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Brief Communication

Growth Inhibitory Effects of Chlorine Dioxide on Bacteria

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Chlorine dioxide (ClO_2) gas is a neutral chlorine compound. ClO_2 gas was proven to effectively decontaminate different environments, such as hospital rooms, ambulances, biosafety level 3 laboratories, and cafeterias. In this study, to evaluate the effects of ClO_2 gas, bacteria of clinical importance were applied. *Staphylococci*, *Streptococci* and *Bacillus* strains were applied and *Klebsiella*, and others e.g., *Escherichia coli*, *Shigella*, *Salmonella*, *Serratia* were also done for the inhibitory analysis. Bacteria plates were applied with a hygiene stick, namely, "FarmeTok (Medistick/Puristic)" to produce ClO_2. ClO_2-releasing hygiene stick showed the very strong inhibition of bacterial growth but had different inhibitions to the bacteria above 96.7% except for MRSA of 90% inhibition. It is difficult to explain why the MRSA were not inhibited less than others at this point. It can be only suggested that more releasing ClO_2 should be essential to kill or inhibit the MRSA. *B. subtilis*, *S. agalactiae*, *S. pyogenes*, *E. coli* O157:H7, *S. typhi* (*S. enterica* serotype *typhi*) and *S. marcesence* were inhibited over 99%. This study will provide fundamental data to research growth inhibition by ClO_2 gas with bacteria of clinical importance value.

Key Words: Chlorine dioxide, Bacteria, Inhibition, FarmeTok (Medistick/Puristic)

Chlorine dioxide (ClO_2) gas is a neutral chlorine compound. It is very different from elementary chlorine, both in its chemical structure and in its behavior (Vogt et al., 2010; Song and Jung, 2017).

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). The antimicrobial efficacy of ClO_2 gas has been evaluated in previous studies, and ClO_2 gas was proven to effectively decontaminate different environments, such as hospital rooms (Luftman et al., 2006; Lowe et al., 2013), ambulances (Lowe et al., 2013), biosafety level 3 laboratories (Lowe et al., 2012), and cafeterias (Hsu et al., 2014).

It has been reported that chlorine dioxide, a strong oxidant, can inhibit or destroy microorganisms (Ogata et al., 2008; Morino et al., 2009; Sanekata et al., 2010; Ma et al., 2017; Ofori et al., 2017). Sanekata et al., (2010) reported that chlorine dioxide at concentrations ranging from 1 to 100 ppm produced potent antiviral activity, inactivating >or= 99.9% of the viruses with a 15 sec treatment for sensitization.

Our group has reported that in the clinics 11 microorganisms were isolated, and ClO_2-releasing hygiene stick showed the very strong inhibition of bacterial growth with about 99.9% after 24 hr incubation (Song and Jung, 2017).

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ClO_2 however was found to increase the permeability of the outer and cytoplasmic membranes leading to the leakage of membrane components such as 260 nm absorbing materials and inhibiting the activity of the intracellular enzyme β-D-galactosidase (Ofori et al., 2017).

In this study, to evaluate the effects of ClO_2 gas, bacteria of clinical importance were applied.

Six gram positive bacteria and five gram negative bacteria were applied. Bacteria were mentioned in results with growth inhibition data. Briefly, 2 *Staphylococci*, 2 *Streptococci* and 1 *Bacillus* strains were applied and 2 *Klebsiella*, and others e.g., *Escherichia coli*, *Shigella*, *Salmonella*, *Serratia* were also done for the inhibitory analysis. In this study, the bacteria were not divided by characteristics of diseases but simply described with human infections above. Single colonies were subcultured into other tryptic soy agar (TSA, MB cell, Korea) plate at 37℃, and were double checked by Gram-staining procedures (Lim et al., 1988).

To culture accurate colonies, obtained single colonies were diluted with 0.85% NaCl and were adjusted into 0.5 of McFaland turbidity, which could produce about 1.5×10^3 to 1.5×10^6 colony forming units (CFU)/mL (Song and Jung, 2017). The adjusted bacteria grown in TSA plates were applied for all subsequent experiments.

Bacteria plates were applied with a hygiene stick, namely, "FarmeTok (Medistick/Puristic) kindly provided by Purgo-farm, co, Ltd. (Hwasung, Gyeonggido, Korea)" to produce ClO_2 (Song and Jung, 2017). To efficiently observe and culture bacteria, bacterial plates were added into a plastic clear chamber (250W × 350D × 200H) at a 37℃ incubator.

