



OPEN In-vitro anti-inflammatory and antagonistic efficacy of polymyxin B capped silver nanoparticles in multi drug resistant *Acinetobacter baumannii*

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Multidrug resistant *Acinetobacter baumannii* is declared as crucial level precedence pathogen by World Health Organization that needs new and upgraded antibiotics for better treatment. Against a vast extent of microbes, many silver nanoparticles have displayed anti-microbial activity because of their numerous methods of antimicrobial actions. This study was aimed to isolate and characterize the *Acinetobacter baumannii* by using standard microbiological technique and to analyze the anti-inflammatory and antagonistic effect of PB capped AgNPs in isolated *A. baumannii*. In antagonistic activity, PB capped AgNPs showed antagonistic effect in 8 out of 20 isolates tested and PB when combined with AgNPs showed antagonistic effect in all isolates tested. In in-vitro anti-inflammatory egg albumin assay, PB capped AgNPs gave inhibition rate of 96% at 12.5 µg, 93% at 25 µg, 82% at 50 µg and 62% at 100 µg whereas in Polymyxin B combined AgNPs it gave inhibition rate of 97% at the dose of 50 µg. In-silico analysis also showed that PB in combination with tri-sodium citrate gave good binding energy than tri-sodium citrate and Polymyxin used alone. Hence, this study represented that silver nanoparticles when used in combination proved to be a good alternative in the treatment of MDR *Acinetobacter baumannii*.

Keywords *A. baumannii*, Multidrug resistant (MDR), AgNPs, Polymyxin B (PB)

The antimicrobial resistance increasing extensiveness is a major risk to human health and it is enhancing mortality rates¹. When studying about treatment of *A. baumannii* infections, awareness should be recompense to the definition of resistance used². More research attempts have been made towards the evolution of substituted antimicrobials with multitargeting methods³. *A. baumannii* is a coco bacillus, Gram-negative bacteria. It is non-motile and aerobic bacterial pathogen. It is a major cause of nosocomial infections³. *A. baumannii* is the most frequent pathogen that causes bloodstream infections and ventilator associated pneumonia. It infects those with debilitating immune system. It is an antibiotic-resistant microbe. To all antimicrobial agents that are presently accessible, *A. baumannii* is found to be the most resistant⁴. To primary antimicrobial treatments, the rapidly increasing resistance of *A. baumannii* has made a lethal combination of AMR and pathogenicity that afflicts

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hospitals. At the time of admissions, Nosocomial infection is not present in patients. It arises within 48 h after infection. Because *A. baumannii* is common in humans, it is called the most common type of infection in nosocomial infections⁵. In critically ill patients, hospital acquired infections are seen and has risk of developing *A. baumannii* infections including burns, traumas, immunosuppression and use of previous antibiotics, while community-acquired infections found in hot and humid climate countries as a serious clinical disorder¹. According to urgent need of new antibiotics, WHO has three categories, The most critical group includes MDR bacteria that pose a serious threat to patients such includes *Acinetobacter*, *E.coli*, *Pseudomonas*, *Klebsiella*, *Proteus* and *Serratia*. Resistance to multiple drugs defined as a great provocation as second line treatment options are less clarified in their potency and accompanies infectivity². It is obligatory to evolve different drugs that can be used against *A. baumannii*⁴.

Although the pharmaceutical market offers a wide range of antibacterial medications, continued research into powerful antimicrobial agents—particularly those with novel mechanisms of action—is vital. This is key to curbing the development of resistance to current antibiotics and ensuring effective treatments for infections caused by antibiotic-resistant bacteria. Against a wide range of microbes many silver nanoparticles have displayed antimicrobial activity because of their numerous methods of antimicrobial action⁴. Silver nanoparticles are used in many fields involving food, health care, and medical purposes. The capability of AgNPs as antibiotics is linked to their numerous action modes which grant them the capability to kill numerous types of bacteria^{6,7}. Silver has been used as an antimicrobial agent in all its forms when used alone as well as in combinations with other mechanisms⁶. Silver nanoparticles (AgNPs) generally pose no harm to human health at low concentrations due to their specific form, size, stability, and biocompatibility. However, prolonged exposure to silver can lead to serious conditions such as argyrosis. The chemical reduction method is employed to synthesize a colloidal solution of silver nanoparticles using silver nitrate as the precursor, with trisodium citrate acting as both the stabilizing and reducing agent in the process⁸. In case of *A. baumannii* high resistance is found in Amoxicillin, ampicillin, ciprofloxacin, imipenem, meropenem, cefuroxime, cefoxitin, cefepime, gentamicin, cefotaxime and nitrofurantoin⁹. Polymyxin B is an antibiotic that is used for the treatment of variety of infections. It is used for treatment of Gram-negative organism infections and all Gram-positive organisms remain resistant. It is used for treatment of blood sepsis and urinary tract infection. It has chemical formula $C_{56}H_{98}N_{16}O_{13}$. Polymyxin B damages the Gram-negative bacteria outer membrane. During bacterial lysis, Polymyxin B coheres and counteracts lipopolysaccharide. Polymyxin B and Colistin (Polymyxin E) are FDA approved drugs. They are considered as last line therapy for inflammation produced by Gram-negative bacteria. Polymyxin B is used as therapy for various infections including meningitis, urinary tract infections and blood stream infections caused by Gram negative bacteria¹⁰. Hence, the capping of Polymyxin B with silver nanoparticles must be studied to eliminate the resistance towards *A. Baumannii*. Capping of Polymyxin B with silver nanoparticles refers to the process in which Polymyxin B replaces the trisodium citrate on the surface of the silver nanoparticles, effectively attaching to it. Antagonistic efficacy and anti-inflammatory study will be done on *A. baumannii* by capping Polymyxin B and Citrate with silver nanoparticles and combining both CitAgNPs and PBAgNPs. The purpose of this study is to analyze the anti-inflammatory and antagonistic effect of PB capped AgNPs in isolated *A. baumannii*.

Materials and methods

Silver nanoparticles preparation (AgNPs)

0.084 g of silver nitrate was added in 50 ml deionized water in a beaker and heat it for 30 min with a stirrer in it. Then in 50 ml distilled water, 1 g tri sodium citrate was added. Then from prepared tri sodium citrate, 5 ml solution was added dropwise in prepared silver nitrate with continuous heating and stirring. Then it was heated for 15 min and cooled at room temperature. Silver nanoparticles was prepared¹¹.

Polymyxin B capped silver nanoparticles preparation (PBAgNPs)

0.084 g of silver nitrate was added in 50 ml deionized water in a beaker and Polymyxin B with different concentrations (12.5 µg, 25 µg, 50 µg and 100 µg) was added in prepared silver nitrate solution and heat it for 30 min with a stirrer in it. Then in 50 ml distilled water, 1 g tri sodium citrate was added. Then from prepared tri sodium citrate, 5 ml solution was added dropwise in prepared silver nitrate with continuous heating and stirring. Then it was heated for 15 min and cool at room temperature. Polymyxin B capped silver nanoparticles was prepared¹².

Characterization of AgNPs and PBAgNPs

The characterization of AgNPs and Polymyxin B capped AgNPs was done using techniques such as Dynamic light scattering (DLS) and Fourier transform infrared (FTIR) to determine their morphology.

Collection *A. baumannii* samples

A total 250 samples of Multidrug resistant *A. baumannii* was collected from tertiary care hospitals of Lahore. The sample was processed for isolation and identification of *A. baumannii* by using standard microbiological technique.

Isolation and identification of *A. baumannii*

The collected isolates of *A. baumannii* was cultured on Macconkey agar and was identified using biochemical tests as urease test, Simon citrate test, indole test, catalase test, Triple sugar iron test and gram staining.

