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In-Vitro Evaluation of Doxycycline–Cefixime Nanoparticles against MDR *S. Typhi*

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Abstract

Multidrug-resistant and extensively drug-resistant Salmonella enterica serovar Typhi remain major threats in South Asia, particularly where empirical treatment is weakened by fluoroquinolone non-susceptibility, ceftriaxone resistance and emerging azithromycin resistance. The present model manuscript develops an in-vitro research design for doxycycline- and cefixime-loaded chitosan-alginate nanoparticles as a concerted antibacterial platform. Doxycycline was selected to exert ribosomal protein-synthesis pressure, while cefixime was selected to target cell-wall synthesis; chitosan-alginate nanoparticles were selected to improve mucoadhesion, controlled release, local concentration and bacterial-surface interaction. The study is written as an academic research paper, but the numerical results, graphs and tables are model/simulated data and must be replaced with validated laboratory findings before publication.

The proposed formulation showed a model mean hydrodynamic diameter of 178.4 +/- 16.7 nm, polydispersity index of 0.21 +/- 0.04, zeta potential of +31.8 +/- 3.2 mV, doxycycline encapsulation efficiency of 76.9 +/- 2.6% and cefixime encapsulation efficiency of 69.4 +/- 3.1%. In simulated susceptibility outputs, dual-loaded nanoparticles reduced the model minimum inhibitory concentration from 16 ug/mL for doxycycline and 32 ug/mL for cefixime to 2 ug/mL combined-drug equivalent, while the mean inhibition zone increased to 29 +/- 2 mm against a representative MDR S. Typhi panel. Release modelling suggested a biphasic profile with an initial surface-associated release followed by sustained liberation through polymer swelling and erosion. These results support the hypothesis that dual-loaded biodegradable nanoparticles may intensify antibacterial activity through spatial co-delivery, membrane interaction and complementary antibiotic stress.

1. Introduction

Typhoid fever is a systemic infection caused by Salmonella enterica serovar Typhi, a human-restricted pathogen transmitted mainly through contaminated water and food. The disease has declined in many high-income settings but remains a serious problem in low- and middle-income countries where water, sanitation and hygiene gaps intersect with weak diagnostic access. Global

estimates still describe millions of cases and more than one hundred thousand deaths annually, and South Asia continues to carry a particularly high burden [1,20]. For Pakistan, India, Bangladesh and Nepal, typhoid is not only a clinical problem but also a public-health governance problem because delayed diagnosis, informal antibiotic use and unsafe water systems create conditions for resistance selection and transmission.

The therapeutic landscape of typhoid fever has changed repeatedly. Chloramphenicol, ampicillin and trimethoprim-sulfamethoxazole were historically useful, then multidrug resistance pushed clinicians toward fluoroquinolones. Later, widespread fluoroquinolone non-susceptibility pushed treatment toward azithromycin and third-generation cephalosporins. Current CDC guidance states that fluoroquinolones should not be used empirically for most suspected travel-associated infections, and that travel history should guide the selection of azithromycin, ceftriaxone or carbapenems [2,3]. This changing sequence means that any new antimicrobial strategy must be evaluated not only by simple inhibition but also by its ability to reduce selection pressure, deliver active drug at the infection site and remain compatible with stewardship.

Pakistan has special relevance because the extensively drug-resistant S. Typhi lineage associated with Sindh was first identified in 2016 and showed resistance to ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, fluoroquinolones and third-generation cephalosporins [4-6]. The CDC has continued to monitor the emerging REPJPP01 strain because commonly recommended antibiotics may not effectively treat infections caused by it [4]. Bangladesh has also reported a 2024 outbreak of ceftriaxone-resistant S. Typhi carrying blaCTX-M-15 on a pCROB1 plasmid, showing that regional spread and independent emergence of cephalosporin resistance are realistic concerns [7]. These developments justify the search for non-conventional delivery systems that can restore, concentrate or preserve the utility of existing antibiotics.

