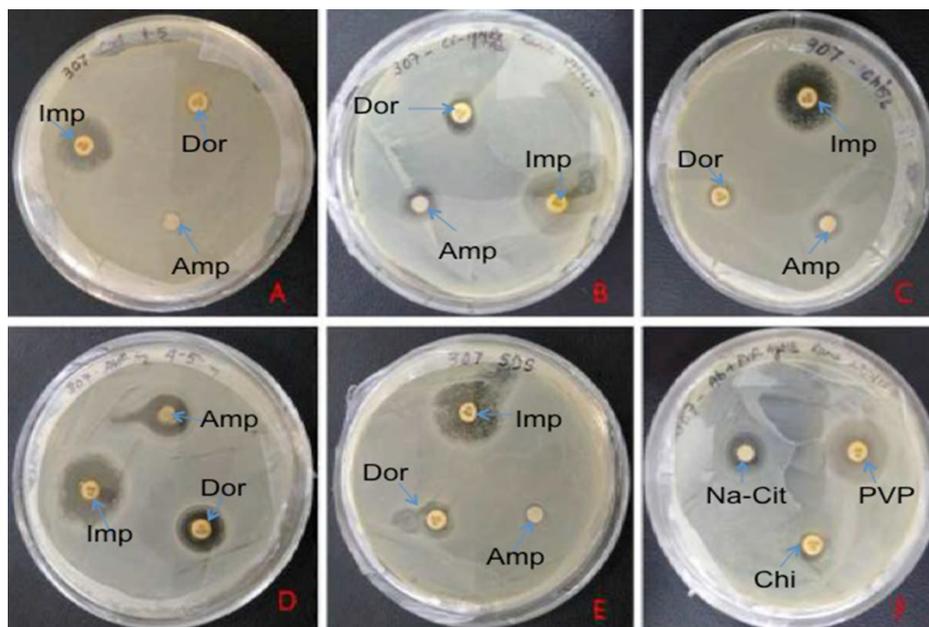


Figure 5: Disc diffusion assay of RS307 strain of *A. baumannii* (A) control (B) Na-Citrate-AgNPs (C) Chitosan-AgNPs (D) PVP-AgNPs (E) SDS-AgNPs (F) Alone AgNPs (PVP, Sodium citrate and Chitosan).

<i>A. baumannii</i> 307	Imipenem	Doripenem	Ampicillin	Alone
Control	1.8 cm	1 cm	Nil	-
PVP-AgNPs	1.8 cm	1.5 cm	1.4 cm	1.4 cm
Sodium-Citrate-AgNPs	2 cm	1.2 cm	1.3 cm	1.3 cm
Chitosan-AgNPs	2 cm	1 cm	1 cm	1.0 cm
SDS-AgNPs	2.3 cm	1 cm	1 cm	No

Table 1: Observation table of antibacterial assay showing zone of inhibition in synergy with AgNPs and antibiotics on the RS307, carbapenem resistant strain of *A. baumannii*.

(Figure 5). PVP and sodium-citrate capped AgNPs showed good zone of inhibition as compared to chitosan and SDS capped AgNPs. When

these AgNPs are given along with these antibiotics, the differentially capped AgNPs showed synergism with different antibiotics but the

behavior is different for different AgNPs. Sodium-citrate showed synergism with imipenem and doripenem while PVP does synergism only with doripenem not with imipenem (Table 1). Chitosan and SDS capped AgNPs also showed synergism with imipenem. Similarly, Figure 6 showed the disc diffusion assay of ATCC. Result shows that all three antibiotics showed some zone of inhibition (Table 2) that further confirmed that ATCC strain is susceptible for all three antibiotics. PVP and sodium citrate capped AgNPs showed good zone of inhibition while chitosan capped AgNPs showed less activity while SDS capped AgNPs were ineffective. Sodium citrate showed synergism with imipenem and doripenem. PVP showed synergism with doripenem and ampicillin while Chitosan showed synergism with imipenem. It is interesting to note that the synergism behavior of the different capped AgNPs and antibiotics are different for carbapenem resistant and sensitive strain of *A. baumannii*. Different resistance mechanism developed in the resistance strain might have some role in this differential behavior.