Antagonistic activity

The Antagonistic activity of AgNPs, Polymyxin B and PBAgNPs at 100 µg, 50 µg, 25 µg and 12.5 µg were evaluated against multidrug *A. baumannii* using well diffusion method. Muller Hinton broth was made for determining the pure culture of bacteria. Then Muller Hinton agar plates were prepared and well has been made with in AgNPs, Polymyxin B and PBAgNPs at 100 µg, 50 µg, 25 µg and 12.5 µg were placed. Wells are created with diameter ranging from 6 to 8 mm using sterile straw and plates are incubated at 37 °C for 24 h. Zone of inhibition was measured in mm and antagonistic activity was evaluated against bacterial strains¹³.

Anti-inflammatory egg albumin

From fresh hen's egg, 0.2 ml of 1–2% aqueous solution of egg albumin will be prepared. At different concentrations of 2 ml of PBAgNPs, AgNPs and Polymyxin B prepared egg albumin solution will be added and then phosphate buffer saline 2.8 ml (PH 7.4) were added and mixed to make volume up to 5 ml. Mixture will be incubated for 20 min and then heat for 20 min at 51°C in water bath. After cooling, absorbance at 590 nm will be done by Elisa reader. A drug Diclofenac sodium (Standard) will be taken as positive control and distilled water as negative control. Then Anti-inflammatory % of inhibition was measured through the equation.

$$\% \text{ INHIBITION} = 100 \times ([V_t/V_c] - 1).$$

V_t is test sample absorbance and V_c is control absorbance¹⁴.

In silico analysis

Selection of ligands and protein

As *Acinetobacter baumannii* causes hospital-acquired infections and is a multidrug-resistant bacteria. For the treatment of *Acinetobacter baumannii*, Polymyxin B an antibiotic is selected coated with silver nanoparticles. Hence ligands selected were tri-sodium citrate and Polymyxin B along with LPS protein (LptE) of *A. baumannii* for the treatment of MDR bacteria¹³.

Ligand preparations

SDS file of ligands (tri-sodium citrate and Polymyxin B) were selected from PUBCHEM and drawn on CHEMSKETCH. Then from PYMOL their 3D structure were obtained as PDB format¹³.

Protein preparations

3D structure of protein was downloaded from Protein data bank in PDB format with PDB ID 5TSE. The selected protein LptE was compressed by UCSF Chimera. All the ions, solvent and ligands were removed and single chain was saved¹³.

Prediction of active site

During a reaction, a site where an enzyme binds to protein is called active site. The active sites were predicted by CAST-p site. Amino acids were selected from chain C¹³.

Docking analysis

All Amino acids were selected in grid box. For LptE protein with Polymyxin B and Tri sodium citrate there were center $x = -0.0313$, center $y = -43.38$, center $z = 1.194$ and size $x = 15.12$, size $y = 21.82$ and size $z = 25.57$. Docking was done using PYRX and then protein ligand binding pattern will be obtained using Chimera. Same process was repeated with single Polymyxin B with LptE protein as there was center $x = -0.0935$, $y = -38.48$, $z = -11.63$ and size $x = 23.05$, $y = 12.02$ and $z = 26.86$. The center for Tri-sodium citrate with LptE protein was $x = 1.057$, $y = -36.94$, $z = -10.89$, and size $x = 25.0$, $y = 13.38$, $z = 27.34$ ¹³.

Results

Collection of samples of *Acinetobacter baumannii*

A total of 250 samples were collected including pus, blood, wound and CSF from the Tertiary care hospital in Lahore. Out of 250 samples only 136 grew, other 114 samples were shown no prominent growth as shown in Fig. 1.

Isolation and identification of *A. baumannii*

The 136 samples were cultured and out of 136 only 14 were multi drug resistant *Acinetobacter baumannii* and 20 were *A. baumannii* which was sensitive against Polymyxin and AgNPs (Figs. 2 and 3). In this study, the 20 out of 136 samples which was sensitive to PB and Citrate capped AgNPs were considered for the antiinflammatory as well as antagonistic activity of Polymyxin B capped silver nanoparticles. Out of 20 MDR *A. baumannii* 6 samples were from wounds, 11 were from pus and 3 were from blood. Out of 20 *A. baumannii* 12 were MDR, 5 were XDR and 3 were PDR (Tables 1 and 2).

Acinetobacter baumannii was identified using biochemical tests including indole test, urease test, TSI test, Simon citrate test, Catalase test and gram staining. TSI results showed pink color that indicates positive test result for *A. baumannii* (Fig. 3). Indole showed no ring formation that indicates negative test results for *A. baumannii*. Urease were negative for *A. baumannii*, Simon citrate were positive as due to the appearance of blue color. Catalase test were positive for *A. baumannii* due to the formation of bubbles.

Synthesis of citrate capped and PB capped silver nanoparticles

A dark grey color obtained by addition of drop wise tri-sodium citrate on prepared silver nitrate solution indicated the formation of Citrate capped AgNPs. Polymyxin B with varying concentration (2 fold) 100 µg, 50



Fig. 1. The isolation of *A. Baumannii* on MacConkey agar.

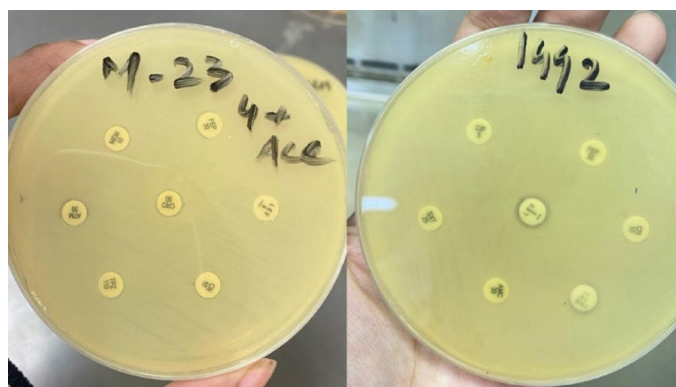


Fig. 2. The resistant isolates of *A. baumannii*. Antibiotics used are Tobramycin, Doripenem, Ceftazidime, Ampicillin, Azithromycin, Cephalosporin, Polymyxin B, Tirapazamine, Ciprofloxacin, Cefepime, Sulphamethoxazole, Levofloxacin, Imipenem, Gentamicin, and Amoxicillin.

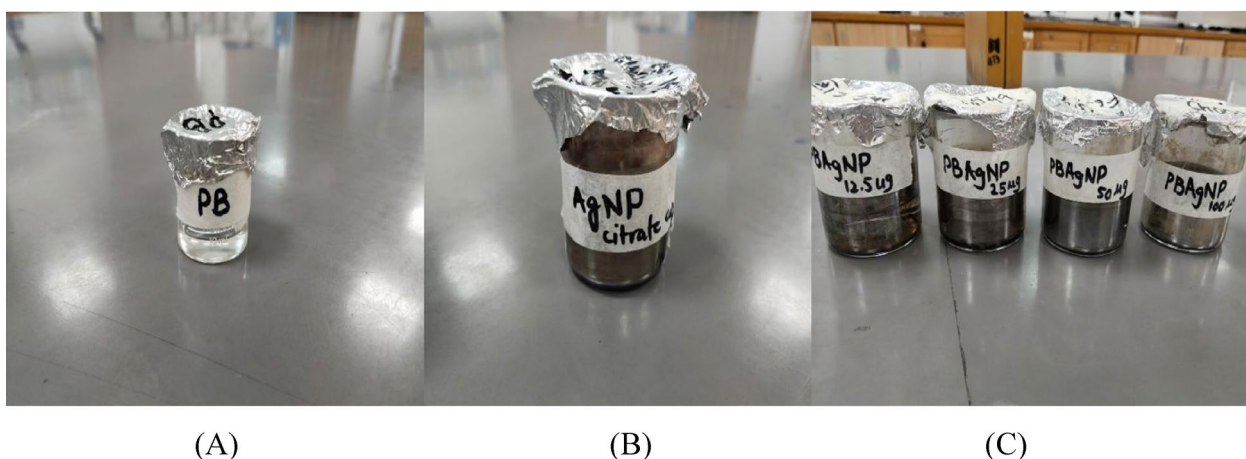


Fig. 3. TSI, indole, urease, simon citrate, and catalase test results for *A. baumannii*.

