J Vet Clin 24(2) : 99-103 (2007)

Journal of Veterinary Clinics
www.ksvc.or.kr

Antibacterial Efficacy of Chitosan against *Staphylococcus intermedius* in Dogs

Hyo-Hoon Jeong*, Keun-Woo Lee and Tae-Ho Oh[1]

College of Veterinary Medicine, Kyungpook National University, Daegu 702-701, Korea
**Korea Racing Association, Gwacheon 427-711, Korea*

(Accepted: June 15, 2007)

Abstract : The antibacterial efficacy of 0.1% (w/v) chitosan solution against *Staphylococcus intermedius* isolated from a dog with superficial pyoderma was evaluated *in vitro* and *in vivo*. The exposure time for the 0.1% chitosan solutions at different pH to be able to eliminate the bacterial cells and the effect of pH of the solutions on antibacterial activity was tested at the same time *in vitro*. The antibacterial activity of chitosan was compared to other antibacterial agents including 2.5% benzoyl peroxide, 0.5% chlorhexidine acetate, 0.1% chitosan solution combined with 2.5% benzoyl peroxide and chitosan combined with 0.5% chlorhexidine using a modified detergent scrub quantitative technique in 10 adult mongrel dogs *in vivo*. They were able to eliminate a number of bacteria after the exposure time of 10 minutes at varying degrees according to the pH of the solutions. The antibacterial activity of chitosan was inversely affected by pH with higher activity at lower pH value. The 0.1% chitosan solution was also efficacious against *Staphylococcus intermedius in vivo*. The combinations of chitosan with benzoyl peroxide and with chlorhexidine were shown to exert higher activity when compared to those of chitosan alone and benzoyl peroxide or chlorhexidine alone. The 0.1% chitosan solution was considered to be efficacious against *Staphylococcus intermedius* isolated from a dog with superficial pyoderma in both *in vivo* and *in vitro* and have a potential for the clinical applications in the treatment of pyoderma in dogs.

Key words **:** chitosan, *Staphylococcus intermedius,* dog, pyoderma.

Introduction

Staphylococcal skin infection, pyoderma is a common clinical problem in dogs (6). Most pyoderma are considered as secondary infections due to their concurrent primary diseases that predispose the skin to allow the growth of abnormally high numbers of pathogenic bacteria. Treatment of primary causes is considered to be the most important part of therapy followed by appropriate systemic antibiotic therapy concurrent with topical treatment. Topical treatments are used to reduce or eliminate the bacterial population in and around area of infection and to remove tissue debris (21). Commonly used agents include chlorhexidine, povidone-iodine, ethyl lactate, benzoyl peroxide, and various antibiotics, especially fusidic acid, mupirocin, and bacitracin. In general, shampoos are the most widely applied because lesions of pyoderma are often widespread. The use of creams, gels and ointments is generally inappropriate because of the dense hair coat of dogs and cats, and the tendency for dogs and cats to lick off medications (5). Although the topical agents mentioned above have been proven to be effective clinically, there are some adverse effects reported occasionally such as erythema, pain, pruritus, staining and irritation.

Chitin, a β-(1.4)-D-linked polymer of N-acetylglucosamine, is a common constituent of crustacean and arthropod cell walls and is extracted commercially from shellfish wastes (23). Chitosan is the deacetylated form of chitin. It is said to be the second most abundant natural biopolymer on earth next to cellulose, being widely distributed in nature, and occurring in arthropods, crustaceans, fungi, and yeast (19). The chemical structure of chitosan is similar to that of cellulose with 2-amino-2-deoxy-D-glucose monomers attached via β-(1.4) linkages. It exhibits various promising biological activities, including hemostatic activity, antimicrobial activity, and biodegradability (8).

The objective of this study was to investigate the antibacterial activity of chitosan against *Staphylococcus intermedius* isolated from a dog with superficial pyoderma *in vivo* and *in vitro* so as to assess the potential for the clinical application of chitosan in the treatment of pyoderma as a natural antibacterial agent.

Materials and Methods

Materials

Chitosan from crab shells having a degree of deacetylation of 85% was purchased from Sigma Chemical Company. Growth media were obtained from Difco. All chemicals were purchased from Sigma Chemical Company unless otherwise

[1]Corresponding author.
E-mail : thoh@knu.ac.kr

specified. The pH meter (MP220 K, Mettler Toledo Inc., USA) was used to measure and adjust the pH of the solutions.

