**Efficacy of intravesical targeting of novel quorum sensing inhibitor nanoparticles against *Pseudomonas aeruginosa* biofilm-associated murine pyelonephritis**

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**ABSTRACT**

*Pseudomonas aeruginosa* biofilm-associated pyelonephritis is a severe infection that can lead to mortality. There are no strategies that can effectively manage this infection since the pathogenesis is controlled by quorum sensing (QS) regulated virulence and recalcitrant biofilms. QS inhibitors (QSIs) are emerging therapeutics against such infections but are associated with cytotoxicity or low bioactivity. Hence, we developed novel quorum sensing inhibitor loaded nanoparticles (QSINPs) using the biopolymers, chitosan (CS) and dextran sulphate (DS) and were intravesically targeted against biofilm-associated murine pyelonephritis. The in-vivo targeting of QSINPs was confirmed by tracking the fluorescein isothiocyanate (FITC) tagged QSINPs in bladder and kidney of mice. On characterising, the QSINPs showed a size of 685.7 nm with a zeta potential of 37.9 and polydispersity index (PDI) of 0.5. Scanning electron microscopy (SEM) indicated spherical shape and bioactivity assays indicated QSI activity till 8 months. Fourier transform infrared (FTIR) analysis indicated possibility of isothiocyanate bonding in CS with DS and with QSI. The QSINPs showed excellent in vitro antivirulence activity by reducing the virulence factors and biofilm of *P. aeruginosa* and in vivo therapeutic efficacy with ciprofloxacin (CIP). Hence, we propose a novel next-generation therapeutic and its appropriate targeting route against biofilm-associated pyelonephritis.

**Introduction**

*P. aeruginosa* biofilm causes chronic infections in human, most of which are hospital associated ailments. Catheter-associated urinary tract infections are one such complicated infection which leads to cystitis and pyelonephritis. Mortality and morbidity rate remain significantly high in such infections although the percentage of occurrence is relatively low. Despite the sufferings, the extra cost and additional stay at the hospital for the treatment cause economic burden and inconvenience to the patient [1]. A variety of approaches other than oral antibiotics, such as the use of probiotics [2,3], bacteriophages [4–6] and catheter coating [7,8] have been tried. However, none of these have been observed to be effective since biofilm are involved during infection.

Biofilm is sessile communities characterised by cells that are irreversibly attached to a substratum and are protected by extracellular polymeric substances. Biofilm formation aids *Pseudomonas* to survive in extreme environments including conditions, such as minimal nutritional availability and make the organism resistant to conventional antibiotics and host immune system [9]. Quorum sensing (QS), a cell density-dependent mechanism exists in *P. aeruginosa* imparts an indispensable role in the transition of planktonic bacteria to biofilm mode of growth [10]. QS operates through chemical molecules (quorum sensing signal molecules [QSSMs]) which coordinate the expression of an array of virulence genes required for pathogenesis and existence of bacteria [11].

In this scenario, the novel antivirulence agents/quorum sensing inhibitors (QSIs) could be alternatives to conventional drugs against *P. aeruginosa*. However, the majority of well-characterised QSIs, which are of eukaryotic origin, are observed to be toxic to human [12]. In this context, attention has been focussed on QSIs from higher plants (specifically dietary plants) as they are generally recognised as safe (GRAS). Ajoene (C₉H₁₄O₃S₃), the phytochemical from garlic (*Allium sativum* L.) bulb is characterised earlier as QSI against *P. aeruginosa* [13,14]. However, availability in very limited quantity, hydrophobicity, instability and comparatively very high MIC values than antibiotics limits the therapeutic applicability of ajoene [14,15]. We presume the use of novel delivery systems and targeted route of administration could overcome these issues. Polymeric nanoparticles which are chitosan (CS) based have such prospects since CS offers mucoadhesiveness, biocompatibility, bio-degradability, coagulation ability, immunostimulating activity and nontoxicity [16]. As pyelonephritis model is used, the intravesical route of administration was adopted since it could offer localised delivery and thereby targeted action. Additionally, ciprofloxacin (CIP), an effective antibiotic against *P. aeruginosa* was used to explore the benefits of combination therapy.

