

Chapter 10

Role of Antibacterial Agents Derived from Chitosan-Based Nanocomposites



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Abstract Chitosan is an abundant naturally sourced polymer that exhibits coveted properties of biodegradability, bioactivity, non-toxicity, and polycationic character and their significant antimicrobial, antitumor, antioxidative, anticholesterolemic, hemostatic, and analgesic ramifications. Chitosan-based nanoparticles have revolutionized antibacterial dispersions owing to their capacity to amplify the action of the parent chitosan's inherent effect. The degree of deacetylation of parent chitosan, molecular weight as well as concentration and size of nanoparticles is the various factors affecting its activity. The proof of its versatility has been its crucial applications in per-oral administration of drugs, delivery of parenteral drugs, delivery of vaccines, delivery of non-viral genes, delivery of brain targeting drugs, delivery of mucosal drugs, delivery of ocular drugs, instability improvement in controlled drug delivery of drugs, and tissue engineering. Chitosan-based system efficacy in both organic and inorganic formulations has been favored by its polycationic character which interacts with the negatively charged residues of macromolecules at the exterior. There are numerous pieces of evidence enumerating chitosan-based nanoparticles' antibacterial properties involving testing against detrimental microbes like *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Currently accelerated development of chitosan-based nanoparticles is being carried out in the medical industry for the creation of improved wound dressings, dental and orthopedic mediums, and drug delivery carriers, in defense against plant pathogens, and in the food packaging industry which has increased its relevance in the mainstream.

Keywords Chitosan · Nanocomposites · Antibacterial · Antimicrobial · Bacteria · Applications

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Abbreviations

APDI	antimicrobial photodynamic inactivation
ChNP	chitosan nanoparticles
CM	cell membrane
CS	chitosan
CS	composite films
CSF-Chi-NPs	colony stimulating factor chitosan-based nanoparticles
CS-NPs	chitosan nanoparticles
DD	degree of deacetylation
DS	degree of substitution
EOs	essential oils
GM-CSF	granulocyte-macrophage colony-stimulating factor
HMw	high-molecular-weight
LDPE	low-density polyethylene
LMw	low-molecular-weight
LPS	lipopolysaccharide
MBC	minimum bacterial concentration
MB	methylene blue
MIC	least inhibitory concentration
MWs	molecular weight
NHCS	N-hexanoyl chitosan
NPN	N-phenyl-1-naphthylamine
OM	outer membrane
OmpA	outer membrane protein A
QCh/PVP	quaternized chitosan/polyvinylpyrrolidone
RR	reactive dye
TA	teichoic acid

1 Introduction of Chitosan-Based Nanocomposites

Public health concerns about pathogenic microorganisms have surged recently. This in turn has increased the demand for safe and effective treatments that are less likely to promote resistance development. Due to this urgent need, nanotechnology has emerged to play a significant role, owing to its potential to amplify the efficacy of antibacterial treatment, molecular biology, pharmaceuticals, cell biology, and detection methods that have all seen significant advances. Chitosan-based nanosystems have piqued interest over the years because of their adaptability, biocompatibility, and biodegradability, particularly for the construction of mixed systems with better attributes. Chitosan's antibacterial action has been used in a variety of applications, spanning from agriculture to biomedicine [1].

An ideal antimicrobial material should be

- (1) synthesized quickly and cheaply,
- (2) robust in repeated applications and preservation at the appropriate application temperature,
- (3) be insoluble for the purpose of water treatment,
- (4) does not breakdown into harmful compounds or create them,
- (5) it should not be poisonous or annoying to individuals who handle it,
- (6) regeneratable when activity is lost, and
- (7) biocidal to a broad spectrum of bacteria [2, 3].

Chitosan has long been known for its extraordinary characteristics, and it has been utilized in agricultural fields, industry, and medicinal drugs. In the field of agriculture, the characterization of chitosan is an antiviral for plants, a component of liquid multicomponent fertilizers, and an agent for metal-recovery in agriculture and industry [4]. It has been used in cosmetics as an agent for film-formation, a dye binder for textile, a paper strengthening addition, as well as a hypolipidemic diet ingredient. There has been a wide use as a biomaterial owing to its immuno-stimulatory qualities, anti-coagulant capabilities, antibacterial and antifungal activity, and activity serving as a wound-healer in the case of surgery.

Chitosan is nature's second most abundant polysaccharide. It has regained appeal to the fact that bacteria have not developed resistance to it. Chitin and its derivatives are water-insoluble as well as hydrophobic natural bio-polymers that have been proven to be biocompatible and biodegradable. Chitin is formed of -(1,4)-linked 2-acetamido-2 deoxy-D-glucose, whereas chitosan is a 1,4-linked 2-amino-deoxy-D-glucan produced from partial N-deacetylation of chitin [5]. Chitin's deacetylation, a polysaccharide abundantly distributed in nature (like in crustaceans, insects, and fungi), is a simple way to get it. Because of its low solubility in aqueous mediums, chitin is less likely to be appropriate for commercial use. It is a polymer constituted up of acetylamino-D-glucose units, whereas its chemical composition is more difficult to determine. It generally refers to a collection of polymers that have their molecular weight and degree of deacetylation determined by the sugar units' number per polymer molecule (n) [6]. Despite the fact that the degree of deacetylation impacts chitosan's solubility in aqueous mediums, it is soluble in acidic solutions and very feebly soluble in weak alkaline solutions [7]. There are many techniques to investigate the antibacterial activity of the polymer and its derivatives that have resulted in a myriad of physical forms of chitosan, ranging from the original solution employed in agriculture activities to film configuration in the food industry and universal medicinal nanostructured substances. The insertion of a polyanion such as triphosphosphate (TPP) under constant stirring in a chitosan solution can easily produce chitosan nanoparticles (ChNP). When compared to the parent chitosan, these nanoparticles show more activity. Chitosan has antimicrobial properties that include bacteria, filamentous fungus, yeast, and even viruses. ChNP has been shown to exhibit antibacterial action against *E. coli*, *S. aureus*, *S. mutans*, *Salmonella typhimurium*, *Salmonella choleraesuis*, and *Pseudomonas aeruginosa* in studies [8–11].

2 Mode of Action

Even though chitosan and its derivatives' exact processes behind the antibacterial characteristics are unknown, its antibacterial action is recognized to also be regulated by several factors that operate sequentially and distinctly. According to common opinion, the existence of amine groups (NH_3^+) in glucosamine is the fundamental property of chitosan's ability to interact with surface components of many bacteria that are negatively charged, producing substantial changes to the cell surface and ultimately cell death [12]. Gram-positive bacteria's teichoic acid and gram-negative bacteria's lipopolysaccharide are both important in chitosan binding in this situation, and disruption of cell wall dynamics resulted in changes and instability in cell membrane function [13]. According to a study by Jeon [14], the antibacterial activity of the chitosan microparticles is executed through microparticle binding to bacteria. In a binding assay examination, the researchers used CM-coated glass slides with the *E. coli* O157:H7 as a bacterial study. Scientists conducted several tests to identify the specific target for chitosan microparticles binding after demonstrating that contact was required for bactericidal effects, hypothesizing that surface-exposed proteins were the primary targets. They discovered that the Omp A protein, which is present on the outer membrane, was involved in the binding process using gene deletion procedures [14].

Antibacterial mechanisms begin at the cell surface and destroy the cell wall or the outer membrane to start with, even though gram-negative and gram-positive bacteria have distinguishable cell walls. Lipoteichoic acid has the potential to form a molecular connection with chitosan on the cell surface, permitting it to damage membrane functions in gram-positive bacteria [15]. Electrostatic exchanges with divalent cations maintain the gram-negative bacteria's outer membrane together, which is required to maintain the outer membrane stable [16]. Because the pH of chitosan and its derivatives is below the pKa, polycations may contest with divalent metals for mixing with polyanions. As the pH climbs over pKa, it shifts to chelation. Mg^{2+} and Ca^{2+} ions exchange in the cell wall is likely to interrupt cell wall stability or alter degradative enzyme function. Several methods have been employed to illustrate the disruption of cell wall integrity. A hydrophobic probe, N-phenyl-1-naphthylamine (NPN), in general, is excluded from the outer membrane. N-phenyl-1-naphthylamine can divide into perturbed outer membrane. When outer membrane is functionally damaged invariably in nanoindentation tests, the slopes of the deflection curves for *E. coli* and *S. aureus* administered with chitosan oligomers were less than for the bacteria which were not treated, indicating that cell indentation or cell compaction had happened as a result of less rigid cells after the treatment [17]. The studies suggest that cell walls are weakened, either as a consequence of cell wall destruction or as a result of cell lysis. When the protective covering of the cell wall is lost, the cell membrane becomes vulnerable to the environment. As a result, the processes of the cell membrane can be modified, and the permeability of the membrane can be substantially compromised [13]. The permeability of the cell membrane, which in itself is a negative charge phospholipid bilayer, can vary

significantly when chitosan comes into touch with it. The bacterial surface charge is soon neutralized and even reversed by the binding [18]. Additional interactions might denature protein molecules and allow phospholipid bilayer invasion. Increased membrane permeability causes cell membrane instability and intracellular substance leakage, resulting in cell death. This significant alteration in the nature of membrane proteins is believed to play a role in antibacterial activities as of yet.

3 Factors Affecting chitosan's Antibacterial Activity

Chitosan's antibacterial action is determined by a variety of intrinsic and extrinsic variables. The degree of deacetylation and molecular weight of parent chitosan, along with the concentration and size of the nanoparticles, is all intrinsic variables. pH, temperature, and reaction time are examples of external variables (Fig. 1).

