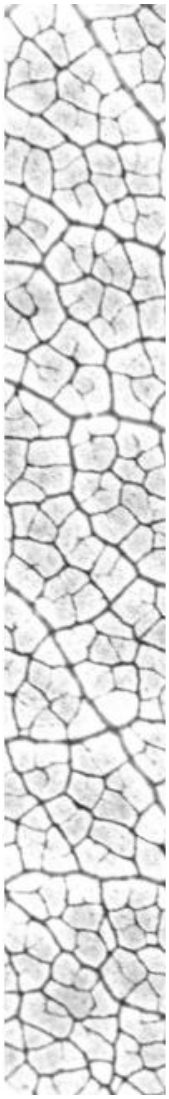

EurAsian Journal of BioSciences
Eurasia J Biosci 14, 3875-3882 (2020)

Antibacterial activities of chlorine gas and chlorine dioxide gas against some pathogenic bacteria

Amal Talib Al-Sa'ady [1*], Habeeb S. Nahar [2], Faisal Fayez Saffah [3]

[1] Dept. Clinical Laboratory Sciences, College of Pharmacy, University of Babylon, Health Security Partners (HSP) fellow, IRAQ
[2] Dept. Pathological Analysis Techniques, AL-Mustaqbal University College, Babylon, IRAQ
[3] B.Sc. Pathological Analysis Techniques, AL-Mustaqbal University College.Babylon, IRAQ
*Corresponding author: amal.atiyah@yahoo.com

Abstract
Infection control in hospitals is the key strategy for prevention of nosocomial infections by breaking down the transmission rout of infection from the source to susceptible patient by using of suitable disinfectants. Chlorine Dioxide Gas and Chlorine Gas are promising alternative to other chlorine-releasing disinfectants. Sixty-five samples were collected from clinical and environmental sources of Babylon Hospital for Pediatric and Gynecology and Al-Hilla General Teaching Hospital through a period from September 2018 to March 2019. An antibacterial efficacy test of ClO_2 and Cl_2 was performed against Streptococcus pneumoniae, Klebsiella pneumoniae, Enterobacter aerogenes, methicillin resistant Staphylococcus aureus (MRSA), Pseudomonas aeruginosa, Escherichia coli, Bacillus sp. and Staphylococcus epidermidis. The results indicated that both of ClO_2 and Cl_2 revealed a remarkable antibacterial potency against all of tested bacteria but ClO_2 was more active compared with Cl_2. This antibacterial activity was a variable with the concentration and the type of bacteria.

Keywords: Chlorine Dioxide Gas, Chlorine Gas, antibacterial activities, pathogenic bacteria

Al-Sa'ady AT, S, Nahar H, Saffah FF (2020) Antibacterial activities of chlorine gas and chlorine dioxide gas against some pathogenic bacteria. Eurasia J Biosci 14: 3875-3882.

INTRODUCTION

It is known that some hospitalized patients, particularly those patients in the Intensive Care Unit(ICU), are at risk for dying not only because of their critical health conditions but also because of other nosocomial infections which are acquired in the hospital after 48 hours of admission, usually associated with immunosuppression, the severity of underlying disease, invasive medical interventions. Nosocomial Infections included bacteremia, septicemia, Urinary Tract Infection, Nosocomial Pneumonia, ventilator-associated pneumonia, etc. (Horan, et al. (2004. Patwardhan, et al.2008. Rahim, et al. 2011) Frequently, Nosocomial Infections are caused by bacteria have resistance against most common antibiotics such as Methicillin Resistant *Staphylococcus aureus* (MRSA), Multi-Drug Resistant Gram-negative bacilli, and Vancomycin Resistant *Enterococcus faecalis*,VRE.(Al-Sa'ady, et al. 2016. Al-Sa'ady, 2019.) On the other hand, Nosocomial Pneumonia caused by *S. pneumoniae, Klebsiella pneumoniae,Enterobacter sp., S. aureus, P. aeruginosa, Haemophilus influenzae, E. coli*, and anaerobes.(Patwardhan, et al.2008. Al-Sa'ady, Amal Talib, 2019. Depuydt & Vogelaers. 2007) All evidences were indicating that increasing bacterial antibiotic resistance is accompanied with the high rate of morbidity, mortality and high cost of health care facilities. Moreover, many nosocomial pathogens may not be eliminated by the usual cleaning, thus, can survive for extended periods on hospital, indoor air and surfaces, and contribute to the transmission of infections.(Al-Sa'ady; et al. 2018. Kramer, et al.2006. Boyce, et al.2008) In a study by Otter and French (Jaiswal, et al.2018). Resistance of nosocomial bacteria *Acinetobacter* sp., MRSA, *K. pneumoniae*, *C. difficile*, and VRE and bacterial spores against the decontamination by Hydrogen Peroxide Vapor (HPV) was reported. Beyond Urinary Tract Infection, Pneumonia ranks at the second stage as a most common nosocomial infection. Ventilator-associated pneumonia (VAP) is the pneumonia associated with mechanical ventilation and represented 86% of nosocomial pneumonia (Otter1, et al. 2009. Warrenet al. 2003) Several good chances to reduce the occurrence of VAP are available instead of Ventilation may be most simple, effective and lest cost, but ventilation is still an

Received: May 2019
Accepted: March 2020
Printed: October 2020

important protocol in critically ill patients. (Nieuwenhoven,et al.2004. Kollef, 2004).

