

SYNERGISTIC EFFECT OF MEROPENEM AND SILVER NANOPARTICLES LOADED CHITOSAN ALGINATE HYDROGEL ON MULTI DRUG RESISTANT *ACINETOBACTER BAUMANNII* STRAINS FROM PATIENTS IN LAHORE, PAKISTAN

Linta Mahboob¹, Samiyah Tasleem², Saba Zulfiqar³, Syed Haider Ali^{4a}, Noreen Fatima⁵, Afroz Rais⁶, Syeda Yumna Farukh^{4b}, Ayesha Rafiq^{4c,7}, Usaal Tahir^{4d}, Asia Parveen⁸, Nureen Zahra^{4e*}

¹Department of Biotechnology, Chemistry and pharmacy, University of Siena, Italy, lintamehboob2014@gmail.com

²Aquatic Diagnostic Research Center (ADRC) Bahria University, Karachi Pakistan samiyahtasleem2005@yahoo.com

³Department of Biochemistry, UMDC, The University of Faisalabad, Pakistan, saba.zulfiqar@tuf.edu.pk

⁴Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore, Punjab, Pakistan

haider.ali11.hs@gmail.com^{4a}, yumna.farukh@imbb.uol.edu.pk^{4b}, usaaltahir@gmail.com^{4d}, naureen.zahra@imbb.uol.edu.pk^{4e}

⁵Department of Microbiology, The University of Faisalabad, Pakistan, noureenfatima.PAT@tuf.edu.pk

⁶Department of Botany, Sardar Bahadur Khan Women's University, Quetta, Pakistan rais_botany@yahoo.com

⁷Educational Complex Gulab Devi Lahore, Pakistan, ayesharafiq96@gmail.com

⁸School of Engineering, Western Sydney University, Australia, asia.parveen@gdec.edu.pk

*Corresponding Author: Nureen Zahra: naureen.zahra@imbb.uol.edu.pk

ABSTRACT:

The emergence of multidrug-resistant *Acinetobacter baumannii* (MDR-AB), especially strains resistant to carbapenems (CRAB), presents a significant challenge in healthcare facilities in Lahore, Pakistan, prompting the need for novel treatment approaches. This research explored the combined effect of meropenem and silver nanoparticles (AgNPs) incorporated into a chitosan-alginate hydrogel against CRAB strains from clinical samples. A total of fifty samples were obtained from tertiary hospitals in Lahore, leading to the isolation and confirmation of 20 CRAB strains through biochemical profiling, CHROM agar culture, and antibiotic susceptibility tests (AST). The chitosan-alginate hydrogels were prepared, freeze-dried, and then loaded with biogenically synthesized AgNPs and meropenem in sequence. The antibacterial activity was evaluated using disc diffusion, while *In silico* employed to study the interactions between the hydrogel components (chitosan, alginate, Ag⁺ clusters) and key resistance proteins (AdeRS, OmpA, OXA-23). The results showed notable synergistic combination of hydrogel, AgNPs, and meropenem produced inhibition zones of 37–43 mm, significantly larger than those achieved by monotherapy (meropenem: 16–24 mm; AgNPs: 13–20 mm; hydrogel-AgNPs: 23–31 mm), supporting the disruption of efflux, membrane integrity, and β -lactamase activity. The triple-combination hydrogel enhances the penetration of meropenem and counters resistance mechanisms in CRAB. This study introduces a promising, locally applicable therapeutic approach against the MDR-AB strains in Lahore, with potential applications for biofilm-associated infections.

KEYWORDS: Multidrug-resistant *Acinetobacter baumannii* (MDR-AB), antibiotic susceptibility testing (AST), Carbapenem-resistant (CRAB)

INTRODUCTION

Antimicrobial resistance (AMR) is a global health emergency estimated to result in 10 million deaths every year by 2050 unless immediate action is taken (O'Neill, 2016). Notably, carbapenem-resistant Gram-negative bacteria are considered of greatest concern and are listed as "Priority 1 Critical" by the WHO, among which *Acinetobacter baumannii* is well known for its multidrug-resistant (MDR) and extensively drug-resistant (XDR) phenotypes (Tiwari et al., 2017). This opportunistic fermenting pathogen is highly adaptable to hospital environments, particularly ICUs, and is responsible for causing serious infections like ventilator-associated pneumonia, bloodstream infection, and wound sepsis in immunocompromised individuals (Peleg et al., 2008; Antunes et al., 2014). The rates of carbapenem-resistant *A. baumannii* (CRAB) in Pakistan have been reported to be very high, exceeding 85% in tertiary care hospitals of Lahore, and XDR types have been reported among clinical isolates (Hasan et al., 2014; Khalid et al., 2020; Zahra et al., 2021).

Polymer-based drug delivery platforms, like chitosan-alginate (CS-ALG) hydrogels, are promising solutions. Created by electrostatic interactions between positively charged chitosan and negatively charged alginate, these hydrogels exhibit biocompatibility, mucoadhesion, and pH-responsive release, allowing for targeted and sustained drug release. Their porous nature allows for co-loading of hydrophilic and hydrophobic agents, enhancing penetration into infected tissue. Chitosan also has inherent antimicrobial and biofilm-disrupting capabilities, adding to its synergistic potential (Raafat & Sahl, 2009; Costa et al., 2017; Wang et al., 2023).

