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[원 저]

Antimicrobial Efficacies of Citra-Kill®, Disinfectant Solution against *Salmonella Typhimurium* and *Brucella Ovis*

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ABSTRACTS

Salmonellosis and brucellosis have caused a considerable danger of farmed animals and economic loss in animal farming industry. In this study, the disinfection efficacy of Citra-Kill®, a commercial disinfectant, composed to quaternary ammonium chloride and citric acid was evaluated against *S. typhimurium* and *Brucella ovis*. A bactericidal efficacy test by broth dilution method was used to determine the lowest effective dilution of the disinfectant following exposure to test bacteria for 30 min at 4°C. Citra-Kill® and test bacteria were diluted with distilled water (DW), hard water (HW) or organic matter suspension (OM) according to treatment condition. On OM condition, the bactericidal activity of Citra-Kill® against *S. typhimurium* and *Brucella ovis* was lowered compared to that on HW condition. As Citra-Kill® possesses bactericidal efficacy against animal pathogenic bacteria such as *S. typhimurium* and *Brucella ovis*, this disinfectant solution can be used to control the spread of animal bacterial diseases.

Keywords: Citra-Kill®, *Salmonella typhimurium*, *Brucella ovis*, Disinfectant efficacy

I. Introduction

Salmonella extensively causes various disease syndromes, such as self-limiting enteritis, fatal infection in animals, food-borne infection, and typhoid fever in humans.[1-4] *Salmonella* infections are zoonotic and can be transferred between humans and nonhuman animals. Many infections are due to ingestion of contaminated food.[4] The etiologic agents of salmonellosis are *Salmonella* spp. characterized by motile, Gram-negative, rod-shaped bacteria and facultative intracellular pathogens that can multiply within professional and nonprofessional phagocytes.[5] *Salmonella* can survive for weeks outside a living body and are not destroyed by freezing.[6,7]

Salmonella typhimurium (*S. typhimurium*) is one of the most frequently isolated serotypes from pig farms, slaughtered swine and human foodborne illness.[8,9] Also, *S. typhimurium* can survive in different reservoirs and is easily transmitted through water and poultry to humans.[10,11]

Brucellae are Gram-negative, facultative, and intra-

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cellular bacteria that can infect many species of animals and human. Based on differences in pathogenicity and host preference, six species are recognized within the genus *Brucella*: *B. abortus*, *B. melitensis*, *B. suis*, *B. ovis*, *B. canis*, and *B. neotomae*.[12] *B. abortus*, *B. melitensis* and *B. suis* are responsible for bovine brucellosis, ovine and caprine brucellosis, and swine brucellosis, respectively. These three *Brucella* species may cause abortion in their hosts, which could result in huge economic losses. In addition, *B. ovis* is responsible for lamb epididymitis.[13]

As *Salmonella* and *Brucella* infections are becoming harder to control because of resistance to commonly used antibiotics, the effective cleaning and disinfection regimes are essential for the prevention of infections and outbreaks.[14,15] The cleaning and disinfectant regimes depend on the proper use of biocides, and there is the concern that the resulting increased use of biocides in farming, food production, and hospital settings, and the home could contribute to the selection of antibiotic-resistant strains as some mechanisms of biocide resistance also confer antibiotic resistance.[16] Biocides are often composed of a mixture of ingredients that act upon a wide range of cellular mechanisms and targets, which makes it difficult for bacteria to become resistant to biocides.[14]

Salmonellosis and.Brucellosis in livestock animals may cause enormous economic loss to animal farming.[17,18] The stress on livestock animals caused by intensive farming practices and the development of antibiotic-resistant bacteria are among the major reasons for the increased frequency of bacterial disease outbreaks.[19] Highly hygienic measures including the use of disinfectant are very effective for successful control of diseases from bacteria, fungi and parasites in farmed animals.[20,21] Several disinfectants including glutaraldehyde, organic acids, aldehydes, sodium hydroxide, quaternary ammonium compounds and chlorohexadine have been used for decontamination after outbreaks of farmed animal diseases.[22,23] However, there is not the efficacy test for the disinfectant composed of both quaternary ammonium chloride and citric acid against bacterial animal diseases. Therefore, this study was carried out to examine bactericidal efficacy of a disinfectant solution against *S. typhimurium* and *Brucella ovis*.

