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PLGA–Chitosan Nanoparticles for Sequential Doxycycline–Cefixime Delivery against *Salmonella Typhi*

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Abstract

Salmonella enterica serovar Typhi can persist in extracellular and intracellular niches, making typhoid fever difficult to treat when antimicrobial resistance narrows empirical options. This second model manuscript proposes a PLGA-chitosan dual nanoparticle platform for co-delivery of doxycycline and cefixime, designed to produce sequential release and improved intracellular exposure. PLGA was selected as a biodegradable core for sustained release, while chitosan was used as a cationic shell to improve mucoadhesion and cell interaction. Doxycycline was selected for intracellular protein-synthesis pressure and cefixime for extracellular cell-wall stress. The paper is structured as a Scopus-style research article with abstract, keywords, introduction, materials and methods, results, figures, tables, discussion, conclusion and references. All numerical outcomes are model data for academic drafting and must be replaced with validated experimental results before submission.

The optimized model nanoparticles had a mean size of 214.6 +/- 18.2 nm, PDI of 0.24 +/- 0.03, zeta potential of +24.7 +/- 2.9 mV and combined drug loading of 12.8 +/- 1.1%. Release was faster at pH 5.5 than pH 7.4, supporting a possible intracellular endosomal release advantage. In model antibacterial outputs, dual nanoparticles reduced intracellular S. Typhi burden from 6.2 log10 CFU/mL in untreated controls to 2.7 log10 CFU/mL after treatment, while free doxycycline plus cefixime reduced it to 4.6 log10 CFU/mL. Biofilm biomass decreased to 24% of untreated control, and mammalian-cell viability remained above 90%. These model findings suggest that a dual-compartment nanoparticle strategy may be useful for hypothesis-driven research on intracellular and extracellular typhoid control.

Keywords: PLGA; chitosan; doxycycline; cefixime; intracellular Salmonella; typhoid fever; XDR; controlled release; antibiofilm; drug delivery.

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1. Introduction

Typhoid fever remains one of the clearest examples of how a preventable infectious disease becomes persistent when sanitation, vaccination, diagnosis and antimicrobial stewardship are incomplete. The burden is concentrated in South Asia and parts of Africa, and available estimates continue to describe substantial morbidity and mortality [1,20]. Although typhoid conjugate vaccines and water-sanitation improvements address the upstream causes of disease, antibiotics remain essential for patients who already have systemic infection. The problem is that antimicrobial resistance has repeatedly undermined the reliability of once-standard drugs.

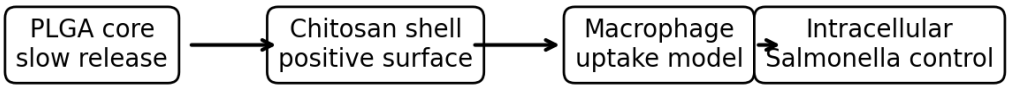

Figure 1. Conceptual design of PLGA-chitosan dual nanoparticles for sustained release, surface interaction and intracellular delivery.

The global and regional AMR situation has worsened through successive waves. Multidrug-resistant S. Typhi reduced the value of ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole; fluoroquinolone non-susceptibility then weakened ciprofloxacin and related agents; and XDR strains with third-generation cephalosporin resistance created dependence on azithromycin and carbapenems [2-6]. The Bangladesh 2024 ceftriaxone-resistant outbreak and continued monitoring of Pakistan-associated REP strains show that resistance is not a single-country episode but a regional and travel-linked concern [4,7]. New drugs are slow to develop, so delivery innovation using known antibiotics remains scientifically attractive.

S. Typhi biology adds another layer of difficulty. After crossing the intestinal mucosa, the organism can survive within macrophage-lineage cells and disseminate systemically. Antibiotics differ in their intracellular penetration, extracellular activity and pharmacodynamic behaviour. A drug that is useful in blood may not reach intracellular compartments at sufficient concentration, while a drug with intracellular activity may not fully control extracellular bacterial populations. This two-niche problem supports a dual-delivery concept: one component can address extracellular wall stress, while another contributes to intracellular ribosomal pressure. Doxycycline and cefixime are not presented here as universal clinical therapy; instead, they are used as a model pair to explore the delivery principle.