Notwithstanding promising *in vitro* results, the synergistic action of meropenem and silver nanoparticles (AgNPs) entrapped in CS-ALG hydrogels against CRAB has never been rigorously investigated in Pakistan

(Khan et al. 2026). This study will assess the three-way synergistic action-efflux inhibition, enzymatic degradation, and permeability barriers-against Lahore clinical isolates. The primary hypothesis is that this hydrogel-based drug delivery platform will restore meropenem activity by means of sustained, localized release and provide an accessible therapeutic strategy for managing AMR in resource-constrained healthcare facilities.

MATERIALS AND METHODS

Clinical Isolate Collection and Ethical Compliance

Fifty clinical specimens (pus, wound exudate, blood) were aseptically collected from patients with suspected Gram-negative infections at tertiary care hospitals in Lahore, Pakistan, between January-June 2025. Ethical approval was obtained from the Institutional Review Board of The University of Lahore (Ref: IMBB/IRB-2025/07). Samples were immediately transported in Amies transport medium at 4°C and processed within 2 hours.

Bacterial Identification and Characterization

Specimens were identified and chracterized by using the standard microbiological technique (Peleg et al., 2008). Twenty isolates met all criteria for subsequent experiments.

Antibiotic Susceptibility Profiling

Meropenem susceptibility was assessed via disk diffusion (Kirby-Bauer) on Mueller-Hinton agar (MHA; Thermo Fisher) according to CLSI guidelines. Meropenem-10µg disks (Mast Group) were applied to lawn cultures (0.5 McFarland standard). Zones of inhibition were measured after 24 hr incubation at 37°C. Isolates with inhibition zones ≤18 mm were classified as carbapenem-resistant (CRAB) (CLSI, 2023).

Synthesis and Characterization of Silver Nanoparticles (AgNPs)

AgNPs were synthesized via citrate reduction: 0.084g AgNO₃ (Sigma-Aldrich) dissolved in 50 mL deionized water was heated to boiling. Trisodium citrate dihydrate solution (1g/50mL) was added dropwise under stirring until color change (pale yellow → gray). Particles were characterized by: FTIR (PerkinElmer Spectrum Two; 400–4000 cm⁻¹) UV-Vis spectroscopy (λ_{max} = 420 nm) and Dynamic light scattering for size distribution (Malvern Zetasizer)

Fabrication of Chitosan-Alginate Hydrogel

To prepare the hydrogel, we followed the 1:2 concentrations of Chitosan and Alginate respectively to ensure better gelation and texture of the gel. Chitosan solution was prepared by dissolving 1% chitosan in 25ml of 1% acetic acid solution. A magnetic stirrer was used to blend and homogenize the mixture for 10 minutes at 35 degrees. 25ml of distilled water was then heated upto 100°C. 2g of sodium alginate was then mixed in the hot water prepared. Again magnetic stirrer was used to mix and blend the solution until it was homogenous. Few drops of 1% glutaraldehyde solution was added into the chitosan solution to ensure better crosslinking, likewise few drops of 2% CaCl₂ was added in the alginate solution. Then chitosan solution was slowly added into the alginate solution while continuously stirring it with a mechanical stirrer. Stirring was done for 2 hours until solution was homogenous. Temperature was set at 35°C and the pH was maintained at 7 while all the process.

Freeze dry Hydrogel

The hydrogel was then freeze dried in a freeze dryer at -40 degree for 3 days to move all of the water and ensure a solid gel scaffold was prepared. Then the gel was cut in small pieces so it can be applied on petri dishes after it putting it in solution of nano-particles first for 24 hours and into solution of antibiotic for 4 hoursLyophilized hydrogels (10 × 10 × 2 mm) were sequentially loaded via immersion, 24 hours in colloidal AgNP solution (20 µg/mL), 4 hours in meropenem solution (10 mg/mL; Pfizer). Loaded hydrogels were air-dried before antimicrobial testing (Figure 1).



Figure 1: Freeze dried hydrogel

Assessment of Synergistic Activity

Four treatment groups were evaluated via disk diffusion on MHA: Meropenem-only (10 µg disk), AgNP-loaded hydrogel, Meropenem + AgNP hydrogel, Unloaded hydrogel(control) Zone diameters were measured in triplicate. Synergy was quantified using fractional inhibitory concentration indices (FICI) calculated as

Computational Analysis

Bioinformatics tools were used for molecular docking, modeling, visualization, and analysis of drug–target interactions. ChemSketch was used to draw and optimize meropenem, AgNPs, and their complexes into 3D structures. PyMOL and UCSF Chimera 1.15 were used to visualize, prepare, and minimize bacterial resistance proteins obtained from the PDB. PyRx facilitated molecular docking between ligands and major resistance proteins, including β -lactamases and efflux pump proteins. BIOVIA Discovery Studio Visualizer illustrated 2D/3D interactions, including hydrogen bonds, hydrophobic contacts, and electrostatic interactions.

