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Brief Communication

Inhibition of Clinical Nosocomial Bacteria by Chlorine Dioxide

Suk-Yul Jung†,*

Department of Biomedical Laboratory Science, Molecular Diagnosis Research Institute, Namseoul University, Cheonan-city, Choongnam 31020, Korea

Chlorine dioxide is an effective chemical to inhibit the growth of bacteria and viruses or to disinfect infected areas. In this study, the effects of chlorine dioxide on several bacteria in hospitals were analyzed. *Alloiococcus otitis*, *Kocuria rosea*, *Leuconostoc mesenteroides* spp. and *Staphylococcus lentus* as gram-positive bacteria and *Acinetobacter lwoffii*, *Aeromonas salmonicida*, *Brucella melitensis*, *Oligella ureolytica* as gram-negative bacteria were done for the inhibitory analysis. The growth and morphology of the bacteria were analyzed by placing a plastic stick which was called "FarmeTok (medistick/ Puristic)" provided by Purgofarm, co, Ltd. to release ClO_2 (13 ppmv/hr) next to the plate where the bacteria were incubated for 24 hours. Less than 10 bacterial colonies were evaluated as having 99% inhibitory effect. The initial bacterial culture concentration of 0.5 McFaland turbidity was good for analyzing the chlorine dioxide inhibitory effect. All bacteria could be easily counted post 24 hr co-incubation with ClO_2, but *A. otitis* and *A. lwoffii* without ClO_2 gas were not countable due to very dispersed colony types which were not affected for result analysis. As shown in this study, the FarmeTok plastic stick, which discharges chlorine dioxide at 13 ppmv / hour, was evaluated to be sufficient to suppress the above bacteria in the hospital. Bacteria existing in the clinic such as this hospital will be used as a data to inhibit the growth of bacteria by using ClO_2, and molecular biology analysis using the gene of bacteria will be possible in the future rather than inhibiting the growth of bacteria itself.

Key Words: Bacteria, Chlorine dioxide, FarmeTok, Growth

Chlorine dioxide, ClO_2, is water-soluble gas of a strong oxidative activity (Moran et al., 1953; Fukayama et al., 1986; Chung et al., 2018), and it is thought to have inhibitory or disinfective effects to bacteria, yeast, molds and viruses (Sy et al., 2005; Morino et al., 2009; Lowe et al., 2013; Kim et al., 2016; Chung et al., 2018). Chlorine dioxide is 10 times more soluble in water than sodium hypochlorite, which is less reactive with organics and used as a disinfectant for food (Jung et al., 2019). ClO_2 gas is an effective disinfectant agent with strong oxidization ability and a broad biocidal spectrum (Gómez-López et al., 2009; Wang et al., 2016). Sanekata et al. (2010) reported that chlorine dioxide was treated at a concentrations of 1 to 100 ppm for 15 seconds and it had a powerful antiviral function that inactivated more than 99% of the virus. In addition, the treatment of chlorine dioxide inhibited the growth of bacteria in crops such as blueberries (Wu and Kim, 2007; Xu et al., 2016) and paprika (Kim and Song, 2017).

In our previous study, microorganisms isolated from nosocomial environment, e.g. *Micrococcus luteus*, *Granulicatella*

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*Professor.

†Corresponding author: Suk-Yul Jung. Department of Biomedical Laboratory Science, Molecular Diagnosis Research Institute, Namseoul University, 91 Daehak-ro, Seonghwan-eup, Seobuk-gu, Cheonan-city, Choongnam 31020, Korea.
Tel: +82-41-580-2723, Fax: +82-41-580-2932, e-mail: syjung@nsu.ac.kr

adiacens, *Staphylococcus caprae*, etc, were inhibited upto 99% by a plastic stick releasing chlor ine dioxide gas provided by Purgofarm company.

In this study, clinical nosocomially shown bacteria which had little infection case and no report until now were evaluated by the effects of ClO_2 gas for inhibition tests. Four gram- positive bacteria and four gram-negative bacteria were applied. Briefly, *Alloiococcus otitis*, *Kocuria rosea*, *Leuconostoc mesenteroides* spp. and *Staphylococcus lentus* as gram-positive bacteria and *Acinetobacter lwoffii*, *Aeromonas salmonicida*, *Brucella melitensis*, *Oligella ureolytica* as gram-negative bacteria were done for the inhibition test. Briefly, *O. ureolytica* is an aerobic gram-negative bacillus in human urinary tracts but *K. rosea* has also been found to cause urinary tract infections in patients with weakened immune systems. *L. mesenteroides* is associated with slime production. *A. otitis* is a species of bacteria first isolated from human middle-ear fluid, and *S. lentus* and *A. lwoffii* are seen as normal flora of skin. *A. salmonicida* is an etiological agent for furunculosis which causes sepsis and *B. melitensis* is a bacterium to cause ovine brucellosis. They were previously isolated from nosocomial environment (Song and Jung, 2017). The isolated bacteria were subcultured into other tryptic soy agar (TSA, MB cell, Korea) plate at 37℃ and were confirmed by Gram-staining procedures (Lim et al., 1988).

