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Biocidal Effects of Chlorine Dioxide on Isolated and Identified Pathogens from Nosocomial Environment – Biochemical and Technical Covergence

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병원내 환경으로부터 분리 및 확인된 병원균에 대한 이산화염소의 살균 효과 – 생화학 및 기술 융합

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Abstract In this study, microorganisms were isolated from nosocomial environment and are identified by biochemical analysis as the part of biochemical and technical convergence. Microorganisms were collected at intense care unit of general hospital located in Pyeongtak (2014.11.28. - 2014. 11. 30). Using a VITEK2 equipment of biochemical approaches, eleven microorganisms e.g., *Micrococcus luteus (or M. lylae), Granulicatella adiacens (M. luteus or M. lylae), Staphylococcus caprae, Sphingomonas paucimobilis, Kocuria kristinae, G elegans, Aerococcus viridans (or Staphylococcus arlettae), Methylobacterium spp., Dermacoccus nishinomiyaensis (or Kytococcus sedentarius), Kocuria kristinae (or M. luteus, M. lylae), Pseudomonas oryzihabitans* were identified. And then identified bacteria plates were applied with a plastic stick, so called with "FarmeTok (medistick/Puristic) to produce ClO2. ClO2-releasing plastic stick showed the very strong inhibition of bacterial growth with about 99.9%. There were no bacterial colonies on the ClO2-incubated plate. Taken together, it is suggested that chlorine dioxide should be very strong inhibitor to microorganisms of nosocomial infections.

Key Words : Chlorine dioxide, Nosocomial environment, Pathogen, Biochemical test, Colony

요 약 본 연구에서는, 생화학 및 기술학 융합 연구의 일환으로, 병원내 환경으로부터 미생물이 추출되고 생화학적 분석으로 통하여 동정되었다. 미생물은 평택 소재 평원 중환자실에서 수집되었다 (2014년 11월 28일부터 2014년 11월 30일). 생화학장비인 VITEK2를 이용하여 11개의 미생물, *Micrococcus luteus (or M. lylae), Granulicatella adiacens (M. luteus or M. lylae), Staphylococcus caprae, Sphingomonas paucimobilis, Kocuria kristinae, G elegans, Aerococcus viridans (or Staphylococcus arlettae), Methylobacterium spp., Dermacoccus nishinomiyaensis (or Kytococcus sedentarius), Kocuria kristinae (or M. luteus, M. lylae), Pseudomonas oryzihabitans*이 동정되었다. 이후 이산화염소가스를 배출하는 팜이톡이라고 하는 플라스틱 막대와 함께 두고 측정해본 결과, 약 99.9% 이상의 매우 강한 미생물 성장 억제가 분석되었다. 팜이톡과 함께 배양된 미생물 플레이트에는 어떠한 세균 집락도 발견되지 않았다. 종합해볼 때, 이산화염소가스를 배출하는 팜이톡은 병원내 감염을 유발하는 미생물에 대한 매우 강한 성장 억제효과를 가지는 것으로 확인되었다.

주제어 : 이산화염소, 병원내 환경, 병원균, 생화학적 검사, 집락

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1. Introduction

Chlorine dioxide (ClO_2) is a neutral chlorine compound. It is very different from elementary chlorine, both in its chemical structure and in its behavior [1]. One of the most important qualities of chlorine dioxide is its high water solubility, especially in cold water. Chlorine dioxide does not hydrolyze when it enters water; it remains a dissolved gas in solution. Chlorine dioxide is approximately 10 times more soluble in water than chlorine [1]. In 1933, L. O. Brockway proposed a structure that involved a three-electron bond [2]. In Pauling's view the latter combination should represent a bond that is slightly weaker than the double bond. In molecular orbital theory this idea is commonplace if the third electron is placed in an anti-bonding orbital [3].