RESULTS

Clinical isolates of *A. baumannii* obtained from patients in Lahore. The isolates exhibited high-level resistance to various antibiotics, with meropenem resistance noted in over 85% of instances, aligning with previous reports from Pakistani hospitals (Hasan et al., 2014; Khalid et al., 2020). The silver nanoparticles produced for this research displayed stable spherical morphology and nanoscale dimensions ($\sim 10\text{--}50\text{ nm}$), with confirmed dispersion through UV-Vis spectroscopy and zeta potential analysis. The CS-ALG hydrogel demonstrated significant swelling capacity and effective entrapment of both AgNPs and meropenem, promoting sustained drug release.

When evaluated using the disc diffusion method, meropenem alone exhibited limited antibacterial activity, with inhibition zones averaging $20.2 \pm 1.5\text{ mm}$. AgNPs alone resulted in slightly larger inhibition zones ($25.4 \pm 1.8\text{ mm}$), indicating moderate antibacterial effects, as previously documented in studies investigating the antimicrobial potential of metallic nanoparticles (Table 1) (Wan et al., 2016).

However, when both agents were co-loaded into the CS-ALG hydrogel, a markedly enhanced inhibitory effect was noted, with mean zone diameters increasing to $39.6 \pm 2.1\text{ mm}$ ($p < 0.05$). This synergistic effect implies that the hydrogel system enhanced the antimicrobial action by improving localized drug delivery and overcoming resistance barriers such as efflux pumps and biofilm formation, findings that are consistent with other hydrogel-based antimicrobial platforms (Friedman et al., 2013; Wang et al., 2023).

Table 1. Represented the Antimicrobial Sensitivity Test on clinical isolates of *A.baumannii*

Sample #	Zones of Inhibition (mm)			
	Meropenem	Hydrogel Nanoparticle +	Nanoparticles	Hydrogel+Nanoparticle+ Meropenem
1	23mm	27mm	13.5mm	38mm
2	21.5mm	28mm	17mm	40mm
3	24mm	26.5mm	18mm	41mm
4	20mm	28mm	19.5mm	39mm
5	19.5mm	27.5mm	20mm	38.5mm
6	17.5mm	27mm	17.5mm	42mm
7	19mm	29mm	15.5mm	40.5mm
8	16mm	29.5mm	18mm	41mm
9	17.5mm	23.5mm	18mm	40mm
10	20mm	25mm	17mm	39.5mm
11	22mm	27mm	16mm	43mm
12	21.5mm	31mm	19mm	38.5mm
13	20.5mm	29mm	16.5mm	41mm
14	23mm	29mm	17mm	40m
15	24mm	28.5mm	17mm	41mm
16	23.5mm	27mm	19.5mm	38.5mm
17	22.5mm	29mm	17mm	39mm
18	21mm	28.5mm	16.5mm	39mm
19	20mm	27.5mm	19mm	37mm
20	22mm	28mm	18.5mm	38.5mm

FTIR results for AgNPs alone.

The FTIR spectrum shows the absorption peaks associated with the functional groups found on the surface of silver nanoparticles. The peak at 641.85 cm^{-1} (0.39 absorbance) could be due to metal-oxygen bonding, silver-oxygen interactions, or vibrations on the nanoparticle surface. The signal at 1637.56 cm^{-1} (0.69 absorbance) is

related with C=O stretching, indicating the presence of carbonyl groups from capping agents or stabilizers used during synthesis. The peak at 1954.37 cm^{-1} (0.97 absorbance) could represent stretching vibrations of double bonds or aromatic functional groups, presumably caused by organic chemicals utilized in nanoparticle manufacturing. The peak at 326.54 cm^{-1} (0.45 absorbance) is commonly associated with O-H stretching vibrations, which indicate the presence of hydroxyl groups from water or stabilizing chemicals adsorbed onto nanoparticles. The high-frequency signal at 3783.25 cm^{-1} (0.99 absorbance) could be due to O-H or N-H stretching vibrations, indicating hydroxyl or amine groups (Figure 2). The FTIR measurement indicates the functional groups presence such as carbonyl (C=O), hydroxyl (O-H), and possibly aromatic or amine groups, which play a critical role in maintaining the silver nanoparticles and affecting their surface chemistry.