Microorganism

Staphylococcus intermedius was isolated from a pustular lesion from a dog with recurrent superficial pyoderma. The isolated microorganism was identified and confirmed to be *S. intermedius* on the bases of gross appearance, pigment, hemolytic pattern, and the results of Gram stain, catalase and coagulase tests, and biotyping using the API STAPH-IDENT System® (bioMerioux Co., France) and Biolog® (Microstation Co., USA). The organism was grown for 18 hours at 37°C in tryptic soy broth.

Antibacterial activity of chitosan *in vitro*

Chitosan solutions were prepared in 1% (v/v) acetic acid at a concentration of 1% (w/v) before being applied to broth and were added to Muller Hinton broth to give a final chitosan concentration of 0.1% (w/v). The pH of chitosan added Muller Hinton broth solutions were adjusted to 4.5, 5.0, 5.5 and 5.9 with 1 N HCl and 1 N NaOH to assess the effect of pH on the antibacterial activity. Fifty μl of test bacterium subcultured in tryptic soy broth at 37°C for 18 hours was inoculated into each 10 ml of chitosan added Muller Hinton broth solutions. The 1% acetic acid added Muller Hinton broth was also inoculated with bacterium in the same manner described above as control. One hundred μl of samples were removed after 10, 30 and 120 minutes of exposure time and subjected to ten-fold serial dilutions. Aliquots (50 μl) were spread on tryptic soy agar plates in triplicate, incubated at 37°C for 48 hours and colony-forming units (CFUs) were counted.

Antibacterial activity of chitosan *in vivo*

Dogs

Ten adult mongrel dogs weighing 3.7 ± 0.7 kg were used. All dogs were free of any visible skin lesion and had normal hair coat as determined by physical examination. Results of a CBC and serum chemistry values were within normal range for each dog. The total T_4, fT_4 and total T_3 also were in normal range. No topical treatment had been applied to any of the dogs for at least 2 weeks prior to the study. They were housed individually in indoor runs, and food and water were available at all times.

Preparation of test antibacterial solutions

The chitosan solution was prepared in the same way as *in vitro* assay except that the diluent was sterile distilled water to give the final concentration of 0.1% (w/v) of chitosan *in vivo* experiment. The pH of chitosan solution was adjusted to 4.5 regarding its relatively stronger antibacterial activity shown in vitro. Benzoyl peroxide (2.5%) and chlorhexidine acetate (0.5%) were chosen to be compared their antibacterial activities with that of chitosan. The combinations of 0.1% chitosan solution with 2.5% benzoyl peroxide shampoo and 0.5% chlorhexidine at the ratio of 9:1 also were tested for synergistic effects of chitosan. The two commercially available shampoos were tested as 1:10 aqueous dilutions of the commercial formulations to mimic more closely their clinical use.

Applications of bacteria and test antibacterial solutions

Seven circular areas on the lateral thorax of each dog were prepared. Five sites were to be tested with the 5 test antibacterial solutions and the rest were used as control sites. Hair on the thorax was clipped using a sterilized No. 40 electric clipper blade. Barrels of 30-ml syringes were cut approximately 2 cm from the flanged end. The syringe barrels were attached with cyanoacrylate adhesive to demarcate the designated test sites. The flanged end attached to skin with cyanoacrylate adhesive provided an air and water-tight seal. 10 μl of subcultured *S. intermedius* suspension was dispensed on each test site and 1 of 2 control sites using a sterile micropipette and spread with the tip of micropipette. Each test and control site was occluded for 5 hours with the sterile rubber plunger without the plunger touching the skin surface. One of the 2 control sites was not inoculated with the bacteria to determine the effect of occlusion alone on growth of resident *S. intermedius* during the occlusion period. 0.3 ml of each test solutions was then applied to the test sites after the 5 hours of occlusion with 1.0 ml pipettes, and the sites were spread evenly with the tip of the pipettes used. The test sites were left for 10 minutes with the solutions left on. The 2 control sites were treated in the same fashion except that normal saline was used instead of the antibacterial solutions during the application period.