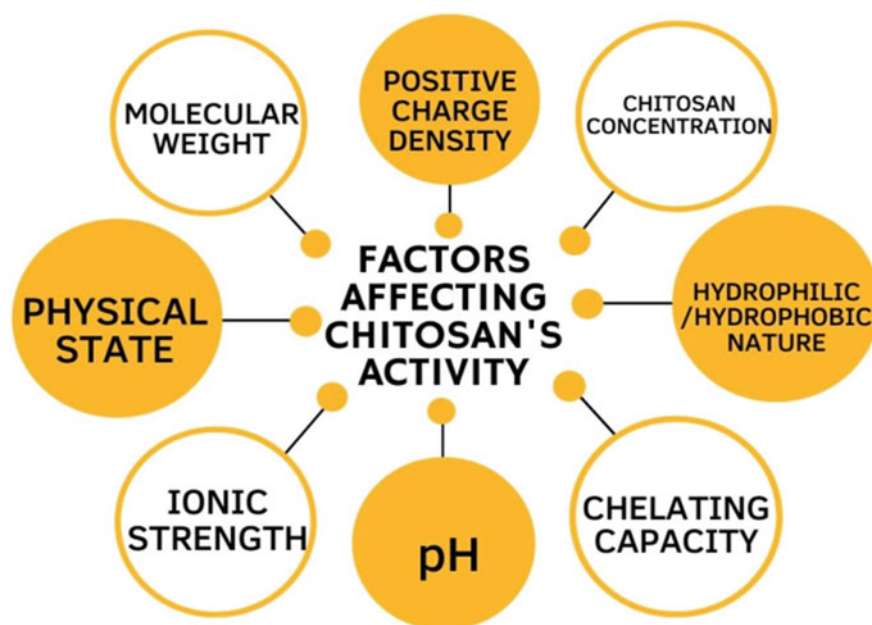


Fig. 1 List of the various factors that affect the activity of chitosan-based nanoparticles

3.1 Concentration of Chitosan

At lower concentrations, chitosan binds to negatively charged cell surfaces and disrupts the cell membrane, enhancing cell death by facilitating intracellular component leakage, whereas, at higher concentrations, protonated chitosan covers the cell surface and blocks intracellular component outflow. Agglutination is also deterred because positively charged bacterial cells resist one other. [19]. By uniformly ingraining 1, 3, and 5% chitosan (w/w) in low-density polyethylene (LDPE) matrix, an antimicrobial packaging material was created [20]. The antibacterial effectiveness of LDPE/chitosan composite (LDPE/CS) films on virgin LDPE films was shown in an *E. coli* assay. Virgin LDPE and 1%, 3%, and 5% LDPE/CS films were tested as chill-stored tilapia packaging films, and samples packaged in LDPE films were rejected by the seventh day, whereas fish sealed in 1%, 3%, and 5% LDPE/CS coatings were fine for up to 15 days. 3% LDPE/CS films had more improved physical and antibacterial qualities and enhanced the maintained quality of tilapia steaks while it was in chilled storage, as compared to other films [21].

3.2 Molecular Weight

When it pertains to the association between bactericidal activity and chitosan's molecular weight, several studies on chitosan's bactericidal effect have generated inconsistent results. In some investigations, increasing chitosan Mw resulted in lower chitosan activity against *E. coli* while in others, high-Mw (HMw) chitosan was found to be more active than low-Mw (LMw) chitosan. Furthermore, regardless of Mw, activity against *E. coli* and *Bacillus subtilis* were shown to be identical [22]. Despite the fact that the limited availability of the results on various bactericidal activity in the case of LMw chitosan was equivalent depending on the bacterial strains used, for the biological testing circumstances, and related chitosan Mw, the results were not consistent. For example, 9.3 kDa chitosan inhibits *E. coli* growth, while 2.2 kDa chitosan promotes it. In addition, LMw chitosan (4.6 kDa) and its derivatives had superior antibacterial, yeast, and fungicidal activities [13].

3.3 Positive Charge Density

The part of the polycationic structure in antibacterial activity has been sufficiently documented. Powerful electrostatic contact is driven by a bigger positive charge density. The positive charge is correlated to the degree of substitution (DS) of chitosan or its derivatives, which influences positive charge density. To some degree, chitosan microspheres with a high DD (97.5%) have a larger positive charge density that causes stronger antibacterial action on *Staphylococcus aureus* at a pH of 5.5 than

those with a moderate degree of deacetylation (83.7%) [23]. According to one study, a greater degree of deacetylation with a more positive charge was notably effective in preventing *S. aureus* growth, implying that chitosan's antibacterial efficacy against *S. aureus* was boosted with greater DD [15]. The antimicrobial action of chitosan derivatives is mainly defined through the grafting groups' DS. The antibacterial activity of water-soluble N-alkylated disaccharide chitosan derivatives against *Escherichia coli* and *Staphylococcus aureus* was examined, and it was found that the DS of disaccharides and the kind of disaccharide present in the molecule affect the antibacterial activity of chitosan derivatives [24]. In regard to the exact study, a DS of 30–40% induced the most powerful antibacterial activity against *E. coli* and *S. aureus*, regardless of the type of disaccharide linked to the chitosan molecule, among the myriad chitosan derivatives, the two bacterias are especially vulnerable to cellobiose chitosan derivative DS 30–40% and maltose chitosan derivative DS 30–40%, respectively [13].

3.4 *Hydrophilic/Hydrophobic Characteristic*

Water is required for antimicrobial agents to act, regardless of their type or amount. For needed to commence contact, completely dry samples are almost impossible to liberate the energy contained within chemical bonds. Hydrophilicity and hydrophobicity are terms that describe how chitosan interacts with bacteria and are also affected by the aqueous environment. Chitosan's hydrophilic properties have a significant impact on its water solubility. Because of its low-water solubility, chitosan's application is restricted [25]. Chemical changes are effective in increasing the solubility of chitosan and its variants in water and expanding its applications [26]. Saccharization, acylation, quaternization, and metallization have all been used to make water-soluble chitosan and its derivatives, which have always been a primary focus of antibacterial studies research. Quaternary ammonium chitosan, for example, could be made by attaching a quaternary ammonium group to a dissociative hydroxyl or amino group. The antibacterial capabilities of chitosan and its derivatives are influenced by the hydrophilic-lipophilic variance. The hydrophobicity of NHCS 0.5 (N-hexanoyl chitosans, with a molar ratio of 0.5 when compared to chitosan residue) is believed to be responsible for its increased inhibitory impact [27, 28]. The inclusion of a long aliphatic chain promoted the absorption and boosted the effects of a substituted LMW chitosan, N/2(3)-(dodec-2enyl) succinoyl/chitosans, onto the cell walls using hydrophobic contact with cell wall proteins in another study [13].

3.5 *Chelating Capacity*

In acidic circumstances, chitosan has a sufficient chelating ability for numerous metal ions (containing Ni^{+2} , Co^{+2} , Zn^{2+} , Mg^{2+} , Fe^{2+} , and Cu^{2+}), and it is been widely

used to remove or recover metal ions in a variety of industries [29]. The metal ions that react with the cell wall constituents of the microorganism are important for the stability of the cell wall. The chelation of these metal ions by chitosan has been proposed as a potential method of antibacterial action [30]. Chelation is competent for blending divalent metal ions in neutral conditions and also in acid conditions. Further, the chitosan metal complex is developed by chelating capacity and has substantial antibacterial action [13].

3.6 pH

Chitosan's antibacterial action is pH-dependent. Since chitosan is readily soluble in acidic conditions, thus it becomes polycationic when the pH goes under the molecule's pKa (6.3–6.5), it may be used in a variety of applications [31]. It is been stated that chitosan possesses antibacterial properties exclusively in an acidic environment, although it has not been proven. At lower pHs, chitosan has a stronger inhibitory impact, and as the pH rises, the inhibitory effect weakens. The presence of a large number of positively uncharged amino groups, and also chitosan's poor solubility, might explain why chitosan is unable to kill bacteria at pH 7 [32]. However, according to some workers, chitosan and its derivatives fully lose their antibacterial activity under neutral conditions, which may not be entirely accurate. Under neutral conditions, a novel technique for antibacterial study using chitosan microspheres (CM) in a solid dispersion system revealed that the CM sample with DD of 62.6% exerted the only inhibitory effect among the three DD (97.5, 83.5, 62.6%) [11]. The characteristics of native chitosan were preserved in the CM samples used in this study. Another study found that the antibacterial activity of N-alkylated chitosan derivatives (DS 30–40%) against *E. coli* increased as the pH climbed from 5.0 to 7.0–7.5, with a peak around 7.0–7.5 [33]. These findings also show that a positive charge on amino groups is not the only thing that causes antibacterial activity. However, nothing is known about chitosan's antibacterial action in alkaline environments [13].

3.7 Ionic Strength

Any changes in the ionic strength of a medium can affect the inhibitory effect of chitosan, which is presumably due to two different mechanisms. Firstly, a gain in metal ions, mainly divalent ions, can lower chitosan's chelating capability's efficacy. The inhibitory ratio of chitosan samples reduced dramatically when 0.05 mol/L magnesium ions were added to the medium, resulting in abrogated antibacterial action [23]. In another study, divalent cation doses of 10 and 25 mM reduced shrimp chitosan's antibacterial effectiveness against *E. coli* in comparison with Ba. Furthermore, when compared to Ba²⁺, Mg²⁺, Ca²⁺, and metal ions decreased, the antibacterial action of chitosan was the most successful when the Zn²⁺ and Ca²⁺ ions were

added [34]. Secondly, along with polycationic chitosan, cations already present in the medium may compete with the negative components that predominate on the bacterium's cell wall, reducing antibacterial efficacy. The antibacterial effectiveness was also modified by the addition of anion [13].

3.8 Physical State

Chitosan's antibacterial activity is the outcome of many reactions rather than the driver of them. Interaction of chitosan molecules with the cell wall helps in producing processes. The morphology of molecules determines the effectiveness of processes. Similarly, chitosan's physical state, which dictates molecular form, has a massive effect on its antibacterial action [13].

3.8.1 Soluble State Antimicrobial Activity

The dissociating form of soluble chitosan in solution as a dissociating form allows for sufficient reactivity with the counterparts and brings all of the possibilities to life. This explains how soluble chitosan and its variations are often more efficient in inhibiting bacterial growth than insoluble chitosan and its variants [34]. The least inhibitory concentration (MIC) of chitosan derivatives is much lower than that of native chitosan against all tested bacteria. Meanwhile, soluble chitosan and its derivatives are readily affected by both external and intrinsic influences due to their substantial contact with the solution. In one study, Maillard reaction-produced chitosan derivatives (chitosan, fructose, glucose, glucosamine, and maltose) improved the solubility of native chitosan [34]. Incorporating hydrophilic groups into the molecule, such as in quaternary ammonium chitosan, is yet another important way to improve chitosan solubility. After quaternization, the derivative exhibits more solubility in water and better antibacterial activity than chitosan [13].

3.8.2 Solid State Antimicrobial Activity

Solid chitosan, unlike soluble chitosan, only comes into contact with a solution through the surface, such as fibers, hydrogels, membranes, microspheres as well as nanoparticles. The hydrogels are created by covalently cross-linking chitosan with itself. Several initiatives have lately been made to produce chitosan particle systems capable of forming large reactive surface areas as a dispersion in solution. As its physical condition alters, its antibacterial potency will surely vary. Nanoparticles have a weaker inhibitory effect on *S. aureus* ATCC 29737 than polymers in free soluble form because they have less positive charge available to attach to the negative bacterial cell wall [35]. Another study discovered that chitosan nanoparticles have more antibacterial activity than chitosan due to the nanoparticles' unique properties,

such as their larger surface area and increased affinity with bacterial cells, resulting in a quantum-size effect [13].