All bacterial strains were stated below: *A. otitis*, *K. rosea*, *L. mesenteroides* spp. and *S. lentus* as gram-positive bacteria and *A. lwoffii*, *A. salmonicida*, *B. melitensis*, *O. ureolytica* as gram-negative bacteria were done for the inhibition test. Prior to the inhibition test, single bacterial colonies were adjusted to 0.5 of McFaland turbidity, diluted with 0.85% NaCl to form approximate 1.5×10^5 to 1.5×10^8 colony forming units (CFU)/mL (Song and Jung, 2017). The bacteria were cultured with TSA medium for the next procedures. Bacteria plates were applied with a plastic stick, namely, "FarmeTok (medistick/Puristic) kindly provided by Purgofarm, co, Ltd. (Hwasung, Gyeonggi-do, Korea)" to produce ClO_2 (13 ppmv/hr) (Song and Jung, 2017). In order to observe the growth and culture of bacteria, bacterial plates were placed in a plastic clear chamber (250W × 350D × 200H) at a 37℃ incubator and observed for 24 hr.

Table 1. The number of bacterial colonies

Gram staining	Bacteria	Groups	Initial numbers (CFU/mL)	Numbers after 24 hr (CFU/mL)	Growth inhibition rate (%)*
+	*A. otitis*	Control	1.5×10^8	Uncountable**	−
+	*A. otitis*	ClO_2	1.5×10^8	0	99.9
+	*K. rosea*	Control	1.5×10^8	43	−
+	*K. rosea*	ClO_2	1.5×10^8	0	99.9
+	*L. mesenteroides* spp.	Control	1.5×10^6	Uncountable**	−
+	*L. mesenteroides* ssp.	ClO_2	1.5×10^6	0	99.9
+	*S. lentus*	Control	1.5×10^5	Uncountable**	−
+	*S. lentus*	ClO_2	1.5×10^5	9	99.9
−	*A. lwoffii*	Control	1.5×10^6	Uncountable**	−
−	*A. lwoffii*	ClO_2	1.5×10^6	0	99.9
−	*A. salmonicida*	Control	1.5×10^5	330	−
−	*A. salmonicida*	ClO_2	1.5×10^5	1	99.9
−	*B. melitensis*	Control	1.5×10^5	420	−
−	*B. melitensis*	ClO_2	1.5×10^5	0	99.9
−	*O. ureolytica*	Control	1.5×10^7	Uncountable**	−
−	*O. ureolytica*	ClO_2	1.5×10^7	0	99.9