The control of indoor air quality has a main task in the prevention of nosocomial infections in order to create protective conditions for both patients and health care workers.(Dodek, et al. 2004. ASHRAE, 2001. Aysa, Noor Hadi;A. et al. 2015a)Thus, special control strategies of the indoor air quality system must design and implement in hospitals, especially for critical areas, such as a patient' s room, delivery room, Intensive care unit, operating theatre, the isolation room (Infectious or Protective isolation), Laboratories and medical equipment. The present study suggests the evaporation (fumigation) with ClO_2 and/or Cl_2 as alternate strategy for hospital decontamination.

Chlorine gas and chlorine dioxide gas are strong oxidizing agents. Both have the reaction of the proteins and amino acids in the bacterial cell structure and alter their chemical characteristics which destroy bacterial cell and disintegrate it. Despite, Cl_2 and ClO_2 have a common name, but their chemistry are completely different. ClO_2 will not produce environmentally dangerous chlorinated organics because ClO_2 can't react with many organic compounds, whereas Cl_2 has the ability for attaching with organic compounds to form a chlorinated highly toxic compounds that persist in the environment long after it is produced.(Körtvélyesi, 2004. Aysa, Noor Hadi;A. et al. 2015a; Manan et al., 2019).

Many studies demonstrated that, very low concentrations of ClO_2 gas(sub-toxic levels) have adequate safety for using as a decontamination agent in places of human activities without evacuation. If the potent antimicrobial activity of ClO_2,at sub-toxic levels, was shown, it would be applicable in the prevention of respiratory infection transmission in many public places without evacuating, such as hospitals, schools, offices, airport buildings and theatres without interrupting their normal activities.(Gray, Nicholas 2014. Ogata, & Shibata, 2008. Aysa, et al. 2015b)

Aim of Study: The study aimed to use the chlorine dioxide gas and chlorine gas in sub-toxic levels to decontaminate the hospital's environments by fumigation as novel and suitable strategy for decontamination and disinfection to control the distribution of bacteria causing nosocomial infections.

MATERIALS AND METHODS

Bacterial Isolation and Identification

Sixty-five samples were collected from different sources included: blood, urine, sputum, skin infection, wound infection, burn infection, the mask of the mechanical ventilator in the emergency unit, cannula, Intensive Care Unit (INCU), disinfectant, fluid sucker, the catheter, operating theatre in Babylon Hospital for Pediatric and Gynecology and Al-Hilla General Teaching Hospital. The bacterial isolates have diagnosed by VITEK 2 Compact System.

Fig. 1. Lower bottom of plane tube was cut and sterilized in order to use as a container for gas production

Table 1. Substances were used to produce ClO_2 Gas in three Different Concentrations

Concentration	First substance: NaClO2(25%) solution(µl)	Second substance: HCl(35%) In µl
First Concentration	70	60
Second Concentration	60	50
Third Concentration	50	50

Table 2. Substances were used to produce Cl_2 Gas in four Different Concentrations

Concentration	First substance: CaClO2 powder (gm)	Second substance: HCl (35%) In µl
First Concentration	0.015	60
Second Concentration	0.012	50
Third Concentration	0.006	50
Fourth Concentration	0.003	50

Antibacterial Activity Test of ClO_2 gas and Cl_2 gas

The bacterial suspension has prepared of turbidity equal to McFarland standard tube. Muller-Hinton plate has inoculated by streaking with the bacterial. A lower bottom of a plane tube(diameter 12 mm) was cut and sterilized by two methods, Ultra-Violate and flame of burner and placed at the center of inoculated plate in order to use it as a container for gas production. The first substance was added in the sterilized plane tube bottom, then, the second substance was added to it carefully. The cover of plate was closed quickly in order to keep the produced gas inside. The addition done with three different concentrations as shown in **Tables 1** and **2**. control plate was inoculated with bacteria for each species without any addition. The Incubation was done at 37°C for 24hrs. After incubation, the absence of growth(no growth) indicated a positive result and the produced gas has antibacterial activity.