Removal of bacteria

Bacteria were removed after 10 minutes of exposure time using the quantitative cup-scrub technique of Williamson and Kligman (7). Triton X-100 was replaced with Tween 80 as the scrubbing detergent. 0.7 ml of 0.1% Tween 80 in 0.075 M phosphate-buffer solution at pH 7.9 was placed into each syringe. Each area was scrubbed for 1 minute with a sterile micropipette tip. The resultant solutions were aspirated and transferred to the sterile vials. From the removed samples of all sites, serial dilutions of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} were made. The tryptic soy agar was inoculated with 50 μl of the full-strength scrub suspension and with each of the serial dilutions. The inoculated agar plates were incubated for 48 hours at 37°C and then total number of colony-forming units was counted using a lighted grid colony counter. Plates with ≥ 30 ≥ and ≥ 300 colonies were used for counting. Total number of *S. intermedius* CFU/cm^2 was calculated by the formula shown below:

$$CFU/cm^2 = (nxV_2)/AV_1$$

Where n = dilution factor used for the colony count, x = CFU for the dilution counted, V_2 = volume of the sample, A = area of the syringe casing, and V_1 = volume used on the plate (9). The colonies were confirmed as *S. intermedius* as described above.

Statistical analysis

Mean values were calculated in the number of *Staphylococcus intermedius* CFU/cm^2 of skin for each of the 5 solutions and

2 controls. Values were expressed $\log_{10}$ of CFU/cm^2. Data were compared by analysis of variance and by the Student-Newman-Keuls test. A probability value of 0.05 was considered to be significant.

Results

Antibacterial activity of chitosan against *S. intermedius in vitro*

All the numbers of the recovered bacteria were taken $\log_{10}$ values. The mean value of the inoculum of *S. intermedius* was 6.04 CFU/ml. The changes in the number of bacteria after the predetermined exposure time with 4 different chitosan added Muller Hinton broth solutions of which the pH adjusted to 4.5, 5.0, 5.5 and 5.9, and with 1% acetic acid added broth solutions are tabulated below.

As seen in the table, all the 0.1% (w/v) chitosan solutions showed significant antibacterial activities. The antibacterial activity was inversely affected by pH of the solutions exerting the highest activity at pH 4.5. It was shown that the inoculated bacteria were decreased significantly in 10 minutes of exposure time and prolonged exposure time enhanced the activity.

Antibacterial activity of chitosan against *S. intermedius in vivo*

The 0.1% chitosan solution was found to be efficacious against *S. intermedius in vivo*. The results are summarized in Table 2. The number of bacteria in the initial inoculum was 6.61 CFU/cm^2 of skin. After 5 hours of occlusion period, the mean value of 6.95 CFU/cm^2 of skin of *S. intermedius* was recovered from the nontreated bacteria-challenged sites. This indicated that the viability of the inoculated organism was actually maintained on skin for the 5 hours of the test period. The mean value of 2.13 CFU/cm^2 was recovered from the non-treated, non-bacteria-challenged control sites. The difference between the numbers of the inoculated bacteria and those recovered from the skin not having been inoculated was significant. Therefore it was assumed that the quantity of preexistent *S. intermedius* on the skin surface did not contribute to the population in the bacteria-challenged sites. All of the recovery rates from the treated sites were significantly lower than that from the non-treated, bacteria-challenged sites. Thus, all of the tested solutions were considered to have significant antibacterial activities against *S. intermedius*. Both of the chitosan solutions combined with benzoyl peroxide and chlorhexidine showed stronger antibacterial activities than that of the chitosan solution alone.

Discussion

The unusual antimicrobial activity of chitin, chitosan and their derivatives against different groups of microorganisms, such as bacteria, yeast and fungi has received considerable attention in recent years (3,4,16,24,30). The antimicrobial action of chitosan has been postulated to occur by several mechanisms (17,28). Chelation is an important action of chitosan. Deprivation of metals, trace elements of essential nutrients by chelation limits the growth of microorganisms. Chitosan

Table 1. Antibacterial activity of Chitosan in vitro. ($\log_{10}$ CFU/ml)

Chitosan added Muller Hinton broth	No. of bacteria in the inoculum	Exposure time		
		10 mins	30 mins	120 mins
pH 4.5	6.04	3.00	2.60	2.30
pH 5.0	6.04	4.15	3.15	3.08
pH 5.5	6.04	4.78	4.30	3.38
pH 5.9	6.04	5.20	5.00	4.82
Control[a)]	6.04	6.30	6.11	5.97

a) 1% acetic acid added Muller Hinton broth

Table 2. Antibacterial efficacy of Chitosan in vivo. ($\log_{10}$ CFU/cm^2)

