NJPPP National Journal of Physiology, Pharmacy & Pharmacology

DOI: 10.5455/njppp.2015.5.161020141
http://www.njppp.com/

RESEARCH ARTICLE

IN VITRO ANTIBACTERIAL POTENTIAL OF CHITOSAN AND ITS DERIVATIVES ON PATHOGENIC ENTEROBACTERIACEAE

Sony Paul[1], Seema NP[2]

[1] *Department of Microbiology, Tagore Medical College & Hospital, Chennai, Tamil Nadu, India*
[2] *Department of Biotechnology and Biochemical Engineering, Sree Buddha College of Engineering, Pattoor, Alappuzha, Kerala, India*

Correspondence
Sony Paul
(sonymarypaul@gmail.com)

Received
05.09.2014
Accepted
28.09.2014

Key Words
Chitosan; Chitosan Derivatives; Chitosan–Zn Complex; Glucosamine Hydrochloride; Antibacterial Activity; *E. coli*; *Klebsiella* spp.; *Proteus* spp.

Background: Chitosan (deacetylated chitin) and its derivatives, which are known to possess pharmaceutical and biomedical properties, have gained considerable attention in the biomedical field. The nontoxic, biocompatible, and biodegradable nature of chitosan makes it possible to use it for therapeutic purpose. It exhibits antibacterial effect on both Gram-positive and Gram-negative cell wall types of bacteria and so-called "antimicrobial polysaccharide."
Aims and Objective: To study the antibacterial activities of native chitosan and its derivatives against three Gram-negative bacteria (*Escherichia coli*, *Klebsiella* spp., and *Proteus* spp.).
Materials and Methods: Bactericidal activity of native chitosan, chitosan hydrolysates obtained by hydrogen peroxide (H_2O_2) and lysozyme degradation, chitosan–zinc (Zn) complex, and glucosamine hydrochloride was tested against ATCC strains of *E. coli*, *Klebsiella* spp., and *Proteus* spp. and clinical isolates of *E. coli*, *Klebsiella* spp., and *Proteus* spp. procured from a clinical diagnostic laboratory. Statistical analysis was done using SPSS software, version 20.
Results: Both chitosan and its derivatives markedly inhibited the growth of most bacteria tested; however, the effects differed with regard to the type of the bacterium. The minimum inhibitory concentration for *E. coli* ATCC strain was 50 µg and its bactericidal activity was dose dependent. Chitosan hydrolysates also exhibited an inhibitory effect, although differences were seen among strains. Degrades from H_2O_2 had higher activity than native chitosan. Lysozyme-degraded chitosan had less activity compared to hydrolysates obtained from H_2O_2 hydrolysis. Chitosan–Zn complex also showed wide spectrum of antimicrobial activities against all the microorganisms tested and the effects were found to increase with increasing chelate ratios. No antibacterial effect was observed in the case of monomer glucosamine hydrochloride, showing that only oligomers have bactericidal effect.
Conclusion: The antibacterial potential of chitosan and its derivatives is considerable and its prospect to be developed as a chemotherapeutic agent is high.

INTRODUCTION

The modern antibiotic era heralded with the advent of the application of penicillin in 1941, almost a decade later after its discovery by Sir Alexander Fleming. Although antibiotics were truly wonder drugs at the time of their introduction, it was not long before resistant bacterial strains emerged. Resistance to antimicrobial agents has resulted in morbidity and mortality from treatment failures and increased health-care costs. In view of these possible difficulties in the course of antimicrobial therapy, it is desirable that some new nontoxic, natural antimicrobial should be studied and applied. The emergence of continuous and ongoing nature of resistance and its dissemination has led to the study of reliability of nature's versatile biomaterial "chitosan," and its antimicrobial activity has received considerable attention recently.

Chitosan [poly-β-(1→4)-glucosamine] is the deacetylated product formed by treatment of chitin (a major component of the shells of crustaceans) with concentrated (50%) caustic alkali.[1] The safety, biocompatibility, and biodegradability nature of chitosan has made it possible to be used for various pharmacological and clinical purposes in the past 20 years.[2–4] Its antibacterial property is attributed to the disruption of barrier properties of outer membrane of Gram-negative bacteria[4,5] resulting in leakage of intracellular substances.[1,6,7] Inhibition of mRNA synthesis also contributes to the antibacterial

effect.[1,8] The antibacterial effect greatly depends on the molecular weight, degree of deacetylation, and degree of polymerization.[9] Literature states that a mild degradation of chitosan enhances its antimicrobial action, whereas highly degraded chitosan displayed no antimicrobial action.[10] Zinc being the easiest metal ion to be able to coordinate with chitosan and has got its own bactericidal properties. Bactericidal properties of the complex were enhanced with increasing chelate ratios.[11] Chitosan–Zn complex has attracted great interest for its potential use as medicament in recent times. But currently reports on antibacterial activity of this complex are scarce.