RESULTS AND DISCUSSION

Bacterial Isolation and Identification

A total of 35 bacterial isolates from different clinical sources, as detailed in **Table 3**, included: Pseudomonas

Table 3. Bacterial Isolates and Their Sources in the Present Study

No.	The Source of Isolation	The Bacterial Isolates
1	Blood (Septicemia)	*K. pneumoniae*; Methicillin Resistant *S. aureus*
2	Urine (UTI)	*K. pneumoniae*; *Enterobacter aerogenes E. coli*
3	Sputum (Pneumonia)	*Streptococcus pneumoniae*; *K. pneumoniae*; *P. aeruginosa*
4	Skin Infection(Nosocomial)	Methicillin Resistant *S. aureus*; *Staphylococcus epidermidis*
5	Wound Infection(Nosocomial)	*Enterobacter aerogenes*; Methicillin Resistant *S. aureus*; *P. aeruginosa*; *E. coli*
6	Burn Infection(Nosocomial)	Methicillin Resistant *S. aureus*; *E. coli*; *Bacillus* sp; *Staphylococcus epidermidis*
7	Mask of Mechanical Ventilator in Emergency Unit (Nosocomial)	*Streptococcus pneumoniae*; *P. aeruginosa*; *Bacillus* sp.
8	Cannula(Nosocomial)	Methicillin Resistant *S. aureus*; *Bacillus* sp.; *P. aeruginosa*
9	Intensive Care Unit(ICU)(Nosocomial)	*P. aeruginosa*; *Bacillus* sp.; *Staphylococcus epidermidis*
10	Fluid Sucker(Nosocomial)	Methicillin Resistant *S. aureus*; *P. aeruginosa*; *Bacillus* sp.
11	Catheter(Nosocomial)	Methicillin Resistant *S. aureus; P. aeruginosa*
12	Operating Theatre (Nosocomial)	*E. coli*; *Bacillus* sp.; *Staphylococcus epidermidis*
13	Disinfectant(Nosocomial)	*P. aeruginosa*

Table 4. Antibacterial activity for three concentrations of Chlorine Dioxide Gas

Bacterial isolates	Source of Isolate	Concentration		
		First Conc*	Second Conc	Third Conc
S. pneumonia	Sputum	No growth	No growth	No growth
K. pneumonia	Sputum	No growth	No growth	No growth
E. aerogenes	Urine	No growth	No growth	No growth
Methicillin Resistant *S. aureus*	Wound Infection	No growth	No growth	No growth
P. aeruginosa	Disinfectant	No growth	No growth	No growth
E. coli	Operating Theatre	No growth	No growth	No growth
Bacillus sp.	Mask of Mechanical Ventilator	No growth	No growth	No growth
S. epidermidis	Catheter	No growth	No growth	No growth

Conc*= Concentration

aeruginosa, Methicillin Resistant Staphylococcus, Streptococcus pneumoniae, Klebsiella pneumoniae, Enterobacter aerogenes, aureus(MRSA), Escherichia coli, Bacillus sp., Staphylococcus epidermidis.

The Antibacterial Activity Test

The isolating between the hospital environment and the sources of infection is most basic procedure to Prevent nosocomial infections which can be done by breaking down the transmission rout of infection and isolating the source of infection from the hospital environment, in addition, improving the patient 's resistance against infection. Using of a suitable disinfectant which is one of the key strategies of infection control in the hospitals. ClO_2 and Cl_2 are promising alternative to other chlorine-releasing disinfectants. So, their antibacterial activity should be understood.

In the present study, the antibacterial activity test was performed for chlorine dioxide gas ClO_2 and chlorine gas Cl_2 gas against 8 species of bacteria included nosocomial pathogens and bacteria causing pneumonia: *Pseudomonas aeruginosa, Streptococcus pneumoniae, Klebsiella pneumoniae, Escherichia coli*, *Enterobacter aerogenes,* methicillin resistant *Staphylococcus aureus*(MRSA)*, Bacillus* sp., *Staphylococcus epidermidis*. The results of this study showed that, both ClO_2 and Cl_2 have clear antibacterial effectiveness against all studied bacterial species with some variation, as detailed in **Tables 4** and **5**.

These results are accordance with other studies who described chlorine dioxide(ClO_2) as a strong oxidizing agent because it has free radicals and unpaired electrons and has broad spectrum of potent antimicrobial activities must be studied.(WHO, 2016. Eleraky, et al. 2002). Studies by Ogata (Eleraky, et al.2002) and Ogata and Shibata(Gray, 2014). emphasized that the antimicrobial efficacy of ClO_2 is ascribable to it' s ability of protein denaturation resulting from the covalent oxidative modification and have shown that ClO_2 denatures the viral envelope' s proteins which decreased it' s infectiveness.