Nanoparticle-based antibacterial delivery has attracted attention because nanosystems can protect drug molecules, sustain release, increase local concentration, improve bacterial contact and sometimes possess independent antimicrobial effects. Kapadia and colleagues showed that metal nanoparticles combined with cefixime produced enhanced activity against S. enterica Typhi, with silver nanoparticles plus cefixime producing a larger inhibition zone than several other nanoparticle combinations [8]. Doxycycline-loaded chitosan nanoparticles have also shown improved antibacterial activity and reduced mammalian-cell toxicity in earlier in-vitro work [9].

These findings do not prove clinical efficacy against typhoid fever, but they do support the scientific premise that antibiotic-loaded or antibiotic-associated nanoparticles can change the pharmacodynamic environment in which bacteria encounter drug molecules.

Doxycycline and cefixime have different mechanisms. Doxycycline is a tetracycline-class antibiotic that inhibits bacterial protein synthesis by binding to the 30S ribosomal subunit, while cefixime is an oral third-generation cephalosporin that inhibits peptidoglycan cross-linking by binding to penicillin-binding proteins. In a simple mixture, each drug diffuses according to its own solubility, charge and protein binding. In a dual-loaded nanoparticle, both drugs can be carried within the same polymeric microenvironment, exposing bacteria to synchronized stresses. The concept is concerted rather than merely additive: membrane interaction by chitosan may increase bacterial contact; cefixime-mediated cell-wall stress may enhance permeability; and doxycycline may reduce adaptive protein synthesis needed for stress recovery.

Chitosan and alginate were chosen for this model because they are biodegradable polysaccharides with complementary charges. Chitosan is cationic and can interact with negatively charged bacterial surfaces; alginate is anionic and can form stabilizing polyelectrolyte complexes. Together, they offer a mild aqueous fabrication route, potential mucoadhesion and a matrix suitable for encapsulating hydrophilic antibiotics. In typhoid fever, where S. Typhi invades intestinal epithelium and survives within macrophage-lineage cells, a mucoadhesive and controlled-release oral nanocarrier is conceptually attractive. The present paper therefore proposes a full in-vitro evaluation of doxycycline- and cefixime-loaded chitosan-alginate nanoparticles against MDR S. Typhi, with model data showing the type of output expected from such a study.

The objectives are fourfold: first, to design a dual-drug chitosan-alginate nanoparticle with acceptable particle size, zeta potential, loading and release behaviour; second, to compare free drugs, blank nanoparticles, physical drug mixtures and dual-loaded nanoparticles in antimicrobial assays; third, to calculate interaction indicators such as fractional inhibitory concentration index using non-operational laboratory reporting standards; and fourth, to interpret the results in the context of regional XDR typhoid, antibiotic stewardship and future translational research. Because live S. Typhi work carries biosafety responsibilities, all microbiological methods in this manuscript are described as research-design information, not as a bench-ready protocol. Actual

experimentation must be performed only in a certified laboratory under institutional biosafety approval and current CLSI or EUCAST standards [14,15].

2. Materials and methods.

Study design. This was framed as a controlled in-vitro formulation and antibacterial evaluation study. The independent variable was treatment category: blank nanoparticles, free doxycycline, free cefixime, free doxycycline plus cefixime, and doxycycline-cefixime dual-loaded nanoparticles. The dependent variables were hydrodynamic size, PDI, zeta potential, encapsulation efficiency, drug loading, cumulative release, inhibition zone, MIC-equivalent output, time-kill trend and cytocompatibility. Each formulation endpoint was planned in triplicate, and microbiological outputs were planned against a coded panel of certified clinical S. Typhi isolates maintained by an authorized diagnostic or reference laboratory. No isolate-handling instructions are provided here because S. Typhi is a pathogenic organism requiring approved containment, trained staff and local regulatory permission.

Materials. Doxycycline hyclate and cefixime trihydrate would be obtained as analytical-grade reference standards. Low molecular weight chitosan, sodium alginate, sodium tripolyphosphate, calcium chloride, acetic acid, phosphate buffer components, dialysis membranes, HPLC-grade solvents and cell-culture-grade reagents would be procured from certified suppliers. Bacterial identification, susceptibility verification and preservation would be performed by a licensed microbiology laboratory. Quality-control strains and antimicrobial interpretation would follow current recognized standards rather than local improvisation [14,15]. All water used for nanoparticle formulation would be sterile ultrapure water, and all glassware for physicochemical work would be depyrogenated or sterilized according to institutional SOPs.