Protein profiling analysis

Growth kinetics and disc diffusion assay showed that PVP and sodium citrate capped AgNPs are very effective. Therefore their effect on the protein level of RS307 and ATCC has been investigated. We wanted to monitor the effect of capped AgNPs in the total protein level change rather than differential expression of individual proteins therefore similar volume approach (discussed in method) has been used. SDS-PAGE profiling (Figure 7) showed that a very light or absence of bands in presence of PVP AgNPs in both the strains while

sodium citrate showed more effect in the ATCC as compare to the RS307. This indicates that PVP is relatively more effective than sodium citrate capped AgNPs on RS307 strain of *A. baumannii* while both are equally effective on the ATCC strain of *A. baumannii*. PVP capped AgNPs are reported to interact with certain proteins and thereby showed its activity [24]. Present SDS PAGE results are also suggesting decrease total protein level is due to that decrease expression of protein or increase degradation of the protein that is our future study.

PVP is a neutral organic stabilizer and is less sensitive to the surface charge screening processes by ionic strength than citrate [19]. Retention of PVP coated AgNP is more than citrate coated AgNP because particle size of PVP coated nanoparticles is less sensitive to changes in pH and ionic strength [19,25,26]. Recent report showed that PVP capped Ag-NPs are more stable than other AgNPs such as sodium-citrate AgNPs and SDS AgNPs [19,20] hence PVP capped AgNPs is less toxic for mammalian cells [27] therefore PVP capped AgNPs can be more favorable as an alternative drug to carbapenem as compared to other capped AgNPs. Similarly, interaction of AgNPs with serum proteins also affects its antimicrobial activity *in-vivo*. It is also reported that PVP-capped AgNPs showed better antimicrobial activity than other capped AgNPs *in-vivo* [28]. Therefore, PVP capped and sodium citrate capped AgNPs could be the future hope for the replacement of carbapenem.

Conclusion

Carbapenems are regularly prescribed by the doctors against

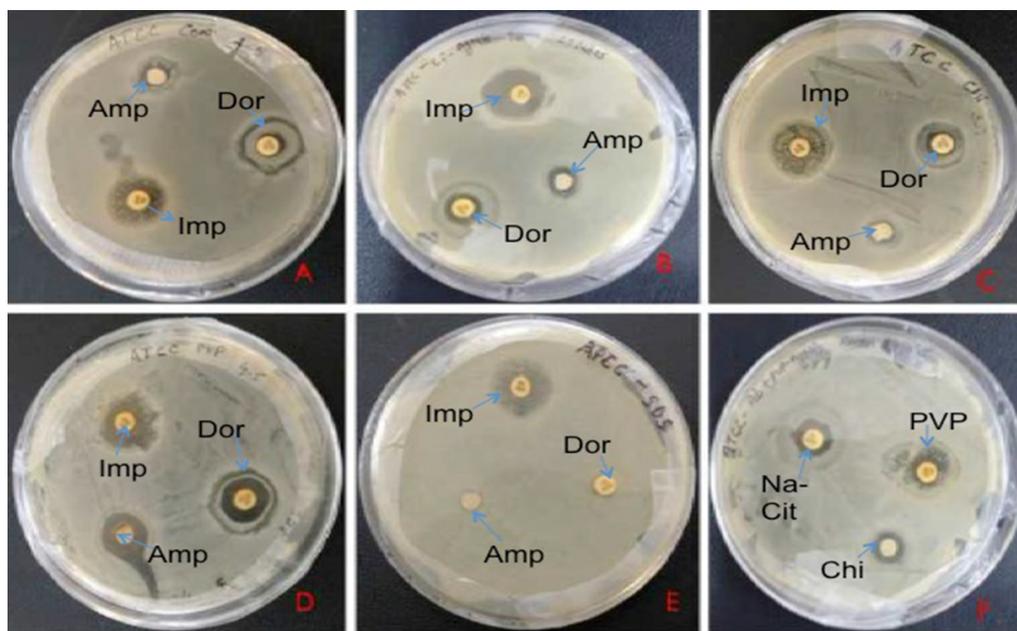


Figure 6: Disc diffusion assay of ATCC strain of *A. baumannii* (A) control (B) Na-Citrate-AgNPs (C) Chitosan-AgNPs (D) PVP-AgNPs (E) SDS-AgNPs (F) Alone AgNPs (PVP, Sodium-citrate and Chitosan).