1. Chlorine(Cl_2) has used widely in water treatment or surface disinfection since 19th century. As a result of his deodorizing and disinfecting properties, today, most people are very familiar with chlorine as disinfectant due to it is a strong oxidizing agent that is highly reactive with bacterial cell components such as DNA, proteins, lipids and carbohydrates. It is noteworthy that, chlorine is readily degenerate of the environment to oxygen and chloride which reduces the chance to develop resistance against it by pathogen·(Ogata, 2007.Windf, 2011. Gilberto, & Eloisa, 2015)

These findings were in agreement with many studies who described chlorine-releasing agents as excellent environmental disinfectants have the ability to inhibit many kinds of microorganisms such as Gram positive and Gram negative bacteria, viruses both enveloped and non-enveloped in the low-concentrations and reducing the risk of nosocomial infections (Gilberto, & Eloisa, 2015. Saheem, et al. 2017. Morino, et al. 2011.Sanekata, et al. 2010)

The Antibacterial Activity Test of chlorine dioxide gas ClO_2

In the present study, Controlled-Releasing Chlorine Dioxide Gas(ClO_2) was tested as antibacterial agent against 8 bacterial species in vitro. The results emphasized that chlorine dioxide gas has absolute antibacterial activity against all studied bacterial species in all concentrations, **Table 4**. All studied bacterial

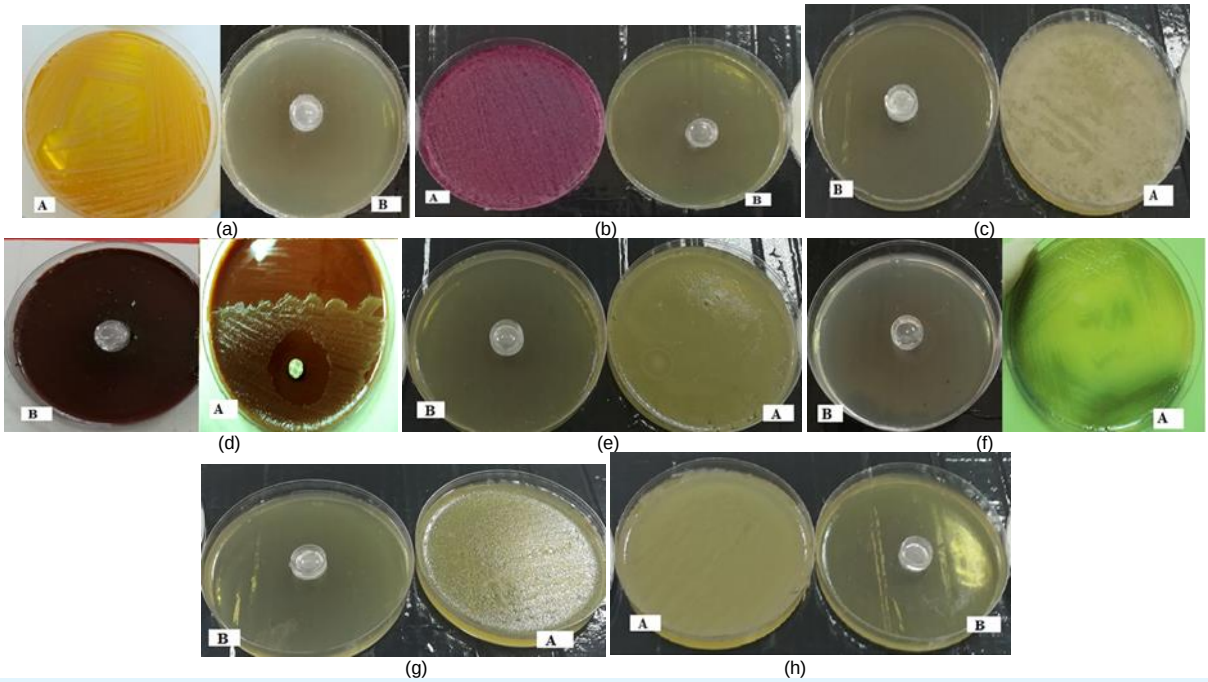

Fig. 2. Antibacterial Activity of Chlorine Dioxide Gas (ClO_2) in the present study **a:** Antibacterial activity of ClO_2 gas against MRSA **b**: Antibacterial activity of ClO_2 against *S. epidermidis* **c:** Antibacterial activity of ClO_2gas against *Bacillus sp.* **d**: Antibacterial activity of ClO_2 gas against *S. pneumoniae* **e:** Antibacterial activity of ClO_2 gas against *E. coli* **f**: Antibacterial activity of ClO_2 gas against *P. aeruginosa* **g:**Antibacterial activity of ClO_2 gas against *E. aerogenes* **h**: Antibacterial activity of ClO_2 gas against *K. pneumoniae*
A: Control without treatment by chlorine dioxide gas
B: No growth after the treatment by chlorine dioxide gas(full inhibition)

Table 5. Antibacterial activity for three concentrations of chlorine gas

Bacterial isolates	Source of Isolate	Concentration			
		First Conc*	Second Conc	Third Conc	Fourth Conc
S. pneumoniae	Sputum	No growth	No growth	No growth	No growth
K. pneumoniae	Sputum	No growth	No growth	Partial Growth**	Partial Growth
E. aerogenes	Urine	No growth	No growth	No growth	No growth
MRSA	Wound Infection	No growth	No growth	Partial Growth	Partial Growth
P. aeruginosa	Disinfectant	No growth	No growth	No growth	No growth
E. coli	Operating Theatre	No growth	No growth	No growth	No growth
Bacillus sp.	Mask of Mechanical Ventilator	No growth	No growth	Partial Growth	Partial Growth
S. epidermidis	Catheter	No growth	No growth	No growth	No growth

Conc*=Concentration; Partial Growth** = Growth at the inner perimeter of the petri dish

isolates have high sensitivity with no growth after the treatment by chlorine dioxide gas under controlled conditions as shown in **Fig. 2**.