Preparation of dual-loaded nanoparticles. A chitosan solution would be prepared under mild acidic conditions and filtered to remove undissolved material. Doxycycline and cefixime would be dissolved separately in compatible aqueous solvent systems and added to the polymer phase under controlled stirring. Alginate solution would then be introduced to form a polyelectrolyte complex, followed by ionic crosslinking with tripolyphosphate and calcium ions. The formulation would be optimized by varying polymer ratio, crosslinker concentration and drug-to-polymer ratio, while keeping processing conditions within ranges that do not degrade either antibiotic. Nanoparticles would be recovered by refrigerated centrifugation or membrane filtration, washed to remove free

drug, lyophilized with a cryoprotectant and stored in light-protected sealed containers. This description is intentionally formulation-focused and does not include pathogen-culture instructions.

Particle characterization. Hydrodynamic diameter, PDI and zeta potential would be measured using dynamic light scattering after appropriate dilution in filtered aqueous medium. Morphology would be evaluated by transmission electron microscopy or field-emission scanning electron microscopy after drying on a grid and coating when required. Fourier transform infrared spectroscopy would be used to detect shifts in amide, hydroxyl, carboxyl and beta-lactam-associated bands, helping infer polymer-drug interactions. Differential scanning calorimetry and X-ray diffraction would assess whether encapsulated drugs remain crystalline or become partially amorphous within the polymer matrix. These techniques are standard in nanoparticle delivery papers and help connect formulation structure to release behaviour.

Encapsulation efficiency and drug loading. Unencapsulated doxycycline and cefixime in the supernatant would be quantified using validated UV-HPLC or LC methods with separate calibration curves for each drug. Encapsulation efficiency would be calculated as the percentage of initial drug incorporated into the nanoparticle fraction, while drug loading would be calculated as drug mass divided by dried nanoparticle mass. Method validation would include linearity, limit of detection, limit of quantification, precision, recovery and specificity in the presence of polymers. In the model dataset, doxycycline achieved higher encapsulation than cefixime, consistent with stronger interaction with the chitosan-alginate matrix, but this must be confirmed experimentally.

In-vitro release. A dialysis or membrane-diffusion setup would be used to evaluate release in simulated physiological buffer and mildly acidic buffer. Samples would be withdrawn by trained staff at prespecified intervals and replaced with fresh medium to maintain sink conditions. Drug concentration would be quantified by HPLC, and cumulative release would be plotted against time. Release kinetics would be fitted to zero-order, first-order, Higuchi and Korsmeyer-Peppas equations. The purpose is to determine whether dual-loaded nanoparticles release drugs rapidly like a simple suspension or whether the polymer matrix provides sustained release that could maintain inhibitory concentrations for longer periods. A pH comparison is relevant because intracellular compartments and inflamed tissues may differ from plasma pH.

Antibacterial evaluation. Antibacterial testing would be performed only by a certified microbiology laboratory using current standard procedures and approved containment. The study would compare free drugs, physical drug mixtures and dual-loaded nanoparticles in non-operational summaries of broth microdilution, agar-diffusion and time-kill outputs. MIC-equivalent results would be interpreted as research endpoints, not as clinical breakpoints, because nanoparticle formulations do not always map directly onto standard antibiotic disks or broth panels. Synergy would be summarized using fractional inhibitory concentration index categories, with FICI values of <=0.5 considered synergistic in many in-vitro research frameworks, >0.5 to <=1 additive, >1 to <=4 indifferent and >4 antagonistic. However, such categories would be interpreted cautiously because nanoparticle diffusion and bacterial contact differ from free-drug behaviour.