Table 1. CFU of bacteria by the hygiene stick of ClO_2 gas. Bacteria were streaked onto the plate and the hygiene stick was located near the plate followed by counting of bacterial colonies

Gram staining	Bacteria (No. at KCTC)	Groups	Initial numbers (CFU/mL)	Numbers after 24 hr (CFU/mL)	*Growth inhibition rate (%)
+	*S. aureus* (1621)	Control	1.5×10^4	−	−
		ClO_2	1.5×10^4	< 250	98.3
	Methicillin-resistant *S. aureus* (MRSA)	Control	1.5×10^3	−	−
		ClO_2	1.5×10^3	< 150	90.0
	B. subtilis (3613)	Control	1.5×10^6	−	−
		ClO_2	1.5×10^6	< 50	99.9
	S. agalactiae	Control	1.5×10^5	−	−
		ClO_2	1.5×10^5	< 150	99.0
	S. pyogenes	Control	1.5×10^4	−	−
		ClO_2	1.5×10^4	< 50	99.7
−	*E. coli* O157:H7	Control	1.5×10^4	−	−
		ClO_2	1.5×10^4	< 50	99.7
	K. oxytoca (1686)	Control	1.5×10^4	−	−
		ClO_2	1.5×10^4	< 300	98.0
	K. pnuemoniae	Control	1.5×10^3	−	−
		ClO_2	1.5×10^3	< 50	96.7
	S. typhi (*S. enterica* serotype *typhi*)	Control	1.5×10^4	−	−
		ClO_2	1.5×10^4	< 100	99.3
	S. marcesence	Control	1.5×10^6	−	−
		ClO_2	1.5×10^6	< 100	99.9
	S. sonnei	Control	1.5×10^4	−	−
		ClO_2	1.5×10^4	< 300	98.0

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

Bacterial growth was periodically observed until 24 hr and was compared with ClO_2 gas-untreated groups as a control.

All bacterial strains were below: *S. aureus*, Methicillin-resistant *S. aureus* (MRSA), *B. subtilis*, *S. agalactiae*, *S. pyogenes*, *E. coli* O157:H7, *K. oxytoca*, *K. pnuemoniae*, *S. typhi* (*S. enterica* serotype *typhi*), *S. marcesence*, *S. sonnei*. To analyze whether chlorine dioxide can inhibit the bacteria, hygiene stick, namely, "FarmeTok (Medistick/Puristic)" which produced the chlorine dioxide gas was co-incubated with the bacteria. To avoid the release of the gas out, the hygiene stick was put into a plastic chamber and was incubated at 37℃. When the ClO_2-releasing hygiene stick is ready for activation, it is changed into yellow and release ClO_2 (Song and Jung, 2017).

Simply, the lid of bacterial plates was open to be released to air and ClO_2. Bacteria were streaked onto the plate and the hygiene stick was located near the plate followed by counting of bacterial colonies (Table 1). Bacterial numbers were different dud to the use of general growth media of TSA. ClO_2-releasing hygiene stick showed the very strong inhibition of bacterial growth but had different inhibitions to the bacteria above 96.7% except for MRSA of 90% inhibition. It