The results in the present study are in agreement with many studies who described ClO_2 gas as an excellent disinfectant has a broad spectrum of antimicrobial efficacy (Aysa, et al. 2015a, Gilberto, & Eloisa, 2015, Sanekata, et al. 2010.

Chlorine dioxide is a water-soluble gas and has been known as an antimicrobial agent has been used in wastewater treatment and water purification because it reveals fast kill a broad range of microbes by oxidation, penetration and killing of bacterial cell through reaction with essential amino acids in the protoplasm. Because of it' s fungicidal and sporicidal activity in solutions, ClO_2 is considered the most effective on hard surfaces compared to other disinfectants.(Young, 2016.Sun X1, et al.2014).

Antibacterial Activity Test of chlorine gas Cl_2 gas

In the present study, Antibacterial Activity of Chlorine Gas(Cl_2) was tested under controlled conditions against 8 bacterial species, in vitro. The results emphasized that chlorine gas has antibacterial activity against all studied bacterial species, but, this activity varies with various concentrations and with various bacterial species. As detailed in **Table 5**, antibacterial activity decreased with the decreasing in concentration, in the lower tow concentrations(third and fourth) three species of studied bacteria (*Bacillus* sp., *K. pneumoniae*, *P. aeruginosa)* have growth at the inner perimeter of the petri dish as shown in **Fig. 3**. Depending on their virulence factors, various bacteria differ in their susceptibility to

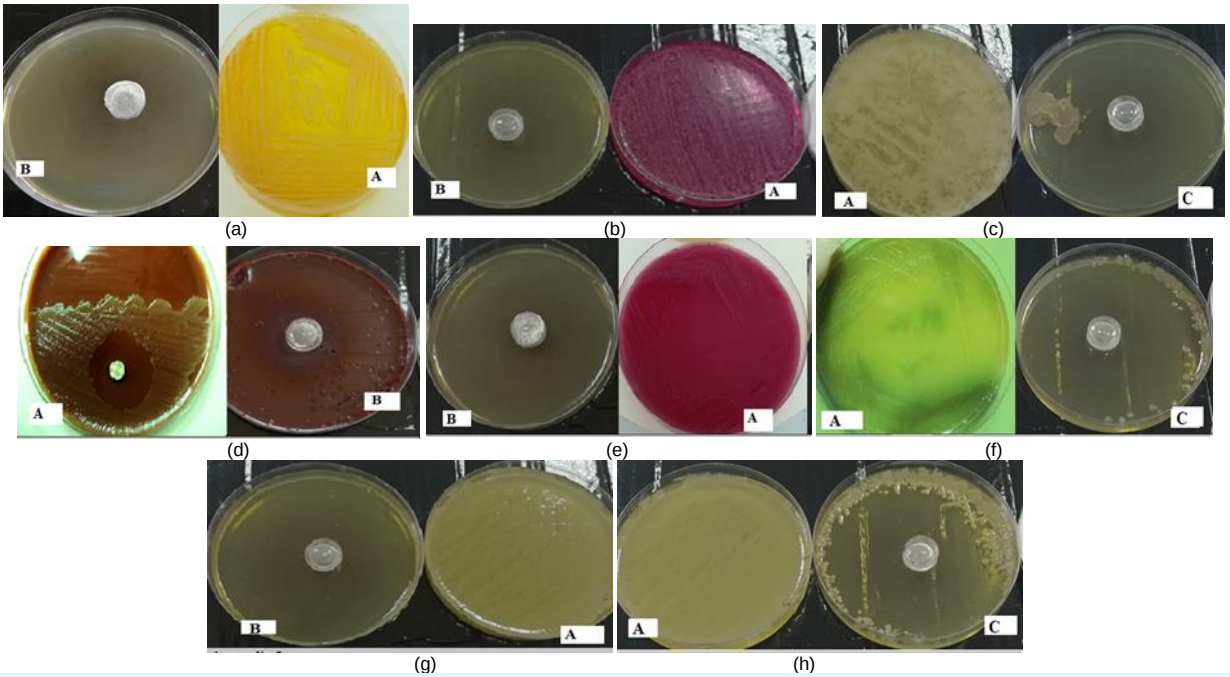

Fig. 3. Antibacterial Activity of Chlorine Gas in the Present **a:** Antibacterial activity of chlorine gas against MRSA **b:** Antibacterial activity of chlorine gas against *S. epidermidis* **c:** Antibacterial activity of chlorine gas against *Bacillus sp.* **d:** Antibacterial activity of chlorine gas against *S. pneumoniae* **e:** Antibacterial activity of chlorine gas against *E. coli* **f:** Antibacterial activity of chlorine gas against *P. aeruginosa* **g:** Antibacterial activity of chlorine gas against *E. aerogenes* **h:** Antibacterial activity of chlorine gas against *K. pneumoniae*
A: Control without treatment by chlorine gas
B: No growth after the treatment by chlorine gas(full inhibition)
C: Growth at the inner perimeter of the petri dish after the treatment by chlorine gas(partial inhibition