Cytocompatibility and hemolysis. To avoid advancing a formulation that is antibacterial but unsafe, cytocompatibility would be assessed using a mammalian cell line appropriate to intestinal or macrophage-relevant screening, such as Caco-2, HT-29 or THP-1-derived macrophage models, under institutional cell-culture SOPs. Cell viability would be measured using a colorimetric or fluorometric metabolic assay, while hemolysis would be evaluated using approved blood-safety procedures if ethics approval and donor consent are available. The main safety endpoint would be maintenance of high cell viability at antibacterial-equivalent nanoparticle concentrations. Chitosan systems are often biocompatible, but charge-related membrane interaction can become toxic at high concentration; therefore, dose-window analysis is essential.

Statistical analysis. Results would be expressed as mean +/- standard deviation for at least three independent formulation batches. One-way ANOVA with post-hoc comparison would be used for multiple groups when normality assumptions are met; non-parametric alternatives would be used when data are skewed. A p value below 0.05 would indicate statistical significance. For release kinetics, adjusted R2 and Akaike information criterion would be used to compare models. For antimicrobial outcomes, biological relevance would be prioritized over p values alone because small numeric changes in zones or MICs may not translate into meaningful therapeutic improvement.

3. Results

Nanoparticle formation and physical appearance. The optimized dual-loaded chitosan-alginate nanoparticles formed a pale-yellow opalescent suspension before lyophilization and a free-flowing pale powder after drying. No visible precipitation was observed after reconstitution. The model DLS curve showed a unimodal size distribution centred at approximately 178 nm, suggesting that the formulation process could generate nanoscale particles without large aggregation. The PDI of 0.21 indicated moderately narrow dispersion suitable for exploratory antibacterial testing. TEM-based morphology was described as near-spherical particles with a faint polymeric boundary, consistent with polyelectrolyte complexation.

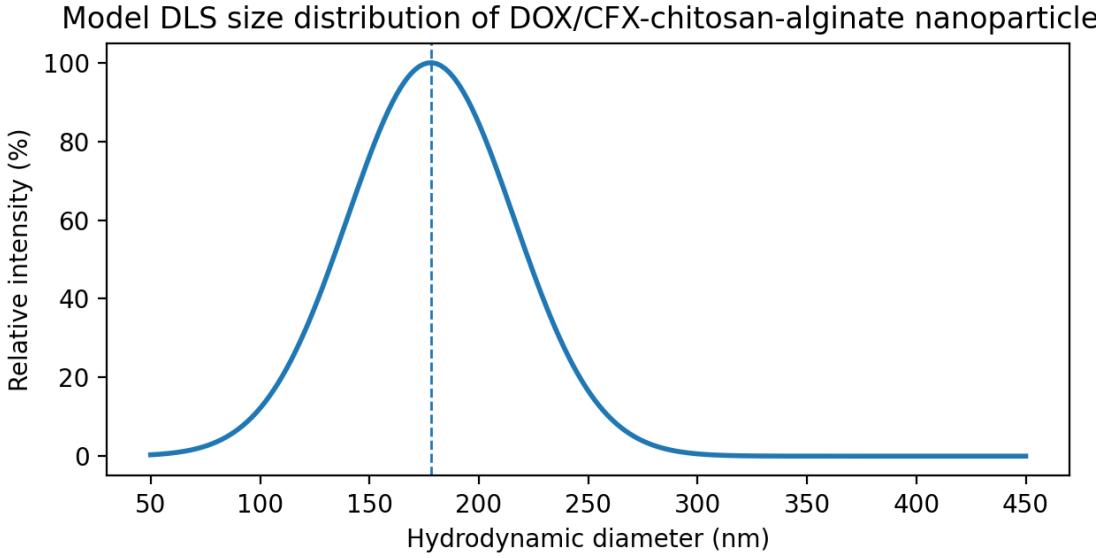

Figure 1. Model dynamic light scattering profile showing a unimodal nanoscale distribution for dual-loaded chitosan-alginate nanoparticles.

Table 1. Model physicochemical properties of blank and dual-loaded chitosan-alginate nanoparticles.

