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# Antibacterial activity of Chitosan Nanoparticles against sea food pathogens

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**Abstract**
The present study was designed to investigate antibacterial activity of chitosan nanoparticles against sea food pathogens. Chitosan is an interesting polymer that has been used extensively in the medical field. It is either partially or fully deacetylated chitin. It is a good antibacterial and antifungal agent. Chitosan is synthesized by STPP method and its active range is evaluated by uv-vis spectroscopy. Then its antibacterial is tested against isolated Vibrio and also tested the antibacterial activity of chloramphenicol against the isolated species for knowing the strength of the strain. Chitosan is a good antibacterial agent that killed Vibrio around its active sites. But, chloramphenicol is a weak antibacterial agent than chitosan.

**Keywords**:Chitosan, Vibrio, Chloramphenicol.

## Introduction

Chitinis a long- chain polymer of a *N*-acetyl glucosamine, a derivative of glucose, and is found in many places throughout the natural world. It is the main component of the cell walls of fungi, the exoskeletons of arthropods suchas crustaceans (e.g., lo bsters and shrimps). The structure of chitin is comparable to the polysaccharide cellulose, forming crystalline nano fibrils or whiskers. In terms of function, it may be compared to the protein keratin. Chitin has also proven useful for several medical and industrial purposes. In butterfly wing scales, chitin is often organized into stacks of nano - layers or nano-sticks made of chitin nano crystals that produce various iridescent colors by thin-film interference: similar, analogous structures made of keratin are found in iridescent bird plumage.

Chitosan has a number of commercial and possible biomedical uses.

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It can be used in agriculture as a seed treatment and bio pesticide, helping plants to fight off fungal infections. In winemaking it can be used as a fining agent, also helping to prevent spoilage. In industry, it can be used in a self-healing polyurethane paint coating. In medicine, it may be useful in bandages to reduce bleeding and as an antibacterial agent; it can also be used to help deliver drugs through the skin.
Chitosan is soluble in acidic conditions- in solution the free amino groups on its polymeric chains can protonate, giving it a positive charge. Chitosan nanoparticles can be formed by incorporating a polyanion such as tripolyphosphate (TPP) in to a chitosan solution under constant stirring. These nanoparticles can then be used for drug delivery and gene therapy applications. Due to its poor solubility at pH more than 6.5, a number of chemically modified chitosan derivatives with improved water solubility can be used as well. The present study was designed to investigate antibacterial activity of chitosan nanoparticles against sea food pathogens.

## Material and Methods

Fish samples were inoculated on to Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar plates. Sample was streak plated on the agar plates and incubated at $37^0$C for 24 hours. Growth was observed and the sample was sub cultured on to nutrient broth.Gram

staining was performed to study & to confirm the isolates.A thin uniform bacterial smear was prepared, the smear was then air dried and heat fixed by passing the slide rapidly over the flame and cooled. The smear was then flooded with crystal violet for 1minute.The crystal violet stain was then washed off with water. The smear was covered with Gram's iodine solution and was left for 30seconds. The grams iodine was washed off. The smear was rinsed with decolourising agent alcohol for 1-2 seconds and then washed with water. Then the counter stain saffranine is added to stand for 45s and washed with tap water to remove excess stain and was air dried. After the slide has been completely dried, it was observed for stained bacterial cells under oil immersion objective of the microscope.

**Chitosan nanoparticles synthesis**

Chitosan/Tripolyphosphate nanoparticles were prepared by ionic gelation process. Different concentrations of chitosan in acetic acid and STPP were mixed drop wise under constant stirring to get a white precipitate indicated by nanoparticle synthesis. Suspension was lyophilized using cryoprotectant 1% (w/v) mannitol. Particles were incubated at $37^0$C for 30minutes. Nanoparticles were separated by centrifugation at 25,000rpm for 30minutes. The supernatant was discarded and the wet pellet was collected. Stored at 4°C for further studies. The dry pellet was dissolved in water and used for further analysis.