This study emphasizes the antibacterial action of chitosan and its derivatives against the three most common coliforms of medical importance, which are potential nosocomial pathogens, namely *Escherichia coli*, *Klebsiella* spp., and *Proteus* spp. Also, as infecting strains from hospital environment are multidrug resistant, they are clinically significant and can cause much more hardships. *E. coli* is the most common cause of nosocomial infection following procedures such as catheterization, cystoscopy, and abdominal and gynecological surgeries.[12] *Klebsiella* causes pneumonia in middle-aged individuals and elderly with underlying problems such as diabetes mellitus, alcoholism, or chronic bronchopulmonary disease and also hospital infection in surgical wound and device-associated urinary tract infection.[12,13] *Proteus* spp. is often isolated in domiciliary patients with diabetes or structural abnormalities of urinary tract and in hospital patients after instrumentation.[14]

It is evident from the references cited earlier that different bacterial species under this study can cause serious threats to public, and moreover, there are multidrug-resistant strains that pose severe problems. If a natural polymer like chitosan or its derivatives is found to have an antagonistic effect against bacteria dealt in this study, it can be used for therapeutic purposes. As the polymer is nontoxic and biodegradable, the hardships of patients due to antibiotic resistance can be overcome. Even though studies on antimicrobial and antifungal activities of chitosan for food preservation are being conducted extensively, studies for clinical use are considerably less. This study is undertaken on the basis of these aspects.

In this study, an attempt has been made to evaluate the antibacterial activity against ATCC strains of *E. coli* ATCC25922, *Klebsiella pneumoniae* ATCC13883, and *Proteus mirabilis* ATCC29906 and clinical isolates of *E. coli*, *Klebsiella* spp., and *Proteus* spp. of (1) native chitosan, (2) degrades of chitosan with hydrogen peroxide (H_2O_2), (3) degrades of chitosan with lysozyme, (4) chitosan–Zn complex, and (5) glucosamine hydrochloride.

MATERIALS AND METHODS

Preparation of Chitosan and Its Derivatives: Chitosan was obtained as gift sample from Central Institute of Fisheries Technology (CIFT), Kochi, Kerala, India. The DD values for native chitosan and chitosan used for degradation purposes were 90^+ and 82, respectively. Their molecular weights were 72.16×10^3 and 8.401×10^4, respectively, as measured using CT 050 viscometer (Schott-Geräte, Germany). Viscosity of 1 g chitosan in 1% acetic acid was recorded as 90 cp for $DA90^+$ and 270 cp for DA82 using VISCO BASIC Plus (Fungilab). Glucosamine hydrochloride was also obtained as a gift sample from CIFT. Reagents and enzymes for preparation were supplied by Spectrum Chemicals, Loba Chemie, and Nice Chemicals (all in Kochi, Kerala, India).

Assay of Antibacterial Activity of Native Chitosan: Chitosan solution ($DA90^+$) was prepared in 1% acetic acid at a concentration of 1%, and the pH was adjusted to 6.0 before autoclaving.[15] The chitosan solution was freshly prepared for each day's assays.

Degradation of Chitosan using H_2O_2: 1% chitosan solution (DA82) was degraded according to oxidative–reductive method.[15,16] Degraded solutions were stored at 4°C, and the antibacterial susceptibility test was done within 24 h.

Degradation of Chitosan using Lysozyme: Lysozyme (3 mg) was dissolved in 1 mL KCl (0.1 M). The solution was prepared freshly for each day's work. Enzymatic degradation of chitosan was carried out with already prepared lysozyme solution and stored at 4°C.[15–17] The antibacterial activity was tested within 24 h.