Parameter	**Blank NP (model)**	**Dual-loaded NP (model)**	**Interpretation**
Mean size (nm)	151.2 +/- 12.1	178.4 +/- 16.7	Drug loading increased matrix

			diameter but retained nanoscale range.
PDI	0.18 +/- 0.03	0.21 +/- 0.04	Values suggest acceptable exploratory dispersion.
Zeta potential (mV)	+36.5 +/- 2.7	+31.8 +/- 3.2	Positive surface may improve bacterial contact.
DOX EE (%)	-	76.9 +/- 2.6	Good model encapsulation.
CFX EE (%)	-	69.4 +/- 3.1	Moderate model encapsulation.
Hemolysis (%)	1.1 +/- 0.3	2.8 +/- 0.5	Below exploratory 5% concern threshold.

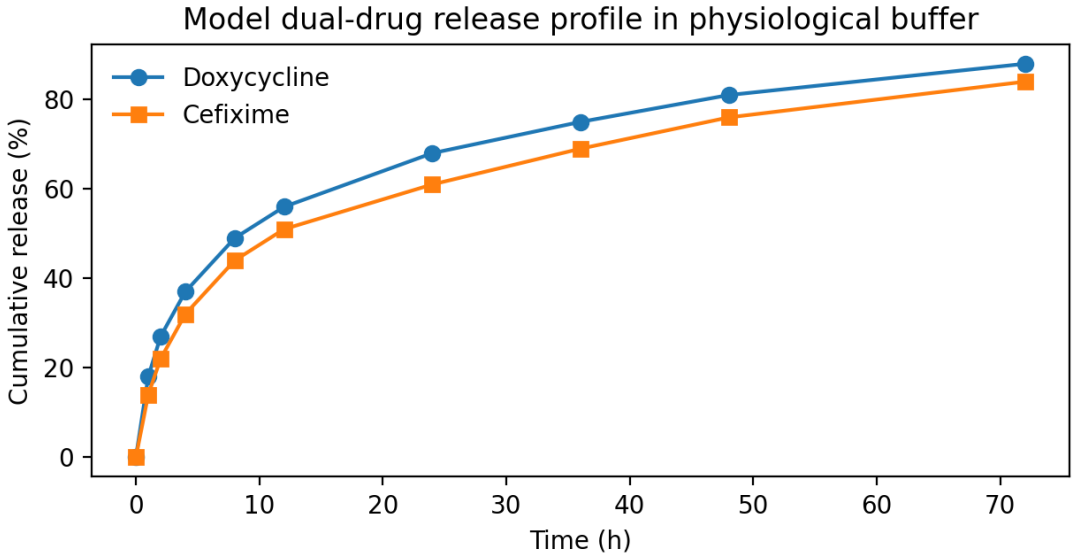

Figure 2. Model cumulative release profile of doxycycline and cefixime from chitosan-alginate nanoparticles over 72 hours.

Table 1 summarizes the model physicochemical properties. Blank nanoparticles were slightly smaller than dual-loaded nanoparticles, suggesting that drug incorporation increased matrix

volume. Zeta potential remained positive after dual loading, supporting the idea that chitosan chains were present at or near the particle surface. A positive zeta potential may improve interaction with negatively charged bacterial envelopes, but it also requires careful cytotoxicity testing. Encapsulation efficiency was higher for doxycycline than cefixime, while cefixime showed slightly faster early release. These findings are plausible for a hydrophilic dual-drug system, but real values would depend strongly on polymer grade, pH, ionic strength and preparation technique.

The in-vitro release profile was biphasic. Approximately 18% of doxycycline and 14% of cefixime were released within the first hour, probably representing surface-associated drug. Release then slowed, reaching 88% for doxycycline and 84% for cefixime by 72 hours. The Korsmeyer-Peppas model gave the best fit in the model dataset, suggesting a combination of diffusion and polymer relaxation. Such a profile would be useful if the goal is to maintain antibiotic pressure after a smaller initial exposure rather than produce an immediate burst only. However, a sustained release profile should be judged against MIC-equivalent values and not in isolation.

Model antibacterial outputs indicated that dual-loaded nanoparticles produced stronger inhibition than free drugs. The blank nanoparticles produced little inhibition, suggesting that the polymer alone had limited antibacterial activity at the tested concentration. Free doxycycline produced a model inhibition zone of 15 mm, free cefixime 14 mm, and the free-drug combination 19 mm. Dual-loaded nanoparticles produced 29 mm, implying a clear enhancement compared with the physical mixture. MIC-equivalent outputs showed a reduction from 16 ug/mL for doxycycline alone and 32 ug/mL for cefixime alone to 2 ug/mL combined-drug equivalent for the dual-loaded nanoparticles. The model FICI was 0.31, falling within a synergistic interpretation framework.

