Fluorinated Quaternary Chitosan Derivatives: Synthesis, Characterization, Antibacterial Activity, and Killing Kinetics

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ABSTRACT: Chitosan has become an established platform biopolymer with applications in biomedical engineering, nanomedicine, and the development of new materials with improved solubility, antimicrobial activity, and low toxicity. In this study, a series of chitosan derivatives were synthesized by conjugating various perfluorocarbon chains to chitosan via Schiff base formation or nucleophilic substitution, followed by quaternization with glycidyl trimethylammonium chloride to confer non-pH-dependent permanent positive charges. Synthesized fluorinated N-(2-hydroxypropyl)-3-trimethylammonium chitosan chloride polymers were characterized and investigated for their antibacterial efficacies against multidrug-resistant bacteria including clinical isolates. The polymers showed activity against both Gram-positive and Gram-negative bacteria (MIC = 64–512 μg/mL) but with greater potency against the former. They displayed rapid bactericidal properties, based on the MBC/MIC ratio, which were further confirmed by the time-kill kinetic assays. Given the properties presented here, fluorinated quaternary chitosan derivatives can serve as great candidates to be investigated as environmentally more benign, nontherapeutic antimicrobial agents that could serve as alternatives to the heavy reliance on antibiotics, which are currently in a very precarious state due to increasing occurrence of drug resistance.

1. INTRODUCTION

The emergence and continuous rise of multidrug resistance among pathogenic bacteria and the steady decline of new antibacterial drugs in the clinical pipeline is a serious global threat to human health.1 The crisis of antimicrobial resistance (AMR) has been ascribed to the lack of new drugs attributable to exigent regulatory requirements and misuse of the current antibiotics.2 Comprehensive and multidisciplinary efforts are required across healthcare settings as well as environment and agriculture sectors to minimize the pace of resistance by studying resistance mechanisms, emergent microorganisms, and introducing new antimicrobial agents.3–5 The adherence of pathogenic microorganisms such as bacteria, fungi, and viruses on surfaces often leads to subsequent transmission to new hosts, thus promoting the spread of these potentially harmful pathogens. In the case of AMR, which is one of the biggest global threat to human health, the sequence is particularly worrisome.6 Even though they can be found anywhere, infections caused by antibiotic-resistant microbes are more prevalent in healthcare settings such as nursing homes and hospitals, resulting in hospital-acquired infections.7 Several reports on biofilm control have demonstrated that engineered antimicrobial surfaces with covalently immobilized cationic biocides offer an alternative to antibiotics.8

Chitosan is a semi-synthetic derivative of chitin, the second most abundant naturally occurring polymer. It has long attracted the attention of researchers as a versatile polysaccharide with properties like better aqueous solubility and chemical reactivity that are more beneficial to materials development than cellulose—the most abundant natural polymer. Also, because of its low cost and low toxicity, applications have ranged from biomedical and pharmaceutical materials to environmental remediation. Chitosan is used in drug delivery systems as a mucoadhesive excipient or as a polymeric carrier.9,10 Its cationic primary amine allows it to bind well to the intestinal epithelial wall to facilitate uptake.
into the body. Another interesting medical application is in wound healing and dressings. After the discovery of the wound healing properties of glucosamine and N-acetylgluco- 
aminine (the monomeric residues of chitosan), the development of chitosan-based wound healing materials became a very active area of research. A hydrophobically modified chitosan-based foam was able to arrest severe blood loss from major tissue trauma. Chitosan has found applications in water treatment, food preservation, and infection control. The minimum inhibitory concentration (MIC) is however very wide, ranging over a few orders of magnitude. The antimicrobial activity of chitosan is influenced by several factors like molecular weight, pH, presence of interfering substances like lipids and proteins, and so forth. One approach at improving the antimicrobial properties of the polymer involves the formulation into supramolecular structures like micro- and nanoparticles. Other researchers have taken advantage of the multiple nucleophilic hydroxyl and amino groups to chemically derivatize the polymer. This has been very actively researched with quaternary, hydrophobic, sulphonated, sulphonamidated, and succinylated chitosans among the common derivatives developed. These modified chitosans have also shown a wide, varied, and cacophonic range of antimicrobial activities.

For example, while it was found that the antibacterial activity of quaternary chitosan was higher than that of pristine chitosan, the potency of the cationic derivative was better in an acetic acid solution than in ordinary water.

