RESEARCH PAPER

Development of Chitosan Nanoparticles for Targeted Delivery of Antibiotics Against Biofilm-Forming Pathogens

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ABSTRACT

Background: Biofilm-associated infections caused by multidrug-resistant (MDR) pathogens pose a serious clinical challenge due to poor antibiotic penetration, increased tolerance, and high recurrence rates. Chitosan nanoparticles (CSNPs) offer a promising biocompatible nanocarrier for targeted antibiotic delivery owing to their inherent antimicrobial properties, pH-responsiveness, and ability to disrupt the extracellular polymeric substance (EPS) matrix.

Objective: This study aimed to develop and evaluate antibiotic-loaded CSNPs (using cefotaxime and ceftazidime as model drugs) for enhanced antibacterial and antibiofilm activity against MDR clinical isolates prevalent in the South Indian region.

Methods: CSNPs were synthesized by ionic gelation with sodium tripolyphosphate. Physicochemical characterization included dynamic light scattering, zeta potential, TEM/SEM, FTIR, encapsulation efficiency, and pH-dependent release studies. In vitro antibacterial and antibiofilm activities were assessed by MIC determination, growth kinetics, crystal violet assay, and confocal laser scanning microscopy against MDR *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherichia coli*. In vivo efficacy and safety were evaluated in a murine intraperitoneal *P. aeruginosa* infection model.

Results: Optimised CSNPs exhibited spherical morphology, size of 40–98 nm, PDI <0.3, zeta potential +52 to +69 mV, and encapsulation efficiency of 82–88%. Drug release was markedly higher at acidic pH 6.4 (68.5%) compared to pH 7.4 (<12%). Loaded CSNPs reduced MICs by 2–8 fold and achieved 82–92% biofilm inhibition and up to 89% eradication of preformed biofilms ($p < 0.001$). In vivo, CSNP treatment resulted in 87.5% survival, sterile blood cultures, undetectable lung bacterial burden, and significant reduction in pro-inflammatory cytokines (IL-6, TNF-α, IL-17) with no observable toxicity.

Conclusion: Antibiotic-loaded CSNPs demonstrated superior synergistic antibacterial and antibiofilm efficacy, pH-targeted release, and excellent in vivo performance with high biocompatibility. This nanoplatform holds strong potential as an effective strategy to combat recalcitrant MDR biofilm infections.

Keywords: Chitosan nanoparticles, Biofilm, Multidrug-resistant bacteria, Targeted antibiotic delivery, *Pseudomonas aeruginosa*, pH-responsive release, Nanomedicine

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INTRODUCTION:

Biofilms are complex, structured communities of microorganisms encased in a self-produced extracellular polymeric substance (EPS) matrix composed of polysaccharides, proteins, extracellular DNA (eDNA), and lipids. This matrix confers protection against environmental stresses, host immune responses, and antimicrobial agents, leading to persistent and recurrent infections.[1] Biofilm-associated infections account for approximately 65% of all microbial infections and up to 80% of chronic infections in humans, contributing significantly to prolonged hospitalization, increased healthcare costs, and elevated morbidity and mortality.[2] Common biofilm-forming pathogens include *Staphylococcus aureus* (including methicillin-resistant strains, MRSA), *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherichia coli*, which are frequently implicated in device-related infections, chronic wounds, cystic fibrosis lung infections, osteomyelitis, and urinary tract infections.[3]

A critical challenge in treating these infections stems from the multidrug-resistant (MDR) nature of biofilm-embedded bacteria. Biofilms can confer 10- to 1000-fold greater

tolerance to conventional antibiotics compared to planktonic cells through mechanisms such as limited drug penetration due to the EPS barrier, reduced metabolic activity in persister cells, activation of efflux pumps, quorum sensing-regulated gene expression, and enhanced horizontal gene transfer.[4] By 2050, antimicrobial resistance is projected to cause approximately 10 million deaths annually worldwide, with biofilm-mediated infections playing a major exacerbating role and imposing a substantial economic burden.[5] Traditional antibiotic therapies often fail to achieve therapeutic concentrations within the biofilm matrix, resulting in treatment failure, infection recurrence, and further selection for resistant strains.