Test antibacterial solutions	No. of bacteriain the inoculums	No. of bacteria recovered
CT[a)]	6.61	3.25
BP[b)]	6.61	0.68
CHX[c)]	6.61	3.14
CT+BP[d)]	6.61	0.48
CT+CHX[e)]	6.61	2.55
CBC[f)]	6.61	6.95
CNBC[g)]	6.61	2.13

a) 0.1% chitosan solution, b) 2.5% benzoyl peroxide, c) 0.5% chlorhexidine
d) 0.1% chitosan solution combined with 2.5% benzoyl peroxide,
e) 0.1% chitosan solution combined with 0.5% chlorhexidine
f) Bacteria-challenged control, g) Nonbacteria-challenged control

has also pH dependent ability to interact with and flocculate proteins (17). In our study, the antibacterial activity of chitosan was shown in only 10 minutes of exposure time. This result is in a good agreement with the initial rapid decrease in the viability of the organisms (2). This indicates that exposure to the chitosan either rapidly killed the cells or rendered them non-culturable by directly attacking their membranes. We examined antibacterial activity of the chitosan only at low concentration after short-exposure times with intention of investigating its potential practical use as an antibacterial agent against *S. intermedius* but the antibacterial activity could have been enhanced by increasing the concentration of chitosan or the exposure time. The upper pH value studied was limited to 5.9 because chitosan is soluble in most organic acid solutions with less than pH 6 (13). As shown in Table 1, antibacterial activity of chitosan was affected by pH, with greater activity being found at lower pH value. Several workers (14,27,29,31) have reported results comparable to that found in present study.

The method used *in vivo* experiment of this study was modified from previous reports and proved to be a simple, quantitative *in vivo* technique for measuring the antibacterial activity (9,15). Although antibacterial activity can be assessed *in vitro* only, *in vivo* models are necessary to account for the complex factors that make up the skin surface microenvironment, which is difficult to achieve *in vitro*. The heat and moisture produced by occlusion are necessary to support viability of the organism on the intact skin surface. Without occlusion the desiccation and death of the microorganism occur quickly (12). In present study the 30-ml syringe barrel attached to the skin with cyanoacrylate adhesive worked well. Cutaneous inflammation or irritation was not evident where the adhesive was applied. The rubber plunger occluded the cylinders almost perfectly providing an excellent strong seal without touching the skin surface and was easily removed after the occlusion period. This method proved to be adequate for maintaining viability of *S. intermedius* on the intact skin surface.

All 5 tested solutions turned out to have significant antibacterial activity against *S. intermedius*. The benzoyl peroxide showed the best antibacterial efficacy among 3 solely used test antibacterial solutions followed by chlorhexidine and chitosan solutions respectively. Benzoyl peroxide has been known as a very effective antibacterial agent, used in treatment of superficial and deep pyodermas in dogs (6,20,21,26). Undesirable side effects such as erythema, pain and pruritus may be observed following treatment with benzoyl peroxide in domestic animals, especially when generic products are used in concentrations over 5% (20). None of the signs of the side effects was observed at the concentration used in this study. Chlorhexidine is bisguanide compound that is effective against gram-positive and gram-negative bacteria, molds, yeasts, and viruses (22,25). Chlorhexidine has a rapid onset of action and reduces the number of bacteria on the skin almost immediately and has a good residual activity (1,22). The antibacterial effect of chlorhexidine is via action on the bacterial cell wall membrane, precipitation of intracellular contents, and inhibition of adenosine triphosphate (11,18). Although it has been used to lavage open wounds, chlorhexidine is cytotoxic to cells involved in the wound healing process (10). However, Chlorhexidine is known to be relatively non-irritating, non-toxic, rarely sensitizing than other antibacterial agents such as benzoyl peroxide or povidone iodine (11). Chlorhexidine has the advantage of being in an emollient formulation for long-term use on dry skin and coat. Thus it is very useful with a concurrent dry scaling disorder or when irritation from benzoyl peroxide is observed.

The antibacterial activity of chitosan solution was shown to be comparable to that of chlorhexidine solution *in vivo* in present study implying that chitosan has an excellent bactericidal activity against *S. intermedius*. Moreover, the antibacterial activities of single solutions were significantly increased when a relatively small volume of benzoyl peroxide or chlorhexidine was added to the 0.1% chitosan solutions. It is suggested that the rapid action of chitosan on the bacterial cell membrane caused better environment for the benzoyl peroxide or chlorhexidine to exert their activities. The combination of chitosan with these two chemical antibacterial agents would provide their application at lower concentrations thus avoiding their unwanted side effects.

