J Prev Vet Med Vol. 37, No. 1: 49-52, March 2013

<Short Communication>

Antimicrobial effect of hypochlorous acid on pathogenic microorganisms

Sang Phil Shin[1,*], Mun Sup Kim[1,*], Sung Hee Cho[2], Ji Hyung Kim[1], Casiano H. Choresca Jr[1], Jee Eun Han[1], Jin Woo Jun[1], Se Chang Park[1,†]

[1]College of Veterinary Medicine and Research Institute for Veterinary Science, Seoul National University, Seoul 151-742, Republic of Korea
[2]R&D Department, Yuhan-Clorox Co. Ltd, Incheon 403-030, Republic of Korea

Abstract : Disinfection is essential in terms of the public health or environmental hygiene. Hypochlorous acid (HOCl) solution was developed as a disinfectant in Korea. We evaluated the germicidal activity of HOCl against various pathogenic microbes. Thirty-one ATCC strains were exposed to HOCl solution at various concentrations (20, 40 and 80 ppm) for 1 minute. All the strains of bacteria, yeasts and mycobacteria were killed at 80 ppm after exposure to HOCl. The results suggest that HOCl solution could be used to effectively disinfect public areas.
Key words : Disinfectant, public health, hypochlorous acid, pathogenic microbes

Public health has been defined as the science and art of preventing disease, prolonging life and promoting health through the organized efforts and informed choices of society, organizations both public and private, communities and individuals [17]. Environmental health (or environmental hygiene) is a branch of public health concerned with all aspects of the natural and built environment that may affect human health. Other phrases that concern or refer to the discipline of environmental health include environmental public health and environmental health and protection. Disinfectants in terms of public health safety have been used to destroy microorganisms on non-living objects and sanitizers are agents that decrease the level of microorganisms present on the surface of an abiotic material [16]. Hypochlorous acid (HOCl) is a weak acid and dissociates to the hypochlorite ion (−OCl) and proton (H^+) depending on the solution pH. Both HOCl and −OCl are strong oxidizing agents and show germicidal activity [10]. Previous studies have reported that HOCl is 80 times more effective against *Escherichia coli* and 40 times more effective to *Pseudomnas* spp. than −OCl [13, 15]. However, until now, HOCl has simply been thought of as a transient by-product of ubiquitous chlorine disinfectants because of the difficulty to isolate and purify it. We made highly concentrated and stable HOCl with patented technology (patent Number 10-1051312). The purpose of this study, therefore, was to evaluate the disinfectant activity of HOCl against various pathogenic microbes. Our results showed that HOCl is an effective disinfectant.

HOCl solution (3,000 ppm) was diluted with phosphate buffered saline (PBS) to 20, 40 and 80 ppm. The following strains were cultured in Tryptic Soy Broth (TSB) at 37℃ (*Bacillus* sp. and *Paenibacillus alvei* at 30℃) for 24 h: 2 *Staphylococcus aureus* subsp. *aureus*, 2 *Enterococcus faecalis*, 2 *Escherichia coli,* 2 *Pseudomonas aeruginosa*, 3 Methicillin resistant *Staphylococcus aureus* (MRSA), 1 *Salmonella enterica* subsp. *enterica* (*typhimurium*), 1 *Shigella sonnei,* 1 *Shigella flexneri* , 1 *Bacillus thuringiensis*, 1 *Bacillus cereus*, 1 *Bacillus cereus,* 1 *Bacillus circulans* and 1 *Paenibacillus alvei*. Eight *Candida albicans* were cultured in Yeast extract-Malt extract Broth (YMB) at 37℃ for 24 h. *Mycobacterium chelonae* subsp. *Chelonae*, *Mycobacterium fortuitum* subsp. *fortuitum* and *Mycobacterium smegmatis* were cultured in 3% OGAWA media at 37℃ for 24 h. *Listonella anguillarum* was cultured in 1% TSB at 25℃ for 24 h. All strains were diluted with PBS to over 10^8 CFU/㎖ and 0.1 ㎖ of the dilution was added to 9.9 ㎖ of HOCl solution. After 1 min, the 0.1 ㎖ mixtures were inoculated on Tryptic soy agar (TSA), Yeast extract-Malt extract Agar (YMA), 3% OGAWA media and 1% NaCl TSA and incubated at the optimal temperatures described above for 24~48 h.