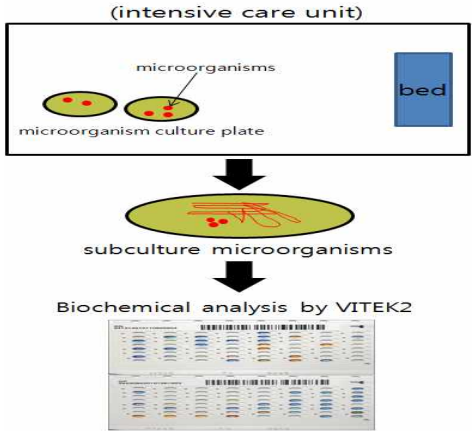
It has been reported that chlorine dioxide, a strong oxidant, can inhibit or destroy microorganisms [4, 5, 6, 7, 8]. Sanekata et. al., reproted that chlorine dioxide at concentrations ranging from 1 to 100 ppm produced potent antiviral activity, inactivating >or= 99.9% of the viruses with a 15 sec treatment for sensitization [4]. Transmission electron microscopy images of the bacteria exposed to lethal concentrations of ClO_2 indicated very little observable morphological damage to the outer membranes of the cells. 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 [8]. An increasing concentration of ClO_2 significantly reduced the microbial population compared with the control. A combined treatment of 50 ppm ClO_2 at 55°C reduced the population of naturally existing bacteria on kale by 3.10 log colony forming units (CFU)/g [9]. Interestingly, the dosage rates of silver micro-particles, hydrogen peroxide, chlorine dioxide and ozone and pH stress to the activated sludge were not able to decrease the number of culturable *Legionella* spp. in the effluent [10]. However, other treatment such as ultraviolet (UV) was also required to reduce the bacterium.

In this study, to analyze nosocomial microorganisms, microorganisms were isolated from nosocomial environment and identified by a biochemical analysis. In particular, it was performed to analyze whether chlorine dioxide inhibit the growth of the microorganisms above.

2. Materials and Methods

2.1 Culture of air-borne microorganisms

Microorganisms were collected at intense care unit of general hospital located in Pyeongtak (2014. 11. 28. - 2014. 11. 30). Isolated single colonies were subcultured into other tryptic soy agar (TSA, MB cell, Korea) plate at 37℃ [Fig. 1]. Isolated microorganisms were observed by traditional Gram-staining procedures, and gram-positive or gram-negative bacteria were separately inoculated into identification kits (Biomerieux, USA) shown at [Fig. 1] as an example for VITEK2 and then moved into a bacteria identification equipment, e.g. VITEK2 (Biomerieux, France) which is applicable for the identification of microorganisms by a biochemical analysis.



[Fig. 1] Illustrated flow chart of collection and identification of microorganisms by biochemical analysis.

Obtained single colonies were diluted with 0.85% NaCl and were adjusted into 0.5 of McFaland turbidity, which could produce about 1.5×10^5 colony forming units (CFU)/㎖. The adjusted bacteria were identified by the VITEK equipment and bacteria grown in TSA plates were applied for all subsequent experiments.