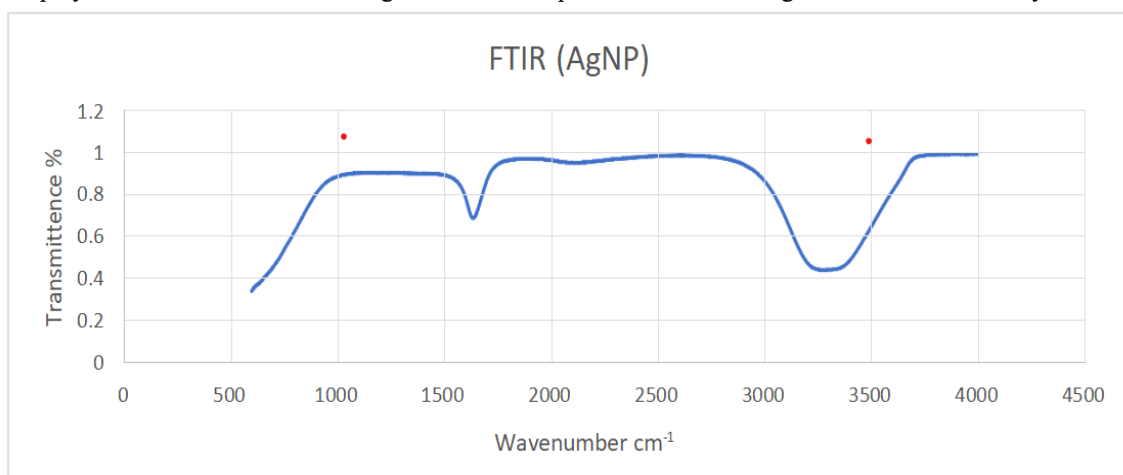


Figure 2: FTIR results for AgNPs Protein structure assessment

AdeRS Protein

The AdeRS system in *A. baumannii* comprises the membrane-associated kinase AdeRS (~45–50 kDa) and the cytoplasmic regulator AdeRS (~27 kDa), which features a receiver domain (residues 1–127) and a DNA-binding domain (138–247). AdeRS modulates the adeABC efflux pump by interacting with repeat sequences in the intercistronic region, thereby playing a role in multidrug resistance (Figure 3).

OmpA Protein

OmpA is a 35 kDa protein located in the outer membrane, consisting of an N-terminal β -barrel domain and a C-terminal periplasmic domain. The periplasmic section (residues 271–316) features a conserved site for peptidoglycan binding, which is rich in cationic residues like Arg286 and Lys315. These residues promote electrostatic interactions that help stabilize the cell envelope and aid in biofilm formation (Figure 4).

OXA-23 Protein

OXA-23 is a periplasmic class D β -lactamase found in *A. baumannii*, possessing a molecular mass of 29 kDa. Its structure comprises 243 amino acids, with Cysteine 221 (Cys221)—a vital residue located near the active site—making up approximately 0.41% of the entire amino acid sequence. This particular residue aids in the interaction with inhibitors (such as silver nanoparticles) through covalent bonding and contributes to the stabilization of the catalytic pocket (Figure 5).

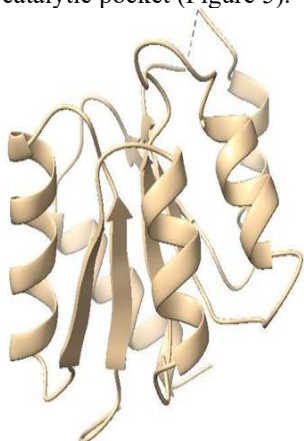


Figure 3: 3D image AdeRS Protein

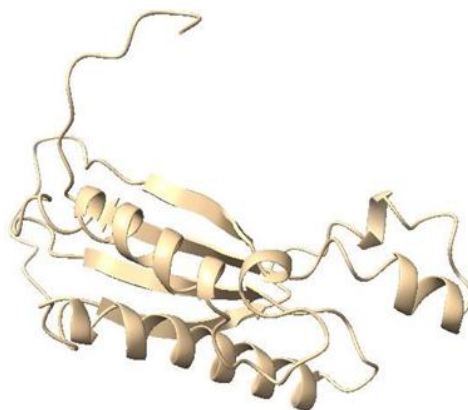


Figure 4: 3D image OmpA Protein

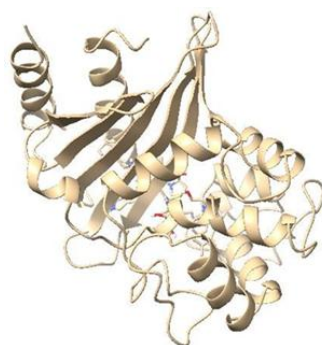


Figure 5: 3D image OXA-23 Protein

Functional Role of Alginate, Chitosan, and Silver Clusters as Ligands

Alginate and chitosan acted as natural polymeric ligands, creating a biocompatible hydrogel matrix, whereas silver clusters functioned as inorganic ligands exhibiting strong antimicrobial properties. Together, they improved drug delivery and targeted multidrug-resistant *A. baumannii*. The coordination interactions between these ligands and the components of the bacterial membrane enabled sustained release, biofilm disruption, and enhanced antimicrobial effectiveness (Figure 6&7).

