

Comparative study of antibacterial activity of vancomycin and chemically treated chitosan prepared from shrimp (*Macrobrachium rosenbergii*) waste

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Abstract: Chitosan is derived from exoskeleton of crustaceans (such as crabs and shrimp) and cell walls of fungi has a number of commercial and possible biomedical uses is produced commercially by deacetylation of chitin is a linear polysaccharide composed of randomly distributed β -(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). The structural element This study was conducted to compare the antibacterial activity of chitosan with the combination of chitosan and vancomycin against gram negative *Escherichia coli* and gram positive *Staphylococcus aureus*. Optical density (OD) value measurement technique was undertaken to measure antibacterial activity. After incubation, turbidimetric measurements of bacterial growth of sets of test tubes were taken after every 4 hours, for 48 hours and then compared. For *S. aureus* and chitosan the average absorbance were found as 0.448, 0.457, 0.492, 0.532, 0.582, 0.645, 0.683, 0.724, 0.751, 0.793, 0.840, and 0.896, while for *S. aureus* with both chitosan and vancomycin, absorbance were 0.267, 0.286, 0.321, 0.346, 0.386, 0.431, 0.467, 0.475, 0.509, 0.541, 0.565, and 0.629. Again for *E. coli* and chitosan the average absorbance were found as 0.407, 0.429, 0.464, 0.506, 0.564, 0.6, 0.645, 0.703, 0.756, 0.815, 0.850, and 0.901, while *E. coli* with both chitosan and vancomycin, absorbance were 0.213, 0.233, 0.322, 0.347, 0.409, 0.446, 0.475, 0.511, 0.545, 0.582, 0.628, and 0.647. Both of these revealed that chitosan and vancomycin together possess higher antibacterial activity against gram positive and gram-negative bacteria than chitosan solitarily used. Statistical analysis of the both study also represented in Mean \pm 3SD. Comparison of the antibacterial activity against *E. coli* and *S. aureus* revealed that chitosan and vancomycin posses similar antibacterial activity against gram positive and gram negative bacteria.

Keywords: Antibacterial activity, Chitosan, Vancomycin

1. Introduction

Chitosan is a linear polysaccharide that consists of (1, 4)-linked 2-amino-deoxy-N- D-glycan. It is a deacetylated form of chitin, the second most abundant polysaccharide found in nature after cellulose [1]. It also exists naturally in a few species of fungi; insects and yeast [2]. Extensive studies have been conducted on the activity of vancomycin on gram positive and gram negative bacteria. To facilitate the genetic investigation, entire genome sequences of the archetypal VRSA (Mu50) and vancomycin-susceptible MRSA strains N315, EMRSA 16 and COL were compared

[3]. Their research helped to understand the mechanism(s) of vancomycin resistance in *S. aureus* Mu50 and other VRSA strains. The antimicrobial activity of chitosan with that of combined chitosan and vancomycin was compared which ultimate goal was to increase effectiveness of narrow spectrum antibiotics applied to preserve food items, may apply as antimicrobial agent in pharmaceuticals and may also find uses in agriculture as a more active seed coating, fertilizer, controlled agrochemical release agent. In drug industry it may be used to formulate weight losing supplement. Chitosan production from shrimp waste certainly save the environment from serious pollution [4] may be used as water purification agent in municipal area

where people always face serious water problem. After considering the applicability of chitosan and its possible development by vancomycin as well as narrow spectrum antibiotic, this research work has been undertaken.

Chitin and chitosan in vitro show antibacterial and anti-yeast activities. One of chitosan derivatives, i.e., N-carboxybutyl chitosan, was tested against 298 cultures of different pathogenic microorganisms that showed bacteriostatic and bactericidal activities, and there were marked morphological alterations in treated microorganisms when examined by electron microscopy [5]. Conversely, growth inhibition and inactivation of mould and yeasts seem to depend on chitosan concentration, pH, and temperature [6].

The antimicrobial activity of chitosan varies depending on their physical properties (degree of deacetylation (DD), and molecular weight), solvent, microorganism species and source. The antimicrobial activity is reported to vary depending on the methods involved in preparation of different DD and molecular weight of chitosan [7, 8]. The antimicrobial action of chitosan is influenced by intrinsic and extrinsic factors such as the type of chitosan (e.g., plain or derivative), degree of chitosan polymerization, host nutrient constituency, substrate chemical and/or nutrient composition, and environmental conditions such as substrate water activity [9]. In an extensive research on the antimicrobial activity of chitosan prepared from shrimp against *E. coli*, it was found that higher temperature and acidic pH of foods increased the bactericidal effect of chitosan [10]. The mechanism of chitosan antibacterial action involving a cross-linkage between polycations of chitosan and the anions on the bacterial surface that changes membrane permeability and has been approved as a food additive in Japan and Korea since 1983 and 1995 respectively [11].