• Received 7 January 2013, revised 20 March 2013, accepted 21 March 2013.
* These authors contributed equally to this work.
† Correspondence author should be addressed.
Tel: +82-2-880-1282; Fax: +82-2-880-1213; E-mail: parksec@snu.ac.kr

Table 1. Antimicrobial effect of hypochlorous acid according to concentration

Bacterial species (ATCC[1] Number)	Initial concentration	20ppm	40ppm	80ppm
Bacillus cereus (11778)	3.26×10^{6}	NE[2]	1.21×10^{3}	<10
Bacillus cereus (21366)	4.18×10^{6}	NE	NE	<10
Bacillus circulans (9500)	2.72×10^{6}	NE	NE	NDC[3]
Bacillus thuringiensis (10792)	5.17×10^{6}	NE	3.57×10^{3}	<10
Candida albicans (10259)	4.26×10^{6}	7.80×10	NDC	NDC
Candida albicans (10261)	7.12×10^{6}	4.68×10^{2}	NDC	NDC
Candida albicans (10231)	1.21×10^{6}	NDC	NDC	NDC
Candida albicans (18804)	3.97×10^{6}	5.12×10^{2}	NDC	NDC
Candida albicans (11006)	1.77×10^{6}	NDC	NDC	NDC
Candida albicans (18814)	3.24×10^{6}	4.10×10	NDC	NDC
Candida albicans (22972)	5.11×10^{6}	2.45×10^{2}	NDC	NDC
Candida albicans (28471)	5.32×10^{6}	3.11×10^{2}	NDC	NDC
Enterococcus faecalis (29212)	1.74×10^{6}	NE	NDC	NDC
Enterococcus faecalis (19433)	4.25×10^{6}	NE	NDC	NDC
Escherichia coli (25922)	6.11×10^{6}	NE	NDC	NDC
Escherichia coli (11105)	3.43×10^{6}	NE	NDC	NDC
Listonella anguillarum (19264)	2.82×10^{6}	NDC	NDC	NDC
Methicillin resistant *Staphylococcus aureus* (33591)	5.39×10^{6}	NE	NDC	NDC
Methicillin resistant *Staphylococcus aureus* (33593)	3.06×10^{6}	NE	NDC	NDC
Methicillin resistant *Staphylococcus aureus* (6538)	2.52×10^{6}	NE	NDC	NDC
Mycobacterium chelonae subsp. *chelonae* (35752)	1.39×10^{6}	NDC	NDC	NDC
Mycobacterium fortuitum subsp. *fortuitum* (6841)	2.11×10^{6}	NDC	NDC	NDC
Mycobacterium smegmatis (19420)	2.26×10^{6}	NDC	NDC	NDC
Paenibacillus alvei (6344)	4.15×10^{6}	NE	NE	NDC
Pseudomonas aeruginosa (27853)	2.02×10^{6}	NE	NDC	NDC
Pseudomonas aeruginosa (25619)	3.55×10^{6}	NE	NDC	NDC
Salmonella enterica subsp. *enterica* (*typhimurium*) (29629)	5.76×10^{6}	NE	NDC	NDC
Shigella flexneri (29903)	3.42×10^{6}	NE	NDC	NDC
Shigella sonnei (25931)	5.79×10^{6}	NE	NDC	NDC
Staphylococcus aureus subsp. *aureus* (29213)	8.15×10^{6}	NE	NDC	NDC
Staphylococcus aureus subsp. *aureus* (25923)	5.68×10^{6}	NE	NDC	NDC

[1]ATCC : American type culture collection.
[2]NDC : not detected colony.
[3]NE : no effect.

Almost all strains were killed at 40 ppm. Especially *Mycobacterium* spp. and *L. anguillarum* were inactivated at 20 ppm while *Bacillus* spp. and *P. alvei* were not recovered at 80 ppm. The results of this study are presented in Table 1. Previous studies have reported that HOCl showed a germicidal effect within 0.5～1 min at 80～100 ppm [6, 7]. Our result indicates that HOCl has good bactericidal effect on *Mycobacterium* spp. over 20 ppm and it is similar to previously reported results [14]. In addition, *S. aureus* subsp. *aureus, E. faecalis, E. coli, P. aeruginosa*, MRSA, *S. typhimurium, S. sonnei, S. flexneri* and *C. albicans* were inactivated at 40 ppm. However, *Bacillus* sp. and *Panibacillus alvei* were killed at 80 ppm. Previous studies [6, 7] mainly showed the germicidal effect on pathogenic microbes at 80～100 ppm HOCl. However, this study demonstrated the germicidal effects of HOCl at lower a concentration with a greater variety of pathogenic strains. In addition, the HOCl used in this study can be made as a neutral pH and at a higher concentration than the previous product [6, 7].

Pathogenic microbes have been isolated from slaughterhouse and feedlots as well as food [5, 11, 12]. Especially, contamination of pathogens such as *Salmonella* spp. and *E. coli* in manufacturing process related meat is known to hazardous factors threaten public health. Therefore, effective disinfectants against pathogenic microbes would be highly desirable.