disinfectants. *Bacillus* sp., is Gram positive spore forming bacteria. The survival of these bacteria in the present test may be ascribable to it' s ability of spore formation because spores are highly resistant for hard chemical and physical conditions and make it is difficult to eliminate(Weaver-Meyers, et al. 2000). *P. aeruginosa* and *K. pneumoniae* are Gram-negative rods have ability to produce fully established biofilms. The survival of these bacteria in the present test may ascribable to it' s ability of biofilm formation which increases the resistance to antibiotic therapy, disinfection and phagocytosis, as well as other parts of the immune system and cause chronic infections. In the present study, *P. aeruginosa* and *K. pneumoniae* have high sensitivity for chlorine dioxide gas more than chlorine gas which may ascribable to the ability of chlorine dioxide to penetrate the biofilm and effectively remove it. While, chlorine is not able to do this.(Zhang, 2007.Block, 2004. Van Laer, et al. 2008.Rasamiravaka, et al. 2015) All reviewed evidences in the present study were indicating that chlorine gas has antibacterial activity less than those of chlorine dioxide gas. This result was in agreement with the fact that, at equivalent doses, chlorine dioxide has 4-7 times more effective as a biocidal agent than chlorine and it is more powerful has clear antibacterial effectiveness. In addition, chlorine dioxide has a broad spectrum of environmental advantages and safety more than chlorine and other antibacterial agents. On the other hand, chlorine dioxide does not produce halogenated, carcinogenic by-products which may be produced when chlorine is used. additionally chlorine dioxide keeps it' s activity under a wide range of pH about 1-10, while the efficacy of chlorine may be significantly reduced above neutral or basic pH(Morino, et al. 2011). In a study by Sadeghi et al(Vuotto et al. 2017) the developed self-releasing sheet was used for releasing ClO2 at suitable concentrations in order to maintain the quality of cherry tomatoes and prolong it' s postharvest life by inactivation of microbial growth and recommended that it can be used for medical or industrial applications.

CONCLUSION

- Chlorine dioxide gas(ClO_2) and chlorine gas (Cl_2) were releasing in the laboratory under controlled conditions.
- Although, both revealed clear antibacterial effectiveness against pathogenic nosocomial bacteria either Gram-negative or Gram-positive, ClO_2 gas has antibacterial activity more than those of Cl_2 gas.
- ClO_2 gas and Cl_2 gas in nontoxic concentrations can be used in the different medical applications for disinfection and sanitization.

ACKNOWLEDGEMENTS

We would like to express our heartfelt thanks to college of Pharmacy/university of Babylon, and AL-mustaqbal University College for their effort during sample collection, analysis and working at their laboratories.

REFERENCES

Ahmad, S., Hamda Kh., Uzma Sh., Shahnawaz R., Zeeshan R., Mohd Y. Kh., Ahsanullah Ansari, Zeba Siddiqui, Jalaluddin Mohammad Ashraf, Saleh M S Abdullah, Safia Habib, Moin Uddin (2017).Protein oxidation: an overview of metabolism of sulphur containing amino acid, cysteine. Frontiers in Bioscience, Scholar,9,71-87.

Al-Sa'ady, A.T (2019). Detection of vancomycin resistance in multidrug-resistant Enterococcus faecalis isolated from burn infections. Drug Invention Today,11(11): 2984-2989.

Al-Sa'ady, A.T (2019). Detection of vancomycin resistance in multidrug-resistant Enterococcus faecalis isolated from burn infections. Drug Invention Today,11(11): 2984-2989.

Al-Sa'ady, A. T. A. & Naher, H. S.(2016). Study of etiologic multi-drug resistant bacteria of neonatal sepsis in Al-Hilla city. American Journal of BioMedicine,AJBM;4(11):513-533.

Al-Sa'ady; A.T. & Naher; H. S. (2018).Study of the Routs of Etiologic Bacteria Causing Neonatal Infections in Al-Hilla City/ Iraq. Biochem. Cell. Arch. Vol. 18, No. 1, 577-586.

ASHRAE, ANSI/ASHRAE Standard 62–2001(2001).Ventilation for acceptable indoor air quality, Atlanta: American Society of Heating, Refrigerating and Air-Conditioning Engineers, Inc.