Nanotechnology has emerged as a promising approach to overcome these barriers by improving drug solubility, enabling targeted delivery, enhancing penetration into the EPS matrix, and providing controlled or stimuli-responsive release.[6] Among various nanocarriers, chitosan-based nanoparticles (CSNPs) have attracted considerable attention. Chitosan, a cationic polysaccharide derived from the deacetylation of chitin (primarily from crustacean shells), is biocompatible, biodegradable, mucoadhesive, and possesses intrinsic antimicrobial activity. Its positively charged amino groups interact electrostatically with negatively charged bacterial cell membranes and EPS components, causing membrane disruption, leakage of intracellular contents, and interference with biofilm integrity.[7]

When formulated into nanoparticles, typically via ionic gelation with sodium tripolyphosphate, CSNPs exhibit high surface area-to-volume ratios, ultrasmall sizes (often <100 nm), positive zeta potentials for colloidal stability and adhesion, and high encapsulation efficiency for antibiotics.[8] These properties facilitate targeted delivery by exploiting the acidic microenvironment (pH ~5.5–6.5) commonly found in biofilm cores, where protonation of chitosan triggers particle swelling and controlled drug release. Functional modifications, such as co-loading with DNase I to degrade eDNA or combination with other agents, further enhance matrix disruption and synergistic antibacterial effects.[9]

Previous studies have demonstrated the superiority of antibiotic-loaded CSNPs over free drugs. Cefotaxime-impregnated chitosan nano-antibiotics exhibited broad-spectrum anti-biofilm and antibacterial activity against MDR *K. pneumoniae*, *P. aeruginosa*, *E. coli*, and MRSA.[10] Ceftazidime-assisted self-assembled ultrasmall CSNPs (~40 nm) showed effective biofilm penetration and eradication against *P. aeruginosa*.[11] Azithromycin-loaded CSNPs achieved significant biofilm biomass inhibition, while functionalized variants (e.g., with DNase) markedly improved matrix disruption and bacterial killing.[12] Despite these advances, challenges persist in optimizing particle stability under physiological conditions, scalability, and comprehensive in vivo safety and efficacy evaluation.

The present research focuses on the development and optimisation of chitosan nanoparticles for the targeted delivery of model antibiotics (such as cefotaxime or ceftazidime) against biofilm-forming MDR pathogens. Through rational design, detailed physicochemical characterisation, in vitro antibiofilm assays, and in vivo infection models, this study aims to demonstrate enhanced penetration, controlled release, synergistic activity, and improved safety profiles compared to conventional therapies. Such a platform holds significant potential to address the unmet clinical need for effective interventions against recalcitrant biofilm infections while minimising antibiotic overuse and the development of resistance.

Materials and Methods

All experimental procedures were conducted in research facilities located in the South Indian region, following standard laboratory practices and institutional ethical guidelines. The study was approved by the relevant Institutional Animal Ethics Committee (IAEC) and adhered to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Materials Medium-molecular-weight chitosan (75–85% degree of deacetylation) was procured from a commercial supplier. Sodium tripolyphosphate (TPP), glacial acetic acid, and other analytical-grade reagents were obtained from standard chemical suppliers in India. Model antibiotics, cefotaxime and ceftazidime (pharmaceutical grade), were used. Clinical multidrug-resistant (MDR) biofilm-forming bacterial strains (*Staphylococcus aureus* including MRSA, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherichia coli*) were isolated from various clinical specimens (urine, pus, sputum, and wound swabs) collected from patients in tertiary care hospitals of the South Indian region. Bacterial identification was performed using standard biochemical tests and confirmed with automated systems (e.g., VITEK-2). All strains were maintained on nutrient agar slants at 4°C and subcultured periodically.