Hence, in this study, the QSI, ajoene (separated from garlic bulb), was made into nanoformulation using CS-dextran sulphate (CS-DS) polymers. They were characterised and assessed for *in vitro* activity along with CIP against *P. aeruginosa*. Further, the QSINPs were delivered intravesically into mice along with CIP (orally) and evaluated for their therapeutic potential against *P. aeruginosa* biofilm-associated murine pyelonephritis. *In vivo* tracking of QSINPs by fluorescent labelling confirmed targeted delivery.
Materials and methods

Materials

Bacteria

The standard strain Pseudomonas aeruginosa PAO1 and reporter strain E. coli MG4 were procured from Barbara H. Iglewski, University of Rochester, Rochester, NY and stored at -80°C in 50% glycerol were used in the study. The fresh culture was used for every new experiment.

Chemicals

QSI (ajoene) was prepared according to previously standardised method [13]. CIP and fluorescein isothiocyanate (FITC) were procured from Himedia Laboratories Pvt. Ltd., Mumbai, India. CS (MMW, 75–85% deacetylated) and DS sodium salt (>500,000 MW) were procured from Sigma-Aldrich Chemical Co. Ltd., St. Louis, MO.

Animals

LACA, Swiss Webster female mice (6–8 weeks old, weight 20–25 g) procured from Central Animal House, Panjab University, Chandigarh, India were used in the study. Mice were housed in clean polypropylene cages and were fed on the standard anti-biotic-free synthetic diet (Hindustan Levers Ltd., Mumbai, India) and water ad libitum. The ethical clearance was approved by Institutional Animal Ethics Committee of Panjab University, Chandigarh, India (Approval ID: IAE/504). All the experimental procedures were followed according to the guidelines of Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Preparation and characterisation of QSINPs

QSNI of concentration (0.1–1% in MeOH) was added to CS (0.075% in 1% HOAc) in 1:5 (w/v) ratio. DS (0.075% in HPLC grade water) in the ratio 3:1 (v/v) was added further. The solution was stirred, washed twice and pelleted at 16,000 rpm. The entrapment efficiency (EE) of NP formulations was calculated according to the following formula. The concentration of QSI was calculated by corroborating the absorbance values with the standard curve of QSI [17].

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\text{% Release} = \frac{\text{Initially entrapped QSI in NPs} - \text{Released QSI from NPs}}{\text{Initially entrapped QSI in NPs}} \times 100
\]

Shelf life of QSINPs was assessed by checking the QSI activity of NPs which were kept at different temperatures [−20, 0, 4 and 28°C] for 8 months. Briefly, fresh Luria agar plates covered with X-gal (Sigma Aldrich, Haverhill, MA) and EtOAc extracted QSSM solution was allowed to dry. Further, reporter strain, E. coli MG4 was spread evenly on the prepared plates under sterile conditions. The NP formulations were loaded in 4 mm wells that were made in Luria agar plates. Plates were incubated at 37°C for 24 h and then assessed for colourless zone diameter (in cm) indicating QSI activity [18].

Preparation of FITC tagged QSINPs

FITC tagging of QSINPs was done using the standard procedure [19]. Briefly, CS solution was mixed with MeOH and FITC. The labelled polymer was precipitated using 0.5 M NaOH and washed by acetonewater (3:1)(v/v). It was dissolved in 0.1 M acetic acid and dialysed in distilled water for 3 d in dark. To the pellet, QSI and DS were added under stirring conditions as previously mentioned.

Establishment of murine acute pyelonephritis model

Peak day (Day 4) biofilm cells of P. aeruginosa PAO1 were inoculated through a soft PE10 (BD Biosciences, San Jose, CA) tubing into bladder of mice according to standard method [20]. Care was taken while inserting and withdrawing the catheter so as to avoid mucosal destruction.