* 100 - (Numbers after 24 hr / Initial numbers) × 100

** Uncountable indicated bacterial colonies to not be easily counted due to very high numbers of their numbers

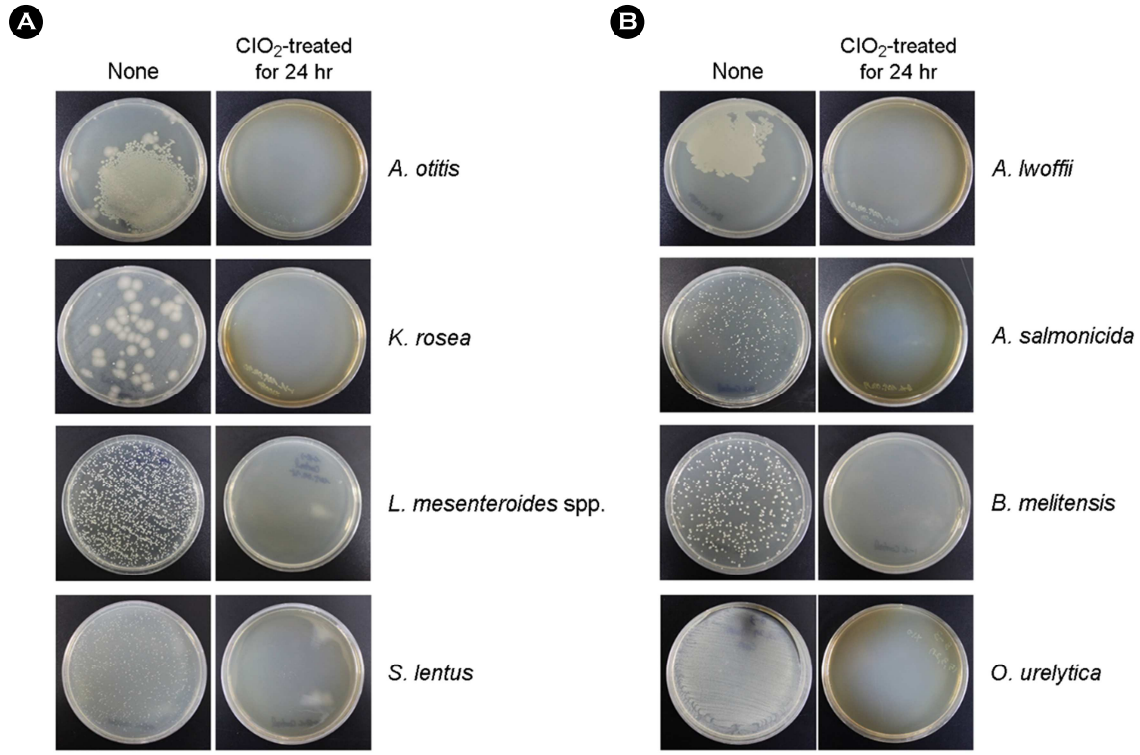

Fig. 1. Bacterial plates treated by chlorine dioxide gas for 24 hr. Four gram-positive (A) and four gram-negative bacteria (B) were applied.

The number of bacterial colonies formed after 24 hours with the plastic stick next to the bacterial plate was calculated (Table 1). The number and morphology of the bacterial colonies formed were visually analyzed, with less than 10 bacterial colonies estimated to be 99% inhibited and the 0.5 of McFaland turbidity used was sufficient to analyze inhibition by ClO_2 gas. Fig. 1 showed bacterial colonies by the treatment of ClO_2 gas. All bacteria could be easily counted, but *A. otitis* and *A. lwoffii* without ClO_2 gas were not countable due to very dispersed colony types which were not affected for result analysis. *A. otitis* and *K. rosea* forming 1.5×10^8 CFU were inhibited with 99% of no colony in treated with ClO_2 gas. Taken together, FarmeTok releasing ClO_2 gas was very effective to inhibit bacterial growth.

This study could give the information of uncommon clinical bacteria and effectiveness of chlorine dioxide-releasing plastic stick to prevent bacterial growth. Eight bacteria in this study were almost completely inhibited for growth by the ClO_2 gas. ClO_2 at concentration of 1.3 and 13 mg/L killed against these pathogens, e.g., *Erwinia carotovora* and *Ralstonia solancearum* (Yao et al., 2010). In our study, the plastic stick of FarmeTok released ClO_2 (13 ppmv/hr) gas and was sufficient to inhibit the bacterial growth.

Analysis of the efficiency and safety of chlorine dioxide showed that chlorine dioxide concentrations of 5 and 20 ppm, respectively, were sufficient to inhibit the growth of bacteria and fungi by 98.2%. Some bacteria can be applied in specific condition and environments. Chlorine dioxide concentrations of 385 ppm have been safely maintained in hospitals of large facilities (Lowe et al., 2013).

The inhibition mechanism of ClO_2 is unclear, but the action of ClO_2 is only known as inhibition. For porcine reproductive and respiratory syndrome virus (PRRSV), ClO_2 could inhibit the first stage of viral life, which inhibited binding itself to cells where PRRSV was not internalized and released (Zhu et al., 2019).

The plastic stick releasing ClO_2 (13 ppmv/hr) gas was enough to inhibit the bacterial growth with 99%. Although the pathogenicity of bacteria is not known to inhibit the growth of chlorine dioxide, the concentration of chlorine

dioxide must be treated higher to inhibit the growth of methicillin-resistant *S. aureus* (MRSA) and *K. pneumoniae*, which are potent pathogenic. According to a research paper by different concentration of ClO_2 (Hsu et al., 2016), after 40 weeks of ClO_2 use by 0.3~0.5 mg/L, the overall non-fermented gram negative bacillus colonies decreased significantly (hot water: 160±143 vs 2±4 cfu/mL).

Bacteria existing in the clinic such as this hospital will be used as a data to inhibit the growth of bacteria by using chlorine dioxide, and molecular biology analysis using the gene of bacteria is possible in the future rather than inhibiting the growth of bacteria itself.

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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