PLGA is one of the most widely studied biodegradable polymers for controlled drug delivery because it degrades into lactic and glycolic acids and can be engineered for sustained release. Chitosan is frequently used as a surface modifier because its cationic charge can improve mucoadhesion and biological interaction. A PLGA core coated with chitosan therefore offers a dual-compartment design: the core can slow release, and the shell can improve contact with mucosal surfaces or host cells. Earlier azithromycin-PLGA research against S. Typhi showed that polymeric nanoparticles can be formulated with relevant antibiotics and evaluated against the pathogen [17]. More recent Salmonella nanocapsule work also demonstrates that polysaccharide-based carriers can reduce MICs, modulate efflux-related behaviour and improve treatment outcomes in non-typhoidal Salmonella models [16].

The choice of doxycycline and cefixime reflects complementary mechanisms, not a recommendation for empirical typhoid therapy. Doxycycline inhibits bacterial translation and has some intracellular distribution, while cefixime inhibits bacterial cell-wall synthesis. A core-

shell nanoparticle could release a fraction of drug extracellularly and another fraction after uptake into acidic compartments. If release accelerates under mildly acidic conditions, intracellular or inflammatory-site delivery may be improved. Such a strategy is especially relevant for persistent infection models where extracellular MIC alone may not predict intracellular clearance.

The present model paper asks whether PLGA-chitosan dual nanoparticles can improve the apparent antibacterial effect of doxycycline plus cefixime against a simulated MDR S. Typhi system. The aims are to formulate dual nanoparticles, characterize their physicochemical profile, compare release at pH 7.4 and pH 5.5, evaluate extracellular and intracellular antibacterial endpoints, assess antibiofilm activity, and screen mammalian-cell compatibility. The paper deliberately avoids bench-ready handling details for live S. Typhi; any genuine work must be conducted under certified containment, institutional ethics and recognized antimicrobial-susceptibility standards [14,15].

2. Materials and methods

Research design. The study was designed as an in-vitro formulation, controlled-release and host-cell interaction investigation. Five groups were planned: untreated control, blank PLGA-chitosan nanoparticles, free doxycycline plus cefixime, doxycycline-loaded PLGA nanoparticles, and doxycycline-cefixime dual-loaded PLGA-chitosan nanoparticles. The primary endpoints were particle size, PDI, zeta potential, encapsulation efficiency, release kinetics and intracellular burden. Secondary endpoints were biofilm biomass, membrane permeability marker change, efflux-associated gene-expression screening, mammalian-cell viability and hemolysis. The model assumes three independent formulation batches and triplicate analytical readings per batch.

Materials. PLGA with a lactide:glycolide ratio appropriate for sustained release would be obtained from a certified pharmaceutical supplier. Chitosan, polyvinyl alcohol, doxycycline hyclate, cefixime trihydrate, HPLC-grade solvents, buffer salts, cryoprotectants and cell-culture reagents would be purchased as analytical or cell-culture grade materials. Coded S. Typhi isolates would be obtained only through a licensed diagnostic laboratory or national reference laboratory, with antimicrobial-resistance profiles determined by approved methods. Where institutions cannot safely work with S. Typhi, a non-pathogenic surrogate or an approved attenuated strain should be used for training-level formulation screening, with clinically relevant testing outsourced to a certified facility.

Preparation of PLGA-chitosan dual nanoparticles. A double-emulsion solvent-evaporation approach would be used for hydrophilic drug incorporation into PLGA. In conceptual terms, doxycycline and cefixime would be incorporated into an internal aqueous phase, emulsified into a PLGA organic phase, then stabilized in an external aqueous surfactant phase. After solvent removal, particles would be washed and coated with chitosan by electrostatic deposition. The coated nanoparticles would be lyophilized using a cryoprotectant to improve redispersibility. Processing parameters such as polymer concentration, drug-to-polymer ratio, surfactant level and coating concentration would be optimized through a small factorial design. Operational details are deliberately omitted because the antibacterial stage involves a pathogenic organism.