Murine treatment groups

The following treatment groups were designed. Group I: infection control (intravesical inoculation of peak day biofilm cells of P. aeruginosa (10^6 CFU/ml); Group II: Intravesical delivery (through PE10 tubing into bladder) of QSI (25 mg/kg body weight [b.wt.]) and Group III: intravesical delivery of QSINPs (600 mg/kg b.wt.) was carried out immediately and same dose was given once a day daily till 4 d post infection (p.i.). Group IV (combination group); intravesical delivery of QSINPs (600 mg/kg b.wt.) and oral delivery of CIP (10 mg/kg b.wt.) till 4 d p.i. Group V (combination group); intravesical delivery of QSINPs (600 mg/kg b.wt.) and oral delivery of CIP (15 mg/kg b.wt.) till 4 d p.i. Each group contained five mice and all the experiments were carried out in duplicates. The animals were sacrificed on third and fifth post-infection day (p.i.d.). The bladder and kidney were aseptically removed and processed. The bacterial load in the organs was enumerated as per the standard protocol. Histopathological examination of renal tissue was carried out according to standard procedure [21].
In vivo tracking of FITC tagged QSINPs

The uninfected mice were administered with FITC tagged QSINP formulation (600 mg/kg b.wt.) for 4 d via intravesical route. Mice were sacrificed on 5th and 10th days and the bladder and kidneys were dissected out. The organ sections were made and visualised under fluorescent microscope for the presence of FITC tagged QSINPs. Sections were stained with H & E and visualised under light microscope for any histopathological changes due to fluorescent QSINPs.

In vitro antivirulence efficacy of QSINPs

P. aeruginosa was grown in absence and presence of sub-MIC of QSI, QSINPs alone and QSINPs in combination with CIP. Cell-free supernatant was extracted by centrifuging these overnight grown bacterial cultures at 10,000 rpm for 10 min at 4 °C. Virulence factors, such as elastase, protease, haemolysins, alginate and rhamnolipid were estimated in the cell-free supernatants according to standardised methods, respectively [22–26].

Further, biofilm formation and eradication assays were performed with the agents (QSI; QSINPs alone and QSINPs in combination with CIP). Briefly, for biofilm formation assay, to the wells of sterile microtiter plate (Laxbro Manufacturing Co., Pune, India), sterile LB or LB with sub-MIC of QSI, QSINPs alone and QSINPs in combination with CIP was added separately. To this, bacterial culture (10^8 cfu/ml) was added and incubated at 37 °C. The wells were drained, washed and replenished with respective fresh media till 4 d. On the 4th day (peak day), wells were extracted for biofilm cells and were counted by plating on MacConkey agar. Biofilm eradication assay was performed by growing overnight biofilm in LB in microtiter wells. Next day, the medium was drained off from the wells and the wells were replenished with LB containing agents (QSI, QSINPs, QSINPs with CIP) at their MIC or above MIC. After incubating overnight, the count was estimated as mentioned above. The concentration which completely eradicated the preformed biofilm was considered as minimum biofilm eradication concentration (MBEC) of the agents [27].

In situ visualisation of biofilm

The untreated peak day (day 4) biofilm (control 1); peak day biofilm grown in presence of sub-MIC of QSINPs with CIP (test 1); 1 d old biofilm (control 2) and biofilm grown in presence of MBEC of QSINPs with CIP (test 2) on separate Foley’s catheter (Rusch, Hagenow, Germany) were visualised using SEM. Briefly, the catheter pieces on which biofilm was grown were cut into longitudinal halves. The biofilm cells were fixed by incubating it with 2.5% glutaraldehyde in 0.1 mM PBS for 1 h at 22 °C followed by washing with sterile PBS (0.1 M, pH 7.2). It was then dehydrated using 50–90% of ethanol and dried in desiccator. The catheters were then mounted (Establishment of murine acute pyelonephritis model section) for visualising under SEM.