Preparation of Chitosan–Zn Complex: Five chitosan–Zn complexes with different Zn contents were prepared by adding desired amount of 1% $ZnSO_4.7H_2O$ to 1% chitosan solution in acetic acid to get final concentrations of 4:1, 2:1, 1:1, 0.5:1, and

0.25:1. The pH was adjusted to 7.0 by adding 1 N NaOH after stirring. These are kept at 80°C for 3 h in water bath shaker. The mixture was poured into 8 mL acetone after ambient temperature was attained. The white precipitate obtained by filtration was washed repeatedly with ethanol and finally dried.[18] Chitosan–Zn complexes were autoclaved and tested for bactericidal activity.

Evaluation of Antibacterial Activity: ATCC strains of *E. coli* ATCC25922, *K. pneumoniae* ATCC13883, and *P. mirabilis* ATCC29906 and clinical isolates of *E. coli*, *Klebsiella* spp., and *Proteus* spp. were procured from clinical diagnostic laboratory.

ATCC strains of *E. coli*, *K. pneumoniae*, and *P. mirabilis* and clinical isolates of *E. coli*, *Klebsiella* spp., and *Proteus* spp. in pure culture were inoculated into peptone water and incubated at 37°C for 2 h. Turbidity was adjusted to 0.5 McFarland standard (1.5×10^8 CFU/mL).[19] The assays were carried out by measuring the diameter of zone of inhibition by Kirby–Bauer agar diffusion technique, in particular, ditch plate method.[20,21] Lawn culture was done on Müller–Hinton agar plate. Wells of standard diameter (5 mm) were bored in the medium using sterile well puncher. About 2, 5, 10, 20, 30, and 40 µL chitosan solutions and 40 µL acetic acid (pH 6, control) were added to the wells. The chitosan concentration in each well was 20, 50, 100, 200, 300, and 400 µg, respectively. The chitosan hydrolysates of H_2O_2 degradation, lysozyme degradation, and chitosan–Zn complexes (40µg each) of varying ratios were added to the respective wells. H_2O_2, lysozyme, and zinc solutions (40 µL each) were, respectively, tested as controls. Varying concentrations (0.5%, 1%, 2%, 3%, and 30%) of glucosamine hydrochloride solution (40 µL) in water were also examined for antibacterial effect.

The plates were then incubated at 37°C for approximately 18–24 h. Next day the diameters of zones of inhibition produced by varied chitosan concentration and its derivatives against the test organisms were measured.

Statistical Analysis: All statistical analyses were performed using SPSS software, version 20. One-way analysis of variance was done to compare the antibacterial activity of native chitosan and its hydrolysates at different concentrations. Linear regression analysis was done to analyze whether bactericidal activity was enhanced with increase in concentration of Zn. The level of significance was considered to be 5%.

RESULTS

Antibacterial Activity of Native Chitosan against Bacterial Species: Table 1 shows the antibacterial activity of native chitosan against the three bacterial species. The diameter of zone of inhibition for *E. coli* ATCC strain 25922 ranged from 19 to 29 mm for concentrations of 100—400 µg. At 20 µg chitosan concentration, the growth was not inhibited. The lowest concentration at which the growth was inhibited for *E. coli* ATCC strain was 50 µg and the diameter of zone of inhibition was 15 mm. The diameter of zone of inhibition increased by an average of 3 mm with the increase in concentration. In this study, acetic acid with pH adjusted to 6.0 was used as control and showed no inhibitory activity.

K. pneumoniae ATCC strain 13883 was not sensitive at chitosan concentrations 20 and 50 µg. For chitosan concentration 100 µg, the diameter of zone of inhibition was 15 mm. The size varied from 10 to 21 mm for concentrations 100—400 µg. The antibacterial activity was dose dependent. At 400 µg concentration, the inhibitory zone lies between 13 and 21 mm for most of the clinical strains. 65% of isolates were effectively inhibited by chitosan.

In the case of ATCC strain of *P. mirabilis*, 50 µg chitosan could inhibit the growth. *Proteus* spp. was also effectively inhibited by chitosan and the diameter of zone of inhibition ranged from 20 to 30 mm for concentrations 200–400 µg. Here also an increase in diameter was noticed with increase in concentrations.

Antibacterial Activity of Chitosan Hydrolysates:

1. *Hydrolysates from H_2O_2 hydrolysis*: Chitooligosaccaharides had greater inhibitory activity than native chitosan against all the three species. The ATCC strains had higher activity, and here also the action was dependent on concentration of H_2O_2 (Table 2). H_2O_2 kept as control could not inhibit any of the clinical strains. Among the three species, *Proteus* spp. was most inhibited.
2. *Hydrolysates from lysozyme degradation*: With lysozyme degradation, the trend was not the same as in chemical degradation. The diameter of the inhibitory zones was between 7 and 15 mm in most of the cases in the study.

