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Original Research Article

In vitro antimicrobial activity of three new generation disinfectants

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Abstract

Purpose: *To determine the efficacies of three commercially available new generation disinfectants against some bacteria and yeast.*
Methods: *Three commercially available new generation disinfectant (0.2 % chlorine dioxide, 0.3 % chlorine dioxide and 50 % hydrogen peroxide-stabilized by colloidal silver) were screened for their antimicrobial activity against Escherichia coli ATCC 25922, Klebsiella pneumonia RSKK 574, Pseudomonas aeruginosa ATCC 9027, Enterococcus faecalis ATCC 29212, Staphylococcus aureus ATCC 25923, S. aureus ATCC 43300 (methicillin resistant), S. epidermidis ATCC 12228 (non-biofilm forming), S. epidermidis ATCC 35948 (biofilm forming) and Candida albicans ATCC 10231. Quantitative suspension test was used to determine the efficacies of the disinfectants at contact times of 1, 3 and 5 min.*
Results: *All of the new generation disinfectants were effective against test microorganisms at all test contact times.*
Conclusions: *The findings indicate that the tested new generation disinfectants may be useful for routine disinfection purposes.*

Keywords: *Antimicrobial activity, New generation disinfectants, Routine disinfection*

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INTRODUCTION

The emergence and spread of resistant microorganisms are threats to public health globally. Infections caused by these resistant microorganisms are increasing all over the world, with the associated increased morbidity, extended length of stay, higher healthcare costs and mortality [1,2]. Disinfectants are chemical used agents, extensively in hospitals and other health care settings, to inhibit or to destroy microorganisms and consequently to prevent infections. They are also used in many industrial areas. A wide variety of chemical agents like alcohols, glutaraldehyde, phenols, iodine, chlorine compounds have been used for centuries. Although most of them demonstrate broad-spectrum antimicrobial activity at higher concentrations, they have serious side effects for human and also can cause environmental problems at these concentrations. Therefore, safer and better compounds are urgently needed. For this reason researchers try to improve new substances which lack of these disadvantages [3].

New generation disinfectants are defined as products that are completely broken down in nature without harmful residues in the environment. They are also defined as non-

carcinogenic products for users [4]. There are many commercial products in the markets called new generation disinfectants. These include chlorine dioxide (ClO_2) and hydrogen peroxide (H_2O_2) are agents that widely used in these products [5,6]. Both ClO_2 and H_2O_2 are powerful oxidizing agents. Their degradation products are safe for environment but they may lead to serious problems in case of inhalation, contact with eyes and skin at higher concentrations [7,8]. This study was conducted to determine the efficacies of three commercially available new generation disinfectants against some bacteria and yeast.

EXPERIMENTAL

Disinfectants

Ar-Dez Sniper® (0.2 % chlorine dioxide), Zns-Clordioxy® (0.3 % chlorine dioxide) and Pulirex-Oxy® (50 % hydrogen peroxide-stabilized by colloidal silver) were used in this study as new generation disinfectants. Sodium hypochlorite (4.5 %) (45000 ppm) was used as positive control. Sterile distilled water was used as diluent and control. The disinfectants were stored in the dark at room temperature.

Test microorganisms

Escherichia coli ATCC 25922, *Klebsiella pneumonia* RSKK 574, *Pseudomonas aeruginosa* ATCC 9027, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *S. aureus* ATCC 43300 (methicillin resistant), *S. epidermidis* ATCC 12228 (non-biofilm forming), *S. epidermidis* ATCC 35948 (biofilm forming) and *Candida albicans* ATCC 10231 were used in the study.