Acknowledgement

This research was supported by Kyungpook National University Research Fund, 2001.

References

1. Amber EI, Wsain SF. An update on common wound antiseptics. Aust Vet Pract 1984; 14: 29-33.
2. Choi BK, Kim KY, Yoo YJ, Oh SJ, Choi JH, Kim CY. *In vitro* antimicrobial activity of a chitooligosaccharide mixture against *Actinobacillus actinomycetemcomitans* and *Streptococcus mutans*. Int J Antimicrob Agents 2001; 18: 553-557.
3. Chung YC, Wang HL, Chen YM, Li SL. Effect of abiotic factors on the antibacterial activity of chitosan against waterborne pathogens. Bioresour Technol 2003; 88: 179-184.
4. Darmadji P, Izumimoto M. Effect of chitosan in meat preservation. Meat Science 1994; 38: 243-354.
5. Guaguere E. Topical treatment of canine and feline pyoderma. Vet Dermatol 1996; **7:** 145-151.
6. Hill PB, Moriello KA. Canine pyoderma. J Am Vet Med Assoc 1994; 204: 334-340.
7. Holland KT, Kearney JN. Microbiology of Skin. In: Skerrow D, Skerrow CJ (eds.). Methods in Skin Research. pp. 433-474, John Wiley & Sons, Chichester, 1985.
8. Kurita K. Chemistry and application of chitin and chitosan. Polym Degrad Stab 1998; 59: 117-120.
9. Kwochika KW, Kowalski JJ. Prophylactic efficacy of four antibacterial shampoos against *Staphylococcus intermedius* in dogs. Am J Vet Res 1991; 52: 115-118.
10. Lemarie RJ, Hosgood G. Antiseptics and disinfectants in small animal practice. Comp Cont Ed Pract Vet 1995; 17:

1339-1351.
11. Lozier SM. Topical wound therapy. In: Hariari J. (ed). Surgical complications and wound healing in the small animal practice. pp. 63-88, WB Saunders, Philadelphia, 1993.
12. Maibach I. Experimentally-induced infections in the skin of mice. In: Maibach HI, Hildick-Smith G. (eds.). Skin bacteria and their role in infections. pp. 85-94, McGraw-Hill Book, New York, 1965.
13. Mathiowitz E. Encyclopedia of controlled drug delivery. pp. 773-774, John Wiley & Sons, New York. 1999.
14. No HK, Park HY, Lee SH, Meyers SP. Antibacterial activity of chitosans and chitosan oligomers with different molecular weights. Int J Food Microbiol 2002; 74: 66-72.
15. Osuna DJ, DeYoung DJ, Walker RL. Comparison of three skin preparation techniques in the dog. Vet Surg 1990; 19: 14-19.
16. Papineau AM, Hoover DG, Knorr D, Farkas DF. Antimicrobial effect of water-soluble chitosans with high hydrostatic pressure. Food Biotechnol 1991; 5: 45-57.
17. Roller S, Covill N. The antifungal properties of chitosan in laboratory media and apple juice. Int J Food Microbial 1999; 47: 67-77.
18. Sanchez IR, Nuxbaum KE, Swaim SF, and et al. Chlorhexidine diacetate and povidone iodine cytotoxicity to canine embryonic fibroblasts and *Staphylococcus aureus*. Vet Surg 1988; 17: 182-185.
19. Sanford PA, Hutchings GP. Industrial polysaccharides: Genetic engineering, structure/property relations and application. pp 363-375, Elsevier, Amsterdam, 1987.
20. Scott DW. Clinical assessment of topical benzoyl peroxide in treatment of canine skin diseases. Vet Med Small Anim Clin 1979; 74: 808-810.
21. Scott DW, Miller WH, Griffin CE. Muller & Kirk' s Small Animal Dermatology. 6th ed. pp. 274-335. WB Saunders, Philadelphia, 2001.
22. Sebben JE. Surgical antiseptics. J Am Acad Dermatol 1983; 9: 759-765.
23. Skjak-Braek G., Anthonsen T, Sandford P. Chitin and chitosan. p 560, Elsevier Applied Science, London, 1989.
24. Sudharshan N., Hoover DG, Knorr D. Antibacterial action of chitosan. Food Biotechnol 1992; 6: 257-272.
25. Swaim SF, Lee AH. Topical wound medications. J Am Vet Med Assoc 1987; 190: 1588-1593.
26. Thompson WD, Mandy SH. Benzoyl peroxide-a new topical agent for canine dermatology. Vet Med Small Anim Clin 1976; 71: 1059-1062.
27. Tsai GJ, Su WH. Antibacterial activity of shrimp chitosan against Escherichia coli. J Food Prot 1999; 62: 239-243.
29. Tsai GJ, Wu ZY, Su WH. Antibacterial activity of a chitooligosaccharide mixture prepared by cellulose digestion of shrimp chitosan and its application to milk preservation. J Food Prot 2000; 63: 747-752.
30. Wang G. Inhibition and inactivation of five species of foodborne pathogens by chitosan. J. Food Prot 1992; 55: 916-919.
31. Yalpani M, Johnson F, Robinson LE. Antimicrobial activity of some chitosan derivatives. In: Brine CJ, Sandford PA, Zikakis JP (eds.). Advances in chitin and chitosan. pp. 543-555, Elsevier Applied Science, London, 1992.
32. Yun YS, Kim KS, Lee YN. Antibacterial and antifungal effect of chitosan. J Chitin Chitosan 1999; 4: 8-14.