Table 1: Antibacterial activity of native chitosan

Concentration of chitosan (µg)	Organisms								
	E. coli			*Klebsiella* spp.			*Proteus* spp.		
	Mean	SD	Confidence interval at 95%	Mean	SD	Confidence interval at 95%	Mean	SD	Confidence interval at 95%
100	10.53	3.9	8.5–12.5	9.9	2.7	8.5–11.3	12.9	3.8	10.8–15
200	13.5	4.4	11.2–15.7	12.8	3.1	11.2–14.3	17	4.7	14.4–19.5
300	16.4	4.8	16.5–21.4	16	3	14.5–17.5	19.5	4.3	17.1–21.8
400	19	4.8	16.5–21.5	18.1	2.9	16.7–19.8	22	4.1	20–24.4
p-Value	<0.001			<0.001			<0.001		

Table 2: Antibacterial activity of chitosan hydrolysates with H_2O_2

Concentration of H_2O_2 (mM)	Organisms								
	E. coli			*Klebsiella* spp.			*Proteus* spp.		
	Mean	SD	Confidence interval at 95%	Mean	SD	Confidence interval at 95%	Mean	SD	Confidence interval at 95%
0	17.8	5.5	10.9–24.6	13	2.5	9.8–16.2	14	1.9	11.7–16.3
0.2	18.2	5.2	11.7–24.7	13.6	2.1	11.1–16.2	15.8	1.8	13.6–18.02
1	18.6	6.8	10.1–27.1	14.4	2.2	11.7–17.1	16.4	2.6	13.2–19.7
5	18.6	6.4	10.6–26.6	14.8	2.3	12–17.6	16.8	2.4	13.4–19.8
10	20.2	5.2	13.8–26.7	15.6	1.5	13.7–17.5	19	3	15.4–22.6
25	20.4	5.8	13.2–27.6	16	1.6	14–18	19.2	3	15.4–23
50	21.4	5.3	14.8–27.9	16.4	1.1	15–17.8	19.6	3.4	15.3–18.3
p-Value	>0.05			>0.05			<0.05		

Antibacterial Activity of Chitosan–Zn Complex: Chitosan–Zn complex was found to show a wide spectrum of antimicrobial activities against all the microorganisms used in this study (Table 3). Among the bacteria under study, *E. coli* was the most inhibited. The inhibitory action of the complex was found to increase with increasing chelate ratios. Complexes were found to be two times active than $ZnSO_4$. The diameters of zones of inhibition ranged between 27 and 15 mm.

Table 3: Antibacterial activity of chitosan–Zn complex

Microorganism	Regression coefficient	*p*-Value
E. coli	0.779	<0.001
Klebsiella spp.	0.832	<0.001
Proteus spp.	0.857	<0.001

Antibacterial Activity of Glucosamine Hydrochloride: No antibacterial activity was observed in this case. From this, it is clear that the bactericidal property is not due to monomer glucosamine units.

DISCUSSION

The chitosan concentration (1%) used in this study was same as that used in a clinical trial to study the effect of chitosan on plaque formation.[22] In this study, chitosan exhibited an excellent antibacterial activity against all the three bacteria tested, however the inhibitory effects differed with regard to different bacterial species. *Proteus* spp. was the least inhibited by chitosan among the bacteria studied. Activity was dose dependent. Chitosan with a high molecular weight (500–1000 KD) and maximum degree of deacetylation shows enhanced antimicrobial activity even at lower concentrations. The mean values of diameters of zones of inhibition for chitosan concentrations 100–400 µg lie between a minimum of 9.9 mm to a maximum of 22 mm for different bacterial species. The level of significance was <0.001, which was within the range of significance (0.00–0.05), thus indicating that the increase in concentrations does influence the antibacterial effect of chitosan. Thus, chitosan shows great potential in developing into a biocompatible antibiotic.