Sr. no.	Sample source	% frequency of sample source
1	Wound (6)	30%
2	Pus (11)	50%
3	Blood (3)	15%

Table 1. Demographical data of MDR *A. baumannii*.

S. no.	No of samples	Pattern of resistance
1	12	MDR
2	5	XDR
3	3	PDR

Table 2. Resistant type of isolates of *A. baumannii*.**Fig. 4.** (A) Polymyxin B (B) Citrate capped AgNPs (C) Polymyxin B capped silver nanoparticles with different concentrations.

μg , 25 μg and 12.5 μg was added to silver nitrate solution and a dark grey color was obtained by addition of few drops of tri-sodium citrate (Fig. 4).

Characterization of AgNPs and PBAgNPs

Dynamic light scattering (DLS)

DLS is used to measure the size of a molecule or a particle. It includes Hydrodynamic diameter which indicates the size of a particle whereas zeta potential indicates the stability of a particle. In silver nanoparticles, the hydrodynamic diameter was 19,835 nm with the size of 1771 nm. Silver nanoparticles when capped with Polymyxin B at 12.5 μg shows hydrodynamic diameter of 296.6 nm with the size of 264.6 nm and at 25 μg hydrodynamic diameter was 18,824 nm with the size of 677 nm. Silver nanoparticles when capped with Polymyxin B at 50 μg shows hydrodynamic diameter of 45,882 nm and size shows 1031 nm and at 100 μg hydrodynamic diameter was 734.7 nm with two peaks, one is with size 159 nm and other is 964 nm. This shows that PB when capped with AgNPs at 50 μg showed increase in hydrodynamic diameter than citrate capped AgNPs with the decrease in size means PB capped AgNPs at 50 μg has a good stability than citrate capped AgNPs (Fig. 5)⁴.

Zeta potential value indicates the repulsion among Polymyxin B and AgNPs and increase stability of composition. Zeta potential of silver nanoparticles shows -1.6 mV whereas Polymyxin B capped silver nanoparticles shows zeta potential of -8.9 mV at 12.5 μg , -0.7 mV at 25 μg , and -1.8 mV at 50 μg and 100 μg . Silver nanoparticles are poly dispersed in nature. As because of negative zeta potential values repulsion of particles helps in aversion of accumulation of nanoparticles and helpful in long term stability (Fig. 6)⁴.

Fourier transform infrared (FTIR) results

FTIR is used for determining the biochemical differences between AgNPs and Polymyxin B capped AgNPs. The spectrum of AgNPs showed frequency within the band 1098 cm^{-1} – 3408 cm^{-1} . The strongest peak was observed in 1577 cm^{-1} , and 3375 cm^{-1} which indicates the functional group of C=C and O-H group. Silver nanoparticles when capped with Polymyxin B showed a slight conformational change and in band intensity.

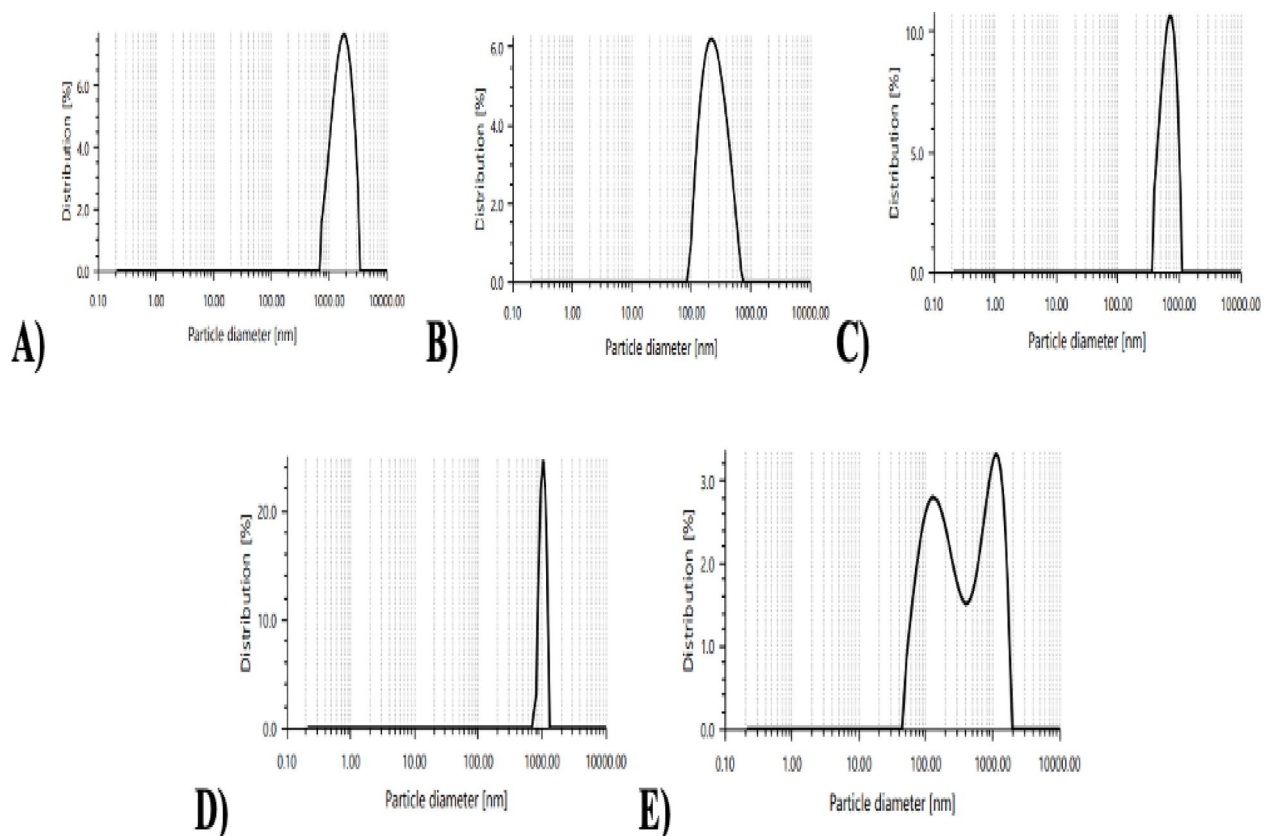


Fig. 5. DLS particle size distribution of (A) AgNPs, (B) 12.5 µg, (C) 25 µg, (D) 50 µg, (E) 100 µg.

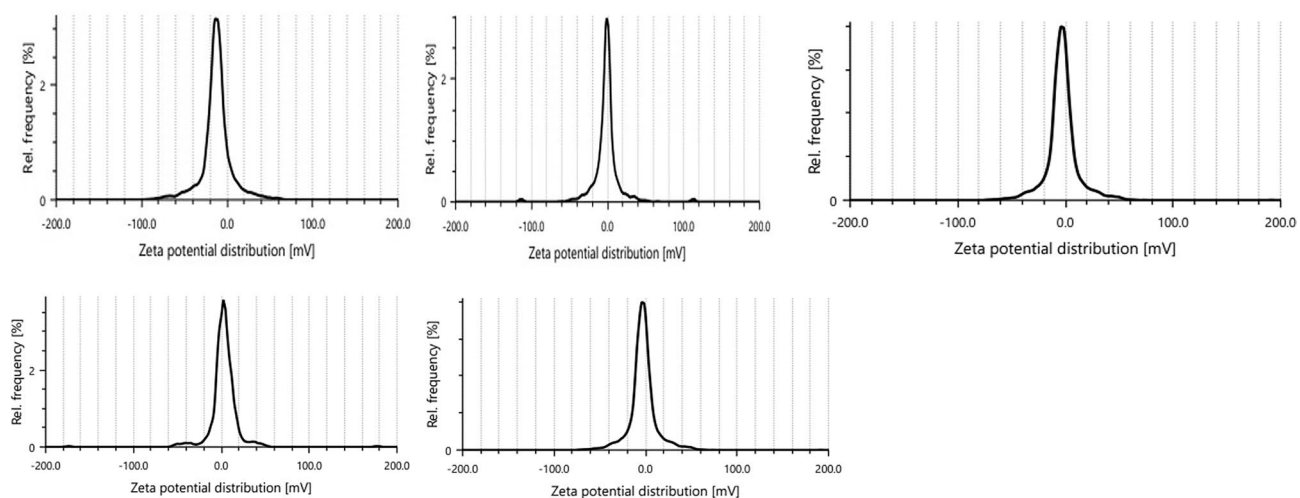


Fig. 6. zeta potential of (A) AgNPs (B) 12.5 µg (C) 25 µg (D) 50 µg and 100 µg.

