Chitosan: A potential biomaterial effective against typhoid

Chitin, a polysaccharide of animal origin, is obtained from waste material of seafood industries. It occurs in the skeletal material of crustaceans such as crabs, lobsters, shrimps, prawns and crayfish. Chitosan is the deacetylated product formed by treatment of chitin with concentrated (50%) caustic alkali. The regulatory and toxicological status of Chitosan has already been established. The oral toxicity of Chitosan has been reported to be 16 g/kg body weight (LD50)[1]. Thus Chitosan is safe (nontoxic), biocompatible and biodegradable.

Chitosan has been subjected to a series of pharmacological and clinical studies[2–6]. For the first time in 1978, Balassa and Prudden[7] studied the use of chitin and its derivatives (including Chitosan) in wound-healing. These studies found that chitin and Chitosan are effective wound healing accelerators in both animal and human tests. Macroporous artificial skin containing antibiotics was prepared by lyophilization of Chitosan/PVA blendmer, which could protect the wound surfaces from bacterial invasions by suppressing bacterial proliferation effectively[8]. Recently, the antimicrobial activity of Chitosan has been studied extensively. It has been shown that Chitosan acts by disrupting the barrier properties of the outer membrane of Gram-negative bacteria[9]. Zivanovic *et al.*[10], while studying antimicrobial efficiency of Chitosan, have shown that addition of 0.1% Chitosan polysaccharide would be sufficient to ensure the microbial safety of oil-in-water emulsions. It has also been found that the antimicrobial activity of Chitosan is influenced by its molecular weight. The water-soluble Chitosan hydrolysate, consisting mainly of low molecular weight Chitosan (LMWC), shows antimicrobial activity against *Escherichia coli*, while Chitooligosaccharides have much weaker antimicrobial activity[11].

Chitosan, obtained as a gift sample from India Seafoods, Cochin was used in this study. Its viscosity (1% solution in 2 M acetic acid) is 325 and the deacetylation degree is 80%. The bacterial strains were procured from Krishna Institute of Medical Sciences and Research Centre, Karad, India.

The antibacterial activity was studied against different strains of bacteria, viz. *Staphylococcus aureus*, *Bacillus subtilis* (Gram-positive), *Pseudomonas aeruginosa*, *E. coli*, *Salmonella enterica*, *S. enterica* var. *Paratyphi-A* and *S. enterica* var. *Paratyphi-B* (Gram-negative) by agar diffusion method in particular, cup-plate method[12,13]. In this method cups of standard diameter are made into the nutrient agar medium containing standard bacterial inoculum. Next 0.1 ml of the test material (Chitosan solution in 2M citric acid) and reference standards (ciprofloxacin, sparfloxacin aqueous solution) were taken into the cups. After incubation of the plates at 37 ± 1°C for 24 h, the diameter of zone of inhibition in millimetres was measured.

Minimum inhibitory concentration (MIC) is the highest dilution of an antimicrobial agent that inhibits the growth of a particular microorganism in a test period. The MIC of Chitosan against *S. enterica*, *S. enterica* var. *Paratyphi-A* and *S. enterica* var. *Paratyphi-B* was determined.

The antimicrobial activity of Chitosan against typhoid organisms was then compared with the reference standard antibiotics. Ciprofloxacin and sparfloxacin were used as standard antibiotics. The zones of inhibition produced by MIC of Chitosan and standard antibiotics were measured. Antimicrobial susceptibility testing with discs for testing bacterial sensitivity to various antibiotics and Chitosan was selected as a method of study[14,15]. Span-combi discs from Span Diagnostics Ltd, Surat, India were used in this study.

Different strains of *S. enterica* (4 strains), *S. enterica* var. *Paratyphi-A* (1 strain) and *S. enterica* var. *Paratyphi-B* (4 strains) resistant to certain antibiotics were procured from Krishna Institute of Medical Sciences and Research. Table 1 gives the list of such strains with their sensitivity to certain antibiotics. Using disc method the sensitivity of resistant strains (viz. *S. enterica-2*, *S. enterica-4*, *S. enterica* var. *Paratyphi-B-3*, *S. enterica* var. *Paratyphi-B-4*, *S. enterica* var. *Paratyphi-A-1*) against standard antibiotics and Chitosan have been studied.

The antibacterial study was carried out by agar diffusion technique, in particular,

Table 1. Resistant strains of typhoid and their sensitivity

		Diameter of zone of inhibition (mm)										
Organism	Resistant strain	Ampicillin 30*	Bactrim 16*	Ceftazidime 18*	Ceftriaxon 21*	Chloramphenicol 18*	Ciprofloxacin 21*	Nalidixic acid 19*	Netylmycin 15*	Pefloxacin 16*	Piperacillin 18*	Tetracyclin 19*
S. enterica	1	27 (R)	30	24	24	30	20 (R)	R	24	20	22	10 (R)
	2	R	R	20	24	R	26	22	22	24	13 (R)	R
	3	25 (R)	34	24	30	R	30	22	20	30	25	R
	4	R	R	26	24	R	28	24	22	26	R	R
S. enterica var. *Paratyphi-B*	1	22 (R)	28	26	24	26	28	22	20	20	22	R
	2	20 (R)	30	24	26	24	26	22	20	28	22	10 (R)
	3	R	22	R	12 (R)	R	20 (R)	R	12 (R)	18	R	R
	4	R	R	R	R	24	R	R	10 (R)	R	R	10 (R)
S. enterica var. *Paratyphi-A-1*	1	20 (R)	34	22	26	26	18 (R)	R	20	14(R)	22	15 (R)

*Minimum zone of sensitivity (mm); R, Resistant.