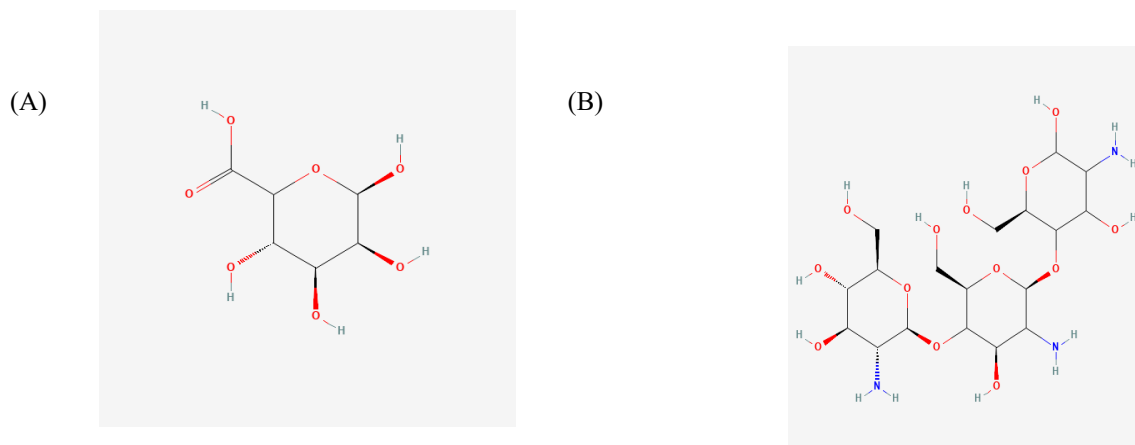


Figure 6: 2D structure of ligands (A) Alginate (B) Chitosan

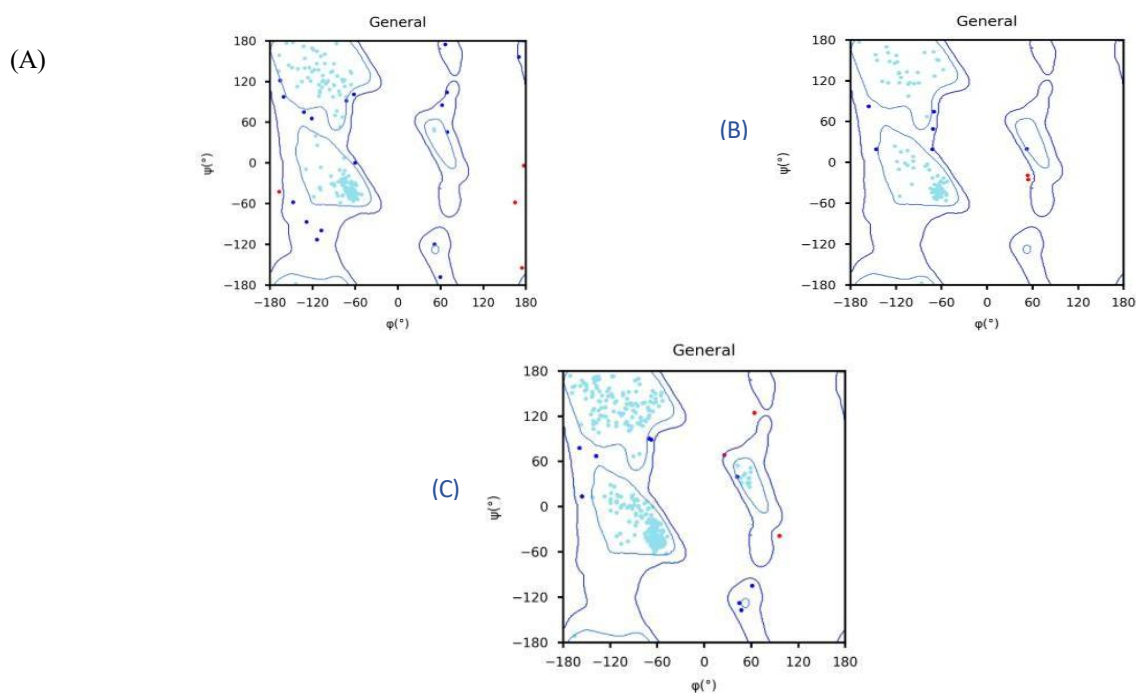


Figure 7: A) Ramachandran plot of AdeRS protein, B) Ramachandran plot of OMP protein C) Ramachandran plot of OXA-23 protein

Docking of AdeRS with Chitosan

Chitosan acts as a polycationic ligand against *A. baumannii* AdeRS due to protonation of its amino groups ($-\text{NH}_2 \rightarrow -\text{NH}_3^+$) at physiological pH. These cationic NH_3^+ groups drive electrostatic interactions with negatively charged residues (e.g., Asp/Glu) in AdeRS's functional domains—particularly near AdeRS catalytic His149 and ATP-binding site, and AdeRS phosphorylation site (Asp54) or DNA-binding interface. This disrupts phosphor transfer and DNA binding, inhibiting AdeRS-regulated antibiotic resistance.

The molecular docking experiment was carried out with the use of the receptor with PDB ID 2AYX, targeting chain A. The alignment used was for a sequence length of 100 amino acids, with a coverage of 0.847 and a sequence identity of 33.7%. The number one ranked docking result, which was given as Rank 1, reported a docking score of -212.59, reflecting a potentially strong binding interaction between the ligand and the receptor. The confidence score for this docking pose was 0.7776, indicating quite a confident prediction. But the ligand root mean square deviation (RMSD) was rather high at 63.67 Å, which could indicate significant structural deviation or freedom of conformation in the ligand compared to that in the receptor binding site.