**Characterization of nanoparticle**

**UV-Vis Spectroscopy:** Nanoparticles were characterized in UV-VIS spectrophotometer (Systronics, Delhi) to know the kinetic behaviour of chitosan nanoparticles. The scanning range for the samples was 340-540nm. The UV Vis absorption spectra of all the samples were recorded and numerical data were plotted.

**Antibacterial activity of chitosan**

Antibacterial study was performed using agar diffusion method against *Vibrio*sp sample on Muller Hinton agar plates. Varying concentration of 1% chitosan (50µl and 100 µl) in water were used for the study. 100 µl of the culture was swabbed on the MHA plate and well was cut on the agar plate, into which chitosan nanaoparticles were added. The plates were incubated at 37°C overnight and the inhibition of the bacteria was viewed.

**Results**

On TCBS Agar, after an incubation of 48 hours at $37^0$C, yellowcolour colonies were obtained. Pink coloured Gram negative rod shaped bacteria were observed on staining.A clear solution with chitosan nanoparticle was obtained after centrifugation.

A graph was obtained with peaks at around 360-380 nm. Those peaks indicate the presence of chitosan nanoparticle in the obtained solution. Using microtitre plate, observed antimicrobial activity of chitosan nanoparticle against *Vibrio sp.*

**Discussion**

Seafood is part of a healthful diet, but seafood consumption is not risk-free. Seafood-associated infections are caused by a variety of bacteria, viruses, and parasites; this diverse group of pathogens results in a wide variety of clinical syndromes, each with its own epidemiology. Some seafood commodities are inherently more risky than others, owing to many factors, including the nature of the environment from which they come, their mode of feeding, the season during which they are harvested, and how they are prepared and served. Prevention of seafood-associated infections requires an understanding not only of the etiologic agents and seafood commodities associated with illness but also of the mechanisms of contamination that are amenable to control (Iwamato, 2010).[1]

Cases of **Vibrio** infections have a marked seasonal distribution; most occur during summer and early fall, corresponding to the period of warmer temperatures. The most frequently isolated *Vibrio species are V.parahaemolyticus,V.vulnificus* and *V.alginolyticus.* Almost all cases of food-borne **Vibrio** infection are associated with a recent history of seafood consumption, primarily raw oyster consumption. Measures to prevent food-borne **Vibrio** infections include consumer education regarding the dangers of eating raw or undercooked shellfish, particularly among persons with medical conditions, such as liver disease, that predispose them to severe illness. Thoroughly cooking fish and preventing raw seafood from cross-contaminating other foods are effective measures for consumers to reduce risk.

Routine screening of isolates on any selective agar relies on the capacity of selection of the target bacteria and on the choice of a few relevant taxonomic traits for presumptive identification. TCBS agar is commonly recommended for the isolation of **Vibrio** spp. (Kobayashi 1963).[2] It eliminates non-bile salt tolerant species and remains the best agar for its ability to isolate vibrios from their natural estuarine

environment (Oliver and Pfeffer, 2003).[3]After all microbiological methods such as plating, gram staining biochemical tests etc*V.parahaemolyticus* was identified and isolated from the seafood sample. It was identified by biochemical results and cultural properties on TCBS plate. TCBS Agar is the specific medium used for *Vibrio* species. The bacterial isolate from the fish sample formed dark green colonies against the yellow background of the TCBS plate and thus it was confirmed as *V.parahaemolyticus*

## Conclusion

This work is comprised antibacterial activity of chitosan nanoparticles against sea food pathogens. The active range of chitosan was evaluated using uv-vis spectroscopy. The chitosan has good antibacterial activity against the isolated Vibrio sp. The result of the present study suggests that selected range of chitosan that can be used a good antibacterial agent against sea food pathogens. Hence, can protect sea food items from infectious agents like Vibrio sp. by the application of chitosan products.

**Source of Support: Nil**
**Conflict of Interest: Nil**

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