3.9 Temperature and Time

Composing chitosan mixtures in mass and reserving them for future use in commercial applications would have been viable. With preservation, chitosan parameters like viscosity and MW may alter. Due to this, a chitosan solution's viscosity should be regulated since it might further change the solution's other operational properties. Chitosan solution's stability (MW of 2025 and 1110 kDa) and its antibacterial effectiveness on gram-positive (*Listeria monocytogenes* and *S. aureus*) and gram-negative (*E. coli* and *Salmonella enteritidis*) bacteria were analyzed after 15 weeks of storage at the temperatures 4 °C and 25 °C [36]. The antibacterial action of chitosan solutions was greater before storing than after 15 weeks. Chitosan solutions held at 25 °C had an antibacterial effect equivalent to or less than those kept at 4 °C. The sensitivity of *E. coli* to chitosan expanded as the temperature was increased from 4 to 37 °C in one investigation, implying that low-temperature stress might change the cell surface configuration in a manner that diminished the number of surface binding sites (or electronegativity) for chitosan derivatives [13].

3.9.1 Microbial Factors

Microbial Species

Despite its broad antibacterial activity, chitosan has varying inhibitory efficacy against fungi, gram-positive, and gram-negative bacteria. They display antifungal properties by inhibiting the process of spore germination as well as sporulation [37]. By contrast, the antibacterial activity mode is a complicated process that differs between gram-positive and gram-negative bacteria caused by changes in cell surface features. In many investigations, pH values of gram-negative bacteria were found to have stronger antibacterial activity than gram-positive bacteria [38, 39], whereas gram-positive bacteria were found to be more vulnerable in another, probably due to the gram-negative outer membrane barrier. Regardless, multiple studies identified no significant differences in antibacterial activity and microbes. Varied initial reaction materials and settings influence the various outcomes. Based on present data, bacteria appear to be less responsive to chitosan's antibacterial activity than fungus. Chitosan has a higher antifungal effect at low-pH values [13, 40].

- Part of Microorganism

Gram [SG1]-negative bacteria have a lipopolysaccharide (LPS)-containing outer membrane (OM). The bacteria have a hydrophilic outer surface as a result of this, electrostatic interactions between divalent cations alter the overall stability of the LPS

layer due to anionic groups (phosphate, carboxyl) in the lipid components and inner core of LPS molecules. When these cations are removed by chelating substances like ethylenediaminetetraacetic acid, the OM is destabilized, resulting in the release of LPS molecules.

Gram-negative bacteria are often resistant to hydrophobic medicines as well as hazardous pharmaceuticals since OM acts as a penetrating barrier against macromolecules and hydrophobic chemicals. As a result, any material that wishes to exhibit bactericidal activity against gram-negative bacteria must first overcome the OM [23] teichoic acid (TA) and peptidoglycan (PG) that make up gram-positive bacteria's cell wall. TA is a polyanionic polymer that traverses the cell wall of gram-positive bacteria to make touch with the PG layer. They can be covalently attached to the N-acetylmuramic acid in the peptidoglycan layer. Teichoic acids and glycolipid remain anchored into the cytoplasmic membrane's outer leaflet forming lipoteichoic acids (LTA).

Because the OM works as a penetrating barrier against macromolecules and hydrophobic substances, gram-negative bacteria are generally immune to hydrophobic medicines and hazardous medications. The poly (glycerol phosphate) anion groups in TA are responsible for the cell wall's structural integrity. Furthermore, it is essential to the functioning of a variety of membrane-bound enzymes. In gram-negative bacteria, TA's counterpart LPS has a comparable effect on the cell wall [15].

Cell Age

The age of a cell can affect antibacterial effectiveness for a certain microbial species. *S. aureus* CCRC 12657, for example, is particularly vulnerable to lactose chitosan derivative mostly in the late exponential phase, with really no viability observed following 10 h of incubation. Cells mostly in mid-exponential and late-stationary stages, on either hand, had less population decline in cell viability. Modifications in cell surface electrical negativity are thought to alter with the growth phase, which may also contribute to variances in cell sensitivity to chitosan. In comparison, mid-exponential *E. coli* O157:H7 cells have been the most vulnerable, while stationary phase cells have been the least sensitive. Because the surface charge of microbial cells depends greatly upon the microbe, the inconsistencies were attributed to the microbial species studied [41].

4 Complexes of Chitosan with Certain Materials

To improve the antibacterial effect, chitosan materials containing certain components might be produced. The use of essential oils (EOs) in chitosan-based coatings has attracted agricultural researchers' attention owing to these volatile molecules' bactericidal and fungicidal properties [42]. EOs like carvacrol, clove, lemongrass, and oregano have recently been properly integrated into the chitosan, demonstrating

high-antibacterial activity. Also, researchers [43] showed that chitosan and cinnamon EOs had unique compatibility, with their integration improving the antibacterial characteristics of chitosan. Cinnamon essential oils are effective for covering highly perishable foods like fish and chicken.

The structure of the chitosan/metal complexes is usually determined by the molar ratio of the chitosan to metal ions, the kind of the molecular weight, metal ion and preparation circumstances, as well as the deacetylation of chitosan [21].

4.1 Antimicrobial Activity of Chitosan/Metal Nanocomposites

Chitosan and metals were combined to create novel nanocomposite materials having modified microbicidal characteristics [44]. Silver, gold, and copper-loaded chitosan nanoparticles, in particular, are shown to have a wide range of activity against gram-positive and gram-negative bacteria. Metal ion solutions were added to chitosan nanosuspension to create these nanoparticles, and a soluble metal salt was reduced in the existence of chitosan solutions [45]. The most commonly studied metal/chitosan complexes are silver-based nanocomposites, researchers integrated chitosan films with Ag nanoparticles or Ag⁺ ions at varying concentrations and examined those for antibacterial activity against *S. aureus* and *E. coli* to see whether the occurrence of silver as metallic nanoparticles or ions had been responsible for the increased antibacterial effect of the silver-based chitosan composite materials. They demonstrated that chitosan films with 1% w/w silver nanoparticles or 2% w/w silver ions had the highest antibacterial effect, indicating that Ag/chitosan nanoparticles seem to be more effective than Zn ions or Ag⁺² [46]. Different types of chitosan-based nanocomposites have been mentioned in Table 1.

4.2 Chitosan Nanoparticles' Antimicrobial Activity in Bacterial Biofilms

In biofilms, microbial populations are encased in a sticky extrinsic polymer matrix. Antimicrobial agents are substantially more resilient to microorganisms in biofilms. Antibiofilm activity of natural biological substances is now being investigated to find alternative preventive or treatment strategies. In this regard, anti-biofilm characteristics of chitosan-streptomycin conjugates/gold nanoparticles then were assessed against gram-negative *S. typhimurium* and *P. aeruginosa* as well as gram-positive *Listeria monocytogenes* and *S. aureus* [47]. Gram-negative and gram-positive bacteria biofilms were disrupted by the chitosan-streptomycin gold nanoparticles, which also inhibited the production of gram-negative bacteria biofilms. Streptomycin was conjugated to chitosan and gold nanoparticles, which made it easier

Table 1 List of all the different forms of chitosan nanocomposites with their various applications in different fields

Name of nanomaterial	Form of chitosan nanocomposites created	Distinct application	Crucial properties	References
Ag nanoparticles	Thin film coating on bandage	Antibacterial activity against <i>E. coli</i> and <i>S. aureus</i>	Inactivation bacterial metabolism	Susilowati and Ashadi [118]
Graphene oxide	Thin film	Antimicrobial against <i>E. coli</i> and <i>R. subtilis</i>	Improved mechanical and antimicrobial properties	Grande et al. [119]
Bentonite and sepiolite	Thin film	Application in winemaking	Increased immobilization of protease although negatively affected catalyzation	Benucci et al. [120]
Sulfamethoxazole	Nanoparticle drug	Antibacterial activity against <i>P. aeruginosa</i>	Synergistic activity with sulfamethoxazole	Tin et al. [121]
Clarithromycin	Nanoparticle drug	Works against <i>S. aureus</i>	Shows antibacterial activity	Golmohamadi et al. [122]
Ciprofloxacin Chlortetracycline Hydrochloride Gentamycin sulfate	Nanoparticle drug	Works against <i>E. coli</i> and <i>S. aureus</i>	Stops the growth of gram-positive and gram-negative bacteria	Sharma et al. [123]
Azithromycin Levofloxacin Tetracycline	Nanoparticle drug	Effective against <i>E. coli</i> <i>S. aureus</i>	Shows great antibacterial effects	Brasil et al. [124]
Rifampicin Ciprofloxacin Vancomycin Doxycycline Gentamicin	Nanoparticle drug	<i>S. epidermidis</i>	Stops bacterial biofilm and demonstrates synergism with antibiotics	Ong et al. [125]

for it to penetrate the biofilm matrix and promote bacterial interaction. Chitosan nanoparticles can also be used for photodynamic activation. The effectiveness of chitosan nanoparticles in methylene blue (MB)-mediated antibiotic photodynamic inactivation (APDI) of *P. aeruginosa* and *S. aureus* biofilms was investigated [48]. Chitosan nanoparticles increased the efficacy of MB-APDI by disturbing biofilm formation and enabling MB to probe deeper and much more efficiently into biofilms of *P. aeruginosa* and *S. aureus* [48]. Biofilm forms on the enamel surface as a result of oral infections, which have been related to human caries, gingivitis, and periodontitis. To combat the biofilm, chitosan nanoparticles were utilized.

4.3 Chitosan Nanoparticles Loaded with Antibiotics or Other Microbicidal Substances Possessing Antimicrobial Activity

Chitosan has been used to transport both synthetic and biological chemicals with the goal of increasing or modulating their antimicrobial properties. Antibiotics were internalized inside cells infected with intracellular bacteria or their efficiency versus multiresistant bacteria was boosted using chitosan nanoparticles. In particular, researchers discovered that ceftriaxone sodium was absorbed better by Caco-2 and J774.2 (macrophages) cells when it was encapsulated in chitosan nanoparticles, as well as that these nanoparticles had a stronger intracellular antibacterial action against *S. typhimurium* than with the drug in the solvent [49]. Tetracycline-loaded O-carboxymethyl chitosan nanoparticles were used in a similar [50] study, and the drug-loaded nanoparticles were reported to boost antibiotic effectiveness versus *S. aureus*. Jamil et al. investigated the efficiency of ceftazolin-loaded chitosan nanoparticles against multiresistant gram-negative bacteria such *K. pneumoniae*, *E. coli*, and *P. aeruginosa*. As per the agar well-shared understanding and microdilution broth test, the drug-loaded chitosan nanoparticles exhibited antibacterial activity against the three pathogens which were stronger than ceftazolin in solution. Antimicrobial peptides and proteins have recently been incorporated into chitosan nanoparticles. Among these compounds, lysozyme has attracted much interest since it is used as a preservative in food and medicines [51], while the amphiphilic peptide temporin B has been demonstrated to be beneficial against various bacterial species [51, 52].