Statistical analysis

The results of in vitro and in vivo experiments were expressed as means ± standard deviation (SD). Statistical significance was calculated using GraphPad Prism version 0.5 (GraphPad Software Inc., La Jolla, CA) software by two-way ANOVA followed by Bonferroni test.

Result and discussion

Development of QSINPs

QSINPs were prepared with 0.1–1% of QSI agent were developed and EE was calculated. It was observed that as the concentration of QSI increased, EE of QSINPs was reduced (Figure S1) indicating reduced interaction of CS with increased concentration of QSI. This might be due to reduced availability of positive charges on CS that could react with thiol groups of QSI. It supports the previous observation that drug-polymer ratio significantly affects EE of NPs [28]. The 1% QSINPs (1% QSI loaded CSDS NPs) exhibited maximum QSI activity by reducing the QSSMs produced by P. aeruginosa (data not shown). Hence, further studies were carried out with this formulation.

Characterisation of QSINPs

Particle size and microscopic analysis

The QSINPs showed a particle size of 685.7 nm. PDI and Zeta potential was found to be 0.5, 37.9, respectively, when analysed using Zetasizer. The PDI value indicates the physical stability and the zeta value indicates the electrostatic stability of the QSINP formulation. Since the QSINPs exhibited Zeta potential of value more than 30 mV and PDI value of 0.5, the formulation was considered to be stable [29]. Surface morphology of nanoparticles was analysed using SEM. QSINPs with spherical morphology were observed in SEM images (Figure 1) indicating their suitability for better cellular uptake [30].

![Figure 1. SEM images of QSINPs at 20,000 × (A) at 100,000 × (B) and at 100,000 × (C) magnifying single nanoparticle.](image-url)
FTIR analysis
On analysing, the characteristic peak wave numbers [31,32] corresponding to functional groups was present in CS and DS. It was observed that when NPs were formed, the peak wave numbers (1657 and 1601 cm\(^{-1}\)) corresponding to acetyl groups in CS and wave numbers, 2948 and 822 cm\(^{-1}\) corresponding to CH\(_2\) and C–O–SO\(_3\) in DS got shifted indicating that there is interaction between positively charged amino groups of CS and negatively charged sulphate groups of DS. The shifts in bare NPs (2948 cm\(^{-1}\) of DS shifted to 2932 cm\(^{-1}\)) and QSI loaded NPs (2121 cm\(^{-1}\) of bare NPs shifted to 2069 cm\(^{-1}\)) indicated bonding of CS with DS and CS with QSI might be through isothiocyanate linkage (N=\(_2\)C=S) (Figure 2) which is observed to be in the range of 2140–1990 cm\(^{-1}\) (isothiocyanate) and 2932 cm\(^{-1}\) (isopropyl thio-cyanate) [33].

In vitro release profile of QSINPs
Release profile of QSI from QSINPs was analysed at acidic pH (4.0) and neutral pH (7.0). Burst release of QSI from the NP formulation was observed at pH 7.0 which showed 61.6% release within 3 h and 100% release in 12 h. At pH 4.0, sustained release of QSI was observed throughout the time period indicating 16.9% release of QSI in 3 h and 100% release in 16 h. The release occurs of the drug molecules that may either loosely be adsorbed at the surface of NPs or of those drug molecules that are residing inside the NPs due to dissolution of matrix activated by release medium at neutral pH [34]. Burst release is advantageous where drug at high concentration is required at the initial stages [35]. Sustained release of drug is preferred in clinical practice since it offers availability of drug for a prolonged period of time and avoids emergence of drug resistance or toxicity due to high concentration of the drug. However, in this study, the formulation employed showed 100% release of QSI at both the tested pH conditions (Figure 3) indicating the efficacy of such preparations at both pH in targeted drug delivery.