HOCl is 80 times more effective as a sanitizing agent than the equivalent concentration of OCL- [13, 15]. HOCl, the most effective form of chlorine compounds, kills microbial cells. Molecules that have highly nucleophilic sites are known to react rapidly with HOCl. Cellular components with these nucleophilic sites include porphyrins and hemes, ferredoxin-like iron-sulfur centers, purine and pyrimidine bases, conjugated polyenes, amines, amino acids and sulfhydryl groups [1]. The oxidation of these components by HOCl results in the loss of physiological functions. During HOCl stress in *E coli*, the loss of catalytic function of sulfhydryl enzymes and a decrease in anti- oxidants such as glutathione have been suggested to be bactericidal events [1, 8]. HOCl has also been found to disrupt oxidative phosphorylation [3], metabolic pathways involved in ATP utilization or generation [2], and other membrane-associated activities [4]. Furthermore, HOCl can cause DNA damage resulting from the formation of chlorinated derivatives of nucleotide bases [9].

Based on this study, HOCl solution is recommended as a disinfectant for environmental hygiene and it can the spread of pathogenic microbes using 80 ppm for 1 min. It is suitable for use in public places such as feedlots, slaughterhouses, hospitals, restaurants, schools, transportation hubs and public bathrooms. Before using HOCl, organic material should be removed from the surface.

REFERENCES

1. **Albrich JM, McCarthy CA, Hurst JK.** Biological reactivity of hypochlorous acid: implications for microbicidal mechanisms of leukocyte myeloperoxidase. Proc Natl Acad Sci USA. 1981, 78(1):210-214.
2. **Barrette WC Jr, Albrich JM, Hurst JK.** Hypochlorous acid-promoted loss of metabolic energy in *Escherichia coli*. lnfect lmmun. 1987, 55(10):2518-2525.
3. **Barrette WC Jr, Hannum DM, Wheeler WD, Hurst JK.** General mechanism for the bacterial toxicity of hypochiorous acid: abolition of ATP production. Biochemistry. 1989, 28(23):9172-9178.
4. **Champer AK, McFeters GA.** Chlorine injury and the enumeration of waterborne coliform bacteria. Appl Environ Microbial. 1979, 37(3):633-641.
5. **Cho JK, Kim KS.** Antimicrobial resistance and distribution of tetracycline resistance genes of *Escherichia coli* isolated from human and livestock in slaughterhouse. Kor J Vet Publ Hlth. 2008, 32(3):213-223.
6. **Choi TY.** Biocidal effect of a sanitizer/disinfectant, food-safe, against bacteria, yeast, and mycobacteria. Korean J Clin Microbiol. 2008, 11(2):117-122.
7. **Choi TY, Jang EY.** Bactericidal effect of medilox-S, an electrolyzed oxidized water generated by medilox against bacteria, fungi and mycobacteria. J Soonchunhyang Med Sci. 2009, 14(3):785-794.
8. **Dukan S, Belkin S, Touati D.** Reactive oxygen species are partially involved in the bacteriocidal action of hypochlorous acid. Arch Biochem Biophys. 1999, 367(2):311-316.
9. **Dukan S, Touati D.** Hypochlorous acid stress in *Escherichia coli*: resistance, DNA damage, and comparison with hydrogen peroxide stress. J Bacteriol. 1996, 178 (21):6145-6150.
10. **Fukuzaki S.** Mechanisms of actions of sodium hypochlorite in cleaning and disinfection processes. Biocontrol Sci. 2006, 11(4):147-157.
11. **Harakeh S, Yassine H, Gharios M, Barbour E, Hajjar S,**

El-Fadel M, Toufeili I, Tannous R. Isolation, molecular characterization and antimicrobial resistance patterns of *Salmonella* and *Escherichia coli* isolates from meat-based fast food in Lebanon. Sci Total Environ. 2005, 341(1-3):33-44.

12. **Kang HJ, Kim JS, Suk JM, Lee SM, Son WG.** Prevalence of *Salmonella* spp., *Escherichia coli* 0157 : H7 and *Listeria monocytogenes* in fresh feces and in drinking water of feedlots. Kor J Vet Publ Hlth. 1998, 22(3):195-200.
13. **LeChevallier MW, Cawthon CD, Lee RG.** Inactivation of biofilm bacteria. Appl Environ Microbiol. 1988, 54(10):2492-2499.
14. **Le Dantec C, Duguet JP, Montiel A, Dumoutier N, Dubrou S, Vincent V.** Chlorine disinfection of atypical mycobacteria isolated from a water distribution system. Appl Environ Microbiol. 2002, 68(3):1025-1032.
15. **Morris JC.** Future of chlorination. J Am Water Assoc. 1966(11), 58:1475-1482.
16. **Rutula WA, Weber DJ.** Modern advances in disinfection, sterilization, and medical waste management. In: Prevention and Control of Nosocomial Infections. Wenzel RP. 4th ed, Philadelphia, Lippincott Williams & Wilkins, 2003. p. 542-574.
17. **Winslow CE.** The untilled fields of public health. Science. 1920, 51(1306):23-33.