Based on the above investigation the present study was undertaken to compare whether chitosan is more active than vancomycin and vice-versa against *E. coli* and Staphylococci. The study of the anti-bacterial activity of chemically deacylated chitosan prepared from shrimp and the comparison of anti bacterial activity of chitosan with the combination of chitosan and a narrow spectrum antibiotic vancomycin

2. Materials and Methods

Chitin is extracted from crustacean shell waste such as crab, shrimp, lobster, and crawfish. We prepared chitin from shrimp shell (*Macrobrachium rosenbergii*) locally known as Galda chinri.

2.1. Chitosan Isolation

Isolation of chitosan from shrimp shell wastes involves four traditional steps demineralization (DM), deproteinization (DP), decolorization (DC), and deacetylation (DA). The method was modified from No and Meyers, (1995) [12].

Escherichia coli and *Staphylococcus aureus* strains were tested for the antimicrobial activity of chitosan and vancomycin. Pure culture of Staphylococcus aureus was previously collected from ICDDR, Dhaka and cultured in Mac Conkey and nutrient agar media respectively and maintained in the lab.

2.2. Experimental Procedure

The study was carried out by broth dilution method and used turbidity estimations to determine the growth characteristics of a bacterial culture with or without chitin or Three percent of concentrated (99%) acetic acid was prepared in a conical flask with distill water to dissolve the powder. 7.5gm chitosan was taken into two sterile test tubes and 50 ml of 3% acetic acid was poured into each of these gradually. To increase solubility of the solution was stirred; heat was applied in water bath at 400 C. The solution was left at room temperature for 3 days. Stirring and heat was applied in this time with a spirit lamp. Nutrient broth (N.B) was used as culture media in test tubes.

Nine ml of nutrient broth media was taken in each of fourteen 14-test tubes. Each test tube was always capped with screw to avoid contamination. Three test tubes were used as standard in spectrophotometer measurement for blank calculation contains media. The upper soluble portion of chitosan sample was added to NB medium in test tube, the pellet portion of the test tube was not taken. 0.5 ml supernatant chitosan solution was taken by micropipette and dispensed in each test tube. The pH of chitosan with medium was also adjusted between 6.2-6.5 range to ensure optimum growth of microorganism using NaOH solution. Five vancomycin discs were added in each test tube.

Two test tubes containing 9 ml N.B media and 0.5ml chitosan solution was mixed. One loop full staphylococci. The loop head was immersed into the broth and mixed well. The same process was followed for other two test tubes with gram negative bacteria. Another two test tubes containing N.B media, chitosan and vancomycin were taken and inoculated them with gram positive bacteria and another two tubes with gram negative bacteria in the same way. Absorbance of Nutrient broth with *S. aureus*, and *E. Coli* was measured separately at 640 nm. Chitosan was included separately with the both strain separately for the measurement of activity. Vancomycin was incorporated in broth chitosan and the two organisms in which equal concentration of inoculum was used. In each case blank was prepared. After successful inoculation the test tubes were incubated in an incubator at 37 °C. After each 4 hours interval measurements were taken by spectrophotometer up to 48 hrs. Absorbance or OD value can also refer to index of refraction [13]. This technique is based on the principle that small particles, such as bacteria, scatter a beam of light passed through the cell suspension. The amount of light scattering is proportional to the concentration of particles in the suspension; because a pure culture of single cells is relatively uniform in size (mass). The number of cells can

be measured from the amount of light that reaches a photoelectric cell after passing through the cell suspension. As the bacterial cell population increases, the amount of transmitted light decreases, increasing the absorbance reading on the spectrophotometer. These sequences were repeated for other samples and the respective blank was changed in blank and the absorbance was recorded. O.D

values were taken after every 4 hours for 48 hours.

3. Results and Discussions

The recorded absorbance taken for *Staphylococcus aureus* and *E. coli* are given in the table 1.

Table1. OD values for *S. aureus* (4h-48h)

Description	Average OD values											
	4	8	12	16	20	24	28	32	36	40	44	48
Chitosan + <i>S. aureus</i>	0.448	0.457	0.492	0.532	0.582	0.645	0.683	0.724	0.751	0.793	0.840	0.896
Chitosan + <i>S. aureus</i> + Antibiotic	0.267	0.286	0.321	0.346	0.386	0.431	0.467	0.475	0.509	0.541	0.565	0.629

The OD value measured for *Staphylococcus aureus* with chitosan demonstrated far higher absorbance than chitosan and vancomycin together. So it is clearly evident that chitosan and vancomycin together possess high level antibacterial property against *S. aureus*, while chitosan alone possess lower level of activity. Graphical presentation

of the measured OD values for *S. aureus* has been shown in figure 1. Where V1 represents absorbance in Chitosan and staphylococcus and V2 represents the same included antibiotic. The error bars shows symmetrical distribution at Mean \pm 3SD level.