5 Applications of Chitosan-Based Nanosystems' Antimicrobial Activity

5.1 Antimicrobial Agent

In the past several years, chitosan-based nano systems have drawn attention for their compatibility, degradability, and diversity, particularly for the formation of mixed systems with better attributes. Chitosan-based systems' antibacterial activity has been used in a variety of applications, spanning from agriculture to biomedicine [53]. The following parts describe the advancements of chitosan-based nanoparticles in the areas of wound healing, textiles, and food packaging. Nanoparticle-based material's antibacterial properties are also useful in a variety of medical applications like burn and wound dressings, filters, medical devices, and materials used in dental plaque reduction [41] (Fig. 2).

When scientists were researching antimicrobials in nature to replace synthetic chemicals, they came across chitosan and chitosan nanoparticles. The antimicrobial

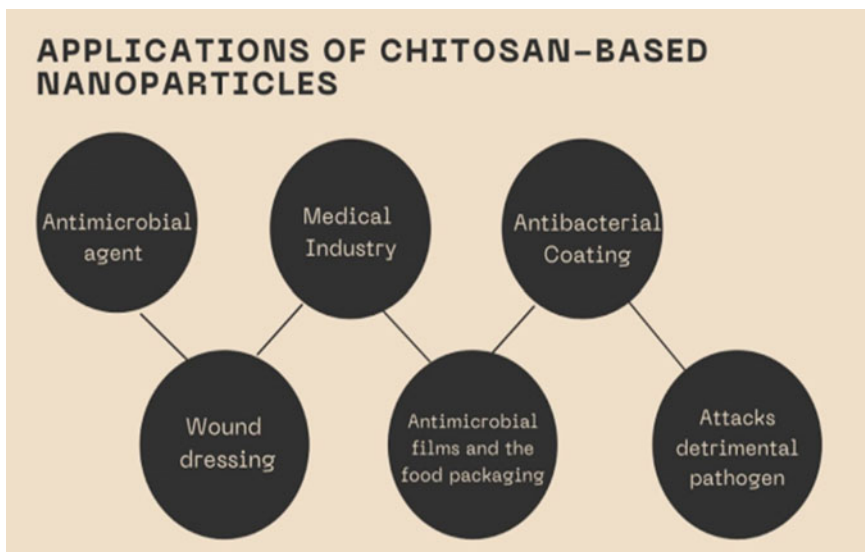


Fig. 2 List of the multifaceted applications of chitosan-based nanoparticles

activity of chitosan is the result of the interaction of negatively charged phospholipid of the plasma membrane with the positively charged chitosan, altering cellular permeability and ultimately resulting in cell death. Chitosan's ability to chelate metal ions, well explains its antibacterial characteristics. Chitosan has the ability to pass through the cell wall and bind with DNA and inhibit transcription. Chitosan nanoparticles can also be formed by decomposing chitosan with hydrogen peroxide. To make antimicrobial paper, after combination with pulp, it was impregnated, dispersed as a coating on hand sheets, and insufflated. *Staphylococcus aureus* and *E. Coli* were discovered to be the most resistant bacterial species to the paper generated by insufflations [54]. Qi et al. (2004) investigated chitosan NP and ChNP loaded with copper against *E. coli*, *S. aureus*, *S. choleraesuis*, and *S. typhimurium*, in vitro [8]. According to the findings, the activity of all the bacteria was suppressed far. Their MBC values exceeded 1 lg/ml, and their MIC values were less than 0.25 lg/ml. *Aspergillus niger* and *Candida albicans* were resistant to low-molecular weight ChNP, and *Fusarium solani* was resistant to high-molecular weight ChNP [55].

5.2 Wound Dressing

Studies that were done in vivo revealed that wounds closed completely when coated with the granulocyte-macrophage colony-stimulating factor and sargramostim chitosan-based nanoparticles composites and complete re-epithelialization after

13 days and only 70% reduction in wound size was observed when the wound was treated with normal saline.

Further, as compared to Chi-NPs composite treated wounds, the GM37 CSF-Chi-NPs treated wounds had enhanced re-epithelialization, lower inflammation, and fast growth of formation of granular tissue. So, GM-CSF was observed to have an enhanced effect on the wound healing process [56].

The activity of the nanoparticles in the wound area depends on the size of Chi-NP. Nanoparticles less than 100 nm can be eliminated from the wound region by migrating to the systemic circulation. As a result, Chi-NPs having particle sizes of more than 100 nm can treat the wound more effectively.

The promotion of healing of wounds in a moist environment at the wound site can be achieved by the application of CSF-Chi-NP. Further, as compared to normal saline and Chi-NPs, the GM 37-CSF-Chi-NPs composite demonstrated fastened wound healing, reduction in inflammation, improved granular tissue synthesis, and reformation of epithelial tissue [56].

5.3 Antibacterial Activity Against Plant Pathogens

Chitosan-based nanoparticles may have a long-term and consistent effect on plant development and protection. CS has been used to lower the severity of phytopathogen diseases and to increase plant innate immunity [57, 58]. CS's antibacterial and immune-stimulating qualities make it an effective antimicrobial product for the control of plant diseases. Nanotechnology is a promising field for developing materials to combat pathogens affecting plants. Cu-CS-NPs are believed to be a promising composite for an increase in plant growth and protection. They are a very effective antibacterial agent because of their outstanding ability to endure pathogenic attacks on plant growth. The inhibition of phytopathogenic bacteria by CS and CS-based NPs has been studied [59, 60]. 1129 kDa, DD 85 peCS-NPs may have a long-term and consistent effect on plant growth and development. The bacteria *Xanthomonas*, which causes bacterial leaf spots on *Euphorbia pulcherrima*, was significantly inhibited when treated with chitosan at a dose of 0.10 (mg/mL) [61]. By treating and soaking of seeds of tomato plants at 10.0 (mg/mL), bacterial wilt caused by *Ralstonia solanacearum* was decreased to 48% and 72%, respectively [62]. Similarly, leaf diseases in rice produced by different species of *X. oryzae* pv. were suppressed by treatment of leaves (0.20 mg/mL) with two distinct CS solutions, i.e., solution-A of MW 1129 kDa, DD 85%, and solution-B of 607 kDa, DD 75% [63]. In another study, it was found that spraying solution-A on leaves of *Acidovorax citrulli* which causes fruit blotch of watermelon reduced the disease severity significantly [64].

The synthesis, evaluation, and testing of the antibacterial efficacy of CS/TiO₂ NPs were done in rice pathogen *X. oryzae* pv. *Oryzae* by Li et al. [60]. Significant inhibition of bacteria *Xanthomonas* species over *E. pulcherrima* was observed [61]. Further, the severity of bacterial speck disease by *P. syringae* pv. in tomato was greatly reduced when tomato seedlings were treated with CS which confirmed the

role of CS as a non-toxic biopesticide [58]. CS-NPs have high-antibacterial action against *P. fluorescens* and *Erwinia carotovora*, two bacteria that cause soft rot [65]. CS-NPs effectiveness was observed by Esyanti et al. in chili peppers for lowering the disease severity of *X. campestris* and so can be considered as a bactericidal agent [66]. Antibacterial activity of three strains of pathogenic bacteria like *E. carotovora* subsp. *carotovora* and one strain of *X. campestris* pv. *vesicatoria* was studied by Oh et al. [67]. The Ag-NP-CS had remarkable antibacterial activity when tested for *R. solanacearum* which causes tomato wilt [68]. It was also observed in another study that NPs derived from CS including Ag-NPs extracted from leaves were a long-term and good alternative in agriculture. These findings substantiate the use of CS-NPs for better crop yield and for the control of phytopathogens without causing any harm to soil properties or the environment [69]. The potential of ChNP to act as a coating material for prolonging the shelf life of tomato, chili, and brinjal was also studied. The addition of ChNP to soil boosts microbial populations while also improving nutrient availability. ChNP has been approved as a growth promoter, speeding up seed germination, plant growth, and agricultural yield. ChNP is an antimicrobial compound that works against a variety of bacteria, viruses, fungi, and bacteria. As a result, it has the potential to be a viable alternative to many chemical fungicides and bactericides in the control of many plant diseases. ChNP is a great fruit and vegetable coating material due to its non-toxicity and biodegradability. Many fruits, including strawberries, papaya, carrot, cucumber, citrus, apple, kiwifruit, pear, peach, sweet cherry, and strawberry, may benefit from ChNP coating to extend storage life and reduce decay. Functional elements such as antibacterial agents and nutraceuticals could also be included in ChNP coating.

5.4 Antimicrobial Films and the Food Packaging

Chitosan has remarkable antibacterial efficacy due to its polycationic characteristic. As a result, films based on chitosan have a usage in the food packaging industry, as they can protect food from microorganisms and ensure food safety. Although chitosan revealed a broad spectrum of antibacterial action, many factors could influence its antimicrobial activity. Organic acid, molecular weight, concentration, temperature, film diameters, microbial type, etc., influenced the antibacterial activity of films made up of chitosan. In certain cases, the inclusion of other materials, such as Ag-NP and propolis extract, was required to inhibit the tested microorganisms [70, 71]. The antibacterial effectiveness of the films formed on chitosan was proved to be different for gram-negative and gram-positive bacteria and observed to be more effective in gram-negative bacteria. Furthermore, some studies claim that an increase in the molecular weight of chitosan enhanced the antibacterial effect of chitosan against gram-positive bacteria by forming a film that hindered the entry of nutrients into the bacterial cell [72]. However, the antibacterial action of low-molecular weight chitosan was increased against gram-negative bacteria because low-MW chitosan

could easily enter the microbial cell and resulted in the breakdown of metabolism [73].

There is evidence that the different gram-positive bacteria showed different antibacterial responses [74]. Due to differences in the outer membrane structure, *Enterococcus faecalis* and *Listeria monocytogenes* were more susceptible than *Staphylococcus aureus* to chitosan-based films [72]. Overall, the produced films demonstrated outstanding antibacterial activity against poisoning microorganisms such as *Lactococcus lactis* [74] and *Listeria innocua* [75], gram-negative bacteria *Pseudomonas spp.* [76–78], *Salmonella spp.* [79–81], fungal mold [82, 83], and *Candida albicans* [84]. There are some distinctions between fungi and bacteria in terms of antibiotic action. At low temperatures, chitosan inhibits bacteria more effectively than fungus, as per some research. Mold spores were more resistant to cold temperatures than bacteria. Continuation of germination of mold spores even at low temperatures was the reason for less fungistasis. Despite the differences, chitosan films were successful in protecting food from mold.