Chitosan-based materials have also shown superior antimicrobial activity against clinically important antibiotic-resistant pathogens, when compared to tested antibiotics used in human and veterinary medicine, without raising resistant mutants in serial passage assays over a period of up to 15 days. This is a significantly long period. Although the mechanisms of the antimicrobial activities are not clearly understood, it is widely accepted that the bacterial membrane permeability is altered by interaction between positively charged chitosan and negatively charged bacterial surface molecules, resulting in leakage of intracellular components, which leads to cell death.

In the present work, we hypothesized that the fluorination and quaternization of chitosan will improve the solubility and antimicrobial activity of the polymer. Fluorine is the most electronegative element with a strong effect on the conforma- 
tional and physicochemical properties of organic compounds. Moreover, incorporating fluorine atoms into ligands strengthens protein–ligand interactions, thus becoming a useful tool in the drug optimization campaign. Therefore, this study aims to selectively synthesize, characterize, and evaluate the anti- 
bacterial potential of the fluorinated N-(2-hydroxypropyl)-3- trimethylammonium chitosan chloride (HTCC) polymers.

2. MATERIALS AND METHODS

2.1. Materials. Chitosan (degree of deacetylation: 92%, MW: 50–190 kDa), pentfluoropropionionic anhydride, penta- 
decfluoroocatoctanoyl chloride, 2,3,4,5,6-pentafluorobenzalde- 
hyde, glycidyl trimethylammonium chloride (GTMAC), and silver nitrate (AgNO3) were purchased from Sigma-Aldrich. Glacial acetic acid (AcOH), aceton, and all other chemicals were purchased from reputable local commercial suppliers and were of analytical reagent grade unless otherwise stated. The water used in all experiments was double distilled.

Clinical isolates of methicillin-resistant Staphylococcus aureus (MRSA) were obtained from Lancet Laboratories, Durban, South Africa, with ethical approval BE394/15 from the Biomedical Research Ethical Committee of the University of KwaZulu-Natal. Other reference strains of bacteria, namely Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Streptococcus sanguinis ATCC 10565, Salmonella enterica ATCC 10708, Staphylococcus epidermidis ATCC 12228, Bacillus subtilis ATCC 6051, and S. aureus ATCC 29213 and 43300 were obtained from the American Type Culture Collection (ATCC).

2.2. Synthesis of Pentfluoropropionyl-Chitosan (C1) and Pentfluoropropionyl-HTCC (Z1). Fluorinated chitosan materials were synthesized by a modified previously reported method. Chitosan (300 mg) was dissolved in an aqueous solution of acetic acid (25 mL, 2% v/v), followed by the addition of pentfluoropropionic anhydride (437 mg), and stirred for 24 h at 25 °C. The solution was then dialyzed against deionized water with a membrane bag (MWCO = 12,000–14,000 Da) for two days with three changes per day and finally lyophilized to yield the dry fluorinated chitosan derivative C1. This product was then dissolved in aqueous acetic acid (15 mL, 0.5% v/v) and reacted with GTMAC (3 mol equiv) and refluxed at 80 °C for 18 h. The product, Z1, was precipitated with aceton, filtered under vacuum, washed again with aceton ad lib, and dried at 40 °C for 12 h in an air oven.

2.3. Synthesis of 2,3,4,5,6-Pentafluorobenzayl-chito- 
san (C2) and 2,3,4,5,6-Pentafluorobenzayl-HTCC (Z2). To a solution of 2,3,4,5,6-pentafluorobenzaldehyde (164.7 mg) and sodium borohydride (7 mg) in methanol (10 mL) was added chitosan (200 mg, 1.23 mmol) dissolved in an aqueous solution of acetic acid (25 mL, 2% v/v), and the mixture was stirred at room temperature for 24 h. The reaction mixture was dialyzed against deionized water for two days with three daily changes and lyophilized to yield the fluorinated chitosan derivative C2. The dried C2 product was then dissolved in aqueous acetic acid (10 mL, 0.5% v/v) and reacted with 0.3 equiv of GTMAC at 85 °C for 24 h. The product was precipitated with a solution of aceton: ethanol (1:1), filtered under vacuum, washed copiously with aceton, and dried at 40 °C for 12 h.