At 12.5 µg dose showed band frequency within 882 cm^{-1} – 3340 cm^{-1} . The strongest peak was observed in 1641 cm^{-1} and 3340 cm^{-1} which reflects the functional group of N–H and O–H group. At 25 µg, 50 µg and 100 µg dose, band frequency ranges within 1639 cm^{-1} – 3330 cm^{-1} which showed increase in intensity which contributes to symmetric and asymmetric stretching. Strongest peak was observed in 1639 cm^{-1} which indicates presence of N–H bond. In AgNPs and Polymyxin B capped silver nanoparticles the results were same except the peak that was observed in 1639 cm^{-1} indicating the amine group as also shown in the study of¹⁵ and a different peak was seen at 12.5 µg dose at 1524 cm^{-1} indicating the functional group N–O bond which were both not present in AgNPs, indicating that Polymyxin B were functionalized with citrate capped AgNPs at N atom of amine group (Fig. 7)¹⁵.

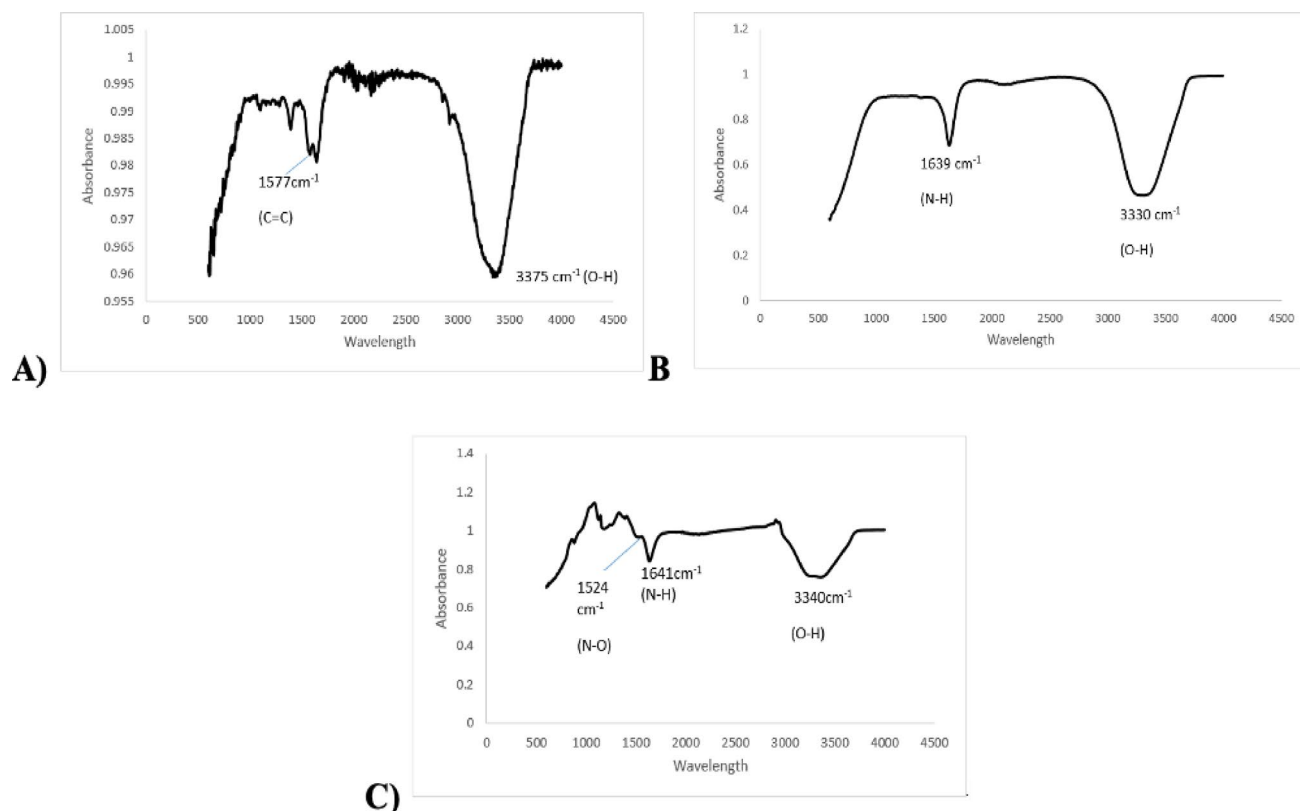


Fig. 7. FTIR spectrum of (A) AgNPs, (B) PB capped AgNPs (12.5 µg), (C) Polymyxin B capped AgNPs (25, 50, and 100 µg).

Combinations	Interaction
PB capped AgNPs	Antagonistic (8)
PB combined AgNPs	Antagonistic (20)

Table 3. Interactions of PB when capped with AgNPs and when combined with silver nanoparticles.

Antagonistic activity

Antagonistic activity was performed on Muller Hinton agar, silver nanoparticles showed no effect, only the Polymyxin B showed the effect. Polymyxin sensitivity pattern were also varied some strains of *A. baumannii* that were resistant against the lowest dose of Polymyxin B and some were sensitive against the longer dose which reflects the pathetic condition of public health because Polymyxin B has many side effects, it cause neurotoxicity and nephrotoxicity. When Polymyxin B capped with AgNPs (Table 3), it showed similar results. Out of 20 samples no sample were sensitive in 12.5 µg dose, 2 samples were sensitive in 25 µg dose, 8 were sensitive in 50 µg dose and 4 were sensitive in 100 µg dose in our results. In combination dose with AgNPs we find that all 20 samples were found sensitive. As a result of antagonistic activity, it is considered from our result that citrate capped silver nanoparticles alone were not a sufficient choice of therapy for the control of MDR *Acinetobacter baumannii* as it is also considered in past studies³ but it can be beneficial when used in combination with Polymyxin B (Table 4; Fig. 8, and Fig. 9).

The antimicrobial susceptibility of Polymyxin B and silver nanoparticles was evaluated using well diffusion method. Zone of inhibition shows sensitivity of Polymyxin B capped silver nanoparticles with different concentrations, AgNPs, and Pure Polymyxin B as shown in previous research.

According to CLSI standards for Polymyxin B, it is resistant at < 11 mm and sensitive when > 12 mm.

Anti-inflammatory egg albumin assay

Anti-inflammatory activity was performed by egg albumin process by Elisa reader. PB Capped AgNPs were taken at various concentrations and it has been compared with standard (Diclofenac sodium). Results showed % inhibition of 96% at the dose of 12.5 µg, 93% at the dose of 25 µg, 82% at 50 µg and 62% at 100 µg whereas Polymyxin B when combined with AgNPs gave the inhibition rate of 97% at the dose of 50 µg. Hence, it is concluded that PB capped AgNPs and PB in combination with AgNPs showed good anti-inflammatory activity in egg albumin assay and it is found that PB capped AgNPs found comparative to the standard set even at all

S. no.	Combinations	No of isolates	Zone of inhibition (mm)
1	AgNPs	20 (4)	12 (S)
2	Polymyxin B (50 µg)	20 (4)	10 (S)
3	100 µg	20 (12)	14 (S)
4	500 µg	20 (20)	18 (S)
5	PB capped AgNPs (100 µg)	20 (4)	24 (S)
6	50 µg	20 (8)	20 (S)
7	25 µg	20 (2)	16 (S)
8	12.5 µg	20 (0)	R
9	PB combined AgNPs (50 µg)	20 (20)	22 (S)

Table 4. Sensitivity pattern used by different combination in *Acinetobacter baumannii*.