Therefore, different antimicrobial theories on food state that

1. Chitosan-based films create cellophane-like structures on the food surface, therefore acting as a protective barrier and shielding the food from microbial attacks [85].
2. Chitosan-based coatings can function as an oxygen barrier. Thus, preventing oxygen transport and so inhibit respiratory activity and bacterial development in food [82].
3. Chitosan can diffuse into a microbial surface and barrier made from polymer and create an obstruction. And neither does the membrane chelate nutrients on bacteria's surfaces, but it also prevents nutrients and critical components from entering the microbial cell. This action of chitosan alters the microbes' physiological activity and thus kill them [86].
4. Chitosan's NH_3^+ groups [87] can disrupt the negative phosphoryl groups on the bacterial cell membrane, causing distortion and then deformation [88, 89].
5. Furthermore, various bacteria were sensitive to the NH_3^+ groups of chitosan in diverse ways. Chitosan can permeate through the cell wall, distort the bacterial membrane, and result in intercellular electrolyte leakage and the death of cells [90, 91].
6. Chitosan can enter the nucleoid, alter the structure of DNA, and prevent DNA replication, RNA transcription, and protein translation [92].
7. Chitosan can easily cause chelation of nutrients and critical metals inside bacteria. The action of these materials can be lost, and the chelation complex will not be available to microorganisms so limiting microbial development. Chitosan can cause the formation of chitinase enzymes in fruits, which destroys the microbial cell walls indirectly. By encouraging the buildup of phenolics and lignin, chitosan-treated wheat seeds were able to promote resistance to *Fusarium graminearum* and increase seed quality [93].

5.5 Application in Medical Industry

Biocidal polymers are disinfectants that may be integrated into fibers, membranes, or hydrogels and employed in wound dressings, orthopedic therapies, hemodialysis, and medication carriers. An authentic dressing material for a wound should be able to soak released liquid from the affected area, allow for controlled water evaporation, and prevent microbiological transmission [94]. The candidate's eligibility and capability are determined by the antimicrobial property assessment, which is a significant measure in wound dressing. The use of polysaccharides with hydrogel-forming properties, such as chitosan, as wound dressing materials, has been deemed beneficial [95]. The products made from chitosan have attracted a lot of attention in this respect.

Chitosan is available in four different forms that provide antibacterial qualities to wound dressing material, i.e., fiber, membrane, hydrogel, and sponge. These methods rely on the chitosan's physicochemical properties. Wound dressings can be made using micro- and nanofiber materials. Electrospinning is one of them, and it is a good way to make continuous polymer threads with nanoscale diameters [96]. Electrospun mats constructed of ultrafine polymer fibers have sparked a lot of attention due to their unusual qualities like nanosize, more porosity, and high-surface-to-volume ratio. In another study, the growth of gram-negative and gram-positive bacteria were found to be inhibited by cross-linked electrospun-like PVC/QCh (polyvinylpyrrolidone/quaternised chitosan) [97]. PVP and PVA both are indeed non-toxic, hydrophilic, and biocompatible, with good complex and film-forming capabilities, making them suitable for wound dressing [14].

5.6 Antibacterial Coating

As discussed earlier, the positive amino group of chitosan can bind with the negatively charged microbial cell membrane. Proteinaceous along with other intracellular substances of the microorganisms pour out as a result. One explanation for chitosan's antibacterial effects could be this [98]. The migration of chitosan on the bacteria's outer surface is another reason. Polycationic chitosan at the concentration of 0.2 mg/ml can bind with negatively charged bacterial surfaces and agglutination can occur. Bacterial surfaces may become positive as a result of the increased number of positive charges, allowing them to remain in suspension at higher concentrations [99, 100]. Chitosan causes *Pythium oarocandrum* to lose a large quantity of protein content when grown at pH 5.8, as per UV absorption measurements [101]. Chitosan's chelating property helps it to bind with metals and inhibits the formation of toxic chemicals and thus stops the multiplication of microbes. It can serve as a water binder and also block certain enzymes by inducing host tissue defense mechanisms. As a result of its penetration into the microorganism's nuclei interference occurs with transcription and translation [20]. The antibacterial activity of chitosan may be influenced by its own structure, degree of its polymerization, nature of the host, the

natural nutrient constituents, the chemical makeup of the substrates, and the variables of the environment. Antimicrobial compounds have been used to cover surfaces in order to prevent the development of pathogenic bacteria.

Chitosan is still used as an antimicrobial film due to its biocompatibility, biodegradability, antibacterial effect, and cytotoxicity [99]. Acidic solutions as compared to neutral and alkaline environments result in the grabbing of dye at higher levels [102]. In acidic solutions, protonation of amino groups of chitosan molecules to produce $-\text{NH}_3^+$ groups can occur easily as a large number of protons are available [103, 104].

According to the researchers, the diameter of pores in chitosan beads or their number was decreased by chemical crosslinking. As a result, the transfer of the dye molecule over chitosan was more challenging [100, 102]. Chitosan was used to transform an acrylic resin into an antibacterial covering material. Chitosan was injected into the polymer matrix in two states: solid (powders) and colloid (liquid). The purpose was to establish a homogeneous particle dispersion and to improve particle influence by increasing the number of contact sites.

5.7 Application Against Various Detrimental Bacteria (Animal Pathogens)

The antimicrobial activity of CS-NPs against many pathogenic microorganisms is broad. Ikono et al. 2009 [105] investigated the effects of CS-NP on *Streptococcus mutans*, a dental caries-associated bacterium. The viability of cells reduced considerably with increasing nanoparticle concentrations after CS-NP treatment. Cu-CS-NPs were also tested for antibacterial activities against *S. mutans* [106]. Cu-CS-NPs outperformed Cu-NPs in terms of MIC and MBC [107, 108]. Cu-CS-NPs were found to be effective at disrupting *S. mutans* adhesion and biofilm formation [109, 110]. Cu-CS-NPs had a bactericidal effect and were more effective at inhibiting the development of *S. mutans* over the surface and disrupting the biofilm of human teeth [105]. Cu-CS-NPs' bactericidal capabilities should make it a good material for further research into tooth plaque therapies. Hipalawins et al. reported the antibacterial action of synthesized CS-NPs against clinically harmful bacterial strains such as *E. coli* MTTC 1687, *Enterobacter aerogenes* MTCC 111, *K. pneumoniae* MTTC 109, *P. fluorescens* MTCC 1748, *Proteus mirabilis* MTCC 1429, and *S. aureus* MTCC 7443 [111]. The most sensitive bacteria were *E. Coli*, *E. aerogenes*, *P. fluorescens*, *K. pneumoniae*, and *P. mirabilis*. The least harmful to the CS-NPs was *S. aureus*. G-bacteria were more effectively suppressed with CS-NP than G+ bacteria in antibacterial experiments. Antibacterial activity against antibiotic-resistant G+ *S. pneumonia* was tested with the use of CS-NPs and a CS-NP–amoxicillin complex by Nguyen et al. [112].

In comparison with CS-NPs and amoxicillin alone, the CS-NP–amoxicillin injection had higher antibacterial activity. CS–NP Amoxicillin complex was observed to

be three times more effective than amoxicillin and prevented the growth of *S. pneumoniae*. Antibacterial action of CS-NP mixed with lime oil was observed against four foodborne pathogens [113], i.e., *S. aureus*, *E. Coli*, *L. monocytogenes*, and *Shigella dysenteriae*. The nanocarriers to treat *S. aureus* were found to be *S. dysenteriae* [114].

According to Tamara et al. [115], CS hybridized with protamine had more antibacterial activity against *E. coli* than *B. cereus*. CS-NPs loaded with curcumin could also be employed to deliver drugs and as a method to specifically activate antibacterial mechanisms. In mice, curcumin-loaded CS-NPs hindered the development of *S. aureus* and *P. aeruginosa* infections [116].

Four clinically isolated *S. epidermidis* were tested with AMP temporin B (TB)-CS-NPs. CS-NPs showed bactericidal activity within 24 h of incubation and significantly reduced the initial inoculum. Following that, Temporin B was spread across the surface of bacteria, significantly reducing cell viability [117]. It was discovered that CS-NPs inhibited the development of mesophilic bacteria when compared to a conventional coating [63]. The CS-based NPs (110 nm) coating was shown to be significantly more effective in inhibiting microbial growth than conventional coatings (300 nm). There were no pathogenic *Salmonella* sp. or fecal coliforms detected as a consequence. This analysis shows the use of CS-NPs as edible coatings to limit bacterial growth in fruits and vegetables. As a result, the smaller the NPs are the greater their mobility and surface contact, resulting in increased antibacterial efficacy against animal diseases.

6 Conclusions and Future Outlook

Due to its variety of potential uses and specific features, chitosan has received much interest and attention in recent years. The number of studies on its antibacterial properties is rapidly increasing. Non-toxic and biodegradable compounds from 'natural sources' will become increasingly desirable as a replacement for synthetic compounds due to their wide range of applications and people's environmental consciousness. Chitosan is different from other antibacterial compounds as it is harmless, non-toxic, and environment-friendly. People will be healthier if we use this stuff at various periods in our lives. As a consequence, due to its antibacterial capabilities, it is required to enhance research on this molecule, which will necessitate a mix of disciplines such as Nanotechnology, Physics, Chemistry, Bioinformatics, and Genetics. This will be advantageous for the production of novel biomedicine and the exploration of new antibacterial drugs. Future research could focus on using chitosan in the form of composites to deter lower pH values, as chitosan in solution is employed in an acidic environment. Clarification of the molecular mechanisms and their performance in chitosan's antibacterial action will be advantageous. To date, most research has been conducted in vitro; thus, in situ investigations are essential to developing solutions and alternatives to the issues which both the agricultural and medical areas confront. It is still not known how chitosan-based nanoparticles act against bacteria; thus, it necessitates continued research in the current scenario. Furthermore, it is

critical to continue to observe and assess the toxicity associated with the use of chitosan-based nanoparticles when acting against bacteria, as well as to dispense guidance on the regulations and procedures that govern their use and application. To fill the information gap in chitosan-based nanoparticles' antibacterial activity, the following sorts of investigations must be conducted: (1) Determining why chitosan-based nanoparticles are more efficient against gram-negative bacteria as compared to gram-positive bacteria and what is the underlying mechanism; (2) determining why chitosan with medium molecular weight is more effective against bacteria than chitosan with high-molecular weight; (3) designing the technique to reduce the toxicity associated with the synthesis chitosan-based nanoparticles attached to metals and hybrid chitosan-based nanoparticles. These findings will help in the creation of a new class of medicines with efficient and novel antibacterial properties that will be applicable to both animal and plant research.