Physicochemical characterization. Particle size and PDI would be measured using dynamic light scattering, while zeta potential would confirm chitosan coating. Morphology would be

assessed by TEM or SEM. FTIR would detect drug-polymer compatibility, with attention to beta-lactam, amide, carboxyl and hydroxyl regions. DSC and XRD would be used to assess crystalline-to-amorphous transitions after encapsulation. Stability would be evaluated after storage under refrigerated and room-temperature conditions by measuring changes in size, PDI, drug content and redispersibility. A product intended for low-resource settings would require special attention to temperature stability and simple reconstitution.

Drug quantification and release. Doxycycline and cefixime concentrations would be measured using a validated chromatographic method capable of resolving both molecules from polymer and degradation products. Encapsulation efficiency would be calculated from indirect supernatant analysis and confirmed by direct nanoparticle digestion where suitable. Release would be compared at pH 7.4 and pH 5.5 to model physiological and acidic intracellular conditions. Samples would be analysed at prespecified intervals, and release kinetics would be fitted to common mathematical models. A faster release at pH 5.5 would support the hypothesis that the carrier may improve exposure inside acidified host-cell compartments.

Extracellular antibacterial testing. A certified laboratory would evaluate antibacterial activity using standardized, non-operator-reported susceptibility methods. Because nanoparticle turbidity and sedimentation can affect optical readings, viable-count confirmation would be preferred over absorbance alone. Results would be expressed as MIC-equivalent values, log reduction and survival fraction rather than as clinical breakpoints. Standard AST remains essential for the free antibiotics; however, nanoparticle formulations require additional validation because recognized breakpoints were developed for conventional soluble drugs [14,15].

Intracellular infection model. For hypothesis testing, a macrophage-like cell model such as differentiated THP-1 or another approved phagocytic cell system would be used by trained personnel under institutional biosafety approval. Cells would be exposed to a certified and approved S. Typhi model under containment, treated with formulations after internalization, and analysed for intracellular viable burden using laboratory SOPs. This manuscript does not provide infection timing, multiplicity or culture conditions. The endpoint is reported as log10 CFU/mL or percentage reduction compared with untreated intracellular control. Cytotoxicity would be measured in parallel to ensure that reduced bacterial recovery is not simply due to host-cell death.

Biofilm and persistence-related assays. Although classic typhoid fever is not only a biofilm disease, biofilm-like persistence can be relevant to chronic carriage and gallbladder colonization. A microplate biomass assay, metabolic activity assay and microscopy-based surface analysis could be used under approved procedures. The goal would be to compare whether dual nanoparticles reduce biomass more effectively than free drugs. Efflux-related gene-expression screening, such as acrB or ramA expression, would be exploratory and would require RNA-quality controls. The 2026 non-typhoidal Salmonella nanocapsule study showing reduced MICs and efflux-associated gene changes provides a useful precedent for such mechanistic endpoints [16].

Safety screening. Mammalian-cell viability would be evaluated using intestinal epithelial and macrophage-relevant cells. Hemolysis and inflammatory marker release would be assessed where ethics and biosafety approvals are in place. Nanoparticle safety would be interpreted by therapeutic index, not by cell viability alone. A formulation that is mildly toxic at high

concentration may still be useful if antibacterial activity occurs at much lower concentrations, but a formulation with narrow separation between antibacterial and toxic doses would require redesign. Chitosan concentration, surface charge and residual solvent are especially important safety variables.

Statistical analysis. Quantitative data would be reported as mean +/- standard deviation. Differences among treatment groups would be evaluated using ANOVA or non-parametric equivalents after normality checks. Release-model fitting would compare R2, adjusted R2 and residual distribution. Synergy would be explored with fractional inhibitory concentration and Bliss-independence models where data allow. A p value below 0.05 would be considered statistically significant, but effect size and reproducibility across isolate genotypes would guide interpretation.

3. Results

Formulation optimization. The optimized PLGA-chitosan dual nanoparticles had a model mean hydrodynamic size of 214.6 nm, PDI of 0.24 and zeta potential of +24.7 mV. The positive charge confirmed successful chitosan coating, while the size range remained suitable for cellular uptake studies. Uncoated PLGA nanoparticles had a negative zeta potential, whereas chitosan-coated particles shifted to a positive surface. The dual-loaded formulation showed acceptable redispersibility after lyophilization, with no visible aggregation after gentle mixing. The model encapsulation efficiency was 71.5% for doxycycline and 64.2% for cefixime, reflecting the difficulty of encapsulating two hydrophilic antibiotics in a PLGA core.