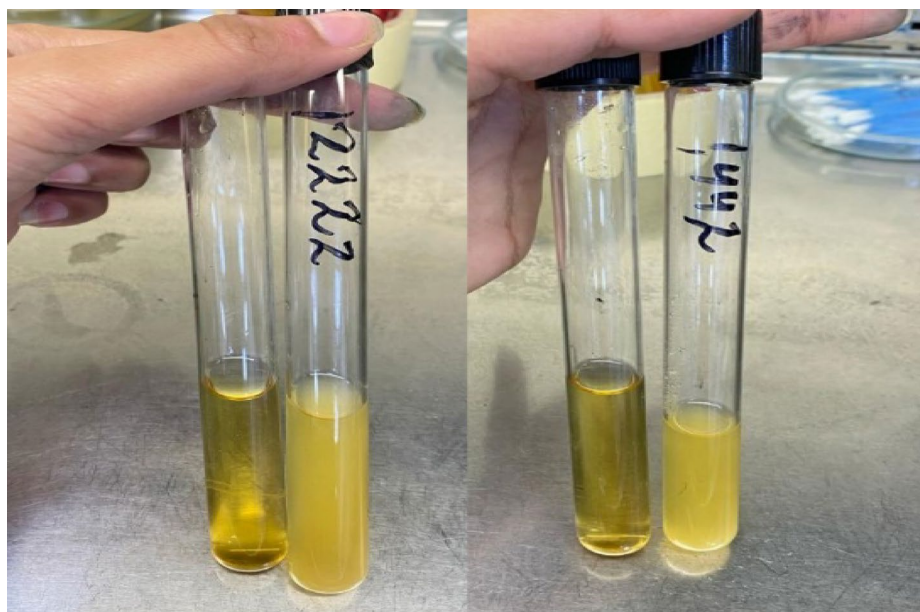


Fig. 8. The pure culture of *A. baumannii* on MH broth.

concentrations. PB capped AgNPs showed good anti-inflammatory activity in the method of egg albumin assay (Table 5, and Figs. 10 and 11).

Computational results

Evaluation of protein structure

LptE protein is a Lipopolysaccharide protein at the surface of outer membrane of *Acinetobacter baumannii*. It has three chains (A –C) and consists of 169 amino acids. Vadar analysis of LptE indicates 31% alpha helix, 46% beta helix, 28% coil and 9% turn. RAMACHANDRAN PLOT of LptE protein contains 95.12% amino acids in preferred region, 4.88% in allowed region and there are 0 outliers remaining (Fig. 12).

Evaluation of ligand structure

Polymyxin B belongs to the category of drugs. It has broad spectrum of activity against gram-negatives¹⁰. Polymyxin B is produced by a gram positive microorganism *Paenibacillus polymyxa*. It is declared as a last line treatment against MDR gram negative organisms. Polymyxins are cyclic polypeptides¹⁶. Its chemical formula is $C_{56}H_{98}N_{16}O_{13}$ and molecular weight is about 1203.477 g/mol¹⁷. Tri-sodium citrate is a sodium salt of citric acid. It acts as a reducing agent in silver nanoparticles¹⁸. It is a white crystalline powder. It has chemical formula $C_6H_5Na_3O_7$ and molecular weight of 258.06 g/mol (Fig. 13).

LptE protein active site

The Binding center of LptE protein consists of 14 amino acids 52 ASP, 56 GLN, 59 VAL, 60 TYR, 124 ARG, 142 TYR, 145 ARG, 146 ILE, 148 ILE, 149 ASP, 150 ASP, 153 GLN, 154 GLN and 157 ARG. These amino acids were selected from chain C (Fig. 14).

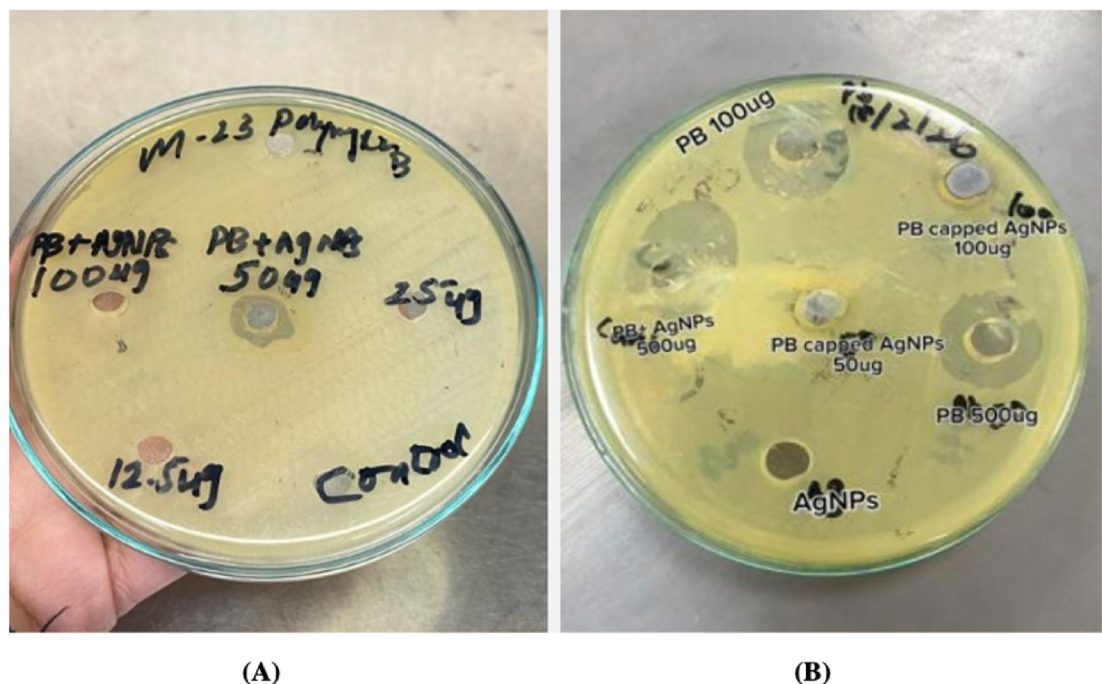


Fig. 9. (A) The antagonistic activity of PB (50 µg), citAgNPs (control), PB capped AgNPs (12.5, 25, 50, 100 µg), (B) PB when combined with AgNPs 500 µg, PB capped AgNPs (50, 100 µg), citAgNPs and PB alone (100, 500 µg) of *A. baumannii*.

Combinations	Concentration	% Inhibition Rate
Diclofenac sodium	50 µg	38%
Polymyxin B	50 µg	65%
AgNPs	20 mg	82%
Polymyxin B capped AgNPs	12.5 µg	96%
	25 µg	93%
	50 µg	82%
	100 µg	62%
Polymyxin B combined AgNPs	50 µg	97%

Table 5. Results of anti-inflammatory egg albumin assay on Elisa.

Binding of protein and ligands

After the docking has been done, both the Ligands occupy in protein binding pocket. All the picked amino acids revealed that both the docked ligands were redundant to gain their binding interaction (Table 6; Figs. 15 and 16).

Docking results of LptE protein with Tri-sodium citrate

When docked in combination with Polymyxin B, after modifying the best possible side for ligand in binding center of LptE protein, the two hydrogen bonds represent binding between Tri-sodium citrate with LptE as ARG at position 157 shows 1.332 Å bond distance and GLN with position 153 shows 1.514 Å (Table 7; Fig. 17). When docked alone, no hydrogen bond formed between tri-sodium citrate and LptE protein of *A. baumannii*. While Table 8 shows opposite results, ARG 157 shows 1.49 Å bond distance when alone.

Docking complex of LptE protein with polymyxin B

When taken in combination, after modifying the best side for ligand in binding center of LptE protein, there is no hydrogen bond formed between Polymyxin B with LptE. When taken alone, Polymyxin B showed hydrogen bond shows binding between Polymyxin B and LptE protein of *A. baumannii* at 153 Å (Fig. 18).