Shelf life of QSINPs
Shelf life of QSINPs in terms of QSI activity of NP formulation kept at different temperatures (−20, 0, 4 and 28 °C) was assessed by qualitative screening using the reporter strain E. coli MG4 till 8 months. It was observed that from 2 to 8 months, the diameter of colourless zone (indicator of QSI activity) remained almost same irrespective of temperature settings. The diameter of 1, 1.2, 0.8 and 0.5 cm was observed at 0, −20, 4 and 28 °C, respectively, after 2 months (Figure S2(a)). After 8 months, the QSI index was 1, 1.1, 0.75, 0.4 cm at the respective temperatures. These results indicated that till 8 months the QSINPs retained the QSI activity at 0 to −20 °C (Figure S2(c)).

Therapeutic efficacy of intravesically targeted QSINPs
The in vivo therapeutic potential of QSINPs against P. aeruginosa associated murine pyelonephritis was evaluated. The targeting of QSINPs was performed through intravesical administration so that maximal activity through localised delivery can be expected. Moreover, when the drug is applied along with mucoadhesive agent (like CS), it might prolong the retention of drug and enhanced bioactivity of the drug can be expected. CS based 5-aminosalicylic acid NP instillation via intravesical delivery into rat bladder increased permeation of the drug and thereby enhanced its bioactivity against interstitial cystitis [36]. Also, there was enhancement in oral bioavailability, intestinal absorption and targeted action of CS-based nano-epigallocatechin gallate

Figure 2. Overlayed FTIR spectra of CS (black), DS (blue), bare NPs (pink) and QSI loaded NPs (green).

Figure 3. Release profile of QSI from QSINPs at pH 4.0 and pH 7.0.
(nano-EGCG) than solo EGCG in prostate cancer cells [37]. Since CS is biodegradable and imparts positive charge, these NPs protected EGCG from endolysosomal degradation as well.

**Bacteriological analysis**

The bacterial load in bladder and kidney of mice was estimated for all the treatment groups. Untreated, infected mice group served as control (Gp.I). When the bladder tissue was analysed, there was significant \((p < .001)\) bacterial reduction in all the groups as compared to Gp.I. Gp.II (QSI treated) showed \(-1\) log reduction in bacterial load as compared to Gp.I. However, Gp.III (QSINPs treated) showed approximately \(2.9\) and \(2\) log reduction in bacterial load on 3rd and 5th p.i.d. This might be due to prolonged retention time of QSINPs in uroepithelium and thereby its maximal exposure to the infected bladder. The dose of QSINPs used was safe for in vivo application since the kidney histopathology of mice did not show any pathological changes corresponding to inflammation. The glomeruli and tubules in renal tissue appeared normal and were similar to uninfected and untreated control mice. Moreover, the LD50 values of CS and DS in mice were found to be 16 and 20.6 g/kg, respectively - [38]. Additionally, the biodegradability and bioactivity of medium molecular weight CS (used in this study) based NPs are very high [39]. Gp.IV (CIP treated) showed around 2 log reductions on 3rd p.i.d and around 1 log reduction in bacterial load on 5th p.i.d as compared to Gp.I. Both Gp.V and VI (combination groups) reduced bacterial load of around \(3–3.5\) log counts as compared to control group on both 3rd and 5th p.i.d. However, the difference in log reduction between Gp. V and VI was not significant \((p > .05)\) (Figure 4(a)). NPs assisted combination therapy imparts significantly improved therapeutic effects. Similar to our results, the combination of redox NPs with doxorubicin showed better anti-cancerous potential in murine colon cancer model, since the side effects of doxorubicin, such as reduction in body weight and oxidative stress due to induction of ROS were controlled by NPs [40].

Similar to bladder, in renal tissue, the log reduction in bacterial load was significant \((p < .001)\) in all the groups as compared to Gp.I. Gp. II exhibited \(-2\) log reduction in bacterial load on 3rd and 5th p.i.d as compared to Gp.I. Gp.III showed around \(3.2–3.75\) log reduction and Gp.IV showed \(2.2–3.3\) log reduction in the respective days as compared to control. When the individual groups were compared with each other, Gp.III exhibited the maximal reduction and it was significant \((p < .001)\) as compared to Gp.II on both the days \((1–1.6\) log cycle). Gp.V showed \(5–5.6\) log reduction and Gp.VI showed \(5.8–6.8\) log reduction on 3rd and 5th p.i.d as compared to Gp.I indicated that the difference in log reduction between Gp.V and VI was significant \((p < .001)\) to each other (Figure 4(b)).