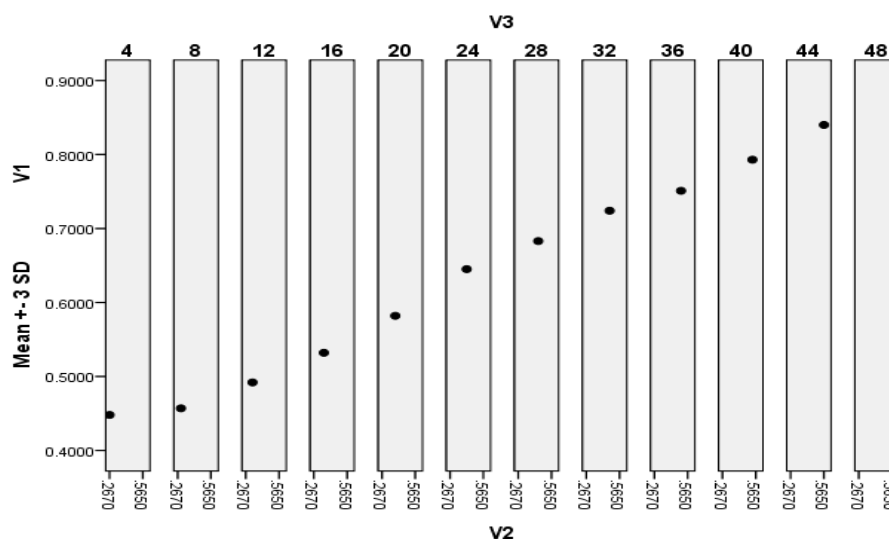


Figure 1. Growth curve for *S. aureus* + chitosan and *S. aureus* + chitosan + vancomycin

Table 2. OD values for *E. coli* (4h-48h)

Description	Average OD values											
	4	8	12	16	20	24	28	32	36	40	44	48
Chitosan + <i>E. coli</i>	0.407	0.429	0.464	0.506	0.564	0.600	0.654	0.703	0.756	0.815	0.85	0.901
Chitosan + <i>E. coli</i> + Antibiotic	0.213	0.233	0.322	0.374	0.409	0.446	0.475	0.511	0.545	0.582	0.628	0.647

The OD value measured for *Escherichia coli* with chitosan demonstrated far higher absorbance than chitosan and vancomycin together. It is clearly evident that chitosan and vancomycin together

possess high level antibacterial property against *E. coli*, while chitosan solitarly possess lower level of activity. Graphical presentation of the measured O.D. values for *E. coli* has been shown in figure 2. Where V1 represents

absorbance in Chitosan and *E. coli* and V2 represents the same included antibiotic. The graph shows symmetrical

distribution at Mean \pm 3SD level.

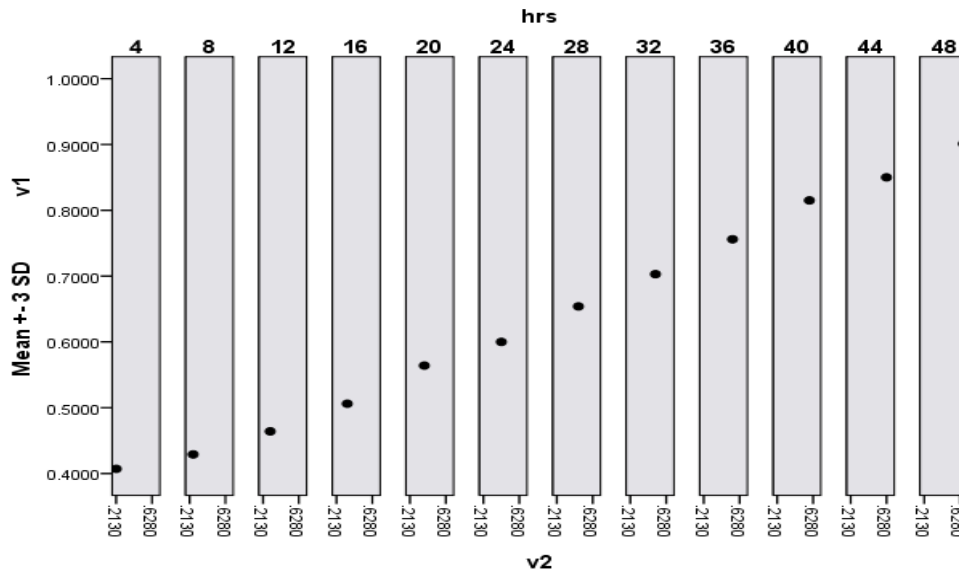


Figure 2. Shows growth curve for *E. coli* + chitosan and *E. coli* + chitosan + vancomycin

The growth of *E. coli* and staphylococcus with chitosan and vancomycin lies far behind than the growth curve of *E. coli* and staphylococcus with chitosan reveals growth of *E. coli* and staphylococcus is retarded more by the addition

of vancomycin with chitosan. Comparative study of antibacterial activity of chitosan and vancomycin against *E. coli* and *S. aureus* has shown in figure. 3.