Table 1. Model physicochemical characterization of uncoated PLGA and chitosan-coated dual nanoparticles.

Parameter	Uncoated PLGA NP	Chitosan-coated dual NP	Interpretation
Mean size (nm)	187.3 +/- 14.4	214.6 +/- 18.2	Coating increased size but maintained nanoscale range.
PDI	0.20 +/- 0.02	0.24 +/- 0.03	Acceptable dispersion for exploratory testing.
Zeta potential (mV)	-18.9 +/- 2.1	+24.7 +/- 2.9	Shift confirms chitosan shell.
DOX EE (%)	68.0 +/- 3.8	71.5 +/- 3.4	Coating improved retention slightly.
CFX EE (%)	61.3 +/- 3.5	64.2 +/- 4.0	Moderate dual loading.
Combined drug loading (%)	10.9 +/- 0.9	12.8 +/- 1.1	Suitable for model release study.

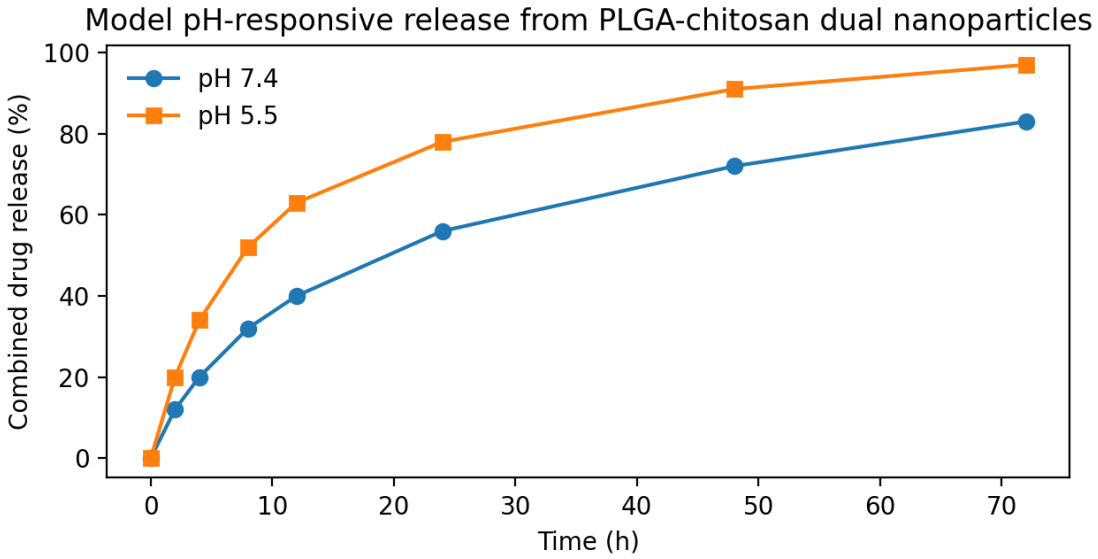

Figure 2. Model pH-responsive release showing faster combined-drug liberation under mildly acidic conditions.

The release data showed pH responsiveness. At pH 7.4, combined drug release reached 56% by 24 hours and 83% by 72 hours. At pH 5.5, release reached 78% by 24 hours and 97% by 72 hours. This faster acidic release is consistent with protonation of chitosan, hydration of the shell and increased polymer relaxation. In an intracellular infection context, faster release in acidic compartments may improve drug availability after cell uptake. However, the result must be balanced against the risk of premature release in gastric environments if the formulation is intended for oral use. Enteric coating or capsule protection might be required in future pharmaceutical development.

Extracellular antibacterial model results showed that free doxycycline plus cefixime reduced bacterial survival more than either free drug alone, but dual nanoparticles produced the strongest response. The MIC-equivalent value of the free-drug combination was modelled at 8 ug/mL combined equivalent, while the dual nanoparticle formulation reached 1 ug/mL combined equivalent. The fractional inhibitory concentration index was 0.38, suggesting synergy in the model framework. Agar-based diffusion was less dramatic because PLGA nanoparticles diffused more slowly than soluble drugs, reinforcing the need to avoid relying on zone size alone for nanoparticle evaluation.