2.2 Application of ClO_2 gas kits to microorganisms

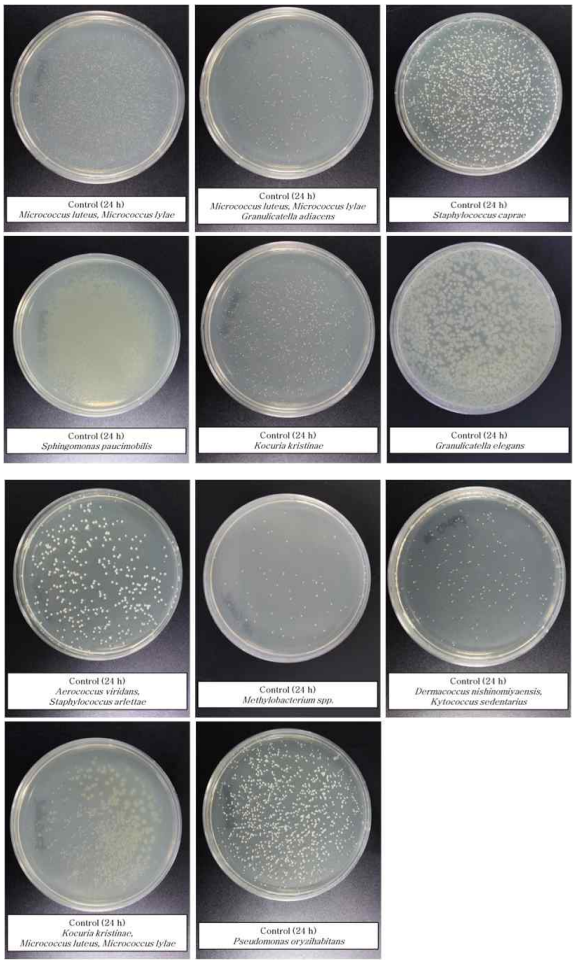
At first, bacterial suspension of 0.5 of McFaland turbidity was diluted with 0.85% NaCl upto 1,000x ratio and was cultured onto TSA plates. Identified bacteria plates were applied with a plastic stick, so called with "FarmeTok(medistick/Puristic) kindly provided by Purgofarm, co, Ltd. (Hwasung, Gyeonggido, Korea)" to produce ClO_2. To efficiently observe and culture bacteria, bacterial plates were added into a plastic clear chamber (250W×350D×200H) and then the closed chamber was incubated into a 37℃ incubator. Bacterial growth was periodically observed until 24 hr and was compared with ClO_2 gas-untreated groups as a control.

3. Results

3.1 Identification of air-borne microorganisms

To identify air-borne microorganisms at the hospital, a VITEK2 equipment was applied and its biochemical kits showed their identification with computer analysis. Eleven microorganisms colonies were isolated and analyzed by the VITEK2 [Fig. 2].

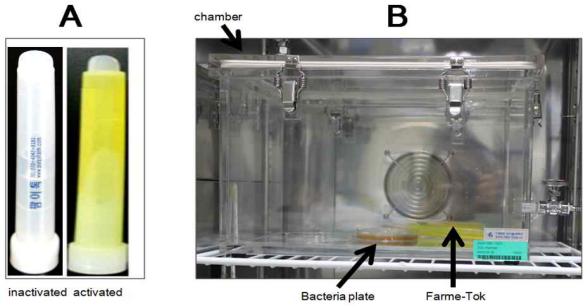
The growth of the microorganisms were different, which showed round or spread. However, a lot of colonies were observed. VITEK2 can provide the their identification by biochemical color changes. As shown [Fig. 2], *Micrococcus luteus (or M. lylae), Granulicatella adiacens (M. luteus or M. lylae), Staphylococcus caprae, Sphingomonas paucimobilis, Kocuria kristinae, G elegans, Aerococcus viridans (or Staphylococcus arlettae), Methylobacterium* spp., *Dermacoccus nishinomiyaensis (or Kytococcus sedentarius), Kocuria kristinae (or M. luteus, M. lylae), Pseudomonas oryzihabitans* were identified. O hr was not illustrated due to no colonies.



[Fig. 2] Microorganisms identified by VIKET2 and cultured by TSA. The cultures were performed upto 24 hr.

3.2 Biocidal effects of microorganisms by fame-Tok

To analyze whether chlorine dioxide can inhibit the identified microorganisms, plastic stick, so called with "FarmeTok(medistick/Puristic)" [Fig. 3A], to produce the chlorine dioxide gas was co-incubated with the microorganisms above. To avoid the release of the gas out, the plastic stick was put into a plastic chamber and was incubated at 37℃ [Fig. 3B].



[Fig. 3] Co-incubation of ClO_2-releasing plastic stick with bacteria. A showed plastic stick releasing ClO_2 and B did chamber of the stick and plate.