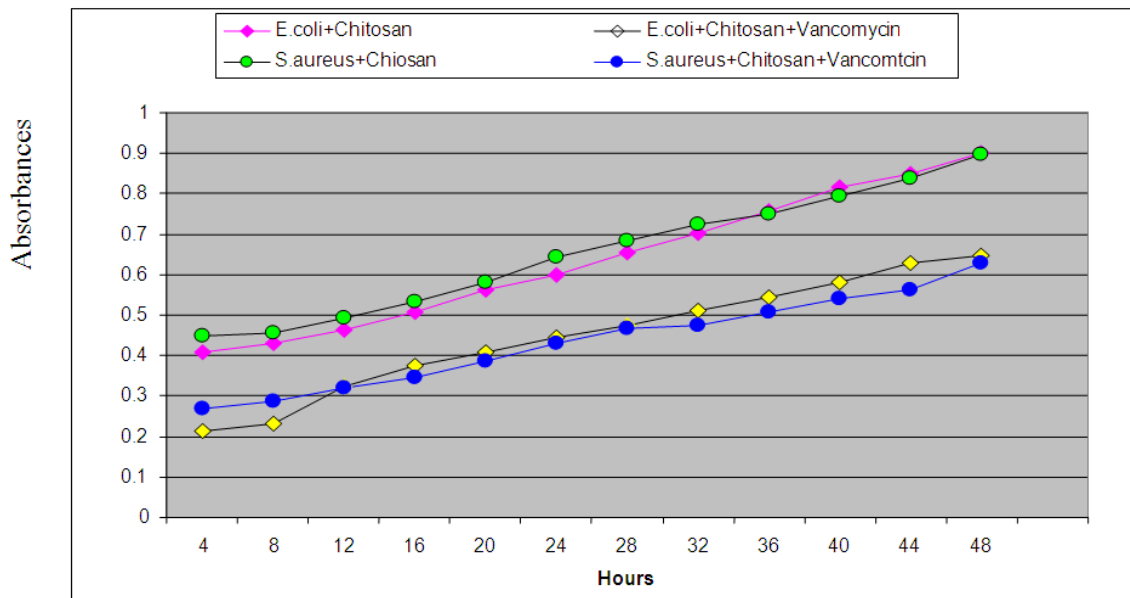


Figure 3. Comparison of the growth curves for *E. coli* and *S. aureus*

The figure 3 reveals that in presence of vancomycin at first the growth of gram negative *E. coli* was lower than that of gram positive *S. aureus*. After 12 hours growth of both the bacteria was same. *E. coli* showed slightly higher growth than *S. aureus*. Although in some points OD value for *E. coli* and *S. aureus* were approximately same. The

efficacy of many antibiotics for treatment of infections has become quite limited due to the development of resistance and the threat from antimicrobial-resistant organisms is accumulating and accelerating [14]. Also, the development of resistance to mono therapy is a common problem and dual antimicrobial coverage is often a necessity in

Pseudomonas infections [15]. Attempts have been made to deal with this problem by using combination therapy with success [16]. Present study will meet the necessary requirement to understand mechanism of activity of natural polysaccharide which will solve best use of antibacterial agents.

4. Conclusion

The activity of chitosan is higher against gram positive bacteria than gram negative bacteria. In this study chitosan and vancomycin together showed slightly raised antibacterial effect against gram negative *E. coli*, the difference between antibacterial activity against both the gram positive *S. aureus* and gram negative *E. coli* very little as negligible. So from this research it has been found that, chitosan and vancomycin possess approximately equal antibacterial activity against gram positive and gram negative bacteria but when they used together as antimicrobial agent they work more effectively than chitosan or vancomycin alone. In table-1 it has been shown that the average OD value of chitosan and aureus is higher than chitosan and aureus and vancomycin together. It is also shown that the existence of a synergistic effect between chitosan and vancomycin antibiotic. Since chitosan itself acts as an antibiotic, it can certainly increase the activity of vancomycin, which would take the antibiotic technology forward one step. Chitosan and vancomycin together can also create advanced ways to produce more effective preservatives.

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