**Histopathological analysis**

Histopathological reports of in vivo studies were corroborated with the bacteriological results. Gp.I showed severe pyelonephritis. It was indicated by foci of neutrophil infiltration in the interstitial tissue, tubular destruction and cystic dilation in the cortex and medulla of murine kidney (severity score of 7) (Figure 5(a)). Although the pathological lesions were less as compared to Gp.I, Gp. II still showed marked interstitial inflammation, cortical destruction and vascular changes which was indicated by the presence of leukocytes (severity score of 4) (Figure 5(b)) whereas Gp. III showed moderate inflammation with distal tubule dilation (severity score of 2) (Figure 5(c)). However, Gp. IV showed dilation of few tubules with the presence of few protein casts and thereby exhibited a high severity score (severity score of 3) than Gp.III (Figure 5(d)). The combination groups, Gp. V and VI, irrespective of difference in concentration of CIP (Figure 5(e,f)) showed recovered renal tissue with normal glomeruli and tubules indicating the beneficiary therapeutic effects of agents when used in combination.

**In vivo tracking of QSINPs**

To assess the efficiency of intravesically targeted QSINPs, in vivo tracking studies were performed in bladder and renal tissue by administering FITC tagged QSINPs to uninjected mice. On 5th day post administration, fluorescence was observed throughout the bladder mucosa and bladder wall (Figure 6(a)) and in kidney, it was observed in outer, inner cortex (Figure 6(c)), medulla (Figure 6(e)), papillae and tubules (Figure 6(d)). It indicated the effective targeting of QSINPs in the urinary system of mice which could be due to the mucosalhesive nature of QSINPs that helped in its absorption into the bladder tissue and then into the kidney. These results were well corroborated with the reduced bacterial load in kidney as was observed on day 5 p.i.d. On 10th day, reduced fluorescence was observed only at the centre of the bladder but no fluorescence was observed on the bladder wall (Figure 6(b)). In kidney, very less fluorescence was observed in medulla (Figure 6(f)) and not in any other parts of the renal tissue. It indicates the clearance of QSINPs from the mouse renal system. Moreover, it was observed that free FITC gets eliminated through urine within 8h after intravesical route of administration [41].
The histopathological analysis of the FITC tagged QSINPs administered bladder (Figure S3(a,b)) and renal tissue (Figure S3(c,d)) indicated that they were intact and there was no deteriorating effect due to FITC tagged QSINPs on both the tissues.

Antivirulence efficacy of QSINPs

Antivirulence activity of agents was assessed by calculating the percent reduction in virulence factors (protease, elastase, pyochelin, cell-free and bound haemolysin, alginate and rhamnolipids) produced by P. aeruginosa PAO1. It was observed that QSINPs exhibited double fold antivirulence activity (Table 1) than solo QSI agent against P. aeruginosa. The beneficiary effects might be related to better solubility, enhanced uptake of QSI by bacterial cells and sustained presence of QSI [42]. However, an excellent antivirulence activity was observed with QSINP-CIP combination as compared to samples treated with QSINP, QSI alone and the untreated control. Around 73–97% reductions (p < .5) in virulence factors was observed in presence of QSINP-CIP combination in comparison with the control (Table 1). These observations point towards the synergistic action of both QSINP formulation and antibiotic against P. aeruginosa.