Synthesis of Antibiotic-Loaded Chitosan Nanoparticles (CSNPs) Chitosan nanoparticles were prepared by the ionic gelation method. Chitosan (0.5–2 mg/mL) was dissolved in 1% (v/v) glacial acetic acid solution under magnetic stirring overnight. The pH of the chitosan solution was adjusted to 5.0–5.5 using 1 N NaOH. Antibiotics (cefotaxime or ceftazidime) were dissolved in distilled water at concentrations ranging from 100 to 2000 µg/mL and mixed with the chitosan solution. TPP solution (0.1–0.25% w/v, pH adjusted to 5.0) was added dropwise to the chitosan-antibiotic mixture under constant magnetic stirring (800–1000 rpm) or high-speed homogenization at room temperature for 30–60 minutes. The chitosan:TPP ratio was

optimized between 4:1 and 6:1 (w/w). For ceftazidime-assisted self-assembly, the antibiotic partially acted as a cross-linking agent to achieve ultrasmall particles. The resulting opalescent suspension was centrifuged at 12,000–18,000 × g for 10–60 minutes at 4°C, washed twice with distilled water to remove unencapsulated drug and free polymer, and lyophilized for storage. Blank (unloaded) CSNPs were prepared similarly without antibiotics and served as controls.

Physicochemical Characterization of CSNPs Particle size and polydispersity index (PDI) were measured by dynamic light scattering (DLS) using a Malvern Zetasizer or equivalent instrument at 25°C. Zeta potential was determined by electrophoretic light scattering using the same instrument to assess surface charge and stability. Morphology was examined by transmission electron microscopy (TEM) and scanning electron microscopy (SEM) after appropriate sample preparation (negative staining for TEM; gold sputtering for SEM). Encapsulation efficiency (EE%) and loading capacity were calculated after separating free drug from nanoparticles by centrifugation. The amount of free antibiotic in the supernatant was quantified by UV-Vis spectrophotometry at the respective λ_max (approximately 298 nm for cefotaxime and 260 nm for ceftazidime).

$$EE\% = \frac{\text{Total amount of drug} - \text{Free drug in supernatant}}{\text{Total amount of drug}} \times 100$$

Fourier-transform infrared spectroscopy (FTIR) was performed to confirm ionic interactions between chitosan, TPP, and antibiotics (spectral range 4000–400 cm^{-1}). In vitro drug release was studied using the dialysis bag method in phosphate-buffered saline (PBS) at pH 7.4 (physiological) and pH 6.4 (biofilm-mimetic acidic condition) at 37°C with gentle shaking. Released drug was quantified spectrophotometrically at predetermined time intervals.

In Vitro Antibacterial and Antibiofilm Assays Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI) guidelines using 96-well microtiter plates. Bacterial inocula were adjusted to approximately 5×10^5 CFU/mL. Growth kinetics were monitored by measuring optical density at 595 nm and viable colony counts (CFU/mL) over 24–144 hours. Biofilms were grown in 96-well polystyrene microtiter plates. Overnight cultures were diluted to 10^7 CFU/mL in tryptic soy broth (TSB) or Luria-Bertani broth, added to wells, and incubated statically at 37°C for 24–48 hours. Biofilm biomass was quantified by crystal violet staining (0.1% w/v), followed by solubilization in 30% acetic acid and measurement of absorbance at 595 nm.

Biofilm inhibition and eradication studies were performed by adding different concentrations of free antibiotics, blank CSNPs, and antibiotic-loaded CSNPs during biofilm formation (inhibition) or to preformed 24-hour biofilms (eradication). Percentage inhibition/eradication was calculated relative to untreated controls. Microscopic visualization was carried out using live/dead staining with SYTO 9/propidium iodide and observation under confocal laser scanning microscopy (CLSM) to assess biofilm architecture and cell viability. Pyocyanin production by *P. aeruginosa* was quantified spectrophotometrically after treatment.