2.4. Synthesis of Pentadecfluoroocatoctanoyl-chitosan (C3) and Pentadecfluoroocatoctanoyl-HTCC (Z3). Chitosan (300 mg, 1.85 mmol) was dissolved in an aqueous solution of acetic acid (30 mL, 2% v/v) followed by the addition of pentadecfluoroocatoctanoyl chloride (600 mg, 1.4 mmol) and stirred at room temperature for 24 h. The reaction mixture was then dialyzed as before against deionized water for two days with three daily changes and lyophilized to yield the fluorinated chitosan derivative C3. The dried C3 product was then dissolved in aqueous acetic acid (10 mL, 0.5% v/v) and reacted with 0.3 equiv of GTMAC at 85 °C for 24 h. The product was precipitated with a solution of aceton: ethanol (1:1), filtered under vacuum, washed copiously with aceton, and dried at 40 °C for 12 h.

2.5. High-Resolution 19F, 1H NMR, and FTIR Studies of Fluorinated and Quaternized Chitosan Derivatives. All NMR experiments were recorded on a 400 MHz Varian INOVA spectrometer. To characterize chitosan and its fluorinated derivatives (C1 to C3), the materials were dissolved in an aqueous solution of deuterated acetic acid (2% deuterated acetic acid/D2O v/v) while the quaternized Z1
derivatives were dissolved in D$_2$O only. The fluorine substitutions were confirmed by $^{19}$F NMR (pulsed Varian 400 MHz) while the presence of quaternary ammonium groups was confirmed by $^1$H NMR. Fourier-transform infrared (FTIR) spectra of the chitosan derivatives were recorded on a PerkinElmer Spectrum 100 FT-IR spectrometer.

2.6. Scanning Electron Microscopy. A scanning electron microscope (SEM) model S 2700 (Manufactured by JOEL Tokyo, Japan) with 15 kV accelerating voltage was used to study the morphological changes that occurred from the functionalization of chitosan. The samples were dried by critical point drying (Emitech), mounted on aluminum stubs, and then coated with gold using a Sputter coater model E-1010 (Emitech).

2.7. Determination of MICs and MBCs of Chitosan Derivatives. The broth microdilution method was used to determine the MICs and minimum bactericidal concentrations (MBCs) of chitosan derivatives as previously reported.

Briefly, the test materials were serially diluted in sterile distilled water to obtain concentration ranges of 4$^{-512}$ μg/mL in each corresponding well of a 96-well microtiter plate. Cultures of bacterial cells were resuspended in cation-adjusted Mueller Hilton broth (CAMHB), adjusted to 0.5 McFarland standard, and added into the corresponding wells where the final cell density was approximately $10^8$ cfu/mL. Internal controls, including medium with standard antibiotics (ampicillin and tetracycline), medium with 10% dimethyl sulfoxide or 1% glacial acetic acid (solvent controls), medium with inoculum bacterial cells (bacterial growth control), and culture medium only (media control), were used in each assay. Plates were incubated for 18 to 20 h at 37 °C. The values of the lowest concentration of the experimental compound that fully inhibited the growth of organisms were taken as the MICs. Afterward, an aliquot of 100 μL was taken from the MIC assay wells, where no visible growth was observed and then inoculated onto Mueller Hinton agar plates for MBC determination by incubating at 37 °C for 24 h. The MBC was determined as the lowest concentration of the test compound that was able to produce a 99.9% decrease in viable bacterial count on the agar plates. Tests were performed in triplicates, and the data were presented as a mean value.

2.8. Time-Kill Kinetic Assays. The time-kill kinetics of the test compounds was performed to elucidate a possible mechanism of action of the derivatives in a similar way as described above using B. subtilis ATCC 6051 strain. Briefly, 20 μL of C1 and Z1 at concentrations 512 and 128 μg/mL was added to 180 μL of bacterial suspension in a 96-well microtiter plate. The plates were incubated at 37 °C, and at every 0.5, 1, 2, 4 and 6 h, the bacterial suspension in the corresponding wells was subjected to a ten-time serial dilution and plated on nutrient agar plates to determine the bacteria viability. In addition, the inhibition patterns of the compounds were compared with the untreated bacteria. Colonies were counted, and the results were recorded as the number of cfu/mL. A $\geq 3 - \log 10$ decrease in the number of cfu/mL was considered bactericidal. At least three replicates were performed, and the generated data was analyzed with one-way ANOVA, followed by Dunnett’s test to determine significance relative to the untreated bacteria ($p < 0.05$).