Figure 1. Antimicrobial activity of Chitosan (central disc) and other standard antibiotics against resistant strains of *S. enterica* var. *Paratyphi-A-1*, *S. enterica* var. *Paratyphi-B-3*, and *S. enterica* var. *Paratyphi-B-4*.

Table 2. Antibacterial activity of Chitosan

Organism	Diameter of zone of inhibition (mm) at 100 µg concentration
Staphylococcus aureus	40
Bacillus subtilis	20
Escherichia coli	18
Pseudomonas aeruginosa	16
Salmonella enterica	32
Salmonella enterica var. *Paratyphi-A*	32
Salmonella enterica var. *Paratyphi-B*	25

Table 3. Antimicrobial activity of Chitosan against *S. enterica*

Chitosan (µg)	Diameter of zone of inhibition (mm)
1000	40
800	40
600	38
400	35
200	30
100	29
50	15
10	Nil

the cup plate method, against selected Gram-positive and Gram-negative organisms. Chitosan had significant activity against these test organisms (Table 2).

Table 4. Comparison of antimicrobial activity of Chitosan and standard antibiotics against *S. enterica*, *S. enterica* var. *Paratyphi-A* and *S. enterica* var. *Paratyphi-B*

Organism	Diameter of zone of inhibition (mm)			
	Ciprofloxacin (25 µg)	Sparfloxacin (25 µg)	Chitosan (50 µg)	Chitosan (100 µg)
S. enterica	43	26	20	39
S. enterica var. *Paratyphi-A*	43	21	18	38
S. enterica var. *Paratyphi-B*	45	24	16	35

The MIC of Chitosan against typhoid producing organisms, *S. enterica* has been determined (Table 3). Our results showed that with increase in dilution, the diameter of zone of inhibition decreased. The MIC of Chitosan was found to be 50 µg.

For comparing the activity of Chitosan with standard antibiotics useful against typhoid organisms, the zones of inhibition produced by MIC of Chitosan and MIC of these antibiotics were measured. Our results reveal that Chitosan has good antibacterial activity against typhoid organisms, which is comparable to the standard antibiotics used in clinical practice today (Table 4).

Further, in our study we have attempted to investigate whether the antimicrobial activity of Chitosan is due to its glucosamine units. In our study we found that glucosamine does not show any antimicrobial activity. Antimicrobial susceptibility testing using discs was performed to assess the ability of Chitosan to act against resistant strains of typhoid-producing organisms and to compare it with the standard antibiotics (Table 5).

Young and co-workers[16,17] have shown that Chitosan affects the membrane permeability of plants and fungi. It has also been shown that Chitosan agglutinates a variety of bacteria and yeasts as well as cells of mammalian origin[18]. Leuba and Stossel[19], while studying the antifungal activity and effect of Chitosan and other polyamines, have concluded that polyamino acids interact with the electronegative bacterial cell surface resulting in displacement of Ca^{++} from anionic membrane sites, making it leaky and thus confirmed the non-specific action of polymines on membrane integrity. In our study we found that glucosamine does not show antimicrobial activity. The polycationic nature of Chitosan might be responsible for interaction with the electronegative

Table 5. Susceptibility testing of resistant strains of typhoid organisms

Resistant strain	Diameter of zone of inhibition (mm) ± SD											
	Amikacin (AK) 17*	Cefaclor (CG) 18*	Cefadxine (CD) 18*	Cefazolin (CF) 18*	Ceftazidime (CZ) 18*	Ceftriaxone (XO) 21*	Ciprofloxacin (CI) 21*	Kanamycin (K) 18*	Netilmicin (NT) 15*	Norfloxacin (NF) 17*	Ofloxacin (OF) 16*	Chitosan (C) 15*
S. enterica-2	17 ± 0.35	26 ± 0.4	R	22 ± 1.47	R	R	R	22 ± 0.4	20 ± 1.6	20 ± 0.81	R	23 ± 0.7
S. enterica-4	R	20 ± 0.4	18 ± 0.8	20 ± 1.6	23 ± 1.41	R	R	R	R	R	R	R
S. enterica var. *Paratyphi-B-3*	R	R	R	R	R	R	24 ± 0.81	R	R	21 ± 0.4	21 ± 0.7	16 ± 1.4
S. enterica var. *Paratyphi-B-4*	R	R	R	R	R	R	R	R	R	R	R	17 ± 1.5
S. enterica var. *Paratyphi-A-1*	R	20 ± 0.8	R	R	20 ± 0.4	R	R	25 ± 1.6	25 ± 0.7	R	R	30 ± 1.5

*Minimum zone of sensitivity (mm); R, Resistant.

bacterial cell surface. Thus, as suggested by Helander *et al.*[9], the disruption of barrier properties of outer membrane of Gram-negative bacteria might be the possible mechanism of antimicrobial action of Chitosan.

Our study confirms and supports the earlier findings regarding usefulness of Chitosan as a wound-healing accelerator, and its effectiveness in protecting wound from bacterial invasion by suppressing bacterial proliferation. Our study further reveals that Chitosan may act effectively against typhoid-producing microorganisms. Moreover, we could establish that Chitosan shows antimicrobial activity; however, glucosamine does not show such activity. Antimicrobial susceptibility testing using discs showed that Chitosan is not effective against one of the resistant strains of *S. enterica* (*S. enterica-4*). However, it is quite effective against resistant strains of *S. enterica* (*S. enterica-2*), *S. enterica* var. *Paratyphi-A* and *S. enterica* var. *Paratyphi-B* (Figure 1).

Further investigations need to be done to develop Chitosan into a useful therapeutic tool for treatment of typhoid. Chitosan shows great potential in this respect, especially on the grounds of limitations such as development of resistance to side effects and toxicity of currently used antibiotics.

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