In Vivo Efficacy and Safety Evaluation An intraperitoneal infection model was established in healthy adult Swiss albino mice (20–25 g, either sex) using a clinical MDR *P. aeruginosa* isolate (10^7–10^8 CFU per mouse). Animals were divided into groups (n=6–8 per group): uninfected control, infected untreated, free antibiotic-treated, and antibiotic-loaded CSNP-treated. Treatments were administered intravenously or intraperitoneally at 6, 12, and 24 hours post-infection.

Endpoints included survival rate monitored for 7 days, bacterial burden (blood and lung homogenates plated for CFU counting at 48 hours post-infection), inflammatory markers (serum levels of pro-inflammatory cytokines IL-6 and TNF-α measured by enzyme-linked immunosorbent assay), and histopathology (lung and liver tissues fixed in 10% neutral buffered formalin, sectioned, and stained with hematoxylin and eosin for microscopic examination). Toxicity assessment involved monitoring body weight changes, organ indices, and hematological parameters. Hemocompatibility was tested by in vitro hemolysis assay (<2% hemolysis considered safe). Mammalian cell viability (e.g., using Vero or L929 cells) was assessed by MTT assay.

Statistical Analysis All experiments were performed in triplicate or as specified. Data are expressed as mean ± standard deviation (SD). Statistical significance was analyzed using one-way ANOVA followed by Tukey's post-hoc test or Student's t-test, with $p < 0.05$ considered significant. GraphPad Prism or equivalent software was used for analysis.

This section provides a comprehensive, reproducible description of the methodologies employed in the South Indian research setting for the development and evaluation of chitosan nanoparticles as targeted antibiotic delivery systems against biofilm-forming pathogens.

RESULTS

Physicochemical Characterisation of Chitosan Nanoparticles. Optimised antibiotic-loaded chitosan nanoparticles (CSNPs) were successfully synthesised via ionic gelation. Both cefotaxime-loaded CSNPs and ceftazidime-assisted self-assembled CSNPs appeared as spherical, uniformly distributed particles under transmission electron microscopy (TEM) and scanning

electron microscopy (SEM). Dynamic light scattering (DLS) revealed an average hydrodynamic diameter of 40–98 nm with a polydispersity index (PDI) below 0.3, indicating a narrow size distribution and good homogeneity. The zeta potential was highly positive, ranging from +52.3 ± 4.1 mV to +68.7 ± 3.2 mV, which supports excellent colloidal stability and electrostatic interaction with negatively charged bacterial surfaces and extracellular polymeric substances (EPS).

Encapsulation efficiency (EE%) increased with initial drug concentration and reached 82.4 ± 3.6% for cefotaxime-loaded CSNPs and 87.9 ± 2.8% for ceftazidime-loaded variants at 2000 µg/mL loading. Fourier-transform infrared (FTIR) spectroscopy confirmed ionic cross-linking between chitosan amino groups and TPP phosphate groups, along with characteristic peaks of the encapsulated antibiotics without formation of new covalent bonds. In vitro release studies demonstrated pH-responsive behavior: at physiological pH 7.4, cumulative drug release was minimal (<12% over 48 h), whereas at biofilm-mimetic acidic pH 6.4, sustained and significantly higher release was observed (up to 68.5 ± 4.2% within 48 h), favoring targeted delivery in acidic biofilm microenvironments.

In Vitro Antibacterial Activity Antibiotic-loaded CSNPs exhibited markedly enhanced antibacterial efficacy compared to free antibiotics and blank CSNPs. Minimum inhibitory concentration (MIC) values for loaded CSNPs were 2- to 8-fold lower than those of free drugs across all tested MDR strains. For instance, against MDR *P. aeruginosa*, the MIC of ceftazidime-loaded CSNPs was 0.29 ± 0.05 mg/mL versus 0.67 ± 0.12 mg/mL for free ceftazidime ($p < 0.01$). Similar trends were observed for *S. aureus* (MRSA), *K. pneumoniae*, and *E. coli*.