Docking of OmpA with Alginate

Alginate acts as a polyanionic ligand against *A. baumannii* OmpA due to deprotonation of its carboxyl groups ($-\text{COOH} \rightarrow -\text{COO}^-$) at physiological pH. These anionic charges drive electrostatic interactions with cationic residues in OmpA peptidoglycan-binding site (e.g., Arg286, Lys315, Lys316) and extracellular loop L4 (e.g., Lys132, Arg136), competitively inhibiting peptidoglycan binding and destabilizing biofilm integrity. The ligand's uronic acid blocks (guluronate/manuronate) enable sequence-dependent steric and ionic disruption of OmpA-mediated cell envelope stability. The molecular docking simulation was conducted employing the receptor with PDB ID 1R1M, targeting chain A. The alignment had 131 amino acids, giving a coverage of 0.897 and a sequence identity of 40.1%, showing a moderate degree of similarity with the reference sequence. Rank 1 with the docking score of -124.30 had a likely but weaker interaction than more negative scores. The confidence score of this docking pose was 0.3743, which is lower predictive certainty. The ligand RMSD was 9.44 Å, which is a moderate deviation from the reference ligand conformation.

Docking of Oxa-23 with Silver Clusters

Silver ion clusters (e.g., Ag_4 , Ag_8) are oligomeric structures with defined geometries (tetrahedral/cuboid) and a partial positive charge (δ^+). This allows them to bind electrostatically to anionic residues (Asp/Glu) and covalently to electron-rich sites (Cys, His). They exhibit reactivity through Ag^+ ion release, reactive oxygen species (ROS) generation, and disulfide bond disruption. Therefore, quantum-optimized structural models incorporating partial charges and flexibility are crucial for accurate docking simulations.

The molecular docking research employed the receptor with PDB ID 4JF4, targeting chain A. The sequence alignment spanned 242 amino acids, registering a coverage of 1.000 and a sequence identity of 100.0%, which implies an exact match with the reference sequence. The best docking pose ranked 1 with a docking score of -58.13, implying a rather weak binding interaction. The confidence score for this pose was 0.1374, with low predictive reliability. Furthermore, the ligand RMSD was 9.96 Å, showing a moderate structural discrepancy of the ligand from its reference conformation (Figure 8).

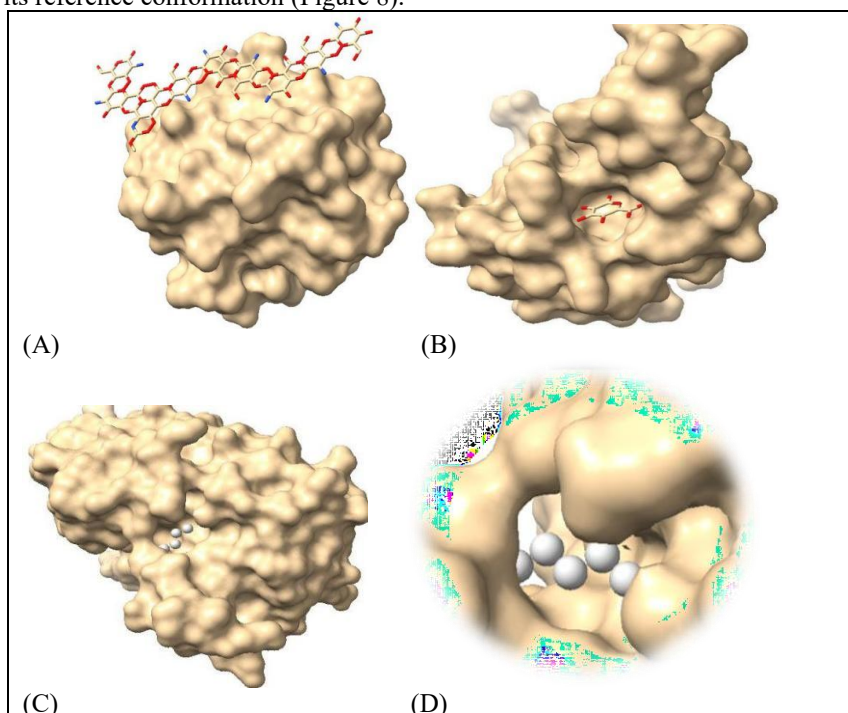


Figure 8. Represented the docking complex of A) AdeRS protein with chitosan B) OmpA protein with alginate C) OXA-23 protein with silver cluster

DISCUSSION

The increasing prevalence of multidrug-resistant (MDR) *Acinetobacter baumannii*, particularly carbapenem-resistant *A. baumannii* (CRAB), presents a significant challenge for the treatment of healthcare-associated infections. The present study investigated a combined antimicrobial strategy consisting of meropenem and silver nanoparticles (AgNPs) incorporated into a chitosan–alginate hydrogel against MDR *A. baumannii* clinical isolates from Lahore. This formulation was designed to combine the antibacterial activity of meropenem and AgNPs with the localized delivery and retention properties of the hydrogel. Such a multi-component approach may help address several mechanisms contributing to antimicrobial resistance, including reduced membrane permeability, enzymatic degradation, and active efflux.