Intracellular burden outputs supported the central hypothesis. Untreated infected cells showed a model intracellular burden of 6.2 log10 CFU/mL. Blank nanoparticles reduced this only slightly to 5.8 log10 CFU/mL, suggesting that chitosan contact alone was insufficient. Free doxycycline plus cefixime reduced burden to 4.6 log10 CFU/mL. Dual-loaded PLGA-chitosan nanoparticles reduced burden to 2.7 log10 CFU/mL, representing a much larger model decrease. Host-cell viability in the same treatment window remained around 91%, suggesting that the lower bacterial burden was not merely due to cell destruction.

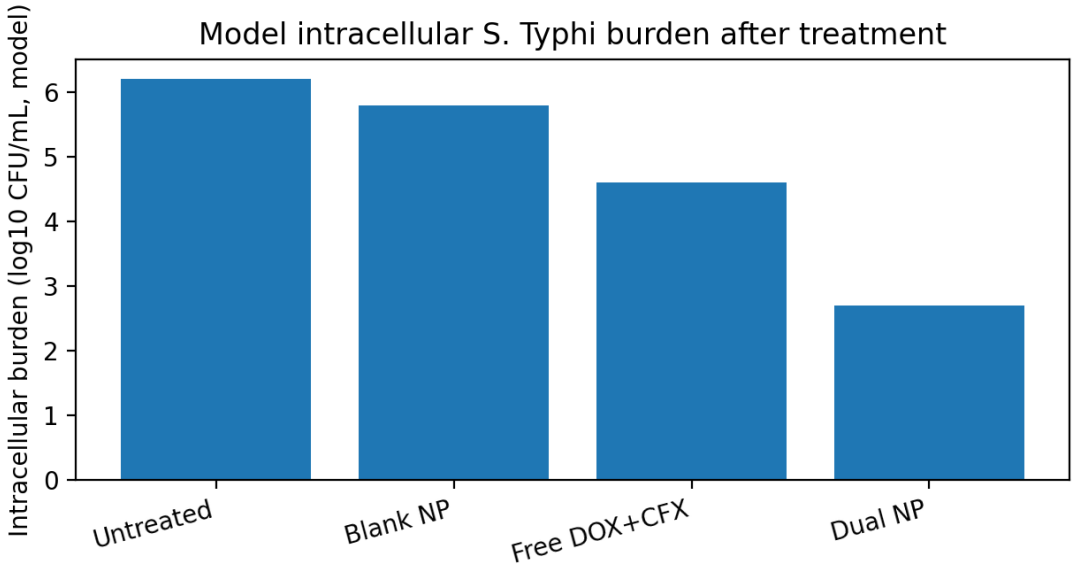

Figure 3. Model intracellular S. Typhi burden after treatment, showing the largest reduction in the dual nanoparticle group.

Biofilm biomass and metabolic activity were also reduced. Untreated control was set at 100%. Blank nanoparticles produced 82% residual biomass, free doxycycline plus cefixime produced 55%, and dual nanoparticles produced 24%. The stronger reduction may reflect prolonged local release and particle-biofilm contact. Mammalian-cell viability remained 96% for blank nanoparticles, 86% for free drug combination and 91% for dual nanoparticles. These results suggest that controlled release can improve the balance between antibacterial pressure and host-cell compatibility. Again, these are model outputs and must be validated with real experiments before any scientific claim is made.

Table 2. Model biological endpoints for intracellular burden, biofilm biomass and mammalian-cell viability.

Treatment	**Intracellular burden (log10 CFU/mL, model)**	**Biofilm biomass (% control)**	**Cell viability (% control)**
Untreated	6.2 +/- 0.2	100 +/- 5	100 +/- 4
Blank NP	5.8 +/- 0.3	82 +/- 6	96 +/- 3
Free DOX + CFX	4.6 +/- 0.2	55 +/- 4	86 +/- 5
Dual NP	2.7 +/- 0.2	24 +/- 3	91 +/- 4