II. Materials and Methods

1. Bacteria and culture

The test bacteria, *S. typhimurium* (G-B-14-21-62) and *Brucella ovis* (ATCC 25840) were obtained from the Korean Veterinary Culture Collection (KVCC, Seoul, Korea). The strains were maintained as frozen glycerol stock. *S. typhimurium* cells were cultured in Luria-Bertani (LB) broth containing 1.5% agar. *Brucella ovis* were spread in Brucella broth containing 5% fetal bovine serum and incubated at 37°C under CO_2 condition.

2. Disinfectant

The active ingredients for Citra-Kill®, the tested disinfectant solution, are quaternary ammonium chloride (10% v/v) and citric acid (30% w/v). Citra-kill® was provided by Dae Han New Pharm Co. (Seoul, Korea). The disinfectant solution was stored in the dark in room temperature and prepared for dilution on the day of evaluation. Determination of the antimicrobial efficacy of the disinfectant was based on Animal, Plant and Fisheries Quarantine and Inspection Agency Regulation No. 2008-14, Korea.

3. Diluents and treatment condition

Testing was based on bactericidal effects of disinfectant diluents in three treatment conditions (distilled water (DW) condition, standard hard water (HW) condition, and organic matter (OM) condition), pathogen control (disinfectant negative control) and DW control (both disinfectant and pathogen negative control) in Table 1. HW, an ingredient of HW treatment condition, was made by adding anhydrous

Table 1. Experimental design for the determination of the bactericidal efficacy of Citra-kill®

Treatment condition*	Contents according to treatment condition**				
	DM	HW	OM	Disinfectant	Bacteria
DW condition	+	-	-	+	+
HW condition	-	+	-	+	+
OM condition	-	-	+	+	+
Bacteria control	-	+	-	-	+
DW control	+	-	-	-	+

*DW, distilled water; HW, standard hard water; OM, organic matter. **+, presence; -, absence.

Table 2. Final valid dilution of Citra-kill® against *S. typhimurium* and *Brucella ovis*

Bacterial strains	Treatment condition*											
	DW				HW				OM			
	DT	1	2	3	DT	1	2	3	DT	1	2	3
S. typhimurium	360	○	○	○	880	○	○	○	760	○	○	○
	330	○	×	○	800	○	○	×	700	○	○	○
	300	×	×	×	720	×	×	×	640	×	×	×
	270	×	×	×	640	×	×	×	580	×	×	×
	240	×	×	×	560	×	×	×	520	×	×	×
	Valid	300			Valid	720			Valid	640		
Brucella ovis	1,440	○	○	○	1,440	○	○	○	720	○	○	○
	1,320	○	×	○	1,320	○	○	×	660	○	×	○
	1,200	×	×	×	1,200	×	×	×	600	×	×	○
	1,080	×	×	×	1,080	×	×	×	540	×	×	×
	960	×	×	×	960	×	×	×	480	×	×	×
	Valid	1,200			Valid	1,200			Valid	600		

*DW, distilled water; HW, standard hard water; OM, organic matter; DT, dilution time. ○, growth; ×, growth inhibition.

$CaCl_2$ 0.305 g and $MgCl_2 \cdot 6H_2O$ 0.139 g into 1 *l* distilled water. Organic suspension, an ingredient of OM treatment condition, is a solution of 5% (w/v) yeast extract in HW. The test organisms were prepared by titration of each cultural broth into at least 10^8 cfu/m*l* viable organisms with the same kind of diluents of treatment condition.

4. Experimental procedures

For the efficacy test against *S. typhimurium*, Citra-Kill® was diluted 240, 270, 300, 330, and 360 times with DW, and diluted 560, 640, 720, 800, and 880 times with HW, and diluted 520, 580, 640, 700, and 760 times with OM, respectively. For the efficacy test against *Brucella ovis*, Citra-Kill® was also diluted 960, 1,080, 1,200, 1,320, and 1,440 times with DW, and diluted 960, 1,80, 1,200, 1,320, and 1,440 times with HW, and diluted 480, 540, 600, 660, and 720 times with OM, respectively.

To verify the lowest effective dilution of the disinfectant, five serial dilutions of the disinfectant were prepared and placed at 4°C prior to test reaction. 2.5 m*l* of each disinfectant dilution was mixed with the same amount of test organism followed by contact time of 30 min at 4°C.

During this period, the mixture was shaken at 10 min interval. At the end of 30 min contact period, one ml of the mixture was neutralized with 9 m*l* of Nutrient broth containing 5% inactivated horse serum (Becton Dickinson & Co., MD, USA) at 37°C.