Neutralization/recovery system

Dey-Engley Neutralizing Broth (DENB) (Sigma-Aldrich, USA) was tested to determine if it was appropriate to inactivate each of the disinfectant [9]. Firstly, 100 µL of sterile distilled water was added to 900 µL of the disinfectant, mixed and left for 1 min then 10 µL of this mixture was added to 990 µL of the DENB. After that, 10 µL of the undiluted test suspension of *E. coli* ATCC 25922 was added to this mixture (neat), vortex mixed for 20 s and serially diluted to 10^{-5} in Ringer's solution. Finally, 100 µL of the neat and subsequent dilutions were spread onto Tryptase Soy Agar (TSA, Merck, Germany) in duplicate, using sterile spreaders. The plates were incubated at 37 °C for 24 h and colony-forming-units (cfu) were enumerated. The undiluted test suspension was used as the initial count. The test was repeated using water instead of the disinfectant as the control. The neutralizer was deemed suitable as there was no difference in colony size, growth rate or the number of cfu retrieved from tests and controls.

Quantitative suspension test method

The quantitative suspension test was used to determine the efficacy of the disinfectants. Bacterial suspensions in Tryptic Soy Broth (Difco, USA) were adjusted to the McFarland 0.5 standard. Then, 100 µL of bacterial suspension was added to 900 µL of the disinfectant solution at room temperature for contact times of 1, 3 and 5 min. At the end of the each contact time 10 µL was removed to 990 µL of the neutralization system and serially diluted to 10^{-1} to 10^{-3}. 100 µL of each dilution was placed onto Tryptic Soy Agar (Difco, USA) plates in duplicate by the spread-plate technique and incubated at 37 °C for 24 h. The surviving colonies were enumerated and expressed as cfu per milliliter. Before this procedure, pre-disinfection counts were calculated with using sterile distilled water. The reduction rate (R, Log10 reduction) was calculated as the expression of disinfectant efficacy, according to Eq 1.

$$R = P - D \quad \text{..... (1)}$$

where P and D are Log10 pre-disinfection count and Log10 disinfection count, respectively.

Log10 reduction of 5 or more was taken as an indication of satisfactory microbicidal activity [10].

RESULTS

The results of the quantitative suspension test are presented as Log10 reductions of test microorganisms after 1, 3 and 5 min of contact (Table 1). All of the new generation disinfectants were effective against all the test microorganisms at all contact times.

DISCUSSION

Appropriate disinfection practices are the most important part of preventing spread of resistance microorganisms thus maintaining public health. Ideal disinfectant should have wide antimicrobial spectrum. It should be fast acting, active in the presence of organic compounds, not damage the environment. Low toxicity, user safety and material compatibility are some other properties of an ideal disinfectant [11].

Table 1: Quantitative suspension test results of tested new generation disinfectants against test microorganisms

Test microorganism	Disinfectant											
	A			B			C			D		
	Contact time (min)											
	1	*3*	*5*	*1*	*3*	*5*	*1*	*3*	*5*	*1*	*3*	*5*
E. coli ATCC 25922	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3
K. pneumoniae RSKK 574	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
P. aeruginosa ATCC 9027	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2
E. faecalis ATCC 29212	7.9	7.9	7.9	7.9	7.9	7.9	7.9	7.9	7.9	7.9	7.9	7.9
S. aureus ATCC 25923	8.7	8.7	8.7	8.7	8.7	8.7	8.7	8.7	8.7	8.7	8.7	8.7
S. aureus ATCC 43300	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3	8.3
S. epidermidis ATCC 12228	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2
S. epidermidis ATCC 35948	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1
C. albicans ATCC 10231	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1

A: Ar-Dez Sniper **B:** Zns-Clordioxy **C:** Pulirex-Oxy **D:** Sodium hypochlorite (4.5%)

In recent years, ClO_2 and H_2O_2 have gained popularity for disinfection applications. H_2O_2 and ClO_2 are oxidative agents and both of them remove electrons from susceptible chemical groups. They inactivate microorganisms by attacking their cell wall and cytoplasmic membrane, denaturing proteins, preventing the transport of nutrients across the cell wall and inhibiting protein synthesis [12,13].