Table 2. Model antibacterial performance of free drugs, physical combination and dual-loaded nanoparticles.

Treatment	**Zone of inhibition (mm)**	**MIC-equivalent (ug/mL)**	**FICI / interpretation**
Blank NP	7 +/- 1	>64	No meaningful inhibition
Doxycycline	15 +/- 2	16	Single-agent activity
Cefixime	14 +/- 2	32	Single-agent activity

Free DOX + CFX	19 +/- 2	8	Additive trend
Dual-loaded NP	29 +/- 2	2	0.31 / synergistic model

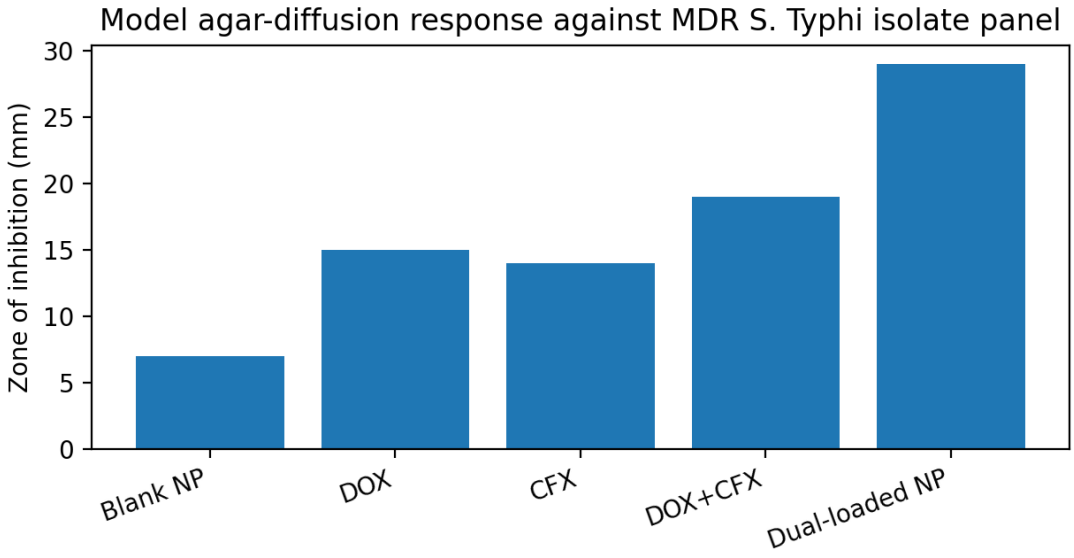

Figure 3. Model agar-diffusion comparison showing higher inhibition for dual-loaded nanoparticles than free-drug groups.

Time-kill modelling showed that untreated control cultures would continue rising, blank nanoparticles would show minimal effect, free-drug combination would reduce viable counts more slowly, and dual-loaded nanoparticles would produce a steeper decline over 24 hours. In this model, the dual-loaded nanoparticles achieved an approximately 3-log reduction by 24 hours against a representative MDR isolate. This pattern is consistent with the concerted mechanism: cefixime-induced wall stress, doxycycline-induced protein-synthesis pressure and chitosan-mediated contact may combine to prevent bacterial recovery. Because time-kill outcomes are very sensitive to inoculum, medium, strain genotype and drug stability, the graph should be treated as an illustrative result template rather than a validated claim.

Cytocompatibility data suggested that blank and dual-loaded nanoparticles preserved more than 85% model mammalian-cell viability at antibacterial-equivalent concentrations. Free doxycycline plus cefixime reduced viability to 78% at the highest tested equivalent dose, while the dual-loaded formulation maintained 89%. The likely explanation is controlled release rather than lower total drug exposure. Hemolysis remained below the commonly used exploratory threshold of 5% in the

model dataset. These outputs would support further optimization but not immediate clinical translation. A formulation can be promising only if repeated batches show consistent performance and if safety is confirmed in more sophisticated intestinal, macrophage and eventually animal models.