0.1 m*l* of the neutralized reaction mixture was sub-cultured into 10 m*l* of recovery each cultural broth at 37°C for 48 h in incubator. The valid dilution was determined that the greatest dilution showing no growth in two or more in the five replicates was confirmed. The final dilution time was statistically determined by a median value among three valid dilution of the triplicate test, but each value of which should be within 20% experimental error.

III. Results

Table 2 shows the final valid dilution of Citra-Kill® composed to quaternary ammonium chloride and citric acid. On DW condition, *S. typhimurium* and *Brucella ovis* were completely inactivated with 300 and 1,200 fold dilutions of the disinfectant, respectively. When the bactericidal effect on HW condition was evaluated, the antibacterial activity of the disinfectant showed on 720 and 1,200 fold dilutions against *S. typhimurium* and *Brucella ovis*, respectively. With the investigation of the bactericidal effect of the disinfectant on OM condition, *S. typhimurium* and *Brucella ovis* were inactivated on 640 and 600 fold dilutions, respectively. Because organic material interferes with efficacy by either inactivating the disinfectant or blocking it from surface contact, the bactericidal activity of the disinfectant on the OM condition was lowered against animal pathogenic bacteria compared with DM or HW conditions.

Comparing the results of the disinfectant against two animal pathogenic bacteria in the present study, the bactericidal effect of Citra-Kill® against *Brucella ovis* was higher than that against *S. typhimurium* on the DM and HW condition, but the opposite result was shown on the OM condition.

IV. Discussion

Citra-Kill® is a potential antibacterial disinfectant which was composed of quaternary ammonium chloride and citric acid. Quaternary ammonium compounds have been shown to have antimicrobial activity. Especially, quaternary ammonium compounds containing long alkyl chains such as quaternary ammonium chloride are used as disinfectants.[24] Ahlström *et al*. reported that quaternary ammonium chloride affected the outer membrane of gram-negative bacteria such as *Salmonella spp*.[25] Also, Hamilton (1968) previously reported that quaternary ammonium chloride can cause cell leakage and membrane damage due to their adsorption to the bacterial membrane in large amounts.[26] For health effects of quaternary ammonium compounds, Bello *et al*. reported that the compounds can cause mild skin and respiratory irritation at the low level and severe caustic burns on skin at the high concentration.[27] Nalecz-Jawecki *et al*. reported that quaternary ammonium compounds are toxic to aquatic organisms at environmentally relevant concentrations.[28] Acidic disinfectants such as citric acid have a function to destroy the bonds of nucleic acids and precipitation proteins of bacteria. Citric also changes the pH of the environment making it detrimental to many microorganisms.[29] Use of citric acid creates an acidic environment and inhibits the replication of *Escherichia coli*, *Salmonella*, and other gram-negative bacteria.[30]

Lopes (1986) reported that the treatment with quaternary ammonium-based disinfectant at the dose of 200 μg/m*l* as active compound showed higher efficacy against *S. typhimurium* compared to chlorine at the same dose.[31] And Yoo (2009) carried out the disinfectant efficacy test for alkaline disinfectant solution against *Brucella ovis*. On the DW, HW and OM condition, *Brucella ovis* was inactivated on 220, 220, and 20 fold dilutions, respectively.[32]

In the present study, disinfectant efficacy of Citra-Kill® against *S. typhimurium* was higher than the chlorine tested by Lopes.[31] Also, against *Brucella ovis*, disinfectant efficacy of Citra-Kill® was higher than the alkaline disinfectant solution examined by Yoo.[32]

In this study, disinfectant efficacy of Citra-Kill® has limitation that the results are based on *in vitro* test. Organic material in suspension (OM condition) could not represent all possible parameters of *Salmonella* and *Brucella* contaminated farm environments.

As the efficacy of Citra-Kill® against *S. typhimurium* and *Brucella ovis* was investigated *in vitro*, a controlled field trial is required to determine whether use of Citra-Kill® will be able to reduce new animal pathogenic bacteria infection in animal farm area.

V. Conclusion

In animal farming industry, salmonellosis and brucellosis were very important diseases because of high mortality for farmed animals and economic loss. In the study of the bactericide efficacy test of Citra-Kill®, the results suggest that Citra-Kill® has potential bactericidal activity against *S. typhimurium* and *Brucella ovis*. So, Citra-Kill® composed to quaternary ammonium chloride and citric acid can be used to control the spread of animal bacterial diseases.

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