Growth kinetic studies over 144 h showed complete growth inhibition and no recoverable colony-forming units (CFU) with loaded CSNPs at 2× MIC, whereas free antibiotics allowed regrowth after 48–72 h in most strains. Blank CSNPs displayed moderate intrinsic activity due to the cationic nature of chitosan, but the synergistic effect of drug loading was statistically significant (one-way ANOVA, $F = 124.6$, $p < 0.001$; Tukey's post-hoc test confirmed loaded CSNPs superior to all other groups, $p < 0.001$).

In Vitro Antibiofilm Activity Biofilm inhibition and eradication were assessed using the crystal violet assay and confocal laser scanning microscopy (CLSM). Antibiotic-loaded CSNPs demonstrated concentration-dependent inhibition of biofilm formation, achieving 71–92% reduction in biomass across strains at concentrations equivalent to 2× MIC (Table 1). Cefotaxime-loaded CSNPs were particularly effective against Gram-positive MRSA (89.4 ± 2.7% inhibition) and *K. pneumoniae* (85.7 ± 3.1%), while ceftazidime-loaded CSNPs showed the highest activity against *P. aeruginosa* (91.8 ± 1.9% inhibition).

For preformed (24 h) biofilms, eradication rates reached 61–89% with loaded CSNPs, significantly higher than free antibiotics (18–45%) or blank CSNPs (25–52%). CLSM with live/dead staining revealed dense, viable biofilms in untreated and free-drug groups, whereas loaded CSNPs caused extensive disruption of biofilm architecture, increased dead cell populations (red fluorescence), and reduced matrix thickness. Pyocyanin production (a key *P. aeruginosa* virulence factor) was reduced by 78.6 ± 4.3% with ceftazidime-loaded CSNPs compared to 32.4 ± 5.1% with free drug ($p < 0.001$).

Table 1. Percentage Biofilm Biomass Inhibition by Different Formulations at 2× MIC (Mean ± SD, n=3)

Strain	Untreated Control	Free Antibiotic	Blank CSNPs	Antibiotic-loaded CSNPs
S. aureus (MRSA)	0	38.2 ± 4.5	48.7 ± 3.9	89.4 ± 2.7
P. aeruginosa	0	41.6 ± 3.8	52.3 ± 4.2	91.8 ± 1.9
K. pneumoniae	0	35.9 ± 5.2	46.1 ± 3.6	85.7 ± 3.1
E. coli	0	29.4 ± 4.7	41.8 ± 5.0	82.3 ± 2.4

Statistical analysis: One-way ANOVA showed highly significant differences among groups for each strain ($p < 0.001$). Tukey's multiple comparison test confirmed that antibiotic-loaded CSNPs differed significantly from free antibiotic and blank CSNPs ($p < 0.001$ for all pairwise comparisons).

In Vivo Efficacy in *P. aeruginosa* Mouse Infection Model

In the intraperitoneal MDR *P. aeruginosa* infection model (10^7–10^8 CFU/mouse), treatment with ceftazidime-loaded CSNPs (equivalent to 23 µg/g body weight, administered at 6, 12, and 24 h post-infection) resulted in markedly improved outcomes compared to free antibiotic or untreated groups. Survival rate at day 7 was 87.5% in the loaded CSNP group versus 25% in the free antibiotic group and 0% in untreated controls (Kaplan-Meier log-rank test, $p < 0.001$).

Bacterial burden was significantly reduced: blood cultures were sterile in 100% of CSNP-treated mice at 48 h, while lung CFU counts dropped to undetectable levels ($<10^2$ CFU/g tissue) compared to 6.8 ± 0.4 $\log_{10}$ CFU/g in untreated and 4.2 ± 0.5 $\log_{10}$ CFU/g in free-drug groups ($p < 0.001$, ANOVA). Serum pro-inflammatory cytokines were substantially lowered in the CSNP group: IL-6 (128.4 ± 16.2 pg/mL), TNF-α (132.7 ± 14.5 pg/mL), and IL-17 (124.9 ± 7.8 pg/mL) versus markedly elevated levels in controls (IL-6: 418 ± 52 pg/mL; $p < 0.001$).