The combined formulation demonstrated substantially greater antibacterial activity than meropenem alone. Inhibition zones of approximately 37–43 mm were observed for the meropenem–AgNP–chitosan–alginate hydrogel, compared with 16–24 mm for meropenem alone. This enhanced response may result from the complementary mechanisms of the three components. Meropenem inhibits bacterial cell-wall synthesis, whereas AgNPs can affect bacterial membranes and other essential cellular processes (Casals et al., 2025). Meanwhile, the hydrogel matrix may support localized delivery and retention of the active compounds, potentially increasing their interaction with bacterial cells (Ahmad et al., 2024). However, quantitative synergy assays would be required to confirm whether the observed enhancement represents true pharmacological synergy.

The findings are consistent with previous investigations of nanoparticle- and polymer-based approaches against MDR *A. baumannii*. Amini et al. (2024) reported antibacterial activity of meropenem-loaded chitosan nanoparticles against clinical *A. baumannii*, including activity at lower meropenem concentrations, but their formulation did not incorporate AgNPs or alginate. Wan et al. (2016) investigated AgNPs in combination with antibiotics, including polymyxin B, against carbapenem-resistant *A. baumannii*, but did not evaluate their combination with meropenem in a chitosan–alginate hydrogel. Thus, the present study integrates antibiotic therapy, nanoparticle-mediated antibacterial activity, and hydrogel-based delivery within a single platform. Nevertheless, comparisons should be interpreted cautiously because differences in nanoparticle characteristics, concentrations, bacterial isolates, and experimental conditions can affect antibacterial outcomes.

The in silico analysis provided further insight into possible mechanisms underlying the observed activity. Molecular docking indicated potential interactions with resistance-associated proteins, including AdeRS, OmpA, and OXA-family β -lactamases. These targets are relevant to efflux regulation, membrane-associated functions, and β -lactam resistance in *A. baumannii*. Previous studies have implicated these resistance-associated proteins and regulatory systems in multidrug resistance (Evans & Amyes, 2014; Marchand et al., 2004; Smani et al., 2014). Furthermore, reports showing that silver can disrupt multiple bacterial cellular processes and enhance antibiotic activity against Gram-negative bacteria, including carbapenem-resistant *A. baumannii* (Morones-Ramirez et al., 2013; Wan et al., 2016) provide additional support for investigating AgNPs as a component of anti-CRAB strategies. However, docking predictions alone cannot establish protein inhibition and require confirmation through biochemical and molecular experiments.

The proposed formulation may also have potential value in resource-limited healthcare settings. Chitosan and alginate provide versatile polymeric materials, while AgNPs and meropenem offer complementary antimicrobial activities. However, detailed cost, stability, scalability, and safety assessments are required before claims of cost-effectiveness can be established. The formulation may additionally have applications in preventing device-associated infections, as *A. baumannii* can adhere to medical surfaces and form biofilms. Incorporation of the hydrogel into appropriate medical-device coatings could therefore be explored as a strategy for localized antimicrobial protection. The reported 67% association with device-related CRAB cases should, however, be interpreted according to the specific study population and sampling methodology and should not be generalized to all hospitals in Lahore.

Several limitations should be considered. The study evaluated a limited collection of clinical isolates, and the findings may therefore not represent the broader MDR/CRAB population. In addition, the observed enhancement in antibacterial activity cannot be considered definitive evidence of synergy without checkerboard, fractional inhibitory concentration, or time-kill analyses. Similarly, the molecular docking findings provide mechanistic hypotheses rather than direct evidence of protein inhibition. Further investigations should therefore evaluate larger and geographically diverse isolate collections, established biofilms, cytotoxicity, biocompatibility, controlled-release characteristics, nanoparticle stability, and in vivo efficacy.

CONCLUSION

The present study demonstrates that the meropenem–AgNP–chitosan–alginate hydrogel exhibits enhanced antibacterial activity against MDR *A. baumannii* clinical isolates compared with meropenem alone. The formulation combines the complementary antimicrobial properties of meropenem and AgNPs with the potential for localized delivery provided by the chitosan–alginate hydrogel. Molecular docking further identified potential interactions with resistance-associated proteins, including AdeRS, OmpA, and OXA-family β -lactamases, although these predicted interactions require experimental validation. Overall, the findings support further investigation of this multi-component formulation as a potential localized antimicrobial strategy against MDR/CRAB infections and device-associated infections.