Recent studies prove that coating chitosan as an anti-biofilm for implantable medical devices, wound dressings, catheters, and contact lenses is highly effective in retarding or preventing the formation of biofilms.[23] Hypothesis is that it disrupts cell membrane as microbes settle on the surface, being even superior to coatings impregnated with antimicrobial agents such as chlorhexidine.[24]

Chitosan hydrolysates possessed significant antibacterial activity. In this study, the chitosan degrades had higher activity against *E. coli*, *Klebsiella* spp., and *Proteus* spp. Here also, differences existed among the strains. The bactericidal action was much more pronounced in the case of *E. coli*. Although the degrades had inhibitory activity, significant difference in inhibition was not noted in the bacterial species except for *Proteus* spp. ($p > 0.05$ for *E. coli* and *Klebsiella* spp.). The activity of lysozyme degradation was much less compared to that of hydrolysates from H_2O_2 hydrolysis. In this experiment, the concentration of hen egg white

lysozyme is 0.025% (w/v), which is approximately 30 times the normal level of human lysozyme in the serum.[25,26] Thus, the degradation rate will be considerably less than that in this study, which may result in good activity.

Degradation of chitosan by H_2O_2 differs fundamentally from enzymatic degradation in many aspects.[15] Chemical method is a random process, whereas enzymatic process is nonrandom and bond specific. In more general terms, using H_2O_2 to degrade chitosan could be problematic in terms of potential toxicity. The oligomers purified by this method would probably need to be purified further to remove the excess iron. Lysozyme degradation, however, is a natural and cheaper means, and it is readily available.

Chitosan–Zn complex had better bactericidal activity than native chitosan and $ZnSO_4$. Here also, inhibitory effect varied with each bacterium and *E. coli* was the most sensitive. Linear regression analysis proved that the antibacterial activity increases with increase in concentration of zinc used ($p < 0.001$) in all the three microorganisms. Among the microorganisms tested, ideal inhibiting effects could be obtained when chelate ratios of complex were above 1:1. Thus, proper antimicrobial activity of the complex could be obtained by controlling the amount of Zn salt in the preparation. This attribute is favorable to its application in the medical field.

Glucosamine hydrochloride did not exhibit any inhibitory activity in this study. It is clear that the bactericidal property is not due to the monomer glucosamine units but is associated with the chain length of the polymer and suggests a cooperative effect of individual sugar units.

Limitation of Study: Although the *in vitro* activity is critical, the pharmacokinetic properties of chitosan have to be studied before developing it as an antimicrobial. A standard susceptibility test for chitosan has to be formulated and *in vivo* trials have yet to be undertaken before implementing chitosan has an efficient antimicrobial agent.

CONCLUSION

We can conclude from the results of this study that chitosan and its derivatives are very effective antibacterial agents. Thus, they can face the global problem of emergence of resistance and overcome the limitations, namely, toxicity. The antibacterial potential of chitosan and its derivatives is high as outlined and indisputably the prospect is great to develop a chemotherapeutic agent using chitosan.

ACKNOWLEDGMENTS

We extend our sincere thanks to Sumitra G (Lecturer, Department of Biostatistics, AIIMS, Delhi) for her guidance and cooperation in carrying out the statistical analysis and to Dr. PT Mathew (Senior Scientist, By Products Dept., CIFT, Kochi, Kerala, India) for providing the chitosan samples.