Binding energies

Binding of tri-sodium citrate and Polymyxin B with LptE, Polymyxin B with LptE and Tri sodium citrate with LptE was done using PYRX. Tri-sodium citrate shows the highest binding energy of -4.0 whereas Polymyxin B shows highest binding energy of 6. When evaluated in combination Polymyxin B showed highest binding energy of -5 and tri sodium citrate showed highest binding energy of -4.8 (Table 9). Higher the negative value higher



Fig. 10. Egg albumin combinations made on 96 well plates for Elisa reading.

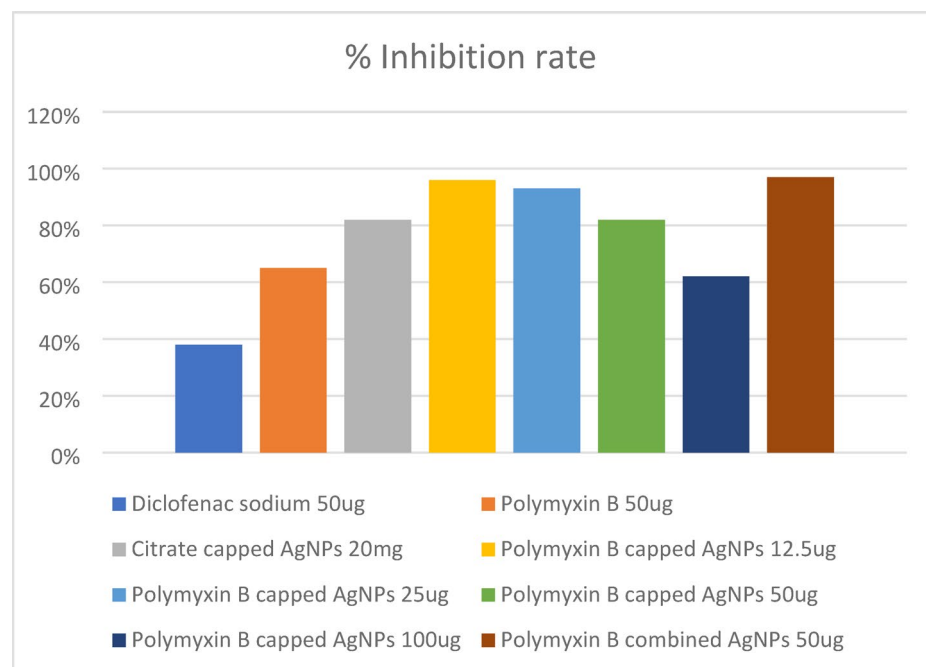


Fig. 11. % inhibition rate of anti-inflammatory egg albumin assay.

is the Bonding. Hence, Polymyxin B and Tri sodium citrate gave good binding energy with LptE protein when used in combination.

Discussion

Multi-drug resistance is a main issue now-a-day. (*A. baumannii*) becoming more and more resistant to multi drugs except Polymyxin B and Tigecycline. Hence, new treatments are required to overcome this issue. Polymyxin B is found to cause side effects like neurotoxicity and nephrotoxicity but it is the last choice of drugs to treat bacterial infections. Hence, our study depends on combinational therapy of Polymyxin B with silver nanoparticles to reduce the toxicity of Polymyxin (B) In our study 8 out of 20 isolates showed antagonistic activity in Polymyxin

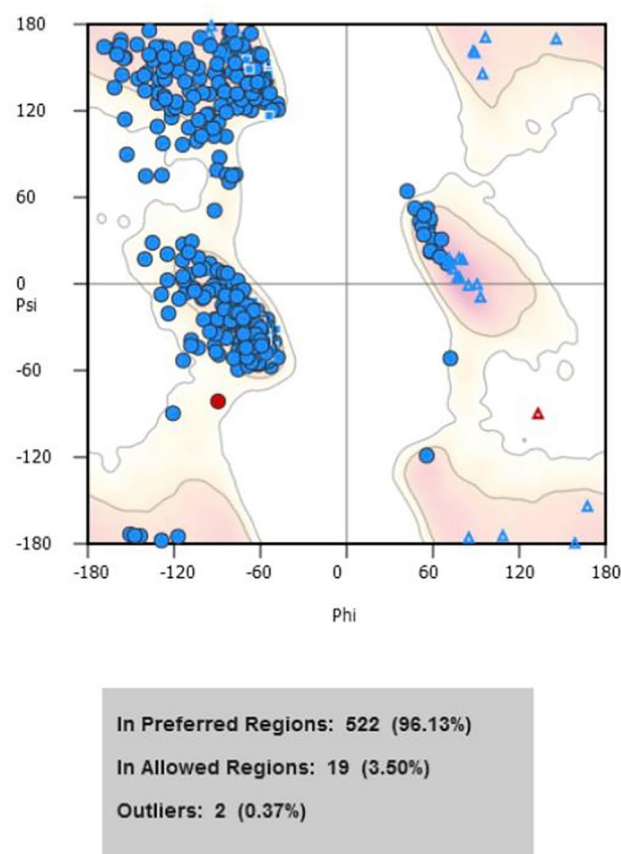


Fig. 12. Ramachandran plot of LptE.

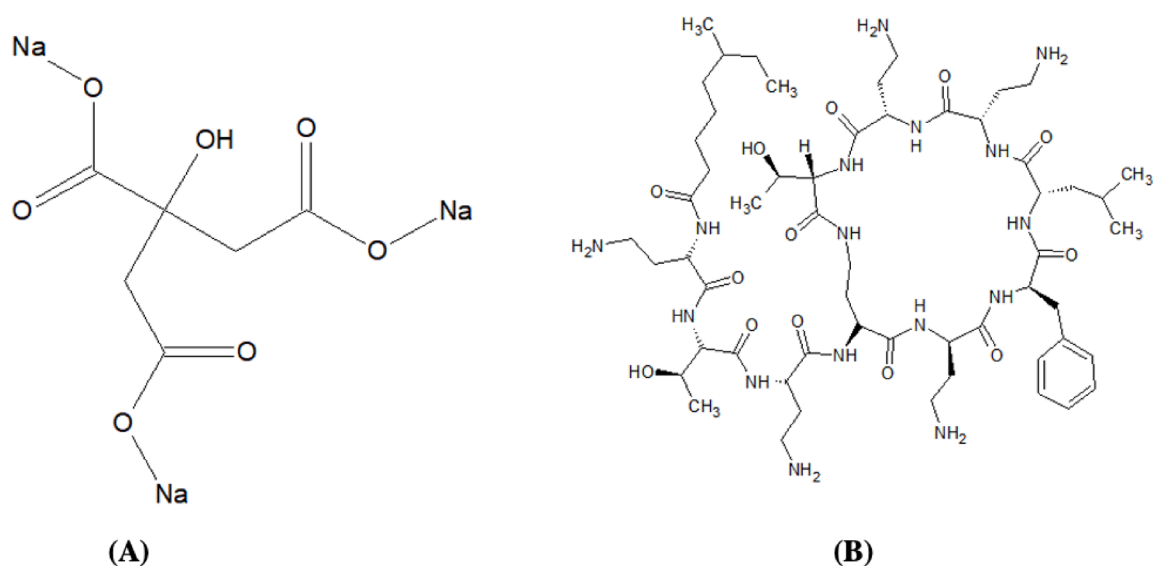


Fig. 13. 2D structure of (A) Tri-sodium citrate (B) Polymyxin B.