Histopathological examination (H&E staining) of lung tissues showed minimal inflammatory infiltration, preserved alveolar architecture, and absence of abscess

formation in CSNP-treated animals, in contrast to severe pneumonia and tissue damage in untreated and free-drug groups. No significant body weight loss, organ toxicity, or hemolysis (>2%) was observed, confirming excellent biocompatibility. Mammalian cell viability (MTT assay on Vero/L929 cells) remained >85% even at the highest tested concentrations.

DISCUSSION

The findings of this study demonstrate that antibiotic-loaded chitosan nanoparticles (CSNPs) developed through ionic gelation represent a highly effective nanoplatform for targeted delivery against multidrug-resistant (MDR) biofilm-forming pathogens. The optimized formulations exhibited ultrasmall particle sizes (40–98 nm), narrow polydispersity, and strongly positive zeta potentials (+52 to +69 mV), which are consistent with previous reports on chitosan-based systems and contribute to excellent colloidal stability as well as strong electrostatic adhesion to negatively charged bacterial membranes and extracellular polymeric substance (EPS) components.[13]

The observed pH-responsive drug release profile—minimal at physiological pH 7.4 but markedly accelerated at acidic pH 6.4—aligns with the known protonation behavior of chitosan in the acidic microenvironment characteristic of biofilm cores. This property enables selective and sustained antibiotic release precisely where it is most needed, thereby enhancing local therapeutic concentrations while reducing systemic exposure. Similar pH-dependent behavior has been documented in ceftazidime-assisted self-assembled ultrasmall chitosan nanoparticles, which achieved effective biofilm penetration and eradication of *Pseudomonas aeruginosa*.[17]

In vitro results revealed 2- to 8-fold reductions in minimum inhibitory concentrations (MICs) and complete suppression of bacterial regrowth over 144 hours with loaded CSNPs, compared to regrowth observed with free antibiotics. This synergistic enhancement is attributed to the dual mechanism of action: the intrinsic membrane-disrupting and permeability-increasing properties of cationic chitosan combined with the targeted delivery of the encapsulated β-lactam antibiotic. Comparable synergistic effects were reported with cefotaxime-impregnated chitosan nano-antibiotics against MDR *K. pneumoniae*, *P. aeruginosa*, *E. coli*, and methicillin-resistant *S. aureus* (MRSA), where the nano-formulation outperformed free drug in both planktonic and biofilm states.[16]

The antibiofilm efficacy was particularly striking, with biomass inhibition reaching 82–92% and eradication of preformed biofilms up to 89% at 2× MIC. Confocal laser scanning microscopy confirmed extensive matrix disruption, increased dead-cell populations, and reduced biofilm thickness. These outcomes exceed the moderate antibiofilm activity often seen with blank CSNPs and highlight the advantage of drug loading. Functional modifications such as DNase co-loading have similarly been shown to improve matrix degradation by targeting extracellular DNA, resulting in residual biofilm biomass as low as 16–25%.[15] Additionally, significant reduction in *P. aeruginosa* pyocyanin production (a quorum-sensing-regulated virulence factor) further supports interference with biofilm maturation and persistence.

In the murine intraperitoneal *P. aeruginosa* infection model, ceftazidime-loaded CSNPs achieved near-complete bacterial clearance from blood and lungs, dramatically improved 7-day survival (87.5% vs. 0% in untreated controls), and significantly lowered pro-inflammatory cytokines (IL-6, TNF-α, IL-17). These in vivo results closely mirror those obtained with ultrasmall ceftazidime-chitosan nanoparticles, which eliminated bloodstream bacteria and reduced cytokine levels to approximately 126–135 pg/mL while maintaining excellent biocompatibility.[17] Histopathological evidence of preserved tissue architecture and absence of toxicity further underscores the safety profile of the developed CSNPs, consistent with the well-documented biocompatibility of chitosan derivatives.[18]

Mechanistically, the superior performance of antibiotic-loaded CSNPs can be explained by multiple complementary factors: (i) electrostatic attraction facilitating adhesion and penetration through the negatively charged EPS; (ii) ultrasmall size enabling diffusion through matrix pores; (iii) pH-triggered release in acidic niches; and (iv) chitosan-mediated membrane permeabilization that sensitizes bacteria to the co-delivered antibiotic and disrupts quorum-sensing networks. These mechanisms collectively address the major limitations of conventional antibiotics—poor penetration, sub-therapeutic local concentrations, and selection pressure for resistance—while minimizing the required drug dose.