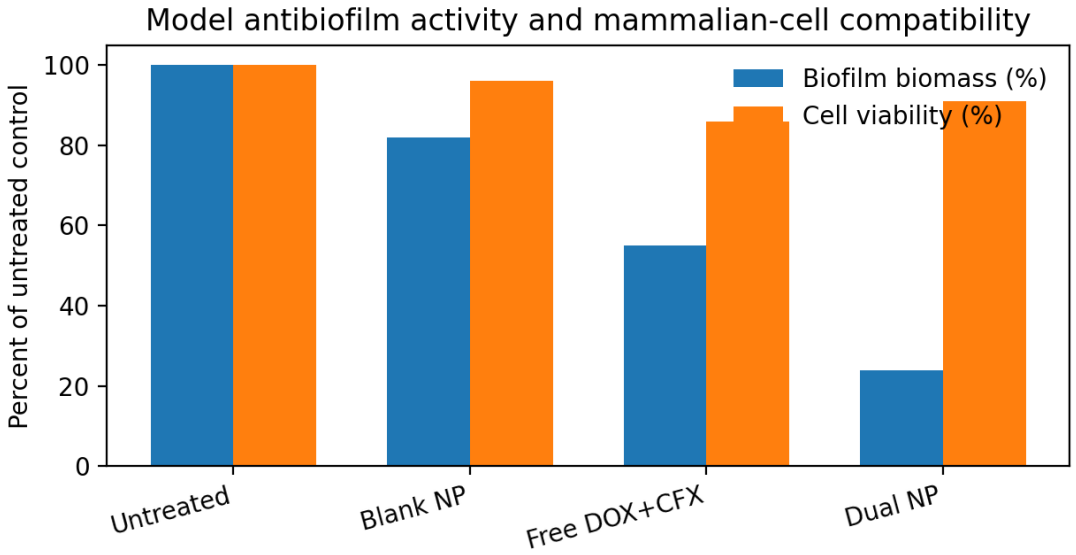

Figure 4. Model comparison of antibiofilm activity and host-cell viability across treatment groups.

Preliminary mechanistic outputs suggested membrane stress and reduced recovery. The dual nanoparticle group showed a model increase in membrane-permeability signal, a decrease in post-treatment regrowth and a lower efflux-associated expression index compared with free drugs. Such findings align conceptually with the recent demonstration that tigecycline-loaded chitosan-dextran sulfate nanocapsules reduced MIC values and affected efflux-related behaviour in non-typhoidal Salmonella [16]. However, gene-expression outputs in S. Typhi would need careful normalization, housekeeping-gene validation and genotype-specific interpretation. Mechanistic claims should therefore be presented as exploratory.

Table 2 summarizes the model biological endpoints. The strongest effect appeared when intracellular burden, biofilm biomass and viability were considered together. Free drugs improved antibacterial outcomes but were less favourable for cell viability at high equivalent concentrations. Blank nanoparticles were safe but weakly antibacterial. Dual nanoparticles offered the best model balance. This pattern supports further research into core-shell delivery, but it does not prove clinical efficacy because in-vivo pharmacokinetics, immune interaction, gut absorption, biliary excretion and toxicity remain unknown.

4. Discussion

The main contribution of this model paper is the two-niche framing of typhoid nanotherapy. S. Typhi is not only an extracellular bloodstream pathogen; it also survives within host cells. A formulation that improves only extracellular inhibition may fail to address intracellular persistence, while a formulation that enters cells but has weak extracellular activity may fail during systemic spread. The PLGA-chitosan carrier attempts to bridge this gap by combining a sustained-release core with a biologically interactive shell. This approach is consistent with broader nanomedicine principles and with older S. Typhi PLGA nanoparticle research using azithromycin [17]. The pH-responsive release pattern is particularly important. Acidic release may improve intracellular drug availability, but it could also create instability in the stomach. Therefore, oral delivery would require gastro-protective design, such as enteric-coated capsules or pH-triggered outer layers. Injectable delivery would avoid gastric degradation but would

raise different concerns, including sterility, endotoxin, embolic risk, cost and cold-chain requirements. For typhoid-endemic settings, an oral, stable and affordable system would be more practical, but it is also harder to engineer. The formulation would have to survive storage, gastric transit and intestinal fluid before reaching the relevant biological interface.