References

1. Perinelli RD, Fagioli L, Campana R, Lam WKJ, Baffone W, Palmieri FG, Casettari L, Bonacucina G (2018) Chitosan-based nanosystems and their exploited antimicrobial activity. *Eur J Pharm Sci* 117:8–20. <https://doi.org/10.1016/j.ejps.2018.01.046>, <https://www.sciencedirect.com/science/article/pii/S0928098718300617>
2. Mahira S, Jain A, Khan W, Domb AJ (2019) Antimicrobial materials—an overview. In: *Antimicrobial materials for biomedical applications*. RSC, pp 1–37. <https://doi.org/10.1039/9781788012638-00001>
3. Kenawy ER, Worley SD, Broughton R (2007) The chemistry and applications of antimicrobial polymers: a state-of-the-art review. *Biomacromol* 8:1359–1384
4. Hadrami AE, Adam LR, Daayf F (2010) Chitosan in plant protection. *Mar Drugs* 8(4):968–987. <https://doi.org/10.3390/md8040968>
5. Badawy MEI, Rabea EI (2011) A biopolymer chitosan and its derivatives as promising antimicrobial agents against plant pathogens and their applications in crop protection. *Int J Carbohydr Chem Article ID 460381*, 29 pages. <https://doi.org/10.1155/2011/460381>
6. Komi EA, Daniel Hamblin MR (2016) Chitin and chitosan: production and application of versatile biomedical nanomaterials. *Int J Adv Res* 4(3):411–427. PMID: PMC5094803
7. Rinaudo M (2006) Chitin and chitosan: properties and applications. *Prog Polym Sci* 31:603–632. <https://doi.org/10.1016/j.progpolymsci.2006.06.001>
8. Qi L, Xu Z, Jiang X, Hu C, Zou X (2004) Preparation and antibacterial activity of chitosan nanoparticles. *Carbohydr Res* 339(16):2693–700. <https://doi.org/10.1016/j.carres.2004.09.007>
9. Thakur R, Kaur P, Chaudhury A (2013) Synthesis of Chitosan-silver nanocomposites and their antibacterial activity. *Int J Sci Eng Res* 4:869–872
10. Huang L, Cheng X, Liu C, Xing K, Zhang J, Sun G, Li X, Chen X (2009) Preparation, characterization, and antibacterial activity of oleic acid-grafted chitosan oligosaccharide nanoparticles. *Front Biol China* 4:321–327. <https://doi.org/10.1007/s11515-009-0027-4>
11. Chávez de Paz LE, Resin A, Howard KA, Sutherland DS, Wejse PL (2011) Antimicrobial effect of chitosan nanoparticles on *streptococcus mutans* biofilms. *Appl Environ Microbiol* 3892–3895. <https://doi.org/10.1128/AEM.02941-10>
12. Nagy A, Harrison A, Sabbani S, Munson RS Jr, Dutta PK, Waldman WJ (2011) Silver nanoparticles embedded in zeolite membranes: release of silver ions and mechanism of antibacterial action. *Int J Nanomed* 6:1833–1852. <https://doi.org/10.2147/IJN.S24019>

13. Kong M, Chen XG, Xing K, Park HJ (2010) Antimicrobial properties of chitosan and mode of action: a state of the art review. *Int J Food Microbiol* 144(1):51–63. <https://doi.org/10.1016/j.ijfoodmicro.2010.09.012> Epub 2010 Oct 15 PMID: 20951455
14. Jeon SJ, Oh M, Yeo W-S, Galvão KN, Jeong KC (2014) Underlying mechanism of antimicrobial activity of chitosan microparticles and implications for the treatment of infectious diseases. *PLoS ONE* 9(3):e92723. <https://doi.org/10.1371/journal.pone.0092723>
15. Raafat D, Bargaen KV, Haas A, Sahl HG (2008) Insights into the mode of action of chitosan as an antibacterial compound. *Appl Environ Microbiol* 74(12):3764–3773. <https://doi.org/10.1128/AEM.00453-08>
16. Sarwar A, Katas H, Samsudin SN, Zin NM (2015) Regioselective sequential modification of Chitosan via Azide-Alkyne click reaction: synthesis, characterization, and antimicrobial activity of chitosan derivatives and nanoparticles. *PLoS*. <https://doi.org/10.1371/journal.pone.0123084>
17. Chen HL, Yen CC, Lu CY, Yu CH, Chen CM (2006) Synthetic porcine lactoferricin with a 20-residue peptide exhibits antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. *J Agric Food Chem* 3;54(9):3277–3282. <https://doi.org/10.1021/jf053031s>
18. Chen CZ, Cooper SL (2002) Interactions between dendrimer biocides and bacterial membranes. *Biomaterials* 23(16):3359–3368. [https://doi.org/10.1016/s0142-9612\(02\)00036-4](https://doi.org/10.1016/s0142-9612(02)00036-4)
19. Lim HS, Hudson MS (2004) Synthesis and antimicrobial activity of water-soluble chitosan derivative with a fiber-reactive group. *Carbohydr Res* 339:313–319. <https://doi.org/10.1016/j.carres.2003.10.024>
20. Reesha VK, Panda KS, Bindu J, Varghese OT (2015) Development and characterization of an LDPE/chitosan composite antimicrobial film for chilled fish storage. *Int J Biol Macromol* 79. <https://doi.org/10.1016/j.ijbiomac.2015.06.016>
21. Hosseinnejad M, Jafari SM (2016) Evaluation of different factors affecting antimicrobial properties of chitosan. *Int J Biol Macromol* 85:467–475. <https://doi.org/10.1016/j.ijbiomac.2016.01.022>
22. Tikhonov EV, Stepnova AE, Babak GV, Yamskov AI, Guerrero PJ, Jansson BH, Lopez-Llorca VL, Salinas J, Gerasimenko VD, Avdienko DI, Varlamov PV (2006) Bactericidal and antifungal activities of a low molecular weight chitosan and its N-/2(3)-(dodecyl-2-enyl)succinoyl-derivatives. *Carbohydr Polym* 64:66–72. <https://doi.org/10.1016/j.carbpol.2005.10.021>
23. Kong M, Xg C, Xue YP (2008) Preparation and antibacterial activity of chitosan microspheres in a solid dispersing system. *Front Mater Sci China* 2:214–220. <https://doi.org/10.1007/s11706-008-0036-2>
24. Wu Y, Yang W, Wang C, Hu J, Fu S (2005) Chitosan nanoparticles as a novel delivery system for ammonium glycyrrhizinate. *Int J Pharm* 295(1–2):235–245. <https://doi.org/10.1016/j.ijpharm.2005.01.042>
25. Dutta J, Dutta PK, Tripathi VS (2004) Chitin and chitosan: chemistry, properties and applications. *JSIR* 63:20–31
26. Xie Y, Liu X, Chen Q (2007) Synthesis and characterization of water-soluble chitosan derivative and its antibacterial activity. *Carbohydr Polym* 69:0144–8617. <https://doi.org/10.1016/j.carbpol.2006.09.010>
27. Hu Y (2007) Synthesis, characterization and antibacterial activity of guanidinylated chitosan. *Carbohydr Polym* 67(1):66–72
28. Hu Y, Du Y, Yang J, Tang Y, Li J, Wang X (2007) Self-aggregation and antibacterial activity of N-acylated chitosan. *Polymer* 48(11):3098–3106
29. Kurita K (1998) Chemistry and application of chitin and chitosan. *Polym Degrad Stab* 59:117–120
30. Rabea EI, Badawy MET, Stevens CV, Smagghe G, Steurbaut W (2003) Chitosan as antimicrobial agent: applications and mode of action. *Biomacromol* 4:1457–1465

31. Lim SH, Hudson SM (2004) Synthesis and antimicrobial activity of a water-soluble chitosan derivative with a fiber-reactive group. *Carbohydr Res* 339:313–319
32. Sudarshan NR, Hoover DG, Knorr D (1992) Antibacterial action of chitosan. *Food Biotechnol* 6:257–272
33. Yang TC, Chou CC, Li CF (2005) Antibacterial activity of N-alkylated disaccharide chitosan derivatives. *Int J Food Microbiol* 97:237–245
34. Chung YC, Kuo CL, Chen CC (2005) Preparation and important functional properties of water soluble chitosan produced through Maillard reaction. *Bioresour Technol* 96:1473–1482
35. Sadeghi AMM, Dorkoosh FA, Avadi MR, Saadat P, Rafiee-Tehrani M, Junginger HE (2008) Preparation, characterization and antibacterial activities of chitosan, N-trimethyl chitosan (TMC) and N-diethylmethyl chitosan (DEMC) nanoparticles loaded with insulin using both the ionotropic gelation and polyelectrolyte complexation methods. *Int J Pharm* 355:299–306
36. No HK, Kim SH, Lee SH, Park NY, Prinyawiwatkul W (2006) Stability and antibacterial activity of chitosan solutions affected by storage temperature and time. *Carbohydr Polym* 65:174–178
37. Hernandez-Lauzardo AN, Bautista-Banos S, Velazquez-del Valle MG, Montealvo MG, SanchezRivera MM, Bello-Perez LA (2008) Antifungal effects of chitosan with different molecular weights on in vitro development of *Rhizopus stolonifer* Vuill. *Carbohydr Polym* 73:541–547
38. Chung YC, Su CC, Chen Y, Jia G, Wang JCG, Wu H, Lin JG (2004) Relationship between antibacterial activity of chitosan and surface characteristics of cell wall. *Acta Pharmacol Sin* 25:932–936
39. No HK, Park NY, Lee SH, Meyers SP (2002) Antibacterial activity of chitosans and chitosan oligomers with different molecular weights. *Int J Food Microbiol* 74(1–2):65–72. [https://doi.org/10.1016/s0168-1605\(01\)00717-6](https://doi.org/10.1016/s0168-1605(01)00717-6)
40. Roller S, Covill N (1999) The antifungal properties of chitosan in laboratory media and apple juice. *Int J Food Microbiol* 47(1–2):67–77. [https://doi.org/10.1016/s0168-1605\(99\)00006-9](https://doi.org/10.1016/s0168-1605(99)00006-9)
41. Atay HY (2020) Antibacterial activity of Chitosan-based systems. In: *Functional Chitosan: drug delivery and biomedical applications*, pp 457–489. https://doi.org/10.1007/978-981-15-0263-7_15
42. Ali A, Noh NM, Mustafa MA (2015) Antimicrobial activity of chitosan enriched with lemon-grass oil against anthracnose of bell pepper. *Food Packag Shelf Life* 3:56–61. <https://doi.org/10.1016/j.foodchem.2010.02.033>
43. Ojagh MS, Rezaei M, Razavi HS, Hashem Hosseini MS (2010) Development and evaluation of a novel biodegradable film made from chitosan and cinnamon essential oil with low affinity toward water. *Food Chem* 122:161–166. <https://doi.org/10.1016/j.foodchem.2010.02.033>
44. Esmaeili A, Ghobadianpour S (2016) Vancomycin loaded superparamagnetic MnFe₂O₄ nanoparticles coated with PEGylated chitosan to enhance antibacterial activity. *Int J Pharm* 501:326–330. <https://doi.org/10.1016/j.ijpharm.2016.02.013>
45. Rhim JW, Hong SI, Park HM, Ng PKW (2006) Preparation and characterization of chitosan-based nanocomposite films with antimicrobial activity. *J Agric Food Chem* 54:5814–5822. <https://doi.org/10.1021/jf060658h>
46. Kumar-Krishnan S, Prokhorov E, Hernández-Iturriaga M, Mota-Morales JD, Vázquez-Lepe M, Kovalenko Y, Sanchez IC, Luna-Bárcenas G (2015) Chitosan/silver nanocomposites: synergistic antibacterial action of silver nanoparticles and silver ions. *Eur Polym J* 67:242–251. <https://doi.org/10.1016/j.eurpolymj.2015.03.066>
47. Mu H, Liu Q, Niu H, Sun Y, Duan J (2016) Gold nanoparticles make chitosan streptomycin conjugates effective towards gram-negative bacterial biofilm. *RSC Adv* 6:8714–8721. <https://doi.org/10.1039/C5RA22803D>
48. Darabpour E, Kashef N, Mashayekhan S (2016) Chitosan nanoparticles enhance the efficiency of methylene blue-mediated antimicrobial photodynamic inactivation of bacterial biofilms: an 15 Antibacterial Activity of Chitosan-Based Systems in vitro study. *Photodiagnosis Photodyn Ther* 14:211–217. <https://doi.org/10.1016/j.pdpdt.2016.04.009>