B capped AgNPs whereas 20 out of 20 isolates showed antagonistic activity in Polymyxin B combined silver nanoparticles. As in Allend et al., showed that bio-AgNPs when combined with Polymyxin B gave synergistic and additive effect and showed decrease in cells of *A. baumannii* with MIC ranges from 0.46 to 1.87 ml. Bio-AgNPs when combined with Polymyxin B gave synergy against 4–5 strains and additivity against one strain¹⁹. In Naser (2018) study showed that 45 *A. baumannii* were isolated. AgNPs when combined with Polymyxin B showed FIC index value of 0.24–0.51 which showed that interaction is synergistic²⁰. PB when used alone was

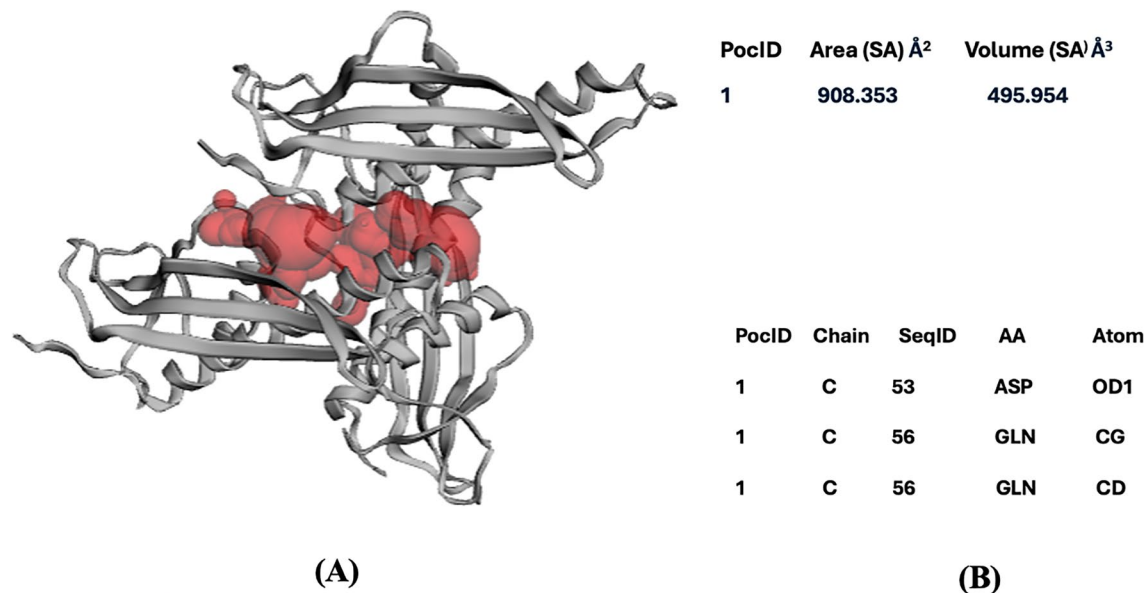


Fig. 14. (A) The binding pocket of LptE from CAST-p. (B) represent the probability of amino acids creating the binding pocket.

LIGANDS		LptE
Polymyxin B	Combination	TYR 60, VAL 59, ARG 157, ARG 124, ASP 150, GLN 154, GLN 153, ILE 146, ASP 149, TYR 142, ILE 148, GLN 56, ASP 52, ARG 145
Tri-sodium citrate		ARG 157, GLN 153, TYR 60, VAL 59, ARG 124, ASP 150, GLN 154, ILE 146, ASP 149, TYR 142, ILE 148, GLN 56, ASP 52, ARG 145
Polymyxin B	Alone	TYR 60, VAL 59, ARG 157, ARG 124, ASP 150, GLN 154, GLN 153, ILE 146, ASP 149, TYR 142, ILE 148, GLN 56, ASP 52, ARG 145
Tri-sodium citrate		TYR 60, VAL 59, ARG 157, ARG 124, ASP 150, GLN 154, GLN 153, ILE 146, ASP 149, TYR 142, ILE 148, GLN 56, ASP 52, ARG 145

Table 6. The amino acids of LptE protein in polymyxin B and tri-sodium citrate and red ones shows hydrogen bonding used in combination and alone.

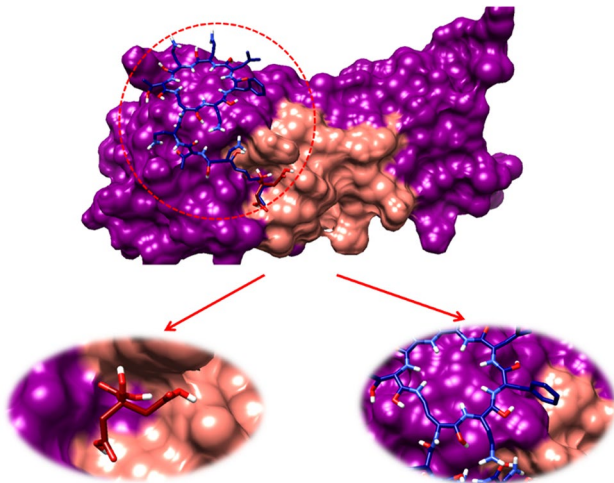


Fig. 15. Binding of tri-sodium citrate and Polymyxin B with LptE protein.

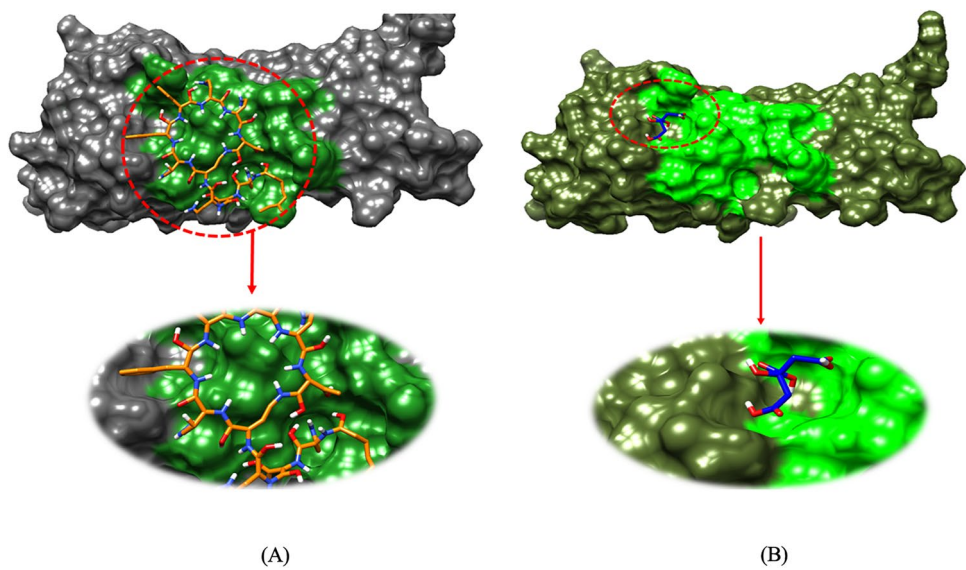


Fig. 16. (A) Binding of Polymyxin B with LptE protein, (B) shows binding of tri-sodium with LptE.

Ligands		Amino acids	Bond distance
Tri-sodium citrate	With LptE	Arginine 157	1.332 Å
		Glutamine 153	1.514 Å
	LptE protein when used alone	No	No

Table 7. The amino acids in Tri-sodium citrate which formed hydrogen bonds with LptE and with LptE protein when used alone.

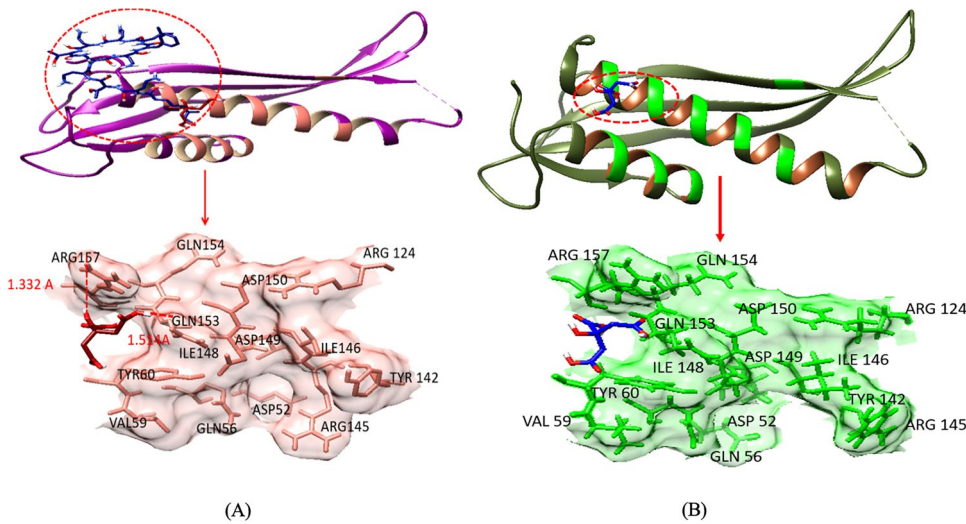


Fig. 17. (A) shows docking complex of LptE protein with Tri-sodium citrate in combination with Polymyxin B (B) shows docking complex of LptE protein with Tri-sodium citrate when used alone.