개의 표재성 농피증에서 분리된 *Staphylococcus intermedius*에 대한 키토산의 항균효과

정효훈 · 이근우 · 오태호[1]

경북대학교 수의과대학 수의내과학교실

요약 : 개의 표재성 농피증으로부터 분리된 *Staphylococcus intermedius*에 대한 0.1%(w/v) 키토산의 항균효과를 조사하였다. *In vitro*에서 0.1% 키토산이 *S. intermedius*에 대한 항균효과를 나타내기 위한 접촉시간과 키토산 용액의 pH가 미치는 영향을 조사하였다. *In vivo*에서 10두의 개의 피부에 인공적으로 *S. intermedius*를 접종하여 0.1% 키토산, 2.5% benzoyl peroxide, 0.5% chlorhexidine, 키토산-benzoyl peroxide 혼합용액, 키토산-chlorhexidine 혼합용액의 항균효과를 modified detergent cup scrub technique을 이용하여 비교하였다. *In vitro* 에서 접종액의 내의 세균수는 6.08 ± 0.20 CFU/ml 이었으며 pH 4.5 용액의 경우 10분, 30분, 120분의 접촉시간 후 각각 3.57 ± 0.51, 2.82 ± 0.24, 2.40 ± 0.17 CFU/ml로 감소하였다. 동일한 각각의 접촉시간후 pH 5.0 용액은 4.22 ± 0.08, 3.44 ± 0.41, 3.16 ± 0.09, pH 5.5 용액은 4.75 ± 0.14, 4.32 ± 0.08, 3.53 ± 0.33, pH 5.9 용액은 5.57 ± 0.36, 5.02 ± 0.42, 4.87 ± 0.12 CFU/ml로 감소하였다. 따라서 pH 에 따라 정도의 차이는 있었으나 모든 키토산 용액은 10분의 접촉시간후 현저한 항균효과를 나타내었으며 ($p<0.05$) pH 4.5에서 가장 높게 나타났다. In vivo에서 접종액내의 세균수는 6.61 ± 0.30 CFU/cm2 이었으며 키토산은 3.25 ± 0.98, benzoyl peroxide는 0.68 ± 1.13, chlorhexidine은 3.14 ± 0.55, 키토산-benzoyl peroxide은 0.48 ± 0.56, 키토산-chlorhexidine은 2.55 ± 0.88 CFU/ml로 각각 현저히 감소하였다 ($p<0.01$). 0.1% 키토산 단독보다는 소량의 benzoyl peroxide 또는 chlorhexidine을 혼합용액의 항균효과가 현저히 증가하였다. 따라서 0.1% 키토산은 *S. intermedius*에 대하여 *in vitro* 및 *in vivo* 에서 항균효과를 나타내었으며 개의 화농성 농피증의 국소제제로 적용가능성이 있을 것으로 사료된다.

주요어 : chitosan, *Staphylococcus intermedius*, dog, pyoderma.