Aysa, N. H; Al-Maamori, Mohamed Hamza; Al-Maamori, Nehad Abdul Ameer(2015a).Studying The Mechanical Properties of Silicone Rubber-AlumComposites using in Manufacturing of Prosthetic Liners. Journal of Natural Sciences Research, Vol.5, No.15: 47-52.

Aysa, N. H; Al-Maamori, Mohamed Hamza; Al-Maamori, Nehad Abdul Ameer(2015b).Effect of the Unmodified and Modified ZnO Nanoparticles on the Mechanical and Antibacterial Properties of Silicone Rubber Using in Medical Applications. Journal of Nanoscience and Nanoengineering, Vol. 1, No. 3: 119-124.

Block, C.(2004).The effect of Perasafe and sodium dichloro-iso cyanurate (NaDCC) against spores of Clostridium difficile and Bacillus atrophaeus on stainless steel and polyvinyl chloride surfaces. J. of Hospital

Boyce, J. M., N. L. Havill, J. A. Otter, L. C. McDonald, N. M. Adams, T. Cooper, A. Thompson, L. Wiggs, G. Killgore, A. Tauman, & J. Noble-Wang(2008).Impact of hydrogen peroxide vapor room decontamination on Clostridium difficile environmental contamination and transmission in a healthcare setting. Infect. Control Hosp. Epidemiol. 29723-729.

Depuydt, P. Vogelaers, D.(2007).Nosocomial pneumonia outside the hospital: health-care associated pneumonia and nursing home pneumonia. Tijdschrift voor Geneeskunde (Belgium) (in Dutch). 63 (5): 174–181.

Dodek, P., S. Keenan, D. Cook, D. Heyland, M. Jacka, L. Hand, J. Muscedere, D. Foster, N. Mehta, R. Hall, & C. Brun-Buisson.(2004).Evidence-based clinical practice guideline for the prevention of ventilator-associated pneumonia. Ann. Intern. Med. 141:305-313.

Eleraky, N. Z., Potgieter, L. N. & Kennedy, M. A.(2002).Virucidal efficacy of four new disinfectants. J Am Anim Hosp Assoc. 38, 231–234.

Gilberto, R. & Eloisa, T. (2015). Sodium Hypochlorite (NaClO): Ruffini Method for the Treatment of Topic Infectious Diseases of the Skin, Mucous Membranes and Systemic Diseases. The Battle Against Microbial Pathogens: Basic Science, Technological Advances and Educational Programs (A. Méndez-Vilas, Ed.).

Gray, N.F. (2014). "Chemistry of Chlorine Dioxide" in " Microbiology of Waterborne Diseases,Second Edition".

Horan, T.C, Gaynes, R.P. Mayhall, C.G, editor. (2004).Hospital epidemiology and infection control. 3. Philadelphia: Lippincott Williams & Wilkins Surveillance of nosocomial infections.1659–702.

International Program on Chemical Safety (IPCS).(2002) Chlorine dioxide (gas). Geneva: World Health Organization, International Programme on Chemical Safety (Concise International Chemical Assessment.

Jaiswal, Swati; Mishra, Prashant (2018).Antimicrobial and antibiofilm activity of curcumin-silver nanoparticles with improved stability and selective toxicity to bacteria over mammalian cells. Medical Microbiology and Immunology, 207(1):pp 39–53.

Kollef, M. H. (2004). Prevention of hospital-associated pneumonia and ventilator-associated pneumonia. Crit. Care Med. 32:1396-1405.

Körtvélyesi, Z. (2004). Analytical Methods for the Measurement of Chlorine Dioxide and Related oxychlorine species in Aqueous Solution. PhD, A thesis. Miami University, Oxford, Ohio.

Kramer, A., Schwebke, I. & Kampf, G.(2006).How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. BMC Infect. Dis. 6130.

Leung, M & Chan, A.H.S. (2006).Control and management of hospital indoor air quality. Med Sci Monit, 12(3): SR17-23.

Manan, S. S., Jianu, O. A., & Rosen, M. A. (2019). Investigation of Heat Extraction Methods from Cuprous Chloride for Improving the Efficiency of the Thermochemical Copper-Chlorine Cycle. European Journal of Sustainable Development Research, 3(1), em0075.

Morino,H.; T. Fukuda, T. Miura & Shibata, T. (2011).Effect of low-concentration chlorine dioxide gas against bacteria and viruses on a glass surface in wet environments. Letters in Applied Microbiology, 53, 628–634.

National water biosecurity manual : poultry production.(2009).English, Book, Illustrated edition. Canberra, A.C.T.] : DAFF.ID 44879460

Nieuwenhoven,V. C. A., E. Buskens, D. C. Bergmans, F. H. van Tiel, G. Ramsay, & M. J. Bonten(2004). Oral decontamination is cost-saving in the prevention of ventilator-associated pneumonia in intensive care units. Crit. Care Med. 32:126-130.