84% sensitive and 15% resistant. This study also proved that AgNPs have better results when combined with Polymyxin B against *Acinetobacter baumannii*. In Wan et al., combination of antibiotics with AgNPs and as well as alone is studied²¹. Study showed that AgNPs when combined with Polymyxin B shows good synergistic effect than used alone.

As in Hetta et al., *A. baumannii* has been inhibited by AgNPs at MIC ranging from 4 to 25 µg/ml by well diffusion method²². In presence of AgNPs, *A. baumannii* has showed very less growth and also showed good antibacterial activity against *A. baumannii* in in-vitro infection model. In study by³ showed that resistant to silver

Ligands		Amino acids	Bond distance
Polymyxin B	Combination	No	No
	Alone	Arginine 157	1.49 Å

Table 8. The amino acids in polymyxin B that formed hydrogen bond with LptE protein when used in combination with tri-sodium citrate and when used alone.

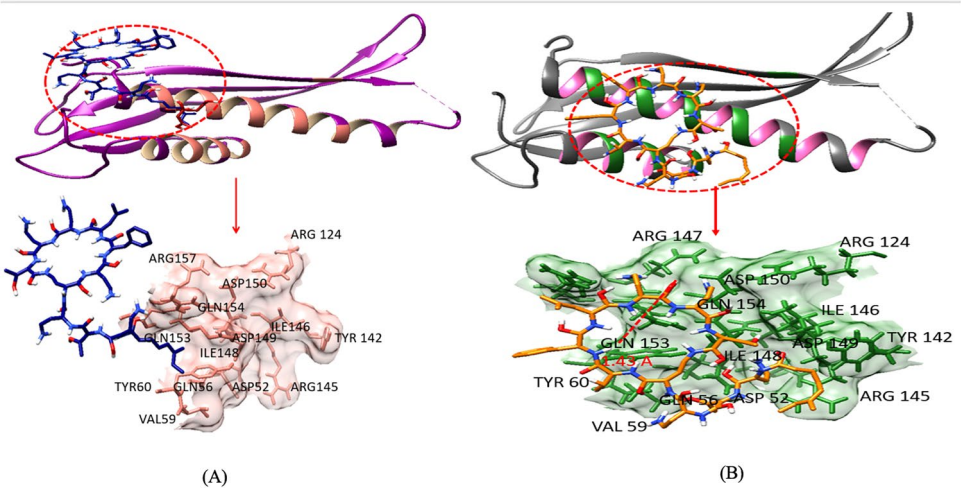


Fig. 18. (A) shows docking complex of LptE protein with Polymyxin B in combination with tri-sodium citrate, (B) shows docking complex of LptE protein with Polymyxin B alone.

Protien	Polymyxin B	Tri-sodium citrate
LptE	6.2	
LptE		− 4.0
LptE	− 5	− 4.8

Table 9. Binding energies of polymyxin B and tri-sodium citrate.

nanoparticles can also be found. This study revealed that silver nanoparticles showed resistance in multidrug resistant strains of *A. baumannii*. In study by Auda et al., showed that citrate capped silver nanoparticles gave better results when given in combination with imipenem than used alone²³. Citrate capped silver nanoparticles gave additive effect when combined with imipenem. MIC and MBC of citrate capped silver nanoparticles were very low against *A. baumannii*.

In our study, Polymyxin B were sensitive at the dose of 100 µg and 500 µg whereas at 50 µg it was found resistant against *A. baumannii*. In study by Thomas et al., showed that Polymyxin B were susceptible to *A. baumannii* that were XDR within MIC ranges from (1–2 µg) whereas PB at > 4 µg were found to be resistant to *A. Baumannii*²⁴. In study by Zhu et al., it was found that all 11 isolates showed resistance to PB and amikacin at the dose of 8 mg against *A. Baumannii*²⁵. In study by da Costa et al., showed that among 38 isolates of *A. baumannii* 13.2% were positive for Polymyxin B and all of them were resistant to PB within MIC value of 32 µg²⁶. In study by Shah et al., showed that 100 isolates of *A. baumannii* were collected from tertiary care hospital and these isolates were recovered from tracheal secretion, CSF and urine²⁷. In antibacterial activity it was found that AgNPs gave zone of 22 mm at 50 mg and 18 mm at 40 mg.

As in Zhang et al., showed that in ten Carbapenem resistant *A. baumannii* isolates Polymyxin B and Tigecycline showed 0% resistance rate and imipenem, meropenem and SUL showed 100% resistance rate⁷. In study by Xi et al., showed that isolates of *A. baumannii* collected were 4.2% and about 94% was found susceptible²⁸. It is reported first time in³ that *A. baumannii* developed resistance to silver nanoparticles. In study by Lambadi et al., showed that Polymyxin B when capped with AgNPs gave good anti-bacterial activity in *Pseudomonas aeruginosa* evaluated first time against *A. Baumannii*¹⁵. Liu et al., showed that Polymyxin B is a good target against *A. baumannii*, it showed a good anti-bacterial activity but antibiotic combining therapies seem to give promising result than Polymyxin B used alone²⁹.

This study Rather et al., also showed that Polymyxin B capped silver nanoparticles showed a good anti-bacterial activity against *Pseudomonas aeruginosa* as tested in a surgical blade³⁰. This study also includes the inhibitory effect of both AgNPs as well as Polymyxin B and this study concludes that Polymyxin B capped AgNPs

has superior anti-microbial effect than citrate capped AgNPs but our study was on Polymyxin B capped AgNPs against *Acinetobacter baumannii* that found sensitive in 8 out of 20 samples tested at the dose of 50 µg which was not studied before.

In our results of in vitro anti-inflammatory activity of egg albumin assay, Polymyxin B when capped with AgNPs gave inhibition rate of 96% at the dose of 12.5 µg, 93% at the dose of 25 µg, 82% at the dose of 50 µg and 62% at the dose of 100 µg. Whereas at 50 µg Polymyxin B gave inhibition rate of 65%, Citrate capped AgNPs gave inhibition rate of 82% and Polymyxin B when combined with silver nanoparticles gave inhibition rate of 97% at the dose of 50 µg. In comparison to our results, a study by Kedi et al., showed that at 0.2 mg silver nanoparticles showed 99% inhibition rate and standard set acetyl salicylic acid gave inhibition rate of 65%^{31–35}. In study by Revathi et al., showed that silver nanoparticles gave inhibition rate of 78.65% and Diclofenac sodium gave inhibition rate of 81.23% at the dose of 500 µg³⁶. In study by Ameena et al., showed that diclofenac sodium gave inhibition rate of 84% at 50 µg and 47% at 10 µg¹⁴.

Conclusion

Consider from our result that in antagonistic activity citrate capped AgNPs alone is not a good choice of therapy against MDR *A. baumannii* but it can be beneficial when used in combination as also proved from in-silico study. In anti-inflammatory activity, PB combined and capped AgNPs found to be comparative than standard use (Diclofenac sodium). Hence Silver nanoparticles when combined with Polymyxin B found to be an excellent choice to the health issues and offered as a good alternative in future use against MDR *Acinetobacter baumannii*.

Data availability

All the data generated in this research work has been included in this manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Ethical approval

Ethical approval for this study was granted by Ethics approval committee Institute of Molecular Biology and Biotechnology under ref no, Ref-IMBB/BBBC/23/2409-C.

Additional information

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