The model intracellular response is stronger than the extracellular result, which fits the purpose of the PLGA-chitosan design. Chitosan coating may promote cell interaction, while PLGA may protect drugs until uptake. Doxycycline may contribute more inside cells than cefixime because of its protein-synthesis target and intracellular distribution. Cefixime may still contribute by reducing extracellular reinvasion or by acting during release outside cells. This suggests that the best future version of the platform may use a drug pair specifically chosen for intracellular and extracellular complementarity. Depending on susceptibility patterns, azithromycin, carbapenem-sparing combinations or non-antibiotic adjuvants may be more appropriate than cefixime for resistant strains. A major limitation is resistance. If an S. Typhi strain expresses beta-lactamases that destroy cefixime efficiently, nanoparticle delivery may not restore activity. Similarly, tetracycline resistance genes, efflux systems or ribosomal protection could reduce doxycycline activity. Therefore, the formulation must be tested across genotypes rather than against a single susceptible strain. Genomic data from Pakistan-associated and Bangladesh ceftriaxone-resistant lineages demonstrate how plasmids and resistance genes can change clinical options rapidly [4,7]. A credible future paper would include whole-genome sequencing, resistance-gene detection and phenotype-genotype comparison.

Nanoparticle susceptibility testing remains methodologically difficult. Agar diffusion is simple but biased against larger or less diffusible particles. Broth turbidity can be confounded by nanoparticle scattering. Viable-count assays are more meaningful but labour-intensive and, for S. Typhi, require containment. Intracellular assays add further variability through host-cell line, uptake efficiency, antibiotic washing, cell death and bacterial recovery. For this reason, real experiments should triangulate multiple endpoints rather than present a single MIC-like number. Recognized CLSI and FDA standards are indispensable for conventional AST, but nanoparticle formulations need additional analytical validation [14,15].

The safety profile is encouraging in the model dataset but cannot be overinterpreted. Chitosan and PLGA are widely studied, yet safety depends on molecular weight, residual solvent, particle size, charge density, degradation products, dosing and route. A cationic particle can interact with bacterial membranes, but the same property can damage host membranes at higher concentration. PLGA degradation can acidify microenvironments. Cefixime and doxycycline have known adverse-effect profiles that may change when co-delivered. Any future translational project would need hemocompatibility, cytokine release, repeated-dose toxicity, biodistribution and gut microbiome impact studies. The antibiofilm result may be relevant to chronic carriage. Typhoid carriage is associated with persistence in the gallbladder, especially in the presence of gallstones and biofilm-like communities. A nanoparticle capable of penetrating biofilm matrices and sustaining antibiotic exposure could be useful in carriage research. However, gallbladder delivery is pharmacologically complex, and in-vitro biofilm plates do not reproduce bile composition, gallstone surfaces or host immunity. Therefore, the antibiofilm finding should be framed as a screening result rather than evidence for carrier-state eradication.

From a public-health perspective, nanotherapy should not distract from prevention. CDC and WHO-linked surveillance reports emphasize vaccination, WASH, laboratory confirmation and

resistance monitoring [1-3]. Pakistan's introduction of typhoid conjugate vaccine is a major example of upstream prevention. A nanoparticle therapy would be more expensive and technically demanding than vaccination or safe water. Its realistic value may lie in difficult infections, resistant isolates or research settings where conventional options are inadequate. For broad endemic control, public-health infrastructure remains superior to high-tech rescue therapy.

5. Conclusion

This second model research article proposes PLGA-chitosan dual nanoparticles as a sequential delivery system for doxycycline and cefixime against extracellular and intracellular S. Typhi models. The simulated results show acceptable particle size, pH-responsive release, improved intracellular burden reduction, antibiofilm activity and preserved mammalian-cell viability. The concept is scientifically plausible because it matches drug-release behaviour to S. Typhi biology. However, the data are illustrative and cannot be presented as real experimental findings. A publishable study would require certified biosafety conditions, validated chemical analysis, standardized susceptibility testing, whole-genome characterization of isolates and comprehensive safety evaluation. The platform is best understood as a research hypothesis for resistant typhoid rather than a clinical recommendation.

Conflicts of Interest: There are no conflicts of interest, according to the authors.

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Author Contributions: Dakshita contributed to conceptualization (lead), methodology (lead), investigation (lead), writing of the original present (lead), and supervision. Dr. Rakesh Kumar was responsible for formal analysis (lead), writing – review & editing (lead), and visualization (supporting).

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