Ogata, N.(2007). Denaturation of Protein by Chlorine Dioxide: Oxidative Modification of Tryptophan and Tyrosine Residues. Biochemistry, 46 (16), pp. 4898–4911. DOI: 10.1021/bi061827u.

Ogata, Norio and Shibata, Takashi (2008)Protective effect of low-concentration chlorine dioxide gas against influenza A virus infection. journal of General Virology. 89: 60–67.

Otter1, J. A. & French, G. L.(2009). Survival of Nosocomial Bacteria and Spores on Surfaces and Inactivation by Hydrogen Peroxide Vapor. J Clin Microbiol.;47(1): 205–207.

Patwardhan, R.B, Dhakephalkar, P.K, Niphadkar, K.B, Chopade, B.A.(2008). A study on nosocomial pathogens in ICU with special reference to multiresistant Acinetobacter baumannii harbouring multiple plasmids. Indian J Med Res.,128:178–87.

Rahim, B. Peyman, M. Davood, N. Hamid, R.K. (2011).An epidemiological study of nosocomial infections in the patients admitted in the intensive care unit of Urmia Imam Reza Hospital: An etiological investigation. Ann Biol Res., 2:172–8.

Rasamiravaka, T., Quentin L., Pierre D.,& Mondher E. J.(2015).The Formation of Biofilms by Pseudomonas aeruginosa: A Review of the Natural and Synthetic Compounds Interfering with Control Mechanisms. BioMed Research International.Volume 2015, ID 759348, 17 pages. http://dx.doi.org/10.1155/2015/759348.

Sadeghi, K.; Gopinath, K.; Phuntheera, K.; Sarinthip, Th.; Sher, B. Kh.; Jongchul S.(2020). A polymeric chlorine dioxide self-releasing sheet to prolong postharvest life of cherry tomatoes. Postharvest Biology and Technology; Volume 161, March 2020, 111040. https://doi.org/10.1016/j.postharvbio.2019.111040.

Sanekata, T., Fukuda, T., Miura, T., Morino, H., Lee, C., Mae-da, K., Araki, K. Otake, T.et al.(2010).Evaluation of the antiviral activity of chlorine dioxide and sodium hypochlo-rite against feline calicivirus, human influenza virus,measles virus, canine distemper virus, human herpesvirus,human adenovirus, canine adenovirus canine parvovi-rus.Biocontrol Sci,15, 45–49.

Seifi, K., Kazemian, H., Heidari, H., Rezagholizadeh, F., Saee, Y., Shirvani, F., & Shirvani, Houri, H. (2016).Evaluation of Biofilm Formation Among Klebsiella pneumoniae Isolates and Molecular Characterization by ERIC-PCR. Jundishapur J Microbiol.; 9(1): e30682. doi: [10.5812/jjm.30682.

Sun, X1., Bai, J., Ference, C., Wang, Z., Zhang, Y., Narciso, J., Zhou, K.(2014).Antimicrobial activity of controlled-release chlorine dioxide gas on fresh blueberries. J Food Prot.;77(7):1127-32. doi: 10.4315/0362-028X.JFP-13-554.

Van Laer, A.F., Verdyck, R., Jansens, H. and Coenen. E.(2008). Disinfectants containing chlorine: an occupational hazard? Infection control and hospital epidemiology.29(5):474.

Vuotto, C, Longo, F, Pascolini, C, Donelli, G, Balice, M.P, Libori, M.F, Tiracchia, V., Salvia, A., Varaldo, P.E.(2017).Biofilm formation and antibiotic resistance in Klebsiella pneumoniae urinary strains. J Appl Microbiol.123(4):1003-1018. doi: 10.1111/jam.13533.

Warren, D. K., S. J. Shukla, M. A. Olsen, M. H. Kollef, C. S. Hollenbeak, M. J. Cox, M. M. Cohen, & V. J. Fraser(2003).Outcome and attributable cost of ventilator-associated pneumonia among intensive care unit patients in a suburban medical center. Crit. Care Med. 31:1312-1317.

Weaver-Meyers,P.L., Stolt,W.A. & Kowa leski, B.(2000).Controlling mould on library materials with chlorine dioxide: An eight-year case study. Abbey newsletter. 24:4.

WHO/FWC/WSH/16.49(2016). Chlorine Dioxide, Chlorite and Chlorate in Drinking-water. Background document for development of WHO Guidelines for Drinking-water Quality.

Windf, C. (2011). The Toxicology of Chlorine. Environmental Research. 85(2): 105-114.

Young, R.O. (2016).Chlorine Dioxide (CLO2) as a Non Toxic Antimicrobial Agent for Virus, Bacteria and Yeast (Candid albicans). International Journal of Vaccines and Vaccination.2(6):00052.

Zhang, Z., McCann, C., Stout, J.E., Piesczynski, S., Hawks, R., Vidic, R. and Yu, V.L. (2007).Safety and efficacy of chlorine dioxide for Legionella control in a hospital water system. Infection Control and Hospital Epidemiology. 28(8):1009-1012.