49. Zaki NM, Hafez MM (2012) Enhanced antibacterial effect of ceftriaxone sodium loaded chitosan nanoparticles against intracellular *Salmonella typhimurium*. AAPS PharmSciTech 13:411–421. <https://doi.org/10.1208/s12249-012-9758-7>
50. Maya S, Indulekha S, Sukhithasri V, Smitha KT, Nair SV, Jayakumar R, Biswas R (2012) Efficacy of tetracycline encapsulated O-carboxymethyl chitosan nanoparticles against intracellular infections of *Staphylococcus aureus*. Int J Biol Macromol 51:392–399. <https://doi.org/10.1016/j.ijbiomac.2012.06.009>
51. Wu T, Wu C, Fu S, Wang L, Yuan C, Chen S, Hu Y (2017) Integration of lysozyme into chitosan nanoparticles for improving antibacterial activity. Carbohydr Polym 155:192–200. <https://doi.org/10.1016/j.carbpol.2016.08.076>
52. Mangoni ME, Nargeot J (2008) Genesis and regulation of the heart automaticity. Physiol Rev 88(3):919–982. <https://doi.org/10.1152/physrev.00018.2007>
53. Perinelli DR, Fagioli L, Campana R, Lam JKW, Baffone W, Palmieri GF, Casettari L, Bonacucina G (2018) Chitosan-based nanosystems and their exploited antimicrobial activity. Eur J Pharm Sci 117:8–20
54. Gómez-Sequeda N, Ruiz J, Ortiz C, Urquiza M, Torres R (2020) Potent and specific antibacterial activity against *Escherichia coli* O157:H7 and methicillin resistant *Staphylococcus aureus* (MRSA) of G17 and G19 peptides encapsulated into Poly-Lactic-Co-Glycolic Acid (PLGA) nanoparticles. Antibiotics 9(7):384. <https://doi.org/10.3390/antibiotics9070384>
55. Ing LY, Zin NM, Sarwar A, Katas H (2012) Antifungal activity of chitosan nanoparticles and correlation with their physical properties. Int J Biomater 632–698. <https://doi.org/10.1155/2012/632698>
56. Dehkordi NK, Minaiyan M, Talebi A, Akbari V, Taheri A (2019) Nanocrystalline cellulose-hyaluronic acid composite enriched with GM-CSF loaded chitosan nanoparticles for enhanced wound healing. Biomed Mater 14(3). <https://doi.org/10.1088/1748-605X/ab026c>
57. Li B, Liu B, Shan CL, Ibrahim M, Lou Y, Wang Y, Xie G, Li HY, Sun G (2012) Antibacterial activity of two chitosan solutions and their effect on rice bacterial leaf blight and leaf streak. Pest Manag Sci 69:312–320
58. Mansilla AY, Albertengo L, Rodriguez MS, Debbaudt A, Zúñiga A, Casalongué CA (2013) Evidence on antimicrobial properties and mode of action of a chitosan obtained from crustacean exoskeletons on *Pseudomonas syringae* pv. tomato. DC3000. Appl Microbiol Biotechnol 97:6957–6966
59. Rabea EI, Steurbaut W (2010) Chemically modified chitosans as antimicrobial agents against some plant pathogenic bacteria and fungi. Plant Protect Sci 46:149–158
60. Li B, Zhang Y, Yang Y, Qiu W, Wang X, Liu B, Wang Y, Sun G (2016) Synthesis, characterization, and antibacterial activity of chitosan/TiO₂ nanocomposite against *Xanthomonas oryzae* pv. *oryzae*. Carbohydr Polym 52:825–831. <https://doi.org/10.1016/j.carbpol.2016.07.070>. Epub . PMID: 27516334
61. Wang Y, Li L, Li B, Wu G, Tang Q, Ibrahim M, Li H, Xie G, Sun G (2012) Action of chitosan against *Xanthomonas* pathogenic bacteria isolated from *Euphorbia pulcherrima*. Molecules 17:7028–7041
62. Algam SAE, Xie G, Li B, Yu S, Su T, Larsen J (2010) Effects of paenibacillus strains and chitosan on plant growth promotion and control of *Ralstonia* wilt in tomato. JPP 92(3):593–600. <http://www.jstor.org/stable/41998847>
63. Pilon L, Spricigo PC, Miranda M, Moura MRD, Assis OBG, Mattoso LHC, Ferreira MD (2014) Chitosan nanoparticle coatings reduce microbial growth on fresh-cut apples while not affecting quality attributes. Int J Food Sci Technol 50:440–448
64. Li B, Shi Y, Shan C, Zhou Q, Ibrahim M, Wang Y, Wu G, Li H, Xie G, Sun G (2013) Effect of chitosan solution on the inhibition of *Acidovorax citrulli* causing bacterial fruit blotch of watermelon. J Sci Food Agric 93:1010–1015
65. Mohammadi A, Hashemi M, Hosseini S (2016) Effect of chitosan molecular weight as micro and nanoparticles on antibacterial activity against some soft rot pathogenic bacteria. LWT 71. <https://doi.org/10.1016/j.lwt.2016.04.010>

66. Esyanti RR, Farah N, Bajra BD, Nofitasari D, Martien R, Sunardi S, Safitri R (2020) Comparative study of nano-chitosan and synthetic bactericide application on chili pepper (*Capsicum annuum* L.) infected by *Xanthomonas campestris*. *Agrivita* 42:13–23. <https://doi.org/10.17503/agrivita.v42i1.1283>
67. Jw OH, Chun SC, Chandrasekaran M (2019) Preparation and in vitro characterization of chitosan nanoparticles and their broad-spectrum antifungal action compared to antibacterial activities against phytopathogens of tomato. *Agronomy* 9:21
68. Santiago TR, Bonatto CC, Rossato M, Lopes CAP, Lopes CA, Mizubuti G, ES, Silva LP, (2019) Green synthesis of silver nanoparticles using tomato leaf extract and their entrapment in chitosan nanoparticles to control bacterial wilt. *J Sci Food Agric* 99:4248–4259
69. Chandrasekaran M, Kim KD, Chun SC (2020) Antibacterial activity of chitosan nanoparticles: a review. *Processes* 8:1173. <https://doi.org/10.3390/pr8091173>
70. Siripatrawan U, Vitchayakitti W (2016) Improving functional properties of chitosan films as active food packaging by incorporating with propolis. *Food Hydrocoll* 61:695–702
71. Nair SB, Alummoottil NJ, Moothandasserry SS (2017) Chitosan-konjac glucomannan-cassava starch-nanosilver composite films with moisture resistant and antimicrobial properties for food-packaging applications. *Starch Stärke* 69:1600210. <https://doi.org/10.1002/STAR.201600210>
72. Wang H, Qian J, Ding F (2018) Emerging Chitosan-based films for food packaging applications. *J Agric Food Chem* 66(2):395–413. <https://doi.org/10.1021/acs.jafc.7b04528>
73. Cruz-Romero MC, Murphy T, Morris M, Cummins E, Kerry JP (2013) Antimicrobial activity of chitosan, organic acids and nano-sized solubilisates for potential use in smart antimicrobially-active packaging for potential food applications. *Food Control* 34:393–397
74. Fernández-Saiz P, Sánchez G, Soler C, Lagaron GM, Ocio MJ (2013) Chitosan films for the microbiological preservation of refrigerated sole and hake filets. *Food Control* 34:61–68
75. Iturriaga L, Olabarieta I, Castellan A, Gardrat C, Coma V (2014) Active naringin-chitosan films: impact of UV irradiation. *Carbohydr Polym* 110:374–381. <https://doi.org/10.1016/j.carbpol.2014.03.062>
76. Genskowsky E, Puente DL, Pérez Alvarez JA, Fernández LJ, Muñoz LA, Viuda Martos M (2016) Assessment of antibacterial and antioxidant properties of chitosan edible films incorporated with maqui berry (*Aristotelia chilensis*). *LWT-Food Sci Technol* 64:1057–1062. <https://doi.org/10.1016/j.lwt.2015.07.026>
77. Song Z, Li F, Guan H, Xu Y, Fu Q, Li D (2017) Combination of nisin and ϵ -polylysine with chitosan coating inhibits the white blush of fresh-cut carrots. *Food Control* 74:34–44. <https://doi.org/10.1016/j.foodcont.2016.11.026>
78. Caro N, Quiñonez ME, Dosque DM, Lopez L, Abugoch EL, Tapia C (2015) Novel active packaging based on films of chitosan and chitosan/quinoa protein printed with chitosan-tripolyphosphate-thymol nanoparticles via thermal ink-jet printing. *Food Hydrocoll* 52. <https://doi.org/10.1016/j.foodhyd.2015.07.028>
79. Siripatrawan U, Vitchayakitti W (2016) Improving functional properties of chitosan films as active food packaging by incorporating with propolis. *Food Hydrocoll* 61:695–702. <https://doi.org/10.1016/j.foodhyd.2016.06.001>
80. Severino R, Ferrari G, Vu DK, Donsì F, Salmieri S, Lacroix M (2015) Antimicrobial effects of modified chitosan-based coating containing nanoemulsion of essential oils, modified atmosphere packaging and gamma irradiation against *Escherichia coli* O157:H7 and *Salmonella Typhimurium* on green beans. *Food Control* 50: 215–222. <https://doi.org/10.1016/j.foodcont.2014.08.029>
81. Al-Naamani L, Dobretsov S, Dutta J (2016) Chitosan-zinc oxide nanoparticle composite coating for active food packaging applications. *Innov Food Sci Emerg Technol* 38:231–237. <https://doi.org/10.1016/j.ifset.2016.10.010>
82. Dotto GL, Vieira MLG, Pinto LAA (2015) Use of chitosan solutions for the microbiological shelf life extension of papaya fruits during storage at room temperature. *Lebensm Wiss Technol* 64:126–130. <https://doi.org/10.1016/j.lwt.2015.05.042>