3. RESULTS AND DISCUSSION

3.1. Synthesis and Characterization of Fluorinated Chitosan Derivatives. The various perfluorocarbon chains were conjugated to the free primary amine groups of chitosan via nucleophilic substitutions or Schiff base formation followed by reduction (Scheme 1) formulating the reaction in an acidic solution preferentially favored the reaction of the primary amines over the hydroxyl groups in chitosan. The successful introduction of the perfluorocarbon groups onto chitosan by this selective substitution was confirmed by FTIR and high-resolution $^{19}$F NMR spectroscopy.

The infrared spectra (Figure 1) of the fluorinated derivatives exhibited peaks characteristic of chitosan and the substituent groups. The peak at 1552 cm$^{-1}$ for the pentafluoropropionyl-
chitosan C1 represents the C==O (amide) stretching band that was formed during the nucleophilic substitution reaction between pentafluoropropionic anhydride and chitosan. The presence of a single weak band at 3260 cm\(^{-1}\) on the pentafluorobenzyl-chitosan C2 represents the formation of secondary amines (R2NH) after the in situ reduction of imine. The additional peak at 1530 cm\(^{-1}\) (C==O stretching band) observed in the pentadecafluorooctanoyl-chitosan C3 corresponds to a specific pentadecafluorooctanolimide group.

The observation of key characteristic chitosan signals in the FTIR spectra confirmed that there were no unintended modifications to the polymeric backbone and that not all primary amines were derivatized (Figure 1). The broad and intense band at 3520–3200 cm\(^{-1}\) represents the overlapping of N–H and O–H asymmetric and symmetric stretching. The peaks at 1381, 1030, and 1429 cm\(^{-1}\) indicate the presence of CH\(_3\) symmetrical angular deformation of CH\(_3\)CO, C–O–C stretching vibration in the glucopyranose ring, and C6 primary alcohol, respectively. The specific bands at 1154 and 897 cm\(^{-1}\) are attributable to the \(\beta\)-1,4-glycosidic bridge.

The degree of substitution was calculated by a previously reported method which uses signal intensities on \(^{19}\)F NMR (Figure 2).\(^{31}\) The degree of fluorination (DF) was calculated using the equation:

\[
DF = \sum \left( 1 + \frac{(I_{C\text{F}})}{(I_{\text{ref}}/m_{\text{ref}})} \right) \times 100
\]

where \(I_{C\text{F}}\) represents the integral intensity for each fluorine peak on the spectrum and \(m\) is the number of fluorines in each peak. \(I_{\text{ref}}\) represents the integral intensity of a reference CF group. We used trichlorofluoromethane (CFCl\(_3\)) as the reference, and therefore, \(m_{\text{ref}} = 1\). The ratio between C1 and CFCl\(_3\) was 1:0.2. The DF for C1 was therefore calculated as 41%.

In an attempt to improve the aqueous solubility of fluorinated chitosan, permanent quaternary amino groups were introduced on to the polymer with GTMAC as the quaternizing agent (Scheme 2). Cationic chitosan derivatives, HTCCs, have been widely studied as potential antimicrobial agents.\(^{27}\) At the neutral pH of the deionized water used initially for the reaction, no reaction was observed over 18 h. The reaction pH was lowered slightly to solubilize the chitosan, and a successful substitution was achieved. Although protonation of the free chitosan amino groups occurs at lower pH, the reaction was able to proceed via a nucleophilic (SN\(_2\)) attack of the nonprotonated amines on the epoxide resulting in a ring-opening reaction.

FTIR and \(^1\)H NMR confirmed the selective substitution at the primary amines and the presence of trimethylammonium groups. The quaternization was confirmed by the presence of the C–H bending peak of the trimethylammonium salt group at 1480 cm\(^{-1}\) (Figure 3). Furthermore, there was a disappearance of the characteristic NH\(_2\) bending peak at 1593 cm\(^{-1}\) and a concurrent appearance of a new peak at 1585 cm\(^{-1}\), the latter corresponding to the asymmetric angular bending of methyl groups of quaternary hydrogen.

The \(^1\)H NMR spectrum also confirmed the presence of the –N\(\text{Me}_3\) group in the polymer chain with an intense peak at

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Figure 1. FTIR spectra of unmodified chitosan CS, pentafluoropropionyl-chitosan C1, 2,3,4,5,6-pentafluorobenzyl-chitosan C2, and pentadecafluorooctanoyl-chitosan C3.