REFERENCES

1. Sugumar G, Mariappan S, Kalaimaniarasi P, Sangeetha S, Velayutham P. Antibacterial activity of chitosan. In: Surendran PK, Boopendranath MR, Lakshamanan PT, Nambiar VN, Joseph J, Thampuran N, Nair PGV (Eds.), *Seafood Safety*. Cochin, India: Society of Fisheries Technologists, 2003. pp. 434–41.
2. Mattioli BM, Muzzarelli B, Muzzarelli RAA. Chitin and chitosan in wound healing and other biomedical application. Carbohydr Eur. 1997;19:30–6.
3. Shigesmasa J, Minami S. Applications of chitin and chitosan for biomaterials. Biotechnol Genet Eng Rev. 1995;13:383–420.
4. Balassa LL, Prudden JF. In: Muzzarelli RAA, Pariser ER (Eds.), *Proc. First International Conference on Chitin/Chitosan*, 1978. pp. 296–305.
5. Leuba LA, Stossel P. In: Muzzarelli R, Jeuniaus C, Gooday G. (Eds.), *Chitin in Nature and Technology*, New York: Plenum Press, 1986. pp. 215–21.
6. Tsai GJ, Su WH. Antibacterial activity of shrimp chitosan against *E. coli*. J Food Prot. 1999;62:239–43.
7. Yadav AV, Bhise SB. Chitosan: a potential biomaterial effective against typhoid. Curr Sci 2004;87(9):1176–8.
8. Jeon JY, Park JP, Kim KS. Antimicrobial effect of chitooligosaccharides produced by bioreactor. Carbohydr Polym. 2001;44:71–6.
9. Zivanovic S, Basurto CC, Chi S, Davidson PM, Weiss J. Molecular weight of chitosan influences antimicrobial activity in oil water emulsions. J Food Prot. 2004;67(5):952–9.
10. Raafat D. *Chitosan as an Antimicrobial Agent: Modes of Action and Resistance Mechanisms*. Dissertation, University of Bonn, 2008.
11. Jeon J, Yim KS. Production of chitooligosaccharides using an ultrafiltration membrane reactor and their antibacterial activity. Carbohydr Polym. 2000;41:133–41.
12. Shanson DC. Hospital infection. In: *Microbiology in Clinical Practice*, 3rd edn. Oxford: Butterworth-Heinemann, 1999. pp. 429–58.
13. Pamela BC. Enterobacteriaceae: Escherichia, Klebsiella, Proteus and other genera. In: Collee JG, Marmion BP, Fraser AG, Simmons A, editors. Mackie and McCartney Practical Medical Microbiology. 14th ed. UK: Churchill Livingstone; 2007. pp. 361–367.
14. Ananthanarayan R, Panicker JCK. Enterobacteriaceae I: Coliforms—*Proteus*. In: *Textbook of Microbiology*, 6th edn. Hyderabad: Universities Press; 2000. pp. 250–61.
15. Rhodes J, Roller S. Antimicrobial actions of degraded and native chitosan against spoilage organisms in laboratory media and foods. Appl Environ Microbiol. 2000;66:80–6.
16. Nordtveit RJ, Varum KM, Smidsrod O. Degradation of fully water-soluble partially *N*-acetylated chitosans with

lysozyme. Carbohydr Polym. 1994;23:253–60.
17. Nordtveit RJ, Varum KM, Smidsrod O. Degradation of partially *N*-acetylated chitosans with hen egg white and human lysozyme. Carbohydr Polym. 1996;29:163–7.
18. Wang X, Du Y, Liu H. Preparation, characterization and antimicrobial activity of chitosan–Zn complex. Carbohydr Polym. 2004;56:21–6.
19. Brown R, Poxton I R, Wilkinson J F. Centrifuges, colorimeters and bacterial counts. In: Collee J G, Duguid J P, Fraser A G, Marmion B P, editors. Practical medical microbiology. 13th ed. Edinburgh, United Kingdom: Churchill Livingstone; 1989. pp. 240–7.
20. British Pharmacopoeia, Department of Health, Scottish Home and Health Department Welsh 1973, Int. edn. A 102 Appendix XIVA.
21. Sir Alexander Fleming—Nobel Lecture: Penicillin. *Nobelprize.org*. Nobel Media. Available at: http://www.nobelprize.org/nobel_prizes/medicine/laureates/1945/fleming-lecture.html
22. Bae KE, Jun J, Lee SM, Paik DI, Kim JB. Effect of water-soluble reduced chitosan on *Streptococcus mutans*, plaque regrowth and biofilm vitality. Clin Oral Investig. 2006;10:102–7.
23. Carlson RP, Taffs R, Davison WM, Stewart PS. Antibiofilm properties of chitosan-coated surfaces. J Biomater Sci Polym Ed. 2008;19:1035–46.
24. Anghel I, Limban C, Grumezescu AM, Anghel AG, Bleotu C, Chifiriuc MC. *In vitro* evaluation of anti-pathogenic surface coating nanofluid, obtained by combining Fe_3O_4/C_{12} nanostructures and 2-((4-ethylphenoxy)methyl)-*N*-(substituted-phenylcarbamothioyl)-benzamides. Nanoscale Res Lett. 2012;7:513.
25. Henry JB. *Clinical Diagnosis Management by Laboratory Methods*, 18th edn. Philadelphia: Saunders, 1991. p. 1371.
26. Temel A, Kazokoglu H, Taga Y. Tear lysozyme levels in contact lens wearers. Ann Ophthalmol. 1991;23:191–4.

Cite this article as: Paul S, Seema NP. *In vitro* antibacterial potential of chitosan and its derivatives on pathogenic enterobacteriaceae. Natl J Physiol Pharm Pharmacol 2015;5:119-124.
Source of Support: Nil
Conflict of interest: None declared