<Table 1> Colony forming units of the microorganisms by the treatment of ClO_2-releasing plastic stick

Microorganism	Treatment	0 hr (CFU/㎖)	24 hr (CFU/㎖)	% Inhibition
Micrococcus luteus *Micrococcus lylae*	Control	1.5×10^5	-	-
	ClO_2	1.5×10^5	< 10	99.9
Micrococcus luteus *Micrococcus lylae* *Granulicatella adiacens*	Control	1.5×10^5	-	-
	ClO_2	1.5×10^5	< 10	99.9
Kocuria kristinae	Control	1.5×10^5	-	-
	ClO_2	1.5×10^5	< 10	99.9
Granulicatella elegans	Control	1.5×10^3	-	-
	ClO_2	1.5×10^3	< 10	99.9
Staphylococcus caprae	Control	1.5×10^5	-	-
	ClO_2	1.5×10^5	< 10	99.9
Sphingomonas paucimobilis	Control	1.5×10^3	-	-
	ClO_2	1.5×10^3	< 10	99.9
Aerococcus viridans *Staphylococcus arlettae*	Control	1.5×10^4	-	-
	ClO_2	1.5×10^4	< 10	99.9
Methylobacterium spp.	Control	1.5×10^3	-	-
	ClO_2	1.5×10^3	< 10	99.9
Dermacoccus nishinomiyaensis *Kytococcus sedentarius*	Control	1.5×10^5	-	-
	ClO_2	1.5×10^5	< 10	99.9
Kocuria kristinae *Micrococcus luteus* *Micrococcus lylae*	Control	1.5×10^3	-	-
	ClO_2	1.5×10^3	< 10	99.9
Pseudomonas oryzihabitans	Control	1.5×10^4	-	-
	ClO_2	1.5×10^4	< 10	99.9

When the ClO_2-releasing plastic stick is ready for activation, it is changed into yellow and release ClO_2. Bacteria was streaked onto the plate and the plastic stick was located near the plate followed by counting of bacterial colonies <Table 1>. ClO_2-releasing plastic stick showed the very strong inhibition of bacterial growth with about 99.9%. There were no bacterial colonies on the ClO_2-incubated plate (data not shown).

4. Discussion

This study could give the information of nosocomial infective microorganisms and usefulness of ClO_2-releasing plastic stick to inhibit bacterial growth. Nosocomial infections are those acquired in or associated with hospitals. They are also known as hospital-acquired or healthcare-associated infections [11, 12, 13, 14, 15]. The related term 'iatrogenic' refers to infection or illness specifically associated with medical devices, procedures or therapies [11]. Nocosomial microorganisms can live in air and be infected into respiratory system. If the microorganisms are infected to immunity-weak patients, they will induce serious conditions. To prevent the nosocomial infections, air filtration and cleaning is strongly required. Patients need to make their body clean and wear protective mask or gloves. Our goal was also to analyze the effect of ClO_2-stick to microorganisms causing nosocomial infections. It was reported that gaseous ClO_2 was effective for sterilizing environmental contamination in a hospital room. Concentrations of ClO_2 up to 385 ppm were safely maintained in a hospital room with enhanced environmental controls [16]. In other report, ClO_2 at concentration of 1.3 and 13 mg L^{-1} has significantly bactericidal efficiency against these pathogens, e.g, *Erwinia carotovora, Ralstonia solancearum* [17]. On the other hand, free chlorine was found to inactivate severe acute respiratory syndrome-associated coronavirus

(SARS- CoV) better than chlorine dioxide [18, 19].

This study may be applied into other areas such as convergence. Health scientists are working at various parts of a hospital. If health scientists are well conscious of hospital safety, they will supplement their activities like nursing [20]. Furthermore, health service and smart health care will be better as based on health safety of the nosocomial infections [21, 22].

Taken together, it is suggested that chlorine dioxide should be very strong inhibitor to microorganisms to nosocomial infections.

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