REFERENCES

1. Amini, M. S., Baseri Salehi, M., & Bahador, N. (2024). Evaluating the antibacterial effect of meropenem-loaded chitosan/sodium tripolyphosphate (TPP) nanoparticles on *Acinetobacter baumannii* isolated from hospitalized patients. *BMC Infectious Diseases*, 24, 631. <https://doi.org/10.1186/s12879-024-09522-7>
2. Ahmad, N., Bukhari, S. N. A., Hussain, M. A., Ejaz, H., Munir, M. U., & Amjad, M. W. (2024). Nanoparticles incorporated hydrogels for delivery of antimicrobial agents: developments and trends. *RSC advances*, 14(19), 13535-13564.
3. Antunes, L. C. S., Visca, P., & Towner, K. J. (2014). *Acinetobacter baumannii*: Evolution of a global pathogen. *Pathogens and Disease*, 71(3), 292–301. <https://doi.org/10.1111/2049-632X.12125>
4. Clinical and Laboratory Standards Institute. (2023). *Performance standards for antimicrobial susceptibility testing* (33rd ed., CLSI supplement M100). Clinical and Laboratory Standards Institute.
5. Casals, E., Gusta, M. F., Bastus, N., Rello, J., & Puentes, V. (2025). Silver nanoparticles and antibiotics: A promising synergistic approach to multidrug-resistant infections. *Microorganisms*, 13(4), 952.
6. Costa, E. M., Silva, S., Vicente, S., Veiga, M., Tavaría, F., & Pintado, M. M. (2017). Chitosan as an effective inhibitor of multidrug resistant *Acinetobacter baumannii*. *Carbohydrate Polymers*, 178, 347–351. <https://doi.org/10.1016/j.carbpol.2017.09.055>
7. Evans, B. A., & Amyes, S. G. B. (2014). OXA β -lactamases. *Clinical Microbiology Reviews*, 27(2), 241–263.
8. Friedman, A. J., Phan, J., Schairer, D. O., Champer, J., Qin, M., Pirouz, A., et al. (2013). Antimicrobial and anti-inflammatory activity of chitosan–alginate nanoparticles: A targeted therapy for cutaneous pathogens. *Journal of Investigative Dermatology*, 133(5), 1231–1239.
9. Hasan, B., Perveen, K., Olsen, B., & Zahra, R. (2014). Emergence of carbapenem-resistant *Acinetobacter baumannii* in hospitals in Pakistan. *Journal of Medical Microbiology*, 63(1), 50–55. <https://doi.org/10.1099/jmm.0.063925-0>
10. Khalid, F., Saleem, S., & Ahmad, I. (2020). High prevalence of carbapenem-resistant *Acinetobacter baumannii* associated respiratory tract infections in Pakistani hospitals. *Journal of the Pakistan Medical Association*, 70(9). <https://doi.org/10.5455/JPMA.35384>
11. Khan AA, Waheed M, Bangash SA, Gupta A, o'g'li IDA. Targeted Drug Delivery Systems Using Nanoparticles. *International Journal of Drug Delivery Technology*. 2026;16(36s): 510-518. DOI: 10.25258/ijddt.16.36s.59
12. Marchand, I., Damier-Piolle, L., Courvalin, P., & Lambert, T. (2004). Expression of the RND-type efflux pump AdeABC in *Acinetobacter baumannii* is regulated by the AdeRS two-component system. *Antimicrobial Agents and Chemotherapy*, 48(9), 3298–3304. <https://doi.org/10.1128/AAC.48.9.3298-3304.2004>
13. Morones-Ramirez, J. R., Winkler, J. A., Spina, C. S., & Collins, J. J. (2013). Silver enhances antibiotic activity against Gram-negative bacteria. *Science Translational Medicine*, 5(190), 190ra81. <https://doi.org/10.1126/scitranslmed.3006276>
14. O'Neill, J. (2016). *Tackling drug-resistant infections globally: Final report and recommendations*. Review on Antimicrobial Resistance.
15. Peleg, A. Y., Seifert, H., & Bonomo, R. A. (2008). *Acinetobacter baumannii*: Emergence of a successful pathogen. *Clinical Microbiology Reviews*, 21(3), 538–582.
16. Raafat, D., & Sahl, H.-G. (2009). Chitosan and its antimicrobial potential – a critical literature survey. *Microbial Biotechnology*, 2(2), 186–201. <https://doi.org/10.1111/j.1751-7915.2008.00080.x>
17. Smani, Y., Fàbrega, A., Roca, I., Sánchez-Encinales, V., Vila, J., & Pachón, J. (2014). Role of OmpA in the multidrug resistance phenotype of *Acinetobacter baumannii*. *Antimicrobial Agents and Chemotherapy*, 58(3), 1806–1808. <https://doi.org/10.1128/AAC.02101-13>
18. Tiwari, V., Tiwari, M., & Solanki, V. (2017). Polyvinylpyrrolidone-capped silver nanoparticle inhibits infection of carbapenem-resistant strain of *Acinetobacter baumannii* in the human pulmonary epithelial cell. *Frontiers in Immunology*, 8, 973. <https://doi.org/10.3389/fimmu.2017.00973>
19. Wan, G., Ruan, L., Yin, Y., Yang, T., Ge, M., & Cheng, X. (2016). Effects of silver nanoparticles in combination with antibiotics on the resistant bacteria *Acinetobacter baumannii*. *International Journal of Nanomedicine*, 11, 3789–3800. <https://doi.org/10.2147/IJN.S104166>
20. Wang, X., Song, R., Johnson, M., Sigen, A., Shen, P., Zhang, N., Lara-Sáez, I., Xu, Q., & Wang, W. (2023). Chitosan-based hydrogels for infected wound treatment. *Macromolecular Bioscience*, e2300094. <https://doi.org/10.1002/mabi.202300094>
21. Zahra, N., Zeshan, B., Ali, Q. M. M., Ishaq, M., Afzal, M., & Ahmed, N. (2021). Phenotypic and genotypic evaluation of antibiotic resistance of *Acinetobacter baumannii* bacteria isolated from surgical intensive care unit patients in Pakistan. *Jundishapur Journal of Microbiology* 14(4):e113008.