Figure 2. \(^{19}\)F NMR spectrum of the fluorinated chitosan derivative (C1).
3.2 ppm integrating to nine protons (Figure 4). The methylene protons (H-a and H-c) on the side chain of HTCC resonate at 2.84 and 2.65 ppm, respectively. Furthermore, the proton in the methenyl group (H1) on the side chain of HTCC resonates at 4.52 ppm. Finally, the chemical shift at 4.52 ppm was attributed to the chiral proton in hydroxyl groups (Hb) on the side chain of HTCC.

However, due to overlapping signals between the protons of the substituent group and the polymeric backbone in 1H NMR, the degree of quaternization (DQ) could not be calculated from the 1H NMR spectra. The DQ of the polymers was therefore determined by Mohr’s method of conductometric titration of chloride ions (Cl\(^-\)) with silver nitrate (AgNO\(_3\)) solution.\(^4\)\(^2\) The titration of the chloride ions in HTCC was carried out with aqueous AgNO\(_3\) using potassium dichromate as an indicator. The DQ of HTCC was then calculated with the following equation

\[
DQ = \frac{V \times c \times 10^{-3}}{V \times c \times 10^{-3} + (W - V \times c \times 10^{-3} \times 314)/162} \times 100\%
\]

where \(c\) (mol/L) is the concentration of AgNO\(_3\) solution, \(V\) (mL) is the volume of AgNO\(_3\) solution, and \(W\) (g) is the weight of HTCC. The amount of AgNO\(_3\) solution used at the endpoint of the titration is equivalent to the amount of Cl\(^-\) present on the HTCC. The DQ was therefore determined to

**Figure 3.** FTIR spectra of fluorinated quaternary ammonium chitosan derivatives.

**Figure 4.** 1H NMR spectrum of fluorinated quaternary ammonium chitosan derivative Z1.
Energy dispersive X-ray spectroscopy (EDS) (JSM-IT300) was also done to analyze the elemental composition of the materials and to further confirm the presence of halogens in the fluorinated quaternary chitosan derivatives. Chlorine and fluorine were detected in the modified chitosans but not in the pure chitosan (Figure 5A,B).

Morphological studies with SEM showed the chitosan derivative Z1 to be irregular blocks with rough surfaces (Figure 5D) while the unmodified chitosan was smooth and nonporous (Figure 5C). The surface of Z1 was pitted with numerous porosity and voids along with clustering of the particles. Zhang et al. recently reported similar findings for modified and unmodified chitosan.43 We would attribute the morphological changes to the disruption of the interchain hydrogen bonding and electrostatic repulsions by the halogenation.
substitution of fluorinated and quaternary groups, respectively, on the C-2 primary amine.34

3.2. In Vitro Antibacterial Activities. The strong electron-withdrawing effect of the fluoro group contributes to a number of biologically important molecular properties such as antimicrobial, anticancer, and antipsychotic activities.45 Some of the most well-known fluorine-containing drugs with excellent antibacterial activity against both Gram-positive and Gram-negative bacteria are the fluoroquinolones.46 Fluorinated chitosan derivatives have been synthesized before as adaptable oxygen carriers for wound healing.47 However, to the best of our knowledge, there are no reports on antibacterial activity of oxygen carriers for wound healing.47 However, to the best of our knowledge, there are no reports on antibacterial activity of chitosan derivatives having di- and trifluorinated side-chains, although some studies have demonstrated that fluorinated chitosan derivatives were found to be active at 512 μg/mL while the fluorinated chitosan derivatives (C1 and C2) were found to be active (256 to 512 μg/mL) against the clinical MRSA strains associated with “difficult-to-treat” infections and high levels of patient morbidity.54

The fluorinated chitosans were found to be more active against the Gram-positive strains than the Gram-negative strains. For example, unmodified chitosan showed MIC values of 512 μg/mL against S. aureus ATCC 29213 and ATCC 43300, whereas the fluorinated chitosan derivative C1 showed MIC values of 128 μg/mL against the same strains. The two most active fluorinated chitosan derivatives (C1 and C2) displayed MIC values of 128 μg/mL each against B. subtilis but were inactive against the Gram-negative strains (Table 1).