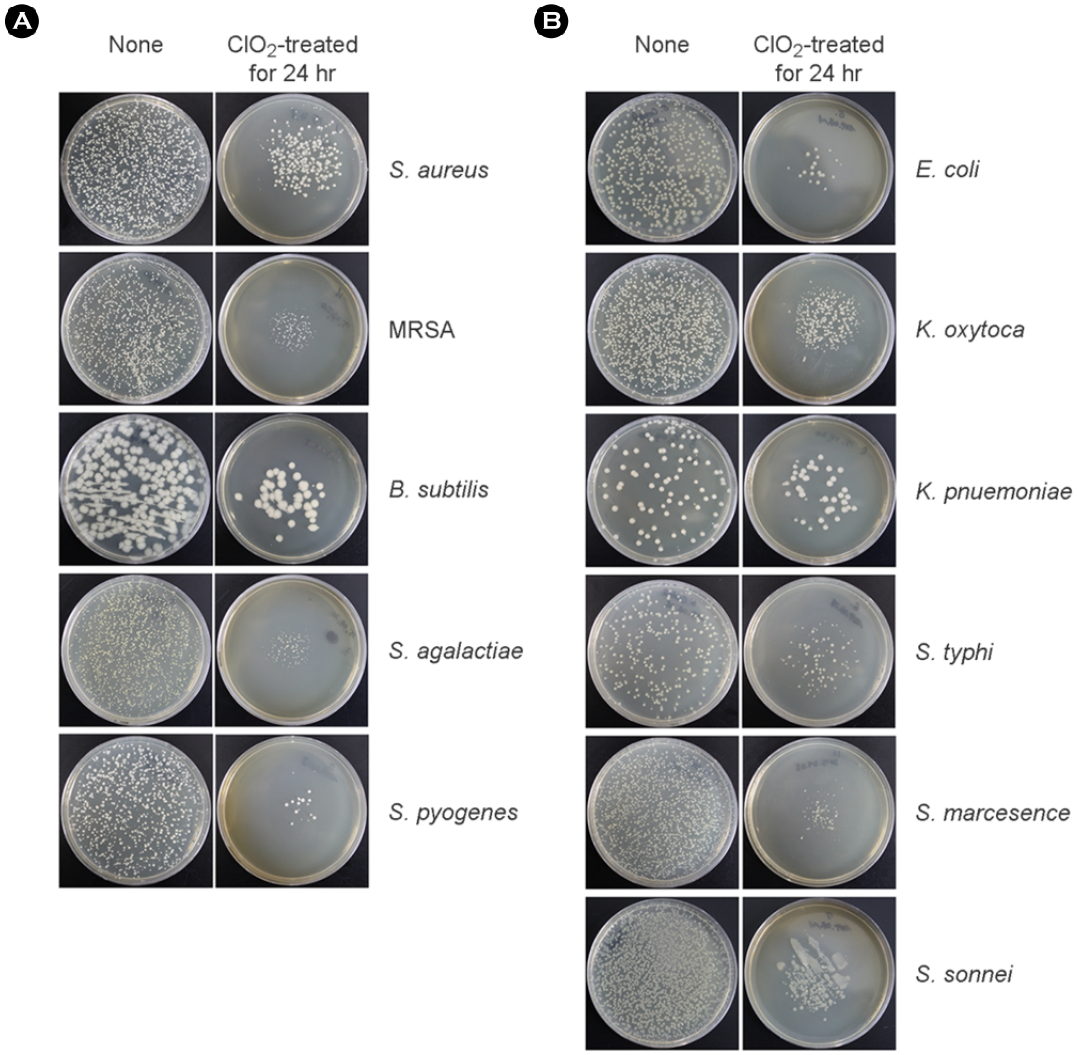

Fig. 1. Bacterial plates by the co-incubation of the hygiene stick of ClO_2 gas. Bacteria plates were applied with a hygiene stick to produce ClO_2. The bacterial plates were added into a plastic clear chamber at a 37℃ incubator. Bacterial growth was periodically observed until 24 hr and was compared with ClO_2 gas-untreated groups as a control.

is difficult to explain why the MRSA were not inhibited less than others at this point. It can be only suggested that more releasing ClO_2 should be essential to kill or inhibit the MRSA. *B. subtilis*, *S. agalactiae*, *S. pyogenes*, *E. coli* O157:H7, *S. typhi* (*S. enterica* serotype *typhi*) and *S. marcesence* were inhibited over 99%. It can also suggest that the inhibition may not be affected by the Gram positivity and Gram negativity.

Fig. 1. represented bacterial plates from the counting of CFU. All bacteria could be easily counted post 24 hr co-incubation with ClO_2, but *S. sonnei* plate showed dispersed patterns due to moisturized surface of the plate. Very interestingly, the areas of growth inhibited plates were peripheral but not the central, implied that diffusion of ClO_2 gas affect the margin and periphery at first and then go to the central region.

ClO_2 gas is required to sanitize a lot of areas and an equipment to release the ClO_2 gas may be necessary in hospitals. The hygiene stick, namely, "FarmeTok (Medistick/Puristic)" kindly provided by Purgofarm would be useful to release ClO_2 gas and were sufficient to inhibit bacterial growth for 24 hr release. In our previous study, 11 microorganisms including *Micrococcus luteus*, *Granulicatella adiacens*, *Staphylococcus caprae*, *Sphingomonas paucimobilis*, *Kocuria kristinae*, etc which were isolated from the clinic were completely inhibited by the hygiene stick of ClO_2 gas (Song and Jung, 2017). Incomplete growth inhibition may be resulted from different pathogenicity of those bacteria and this applied bacteria.

All 11 bacterial strains in this study possess different pathogenicity and require different growth media. TSA medium was only used to check the bacterial growth, even if the bacteria grew faster or slower. Interestingly, MRSA was not completely inhibited by the hygiene stick of ClO_2 gas, in view of the 90% inhibition. The difference of its pathogenicity might be definitely described, but MRSA was antibiotics-resistant bacterium of interests. Other 10 bacteria are killed by broad antibiotics, but MRSA is characterized by resistance. Even though only one antibiotics-resistant bacterium was applied here, it implied that antibiotics-resistant bacteria require more dose of ClO_2 gas to be killed or growth-inhibited.

Some bacteria can be applied in specific condition and environments. No detectable levels of *E. coli* (limit of detection 5 log) were determined in the water within 1 min after *E. coli* was added to the ClO_2 containing wash water (Banach et al., 2018). And Five mg/L of ClO_2, *E. coli* was reduced >5 orders of magnitude after 3 min (COD 1,130 mg O_2/L) (Haute et al., 2017). Concentrations of ClO_2 up to 385 ppm were safely maintained in a hospital room with enhanced environmental controls (Lowe et al., 2013). In this study, the released ClO_2 gas concentration was 13 ppmv/hr (data not shown), so we suggest that this 'ready-to-use- ClO_2 stick' maybe useful tool for inhibition of nosocomial infection.

This study will provide fundamental data to research growth inhibition by ClO_2 gas with bacteria of clinical importance value.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to disclose.

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