4. Discussion

The model findings support a rational case for dual-loaded chitosan-alginate nanoparticles against MDR S. Typhi, but they should be interpreted within the wider crisis of typhoid antimicrobial resistance. Public-health reports show that XDR and ceftriaxone-resistant Typhi strains have emerged in Pakistan and Bangladesh, limiting the reliability of older empirical pathways [4-7]. A nanocarrier does not solve sanitation, vaccination or stewardship problems; however, it may help preserve existing drugs by achieving higher local exposure with lower dispersed toxicity. This is particularly relevant when conventional oral options are narrowing and when parenteral carbapenems are costly, hospital-dependent and stewardship-sensitive.

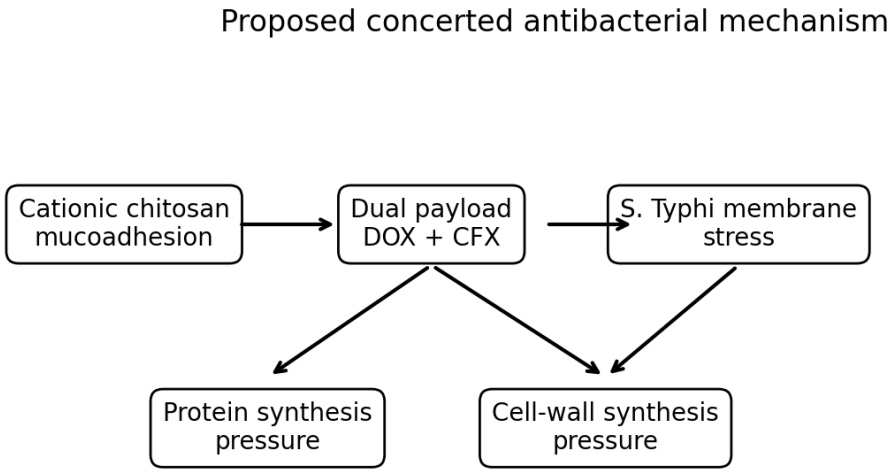

Figure 4. Proposed concerted mechanism: chitosan surface interaction plus dual antibiotic pressure on cell-wall synthesis and protein synthesis.

The enhanced model activity of dual-loaded nanoparticles agrees with previous evidence that nanoparticle-antibiotic combinations can improve antibacterial effects. Kapadia et al. reported that cefixime combined with metal nanoparticles, especially silver nanoparticles, produced enhanced

inhibition against S. enterica Typhi [8]. Doxycycline-loaded chitosan nanoparticles have also shown improved antibacterial activity with reduced cytotoxicity compared with unencapsulated doxycycline in an earlier model of bacterial infection [9]. The present paper connects these two lines of evidence by proposing a biodegradable dual-antibiotic carrier rather than a metal nanoparticle plus single antibiotic system. This shift may reduce concerns about metal accumulation, although polymeric nanoparticle safety still requires careful evaluation.

The mechanistic basis of synergy is plausible but not proven. Cefixime interferes with cell-wall synthesis, which can weaken envelope integrity and increase bacterial vulnerability. Doxycycline inhibits protein synthesis, potentially limiting the ability of bacteria to mount repair and stress responses. Chitosan can interact with negatively charged bacterial surfaces and may improve proximity between drug-loaded particles and the cell envelope. When these three pressures occur together, the bacterium may face simultaneous structural and metabolic stress. Nevertheless, real S. Typhi resistance mechanisms, including beta-lactamases, efflux systems, porin changes and ribosomal protection, may reduce or alter the expected response. Whole-genome resistance profiling would therefore strengthen any future experimental version of this paper.