The effect of QSINPs against biofilm was evaluated further. The results indicated that QSINPs has better antibiofilm effect than solo QSI. QSINPs showed around 1.75 log reductions in biofilm count as compared to solo QSI on day 4 (peak day) (Table 1). This may be attributed to the capability of QSINPs to maintain high dose of QSI
at the proximity of biofilm formation. Moreover, CS based NPs have the capacity to depolarise cell membrane of biofilm cells which can in turn increase its permeability to NPs [43]. In addition to this, a fast burst release (as was observed in in vitro release profile at pH 7.0) could have contributed towards better antibiofilm activity of QSINPs. The positive charge of QSINPs also might help in its attachment with biofilm matrix polymers and extracellular DNA in the biofilm [43]. It was also noted that QSINPs led to inhibition of biofilm development rather than killing of bacterial cells in biofilm. It might be by inhibiting the synthesis of exopolysaccharide matrix of biofilm as was observed with combination of silver NPs and Drosera binata extract against resistant S. aureus biofilm [44].

However, the combination of QSINP and CIP showed better antibiofilm effect by showing 4.3 log reduction ($p < 0.05$) on the peak day (day 4) as compared to untreated control (Table 1). The SEM images were well corroborated with these observations since negligible isolated cells were observed on peak day in presence of QSINP and CIP combination (Figure 7(a,b)). We hypothesise that QSINPs could easily penetrate into the biofilm matrix and hence could facilitate the antiquorum sensing and antibacterial action of both QSI and CIP against biofilm as observed with the combination of silver NPs and aztreonam against P. aeruginosa [45].

The MBEC or the complete eradication (>99%) of biofilm was achieved by the combination of QSINPs (at 2MIC) and CIP (at MIC). Visualisation of preformed biofilms (24 h old) under SEM showed negligible bacterial cells indicating the complete removal of biofilm cells (Figure 7(c,d)).

### Conclusions

The study discusses the development and application of QSINPs against biofilm of P. aeruginosa. They exhibited excellent in vitro and in vivo activity by reducing the pathogenesis of P. aeruginosa. The relevance of targeted administration in improving the bioactivity of QSINPs was highlighted. It was further emphasised by visualising the presence and clearance of FITC-QSINPs from murine bladder and kidney. The significance of combination effect of QSINPs and CIP was also projected. To conclude, we hypothesise, these novel QSINPs has the potential to become the next-

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**Table 1.** Antivirulence activity of QSI, QSINPs alone and in combination with CIP.

| Sr. no. | Virulence markers | Percent reduction in virulence factors of samples treated with different agents |
|---------|-------------------|---------------------------------------------------------------------|
| a.      | Protease         | 58.75 ± 1.76 | 78.8 ± 4 | 94.25 ± 2.47 |
| b.      | Elastase         | 32.05 ± 1.81 | 56.08 ± 3.17 | 84.13 ± 4.75 |
| c.      | Pyochelin        | 69.04 ± 3.36 | 76.42 ± 9.09 | 92.61 ± 7.4 |
| d.      | Cell-free haemolysin | 31.47 ± 8.01 | 48.11 ± 8.73 | 82.30 ± 11.3 |
| e.      | Cell-bound haemolysin | 57.0 ± 4.2 | 79.12 ± 1.24 | 86.88 ± 1.57 |
| f.      | Alginate         | 41.85 ± 6.8 | 95.40 ± 0.83 | 97.38 ± 0.54 |
| g.      | Rhamnolipid      | 41.87 ± 2.65 | 81.62 ± 0.53 | 92.37 ± 5.12 |
| Biofilm count (log count) on peak day (day 4) treated with different agents | Control | QSI | QSINPs | QSINPs + CIP |
|         |                   | 9.24 ± 0.57 | 7.75 ± 0.09 | 6.005 ± 0.19 | 4.9 ± 0.16 |

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**Figure 6.** Fluorescent photomicrograph of bladder (a, b) and kidney (c–f) of mice sacrificed on 5th and on 10th day after intravesical delivery of FITC tagged QSINPs for 4 d. (a, b) Bladder tissue of mice sacrificed on 5th and 10th day at 100×, respectively. Renal tissue of mice sacrificed on 5th day at 400×, (c) inner cortex (d) tubules and (e) medulla. (f) Renal tissue (medulla) of mice sacrificed on 10th day, at 400×.
generation therapeutic and can offer translational advancements in clinical settings through targeted applications.

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**Disclosure statement**

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