83. Vimaladevi S, Panda SK, Xavier KA, Bindu J (2015) Packaging performance of organic acid incorporated chitosan films on dried anchovy (*Stolephorus indicus*). Carbohydr Polym 127:189–194. <https://doi.org/10.1016/j.carbpol.2015.03.065>
84. Xiao G, Zhang X, Zhao Y, Su H, Tan T (2014) The behavior of active bactericidal and antifungal coating under visible light irradiation. Appl Surf Sci 292:756–763
85. Pushpa K, Barman K, Patel VB, Siddiqui MW, Koley B (2015) Reducing postharvest pericarp browning and preserving health promoting compounds of litchi fruit by combination treatment of salicylic acid and chitosan. Sci Hortic 197:555–563. <https://doi.org/10.1016/j.scienta.2015.10.017>
86. Díez-Pascual AM, Díez-Vicente AL (2015) Antimicrobial and sustainable food packaging based on poly(butylene adipate-co-terephthalate) and electrospun chitosan nanofibers. RSC Adv 5:93095–93107. <https://doi.org/10.1039/C5RA14359D>
87. De Aquino BA, Blank FA, Santana LCL (2015) Impact of edible chitosan-cassava starch coatings enriched with *Lippia gracilis* Schauer genotype mixtures on the shelf life of guavas (*Psidium guajava* L.) during storage at room temperature. Food Chem 171:108–116. <https://doi.org/10.1016/j.foodchem.2014.08.077>
88. Latou E, Mexis SF, Badeka AV, Kontakos S, Kontominas MG (2014) Combined effect of chitosan and modified atmosphere packaging for shelf life extension of chicken breast fillets. LWT-Food. Sci Technol 55:263–268. <https://doi.org/10.1016/j.lwt.2013.09.010>
89. Hoyo-Gallego DS, Álvarez PL, Galván GF, Lizundia E, Kuritka I, Sedlarik V, Laza MJ, Vila-Vilela LJ (2016) Construction of antibacterial poly(ethylene terephthalate) films via layer by layer assembly of chitosan and hyaluronic acid. Carbohydr Polym 143:35–43. <https://doi.org/10.1016/j.carbpol.2016.02.008>
90. Shao X, Cao B, Xie S, Yu D, Wang H (2015) Effect of postharvest application of chitosan combined with clove oil against citrus green mold. Postharvest Biol Technol 99:37–43. <https://doi.org/10.1016/j.postharvbio.2014.07.014>
91. Youssef AM, El-Sayed SM, El-Sayed HS, Salama HH, Dufresne A (2016) Enhancement of Egyptian soft white cheese shelf life using a novel chitosan/carboxymethyl cellulose/zinc oxide bionanocomposite film. Carbohydr Polym 151:9–19. <https://doi.org/10.1016/j.carbpol.2016.05.023>
92. Youssef AM, Abou-Yousef H, El-Sayed SM, Kamel S (2015) Mechanical and antibacterial properties of novel high performance chitosan/nanocomposite films. Int J Biol Macromol 76:25–32. <https://doi.org/10.1016/j.ijbiomac.2015.02.016>
93. Bhaskara Reddy MV, Arul J, Angers P, Couture L (1999) Chitosan treatment of wheat seeds induces resistance to *Fusarium graminearum* and improves seed quality. J Agric Food Chem 47(3):1208–1216. <https://doi.org/10.1021/jf981225k>
94. Yang JM, Lin HT (2004) Properties of chitosan containing PP-g-AA-g-NIPAAm bigraft nonwoven fabric for wound dressing. J Membr Sci 243:1–7
95. Chen S, Wu G, Zeng H (2005) Preparation of high antimicrobial activity thioureachitosan-Ag⁺ complex. Carbohydr Polym 60:33–38
96. Deitzel JM, Kleinmeyer J, Harris D, Beck TNC (2001) The effect of processing variables on the morphology of electrospun nanofibers and textiles. Polymer 42:261–272
97. Ignatova M, Manolova N, Rashkov I (2007) Novel antibacterial fibers of quaternized chitosan and poly(vinyl pyrrolidone) prepared by electrospinning. Eur Polym J 43:1112–1122
98. Shahidi F, Arachchi JKV, Jeon YJ (1999) Food application of chitin and chitosans. Trends Food Sci Technol 10:37–51
99. Dutta PK, Tripathi S, Mehrotra GK, Dutta J (2009) Perspectives for chitosan based antimicrobial films in food applications. Food Chem 114:1173–1182
100. Atay HY, Çelik E (2016) Investigations of antibacterial activity of chitosan in the polymeric composite coatings. Prog Org Coat. <https://doi.org/10.1016/j.porgcoat.2016.10.013>
101. Liu H, Du Y, Wang X, Sun L (2004) Chitosan kills bacteria through cell membrane damage. Int J Food Microbiol 95:147–155
102. Hasan M, Ahmad AL, Hameed BH (2008) Adsorption of reactive dye onto cross-linked chitosan/oil palm ash composite beads. Chem Eng J 136:164–172

103. Yoshida H, Fukuda S, Okamoto A, Kataoka T (1991) Recovery of direct dye and acid dye by adsorption on chitosan fiber-equilibria. *Water Sci Technol* 23:1667–1676
104. Chiou MS, Li HY (2003) Adsorption behavior of reactive dye in aqueous solution on chemical cross-linked chitosan beads. *Chemosphere* 50:1095–1105
105. Wei D, Sun W, Qian W, Ye Y, Ma X (2009) The synthesis of chitosan-based silver nanoparticles and their antibacterial activity. *Carbohydr Res* 344:2375–2382
106. Covarrubias C, Trepiana D, Corral C (2018) Synthesis of hybrid copper-chitosan nanoparticles with antibacterial activity against cariogenic *Streptococcus mutans*. *Dent Mater J* 37:379–384
107. Martínez-Robles ÁM, Loyola-Rodríguez JP, Zavala-Alonso NV, Martínez-Martínez RE, Ruiz F, Lara-Castro RH, Donohué-Cornejo A, Reyes-López SY, Espinosa-Cristóbal LF (2009) Antibacterial effect of silver nanoparticles against *Streptococcus mutans*. *Mater Lett* 63:2603–2606
108. Besinis A, De Peralta T, Handy RD (2014) The antibacterial effects of silver, titanium dioxide and silica dioxide nanoparticles compared to the dental disinfectant chlorhexidine on *Streptococcus mutans* using a suite of bioassays. *Nanotoxicology* 8:1–16
109. Aliasghari A, Rabhani Khorasgani M, Vaezifar S, Rahimi F, Younesi H, Khoroushi M (2016) Evaluation of antibacterial efficiency of chitosan and chitosan nanoparticles on cariogenic *streptococci*: an in vitro study. *Iran J Microbiol* 8:93–100
110. Costa EM, Silva S, Tavaría FK, Pintado MM (2013) Study of the effects of chitosan upon *Streptococcus mutans* adherence and biofilm formation. *Anaerobe* 20:27–31
111. Hipalawins WM, Balakumaran M, Jagadeeswari S (2016) Synthesis, characterization and antibacterial activity of chitosan nanoparticles and its impact on seed germination. *J Acad Ind Res* 5:65–71
112. Nguyen TV, Nguyen TTH, Wang SL, Vo TPK, Nguyen AD (2016) Preparation of chitosan nanoparticles by TPP ionic gelation combined with spray drying, and the antibacterial activity of chitosan nanoparticles and a chitosan nanoparticle–amoxicillin complex. *Res Chem Intermed* 43:3527–3537. <https://doi.org/10.1007/s11164-016-2428-8>
113. Sotelo-Boyás ME, Correa-Pacheco ZN, Bautista-Baños S, Corona-Rangel ML (2017) Physicochemical characterization of chitosan nanoparticles and nanocapsules incorporated with lime essential oil and their antibacterial activity against food-borne pathogens. *LWT* 77:15–20
114. Taponen S, Pyörälä S (2009) Coagulase-negative staphylococci as cause of bovine mastitis— not so different from *Staphylococcus aureus*. *Veter Microbiol* 134:29–36
115. Tamara FR, Lin C, Mi FL, Ho YC (2018) Antibacterial effects of chitosan/cationic peptide nanoparticles. *Nanomaterials* 8:88
116. Tran HV, Tran LD, Ba CT, Vu HD, Nguyen TN, Pham DG, Nguyen PX (2010) Synthesis, characterization, antibacterial and antiproliferative activities of monodisperse chitosan-based silver nanoparticles. *Colloids Surf A Physicochem Eng Asp* 360:32–40
117. Piras AM, Maisetta G, Sandreschi S, Gazzari M, Bartoli C, Grassi L, Esin S, Chiellini F, Batoni G (2015) Chitosan nanoparticles loaded with the antimicrobial peptide temporin B exert a long-term antibacterial activity in vitro against clinical isolates of *Staphylococcus epidermidis*. *Front Microbiol* 6:372
118. Susilowati E, Ashadi M (2016) Preparation of silver-chitosan nanocomposites and coating on bandage for antibacterial wound dressing application. *AIP Conf Proc* 1710:030015
119. Grande CD, Mangadlao J, Fan J, De Leon A, Delgado-Ospina J, Rojas JG, Rodrigues DF, Advincula R (2017) Chitosan cross-linked graphene oxide nanocomposite films with antimicrobial activity for application in food industry. *Macromol Symp* 374:1600114
120. Benucci I, Liburdi K, Cacciotti I, Lombardelli C, Zappino M, Nanni F, Esti M (2018) Chitosan/clay nanocomposite films as supports for enzyme immobilization: an innovative green approach for winemaking applications. *Food Hydrocoll* 74:124–131
121. Tin S, Sakharkar KR, Lim CS, Sakharkar MK (2009) Activity of chitosans in combination with antibiotics in *Pseudomonas aeruginosa*. *Int J Biol Sci* 5:153–160. <https://doi.org/10.7150/ijbs.5.153>
122. Golmohamadi M, Ghorbani HR, Otadi M (2019) Synthesis of chitosan nanoparticles loaded with antibiotics as drug carriers and the study of antibacterial activity. *J Nanoanal*. <https://doi.org/10.22034/JNA.2019.664506>

123. Sharma PK, Halder M, Srivastava U, Singh Y (2019) Antibacterial PEG-chitosan hydrogels for controlled antibiotic/protein delivery. *ACS Appl Bio Mater* 2:5313–5322. <https://doi.org/10.1021/acsabm.9b00570>
124. Brasil M, Filgueiras A, Campos M, Neves M, Eugênio M, Sena L, Sant'Anna C, da Silva V, Diniz C, Sant'Ana A (2018) Synergism in the antibacterial action of ternary mixtures involving silver nanoparticles, chitosan and antibiotics. *J Braz Chem Soc* 29:2026–2033. <https://doi.org/10.21577/0103-5053.20180077>
125. Ong T.H, Chitra E, Ramamurthy S, Ling CCS, Ambu SP, Davamani F (2019) Cationic chitosan-propolis nanoparticles alter the zeta potential of *S. epidermidis*, inhibit biofilm by modulating gene expression and exhibit synergism with antibiotics. *PLoS ONE* 14:e0213079. <https://doi.org/10.1371/journal.pone.0213079>