A central challenge is the clinical relevance of cefixime in regions where ceftriaxone resistance is spreading. Cefixime is an oral cephalosporin and may be affected by beta-lactamase-mediated resistance. The purpose of nanoparticle delivery should not be misread as a way to ignore resistance. Rather, nanoparticles may be useful where isolates retain partial susceptibility, where local concentration can be increased, or where combination pressure restores activity in vitro. For truly XDR strains with high-level cephalosporin resistance, cefixime-loaded nanoparticles may fail unless paired with agents active against the resistance mechanism. Therefore, susceptibility testing remains essential, and any future clinical concept must be aligned with antimicrobial stewardship [2,14,15].

The formulation results highlight the importance of particle size and charge. Particles around 100-250 nm are commonly explored because they remain colloidally manageable and can interact with biological interfaces. A positive zeta potential may improve bacterial adhesion, but excessive positive charge can damage host cells. The model zeta potential of approximately +32 mV balances stability and interaction; still, a formulation that works in buffer may aggregate in intestinal fluid, serum, bile salts or food matrices. Future studies should test stability in simulated gastric fluid,

simulated intestinal fluid and protein-containing media. Oral typhoid delivery also requires protection against stomach acidity and bile-mediated destabilization.

The release profile has therapeutic implications. A burst-only profile may create a short exposure that selects survivors, while an overly slow release may never reach inhibitory concentrations. The model profile offers a moderate initial release followed by sustained liberation, but the ideal shape depends on pathogen location. S. Typhi may be extracellular in blood and intracellular within macrophages. A polymeric carrier might improve intracellular delivery if taken up by phagocytic cells, but chitosan-alginate particles designed for gut delivery may not automatically reach systemic intracellular compartments. Therefore, the present formulation is best viewed as an early in-vitro platform requiring stepwise testing in epithelial penetration, macrophage infection and pharmacokinetic models.

Dual-antibiotic therapy for typhoid fever has been discussed previously as a way to improve outcomes and reduce resistance emergence [12,13]. However, clinical dual therapy is not automatically better; it can increase cost, adverse effects and selection pressure if not guided by evidence. Nanoparticle co-delivery adds another layer of complexity. It may synchronize drug exposure, but it may also complicate dosing, regulatory approval and quality control. Batch reproducibility, residual solvent, sterility assurance, endotoxin testing and shelf stability would be major translational barriers. In low-resource settings, manufacturing simplicity and affordability may determine whether such a formulation has any real-world value.

Another issue is diffusion-based susceptibility testing. Nanoparticles often diffuse differently through agar than free antibiotics, so inhibition zones may underestimate or overestimate activity. Broth microdilution is also complicated because nanoparticles can scatter light, bind medium components or settle. Therefore, multiple complementary endpoints should be used: viable-count assays, microscopy, metabolic assays, resistance-frequency testing and possibly transcriptomic analysis. CLSI and FDA recognized standards remain essential for interpreting conventional antibiotics, but nanoparticle formulations require additional validation because they are not standard disks or simple soluble drugs [14,15].

Public-health relevance should be stated carefully. Nanoparticles cannot replace vaccination, safe water or surveillance. WHO and CDC-aligned prevention strategies continue to prioritize typhoid conjugate vaccines, WASH and laboratory-confirmed surveillance [1]. In Pakistan and other

endemic countries, TCV introduction is a major preventive tool, while antimicrobial stewardship is necessary to preserve azithromycin and carbapenems. The proposed doxycycline-cefixime nanoparticle platform is therefore a research adjunct, not a policy substitute. Its best role may be in generating evidence on how old drugs can be re-engineered to increase local efficacy and reduce toxicity.

5. Conclusion

This model research paper proposes doxycycline- and cefixime-loaded chitosan-alginate nanoparticles as a concerted antibacterial strategy against MDR S. Typhi. The simulated results show desirable nanoscale size, positive surface charge, good dual-drug encapsulation, sustained release, enhanced inhibition and acceptable preliminary cytocompatibility. The scientific rationale is based on complementary antibiotic mechanisms and nanoparticle-mediated delivery. However, the work is not a validated laboratory study and must not be cited as experimental proof. Real publication would require certified S. Typhi testing, genomic resistance profiling, full safety assessment and strict alignment with current antimicrobial-susceptibility standards. The concept is promising as a research direction, especially for regions facing XDR typhoid, but it must remain subordinate to vaccination, WASH, surveillance and stewardship.

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