<i>A. baumannii</i> ATCC	Imipenem	Doripenem	Ampicillin	Alone
Control	2 cm	1.2 cm	1.1 cm	-
PVP-AgNPs	2 cm	1.5 cm	1.5 cm	1.5 cm
Sodium-Citrate -AgNPs	2.3 cm	1.3 cm	1.1 cm	1.4 cm
Chitosan-AgNPs	2.3 cm	1.2 cm	1.1 cm	1.2 cm
SDS-AgNPs	2 cm	1.2 cm	1.1 cm	No

Table 2: Observation table of antibacterial assay showing zone of inhibition in synergy with AgNPs and antibiotics on the ATCC, carbapenem sensitive strain of *A. baumannii*.

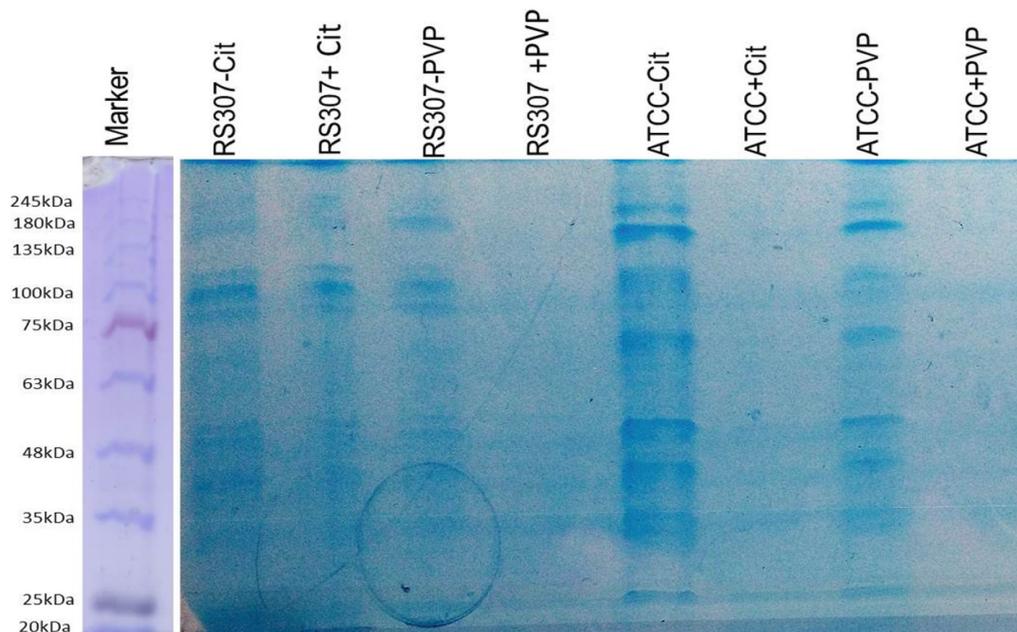


Figure 7: Protein profiling analysis by SDS-PAGE in the absence (-) and presence (+) of sodium citrate-capped AgNPs and PVP-capped AgNPs.

A. baumannii. Reports showed that *Acinetobacter* has developed resistance against carbapenem, which increases the mortality and morbidity caused by this pathogen. Present study highlighted that PVP and sodium citrate capped AgNPs are more effective as compared to chitosan and SDS capped AgNPs, on the carbapenem resistant and sensitive strains of *A. baumannii*. The result also highlights the synergism between differentially capped AgNPs with carbapenem. Interestingly, we found that the synergism between AgNPs and antibiotics is also dependent on the resistant level of pathogen which also concluded that resistant machinery of pathogen might have some role in this differential effect. Result enlighten that PVP and sodium-citrate AgNPs might be suitable replacement of carbapenem or can be used along with carbapenem to cure the infection caused by carbapenem resistant strain of *A. baumannii*.

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Author Disclosure Statement

The authors have declared that no competing interests exist.

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