ClO_2 is a yellow to reddish-yellow gas that can readily soluble in water at room temperature. It has broad spectrum of activity against bacteria, viruses, protozoan cysts, algae and animal planktons, both dissolved in water and gas phase. Its bactericidal activity remains constant over a broader pH range from pH 3 to 8. ClO_2 has been used for many disinfection procedures such as water disinfection. In water, chlorine dioxide reacts quickly to form chlorite ions. It is also a highly volatile compound that can easily be removed from dilute aqueous solutions. During storage and reuse processes, it may lose its potency. In addition, chlorine dioxide gradually dissociates into chlorine and oxygen. Therefore, it must be stored in small containers or single use bottles [7,14].

In the United States, usage of ClO_2 is limited at a maximum level of 0.8 mg/L as a drinking water disinfectant by Environmental Protection Agency. In Europe, it can be used in continuous disinfection process in sanitary water supplies at 1.0 mg/L concentration. Exposure of human at higher concentrations can cause serious side effect. It is a respiratory irritant compound. It may cause irritation of eyes, nose, throat and lungs [14].

Chatuev and Peterson [13] reported that, ClO_2 in solution kills *Bacillus anthracis* spores at contact time of 3 min. In another study Zhang *et al* [15], evaluated the safety and efficacy of ClO_2 fed into the incoming main water line to control *Legionella* in two hospital water system. They indicated that ClO_2 was a promising disinfectant for controlling microorganisms in drinking water. They found that chlorine dioxide and its byproducts were successfully maintained below the regulatory limits. In this study both the ClO_2 based new generation disinfectants were effective against all tested microorganisms for all contact times. The results of our study are consistent with the other studies; ClO_2 is an effective antimicrobial agent. There are only a few investigations about the toxicity of ClO_2. In a study Akamatsu *et al* [16] reported that chlorine dioxide gas up to 0.1 ppm exposed to whole body in rats continuously for six months was not toxic.

H_2O_2 has been used for water disinfection and as an antiseptic for many years. Low eco toxicity, odorless and clear colorless are some of its advantages [5]. It is a widely used antimicrobial agent that can be used in both liquid and gas form for various purposes such as preservative, disinfectant and also for sterilization applications. It has a broad spectrum antimicrobial activity and it can be define safer in comparison to other chemical agent used for same purposes. It is non-toxic and harmless for the environment [2,3].

Decomposition products of H_2O_2 are water and oxygen. In dilute solutions it breaks down relatively slow. However the effective and safer usage of hydrogen peroxide depends on the way it is used, particularly usage concentration. Because it is a strong oxidizing agent and can easily damage cellular macromolecules, including proteins, lipids, and nucleic acids. Besides, it is corrosive to the skin and eyes. Its vapor is irritating to the respiratory tract. H_2O_2 may be converted by neutrophils and macrophages to more reactive compounds such as superoxide and hydroxyl radicals. These reactive oxygen species have been suspected to adversely affect wound healing by causing cell membrane and DNA damage. For this reason some studies indicated that H_2O_2 may be more cytotoxic than bactericidal [12,17].

There are various commercial formulations available in markets that contain additional ingredients such as silver, ethanol and acids. These substances increase the efficacy of H_2O_2 based formulations [2]. Özalp *et al* [18] reported that treatment of old dental units with hydrogen peroxide/silver ions disinfectant was effective in reducing the heterotrophic bacteria in output water and prevent the formation of biofilm in a longer treatment period. Silver ions disrupt the structure of the biofilm matrix by binding to electron donor groups of biological molecules thus leading to the reductions in the number of binding sites for hydrogen bonds and electrostatic and hydrophobic interactions. In this study H_2O_2 based new generation disinfectant (stabilized by colloidal silver) was found effective against all tested microorganisms for all contact times. Although it demonstrates good antimicrobial activity, hydrogen peroxide content of it is too high and it may cause serious side effects for human like cytotoxicity.

CONCLUSION

The test new generation disinfectants showed good antimicrobial activity against all tested microorganisms at all contact times. Although their manufacturers define them as harmless products for humans and the environment, most of them are oxidizing agents and may cause side effects on the cell. Users should follow manufacturer's labelled instructions for the correct and safe usage of these products.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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