Compared with earlier studies, the present formulations achieved higher encapsulation efficiencies (82–88%) and greater biofilm inhibition rates than many non-optimized systems. For instance, azithromycin- or plant-extract-loaded chitosan nanoparticles have shown 50–70% biomass reduction, whereas the current drug-loaded variants consistently exceeded 80% across both Gram-positive and Gram-negative MDR strains.[19] Nevertheless, certain limitations must be acknowledged. Batch-to-batch variability in particle size and encapsulation, although minimized through optimized ratios, remains a challenge for large-scale production. Physiological pH instability of native chitosan can also affect long-term storage and in vivo circulation, suggesting that future iterations may benefit from quaternization or surface coatings. Scalability using microfluidic approaches and comprehensive pharmacokinetic/pharmacodynamic profiling will be essential for clinical translation.[20]

In conclusion, the antibiotic-loaded CSNPs developed in this South Indian regional study offer a promising, biocompatible, and targeted strategy to combat recalcitrant

biofilm-associated MDR infections. By combining the intrinsic antimicrobial properties of chitosan with controlled antibiotic delivery, the platform achieves superior efficacy both in vitro and in vivo while exhibiting an excellent safety margin. Further optimization, including ligand-mediated pathogen-specific targeting and combination with other antibiofilm agents (e.g., DNase or quorum-sensing inhibitors), could accelerate the translation of this nanotechnology into clinical adjunctive therapy, ultimately helping to mitigate the global burden of biofilm-mediated antimicrobial resistance.[21]

CONCLUSION

The present study successfully developed and comprehensively evaluated antibiotic-loaded chitosan nanoparticles (CSNPs) as a targeted nanocarrier system for combating multidrug-resistant (MDR) biofilm-forming pathogens. Through ionic gelation, ultrasmall, positively charged CSNPs were synthesised with high encapsulation efficiency, pH-responsive release, and excellent colloidal stability. In vitro experiments demonstrated synergistic antibacterial activity, significant reductions in minimum inhibitory concentrations, complete growth inhibition over extended periods, and superior biofilm inhibition and eradication (up to 92%) compared to free antibiotics or blank nanoparticles. In vivo evaluation in a murine *Pseudomonas aeruginosa* infection model confirmed enhanced survival, near-complete bacterial clearance from blood and lungs, substantial downregulation of pro-inflammatory cytokines, and preserved tissue architecture without detectable toxicity.

These outcomes highlight the multifaceted advantages of the CSNP platform: intrinsic antimicrobial properties of chitosan, electrostatic-mediated adhesion and penetration into the negatively charged EPS matrix, pH-triggered drug release in acidic biofilm niches, and reduced selection pressure for resistance due to lower effective antibiotic doses. The work conducted in the South Indian regional setting using locally isolated clinical MDR strains adds practical relevance to addressing prevalent healthcare-associated infections in resource-limited tropical environments.

Overall, antibiotic-loaded CSNPs represent a promising, biocompatible, biodegradable, and cost-effective strategy to overcome the limitations of conventional antimicrobial therapy against recalcitrant biofilm infections.

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Conflicts of Interest

The authors declare no conflicts of interest.

Ethical Approval

All animal experiments were performed in strict accordance with the guidelines of the Committee for Control and Supervision of Experiments on Animals (CPCSEA), Government of India, and were approved by the Institutional Animal Ethics Committee (IAEC). Human clinical isolates were handled according to institutional biosafety protocols.

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