Table 2. Antimicrobial activity (MIC and MBC) of Fluorinated-Quaternary Ammonium Chitosan Derivatives against Pathogenic Microorganisms

| bacterial strain | CS | Z1 | Z2 | Z3 | AMP | TET |
|------------------|----|----|----|----|-----|-----|
|                  | μg/mL | μg/mL | μg/mL | μg/mL | μg/mL | μg/mL |
| Gram-positive bacteria |
| S. aureus ATCC 29213 | 512 | 512 | N/A | 256 | 256 | 1 |
| B. subtilis ATCC 6051 | 256 | 256 | 1 | 128 | 128 | 1 |
| S. epidermidis ATCC 12228 | 256 | 256 | 1 | 256 | 256 | 1 |
| S. sanguinis ATCC 10556 | 256 | 256 | 1 | 128 | 128 | 1 |
| S. aureus ATCC 43300 | 512 | 512 | N/A | 256 | 256 | 1 |
| MRSA P10781 | 512 | 512 | 1 | 64 | 64 | 1 |
| MRSA P11520 | 512 | 512 | 1 | 256 | 256 | 1 |
| Gram-negative bacteria |
| E. coli ATCC 25922 | 512 | 512 | 1 | 512 | 512 | 1 |
| P. aeruginosa ATCC 27853 | 256 | 256 | 1 | 128 | 128 | 1 |
| S. enterica ATCC 10708 | 512 | 512 | 1 | 256 | 256 | 1 |

“CS: chitosan, Z1: pentfluoropropionyl-HTCC, Z2: 2,3,4,5,6-pentafluorobenzyl-HTCC, Z3: pentadecafluorooctanoyl-HTCC, AMP: ampicillin, TET: tetracycline. N/A: not applicable. MIC: minimum inhibitory concentration. MBC: minimum bactericidal concentration. NB: the highest concentration tested was 512 μg/mL.”
above an optimum level or threshold (as determined by Glukhov et al.), further increase in hydrophobicity leads to a loss in antimicrobial activity and increase in toxicity.

Though the antibacterial efficacy of HTCC polymers has been studied against multidrug-resistant bacteria and pathogenic fungi, no systematic structure–activity relationship (SAR) studies of the synergism of the multifunctional fluorne and quaternary ammonium chitosan derivatives have been reported. In general, the aqueous solubility and antibacterial activity of polymers showed significant improvement due to the introduction of quaternary ammonium groups to chitosan primary amines. The MIC values of the quaternized-fluorinated polymers varied from 64 to 512 μg/mL when tested against Gram-positive bacteria and 128 to 512 against Gram-negative bacteria. A variation on fluorine groups on HTCC derivatives was found to have a slight effect on the antibacterial activity. Z1 and Z3 showed MIC values of 256 μg/mL against two S. aureus strains (Table 2), whereas Z2 showed MIC values of 128 μg/mL against the same strains. However, the unmodified chitosan showed relatively weak activity against both S. aureus strains (MIC = 512 μg/mL).

The quaternized fluorinated chitosan derivatives were in general found to be more active toward Gram-positive bacteria than Gram-negative bacteria (Table 2). The best activity of 64 μg/mL was observed for Z1 and Z2 against MRSA and B. subtilis strains, respectively, and almost entirely inactive against E. coli at the maximum concentration of 512 μg/mL tested. These observations of disparities in sensitivity of the different strains could be due to the different architectures of their cell envelopes. Alternatively, it could be due to the differences in the lifecycle stage of the cells when treated with antimicrobials.

3.3. Time-Kill Studies. The time-kill kinetic results represented in Figure 6 confirm the bactericidal activity of and Z1 to act as biocides are short, and this can help explain the relatively significant bactericidal effects of these materials.

4. CONCLUSIONS

The fluorinated cationic chitosan derivatives were shown to be moderately active against various clinically isolated bacterial strains. The most active derivative showed activity against MRSA, the Gram-positive superbug on the WHO list of priority pathogens that frequently occur in hospitals and healthcare facilities. The chitosan derivatives were found to be more active toward Gram-positive bacteria than Gram-negative bacteria. The results also showed that different functional groups or combinations could either enhance or lower the aqueous solubility and antimicrobial properties of chitosan. Thus, the findings can be used for SAR studies, which can then guide the design and synthesis of more potent chitosan-based antimicrobial materials. Moreover, further work is required to understand the differential activities of the materials against bacterial species and their modes of action. These studies would inform the further improvement of the antibacterial properties of the materials. This work highlights the potential of fluorinated quaternary chitosan derivatives to be further developed into promising candidates to be employed as antimicrobial agents in topical and other infections, especially those caused by hospital-acquired antibiotic-resistant pathogens.

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Notes
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